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I. Biomedical session

Neuroimaging techniques and biomarkers in contemporary psychiatry

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The search for objective biomarkers in psychiatry is crucial due to the subjective diagnostic process in the practice. Psychiatry is the only medical discipline that routinely does not perform any objective examination on the damaged organ. Imaging methods are widely used across all other medical fields and they are among the most valuable sources of information about the pathogenetic mechanisms of the diseases. Behavioral disorders result from abnormal processes in the central nervous system that have underlying biological substrates and can be localized using neuroimaging techniques. Magnetic resonance imaging (MRI) gained tremendous advance in the field of neuroscience by demonstrating the structural and functional brain aberrations in the etiology of mental disorders. Functional MRI (fMRI) contributed to the identification of abnormal activations and changes of brain connectivity in patients with mental disorders. This review will focus on the translational neuroscience approach and is contribution for the field of psychiatry.

Keywords: psychiatry, functional magnetic resonance imaging, biological validity, biomarkers

1. Introduction

Behavioral disorders are one of the leading causes of disability, with an estimated 970 million people worldwide affected. Furthermore, certain characteristics of psychiatric disorders, such as early onset and high prevalence among the working-age population, are important determinants of their negative socioeconomic effects [1–3]. Patients with serious mental illnesses have a mortality rate that is reported to be 2–3 times higher than the general population, decreasing their lifetime by 10 to 20 years [4].

Current diagnostic manuals in psychiatry are the International Classification of Diseases – ICD [5] and the Diagnostic and Statistical Manual of Mental Disorders – DSM [7] which are widely used among clinicians worldwide. However, according to those manuals mental disorders are classified only on the basis of clinical characteristics, without taking into account their etiopatho-

genetic mechanisms. The assessment methods in psychiatry remain outside of the conventional medical framework due to their low biological validity in relation to the etiological cause due to lack of knowledge about the exact pathological mechanisms [7]. In contrast to other fields of medicine where the clinicians can rely on objective measures (blood tests, imaging tools, etc.), diagnosis in psychiatry is influenced by subjectivism. This determines the “crisis” in psychiatry, which has two dimensions – a crisis of identity (that doubts about the legitimacy of psychiatry as a medical discipline) and a crisis of confidence (doubtful validity and reliability of diagnostic methods) [8].

In this regard, the most important goal in modern psychiatry is to identify biomarkers for severe psychiatric disorders to serve as an objective diagnostic tool which is key to the therapeutic process. The focus is on the translational approach in neuroscience,

which aims to integrate scientific interdisciplinary data about mind-brain interactions with their behavioral manifestations and the application of the data in clinical practice.

2. Translational neuroscience

The concept of translation can be defined as a mechanism for achieving clinical results by transforming research observations into clinically applicable methods [9]. A suitable tool in achieving this goal for psychiatry seems to be neuroimaging. Neuroimaging techniques enable the detection of disease-specific anatomical and functional brain defects, allowing for non-invasive examination with the required precision [10]. Translational techniques applicable in psychiatry are quantitative structural imaging, voxel-based neuromorphometry, functional neuroimaging and spectroscopy using MRI techniques, Positron emission tomography, and Electroencephalography.

Neuroimaging techniques and the knowledge gained so far about the underlying genetic causes, neurochemical dysfunctions and neuroinflammatory mechanisms have the potential to change the current state of “crisis” in psychiatry through the use of evidence-based methods, biomarkers and insight into the etiology of mental disorders [11].

The progress in identifying and understanding the main neurobiological substrates of psychiatric phenomena is due to initiatives such as the Research Domain Criteria (RDoC) from the National Institute of Mental Health (NIMH) [12]. The aim of RDoC is to integrate the results from neuroscience research and neuropathophysiological substrates causing specific behavioral abnormalities, thus expanding knowledge of the brain-behavioral connection and incorporating the gained knowledge into clin-

ical practice [9]. For example, MRI studies of schizophrenia make it possible to detect anatomical and functional aberrations in the brain such as disruptions in brain plasticity and connectivity, which clinically manifest themselves as cognitive disorders. Another example of an accessible translational method is pharmacological fMRI [13], which is advancement in the treatment of psychiatric conditions by examining drug interactions with the brain by searching for accurate neuroimaging biomarkers [14].

3. Does the translation approach contribute to clinical practice?

Translational approach in psychiatry includes a variety of research domains and strategies aimed at incorporating and converting scientific data into clinical practice. Important questions in this regard are whether advances in this field have contributed to the creation of more successful diagnostic or therapeutic methods, and whether biomarkers could be integrated into daily practice.

Despite years of effort and multiple trials, the findings of structural and functional abnormalities in psychiatric disorders are inconsistent [15]. The reason for this discrepancy can be found in the design of the studies themselves. Often, fMRI task-related studies are performed with cognitive tests during neuroimaging that have little or no diagnostic value. Another obstacle may be the assessment of the patient's condition carried out before or after the imaging examination itself, which may adversely affect the end result (for example, in some rapid-cycling mood disorders or in psychotic patients with florid hallucinatory symptoms) [16]. In order to correct these contradictions, in addition to combining several fMRI modalities (e.g., resting-state fMRI and task-related fMRI), it is appropriate to

conduct a diagnostic-specific assessment method during the neuroimaging itself. In this way, a biologically validated method can be established, which psychiatrists can implement in their daily practice with trust in its accuracy. Such initiative could have a positive impact on diagnosis, treatment, and monitoring of treatment response, as well as improving the selection of therapeutic method [9].

4. Establishing a novel paradigm

As a scientific effort to apply the translation model in psychiatry, our research group is concentrating on the development of a new paradigm that integrates resting-state and task-related neuroimaging simultaneously administered with diagnostically-specific clinical self-assessment scale (von Zerssen's Paranoid-Depressive Scale – PDS [17]) in two groups of patients – with paranoid syndrome in the context of schizophrenia and with depressive syndrome, in the context of recurrent depressive disorder or bipolar disorder [16, 18–21]. Our goal is to investigate whether differences in brain activation during the task could be explained by some structural or functional changes in the connectivity at rest. Our focus is on the Default mode network (DMN) and Salience network (SN) which are significant brain networks that represent specific patterns of synchronous neuronal ac-

tivity. This research is drawing on the contemporary concept that some of the psychiatric illnesses can be explained as “dysconnectivity syndromes” [22].

The DMN is the most widely studied network. It consists of the medial prefrontal cortex (PFC), posterior cingulate gyrus, precuneus and angular gyrus. It is activated when the individual focuses on their inner experiences – dreaming, predicting the future and reproducing memories. DMN is negatively correlated with brain networks that focus on external stimuli [23–25]. The SN participates in complex functions such as communication, social behavior, and self-awareness by integrating sensory, emotional and cognitive information [26]. The components of the SN are the anterior insula and the anterior cingulate gyrus. This network is associated with modulating the switching between anti-correlated networks DMN and Central executive network.

The results of our studies demonstrate strong participation of DMN structures in the schizophrenia group in responses to paranoid items on the self-assessment scale (figure 1). Because DMN is a brain network that is active at rest in healthy individuals [27], its involvement in a task observed in the schizophrenia group indicates a violation of some fundamental domains of human behavior.

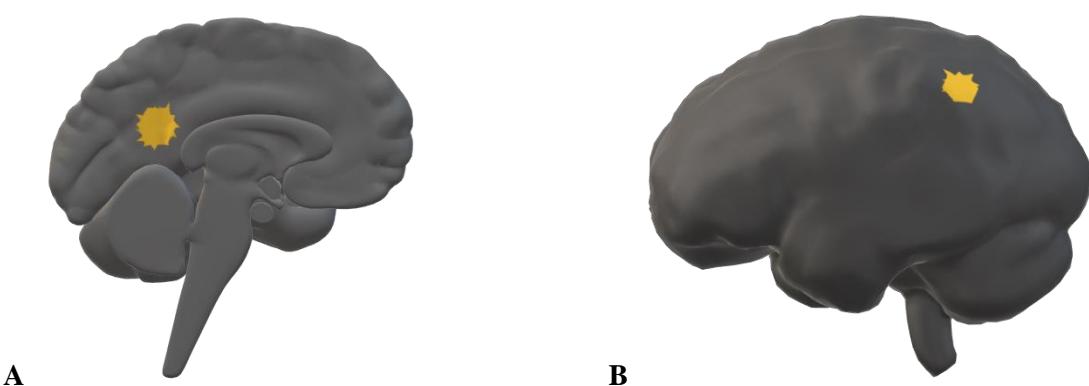


Figure 1. Clusters of activations significantly higher in schizophrenia. DMN structures. (A) – left precuneus; (B) – right posterior parietal lobule.

The results from the effective connectivity at rest show an engagement between SN and the PFC, more specifically an inhibitory effect from the lateral PFC to the anterior insula in the schizophrenia group which is completely absent in the group of depressive patients [19] (figure 2). These findings suggest the involvement of SN and DMN and abnormal interactions between them in the etiology of schizophrenia.

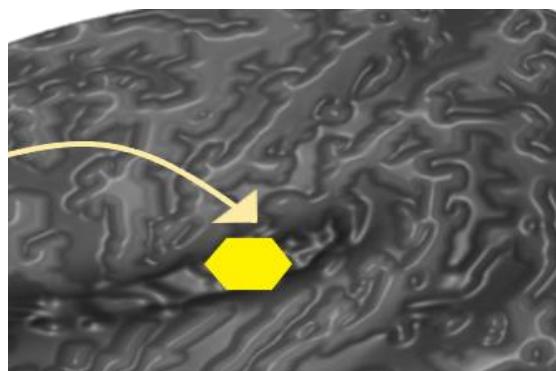


Figure 2. Inhibitory connection from the PFC to anterior insula in the schizophrenic group. Results from effective connectivity analysis.

Additionally, we investigate whether and to what degree clinical diagnostic class prediction can be strengthened by superimposing different MRI measurements (structural, task-related fMRI, and resting-state fMRI) compared to a single modality using a multivariate linear model (MLM) [21]. Important brain signatures with strong discriminatory power can be detected using MLM in patients with schizophrenia and depression. The major dopamine-ergic and noradrenaline-ergic pathways with DMN nodes appear significant when the three imaging modalities are used at the same load. The first brain signature illustrated equal loadings of the different imaging techniques, indicating that the regions involved (temporal, insular and frontal areas) have structural and functional characteristics that can distinguish between the two classes of disorders. The second signature involves

zones that are part of the Effort mode network and DMN and have high discriminative power in functional modalities, including task-related vs. resting-state fMRI. The third signature illustrates the opposing loadings of structural and functional imaging modalities and is primarily composed of temporo-occipital and motor areas [21].

The most significant structure in our MLM study was located in the Superior temporal gyrus (STG), which is involved in auditory and linguistic processing, but has been also shown to be a major structure along with the amygdala and PFC, involved in social cognition processes [28]. It is suggested that this structure plays a key role in causing auditory perceptual disturbances and disorganization of the thought [29] [30]. There is ample evidence that auditory hallucinations are associated with a functional brain networks, namely the auditory and linguistic areas of STG, as well as speech motor areas in Inferior frontal gyrus [31–33], which is also significant region in our study.

5. Conclusion

Severe psychiatric illnesses, such as schizophrenia and depression, affect a large number of people worldwide, with serious negative effects for patients, their families, society, and the financial system. It has become necessary to adjust the diagnostic and therapeutic strategy to improve the lives of those affected and to enhance their prognosis. The translational approach is emphasized, as it could provide the missing link between psychiatry and other medical disciplines that use evidence-based approaches. Many scientists around the world are focusing on neuroimaging as an appropriate tool for detecting potential biomarkers, however, difficulties in translating research data into practice still place psychiatry in

isolation from the rest of medical science. The limitations of the studies are related to the heterogeneity of the studied populations and the inappropriate design of the paradigms used, which leads to difficulties in trying to compare the obtained results.

Such shortcomings can be addressed by extending translational neuroimaging studies through separate centers using similar approaches to detect the functional MRI substrate consistent with clinical assessment and self-assessment tools in replication protocols, as well as investigating different neuroimaging modalities. This methodology could potentially lead to the re-validation of psychiatric classifications and assessment methods based on more accurate evidence-based neurobiological markers.

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The health implication of hypersensitivity reactions in administration of vaccines and the possible impact on COVID-19 vaccination

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Hypersensitivity is the exaggerated reaction observed in many individuals to different types of allergens. It causes an immune response which can lead to clinical symptoms according to the type of hypersensitivity that takes place, for example, in type 1 hypersensitivity, anaphylaxis is observed. This can cause problems when administering vaccines in individuals that suffer from severe allergic reactions. Data obtained suggests that components in vaccines can cause hypersensitivity. The function of vaccines is to promote active immunisation against a certain antigen, in this case coronavirus, helping to decrease the severity of illness caused by the virus due to the faster response by the immune system and to create a level of immunity in individuals and gradually accomplish herd immunity. It is difficult to control the virus because the main mode of transmission being airborne, therefore, vaccines present the best solution to gain immunity against the severity of the health implication from the virus. Therefore, the risk of allergic reaction from the vaccine must be investigated to protect patients when they are being immunised and to ensure safety measures are placed to decrease the potential health hazard. Individuals with a history of severe allergic reactions are required to be monitored by a healthcare professional, so that in the event of an allergic reaction, capable staff are available to manage the situation. Currently there is not enough data to suggest a significant risk of allergic reaction from the vaccine however, this requires constant evaluation and investigation to ensure the safety of patients.

Introduction

Vaccines are able to cause hypersensitivity reactions in individuals, especially those that have a history of severe allergic reaction. Hypersensitivity can occur due to the active antigen or components included in the vaccine. This has led to recent studies suggesting that the Pfizer vaccine for coronavirus, may cause allergic reaction. Therefore, this requires research and evaluation to ensure that vaccinations can be provided in a safe and secure manner, with few possible health complications.

Purpose and task

Hypersensitivity reactions

Hypersensitivity is an exaggerated immunological response to an antigen or allergen. There are four main types of hypersensitivity reactions, type 1, 2, 3 and 4. These 4 types differ due to the outcome, time of development and immunological mechanisms that are involved.

Type 1 hypersensitivity reactions

Type 1 hypersensitivity is a classical allergic reaction known as anaphylaxis caused by the admission of an allergen into the body. Allergens can vary in types and forms, which determines their entry into the body such as pollen which is inhaled and medication, for example, antibiotics, that can be ingested or injected.

There are 2 phases in hypersensitivity type 1, the sensitisation phase and clinical phase. The sensitisation phase starts with the recognition of the allergen, by macrophages and endothelial cells. Once they are engulfed, they are broken down by phagocytes and bound to proteins forming a major histocompatibility complex, MHC 2, which is then presented on the surface of these cells, becoming antigen presenting cells. T helper cells type 2, Th2, recognise and bind to the MHC2 with specific receptors, which cause the Th2 to become active and produce cytokines; interleukin 4 and 5.

The cytokines activate B cells, which proliferate and differentiate into plasma cells, these are able to produce specific monoclonal antibodies, of class immunoglobulin E, IgE, for the specific antigen. The IgE antibodies attach to the surface of mast cells, eosinophils and basophils. The clinical phase begins when the allergen binds to the antibodies and form bridges. This binding is a signal for the mast cells to release granules which contain pre-formed mediators such as histamine, heparin, and also de-novo synthesized leukotrienes and prostaglandins, this causes anaphylactic reactions. Symptoms of anaphylactic reactions include; bronchial constriction causing problems with breathing and rashes. This is due to the release of histamine and bradykinins, causing vasodilation. Due to the degranulation, there may be local activation of the phospholipase. This means arachidonic acids may be released. Depending on the pathway cyclooxygenase, COX, or lipoxygenase, LOX, production of prostaglandins and leukotrienes can occur which will affect the blood vessel wall, affecting the vessel permeability. Some of the leukotrienes secreted are known to affect the smooth muscle of the bronchial tree. It may be accompanied by increased leakage of fluid from the blood vessel into the interstitial space, therefore causing oedema. All the symptoms related to anaphylaxis are due to the presence of various mediators.

Type 2 hypersensitivity reactions

Type 2 hypersensitivity reaction refers to the cytotoxic antibody-mediated reaction causing cellular destruction due to the contact of antibodies, IgG and IgM, and the antigen on the cell or in the extracellular matrix. This can result in damage to the tissues involved.

There are three main mechanisms

through which type 2 hypersensitivity reactions are carried out. The first one is predominantly attributed to the participation of the complement system, referred to as, the complement mediated cytotoxicity/cytolysis. The second mechanism is via antibody dependent cell mediated cytotoxicity. In this mechanism the antigen-antibody complex is present on the surface of the cell and the antibody is recognized by a specific receptor on a natural killer cell which binds to the fragment crystallisable region of the antibodies. This has an opsonizing effect, promotes phagocytosis or activates intracellular apoptotic mechanisms resulting in the destruction of the cell. The third mechanism does not result in cellular destruction but rather modulation of the function of the cell.

Type 3 hypersensitivity reactions

Type 3 hypersensitivity reactions are also known as the immune complex reactions. This relies on the complement system and production of immunoglobulin classes G and M, IgG and IgM, interacting with antigens that are freely circulating in the bloodstream, forming antigen-antibody complexes. These complexes vary in size and smaller complexes can evade macrophages, therefore can be deposited in the blood vessels. There are some target organs for these deposits such as the joints and glomeruli of the kidneys. Neutrophils are attracted to the area where damage to the tissues occurs and they begin to phagocytize the cell that is injured. This leads to the release of cellular enzymes such as lysosomes which induce local inflammation and lead to vasculitis.

Type 4 hypersensitivity reactions

Type 4 hypersensitivity reactions, also known as the delayed type of hypersensitivity reactions, are T-cell mediated, specifi-

cally helper, CD4 T-cells, or cytotoxic CD8 T-cells. There are a small number of subcategories regarding type 4 hypersensitivity reactions. The first subcategory is CD4 T-cell mediated, and is present during acute reactions such as contact dermatitis. The CD4 cell mediated hypersensitivity reactions are delayed and can take up to 72 hours to develop. Another example is the reaction to poison ivy where there would be pro-inflammatory cytokines produced which would lead to macrophages being stimulated to start phagocytosis. Eventually, there is localized oedema and skin reaction. There is also a subcategory which involves CD8 T-cells and it is associated with tissue transplant rejection. The Mantoux, tuberculin skin test, is a method used to determine whether there is a presence of delayed cell-mediated immunity against *Mycobacterium tuberculosis*.

Social implication on allergies and vaccines

In modern society, allergy has become a prominent health concern in the growing population. Rates of allergies such as asthma, rhinitis and atopic dermatitis, have increased exponentially in recent decades, which coincides with the enhancement in hygiene and socioeconomic conditions. The increase in the hygiene level means that the level of exposure to microbial agents has decreased, however, such exposure is detrimental to the priming of the immune system, especially in children. The lack of exposure hinders the development of immunological tolerance to ordinary/common microbes, causing an increase in allergies. Immunologically it can be seen that the activity of the protective regulatory T cells towards allergens has decreased.

Currently there is no significant evidence to support that infant immunisation increases

the risk of atopic diseases, nor that immunisation decreases the risk of atopic diseases later in life. This suggests that there is dire need of research in this area to determine the “potential effects of timing of vaccination and of the number of antigens administered by means of vaccination on the risk of atopic diseases” (1). Evidence suggests that immunisation introduced in early life has the potential to deviate the “immune system towards a more or less allergic phenotype” (1). The timing of the administration of the vaccine to an infant can affect the child's susceptibility to allergic diseases, this can be the result of the change in susceptibility towards infections and allergies after a non-targeted vaccine.

Materials and methods

Vaccines

Vaccines are an effective and efficient method of active immunisation against certain diseases, working to decrease the death and health complications caused by such diseases and to prevent the spreading of the disease. An example of this is, the influenza and the common cold viruses. Vaccines are created with a weakened or killed antigen, which causes an immune response. This initiates the production of specific antibodies to combat the antigen and upon second exposure the memory t-cells produced are able to induce a faster and more efficient production of antibodies. Even though allergic reactions from the microbial agent itself is rare, it has been suggested to affect patients “with systemic allergic reactions after immunisation” (1). This is seen in vaccines such as the DTP vaccine. However, in the vast majority of evidence gathered, there has been a lack of extensive allergy monitoring to validate an immune-mediated allergic reaction.

Vaccines and hypersensitivity reactions

Vaccines, depending on the microbe, require different media and components to gain nutrients for growth. The components used may be residuals in the vaccine such as egg, which causes allergic reaction to individuals with egg allergy. Hypersensitivity reactions from vaccines are not uncommon, however serious acute-onset reactions are exceptionally rare. Hypersensitivity may be induced by active antigen or other components in the vaccine. Following vaccine administration clinical symptoms of hypersensitivity reaction include, “self-limited localized adverse events and, rarely, systemic reactions ranging from urticaria/angioedema to full-blown anaphylaxis with multisystem involvement” (1). The estimated probability of an anaphylaxis from the vaccine is to be “1.31 (95% CI, 0.90–1.84) per million vaccine doses” (1). This would be a major consideration when evaluating vaccines such as the influenza vaccines because this is administered yearly to the public. Influenza vaccines are prone to annual changes to its antigenic composition to accommodate to constantly evolving strains of the influenza virus. Hence, according to published evidence, providers should be mindful of the changing guidelines, especially concerning individuals with a history of egg allergy, due the involvement of embryonated eggs in the production of the vaccine.

Mechanism of hypersensitivity reactions with vaccines

To help evaluate the cause or mechanisms of the reaction, assessment of the potential immunological, immunisation-associated hypersensitivity is essential. If the assessment confirms the acute hypersensitivity then exposure to the vaccine must be through desensitisation, or depend-

ing on the level of risk, in split doses. Patients incorrectly identified as intolerant to the vaccine may be inadequately immunised and suffer from preventable diseases. Careful analysis of the adverse reaction timing and the clinical significance, along with adequate monitoring of “IgE-mediated allergy, mast cell activation, vaccine-specific IgG and complement activation, and T cell-mediated delayed-type hypersensitivity” (1) can allow healthy re-exposure to the vaccine in cases of second dosage.

Allergic reactions from vaccines are generally either acute or delayed. In the cases of acute onset, the reactions are typically type 1 hypersensitivity reactions which appear immediately to few hours after exposure, and the production of IgE antibodies. This can develop into severe life-threatening anaphylaxis, which can involve multiple organ systems, therefore, requires monitoring.

On the other hand, delayed type reaction occurs within hours or days after initial exposure, some clinical symptoms can be delayed up to 2 to 3 weeks, most common being rashes. However, delayed type hypersensitivity is not always an immunologically mediated response. Non-specific inflammation or irritant reactions normally caused by adjuvants, such as aluminium, can include persistent hard nodules at the injection site and do not generally indicate immunological hypersensitivity to vaccine constituents. The reaction is usually self-limited and does not affect the admission of second dosage.

Discussion of COVID-19

COVID-19 Pandemic

Towards the end of 2019 a new virus arose in China, which rapidly spread across countries. It was named coronavirus; it

causes severe acute respiratory syndrome hence why it was called SARS-CoV 2. Coronavirus is transmitted between people through respiratory droplets and by contact from other people. It soon became evident that the countries that entered lockdown earlier faced less of an impact by the virus, compared to those countries that delayed to implement a national lockdown. The first case of COVID-19 in Bulgaria was on the 7th of March 2020 (2) and by the 11th of March, the World Health Organisation declared that coronavirus was a pandemic (3). By the 20th of March 2020, Bulgaria began to close its borders to certain countries in order to enable close monitoring and control of people entering and leaving the country (4). Due to the prompt and effective implementation of these guidelines, the number of cases in Bulgaria were comparatively low to neighbouring countries and the first wave of cases hit much later.

Restrictions to protect against COVID-19 and importance of vaccination

Countries including Bulgaria, relaxed and tightened their restrictions according to the number of cases and deaths, in the hopes to return to normality. During the first week of October the number of cases grew exponentially in Bulgaria, which coincided with the opening of school, universities and local businesses. The peak of the wave was reached on the 17th of November 2020 reaching a record number of new cases 4,828 (2). The total number of cases in Bulgaria as of the 30th of January 2020 were 217, 574 and the total number of deaths were 8,973 (2). Vaccines are the most effective method to decrease the chances of being infected by coronavirus, in comparison to masks and social distancing which remains the primary preventive gov-

ernmental measure. Vaccination not only protects the individual but may also protect surrounding people, herd immunity. The prime reason for the COVID-19 vaccination is so that the body is triggered to produce antibodies so if future contact with COVID-19 takes place, the immune response is much faster, more specific and the antibodies remain in the blood for a longer period of time. The proverb “Prevention is better than cure” from the Dutch philosopher Desiderius Erasmus is the most appropriate for this situation.

SARS-CoV structure and Pfizer vaccine

The SARS-CoV 2 belongs to the family of viruses named Coronaviridae. Its structure consists of a positively charged single stranded RNA molecule, +ssRNA. The virus particle size is between 70–90 nanometres. It consists of surface viral protein spikes, membrane glycoproteins and nucleocapsid proteins. The envelope is embedded in the host membrane and is derived from a lipid bilayer.

The Pfizer vaccine, the first to reach the market, was a collaborative vaccine by 2 pharmaceutical companies based in the USA and Germany. This vaccine was given to over 43,000 people (5) and data has shown that “the Pfizer vaccine is 95% effective at preventing disease” (5). There is no data to indicate how long the antibodies will remain in the bloodstream, how severe the symptoms of reinfection after vaccination will be and for what duration of time does the vaccine reduce the symptoms. The Pfizer vaccine consists of RNA and is transported in a lipid particle, which is then combined with polyethylene glycol, PEG, in a step known as PEGylation. This renders it hydrophilic while ensuring that the compound has increased stability and life

span. The immune system is stimulated by the RNA template of the spike protein. This results in the activation of monoclonal antibodies against the spike proteins that are native to SARS CoV 2. It is mandatory for this vaccine to be stored at low temperatures of -70°C which poses difficulties. The vaccine is given as 2 doses; the second dose is given at least 3 weeks after the first.

Hypersensitivity reaction speculation due to polyethylene glycol

There is speculation regarding which component within the vaccine may be responsible for the allergic reaction. Some scientists suspect that this could be due to the PEG being present as this has never been used in an approved vaccine before. PEGs are present in many everyday products including shampoo, cosmetics, baby wipes, cleaners and toothpaste and their main uses are as surfactants, cleaning agents and emulsifiers (6). However, it is present in a range of drugs that have been found to occasionally trigger anaphylactoid reactions (7). As with type 1 hypersensitivity reactions, some allergists and immunologists believe that prior sensitisation to PEG leads to a higher volume of anti-PEG antibodies present in the blood of the patients. This means that they have an increased risk of an anaphylactic reaction. In general, the rate of anaphylaxis to any vaccine is “1.31 per million vaccine doses” (8) as mentioned previously which suggests that this is a very rare phenomenon. It is thought that PEGs are biologically torpid, but recent studies have shown “with 90% confidence, detectable levels of anti-PEG antibodies in 72%” are present in the global population (9). PEG seems to increase the serum levels of the immunoglobulin groups M and G, IgM and IgG, rather than immunoglobulin E, IgE, which is responsible for triggering allergic reactions.

Allergies with Pfizer vaccine

The clinical trials that took place in America consisted of several thousands of people but abnormal reactions were not recorded. The trials did not include those individuals that previously had drastic reactions to vaccines and to those that had a history of serious allergies. Those with food or drug allergies were included, but it is not clear as to whether this group was represented proportionally to the population or whether this group was underrepresented.

When comparing the vaccine to the seasonal flu vaccine, several individuals experienced allergic reaction to the Pfizer vaccine. The chance of having an allergic reaction from the Pfizer vaccine is approximately 8.5 times higher (10). For this reason, those that carry adrenaline injections, and known allergies are advised by the UK’s medicine regulator not to have the Pfizer vaccination (11). The Medicines and Healthcare Products Regulatory Agency, MHRA, are the organisation that approved the vaccine to be used in the UK. On the 10th of December 2020, the MHRA were investigating 2 cases of anaphylactoid reactions in NHS staff, after being administered with the vaccine on the 8th of December. Upon review it was discovered that both individuals have a history of severe allergies which requires them to carry adrenaline injection on their person. Despite this, both fully recovered after the reaction (11).

Guidelines for the Pfizer vaccine state that those that have an allergy to any substance (11) included in the vaccine or to a substance that resembles polyethylene glycol, such as polysorbates, should not be subjected to this vaccination (10). Furthermore, people with severe allergic reactions were excluded from clinical trials. Peter Openshaw, a professor at Imperial College London and a previous president of the

British Society for Immunology stated “As with all food and medications, there is a very small chance of an allergic reaction to any vaccine... Similar to the rollout of all new vaccines and medications, this new COVID-19 vaccine is being monitored closely by the MHRA” (11). The cases will be investigated “in more detail to understand if the allergic reactions were linked to the vaccine or were incidental” (11). However, the fact that these allergic reactions were recognised, recorded and acted upon rapidly means that the monitoring system “is working well” (11).

Safety precautions after vaccination

For individuals that suffer from severe allergic reactions, it is imperative that they remain with a clinician for approximately 30 minutes after administration of the vaccination, so that should a reaction occur, there is a capable healthcare member available to manage the situation. As the vaccination for COVID-19 is at its early stages it is difficult to evaluate and understand the effect on the population due to the limited data available. Therefore, it is paramount that the data is consistently reviewed and evaluated to ensure the safety and security of the population's health while administering the vaccine. It is important to reduce any further health implications from administering the vaccine, while promoting active immunity against coronavirus.

Conclusion

Allergic reactions are not uncommon in a number of vaccines. There is a general increase in the rate of individuals with allergies, which has been affiliated to the “hygiene hypothesis” which suggests that the development in hygiene and socioeconomic conditions has prevented the immunological tolerance of common microbes in children. This has resulted in the lack of

immunological development and higher susceptibility to allergies and illnesses (12). Anaphylactoid reactions are uncommon in general, but may occur in other vaccines, such as the annual flu vaccine. The evidence suggesting anaphylactoid reaction to the Pfizer vaccine suggests that monitoring patients while administering the vaccine is mandatory. Professor Stephen Powis, medical director for NHS England states it is “common with new vaccines” (13) to observe unpredicted reactions taking place. It is mandatory for clinicians to provide a safe environment and product, to ensure optimum and effective immunisation ensuring that patient safety is never compromised. Patients at higher risk of health complication and those who have a history of severe allergic reaction, must be monitored. The MHRA advise these individuals to carry their adrenaline injection as a precaution. Therefore, it is vital for clinicians to be to weigh the effectiveness of administering the COVID-19 vaccine in an individual after checking for health conditions, that may cause potential risks of health complications after administration. It is also important to create awareness of the potential health complication after administration of the vaccine, especially to those individuals that are most susceptible and vulnerable. Moreover, further research and data are required to evaluate the significance of allergic reaction after administration of the current COVID-19 vaccinations.

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Long-term effects of Δ^9 -tetrahydrocannabinol in hemp (*Cannabis sativa*) on the endocannabinoid system and the resulting effects on the brain and epigenome

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Cannabis is one of the most used narcotics, especially by adolescents, and it's well established, that the main effect concerns the nervous system. This review aims to summarize the available information about the long-term consequences of marijuana on the brain, focusing on the interaction between Δ^9 -tetrahydrocannabinol and the endocannabinoid system, as well as to examine a lesser-known effect of cannabis – the one on the epigenome. The endocannabinoid system is a signaling system with a regulatory function, comprising of the endocannabinoids, their receptors (CB1, CB2), and the enzymes for their manufacturing and degradation. Δ^9 -THC is the main psychotropic cannabinoid in hemp, acting as a CB1-agonist. Long-term cannabis use leads to a decrease in the receptor density in certain brain areas, wherefrom the normal action of the endocannabinoids is disturbed. Resulting are changes in brain morphology and disturbances in glutamate and dopamine signaling, which leads to low motivation. Observed is a higher disposition for addiction and a higher risk for psychosis or worsening of an already developed such. The epigenetic effects of Δ^9 -THC consist of disturbances in the expression of genes, which act in attention deficit, addiction, cancerogenesis, and others. Part of these changes is inherited by following generations, even if they themselves haven't been exposed to Δ^9 -THC. All of this proves the negative effect of chronic cannabis use. Limitations pose the differences between human and animal models, as well as the lack of a unanimous standard for the study design. Cannabis legalization would make the clarification of these questions easier.

Introduction

The endocannabinoid system (ES) belongs to the important mechanisms, thanks to which synaptic conduction, the normal functioning of the central nervous system and many other physiological processes are regulated. It comprises of the endogenous cannabinoids, their receptors, and the enzymes, responsible for their manufacturing and degradation (1). Because of the important function of the ES, every defect in the normal functioning of any of its elements will lead to serious disturbances in neural activity (1), (2), (3). Observed in the latest years is a tendency towards an increase in marijuana use, whereas 28 Million adults (15–64 years) in Europe have used it just in 2016. That is equivalent to about 5,1% the continent's population (4). The acute psychoactive and other physiological effects of cannabis owe to the activity of some its substances (the most active of which is Δ^9 -tetrahydrocannabinol/THC) as

partial agonists of the endocannabinoid receptors (3). Because they are shared both by endogenous as by exogenous cannabinoids, the observed tendency towards increased cannabis use asks the question about the interaction between these two groups of substances.

Aim of the review

The aim of the review is to summarize some of the potential effects of long-term cannabis use on the brain.

The endocannabinoid system

The endocannabinoid system is an ancient signaling system that plays a role in the normal functioning of the nervous, the immune, the cardiovascular and other systems.

Cannabinoid receptors

There are 2 types – CB1 and CB2, and both of them are associated primarily with inhibitory G-proteins (1). CB1 receptors are

expressed in greatest numbers in the central nervous system, but also by adipocytes and others. CB₁ is the point of binding for THC and is especially abundant in brain areas which play a role in information processing and the centers for reward – the frontal and the limbic neocortex, the hippocampus, amygdala and others (5). CB₂ receptors are located on the membranes of immune cells, and their activation is associated primarily with anti-inflammatory reactions.

Endocannabinoids

The endocannabinoids are a group of signaling lipids with an affinity towards the CB₁ and CB₂ receptors. The two most famous representatives are 2-arachidonoylglycerol (2-AG) and N-arachidonoyl-ethanolamine (anandamide or AEA). Because of their lipid nature, they are not stored in vesicles, but are rather produced and released when needed, acting for a couple of seconds. The endocannabinoids exert their effect mainly acting retrogradely. After excitation of the postsynaptic neuron, 2-AG and AEA are released in the synaptic space, after which they bind with CB₁-receptors on the presynaptic membrane. This leads to a decrease in the amount of released neurotransmitter by the presynaptic neuron, the result of which is either long-term or short-term suppression of excitation or suppression of inhibition. This demonstrates the important role, that endocannabinoids play in synaptic plasticity and in the regulation of neural activity (1).

Hemp (*C. sativa*)

Hemp (cannabis) is a remarkable plant, cultivated worldwide and with great economic importance. Because of its especially strong fibers, apart as a narcotic, cannabis has been used for more than 12 000 years in the manufacturing of more than 30 000 dif-

ferent products such as fishing nets, textile, cement and others (6). Used for that are plants with a low concentration of the psychoactive substances. However, in hemp, cultivated for medicinal purposes or for use as a narcotic, the concentration of Δ⁹-tetrahydrocannabinol is significantly higher (6).

Cannabinoids in *C. sativa* and their biological activity

So far 538 active substances have been isolated from hemp extracts, from which about 100 have been identified as phytocannabinoids (7). They are a diverse group of isoprenylated resorcinyl polyketides from which only Δ⁹-THC, its isomer Δ⁸-THC and to a lesser extent cannabinol (CBN) act as CB₁-receptor agonists (Fig. 1).

Δ⁹-tetrahydrocannabinol (Δ⁹-THC)

Most of the psychoactive effect of cannabis owes to Δ⁹-tetrahydrocannabinol. Its acute effect consists in the manifestation of symptoms such as hallucinations, anxiety and euphoria (5). MRI-studies show that Δ⁹-THC increases the activity in the brain systems, responsible for the comprehension of behaviorally important information, which leads to the observed alterations in time perception and sensory input (8).

Cannabidiol (CBD)

Cannabidiol is the other main cannabinoid in hemp that however does not have a psychoactive effect. On the contrary, CBD acts as a CB₁-receptor antagonist, lowering its affinity towards THC, thus preventing some of its acute effect (9). A tendency is observed in the latest years towards a *very serious reduction in CBD content in cannabis, grown as a narcotic, at the expense of increasing the THC concentration* (10).

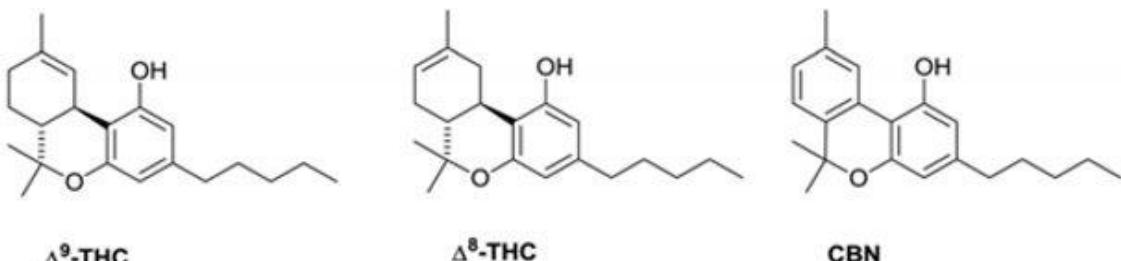


Fig. 1. Phytocannabinoids with a high affinity towards the CB₁ receptor (7).

Interaction between Δ⁹-THC and the CB₁ receptor

PET-scan analyses show that, observed in chronic cannabis users, is a decrease in CB₁-receptor density in the cortical brain areas and a subsequent down-regulation (11). This process appears to be reversible after 4 weeks of discontinued cannabis use, from which follows that the long-term effect of hemp on the brain may owe to a big extent to Δ⁹-THC addiction (Fig. 2). Another possible consequence of long-term use is the *stimulated expression of the gene for the enzyme, responsible for AEA degradation*, which additionally suppresses the effect of the endocannabinoids in the human organism (3). This may mean that some of the long-term effects on brain functioning may appear due to alterations in the epigenome – an important molecular mechanism, capable of preserving changes in gene expression, that occur in response to external factors.

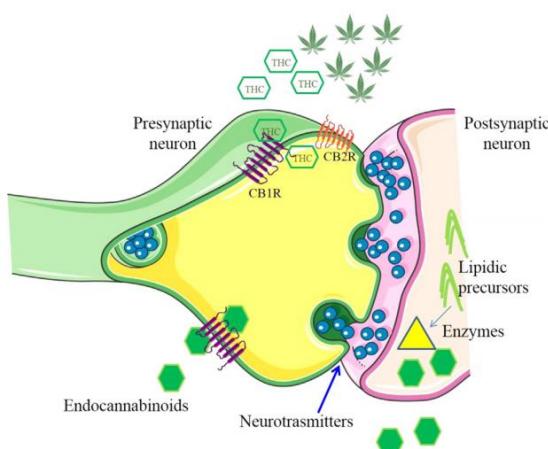


Fig. 2. Exo- and endocannabinoids (6).

Long-term effects of Δ⁹-THC

Effect on brain morphology

Unlike with animal studies, the results from human studies for the moment are inconclusive (12). Some of them prove statistically important differences in some indexes between control groups and users, such as lowered volume of the orbitofrontal cortex and higher functional connectivity in the minor forceps, innervating this area of the brain (13). Such changes could be explained with the disturbed effect of the endocannabinoids on synaptic modulation, which is amplified by the particular density of CB₁ receptors in these areas. This is not however demonstrated by all research (12).

Effect on glutamate signaling

Many studies show, that chronic Δ⁹-THC use alters glutamate signaling in the brain on many levels (14). The effects have to do with desensitization and disturbed pruning of glutamatergic synapses, alterations in glutamate receptors, transporters and others. Again however, there are discrepancies between human and animal work, which points to the need for more research in this area.

Effect on dopaminergic neurons

Conclusive is the evidence for the negative effect of chronic Δ⁹-THC use on the dopamine secretion in the ventral striatum from the neurons in the ventral tegmental area, which plays an important role in motivation and reward (15). The acute psycho-

tropic effects of cannabis owe to the disinhibition of these dopaminergic neurons, which in the long-term lowers their reaction in response to stimuli. In clinical terms this translates in lowered motivation, depression, and an increased likelihood for addiction.

Risk for psychosis

The association between the effect of cannabis on the brain and an increased risk for the development of psychosis was established early-on in the studies on the subject (16). This connection persists even when accounting for reverse causation, genetic risks and other factors. Apart from higher risk for development of psychosis, chronic use is connected to higher relapse probability, worsening of symptoms and others (15). The main hypothesis for the cause of this association has to do with the previously mentioned disturbances in glutamate and dopamine signaling in the hippocampal cortex, observed also as one of the most characteristic symptoms of schizophrenia. Another hypothesis has to do with lower AEA levels in the cerebrospinal fluid, present also in patients with psychosis (3). This possibly owes to *a decrease in the expression of the genes*, that play a role in anandamide synthesis – an example of the hypothesized epigenetic effects of Δ^9 -THC.

Conclusion

Many studies prove the diverse negative effects of chronic cannabis use, and specifically its active substance Δ^9 -THC, on the brain. Affected are the mechanisms of synaptic transmission, especially in the brain areas, rich in CB₁ receptors, which play an important role in the development of *psychosis and addiction*. More studies are necessary to clarify the molecular mechanism of these effects and the potential *differences between human and animal models*, as well

as the introduction of unified standards for the study design. Especially important remains the determination of the *risk for adolescents*, who are particularly vulnerable to the negative effects. The tendency towards cannabis legalization in multiple countries worldwide should provide better opportunities for the conduction of studies that clarify these questions.

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Don't be Sex 'XY' with SARS-CoV-2

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As sex differences increase, it becomes harder to ignore that it is a trait that has already been observed and reported in MERS-CoV and SARS-CoV-1. The difference we focus on is the ICU admission and mortality statistics and why it is greater with men as oppose to women. The reasons are related to levels of ACE2 enzymes where high quantity would open more chances between the S-protein of the COVID-19 and the ACE2 receptors. However low quantities of ACE2 enzyme would lead to cardiological problems. Disability of the ACE2 enzyme would lead to lowered testosterone secretion and further disability of the male immune system. Analyses show that women have a greater humoral and cell-mediated immune response. Women have a better immune system thanks to the X chromosome as it accommodates for many immune related genes. The expression of TLR3, TLR7 and TLR9 (toll-like receptors) is elevated in females. They actively recognise the viral RNA and DNA providing with a stronger surveillance and thus a better immunological defence. This phenomenon is also demonstrated in Klinefelter patients. Oestrogen levels particularly 17b-oestradiol (E2) activates in low and high concentrations, providing TH1-type and TH2-type responses respectively. The acknowledgement of the sexual differences leads to the ideas of using sex-specific vaccines such as mRNA-1273 and other vaccines in exploring new ways to combat past and future members of the SARS-CoV family.

Introduction

SARS-CoV-2 otherwise known as COVID-19 is the recent epidemic that is responsible for over 96.8 million cases and 2 million deaths across the globe and this number continues to rise (1). It falls in the same family as (MERS)-CoV and (SARS)-CoV-1 which caused epidemics in 2012 and 2003 respectively (2). COVID-19 enters the nasal cavity thus affecting the mucosal epithelium of the upper Gastric Intestinal tract similar to (SARS)-CoV-1 however this penetration extends deeper into the lungs. Spike binding occurs between the S protein and the Angiotensin-Converting Enzyme 2 (ACE-2 receptor) and the transmembrane protease serine 2 TMPRSS2, both of which appear on the cell surface. After gaining access into the cell, the disease hijacks the cell production capabilities and induces specific inflammatory mediators which in turn activates the macrophages causing a cytokine storm IL-1, IL-6 and TNF α . This causes endothelial cells to dilate, permeability increase and vasodilation and ultimately alveolar oedema.

Aim of the review

The aim of the review is to examine the differences between males and females with Covid-19 and the importance of that in the development of vaccines.

Differences between sexes

Between all three epidemics there are similar trends, one of which arises as differences between male and female sexes (3). In assessing the statistics, we will analyse three viewpoints – confirmed cases, ICU admissions and deaths. A study conducted by China's Infectious Disease Information System on February 11, 2020 concluded that 51.4% of patients out of 44,672 confirmed cases were male (4). A parallel analysis was conducted in United States, this time with a larger scale of 1,320,488 confirmed cases of COVID-19 across a time span of January to May 2020 and it yielded similar results to the analysis done by China's Infectious Disease Information System with a statistical conclusion in America of 51.1% female and 48.9% male (4). In a selection of European countries ratio between

male and female confirmed cases remained as 1:1 (Fig. 1) (5).

Male:female ratios Covid-19

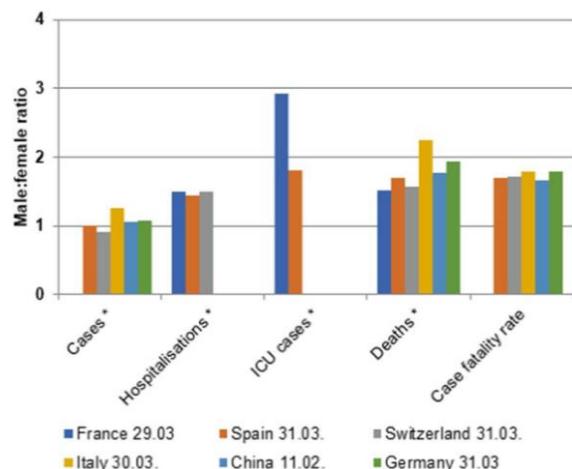


Figure 1. Male to Female ratios of COVID-19 confirmed cases, hospitalization, ICU admission, death and case fatality in China and European countries as of April 2, 2020 (7).

More up to date statistics as of the start of 2021 would show that there may be a sex-linked difference with 16 countries having confirmed cases for males which were over 70% (1). Majority of these countries being Southern East Asia, Middle East Asia and African. An earlier study was conducted in January of 2020 in China at the outbreak of COVID-19 and the trend of sex difference was immediately acknowledged for mortality and ICU admission. The result of this study concluded that “men had much more serious cases of COVID-19 than female in a classification of severity including Mild+ Moderate, Severe and Critical”. In the study of the first 37 cases of deceased patients of the same study, 70% were men whereas 29% were woman. It is to be acknowledged that both men and women had the same susceptibility and none of the patients were pediatric patients under 14 years. In fact, further information would have us acknowledge that the deceased patients were in age range from 65–81 years,

but the conclusion remains to be that men are 2.4 times more prone to death (6). To corroborate the results that men are more prone to greater fatality and mortality from COVID-19, studies from countries around Europe namely Italy, France, Switzerland and United Kingdom yielded similar conclusions (7). The Global Health 50/50 research initiative provides another collection of data (1) proving that confirmed COVID-19 cases show no sex differences however there were sex linked differences when it came to ICU hospitalization and death results. The Kaplan and Meier curve shows that mortality for men is greater than for women and this correlation became increasingly stronger as the groups increased (8). The Global Health 50/50 data initiative shows that 8 of the 10 countries with the highest confirmed cases reported a sex differentiated pattern on ICU cases and deaths (1).

Mechanism of the ACE2 and TMPRSS2

As we have already discussed in order for SARS-CoV-2 and SARS-CoV to gain entry into the cell, they require the ACE-2. Normally this enzyme is actively engaged in the renin-angiotensin aldosterone system (RAS) which controls blood regulation and actions of vasodilation and vasoconstriction. It also plays an active role in regulating cardiomyocytes, cardiac fibroblast and coronary endothelial cells in heart failure with reduced ejection fraction and heart failure with preserved ejection fraction (9). In the creation of a cytokine storm, high levels of IL-1 and IL-6 are released causing an inflammatory reaction in the alveoli. The attack on the ACE2 causes major respiratory problems but also on the heart. ACE2 is 2-fold, too many ACE2 and this would increase chances of COVID-19 binding how-

ever less ACE2 will lead to HF. After many pharmacological and clinical studies, it is found that ACE2 plays major counter regulatory roles of ACE2/Ang 1–7 in the activated RAS which increases susceptibility of heart failure with preserved ejection fraction over time. If levels of operating ACE2 have been reduced, patient's susceptibility of HF would be severely enhanced due to the activation of NADPH oxidase system in a pressure overload-induced heart failure (10). Further consequences of low ACE2 quantities would be the increased Ang II levels and decreased Ang 1–7 levels in the infarct zones which would generate high reactive oxygen species. Further effects of lack of ACE2 are the increased expression of matrix metalloproteinase 2 and 9 which combines with increased gelatinase levels and lead to a disrupted extracellular matrix structure post MI (9). Of course, the sensible treatment to this would be to increase the ACE2 levels with a supplement post MI however this may increase the likelihood of a secondary attack by COVID-19 in taking advantage of the extra ACE2 receptors (11). Relationship between high expression of ACE2 receptor and organ failure were acknowledged in SARS 2002/2003 and 35% of myocardial tissue from deceased patients caused by SARS had low ACE2 protein express and viral RNA (12). A hypothesis as to why males, particularly in an older age range, are more susceptible to ICU admission and mortality could be due to the low levels of testosterone. The strength and quantity of testosterone decreases as age increases (13). As well as appearing in pulmonary organs and in many other vital organs, TMPRSS2 is notably expressed in Leydig and Sertoli cells of the testis (14). In fact, TMPRSS2 is expressed higher in the prostate than any other human

tissue, by several folds even though TMPRSS2 functions are not exactly clear. Testosterone and androgens control gene expression of the TMPRSS2 and seeing that men have up 7–8 times more testosterone levels than women, by deduction TMPRSS2 expression would be greater in men this would open up greater chances for viral entry into target hosts (15). With lower testosterone this indeed would mean lower ACE-2/SARS-CoV-2 binding however, in the increase of Angiotensin-II and decrease of angiotensin 1–7, the result would be the build-up of superoxide species. ACE2 is the main pathway for COVID-19 invasion and men have much higher ACE2 expression compared to women and with this higher ACE2 expression means enhanced chances of contracting COVID-19. The ACE2 gene is found on the X chromosome and women have two XX chromosomes even though only one is activated at any one time to maintain equal gene expression by law of X-inactivation. However, the gene location of ACE2 is activated in both (16) meaning that the phenotype expression is different (17). When COVID-19 bind to ACE 2, COVID-19 takes up most of the available residues with its RBM (18). This is true for men and women alike. However, the same residue sequences for the linkage between COVID-19 and the ACE2 of the second X chromosome is low and allows for the ACE2 to continue its functional duty of preventing oedema by cleaving Ang II to Ang 1–7. Essentially the two X chromosome have two separate mechanisms, yet they were work together like a combination lock. Male chromosomes represent the lock and key with only one mechanism and one key. The female still has a backup failsafe mechanism.

Differences in the immune response

The X chromosome has greater immune response again owing to the extra chromosome but also to the secretion of oestrogen which compared to the earlier explanation of testosterone proves to be relatively less affected by the presence of COVID-19. As with men, both sexes have decreased immunity as age increases. In the analysis of the statistics of the immune response women have greater humoral and cell mediated response (19). In the parameters of immunoglobulin, B cell expression, antibody response females are higher (20). Results have further proven women having higher CD3+ and CD4+ percentages than males who in turn had higher NK and CD8+ values. This track record of performing a better humoral immune response in females has been established from the statistics in malignant cancer, Treponema Pallidum (syphilis) and Influenza A as well as Hepatitis C which are more common in males. This again is due to the extra female X chromosome which accommodates for many immune related genes. We talk specifically of the elevated expression of TLR3, TLR7 and TLR9 in females. Men have higher expression of TLR 2 and TLR4. TLR 2 and TLR4 work by recognizing pathogen-associated molecular patterns (PAMPs) in the cell wall however TLR3, TLR7 and TLR9 actively recognise the viral RNA and DNA providing with a stronger surveillance and thus a better immunological defence. Of the toll-like receptors that have higher expression in females, TLR 7 has especially high expression as it escapes X-inactivation, and this allows for the greater activation of IFN- α and plasmacytoid DC which supports a stronger immune response. The power of the X can be further extended in cases of Klinefelter men who have the trisomy expression of XXY which have also ex-

pressed greater TLR-7 levels as oppose to men (21). The overall immunological strength and resistance to COVID-19 is also owing to female specific hormones, namely progesterone and oestrogen. Estrogenic acts through its receptors known as ER α and ER β . ER α are expressed on predominant forms of CD4+ cells whereas ER β were expressed on predominant forms of B-cells. The binding between hormone and receptor would immediately influence production of cytokines and chemokines. The second oestrogen form 17 β -oestradiol (E2) plays a major factor in immunity particularly in innate immunity and in adaptive immune response in an indirect way by activating innate immune cells first. Many trials in humans and mice show increase in neutrophil levels in the blood and the lungs and in an interaction between E2 and NK there is an elevated production of interferon gamma. In assessing the peritoneal macrophages in rodents, expression of PRRs and TLR4 were increased (22). E2 acts two-fold in terms of concentration. In low concentration of E2, TH1-type response is increased which is responsible for stimulation of cellular mediated immune response and B-cells which produces an IgM and IgG1 however in high E2 concentrations, TH2-type responses and humoral immunity are increased. This causes B-cell proliferation and increases IL-4 levels. It was also found that transgender men had received oestrogen therapy and androgen antagonists had ability to express ACE2 in the testicular cells which reduced the build-up of Ang II (23).

Importance for vaccines

The biological differences in sex are great when it comes to differences in tackling COVID-19 and indeed many other viral diseases. This leads onto the idea sex-based/specific vaccines. Reports from a

vaccine trial of mRNA-1273 where 22 men and 23 women participated by receiving 2 injections of 3 dosage levels showed safer and stronger immunogenicity outcomes with mild injection site reactions. The immunogenicity and adverse events for sex was not disaggregated thus promising gender equality in outcome. Phases 2 and 3 of the mRNAs – 1273 are still ongoing. Other promising vaccine trials include ChAdOx1 again showing no disaggregated differences and acceptable T-cell immune response in all 1077 participants. A trial of adenovirus-vector vaccine candidate Ad5 found that females experience fever as an adverse event. There have already been trials set in place for an oestrogen booster in post-menopausal women also opening the doors of tailored vaccination and oestrogen related vaccines for men and for women.

Conclusion

Given the immune differences, biological function and statistical analysis, there is a clear and defined difference between the sexes. We believe that further discussion on gender specific behaviour, psychiatry, psychoneurobiology and psycho-neuroendocrine immune system, role of progesterone, all of which has not been discussed in this review, would, in prediction, only broaden the difference between men and women's response to COVID-19. "Will men need a booster vaccine?" "Will females need follow up dosage?" "How much stronger should the dosage be for someone with chromosome XY compared to another with XXY?" These questions must be brought to the panel. As the world moves into a direction of desiring gender diversity and at the same time equality, we believe that we should model are vaccines in such a manner too. As to why sex specific vaccines are not yet in major discussion as a

subset of tailored vaccines is simply due to the lack of awareness and lack of statistical evidence too in order to bring this sex-differentiation problem to the centre stage.

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Diagnostic imaging of lung changes at different stages of COVID-19 infection

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Introduction: Changes in the lung parenchyma caused by COVID-19 infection, although found in other diseases, have been shown to be early and specific to the newly emerged in 2020 disease, and these changes can be seen even in asymptomatic patients. Currently, more than 90 million people have encountered the virus and the number is constantly growing. The pulmonary changes at different stages have different characteristics. Knowing these changes is the key to properly diagnose patients, especially when they are an accidental finding. In the present review article, we would like to show our experience in the diagnosis of pulmonary changes, related to COVID-19 infection, performed by high-resolution computed tomography (HRCT) of the thorax and radiography, in outpatients and hospitalized patient at different stages of the disease, as well as control imaging one month after recovery.

Keywords: HRCT, Chest X-ray, COVID-19, pulmonary changes, ground-glass opacities

Background

COVID-19 infection is spreading worldwide reaching a pandemic stage with over 90 million people being affected.

Reverse transcription polymerase chain reaction (RT-PCR) tests are the first step in detecting currently infected patients due to their high sensitivity and over 99% specificity. Unfortunately, results might be affected by a few factors – for example, specimen adequacy, size or acquisition time [1, 2]. Due to the human factor, it turned out that if the result is repeated, there may be a discrepancy and the patient may be actually COVID-19-positive. [3]. The main imaging studies that are performed for diagnosis at different stages of the disease are HR-CT and Chest X-ray. In two studies by Ai et al [4] and Caruso et al [5] the chest computed tomography was reported to have over 97% sensitivity. This means that the diagnostic imaging plays key role in coronavirus fight. Radiologists will be able not only to diagnose COVID-19 infection, but also to follow-up the patient's condition.

Pulmonary changes in different stages of the disease vary. Detailed knowledge of the

changes is required, aiming to distinguish patients in the acute stage of the disease from those in the post-recovery period. This is especially important in the asymptomatic course. In this article we would like to show our diagnostic and clinical experience with patients, passed through Department of Diagnostic imaging in University Hospital Kaspela, Plovdiv.

Pulmonary changes in the early/initial stage (0–4 days) There are no lung changes in the first few days after the first symptom of the patient. Performing a CT scan is not appropriate. A normal CT does not mean that the patient is healthy. Having a positive RT-PCR or a negative one with only symptoms of the disease also means that the patient should be quarantined and treated properly. However, X-ray might be performed in the early stages in order to exclude other pathologies. Features like pleural effusion, pneumothorax or lymphadenopathy are not typical for COVID-19. If they are discovered, the patient should undergo further examination to rule out other disease. [6]

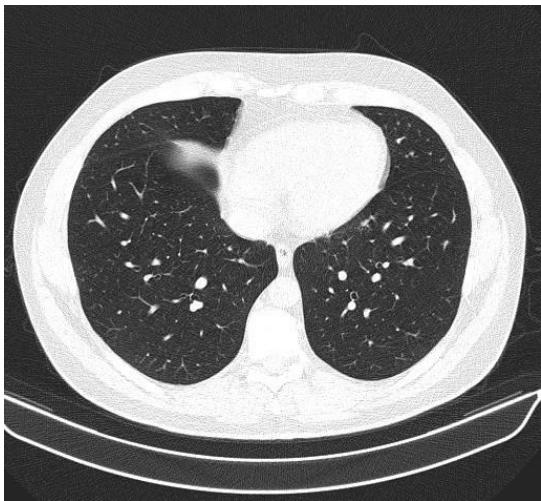


Figure 1. 35-year-old male patient with clinical symptoms and positive RT-PCR test. HRCT does not show any changes in lung parenchyma.

Progressive stage (5–8 days) At the progressive stage, specific findings appear, which are now considered even pathognomonic in COVID-19. The first one of them are the so called ground glass opacities. They represent the inflammation of the alveolar septa with partially filled with liquid alveoli. As a feature in the lung diagnostic imaging they have plenty of etiologies – infection, acute respiratory distress syndrome, edema, neoplasm. On the one hand, the ground glass opacities by a hydrostatic

pulmonary edema are more diffuse, with predominance in the parahilar regions, around the vessels. On the other hand, the ground glass opacities in COVID-19 have very typical characteristics. They are seen as patchy, usually at the basis of the lungs, bilateral with poorly defined margins. Almost all of them are located in the periphery of the lungs, subpleural.

A retrospective analyse of the imaging features by Guan et al. reported the presence of ground glass opacities in all 53 patients (100%). [7] They also evolve during the process. Up to the 10th day the opacifications have a low density and a small size. Typical patterns of the ground glass opacities (GGO) are: rounded, linear, crazy paving sign. Crazy paving sign – superimposition of thickened interlobular septa on the ground glass opacities.

A meta-analyses show that the ground glass opacities are not only the most common feature, but they are also the first one. What's more they can be seen on an X-ray as well as on a CT scan. One X-ray is up to six times cheaper than a HRCT which makes it available for a bigger part of the population.

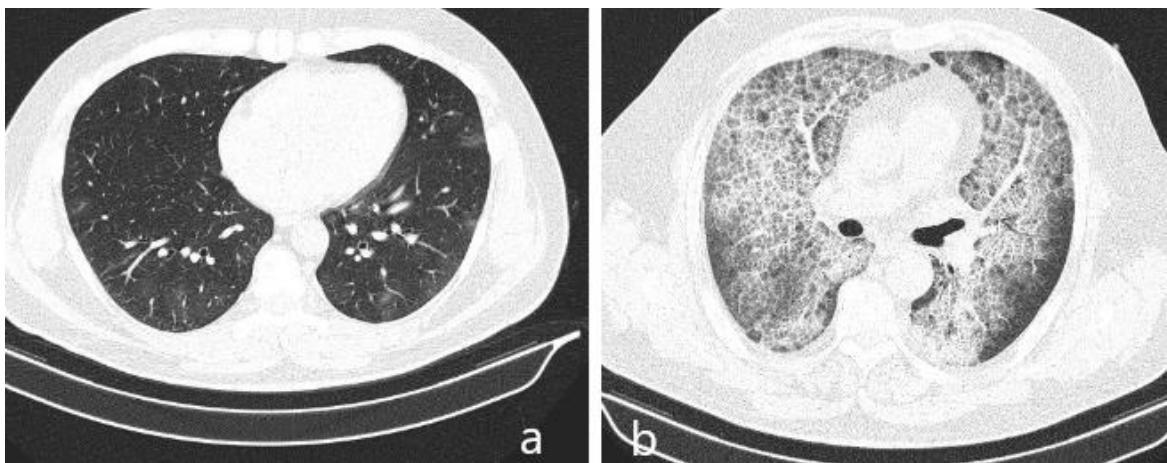


Figure 2. a – 57-year-old patient with GGO, HRCT was performed on 5th day after positive RT-PCR. b – 62-year-old patient with crazy-paving on 9th day of positive RT-PCR test. The HRCT show diffuse bilateral crazy-paving opacification.

Peak stage (10–13 days) The GGO are a typical feature in this period of the disease as well. Compared with a previous study they will be greater, with increased density. The consolidations represent the exudates in the alveoli. They can be usually found in the peak stage with a density higher than the GGO. In severe cases the whole parenchyma of the lungs might be presented white. There is documented correlation between appearance of consolidation and worsening of the clinical presentation of patients. Another feature that shows progression of the disease is the presence of reticulations.

The coronavirus binds to the zinc peptidase angiotensin converting enzyme on the endothelium of the vessels. The suppression of ACE leads to enlargement of the vessels that might be seen on the CT scan near the ground glass opacities. [8] Rare radiological signs of COVID-19 include bronchiectasis, cavitation, pleural or pericardial effusion, lymphadenopathy. The enlargement of lymph nodes (short axial diameter > 1 cm) is known to be connected with inflammation or necrosis. For that reason, a progression of COVID-19 infection or superimposition of bacterial infection should be considered. [9]



Figure 3. 63-year-old female with GGO, crazy paving and consolidation of left lung parenchyma.

Absorption stage (> 14 days) The crazy paving sign is the first to be resolved, followed by disappearance of the consolidations and ground glass opacities. There are two ways of healing – total clearing of the lungs without consequences or the formation of fibrosis at the site of changes. The formation of fibrosis is controversial – some studies suggest that it is a sign of healing or staging of the disease. Others, controversially suggest that it shows higher severity of the process. The clinical practice showed that a few months after the beginning of the infection no changes are found on X-ray or HRCT.

Discussion

To detect COVID-19 infection, patients undergo clinical examination, RT-PCR and imaging. The high sensitivity of CT necessitated its usage in the early stages of the disease, when the result of RT-PCR is uncertain. Although the findings have a relatively low specificity for covid infection, some of them have characteristics that in the epidemic situation are considered positive. [10] In this way, isolation in a timely-manner of suspected patients is achieved, thus preventing the further spread of the infection.

Imaging departments face a huge challenge. In hospitals with a single CT scan, there is a huge risk of mixing healthy and infected patients. After each suspected COVID-19 infected patient, a disinfection of the technique is required, which is expensive and time-consuming. In hospitals with two computed tomographs such as University hospital “Kaspela” this problem was overcomed by dividing the flow of patient – one of the them is used only for patients from the COVID-19-department. [11]

The sensitivity of imaging methods depends on the stage of the disease. Perform-

ing an X-ray in the first few days would help to exclude another reason for the symptoms. When the changes are discrete, we get more reliable results from HRCT. The presence of bilateral, peripheral, subpleural ground – glass opacities in this pandemic situation is almost always connected with lung changes due to COVID-19 infection.

Conclusion

Early detection of Covid-19 patients and their isolation have been shown to effectively control the pandemic. For this purpose, integration of all available resources

is used. HR-CT showed specific features in the different stages of the infection. The ground glass opacities are the most typical one and the first to be seen. Later, we can discover crazy paving sign, consolidation, reticulation, vessel enlargement. The presence of pleural or pericardial effusion, caviation, bronchiectasis or lymphadenopathy is very rare. There is a full recovery of the lungs with no long-term consequence. In conclusion, the diagnostic imaging is very important for the detection, management and follow-up of patient affected by Covid-19 infection.

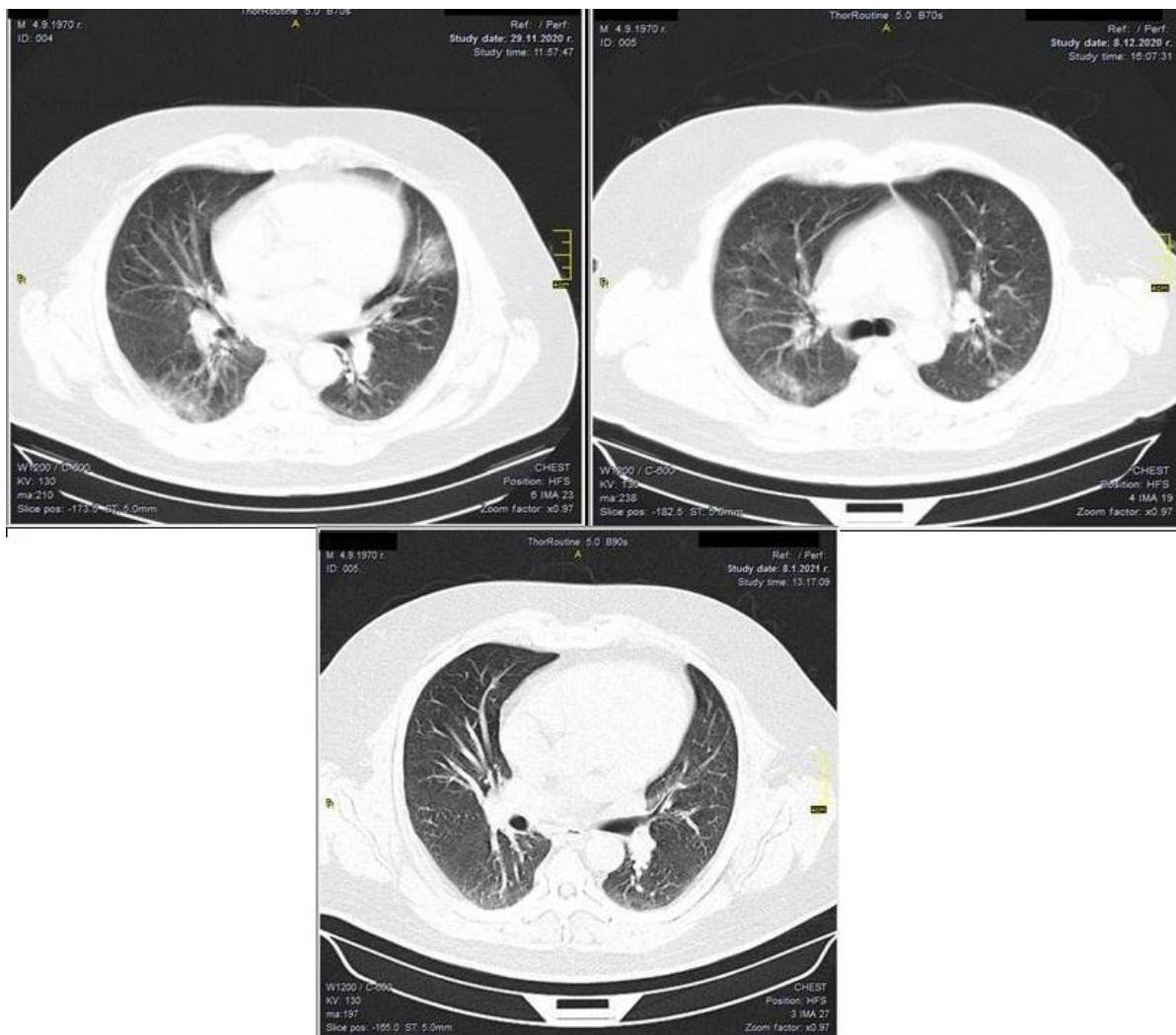


Figure 4. 50-year-old patient with CT scan in dynamic in different stages of the disease – 5th day (GGO), 15th day (consolidation); 45th day (no lung changes).

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CT-coronarography imaging in suspected ischemic heart disease: a case report of LCA narrowing

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CT is a reliable diagnostic imaging tool for noninvasive evaluation of the coronary arteries. In this article we want to present a case of 75 old male with chest pain and suspected ischemic heart disease. The results of CT-coronary angiography show atherosclerotic plaques with calcifications in the left anterior descending artery and the left circumflex artery. CT-coronarography is considered especially helpful in patients with low or intermediate probability for coronary artery stenosis and may be an alternative to invasive coronary angiography.

Keywords: coronary artery disease, atherosclerosis, chest pain, CT-coronarography

Introduction

Coronary artery disease is a severe condition and a leading cause of mortality [1, 2]. Myocardial ischemia is a disorder that may cause stable or unstable symptoms of angina, cardiac arrhythmias, and, less frequently, shortness of breath secondary to ischemic left ventricular dysfunction [3]. The current paper will describe a male patient with suspected ischemic heart disease and CT's role as a diagnostic tool.

Case report

Background

The presented case is of a 75 old male with suspected coronary disease.

The patient has pain behind the sternum for nearly two months before the examination and shortness of breath.

Methods

ECG; Laboratory tests; Echocardiography and abdominal Ultrasound; Chest X-ray; native chest CT scan followed by a CT-coronarography, and digital subtraction angiography.

Results

ECG: Sinus rhythm, left type.

The patient has dyslipidemia with elevated cholesterol levels – 7,5 mmol/l and triglycerides – 4,4 mmol/l, and concomitant arterial hypertension/III grade.

Echocardiography: LA – 3,41 sm.; Ao

sinus – 3,31 sm. End-diastolic volume of LV 97 ml; End-systolic volume of LV – 49 ml; Ejection fraction – 48%; low hypokinesis of the left ventricle with hypertrophy. The Abdominal Ultrasound shows steatosis of the liver. The patient has shortness of breath, and because of COVID 19 pandemics, an X-ray followed by a native chest CT scan is performed. The results show a normal lung. CT-coronarography is performed on 64 slice CT with iodine contrast. The coronary CT angiography results indicate atherosclerotic plaques with calcifications in the left anterior descending artery's proximal segment and the left circumflex artery's proximal and middle segments. (Figures 1; 2; 2.1; 3; 4; 5). After the examination, invasive coronary angiography is performed. Conservative treatment is chosen in the specific case.



Figure 1. Curved reformat of CTCA demonstrating calcified plaques in the proximal segment of the left anterior descending artery.



Figure 2. Curved reformat of CTCA demonstrating calcified plaques in the proximal segment of circumflex artery.

Figure 2.1. Curved reformat of CTCA demonstrating calcified plaques in the proximal and middle segments of circumflex artery.

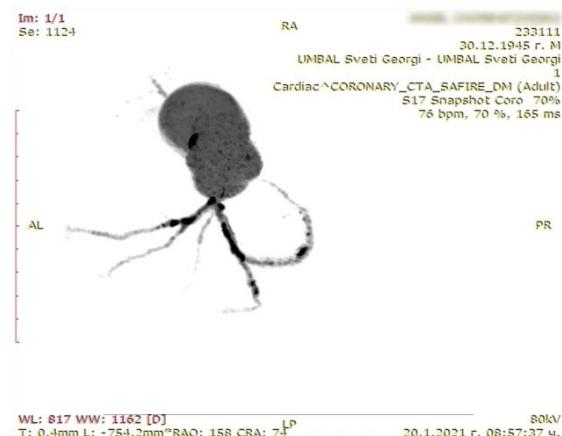


Figure 3. 3D-image of CT-coronary angiography demonstrating calcified plaques in the left anterior descending artery and circumflex artery.



Figure 4. Axial CT-scan MIP (maximum intensity projection) reconstruction demonstrating the left anterior descending artery and circumflex artery with visible calcified plaques.



Figure 5. Volume-rendered 3-D reconstruction showing the left anterior descending coronary artery as well as the circumflex coronary artery.

Discussion

In recent years, several diagnostic methods are developed for coronary atherosclerotic disease. Notably, cardiac CT is more efficient than electrocardiogram and intravascular ultrasound in the diagnosis of coronary atherosclerosis. In particular cases, CT-coronary angiography may be an alternative to invasive coronary angiography and a final test to exclude coronary artery stenosis [4]. CT-coronarography is a noninvasive diagnostic modality that can provide detailed diagnostic information in patients with intermediate or low probability for obstructive coronary artery disease and non-acute symptoms with stable chest pain [2].

In patients with a high probability of severe cardiac ischemia, invasive coronary angiography is recommended to visualize the coronary arteries and detect their pathology, allowing interventional therapy and revascularization. CT should be considered to rule out alternative diagnoses such as pulmonary embolism or aortic dissection [2].

CT-coronarography identifies calcified and non-calcified plaques, high-risk plaques, the presence of positive remodeling, congenital coronary artery anomalies, and evaluates coronary artery bypass grafts /location and patency of grafts/ [2, 4, 5].

Performing coronary CT angiography in some instances require patients premedication in order to reduce the heart rate/often beta-blockers/; ECG-synchronization and at least 64-slice CT. Avoiding potential complications of invasive coronary angiography; providing low dose scanning and short examination times; dual-source modern CT-machines could avoid even ECG-gating.

Limitations include irregular heart rhythm; fast heart rates > 70 bpm/; severe calcifications; contrast nephrotoxicity; allergy to iodine-containing contrast [6].

Conclusions

CT coronary angiography is an excellent method for detecting cardiovascular disease and, more notably, coronary atherosclerosis. Coronary CT-angiography provides high-quality visualization of the coronary arteries' anatomy and is established as the

preferred modality to exclude coronary artery pathology in patients with suspected ischemic heart disease and with a clinical risk profile. CT can identify vascular pathology to motivate prevention and facilitate treatment.

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Basal cell carcinoma – one tumor with many faces. Epidemiological, morphological and clinical implications

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Introduction: Basal cell carcinoma is the most common malignancy among people. In the past years its incidence is increasing progressively. Usually it occurs after the fourth decade, reaching its peak after the age of 60. Different histological subtypes of BCC have been described: superficial, nodular, micronodular, adenoid-cystic, pigmented, infiltrative, basosquamous and others. The last two mentioned are considered more aggressive variants with higher recurrence rates. **Aim and methods:** We selected retrospectively 1303 BCC cases, newly diagnosed in Complex Oncologic Center – Plovdiv for the period between 2017 and 2019 and we analyzed if there are any correlations between sex, age, localization and histologic subtype. **Results and discussion:** 54% of the patients were men, 46% – women. The mean age was 72 years. The majority of the cases were localized in the head and neck region. In 12% of the patients multiple lesion were observed. Nodular variant was the most common histologic subtype. The incidence of BCC increased with age in both sexes, but for patients, under the age of 40, predominated the female gender. With age, the frequency of nodular and aggressive subtypes was increasing, while the frequency of superficial variants was decreasing. Superficial histological subtype was registered more often in the area of the thorax and upper limb, while the incidence of aggressive subtypes was higher in the head and neck region. **Conclusion:** In the era of the personalized therapy, making histological differentiation between the many types and subtypes of BCC is crucial for optimizing diagnostic and treatment strategies.

Introduction

Basal cell carcinoma is the most common cutaneous neoplasm, accounting for 70% of primary malignancies of the skin.^{1,2,3} Although it rarely metastasizes and has a low morbidity, it constitutes a significant financial burden for the health care systems, due to its high incidence.

Risk factors for developing basal cell carcinoma include: fair skin type, blue eyes, red hair, easy freckling, pronounced and prolonged exposure to ultraviolet light, tendency to burn rather to tan, northern European ethnic origin, use of tanning beds, exposure to therapeutic ionizing radiation, HIV seropositivity, immunosuppression in organ transplant recipients and genetic syndromes, like nevoid basal cell carcinoma syndrome, xeroderma pigmentosum and others^{2,4}. Typically, basal cell carcinomas arise after the fourth decade of life and there is a slightly greater incidence among males^{2,3}.

Clinically BCC usually presents as pearly translucent plaque, papule or nodule with teleangiectasias. Ulceration and overlying crusting are common findings. Pigmented variants may occur as a brown, blue or black lesion, raising differential concern for melanocytic lesion^{1,3}.

Majority of basal cell carcinomas are slowly growing with local destructive characteristics. Overall risk of metastasis is estimated to be 0.05%³. Management is predominantly surgical in the form of complete excision, electrodesiccation and curettage or Mohs surgery⁴. Topical treatment can be successfully used for certain non-aggressive histologic subtypes. In cases of inoperable cases, radiation therapy is a great alternative.⁴ Also in the recent years hedgehog pathway inhibitors (sonidegib and modeglib) have been approved as a target therapy for basal cell carcinoma.⁵

Recent studies have shown differences in the distribution of superficial basal cell car-

cinoma compared to the other histologic subtypes with respect mainly to localization and age.

With this study, we aim to analyze the epidemiological pattern of basal cell carcinoma for our region and to verify a possible association between histologic subtypes and age, sex and localization.

Materials and methods

We selected all cases of newly diagnosed basal cell carcinoma for the period of three consecutive years – from 2017 to 2019. Data about the sex and age at excision of the patients and about the size, histologic subtype and anatomic localization of the tumors were included. The information was derived from the pathologic reports. The study included individuals of both genders with no age restrictions. Patients, diagnosed with two or more BCCs, were counted as one BCC case with multiple lesions.

The topographic distribution was categorized as follows: head and neck, trunk, upper limb, lower limb and genitals. The head region was further subdivided into face, ear, nose, lips, eyelid and scalp.

Data regarding the size were analyzed in a continuous manner and further categorized into 3 groups: tumors smaller than 2 cm, tumors greater than 2 cm, but smaller than 5 cm, and giant tumors larger than 5 cm.

Basal cell carcinomas were classified into one of the following histologic subtypes:²

- superficial BCC, when nests of atypical basaloid cells attached to the epidermis separated by areas of uninvolved epidermis

were present; (Fig. 1-B)

- nodular BCC – large, rounded, predominantly dermal-based nests with prominent peripheral palisading (Fig. 1-A)
- infiltrative – small cords and nests of atypical basaloid cells, often deeply invasive
- micronodular – predominantly dermal-based small nests of atypical basaloid cells with infiltrative growth and marked peripheral palisading (Fig. 1-C)
- basosquamous/metatypical – basal cell carcinoma with prominent areas of squamous differentiation
- morpheaform/desmoplastic – infiltrative strands and nests associated with dense sclerotic stroma
- adenoid-cystic – nests and larger nodules of atypical basaloid cells with mucous-containing pools and peripheral palisading (Fig. 1-D)
- pigmented – basal cell carcinomas with abundant amount of melanin pigment
- mixed – basal cell carcinomas with admixed features of two or more of the above mentioned histologic subtypes
- other rare variants, including fibroepithelioma of Pinkus, clear cell and basoskeletal variant.

Infiltrative, morpheaform and metatypical histologic variants were further subcategorized as aggressive type BCCs, while all others histologic variants were grouped as less aggressive BCCs.

Statistical analysis of the data was performed using IBM SPSS Statistics 19. Statistical differences between proportions were determined by χ^2 analysis. $P < 0.05$ was considered significant.

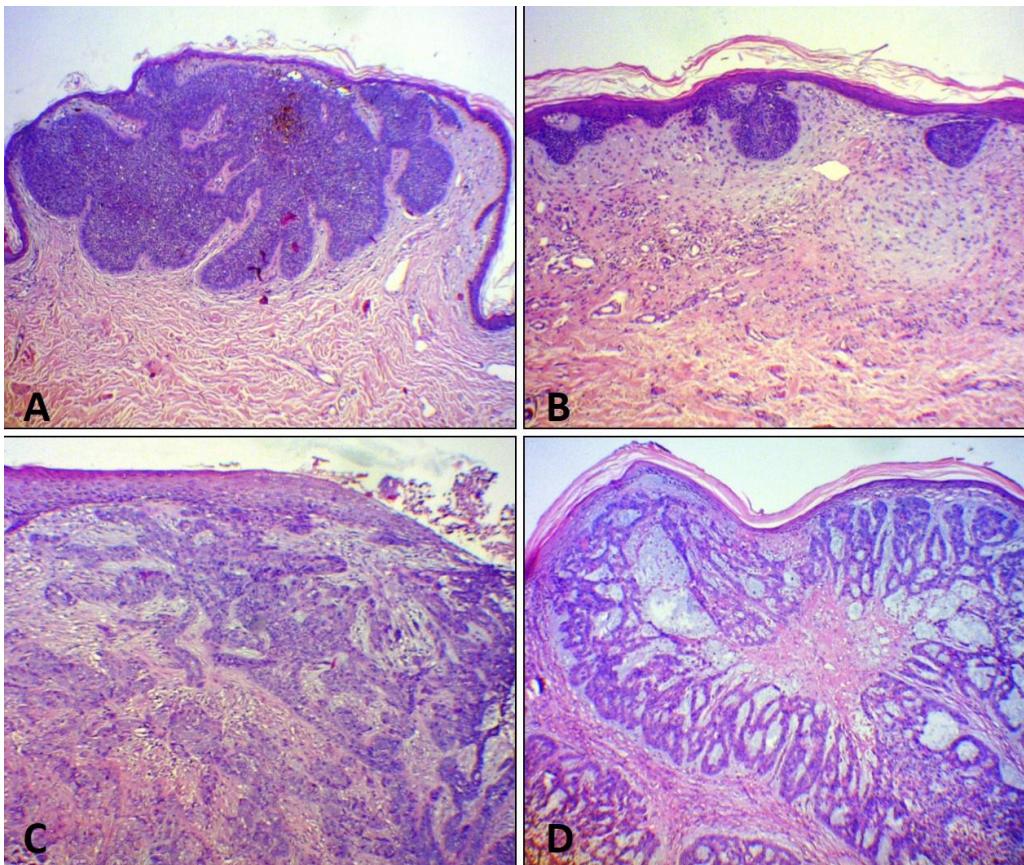


Figure 1. A/ Nodular basal cell carcinoma. B/ Superficial basal cell carcinoma. C/ Infiltrative basal cell carcinoma. D/ Adenoid-cystic basal cell carcinoma.

Results

Among 1303 patients 702 (53,9%) were males and 601 (46,1%) were females with mean age of 72 (SD = 11 years). Minimum age was 23 years, maximum age was 93 years. Most cases (86,2%) involved patients older than 60 years. Only 16 cases were detected in patients younger than 40 years. In both sexes incidence of basal cell carcinoma increased with age, but it is interesting to mention, that among the patients under 40 years, 75% were females.

All the patients underwent excisional biopsy. Mean diameter of the lesions was 11 mm (SD = 7 mm). Majority of cases (93.3%) were smaller than 2 cm and only 4 cases (0.4%) were larger than 5 cm.

In 154 (11,8%) patients multiple lesions were observed at the time of diagnosis. The association between gender and number of

lesions showed that multiple lesions are more often observed in males. ($p = 0.001$) Incidence of multiple lesions increased progressively with age. ($p = 0.002$) Under the age of 40 there are no cases, between 40 and 60 years the percent of patients with more than one lesion is 4.3%, while in the patient group over 80 it is 15.9%. Also among aggressive histologic subtypes multiple lesions are more often observed. (15.6% vs. 10.6%).

In 1016 (78%) patients the tumor was located in the head and neck. The second most common site was the trunk (16.3%), followed by upper (3.3%) and lower limbs (1.6%). There were 4 cases (0.3%) in the genital area. In 6 cases (0.5%) topography was unspecified. Regarding the cephalic region, the most cases (30.2%) were located on the face, followed by the nasal area

(21.9%). There was low predominance of basal cell carcinomas in eyelid (1.9%), lip (2.9%) and neck (3.3%) regions.

Localization on the lips was observed more frequently among women (63%). While the prevalence of basal cell carcinomas on the ears (77%) and in the upper limb region (67%) was greater among men. Interesting to mention is that all 4 patients with genital basal cell carcinoma were females. The association between topographic site and age showed that with age the incidence of basal cell carcinomas in the trunk region decreases, while in the head and neck region increases. ($p = 0.001$)

With regard to histologic subtype, the nodular variant predominated, accounting for 40% of the cases, followed by infiltrative (16.9%) and superficial (15.1%) variants. Aggressive subtypes accounted for 28.5% of the cases.

Superficial BCC occurred slightly more often in females (54%), while micronodular (63.6%) and nodular (57.9%) variants were significantly more often present in males. Superficial BCC showed prevalent distribution in younger patients with almost 70% of the cases under the age of 60. The prevalence of infiltrative subtype increased progressively with age. It accounted for 6.7% of BCCs among patients under the age of 40, 11.3% among patients aged 40–59, 16.7% among patients aged 60–79 and for 21.1% of the cases among patients over 80 years.

Correlations between histologic variant and topographic localization revealed that in the head and neck region predominant was the nodular basal cell carcinoma, while in the area of the trunk, upper and lower extremities predominant was the superficial basal cell carcinoma. ($p = 0.001$) The percentage of aggressive histologic subtypes was greater in the head and neck (30.8%) and lower limb (28.6%) regions, compared

to the trunk (19.7%) and upper limbs (20.9%). ($p = 0.023$)

Discussion

McCormack et al. were the first ones to investigate the differences between the various histologic variants of BCC. Their study showed that superficial basal cell carcinoma showed predilection for younger patient and trunk and upper limb localization. They proposed the theory that there may be correlation between the origin of superficial BCC and superficial spreading melanoma, because both clinical entities show the same topographic distribution and association with rather intermittent than prolonged sun exposure.⁶

Other authors have described differences between nodular and superficial basal cell carcinoma as well.^{7–12} In particular, superficial BCCs occur more often on the trunk and in younger patients. This supports the thesis that chronic sun exposure has greater impact for developing nodular BCC, because it shows strong association with sun-exposed areas, like head and neck, and its incidence increase with age. Concerning the superficial BCC it is quite the opposite – its incidence decrease with age and it shows predilection for sun-protected areas, like the trunk.^{7–10,12}

Our study confirms that there are important differences between some histological types of basal cell carcinoma. Superficial BCCs were more common in younger people predominantly in females. This type of tumor developed on the trunk, whereas other subtypes predominantly occur in head and neck region. The distribution of histological subtypes in our study is in concordance with the literature, the nodular form being the most common subtype, followed by the infiltrative and superficial types.

On the other hand, in a study conducted

on more than five thousands patients for a period of 3 years in Australia, Raasch et al. received different results, showing less clear predilection for superficial basal cell carcinoma to appear on the trunk.¹¹ Therefore, this topic remains controversial and further investigations are needed.

Interesting to mention is also that aggressive histologic subtypes were observed with greater incidence in the head and neck region by other researchers as well.¹¹ In our study this form is with a rate of 28,5% and predominantly located in the head and neck region. Aggressive histological variants are namely the ones with higher rates of deep invasion into the subcutis and adjacent structures and have greater recurrence risk. That can cause surgical difficulties and aesthetic problems for the patient and indicate a need for a new target therapies.

Conclusion

In the era of the personalized medicine, it is crucial to acknowledge the importance of accurate pathological subtyping for the purpose of management of basal cell carcinoma. With the many treatment options available nowadays, clinicians should take into consideration all the clinical and morphological characteristics of BCC in order to provide the optimal treatment for their patients.

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Hydrocephalus, leading to significant alterations in the anthropometric indexes of the brain and skull – presentation of two anatomical cases

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Hydrocephalus is a complex neurological condition, characterized by an increase of the size of the brain ventricles due to an accumulation of cerebrospinal fluid in them, as a result of pathology of the liquor system in terms of the processes of production, circulation and resorption. Depending on the point of the obstruction hydrocephalus can be divided in communicating and non-communicating, and depending on the etiology – in congenital, acquired or idiopathic. Presented are two anatomical cases of communicating hydrocephalus in children, discovered during dissection on cadaver material in the department of Anatomy, histology and embryology of the Faculty of medicine at Plovdiv Medical university, in which due to the early age of onset, significant alterations in many of the anthropometric indexes of the skull and brain have occurred. Other concomitant morphological abnormalities such as porencephaly, agenesis of the corpus callosum and others are also observed. Determination of the premises for the development of such conditions provides more precise guidelines in choosing the correct approach in their treatment.

Introduction

Hydrocephalus is a complex neurological condition, occurring more often as a result of another concomitant pathology or more rarely developing idiopathically. Generally, it consists in an increase in cerebrospinal fluid volume (CST) and an accompanying increase of the volume of the ventricular system. Multiple definitions for these conditions have been proposed over the years, neither of which has met universal support and each one with their own limitations (1). Some focus on the increase in the intracranial CST volume, other on the ventricular dilation, occurring due to the inadequate passing of CST from the point of production to the place of resorption and others.

Classification and pathogenesis

The question of the classification of the types of hydrocephalus is a complex one and several systems have been proposed. Pathogenetically speaking, there is a disturbance in the balance between CST production and resorption, based on which the present classification has been established (Fig. 1).

The main point in the classification of

this multi-faceted condition is the location of the obstruction along the course of CST from the place, where it is produced (choroid plexus) and the place where it is resorbed. In the case of free flow and transmission of CST pressure, hydrocephalus is labeled as communicating (2). In most cases it owes to the disturbed resorption of the arachnoid villi, common reasons for which are meningitis, subarachnoid hemorrhages and others. Rarely it occurs due to CST overproduction (for example in choroid plexus papilloma). When the disturbed CST flow is caused by an obstruction in any of the points along the course of the ventricular system, the hydrocephalus is labeled as non-communicating. This occurs as a result of tumor growth, intracerebral hemorrhage and the development of aqueductal stenosis (as a result of neonatal infection, myxoviruses infecting the ependymal cells, hypo- and hypervitaminosis A and others). Observed occasionally is the transition from one type to the other – as in the case of communicating hydrocephalus due to meningitis turning into non-communicating because of the pressing of the cerebral aqueduct from the lateral ventricles.

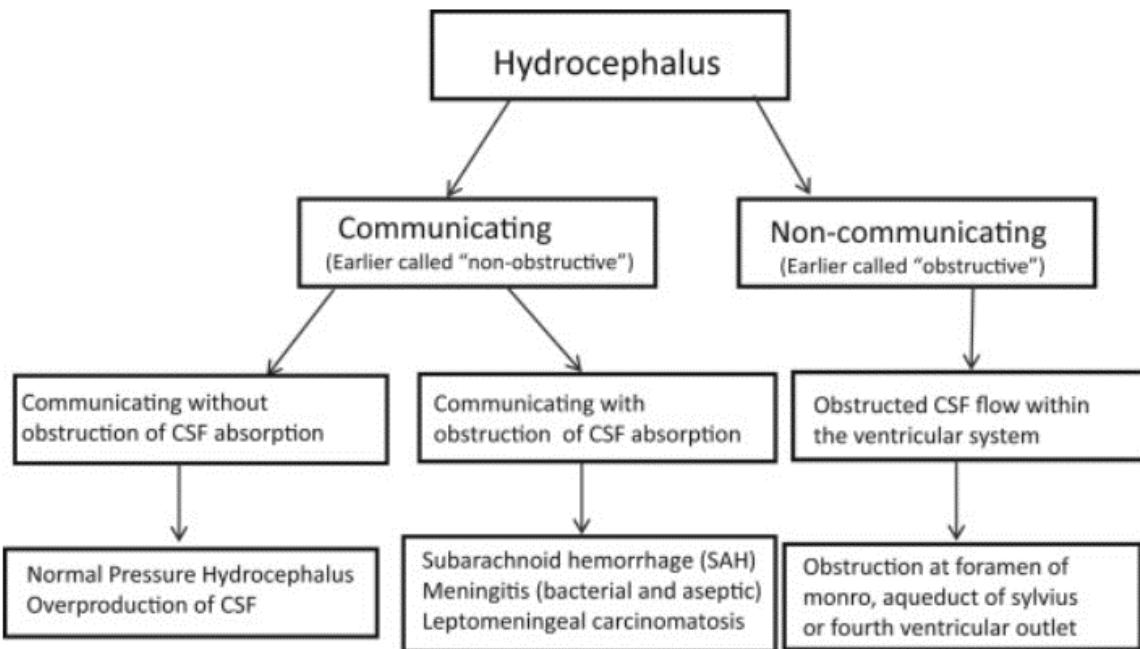


Fig. 1. Classification of hydrocephalus (1).

Important is also the underlying reason for the hydrocephalus. According to the etiology the cases can be classified in three groups – congenital, acquired and idiopathic (3).

The congenital type usually presents in the neonatal period and often occurs along Type 2 Arnold-Chiari malformation, Dandy-Walker syndrome and others. Recent studies find genes in chromosomes 18 and 22, mutations in which are connected with the development of this type of hydrocephalus (4). The pathophysiology of the congenital type is not completely clear to this day. One hypothesis has to do with the abnormal development of the cerebral aqueduct, which is an important event in the development of several types of congenital hydrocephalus. Studies with rats show, that the lack of the subcommissural organ (SO) in the new generations, that is observed when the diet of the parents lacks in vitamins B₉ and B₁₂, is accompanied by hydrocephalus. SO develops early in the ontogenesis and secretes sialoglycoproteins in CST, which play an important role in neuronal differentiation and normal CST flow.

The immunological blockage of SO secretion always leads to aqueductal stenosis and resulting hydrocephalus (5). Other hypotheses include the development of gliosis, neuroinflammatory processes (6) and others.

Frequently the specific reason, leading to the condition, cannot be identified. In these cases, the hydrocephalus is labeled as idiopathic.

Acquired hydrocephalus occurs as a result of other pathological processes. A specific type is labeled as normal pressure hydrocephalus, that presents in adults, most often after sustained cranio-cerebral trauma, subarachnoid hemorrhage, meningoencephalitis or a neurosurgical intervention. The diagnosis includes ventriculomegaly with no/small increase in intracranial pressure, together with the Hakim/Adams triad – dementia, gait disturbance and urinary incontinence (7).

During dissection on cadaver material in the Department of Anatomy, histology, and embryology of the Faculty of medicine at Plovdiv Medical University, the following two hydrocephalus cases were found:

Case 1:

Provided is photographic material of a sagittal section of the head of an approximately five-year-old (Case 1) and a nine-year-old child (Case 2). Presented in Table 1 are some of the anthropometric indexes of both cases.

Observed is visible dilation of the whole ventricular system, resulting in the cerebral aqueduct resembling an opening. The case can be classified as communicating hydrocephalus, which has developed early

enough in the ontogenesis as to lead to macrocephaly. The data shows, that the cranial volume is double (8) and the head circumference 20% bigger than WHO-standards for the age. An interesting finding here is a maldeveloped corpus callosum, which in 46% of cases presents along other structural cerebral defects (9). This anomaly accompanies many of the congenital syndromes such as the Arnold-Chiari malformation, Dandy-Walker syndrome and associates with hydrocephalus.

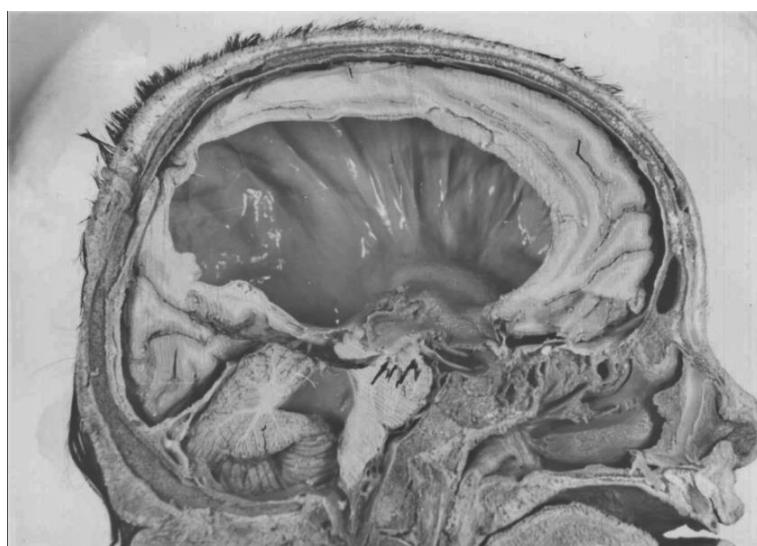


Fig. 2. Case 1 (right half). Observed is agenesis of the corpus callosum.



Fig. 3. Case 1 (left half).

Case 2:

Similarly to the first case, observed here is ventricular dilation, affecting primarily the lateral ventricles, and to a lesser extent the third and fourth ones. Present is a less-pronounced macrocephaly, compared to the first case – the cranial cavity volume is about 20% bigger than the average for the age. Presumably this is another case of communicating hydrocephalus. Several interesting findings are present. Observed on the right side is a big frontal cavity, filled with liquor (**Fig. 4**). Presumably this is also a case of porencephaly, where abnormal cavities in the brain develop as a result of

the destruction of brain tissue, defects in neuronal migration and other factors. In this case the cavity is in contact with the frontal horn of the right lateral ventricle. Possible is a progressive course with manifestation of increased intracranial pressure, which can partly explain the macrocephaly. Established is also a thickening of the flat bones of the calvaria, which can have an idiopathic origin or likelier has occurred secondarily as a compensatory mechanism as a result of the longer period of action of the increased intracranial pressure, in correlation with the later age in the second case.

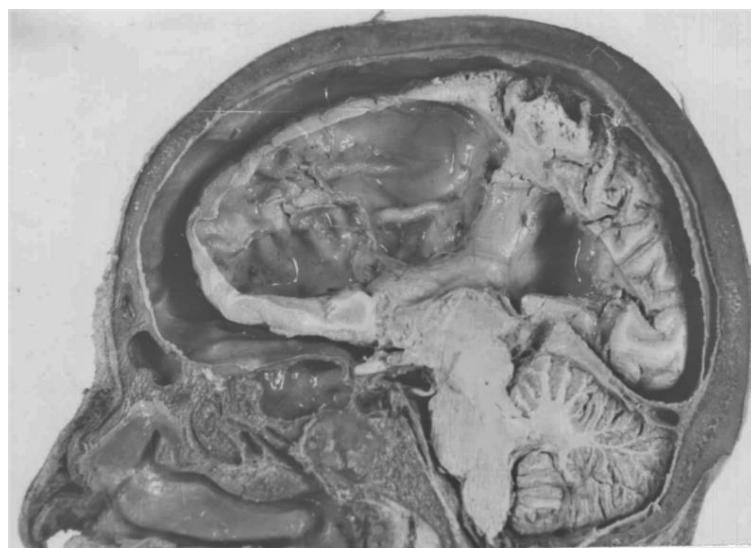


Fig. 4. Case 2 (right half). Removed is a part of the medial cerebral cortex, in order to demonstrate the sizable frontal cavity, filled with liquor.

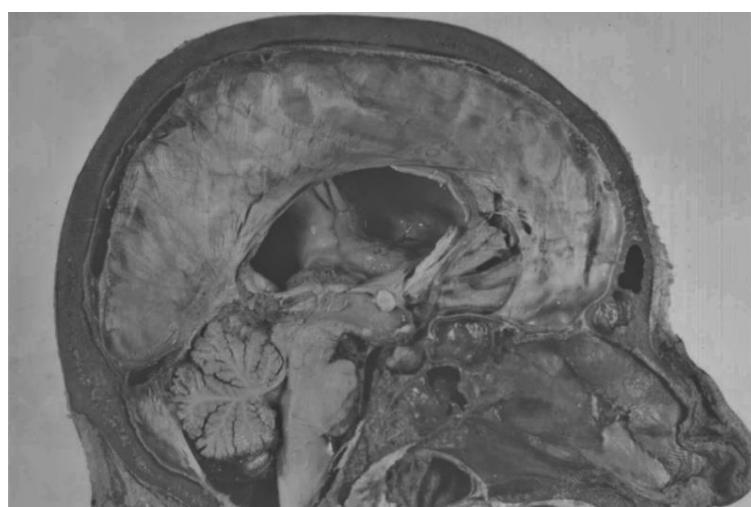


Fig. 5. Case 2 (left half).

Table 1. Anthropometric indexes of the two cases

Anthropometric index	Case 1	Case 2
Head circumference (on glabella)	601 mm	528 mm
Cranial volume	2010 ml	1200 ml
Left lateral ventricle volume	400 ml	115 ml
Right lateral ventricle volume	360 ml	110 ml
Interventricular foramen diameter	21 mm	52 mm
Third ventricle anteroposterior size	61 mm	38 mm
Third ventricle vertical size	34 mm	17 mm
Cerebral aqueduct form	Opening	Canal
Fourth ventricle vertical size	25 mm	21 mm
Fourth ventricle anteroposterior size	28 mm	8 mm
Thickness of the calvaria	2,5 mm	7,3 mm

During comparison of the indexes of both cases, it is established, that the volume of the cranium, ventricular system and the head circumference in the first case are significantly bigger than in the second case, which could be partly explained by the earlier age, the lower degree of cerebral suture adhesion and the greater elasticity of the cranial bones. Apparent in the second case is the almost three times bigger thickness of the bones of the cranial vault, that probably developed compensatorily because of the longer period of increased intracranial volume.

Conclusion

Hydrocephalus in children is one of the most common pathologies, treated by pediatric neurosurgeons worldwide, with a frequency of about one in a thousand births (3). Because of its prevalence, the understanding of the classification of the different types of hydrocephalus is absolutely necessary for its proper management. Depending on the age of onset and the etiology, observed are diverse alterations in many of the anthropometric indexes of the head, which are in direct connection with the underlying acting pathogenetic mechanisms. As the presented anatomic cases show, this condi-

tion presents commonly with other morphological abnormalities in the brain and skull, which demonstrates the association between similar symptoms. The establishment of the risk factors and the pathologic premises for their development, as well as the eventual genes, that play a role in the development of the ventricular system, will provide useful guidelines for more successful treatment and therapeutic action.

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Melatonin – for healthy and peaceful sleep, but not only!

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Melatonin (N-acetyl-methoxytryptamine) is called the “hormone of darkness”, because of its contribution to the regulation of the circadian rhythm. It is synthesized within the pinealocytes (cells of the pineal gland) from the amino acid tryptophan, as the synthesis goes through the intermediate serotonin. Serotonin N-acetyltransferase is the key regulatory enzyme for melatonin synthesis, as noradrenaline, released from the postganglionic sympathetic nerves during the dark, by a cAMP dependent-protein kinase A intracellular signaling pathway activates the enzyme and thus stimulates the synthesis of melatonin. On the other hand, the photopigment, called melanopsin, present in retinal ganglion cells detects the light during the day and by phospholipase C signaling pathway it passes the signal through the nerves until it reaches the pineal gland, where it inhibits melatonin synthesis. In addition to sleep regulation, melatonin also regulates intraocular pressure, glucose homeostasis and the secretion of insulin, it posses also anti-oxidant and immunomodulatory effects.

Melatonin is a neurohormone, known as “the hormone of darkness”, because of its contribution to the regulation of the circadian rhythm and its peak release at night. It was identified in a wide variety of animals and plants. The extensive distribution of melatonin, especially in the primitive bacteria (α -proteobacteria and cyanobacteria) indicates that it is an ancient molecule that has been retained throughout the evolution of all organisms (1, 2).

Melatonin (N-acetyl-5-methoxytryptamine) is synthesized within the pinealocytes (the cells of the pineal gland) from the amino acid tryptophan. Although the pineal gland is the main site for the synthesis of melatonin, other sites such as the gastrointestinal tract and retina contribute, to a lesser extent, to the circulating levels of melatonin. Several studies show that melatonin synthesizing enzymes are found in other ocular structures like the iris and ciliary body (3, 4, 5). An interesting observation is that there is synchronization between melatonin levels and the intraocular pressure, where the later reaches its lowest levels at night while melatonin is at the highest (6, 7). Melatonin is first released around 6–8 weeks after birth with peak secretion at 3–5

year, followed by a steady-state phase during puberty and a progressive decline through the years (8).

The French philosopher Descartes described the pineal gland as the seat of the soul and the region where our thoughts are formed (9). In 1958, the dermatologist Aaron Lerner while looking for a cure for vitiligo, discovered the hormone secreted by the gland – melatonin. Therefore, this hormone was given the name melatonin – “mel-a” from melanin and “toni” from serotonin (10).

The synthesis of melatonin starts from tryptophan which is converted into serotonin and then to melatonin (Fig. 1).

The regulatory enzyme of melatonin synthesis is serotonin N-acetyltransferase (arylalkylamine N-acetyl-transferase, AA-NAT). It’s activation and further secretion of melatonin are mainly regulated by post-ganglionic sympathetic innervation by norepinephrine (NE). NE binds to β_1 -adrenergic receptors, increases the intracellular level of cAMP (cyclic AMP) and activates PKA (protein kinase A). Activated PKA by means of CREB (cAMP response element-binding protein) activates serotonin N-acetyltransferase (11, 12), (Fig. 2).

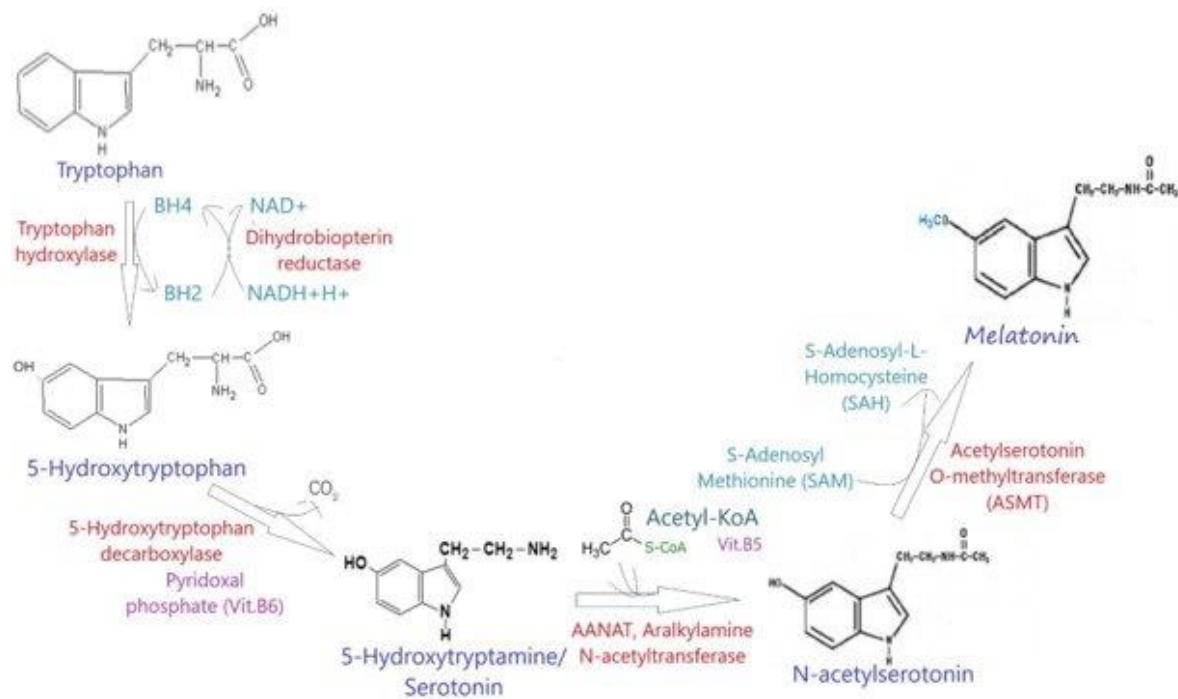


Figure 1. Synthesis of serotonin and melatonin.

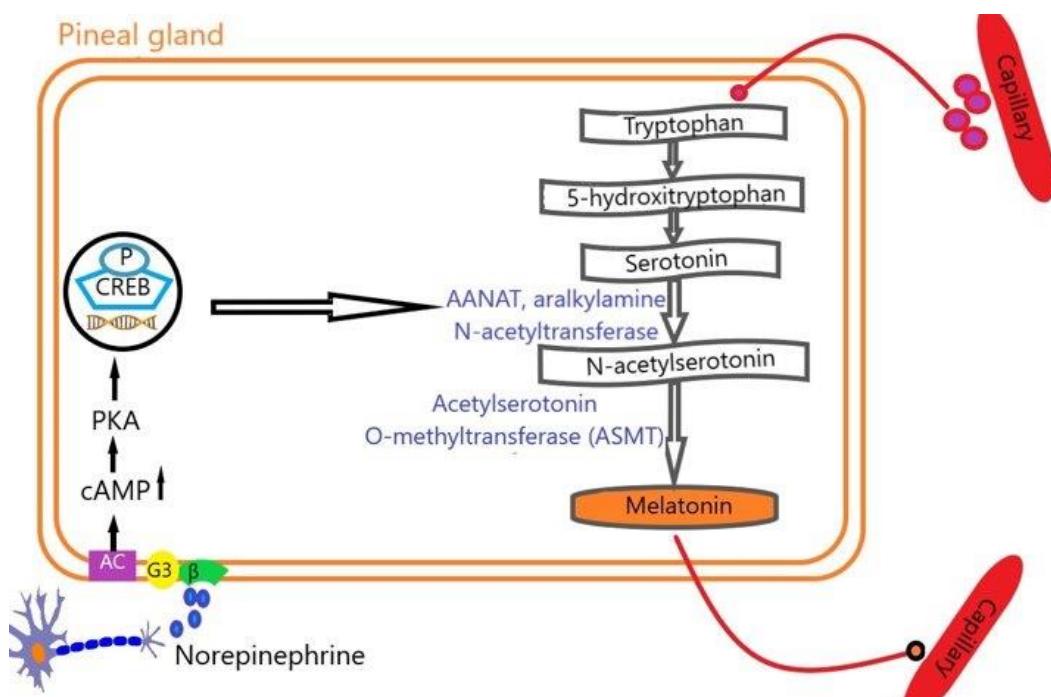


Figure 2. Schematic representation of melatonin synthesis in the pineal gland and regulation of key enzymes; (AC – Adenylate cyclase; CREB – cAMP binding element protein).

Light decreases melatonin production by inducing proteolysis of AA-NAT. Clock genes control the synthesis of melatonin producing complexes of two proteins – Period protein (PER1) and Cryptochrom. Light leads to a decline in the levels of

these clock-proteins which triggers gene transcription and a new cycle of melatonin synthesis with peak activation at night (13). But how the internal environment of the human body receives information about the day/night cycle?

In order to maintain the relevance between the body and the environment as well as the perception of the light and the objects around us, the eye contains photoreceptors called rod cells and cone cells, but there are other cells in the retina of the eye such as ganglion cells that contain a photopigment, called melanopsin (14), extremely sensitive to short wavelength blue light. These photosensitive retinal ganglion cells participate in the physiological response to light (from regulation of the circadian rhythms to pupil constriction). This photopigment melanopsin is responsible for detecting the light and then passing the signal through the nerves until it reaches the pineal gland, where it suppresses melatonin synthesis (15). The transduction of the signal begins with the activation of G_q protein coupled receptor, which in turn activates phospholipase C (PLC). Hydrolysis of phosphatidylinositol 4,5-bisphosphate by PLC generates the second messengers diacylglycerol and inositol 1,4,5-triphosphate (IP₃). IP₃ increases the cytosolic calcium concentration, which leads to membrane depolarization

(16), (Fig. 3). The signal travels down the retinal-hypothalamic tract to the suprachiasmatic nucleus (SCN) of the hypothalamus, which also serves as a major modulator of circadian rhythmicity. The signal then continues on to another synapse in the paraventricular nucleus (PVN) of the hypothalamus, which serves as the center of production of several major hormones, as well as regulates some other important functions such as the physiological response during stressful situations. From PVN, the signal travels through the brainstem to the spinal cord to the superior cervical ganglion (SCG) which reduces the activity of sympathetic nerve fibers that innervate the pineal gland (Fig. 3). Therefore, not enough norepinephrine is being released from the postganglionic sympathetic nerves, which results in a decrease of cAMP in the pineal gland that lowers the function of serotonin N-acetyltransferase, i.e. the production of melatonin is inhibited (17). On the other hand, darkness stimulates the postganglionic sympathetic nerves, i.e. stimulates the production of melatonin.

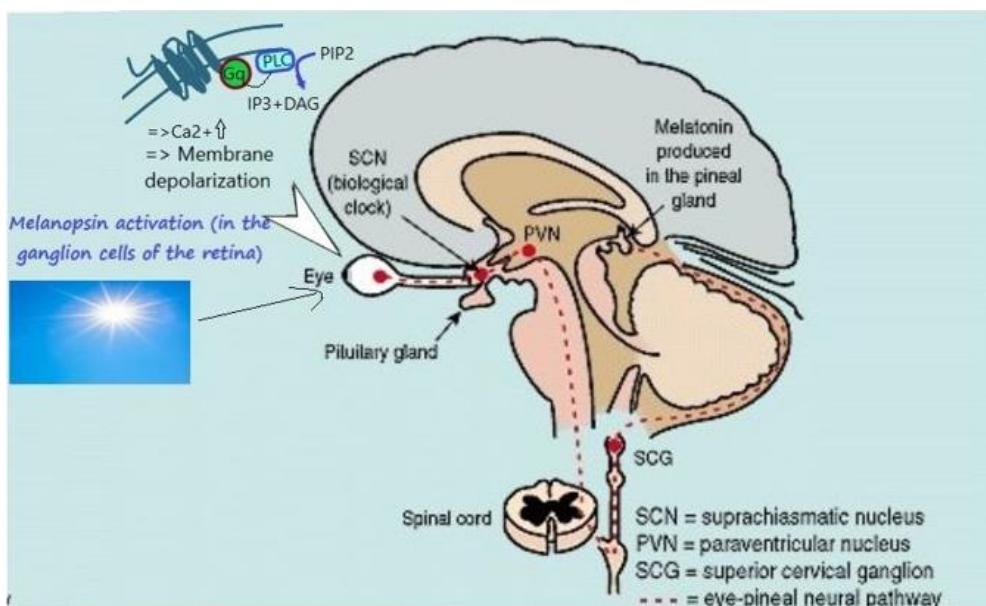


Figure 3. Retina – hypothalamus – spinal cord – upper cervical node – pineal gland.

Biological effects of melatonin

Adaptation to environmental changes and neuroendocrine effects

Melatonin modulates seasonal changes in physiology, core body thermoregulation, the timing of puberty, circadian rhythms (18, 19). It has an inhibitory effect on all endocrine glands but especially known is its antigonadotropic effect. High doses of melatonin can inhibit reproductive function via testosterone-induced luteinizing hormone suppression, while lower doses have beneficial effect on circadian activity, possibly improving fertility (20). The destruction of the pineal gland (e.g. from tumor) in kids leads to precocious puberty (*pubertas praecox*).

Anti-oxidant activity

Melatonin has similar anti-oxidant effects to those of glutathione and vitamin E, which scavenge reactive oxygen species, thus reducing cellular damage (21, 22, 23). It has been found that the anti-oxidant role of melatonin is especially important in brain tissue in order to limit free radical damage and potentially the development of neurodegenerative diseases (24). Moreover, melatonin has shown to inhibit lipid peroxidation (25) and slows down the degenerative changes and clinical progression of Alzheimer's disease (26). Melatonin also inhibits the oxidation of dopamine (27), which has similar protective effects in other organs.

Immune regulation

Dependent on the type and site of its release, melatonin can have paracrine, autocrine, and intracrine action. It can be peripherally released from leucocytes, bone marrow, mast cells, thymocytes, which can lead to a wide variety of immunomodulatory effects (28).

Sleep regulation

Melatonin regulates sleep but the correlation between its levels and the different sleep phases is weak (29, 30, 31). Sleep benefits associated with the use of melatonin are an increase in total sleep time, sleep efficiency and stage 2 sleep with a reduction in slow wave sleep (32). Endogenous melatonin is released at night beginning around 21:00 with peak release between 2:00 and 4:00. Its release in the morning after 7:00 is inhibited, coinciding with the peak release of endogenous cortisol (33).

Glucose homeostasis

Increased blood glucose level induces insulin secretion from the pancreatic β -cells. Melatonin can regulate glucose homeostasis by inhibiting this secretion on one hand and transduce information for the day/night cycle to the islets of Langerhans (pancreatic islets) on the other. By this mechanism of melatonin-dependent synchronization the secretion of insulin adapts to the day/night cycle. Lack of such mutualism can increase the risk for type 2 diabetes. The effect of melatonin on insulin secretion is established through membrane receptors MT₁ and MT₂ and the second messengers cAMP, cyclic GMP (cGMP) and IP₃. Therefore, defects in some of the receptors can also be associated with high risk for developing type 2 diabetes (34, 35).

Observations indicate that the pineal gland and pancreatic islets are related because not only melatonin receptors are present in β -cells but there is also an insulin receptor on the cell membrane of the pinealocytes (36), which means that the melatonin-synthesizing process is sensitive to changes in insulin levels.

Melatonin is a hormone with great significance. It regulates numerous essential for the normal human body functioning

processes. It is also called “hormone of darkness” and “hormone of youth” (37), because of its variety of biological effects.

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Sarcoidosis – a clinical case with pulmonary symptoms

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Sarcoidosis is an idiopathic granulomatous disease characterized by the formation of epithelioid and giant cell granulomas without caseous necrosis. It has diverse presentation, outcome, severity and need for treatment. It is often considered a pulmonary disease (90% of cases) with cough, dyspnea and chest pain observed, but it can affect any extrapulmonary organ in the body, most commonly the mediastinal lymphatic system, skin, joints and eyes. Necrotising granulomas can also be associated with sarcoidosis but is scarcely reported in the medical literature. Necrotising sarcoid granulomatosis is challenging to diagnose due to its rarity and similarity with other necrotizing disorders as tuberculosis. We report a clinical case of sarcoidosis with pulmonary involvement in a 28-year-old young man with caseous necrosis and calcifications identified.

Keywords: *Sarcoidosis, Necrotising sarcoid granulomatosis, Pulmonary involvement*

Introduction

Sarcoidosis is a chronic inflammatory granulomatous disease characterised by granuloma without caseous necrosis, which most often involves the lungs and the lymphatic system, although multiorgan involvement is common. Bilateral hilar lymphadenopathy and interstitial lung disease are the most typical changes in chest imaging. (1) The etiology is not fully understood. The association between sarcoidosis and tuberculosis remains controversial. (2)

Case Report

A 28-year-old man from Greece presented with complaints of fatigue, dyspnea and weight loss. He reported for a non-obstructing left nephrolithiasis diagnosed in February 2020. A CT of the kidneys had been performed. It had showed a disturbing image of the base of his lungs. The nephrolithiasis had been resolved with medication treatment.

In March a CT of the lungs was performed. The results showed bilateral consolidation involving the dorsal segments of both upper lobes and partially the superior segments of the lower lobes. Thickening of some interlobular septa was also noted. Numerous micro-nodules were found in the

rest of the lungs as well as multiple enlarged mediastinal lymph nodes. The right paratracheal lymph node and subcarinal lymph nodes formed conglomerates of 3 cm and 2 cm respectively. A single 1.2 cm enlarged lymph node was noted in the left axilla.

A tuberculosis test (T-spot) was performed but came back negative. The patient was referred to a hematologist because of a suspected lymphoma. Video-assisted thoracoscopic surgery (VATS) was performed. The results of the biopsy led to probable diagnosis of tuberculosis or sarcoidosis.

In May a standard follow-up procedure was requested, for the biopsy material to be evaluated by a second pathologist who concluded on tuberculosis because of caseous necrosis with calcifications identified. A treatment for tuberculosis started. Two months later a new CT was performed and it turned out the treatment showed negative results so tuberculosis was finally excluded as a possible diagnosis. The probable one was a rare form of sarcoidosis – Necrotising sarcoid granulomatosis, where the presence of necrosis may be misleading. In July a treatment for sarcoidosis was prescribed, starting with 30 mg of prednisolone daily.

Because of the high dosage the patient soon was hospitalized with community-acquired pneumonia and the dosage had to be changed.

The results from the pulmonary function

tests from October and December showed significant improvement with a notable change in FVC, FEV1 and DLco. We can conclude that treatment for sarcoidosis showed positive results.

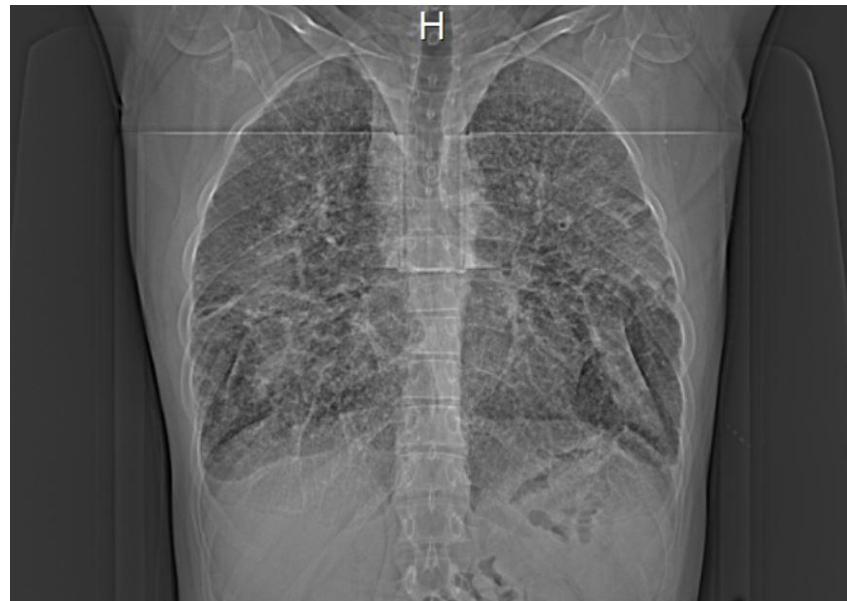


Figure 1. CT Topogram non-contrast demonstrating bilateral consolidation.

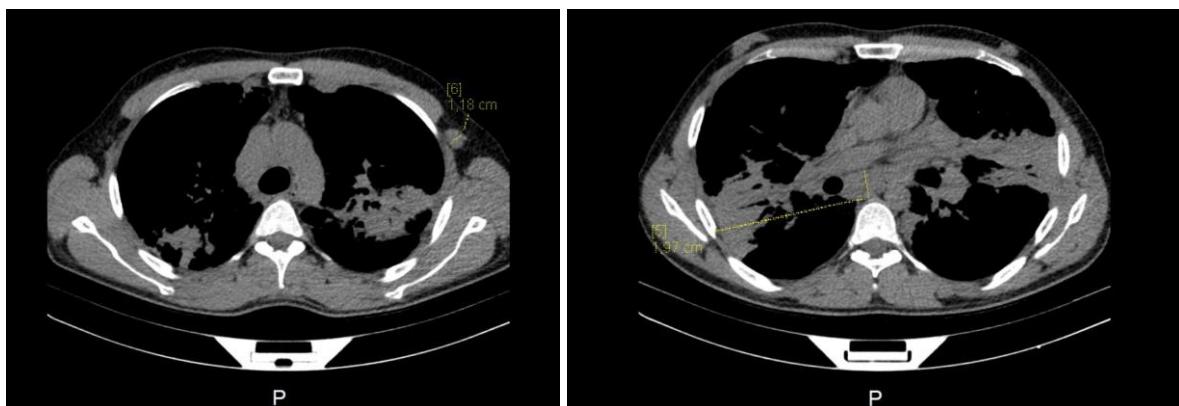


Figure 2. CT Axial non-contrast demonstrating bilateral consolidation, numerous military and micro-nodules and enlarged mediastinal lymph nodes.

Table 1. Pulmonary function testing – follow up

Parameter	July 2020		October 2020		December 2020		
VC	L	3,17	60%	3,96	75%	4,14	79%
FVC		3,17	63,3%	3,96	79%	4,04	80%
FEV1		2,26	53,7%	2,92	69%	2,93	70%
FEV1/FVC			71,16%		73,87%		70,90%
DLco		5,12	44,3%	7,51	65%	8,15	71%
DLco/VA - KCO		1,49	90,2%	1,57	95%	1,63	98%

Additional tests were performed: pulmonary function tests (results showed decreased PFT and DLCO), ophthalmology check-up (results normal), ECG and MRI of the heart (results normal), PET CT of the whole body (results showed sarcoidosis only affected the lungs). Symptoms for any other manifestations of sarcoidosis known (for example ocular, renal and cardiac) weren't found.

Discussion

Sarcoidosis is a systemic disease of unknown cause, characterised by the formation of epithelioid and giant cell granulomas without caseous necrosis. It has diverse presentations and no single criterion allowing a definitive diagnosis. (3) That is why it remains a diagnosis of exclusion – it requires the biopsy finding of noncaseating granulomas not caused by any other etiology. A classic finding on chest radiograph is the bilateral hilar adenopathy. In practice, a combination of clinical, paraclinical and histologic findings is used. It is considered a pulmonary disease with changes in chest imaging in 90% of the cases and clinical manifestation of dyspnea, cough and chest pain. However, multiorgan involvement is common. There may be found ECG abnormalities, anemia, hypercalciuria and elevated liver enzymes.

The exact etiology of sarcoidosis is unknown. Evidence points to a possible genetic component, infectious etiology, or potential exposure to environmental agents. (4) The onset can be insidious and findings may be discovered only on routine chest radiographs. Certain genotypes have been linked to sarcoidosis. The disease affects predominantly people between the age of 20 and 40, often women, showing high prevalence in Scandinavian and African American populations. (5)

Oral corticosteroids with a usual dosage of 30–40 mg prednisone daily are the main treatment for pulmonary sarcoidosis. Patients should be evaluated after one to three months for response. Those who fail treatment after initial corticosteroid therapy usually will not respond to a more protracted course of treatment. In responders, the prednisone dosage should be tapered to 5–10 mg per day or to an every-other day regimen, and therapy should continue for up to a year. Patients must be monitored after cessation of treatment for possible relapse as some patients will require long-term low-dose therapy to prevent recurrent disease. (6)

The course of the disease is variable, but spontaneous remission occurs in 60–70% of patients, while most improve with corticosteroid treatment. About 10–30% of patients have chronic or progressive disease. The mortality rate is approximately 1–6%. (7)

Conclusion

The diagnostic process for sarcoidosis is sometimes long and difficult and can be misleading. There are forms of sarcoidosis that can lead to misdiagnosis of tuberculosis, leading to a consequent delay in treatment. Sarcoidosis is a diagnosis of exclusion.

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Atypical course of the median nerve in the axillary fossa

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The course and branches of the median nerve may have different anatomical variations. The knowledge of these variations is important for the neurosurgical practice and also for any surgical procedures in the region of the median nerve. The topic of this report is the atypical course of the median nerve in the axillary fossa. Normally the median nerve is formed by two radices – radix lateralis and radix medialis, emerging from the lateral and median cord of plexus brachialis. They merge anterior to the axillary artery in the axillary fossa. In the presented case the merge of the two radices and the formation of the median nerve happens posterior to the axillary artery. This anomaly may lead to both neurological and surgical complications as well as compression of the axillary artery and interfered blood flow of the upper limb. Therefore in case of pathology and unspecific symptoms in the upper limb, anatomical variations of the brachial plexus should not be excluded as a possible cause.

Introduction

The course and branches of the median nerve may have different anatomical variations. The knowledge of these variations is important for the neurosurgical practice and also for any surgical procedures in the region.

The median nerve is a branch of the brachial plexus and is composed of fibers from the sixth, seventh and eighth cervical nerves. As with most other nerves, the median nerve may receive contributions from the fifth cervical nerve above or, on occasion, from the first thoracic nerve below (1).

Normally the median nerve is formed by two radices – radix lateralis and radix medialis, emerging from the lateral and medial cord of plexus brachialis. The divisions of the trunks and the formation of the cords represent an important anatomical and functional differentiation. The formation and relations of the cords are variable and, indeed their designations somewhat misleading. The medial and lateral roots merge and assume their appropriate relations anterior or anterolateral to the third of the axillary artery and lateral to the coracobrachialis muscle in the axillary fossa (1), (2), (3). This is equivalent to the surgical neck of the humerus when the arm is abducted to 90 degrees.

Materials and methods

In this report we present and describe a variation of the course of the median nerve found in the left axillary fossa of a cadaver, treated with a normal formaldehyde methodology, in the Department of Anatomy, Histology and Embryology during students dissection. We also describe some other minor variations found in the right axillary fossa of the same cadaver.

Standard anatomical techniques were used during the students dissection.

Results

In the right axillary fossa we observed some minor and not clinically significant variations (**Fig. 1**).

The musculocutaneus nerve is not separated from the lateral root, but from the median nerve, 41 mm after its formation. The other nerves have a normal course and onset. The posterior humeral circumflex artery and the subscapular artery originate from a common trunk. The thickness of the musculocutaneus nerve is 4 mm, of the median nerve – 7 mm, of the subscapular artery – 5 mm, of the posterior humeral circumflex artery – 4 mm and of the common arterial trunk – 7 mm.

In the left axillary fossa we observed the variations which are topic of this report (**Fig. 2**).

The median nerve does not form the usual fork in front of the axillary artery. There are in total three branches separating from the lateral cord:

1) First is the the musculocutaneus nerve with normal course and size, with thickness of 3 mm and length to the breakthrough of coracobrachialis – 32 mm.

2) Second, radix lateralis, continuing parallel to the axillary artery with thickness of 4 mm.

3) Third is detached at the tip of the axillary fossa with thickness of 3.5 mm. It is directed medially and first forms a fork

with radix medialis in front of the axillary artery and then they continue and fuse together distally behind the artery.

The abnormality here is that the medial and the lateral radices form a fork behind the brachial artery and the median nerve continues behind it to the cubital fossa.

Nerves separated from the medial cord:

- radix medialis n. medianus (up to the first fork) with thickness – 3.5 mm

- n. ulnaris – 3.5 mm

- n. cutaneus antebrachii medialis

- n. cutaneus brachii medialis

N. axillaris and n. radialis are normally separated from the posterior fasciculus.

All the vessels are typical for the axillary fossa.



Fig. 1.



Fig. 2.

Discussion

The median nerve is formed by the union of the lateral root from the lateral cord and the medial root from the medial cord, which meet anterior to the third part of the axillary artery. Fibres in the lateral root innervate the palmar skin of the thumb, index and most of the middle fingers, pronator teres, flexor carpi radialis and some of flexor digitorum superficialis. The lateral root conveys most of the sympathetic fibres to the median distribution in the hand. The medial root carries fibres to the skin of the medial side of the middle and the lateral side of the ring finger, and also fibres to palmaris longus, flexor digitorum superficialis and the lateral part of flexor digitorum profundus, flexor pollicis longus, pronator quadratus and the median innervated muscles within the hand.

In the presented report the merge of the two radices and the formation of the median nerve happens posterior to the axillary artery, which is not a common occurrence and may lead to different medical complications (3).

Conclusion

The fusion of the two roots from the medial and lateral cords most commonly occurs superficial to the axillary artery (3). There are categorized variations associated with both abnormal compositions of the median nerve and pattern of the axillary artery, as the artery and nerve have been found to develop in concreted relationship. There are studies showing conjoined arterial and neural variations existing in 8% of the specimens. Of these, the axillary artery being superficial to the median nerve (3.5%) is the most common variation (4).

This anomaly may lead to both neurological and surgical complications as well as compression of the axillary artery and interfered blood flow of the upper limb.

Therefore in case of pathology and unspecific symptoms in the upper limb, anatomical variations of the brachial plexus should not be excluded as a possible cause.

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Adrenal gland rim enhancement – an imaging prognostic factor in septic patients

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The adrenal glands are deeply situated in the retroperitoneal space but have a central role in homeostasis. Their location midway between thorax and abdomen means that they are present on most imaging studies, encompassing these regions. Incidental adrenal findings are common in patients examined for other indications. Adrenal rim enhancement is an uncommon finding encountered in local processes such as adrenal gland inflammation (Addison's disease, tuberculosis) and infarction, or systemic conditions such as sepsis or shock. We report a series of three patients with sepsis of different origin, who demonstrated adrenal enlargement and transient or continuous rim enhancement on contrast-enhanced CT. Patients were a 66-year old woman with a perforated ulcer and peritonitis, a 65-year old woman with a pelvic abscess, and a 41-year old man with a lung abscess. All patients succumbed to their illness in the days following the examination (1–9 days, mean 4.6 days). Adrenal enlargement and rim enhancement observed on contrast-enhanced CT in patients with sepsis could prove to be a prognostic factor for short-term adverse outcome.

Introduction

The adrenal glands are deeply situated in the retroperitoneal space but have a central role in homeostasis. Their location midway between thorax and abdomen means that they are present on most imaging studies, encompassing these regions. Incidental adrenal findings are common in patients examined for other indications.

Background

Adrenal rim enhancement is an uncommon finding which is encountered in local processes such as adrenal gland inflamma-

tion (Addison's disease, tuberculosis) and infarction, or systemic conditions such as sepsis or shock.

Findings

We report a series of three patients with sepsis of different origin, who demonstrated adrenal enlargement and transient or continuous rim enhancement on contrast-enhanced CT. Patients were 66-year old women with a perforated ulcer and peritonitis (Case 1), a 65-year old woman with a pelvic abscess (Case 2), and a 48-year old man with a lung abscess (Case 3).



Fig. 1. Enlarged adrenal glands with transient peripheral rim enhancement and intensive homogenous venous phase enhancement in a patient with peritonitis.



Fig. 2. Rim enhancement of enlarged adrenals without significant difference in density between arterial and venous phase, observed in a patient with a pelvic abscess.

The adrenal glands were moderately enlarged with a mean limb thickness of 7.7 mm (reference < 5 mm) and body thickness of 10.7 mm (reference < 10 mm). There was transient peripheral gland enhancement in two cases, and continuous in one case. In two patients the glands became homogenously contrasted with intense enhancement approximating the density of the inferior vena cava on venous phase (Table 1). No prior images for comparison of baseline adrenal size or hormonal work-up were available.

All patients succumbed to their illness in the days following the examination (1–9 days, mean 4.6 days).

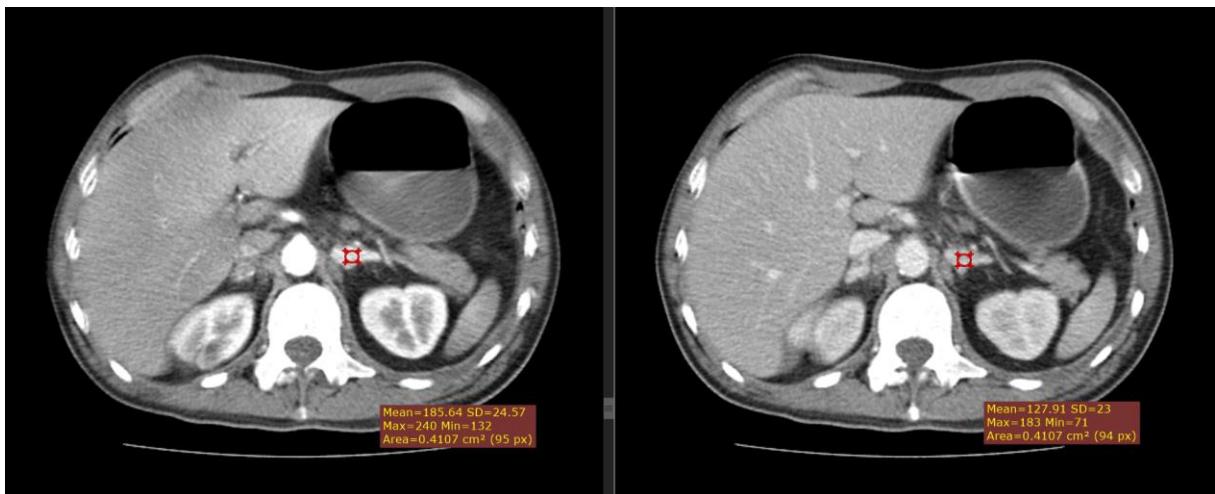


Fig. 3. Transient peripheral rim enhancement and intensive and homogenous venous phase enhancement in a patient with a pulmonary abscess.

Table 1.

	Left Lateral Limb	Left Medial Limb	Left Body	Right Lateral Limb	Right Medial Limb	Right Body	Venous phase gland density	Peripheral enhancement
Case 1	11 mm	6,6 mm	10 mm	7,6 mm	7,6 mm	13 mm	143 HU	transient
Case 2	7,6 mm	10 mm	11 mm	6,2 mm	8 mm	12 mm	35 HU	continuous
Case 2	5,6 mm	9,75 mm	8,45 mm	6,7 mm	6,4 mm	10 mm	127 HU	transient

Discussion

Intensive adrenal enhancement was first described by Taylor, followed by Sivit et al. as part of the so-called “hypoperfusion complex” in pediatric patients who had sustained blunt trauma and subsequently developed hypovolemic shock (1) (2). The authors hypothesized that the described imaging findings were due to tenuous hemodynamic stability. They observed a high rate of adverse outcomes in these patients – 85% of patients died in the following days.

Several other authors have observed adrenal enlargement and/or intense glandular enhancement in patients with sepsis and shock. Data exists that in sepsis there is a relative adrenal insufficiency due to resistance of the peripheral tissues to the effects of glucocorticosteroids and corticotropin hormone. This leads to activation of the hypothalamic–pituitary–adrenal axis (HPA), ACTH release, and compensatory enlargement of the glands (3), (4). In their study Nougaret and al. compare the adrenal volume in a series of 104 septic patients and 40 healthy controls. They discover that the size of the glands in the group of septic patients is nearly double that of the control group, and this could not be explained by differences in weight and body surface area. The authors also find a strong positive correlation between gland volume and survival, with patients with normal-sized glands having a higher rate of adverse outcomes. They did not however assess enhancement patterns of the glands in their study (5).

Bollen et col. report on the imaging findings of 38 patients with pancreatitis (12 of which severe). They found normal to enlarged glands with intensive adrenal gland enhancement (approaching that of the inferior vena cava) in 3/12 severe patients. All three developed multi-organ failure and death ensued within 4,5 days (6).

The intense enhancement of the adrenal glands observed in our series could be a transient sign of hyperperfusion preceding homogenous contrast distribution when hypoperfusion ensues in the setting of shock, as postulated by Cheung (7). A control scan obtained in one of our patients 7 days after the first one did not demonstrate any difference in adrenal gland appearance. Rim enhancement and glandular hyperplasia are probably associated with cortical stimulation in patients with HPA activation and/or unstable hemodynamics. Similarly Cheung observed persistent medullary enhancement of the glands in one of two septic patients with intensive contrast uptake (7).

Conclusion

Adrenal enlargement and rim enhancement observed on contrast-enhanced CT in patients with sepsis could prove to be a prognostic factor for short-term adverse outcome.

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Postmortem destruction of the human body by animal activity.

Case report

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After death, depending on the location of the corpse, geographic conditions, and season, different types of insects, arthropods, or animals (domesticated, stray, or wild ones) may invade the body. Their actions produce injuries over the human body that can vary in size, shape, and localization. Usually, they are situated in the exposed, not covered with clothes parts of the body. Those artifacts may be mistaken for traumatic injuries associated with blunt or sharp force trauma, or another forcible act against the victim's body, which can lead to misinterpretation about the manner of death. A 90 years old woman was sent for an autopsy at the Department of Forensic medicine and Deontology, Sofia, with massive destructive changes in the region of the head, neck, and extremities. The soft tissues of the head were almost completely missing, the skull was partly skeletonized. Tissue samples for further histopathologic evaluation and blood samples for toxicology analyses were taken during the autopsy to precise the cause of death. Devouring the remains of human corpses by insects, arthropods or animals is commonly observed in the practice of forensic doctors. Those findings have to be carefully examined, to determine if they are antemortem or postmortem in origin, depending on their localization, some difficulties may occur in the process of identification of the deceased and sometimes about the cause and manner of death.

Keywords: *postmortem scavenging, devouring, animal activity, destruction, human remains*

Introduction

After death, depending on the location of the corpse (indoor or outdoor – in the open air, underground, or under water), geographic conditions, and season, different types of insects, arthropods, or animals (domesticated, stray, or wild ones) may invade the body (1, 2). This could happen immediately after death, a few hours later, or after several days. Postmortem depredation may produce injuries over the human body that can vary in size, shape, and localization. Those artifacts may be mistaken for traumatic injuries associated with blunt or sharp force trauma, or another forcible act against the victim's body, which can lead to misinterpretation about the manner of death, since thus raise the possibility of homicide (3-5).

Case presentation

A 90 years old woman was found dead early in the morning in the garden near her house. According to the police report, the body was with severe injuries over different surfaces of her body. The previous night it

was raining outside, and the weather forecast showed minimum temperatures for the day from 0 to 1°C, and maximum temperatures from 7 to 9°C. The cadaver was sent for an autopsy at the Department of Forensic medicine and Deontology, Sofia. The external examination of the body at autopsy showed the following findings. A large soft-tissue defect on the face and neck area. The skin and the soft tissues were almost completely missing, in some places the skull was partly skeletonized, exposing the facial bones, which were intact. The remnant skin margins were finely scalloped and serrated. The lesions were dried with no evidence of vital reaction or hemorrhages (figure 1). The skin of the palms and the foot were bleached, wrinkled, and soddened. On the palmar surface of the right hand, there were multiple superficial linear wounds with different orientations (figure 3). The first and second fingers on the left hand were missing, the adjacent skin margins were finely serrated (figure 2). On the posterior surface of the left shank, there was a relatively circular superficial soft tissue

defect. The skin margins were finely notched, without any signs of vital reaction. Next to the internal skin margin, it was noticed the presence of rodent feces (figure 4). The tips and the distal phalanges of the fingers on the left foot were missing. The remnant anatomical structures and soft tissues were gnawed. Tissue samples for further histopathologic evaluation and blood samples for toxicology analyses were taken during the autopsy to precise the cause of death. During an additional talking with the police about the circumstances at the crime scene it was reported that several rats were seen in the vicinity of the house during the inspection of the crime scene. The toxicological analysis found no traces of alcohol or other substances in the blood. According to the autopsy and histological findings, death was attributed to ischaemic heart disease and hypothermia, with postmortem destruction of the remains by the action of rats.

Discussion and conclusion

Postmortem deprivation and scavenging by different animals, arthropods, or insects, are well-known phenomenon in forensic pathology practice (3, 4, 6, 7). Usually, the

postmortem artifacts are situated in the exposed, not covered with clothes parts of the body, such as face, hands, and legs, as they are easily accessible (4, 7, 8). There are some exceptions reported in the literature, where such lesions are found below clothes, and even in the genital areas, but they are rare (7, 9). These findings have to be carefully examined and interpreted if they are antemortem or postmortem in origin. They may lead to difficulties in the process of identification, especially when affecting the soft tissues of the face, and when destroying marks like tattoos, or scars (1, 2, 10, 11). In some cases, when large parts of the body – tissues, bones, different types of anatomical structures, and internal organs are being affected by the process, the precise cause of death cannot be identified (2, 12, 13).

Determining the exact species that has caused these injuries only based on the morphological appearance of the tissues in the affected anatomical areas is difficult and unreliable. In several cases it was reported that the connecting link for the precise diagnosis of postmortem rodent interference is finding their excrements in the lesions (3, 14).



Figure 1, 2, 3, 4. Morphological appearance of the described artifacts.

In the present case, the diagnosis of postmortem destruction by rats was confirmed by the following findings. 1) The characteristic morphological appearance of the injuries with irregular, finely scalloped skin margins. The lesions were dry, without bruising, and hemorrhages – typical gnaw marks. The palmar surface of the right hand was with multiple superficial linear wounds, which can be associated by claw marks. 2) The presence of rodent excrements in one of the injuries. 3) The data from the crime scene – the presence of rats near the deceased. The appearance of the skin of the palms and the foot – bleached, wrinkled, and soddened is typical for continuous stay in moist conditions which corresponds to the given information that the previous night it was raining.

This study aims to present a case of postmortem rodent activity over the human body – the topographical distribution of the artifacts and their morphological appearance. Although it is commonly observed in the forensic pathology practice, those lesions could be mistaken from the investigators, and inexperienced physicians who happen to be at the crime scene, and this could raise suspicions for homicide and could lead to the wrong accusations against somebody.

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Congenital renal anomaly – Horseshoe kidney. Case report

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Horseshoe kidney is one of the most commonly observed congenital abnormalities associated with kidneys. It consists of two kidneys that are connected at the level of their poles. The variations may be different. The body of a 59 years old man, was brought for an autopsy at the Department of Forensic medicine and Deontology, Sofia. During the standard and routine forensic medical examination of the cadaver's body, we found that the deceased was with horseshoe kidney, with an established connection between the kidneys at the level of their lower poles by a thin layer of parenchymatous tissue. The cause of death was established. No other abnormalities were noticed during the autopsy. All the needed samples for further toxicology analysis and histopathology evaluation were being taken. This study aims to present a congenital anomaly that could be a prepossessing factor for future renal failure. Horseshoe kidney is not a rare condition but is usually asymptomatic, which increases the risk of unexpected complications as stones or infectious disease. There are changes in the normal anatomy of the kidneys, especially connected with the blood supply, there are different variations in the number and localization of the blood vessels. Clinicians and surgeons need to be aware of these types of congenital anomaly, to diminish the potential risk for worsening patient health condition.

Keywords: Horseshoe kidney, congenital renal anomaly, autopsy

Introduction

Horseshoe kidney is one of the most commonly observed congenital abnormalities associated with kidneys (1, 2). There are different variations of fused kidneys – L-shaped kidney, sigmoid kidney, or pancake kidney, and horseshoe kidney is regarded as a subtype of the group of fused kidneys (3). It consists of two kidneys connected at the level of their poles, most commonly the connection is at their lower poles. Horseshoe kidney is characterized by three anatomic anomalies: ectopia, malrotation, and vascular changes (4). Such condition occurs in approximately 1 of 400–600 individuals, having male preponderance by 2:1 (2, 4, 5–8). Horseshoe kidney may be sub classified by its shape into two types – U-shaped and L-shaped. The first one is placed symmetrically on either side of the vertebra, and in the second case, the isthmus of the L-shaped kidney lies lateral to the midline (2). There are variations of the arterial supply to horseshoe kidneys, associated with the number of renal arteries and from where they arise – the abdominal aorta and from the common iliac arteries. A classification with six basic patterns of arterial

supply for each horseshoe kidney segment has been proposed, based on reported cases (9).

Case presentation

The body of a 59 years old man, was brought for an autopsy at the Department of Forensic medicine and Deontology, Sofia. According to the police report, he was found dead in the morning at his apartment. There was information that the previous night, he had been feeling sick, and he had vomited. During the standard and routine forensic medical examination of the cadaver's body, it was established that the deceased was with a U-shaped horseshoe kidney, with a connection between the kidneys at the level of their lower poles by a thin layer of parenchymatous isthmus. It was found in a lower lumbar position than the usual position of the kidneys. The pattern of the arterial supply in the presented case was as follows – the upper and the middle segments were supplied by a single renal artery on either side, and the arteries to the lower segment arise from the abdominal aorta by a common trunk. The accessory renal artery was observed to have an equal diameter

compared to the corresponding right and left renal arteries. There was one renal vein and one ureter for each side. Samples for toxicology and histological analyses were

being taken for further examination. Based on the result from the toxicology analyses the cause of death was established as acute intoxication with methyl alcohol.



Figure 1. Horseshoe kidney.

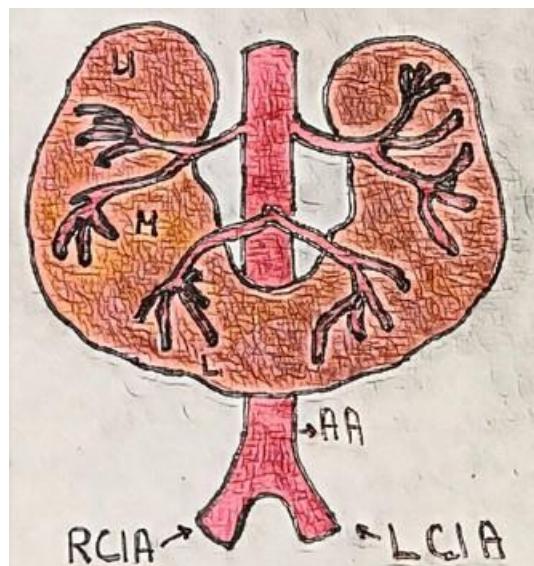


Figure 2. Pattern of the arterial supply in the presented case according to the one described by Graves: AA – Abdominal aorta; RCIA and LCIA – right and left common iliac artery.

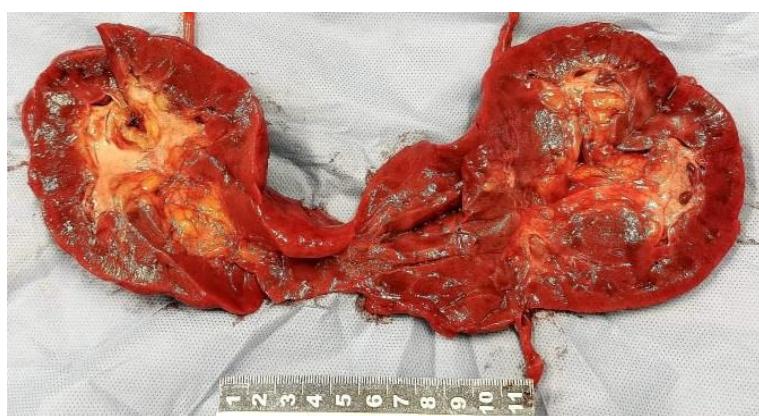


Figure 3. Longitudinal section of a horseshoe kidney.

Discussion and conclusion

This study aims to present a congenital kidney anomaly, and the changes associated with it, as this condition could be a prepossessing factor for future renal failure. Horseshoe kidney is not a rare condition but is usually asymptomatic, and most commonly is an incidental finding, which increases the risk of unexpected complications as stones or infectious disease (2, 4). Its abnormal position leads to compression of the fused kidney against the lumbar vertebral spine, and there is a chance of serious injuries following an abdominal trauma, as in this case, the kidney is not protected from the ribs (4).

Horseshoe kidneys are being used in cases of renal transplantation – from both living and deceased donors. Renal transplantation remains the definitive treatment for patients with end-stage kidney disease (10, 11). As the horseshoe kidney is associated with changes in its position and variations of the renal arteries, this procedure requires great skill from the surgeon and is considered to be a contraindication (4, 12). As reported by Murat Sevmis et al. a 28-years old female recipient with end-stage renal failure had been rejected from 3 different hospitals because the only viable living donor – her mother – was with horseshoe kidney (11).

Horseshoe kidneys manifest numerous anatomical variations, with emphasis on the blood supply (13), there are different variations in the number and localization of the blood vessels. Clinicians and surgeons need to be aware of these types of congenital anomaly, to diminish the potential risk for worsening patient health condition.

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Determination of the average lethal dose of *Tanacetum vulgare* essential oil on rats

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Introduction: *Tanacetum vulgare* is a widespread perennial plant of the *Astereaceae* family in the temperate zone of Europe and North America. The plant is used in Bulgarian traditional medicine for treatment of parasitic diseases of the digestive tract, diabetes, and high blood pressure. Recent search in the scientific literature showed contradictory data on the toxicity of the plant. **The aim** of the present study is to determine the mean Lethal Dose (LD_{50}) of essential oil derived from *Tanacetum vulgare* flowers in rats. **Materials and methods:** Thirty male albino Wistar rats were divided into groups of 6 animals. The groups were injected intraperitoneally with single doses of the essential oil as follows: 1000 mg, 1500 mg, 1600 mg, 1800 mg and 2000 mg. Behavioral reactions and mortality were reported within 24 hours of the start of the experiment. The LD_{50} was calculated by the Litchfield & Wilcoxon method. **Results:** *T. vulgare* essential oil causes dose-dependent behavioral reactions and toxicity: reduced motor activity, asthenia, tremor, difficulty in breathing, polyuria, gastrointestinal spasms. Mortality at the 24-th hour after the injection is as follows: dose 1000 mg – 0%; 1500 mg – 66.7%; 1600 mg – 50%; 1800 mg – 83.3% and 2000 mg – 83.3%. **Conclusion:** The estimated LD_{50} of *T. vulgare* essential oil in rats after intraperitoneal administration was 1494.6 mg/kg b.w. ($1273.9 \div 1753.6$). According to the Hodge and Sterner scale, the essential oil of the plant is slightly toxic to experimental animals.

Keywords: *Tanacetum vulgare oleum*, LD_{50} , rats, acute toxicity

Introduction

Tanacetum vulgare L. (Asteraceae, syn. *Chrysanthemum vulgare* L.) is an aromatic perennial plant, widespread in Europe and Asia. The plant could reach the high of 1 m, and is often found as wild weed near roadsides or water. The leaves are finely divided leaves and, in summer, umbels of small, button-shaped yellow flowers, which do not wilt during dry weather (1, 3, 4). Its common name (“Tansy”), comes from the Greek word for “immortality”: “athanasia” (4).

The main compounds isolated from Tansy are sesquiterpene lactones (parthenolide), flavonoids (methyl ethers of scutellarein and 6-hydroxyluteolin), 3 phenols, coumarins, and polysaccharides (tanacetan TVF). The essential oil has chemical composition, which depends on the geographic region and could be highly diverse, the most prominent variations in-

clude monoterpene and sesquiterpene content. This diversity allows the assignment to different chemotypes. At least 15 chemotypes are isolated, the most common of them are camphor and thujone (Judzentiene & Mockute 2005; Wolf et al. 2012; Mockute & Judzentiene 2013, 7, 4, Barceloux, 10, 11 Kurkina).

Tanacetum vulgare was used in the traditional medicine against migraine, neuralgia, rheumatism and digestion diseases. Essential oil from *T. vulgare* is known as antimicrobial and attractant agent (Raal 2). The high phenolic content of *T. vulgare* is related to its antioxidant properties [10]. Antiviral activity against the herpes simplex viruses HSV-1 and HSV-2 is also reported, probably due to the presence of 3,5-dicaffeoylquinic acid [12Álvarez, A.L].

Recent studies have also shown that the anti-inflammatory activity of essential oil or

extract of tansy (Keskitalo et al. 2001, 5). Other different kinds of bioactivity depending on the chemical composition (Mockute & Judzentiene 2013, 6).

Purpose

The aim of the study was to determine the average Lethal Dose of *Tanacetum vulgare* essential oil in rats.

Materials and methods

Animals

Thirty male Wistar rats, body weight 180–240 g were used for the experiments. They were weighed, numbered and divided in 5 groups of 6. Rats were kept under standard laboratory conditions ($22 \pm 1^\circ\text{C}$, humidity 45% and 12-h light cycle). The rodents received food and water ad libitum. All experiments were approved by the Bulgarian Food Safety Agency.

Preparation of the essential oil

Essential oils were extracted from 200 g of air-dried aerial parts of the plants by hydrodistillation method using Clevenger-type apparatus for 3 hours. The obtained oil was dried over anhydrous sodium sulfate and storage at 4°C .

Experimental

Acute toxicity experiments were executed with single doses of the essential oil, administered intraperitoneally as follows: group 1 – 1000 mg/kg bw; group 2 – 1500 mg/kg bw; group 3 – 1600 mg/kg bw; group 4 – 1800 mg/kg bw; group 5 – 2000 mg/kg bw.

Behavioral reactions and mortality in the experimental groups were recorded up to 24 hours after administration of the extract. The experiments were performed according to the principles of Good Laboratory Prac-

tice (GLP). LD₅₀ values were determined by the Litchfield and Wilcoxon method (14).

Results and discussion

T. vulgare essential oil causes dose-dependent behavioral reactions and toxicity: reduced motor activity, asthenia, tremor, difficulty in breathing, polyuria, gastrointestinal spasms. Mortality at the 24-th hour after the injection was as follows: dose 1000 mg – 0%; 1500 mg – 66.7%; 1600 mg – 50%; 1800 mg – 83.3% and 2000 mg – 83.3%.

The estimated LD₅₀ of *T. vulgare* essential oil in rats after intraperitoneal administration was 1494.6 mg/kg b.w. (1273.9 ÷ 1753.6), as shown in Fig. 1.

After a throughout search of the scientific literature, we failed to detect other information on the LD₅₀ of *Tanacetum vulgare* essential oil. Our previous research showed that the LD₅₀ of *T. vulgare* water extract was 22 g/kg bw (13.5 ÷ 35.5) when administered intraperitoneally.

In the available literature, we found only one team of authors who conducted toxicological studies on the plant *T. vulgare*. S. Lahlou et al. (16) determined the acute and chronic toxicity of aqueous extract of *T. vulgare* leaves on mice weighing 18–30 g. The authors administered single oral doses in the range of 0 to 13 g/kg b.w., administered per axis, as well as single intraperitoneal doses in the range of 0 to 4.5 g/kg b.w. They observed behavioral toxic effects at doses of 9–13 g/kg b.w. administered orally, and at doses of 1.5–4.5 g/kg b.w. administered intraperitoneally. The authors determined the LD₅₀ = 9.9 g/kg bw in mice for oral administration, and LD₅₀ = 2.8 g/kg bw for intraperitoneal administration.

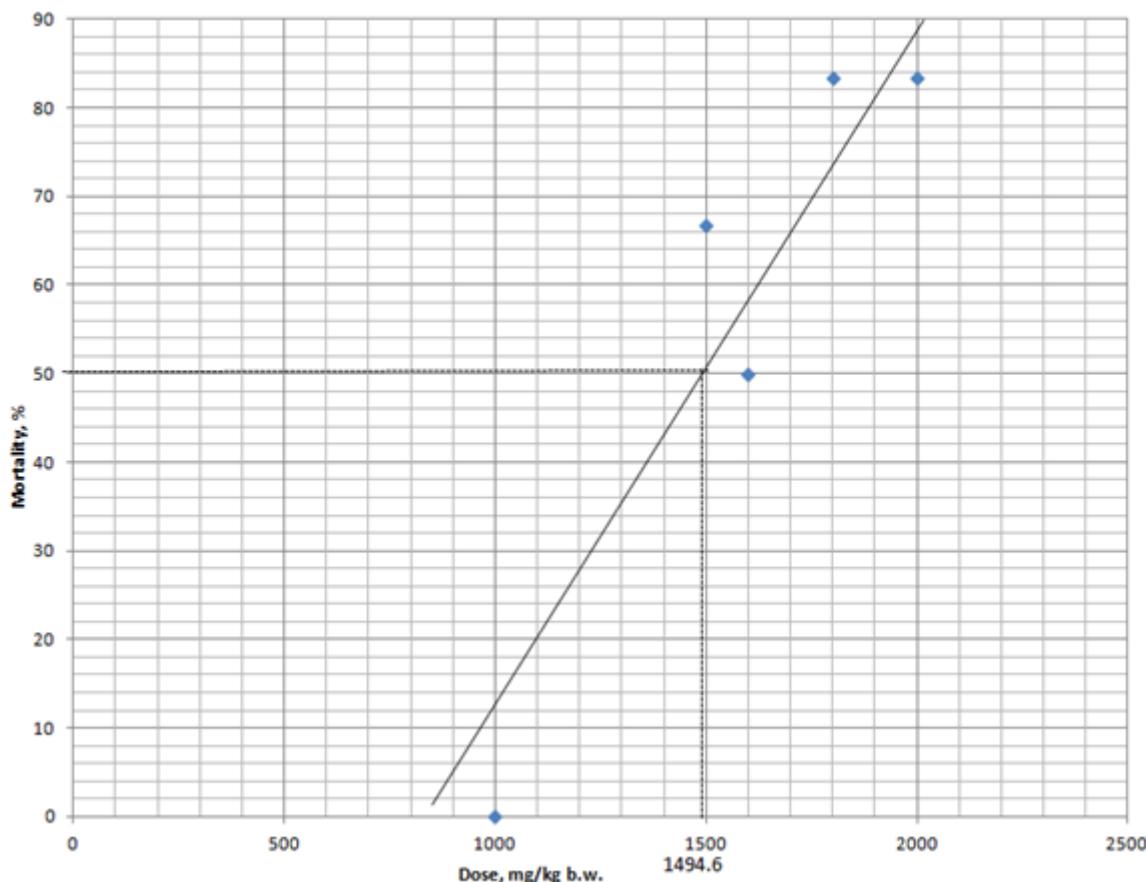


Figure 1. Evaluation of LD₅₀ of *Tanacetum vulgare* essential oil in rats.

Chemical analysis of *Tanacetum vulgare* extracts reveals β -thujone as main constituent of the essential oil (exceeding 87.6%). This compound contributes significantly to the acaricidal activity of the plant (17). It has been found that the anti-inflammatory activity of the plant is due to several compounds in the oil: α -humulene, camphor, borneol and 1,8-cineole (18). They inhibit the production of NO at the inflammatory site. Evidence in the literature shows a positive correlation between the therapeutic effects of polyphenols, α -pinene and caryophyll oxide contained in *T. vulgare* extract and their antioxidant activity (19).

Conclusions

The estimated LD₅₀ of *T. vulgare* essential oil in rats after intraperitoneal administration was 1494.6 mg/kg b.w. (1273.9 \div

1753.6). According to the Hodge and Sternier scale, the essential oil of the plant is slightly toxic to experimental animals.

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Interaction of exogenous neurotransmitters and isoquinoline derivatives upon action on isolated smooth muscle preparations from rat stomach

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The development of a new pharmacological product is a complex, consistent process that requires the determination of the degree, nature and strength of biological activity and in the long run, the evaluation of its efficacy and safety on both animals and humans. Circular rat smooth muscle preparations (SMPs) are a suitable subject for *in vitro* experiments to demonstrate a biological effect. They have a rich set of receptors, which makes it possible to study different in their nature and mechanism of reactions. *In vitro* studies of newly synthesized molecules, in their capacity as chemical or pharmacological agents, must also cover their interaction with major neurotransmitters. In this case ACh and 5-HT as factors in neuromuscular transmission by affecting cholinergic transmission. The subjects of present study are two newly synthesized isoquinoline derivatives – [2-chloro-N-(1-(3,4-dimethoxyphenyl) propan-2-yl)-2-phenylacetamide and 1-(2-chlorophenyl)-6,7-dimethoxy-3-methyl-3,4-dihydroisoquinoline] – tentatively named IQP and DIQ. Despite the similarities in their chemical structure – the presence of an isoquinoline ring, we found significant differences in the influence of the nature and strength of spontaneous contractile activity in rat gastric SMPs, characterized by opposing action and specifics in modulating ACh and 5-HT levels. The data shows mutual influence between SM effects in exogenously administered neurotransmitters and newly synthesized isoquinoline derivatives, which predetermines the information obtained in this type of study as a basis for subsequent research to determine the details and specificity of receptor and enzyme response.

Introduction

From Fundamental Pharmacology's point of view, the main task of technology of medicinal substances is the production of a medicine which can be characterized as highly efficient, stable, and safe.

The circular rat smooth muscle preparations (SMPs) are a suitable object when researching a potential biological effect of a newly synthesized molecule on isolated tissues. The SMPs have a vast supply of receptors, which offers the opportunity to study the nature and mechanism of various reactions.

The studied substances in *ex vivo* experiments can lead to a decrease or increase of the reaction of the tissues towards them, by developing a tolerance or tachyphylaxis⁽¹⁾. This process can develop both after the initial or several administrations in small doses. The most common cause for developing tolerance towards barbiturates and analgesics (such as Papaverine) is the cholinergic

receptors' low sensitivity towards acetylcholine (ACh). It's probable that the same pathway of activity is followed by the newly synthesized isoquinoline derivatives IQP (2-chloro-N-(1-(3,4-dimethoxyphenyl) propan-2-yl)-2-phenylacetamide) and DIQ (1-(2-chlorophenyl)-6,7-dimethoxy-3-methyl-3,4-dihydroisoquinoline). What is also interesting in their capacity as chemical or pharmacological agents, is their mutual influence with major neurotransmitters. As in our case, ACh and 5-HT work as factors in the neuromuscular transmission by affecting the cholinergic transmission.

ACh is a primary neurotransmitter which executes the neuronal control over the functions of neurons and various types of muscular cells^(2,3). It regulates a number of functions in the digestive system⁽⁴⁾ mostly affecting M₂ and M₃ muscarinic receptors over smooth muscle cells⁽⁵⁾.

The functions in the digestive system can also be affected by another neurotrans-

mitter or hormone such as 5-HT. Approximately 90% of 5-HT monoamines are located in the tissues of the Gastrointestinal tract⁽⁶⁾ and their release affects its motility^(7,8). The 5-HT levels can be modulated by several types of antidepressants⁽⁹⁾.

Aim

Having in mind the high level of structural similarity of IQP & DIQ with papaverine, we need to study the interaction of exogenous neurotransmitters and isoquinoline derivatives upon action on isolated SMPs from rat stomach.

Tasks

- To observe the effects which IQP & DIQ have on the contractile activity of isolated smooth muscles following multiple consecutive administrations.
- To research the mutual influence in reactivity between the examined compounds and the neurotransmitters 5-HT and ACh.

Materials and methods

Animals and anaesthetic protocol

Male Wistar rats with body weight in the range 220–280 g were provided by the Animal house of Medical University of Plovdiv, Bulgaria. At the beginning of the *ex vivo* experiments animals were euthanized by overdose anesthesia (Ketamin and Xylazin). All experiments were carried out according to the guidelines for using laboratory animals: directive 2010/63/EU. SMPs of circular dissection, 12–13 mm in length and 1.0–1.1 mm in width were used to record isometrically the contractile activity (CA).

Isometrically registration of SM contractile activity

CA of the preparations was registered isometrically⁽¹⁰⁾ by detectors Tenzo “Swe-

ma” – Sweden. Drug-caused reactivity of SMPs was counted and registered by gain stage “Microtechna” (Czech Republic) and recorded on paper recorder “Linseis” (Germany).

Pharmaceuticals and chemicals

The following chemicals were used: 5-HT and ACh (Sigma), Papaverine hydrochloride Sophsarma and the newly synthesized isoquinoline derivatives (IQP and DIQ) following Grignard reaction and cyclisation.

Statistical analyses

The data obtained were expressed as the mean ± standard error of the mean (SEM). The number of tissue preparations used in each experiment is indicated by n. Statistical differences were tested using Student's t-test, and a probability ($p < 0.05$) was considered significant. All statistical analyses were performed using a specialized software SPSS, version 23.0 (SPSS Inc. Chicago, IL)

Results and discussion

Occurrence of tachyphylaxis after consecutive administrations of IQP, DIQ and papaverine

In our previously conducted researches⁽¹¹⁾, we determined the working concentration of 5×10^{-5} mol/l for both studied newly-synthesized isoquinoline derivatives. In the above-mentioned concentration, we checked the capacity for developing tachyphylaxis after four consecutive administrations of IQP, DIQ and papaverine 1×10^{-6} mol/l, followed by changing the Krebs solution and restoring the CA of the SMPs.

We did not register any significant tachyphylaxis after treatment with papaverine and IQP. However, following DIQ treatment, we established there was a notable difference in the magnitude of pharmacological effect.

logical response after the first application (Fig. 1).

Each tachyphylaxis occurrence is interesting, as normally it links to desensitization of the system which procures the feedback from the used medicine. We consider this phenomenon to be specific to DIQ only because it does not occur during trials with IQP and papaverine, regardless of the similarities in their chemical structures. Thus, this predetermines particularities in the mechanisms of influencing the neurotransmission in the newly synthesized isoquinoline derivatives.

Mutual influence between exogenous neurotransmitters (ACh, 5-HT) on the IQP-induced muscle relaxation and DIQ-induced contraction

The action of exogenous ACh or 5-HT at a concentration of 1×10^{-6} mol/l on SMPs causes a contractile effect. It is known that the cholinergic, as well as the 5-HT receptors, affect the contractile activity by increasing the intracellular Ca^{2+} . This process involves the release of Ca^{2+} from IP_3 depot and its subsequent move from the extracellular space⁽²⁾.

ACh at concentrations of 1×10^{-6} mol/l, accomplished after 5×10^{-5} mol/l IQP or after 5×10^{-5} mol/l DIQ does not change the magnitude of its response (Table 1). Regardless of the similar preconditions, 5-HT is significantly affected by the presence of 5×10^{-5} mol/l IQP, and the tonic component of its contractile effect is reduced by almost 50%. This shows that IQP holds an additional ability which particularly affects the serotonergic intracellular signal pathways, at the same time without affecting the ACh modulating action on the spontaneous CA.

The similar in their chemical structure IQP and DIQ (both have established isoquinoline rings) exhibit totally opposite effect to the contractile activity of the SMPs – these are respectively relaxation and contractile effects⁽¹¹⁾. The relaxation of IQP is significantly increased only in the presence of 5-HT; whilst the DIQ contraction is significantly decreased only in the presence of ACh (Table 1). These observed particularities can be discussed as a consequence of the occurrence of additionally induced parallel pathways of influence of the two newly synthesized molecules on the contractility of the SMPs from rat stomach.

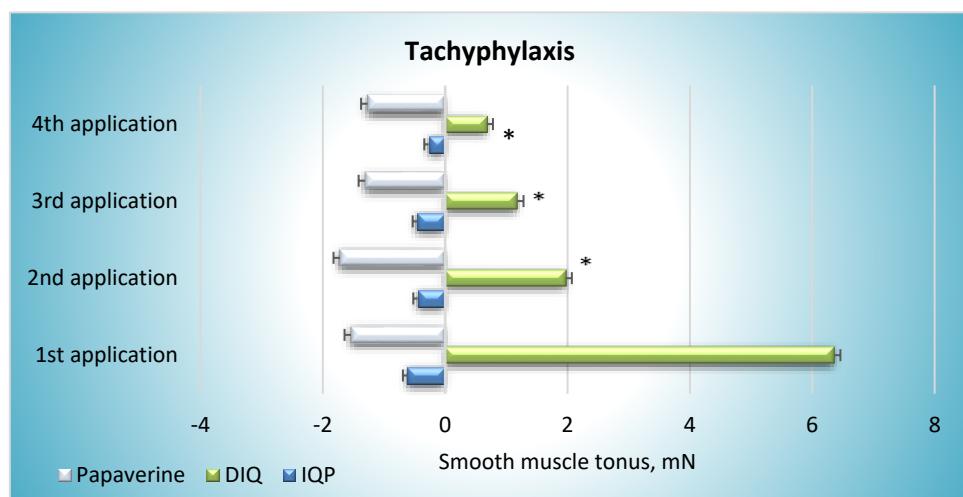


Figure 1. Changes in the smooth muscle tonus following 4 consecutive applications of papaverine, IQP and DIQ. The degree of each reaction has been compared to that of the first interaction. *- $p < 0.05$.

Table 1. Mutual influence on the contractile activity caused by IQP and DIQ on rat circular gastric smooth muscles (autocontrol). The comparison is between the autocontrol and interaction of exogenous neurotransmitters ACh and 5-HT: *-p < 0.05. The number of muscle preparations used for each data point was indicated by n.

<i>Impact agent, mol/l</i>	<i>Autocontrol, mN</i>	<i>Tonus of SMPs (mN) in the presence of:</i>	<i>n</i>	<i>p</i>
<i>IQP</i>	<i>-1.08 ± 0.14</i>	<i>ACh → -0.76 ± 0.12</i>	14	0.090
		<i>5-HT → -3.01 ± 0.59*</i>	12	0.001
<i>DIQ</i>	<i>6.39 ± 1.12</i>	<i>ACh → 2.95 ± 0.61*</i>	12	0.001
		<i>5-HT → 5.86 ± 0.17</i>	16	0.530
<i>ACh</i>	<i>4.91 ± 1.96</i>	<i>IQP → 5.18 ± 0.93</i>	18	0.420
		<i>DIQ → 4.85 ± 0.76</i>	18	0.640
<i>5-HT</i>	<i>4.84 ± 2.51</i>	<i>IQP → 1.57 ± 0.21*</i>	16	0.002
		<i>DIQ → 3.46 ± 0.83*</i>	12	0.037

Conclusions

Despite all similarities in the chemical structure of the two newly synthesized isoquinoline substances – IQP & DIQ, we have found significant differences in the influence over the character and strength of spontaneous CA of rat stomach SMPs. These differences are characterized by opposing action as well as by specifics in modulating the levels of ACh and 5-HT. The data demonstrates mutual influence between smooth muscle effects in the exogenously administered neurotransmitters and newly synthesized isoquinoline derivatives, which predetermines the information derived in this type of research as the basis for subsequent researches to identify the details and specifications of the receptor and enzyme influence when it comes to contractility of the smooth muscles.

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Fulminant hepatitis E

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From exotic travel-related, hepatitis E virus infection (HEV), mainly HEV-3, has recently become a locally acquired zoonosis in Europe. It is often asymptomatic, but can be severe in immunocompromised patients or those with underlying chronic liver disease. Cases of fulminant hepatitis (fulminant liver failure) have been reported even less frequently. We report a case of a young patient with immune thrombocytopenia and acute HEV hepatitis progressing to fulminant liver failure. The patient was transferred to another hospital for further treatment and transplant consideration. Unfortunately, he developed multiple organ failure and died before the transplant became feasible. HEV was subsequently confirmed in archived serum by detection of HEV RNA by commercial RT-PCR. The results of this study confirm that HEV test should be included in the initial assessment of any acute liver failure. Moreover, RNA-bases testing has to be performed in case of fulminant hepatitis to support clinical decisions and enable the use of potential antiviral therapy.

Introduction

From an exotic disease associated with trips to Asia, hepatitis E virus (HEV) infection, genotype HEV-3, has become a locally acquired zoonosis in Europe. Infection occurs mainly through the consumption of contaminated pork. It is often asymptomatic followed by a full recovery. Overt hepatitis is usually diagnosed in older men. Some vulnerable groups of patients bear the burden of the infection. In organ transplant patients, HEV often becomes chronic with the rapid development of cirrhosis. Hepatic decompensation may occur in those with underlying chronic liver diseases (1, 2). Cases of fulminant hepatitis (fulminant hepatic failure) (FHF) have been reported even less frequently (3). We present a case of a young patient with symptomatic acute HEV hepatitis who progressed to FHF.

Case presentation

A forty-two-year-old man was admitted to St. George University Hospital in Plovdiv in June 2019 with a 2-week history of increasing fatigue, abdominal discomfort

and dark urine of the last 2 days. The patient's medical history was notable for childhood immune thrombocytopenia and spondyloarthritis. His medications were celebrex 200 mg/day, piroxicam 20 mg/day and sulfasalazine 1000 mg/day for the last 2 months. The patient didn't report sexual risky behavior, blood transfusions in the last 6 months, recent trips abroad, consumption of raw meat, seafood and alcohol abuse. On admission his temperature was 37.2°C, heart rate 88 beats per minute, blood pressure 134/81 mm Hg, respiratory rate 20 breaths per minute and oxygen saturation 98% while he was breathing ambient air. He appeared ill. His skin was jaundiced. The examination revealed tender hepatomegaly and splenomegaly. There were no signs of chronic liver disease. The remainder of his examination was normal. The initial investigations showed significantly elevated aminotransferases and total bilirubin and impaired hepatic synthetic function (INR – international normalized ratio, INR 1.7) (Table 1). Serological markers of hepatitis A, B and C, D, Cytomegalovirus and

Epstein-Barr virus were negative or indicated past infection. Serum antinuclear and hepatic autoantibodies (anti-ANA IgG, anti-LKM-1 (IgG), anti-SLA (IgG) were all negative. The hepatitis E serological testing showed anti-HEV IgM (+) but anti-HEV IgG (-) (HEV ELISA, Euroimmune, Germany). Acute HEV hepatitis was diagnosed serologically (Table 2).

In the next few days jaundice increased and the patient's liver function tests progressively worsened. Computed tomography of the abdomen showed an enlarged liver with steatosis, enlarged spleen, lack of bile duct dilatation and preserved hepatic vascularity. Fluid was found around the

pancreas, perihepatally and perisplenally, in the pelvis as well as pleural effusion in the right.

On day 7th, hepatitis evolved into FHF with the development of encephalopathy, persistent impaired hepatic synthetic function, decreased aminotransferases level, and increased bilirubin level. Methylprednisolone was added to the maintenance treatment at a daily dose of 300 mg without improvement. The next day the patient was transferred to another hospital in Sofia for further management and consideration for liver transplantation. Unfortunately, he developed multiple organ failure and died before the transplantation became feasible.

Table 1. Investigations of the patient during his hospital treatment

Parameter	Admission	Day 2	Day 5	Day 7	Day 8 On discharge	Reference range
Hb, g/l	144	114	102	112	120	140–160
Lc x10 ⁹ /l	5,57	3,18	7,57	2,17	2,7	3,5–10,5
Tc x10 ⁹ /l	65	56	40	45	40	150–350
INR	1,7	1,7	3,68	4,12	2,2	< 1,2
bilurubin, µmol/l	280	350	926	989	1018,6	5–21
ALT, U/l	5482	4855	787	560	236	10–50
AST, U/l	7407	6820	361	410	412	10–60
protein, g/l	55	60	39	35	39	60–82
albumin, g/l	28	43	33	38	26	33–52
SHE, U/l	5550	-	4300	3100	3600	3500–11500

AST aspartate aminotransferase, ALT alanine aminotransferase, INR international ratio, SHE serum cholinesterase

Table 2. Serological investigations of the patient

Parameter	
Anti-HAVIgM	-
Anti-HCV	-
HBsAg	-
HBeAg	-
Anti-HBcIgM	-
Anti-HBe	-
Anti-HBs	-
Anti-HEVIgM	+
Ani-HEVIgG	-
HEV RNA	5 057 500 IU/ml (in archived serum)

Hepatitis E was subsequently confirmed by different working group in archived serum collected on admission by detection of HEV RNA (5 057 500 IU/ml) by commercial RT-PCR (Altona, Germany). It was done by another team a year later.

Discussion

A case of a young patient with acute HEV hepatitis progressing to FLF is presented. This is the first report with such a condition in Bulgaria. In developing countries a significant proportion of pregnant

women develop this complication. In contrast, in industrialized countries a small number of cases with this condition have been reported. In a multicenter study in Germany, 4% of patients with ALF had acute HEV-3 infection, and 5% of those in Scotland reported acute HEV in archived sera. It remains unclear whether HEV infection was a cause or was associated with liver damage [4, 5]. Many of the known negative prognostic factors were not present in our patient (old age, chronic liver disease, overuse of acetaminophen). We hypothesize that the autoimmune background (immune thrombocytopenia, spondyloarthritis) combined with hepatic steatosis have contributed to his fatal outcome.

There have been isolated reports on treatment of HEV-associated ALF with ribavirin [6]. The medication leads to normalization of liver function tests and undetectable HEV RNA shortly after the initiation of treatment. According to the European Association for the Study of the Liver (EASL), ribavirin is a component of the treatment of chronic HEV hepatitis (7). However, the optimal dosing and treatment duration remain to be established in the setting of acute HEV. Nevertheless, this drug is the only established therapeutic option for HEV infection. Thus timely diagnosis of HEV infection is important for considering

the treatment of severe cases, according to its effectiveness.

Conclusion

The result of this study has confirmed that HEV testing should be included in the initial assessment of any FLF liver failure. Molecular research may support clinical decisions and allow the use of potential antiviral therapy.

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Molecular anatomy of the circadian clock and its clinical significance – a literature review

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Aim: The aim of this article is to review the existing literature on the topic of circadian rhythms and their molecular substrate in the human physiology as well as human pathology. **Material and methods:** Numerous articles examine knockout mutations of the genes responsible for the functioning of the circadian clock namely CLOCK; BMAL1; PER; CRY etc. hence the causative mechanism of inducing severe dysregulation of metabolism is illuminated. The multimodal nature of the observed consequences of the desynchronized clock is explained by the existence of cell autonomous clock in the cells of different organs although all of them are under the control of the “master conductor” – suprachiasmatic nucleus. Endocrine (including reproductive) and metabolic injuries caused by perturbations of the clock are known for relatively long now but immune modulation (pro- and anti-inflammatory effects; repair of tissues; response to infection) is a new aspect of the functions of circadian clock genes. FASPS is the first condition to show an unequivocal association between known core clock genes and human circadian sleep disorders. **Results:** Confirmation of the indisputable role of the intact circadian rhythm in the normal human physiology and its derangements respectably – in disease is achieved. **Conclusion:** Chronobiology, although in its relative infancy as a discipline, harbors a myriad of potential advancements and recommendations to be made concerning the effectiveness of therapies for a number of diverse conditions and diseases.

The preservation of advantageous behavioural and physiological mechanisms which allow precise adaptation of the organisms to specific environmental changes is the hallmark of the evolutionary process – “as natural selection works solely by and for the good of each being, all corporeal and mental endowments will tend to progress towards perfection” (1). One of the relatively unchanging environmental factors acting as a driving force for natural selection is Earth's day-night cycle (2). This factor's considerable stability and the respective consequential variability in the multitude of environmental determinants are a potent impulse for the evolutionary process. The contrivance of a mechanism that can anticipate and respond to the cyclic changes in the environmental stimuli would hypothetically lead to improved organisms' adaptation. The existence of such endogenous mechanism independent of external cues has been discovered not only in multicellular organisms but also in prokaryotes and eukaryotes (3), which attests to the evolutionary advantage it provides.

Short history of key discoveries

In 1971 Ronald Konopka and Seymour Benzer induced mutagenesis in *Drosophila melanogaster* (*D. melanogaster*) using ethyl-methane-sulfonate (4). Employing the utilization of actograms (a graphical representation of an organism's phases of activity and rest over the course of a day) recorded in the state of constant darkness (24-hour darkness), they managed to isolate three mutant strands that differ in the rhythmicity and the duration of their circadian rhythm – perturbations were observed in the locomotor activity as well as the eclosion (the emergence of an adult insect from a pupal case) rhythm of the population (4). The hypothetical gene responsible for these alterations was named Period. The mutants were named according to their circadian cycle duration or the absence of such – *per*¹ – 28 h, *per*^s – 19 h, *per*⁰ – absolute arrhythmicity (4). A differential attribute of the circadian rhythm is its persistence despite the absence of external cues (5) (6). Before the discovery of Konopka and Benzer, it was already repeatedly speculated that an endogenous autonomous molecular apparatus was at

play supporting the stability of the circadian behavioural and metabolic changes in an organism. However, only after their publication, the fundamentals for the genomics and the molecular architecture of the circadian clock were laid down.

The following 50 years of research on the topic illuminated the intimate mechanism in which this intrinsic property of the organisms operates. Numerous articles utilizing a myriad of approaches explicate the subtle molecular interactions between the DNA, RNA and proteins in the cells of *D. melanogaster*. Gene transfer and P-element (plasmid) transformation were used to restore rhythmic behaviour in *per⁰* mutant fruit flies (7) (8). Antibodies directed against the period gene's product revealed its presence in diverse tissues (9) and hinted towards the localization of a central circadian clock – “master conductor” of the circadian rhythm. Distinct diurnal oscillations in the level of period RNA imply an underlying feedback loop (10). This hypothesis was supported by the discovery that the period protein (PER) is predominantly with nuclear localization (11), and evidence was found that it affects its own transcription (11) (12). In 1994 Vosshall et al. confirmed that a cytoplasmic-only model of PER could not form a rhythmic circadian pattern, which solidifies the previously made proposition that a transfer of PER should exist from the cytoplasm to the nucleus of the cell (10) (13). The advancements mentioned above in the understanding of the circadian clock's molecular anatomy formulate the modern view on its *modus operandi*, namely in the manner of transcription-translation based feedback loops (14).

Anatomic and molecular substrate of the circadian clock

Although the description of the circadian clock's machinery in mammals idled in the

dawn of research on the topic, a remarkable part of it has been elucidated nowadays (15). The recognition of a hierarchical model of organization and control of circadian rhythms has been made despite the comprehension of the existence of a relatively autonomous tissue-specific circadian clocks (16). The suprachiasmatic nucleus (SCN) plays the role of the maestro of the internal clock in mammals in the anteroventral hypothalamus (17) (18) (19). Multiple studies have supported this theory – lesions to the SCN in experimental animals lead to circadian arrhythmicity, and restoration of rhythmicity was observed in animals undergone fetal SCN tissue implantation (20) (21). The SCN neurons are capable of single-cell autonomy as circadian oscillators, but the tight intercellular connections in this population of neurons provide the framework for accurate coupling and synchronization of oscillations (22). Regardless of the exceptional stability of circadian rhythms in a “free-running state”, the precise temporal management of organ function is attained through the assistance of the external cues – Zeitgebers (19). With their aid, the internal clock's entrainment is achieved, which appears as a fundamental property, ensuring synchronization of the phases of the endogenous clock period and that of the external astronomical cycle of day and night (19). Temperature is frequently mentioned as one of the Zeitgebers for organisms; however, in mammals, it is recognized as a rather weak entraining signal due to the observed resistance of SCN to temperature impulses (23) (24). Feeding is another cue that nonetheless shows more effect on the workings of peripheral tissue's internal clocks (25). The most potent Zeitgeber in mammals, including humans, is light (26). The anatomical substrate conveying the information from this cardinal cue is the retinohypothalamic tract, which origin is in a specific

population of retinal cells – ipRGC (intrinsically photosensitive retinal ganglion cells) containing the photopigment melanopsin (27). Vasoactive intestinal peptide-energic neurons in the SCN receive the afferent signals from the retinohypothalamic tract and integrate the stimulus for phase shifting of the internal clock with astrocytes' assistance (28, 29). The intracellular transduction of the signal is conveyed by glutamate receptors as NMDAR (N-methyl-D-aspartate receptor) and AMPAR (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor); also, consequent calcium-mediated activation of CaMKII (Ca²⁺/calmodulin-dependent protein kinase II) and gene transcription are observed (30). The latter is achieved by the phosphorylation of CREB (cAMP response element-binding protein), leading to induction of the transcription by binding to calcium/cAMP response elements (19). Per1 and Per2 genes are targets of CREB's activation and are recognized as the two most sensitive to light impulse genes (31) (19).

Sine qua non principle of the circadian clock apparatus is the transcription-translation based feedback loops (Fig. 1) (31). The robustness of these mechanisms with oscillatory nature is maintained by three main interlocking autoregulatory loops using specific proteins' distinct property to regulate DNA expression. The paramount negative feedback loop is the one that involves the transcription factors CLOCK (Circadian locomoter output cycles protein kaput) (32, 33) and BMAL1 (Brain and Muscle ARNT-Like 1) (34). They dimerize, bind to E-box enhancer and promote the transcription of both Period gene (per1, per2, per3) and Cryptochromes gene (cry1, cry2) (14). When the concentration of the protein products of these genes have reached critical levels in the cytoplasm,

they form heterodimers after binding casein kinase 1 ϵ/δ (CK1 ϵ/δ) and translocate to the nucleus where BMAL1:CLOCK dimers are repressed (14). The latter leads to decreased translation and transcription of PER and CRY and subsequent disinhibition of BMAL1:CLOCK activity (35). Thus, the loop is closed, giving rise to the participating molecular agents' oscillation which is observed. Pivotal participants determining the duration of those oscillations are CK1 ϵ/δ and FBXl21 (F-Box And Leucine-Rich Repeat Protein 21) and β TrCP (beta-transducin repeat containing E3 ubiquitin protein ligase) (14).

The second loop guarantees the stability of the first one by exerting both positive (through the transcription of genes for the retinoic acid-related orphan receptors ROR) and negative (through the nuclear receptor REV-ERB) feedback on the Bmal1 gene promoter (14). Both of these receptors synthesis is directly under the control of BMAL1:CLOCK dimer. A third feedback loop is responsible for additional layers of control, but these are accomplished through the D-box element binding (36).

A strict control of the above-mentioned mechanisms over the activity of BMAL1:CLOCK dimer ensues. This is explained by the major impact that BMAL1:CLOCK has over the "Clock Output" (37) (Fig. 1), maintaining biological processes' rhythmicity (38) (39). Nowadays, it has been acknowledged that despite SCN being on the top of the hierarchy as controller of circadian rhythms, various tissues are capable of exhibiting semi-autonomous "clocks". Output signals from the circadian clock's molecular machinery regulate over three thousand genes in the rodent's liver (37), and over half of the protein-coding genome displays circadian oscillations (40).

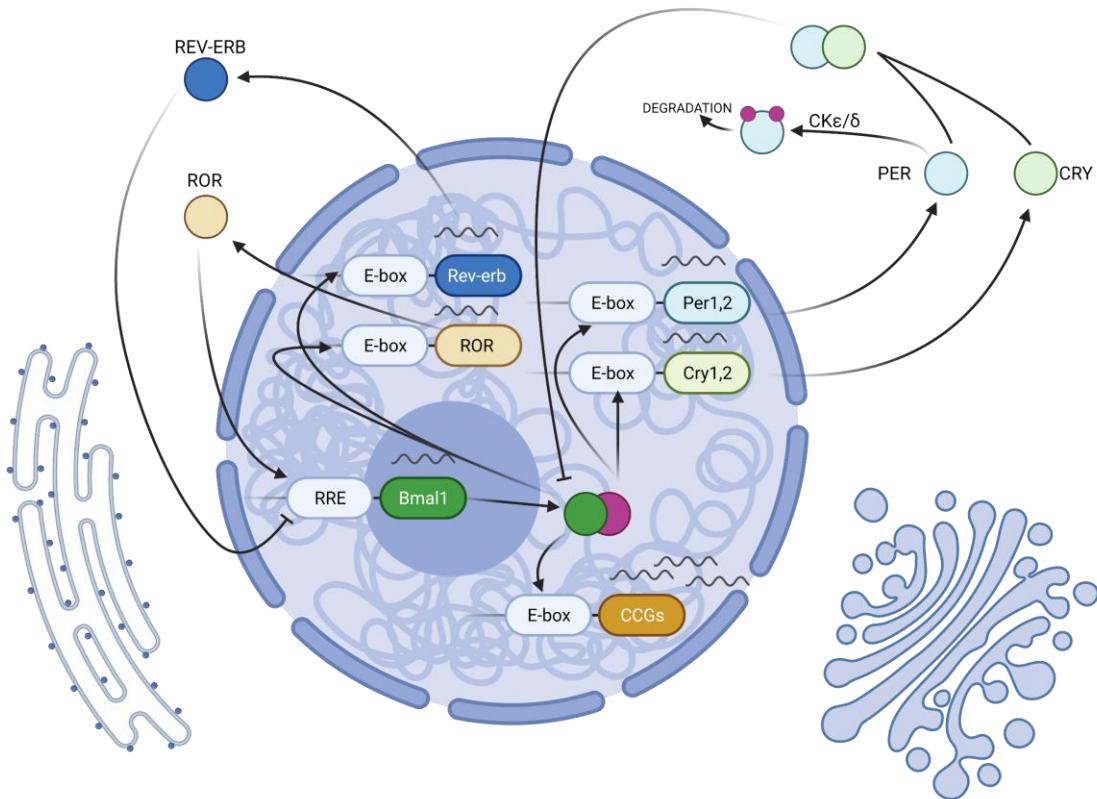


Figure 1. The circadian gene network showing two of the interlocking autoregulatory loops based on negative transcription-translation feedback. Core components of the circadian clock are shown: genes (ellipsoids) – circadian locomotor output cycles kaput (CLOCK) purple; Brain and Muscle ARNT-Like 1 (Bmal1); period homologue (Per1, Per2); cryptochrome (Cry1, Cry2); gene products (circles). Circadian Clock Output Genes (CCGs). – Created with BioRender.

Circadian rhythm and sleep disorder

Familial advanced sleep phase syndrome (FASPS) is considered the first disease described in humans, which is a direct consequence of a primary impairment in the translational-transcriptional feedback (41). FASPS patients show sleep phases, cortical body temperature, and melatonin rhythms that are 4–5 hours earlier than usual (42). A defect in casein kinase I ϵ (CKI ϵ) has been discovered which is associated with post-translational modification of PER proteins – the inability to phosphorylate the region responsible for the nuclear retention of the protein, leading to premature export and earlier cytosolic degradation hence the faster circadian cycle (42). Alteration in the half-life of CRY2, marked by faster degra-

dation, has also been observed as an additional cause for developing this circadian phenotype (43).

Circadian clock and metabolism

An abnormal metabolic phenotype has been repeatedly observed in experimental settings when knockout mutations of core clock genes have been induced. In part, this is to be explained by the confirmed property of diverse tissues to retain their intrinsic “peripheral clock”. Mice that are CLOCK:BMAL1 mutants show obesity and metabolic syndrome features, such as impaired glucose tolerance and compromised skeletal muscle metabolism (44, 45). Significantly attenuated diurnal feeding rhythm as well as being hyperphagic were the be-

havioural changes observed in those mutants (46). The reciprocal effect was observed as a time-restricted high fat diet restored the expression phase of clock genes compared to ad libitum diet (46). The latter statement comes to display the two-way interactions between metabolism and expression of clock genes.

Manifesting as a cardio-metabolic cluster prominently including known risk factors for cardiovascular disease, metabolic syndrome is to this day perceived as an invincible entity due to the lack of central aetiology (47). In 2019 Zimmet et al. propose that the derangements of the circadian clock are the unifying cause behind the tendency for clustering of those symptoms, and a suggestion has been made for the existence of “Circadian Syndrome” (47).

By the induction of tissue-specific epigenomic rhythms, the REV-ERB component of the circadian clock is also associated with the regulation of metabolic pathways of carbohydrates and fatty acids (48). REV-ERB agonist is able to reduce the severity of atherosclerotic plaques in mice (49). This hints at pharmacological targeting of the circadian clock's substrates to become a viable option for therapeutic strategies. On the other hand, deletion of *Bmal1* in myeloid cells increases monocyte involvement and plaque size (50). The attachment of myeloid cells to the microvascular wall peaks during the early active phase (51). Simultaneously, an increase in the number of adhesion molecules on endothelial cells and the presence of immobilized chemokines on the arterial wall was observed (51).

Circadian clock and immunity

As early as the 1960s, it has become clear that there was a diurnal variation in the endotoxin sensitivity in organisms (52).

Although numerous studies have shown circadian rhythms in the migration of immune cells to different loci of organs part of the immune system (53–55), anti-inflammatory effects of BMAL1 and REV-ERB α remain the best-studied components of the circadian clock (56). *Bmal1*^{-/-} macrophages in mice lose their ability to protect against sepsis (57). Furthermore, endotoxins inhibit BMAL1 by targeting the proinflammatory miR-155, whose induction correlates inversely with BMAL1 levels. In wild-type mice, BMAL1 inhibits miR-155 and prevents the development of lipopolysaccharide-induced sepsis (36). Using an autoimmune experimental model of autoimmune encephalitis, Sutton et al. showed that the loss of myeloid BMAL1 creates a proinflammatory environment in the central nervous system through activation and infiltration of IL-1 β -secreting monocytes (58). Nrf2 (nuclear factor erythroid 2 – related factor 2) is an “antioxidant gene” that also appears to be associated with BMAL1. Pharmacological or genetic activation of Nrf2 manages to save the proinflammatory phenotype of *Bmal1*^{-/-} macrophages (59). CLOCK is found to impact immune signalling by enhancing transcriptional activity of NF- κ B complex (60). However this can be attenuated by BMAL1, probably by sequestering CLOCK (60).

A growing body of data shows the well-established relationship between circadian genes' activity, pineal melatonin production, and a viral infection's progression in the organism (36)(61). BMAL1 expression is in positive correlation to pineal melatonin production. Melatonin-induced BMAL1 activation causes disinhibition of the pyruvate dehydrogenase kinase (PDK) and increases Acetyl-coenzyme (acetyl-CoA) levels in the mitochondria, promotes Krebs-cycle, oxidative phosphorylation, and ATP

production (62). This process is well explored in immune cells, which gives grounds to assume a causal connection between viral suppression of the circadian genes and alterations in immune cells' mitochondrial metabolism. Pineal melatonin plays an essential role in the nocturnal immune cell dampening (62).

The findings mentioned above give researchers merit to investigate COVID-19 infection and its effect on the circadian clock genes. It is found that SARS-COVID-19 disrupts melatonin production by the pineal gland, which results in an altered nocturnal decline in the immune activity with subsequent production of cytokines, mediating the inflammatory process ("cytokine storm") (62). The latter is thought to be the primary pathogenic mechanism, accounting for the severe COVID-19 complications (63, 64).

The SARS-COVID-19 interacts with the angiotensin-converting-enzyme-2-receptor (ACEIIR), which serves as an entry for the viral particles. On the other hand, ACEIIR is associated with angiotensin II breakdown and its degradation (61). By blocking the ACEIIR, SARS-COVID-19 raises ATII serum levels, which in turn disrupts the physiological oscillations in the Per2 gene (61). In addition, variations in BMAL1 expression are causing a surge in ATII serum levels, suggesting the bidirectional connection between ACEIIR-ATII-ATG and the circadian clock genes (61).

Circadian clock and nervous system

The fluctuations in the permeability of the blood-brain barrier (BBB) are also related to the circadian rhythm (65). Bmal1 gene deletion causes derangements in the normal functioning of pericytes, and evidence suggests that this might be due to improper transcription of platelet-derived

growth factor receptor β gene – pdgfrβ (65). The latter has been recognized as a crucial component in the integrity of BBB (66). Decreased efflux of xenobiotics has been observed during nighttime administration of the anti-epileptic drug phenytoin in a *D. melanogaster* seizure model (67). Furthermore, it is consistent with the better efficacy of the drug. The oscillations were abolished in *per⁰* flies (deficient in a core component of the circadian clock) (67). These findings imply that adopting therapeutic strategies which incorporate the temporal variations in the BBB physiology holds promises for more efficacious treatment (67).

Circadian clock and oncogenesis

It has been demonstrated that both physiologic perturbations, e.g. jet lag and experimentally induced genetic mutations affecting principal elements of the molecular circadian clock, decreased survival and potentiated neoplastic growth (15). Deletion of Per2 and Bmal1 leads to promoting tumorigenesis in the lung through increased expression of c-Myc (68). An interplay between Bmal1 and p53 has been suggested as a potential pathophysiological hypothesis (68). Systemic derangement of the circadian rhythm instigates global gene dysregulation and metabolic disruption. Kettner et al. concluded that hepatocellular carcinoma (HCC) could be induced by chronic jet lag in wild type mice after 78 weeks of age, corresponding to the median age of HCC diagnosis at 67–72 years in humans (69). Despite the culmination of the circadian disruptions being the neoplasm, the progression to this stage was marked by the occurrence of non-alcoholic fatty liver disease (NAFLD) and then steatohepatitis (69). β-catenin activation and mutation mediated by the constitutive androstane recep-

tor (CAR, NR1I3) are speculated to play a significant role in the development of the neoplasm (70). CAR activation is promoted by intrahepatic cholestasis, which results from mutations of both Per1 and Per2 or phase shifts of the liver clock by restricted feeding (69) (70). Lymphoma is the prevalent malignancy in circadian gene mutant mouse models, and HCC takes the second place (71).

Conclusion

The circadian clock machinery provides a temporal layer of transcriptional regulation to the genome. While not immediately lethal, deletions in core clock genes are crucial for organismal health. A considerable amount of indisputable data accumulates on the close correlation between a properly functioning circadian clock and human health. Incorporating the time axis of human physiology into new strategies for effective prevention and comprehensive therapy of a number of diseases would provide promising opportunities for decreasing incidence as well as improving prognosis. However, it is becoming crystal clear that an interdisciplinary team is needed to interpret the data, and trends in this approach to treatment need to be encouraged. Although Chronobiology is still in its infancy as a science, it is casting light on the dark fields of human pathophysiology and shows an enviable potential for a strong influence on other medical disciplines.

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II. Therapeutic session

Lipid profile changes after thyroidectomy

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Thyroid hormones has profound impact on the basal metabolism, energy expenditure, carbohydrate, lipid, protein and calcium metabolism, as well as cardiovascular, hemathopoetic, pulmonary and neuromuscular system. Monitoring the variations in lipid profile and the avoiding the development of metabolic complications after thyroidectomy is an important aspect of the medical care in the postoperative period. Hypothyroidism is a risk factor for atherosclerotic cardiovascular disease. Therefore it is important to investigate the effects of Levothyroxine replacement therapy on lipid profile and endothelial function after thyroidectomy in patients with overt transient non-autoimmune hypothyroidism. Adipose tissue is an endocrine organ which secretes various adipokines and is regulated by hormonal signals, including insulin, catecholamines and glucocorticoids. Leptin, adiponectin and resistin seem to have a role in energy metabolism. The short-term effects of thyroidectomy-induced hypothyroidism on body mass index, lipid profile and the concentrations of adipokines are sparsely studied. Metabolic syndrome (MS) is an epidemic problem of public health affecting between 15 and 30% of the adult population in Europe. Dyslipidemia is a major component of MS along with insulin-resistant abdominal obesity, glucose intolerance or diabetes mellitus, and hypertension. The effect of poorly controlled postoperative hypothyroidism on the components of MS is not adequately studied which emphasis the need in further researches.

Introduction

Thyroid hormones (TH) has profound impact on the basal metabolism, energy expenditure, carbohydrate, lipid, protein and calcium metabolism, as well as cardiovascular, hemathopoetic, pulmonary and neuromuscular system. Monitoring the variations in lipid profile and the avoiding the development of metabolic complications after thyroidectomy is an important aspect of the medical care in the postoperative period. Hypothyroidism is a risk factor for atherosclerotic cardiovascular disease. Therefore it is important to investigate the effects of Levothyroxine replacement therapy on lipid profile and endothelial function after thyroidectomy in patients with overt transient non-autoimmune hypothyroidism. Adipose tissue is an endocrine organ which secretes various adipokines and is regulated by hormonal signals, including insulin, catecholamines and glucocorticoids. Leptin,

adiponectin and resistin seem to have a role in energy metabolism (1, 2). The short-term effects of thyroidectomy-induced hypothyroidism on body mass index, lipid profile and the concentrations of adipokines are sparsely studied. Metabolic syndrome (MS) is an epidemic problem of public health affecting between 15 and 30% of the adult population in Europe. Dyslipidemia is a major component of MS along with insulin-resistant abdominal obesity, glucose intolerance or diabetes mellitus, and hypertension. TH increases the synthesis of cholesterol and bile acids (BA) flow, resulting in depletion of hepatic cholesterol and enhancement of cholesterol uptake from the circulation to the liver. By contrast, in hypothyroidism, diminution of TH results in slowing of BA flow, marked diminution in the rate of cholesterol secretion into the bile, increase of intrahepatic cholesterol despite the decrease in cholesterol biosynthe-

sis, and decrease of hepatic uptake of cholesterol from the circulation (3). Long-term overt hypothyroidism, as well as subclinical hypothyroidism to a lesser degree, when left untreated cause dyslipidemia and arterial hypertension (diastolic), as well as inflammation characterized by oxidative stress and generation of reactive oxygen species, which may induce endothelial dysfunction, thereby promoting atherosclerosis (4). The effect of poorly controlled postoperative hypothyroidism on the components of MS is not adequately studied which emphasizes the need in further researches.

Aim

The aim of this study is to review:

The short-term effects of thyroidectomy-induced hypothyroidism on body mass index, lipid profile and the concentrations of adipokines.

The effects of Levothyroxine replacement therapy on lipid profile and endothelial function after thyroidectomy in patients with overt transient non-autoimmune hypothyroidism.

Materials and methods

The literature review was based on publications related to the topic over the past 15 years, indexed and referenced in the world-famous Scopus database and Web of science by keywords: lipid profile, metabolic syndrome, thyroidectomy, thyroid hormone, adipocytokines.

Results and discussion

Kaplan O et al were evaluated short term effect of total thyroidectomy on lipid profile and adipokines (5). None of the patients had subclinical hyperthyroidism, hypothyroidism, or thyroid antibody positivity before thyroidectomy. Study variables of the 30 women before and 3 weeks after thyroidectomy are shown in Table 1.

Table 1. Study Variables Before Thyroidectomy (Euthyroid State) and After Thyroidectomy (Hypothyroid State)^a, according to Kaplan O et al., 2012 (5).

Body weight, BMI, waist and hip circumferences, and body fat mass (in percentage of body weight and in kilograms) increased significantly during the hypothyroid period (Table 1). The study group had a relatively high (> 2) HOMA-IR index, indicating insulin resistance in both the euthyroid period and the hypothyroid period. Total cholesterol, HDL-C, LDL-C, and triglyceride concentrations increased significantly after a relatively short period of hypothyroidism. No statistically significant difference was evident between preoperative and postoperative serum adiponectin, leptin, and resistin concentrations. Similarly, fat tissue mass-corrected leptin (leptin/fat mass in kg), adiponectin (adiponectin/fat mass in kg), and resistin (resistin/fat mass in kg) concentrations also did not differ significantly between euthyroid and hypothyroid periods. BMI corrected adipokine concentrations did not change except for a significant decline in leptin/BMI ratio during the hypothyroid period in comparison with the euthyroid state. The patients were further assessed on the basis of their BMI. Normal weight women ($n = 10$) showed significant increases with respect to weight and BMI ($P = .047$ and $P = .047$, respectively), significant increases in total cholesterol, LDL-C, and triglyceride concentrations, and a significantly decreased leptin/BMI ratio. In the overweight group, a significant increase was observed in waist circumference. The lipid profile showed changes similar to those in the overall study group. A near-significant increase in adiponectin/BMI ratio was evident. In the obese women, body weight, BMI, and fat mass (in kg) increased significantly in association with a significantly decreased leptin/

BMI ratio postoperatively. Significant correlations between adiponectin, leptin, and resistin levels and the study variables were as follows. Leptin showed significant positive correlations with weight, BMI, waist and hip circumferences, WHR, fat mass (in kg), insulin, and HOMA-IR during both euthyroid and hypothyroid states. Adiponectin showed significant negative correlations with weight, BMI, waist circumference, WHR, and triglyceride levels during both euthyroid and hypothyroid periods. In contrast, the negative correlation between adiponectin and hs-CRP during the euthyroid period disappeared during the hypothyroid period. Only resistin showed a significant positive correlation with hsCRP during the hypothyroid period. No signifi-

cant correlation was evident between thyroid hormones and adipokine concentrations during both hypothyroid and euthyroid status (data not shown). The main finding in this study indicated that, during short-term thyroidectomy-induced hypothyroidism, no significant changes in serum adipokine concentrations developed, although weight, BMI, and body fat mass increased significantly. Therefore, changes in adipokine concentrations may not directly contribute to body composition alterations during acute, surgically induced hypothyroidism in previously euthyroid patients. Their findings also indicated that a relatively short period of hypothyroidism (3 weeks) is sufficient to induce significant alterations in the lipid profile (5).

Table 1. Study Variables Before Thyroidectomy (Euthyroid State) and After Thyroidectomy (Hypothyroid State)^a

Variable	Euthyroid	Hypothyroid	P value
Weight (kg)	73.9 ± 17.6	75.1 ± 18.0	.001
Body mass index (kg/m ²)	28.6 ± 5.9	29.1 ± 6.1	.001
Waist (cm)	92.4 ± 14.6	93.2 ± 14.5	.047
Hip (cm)	108.7 ± 10.5	110.0 ± 11.1	.005
Waist-to-hip ratio	0.9 ± 0.1	0.9 ± 0.1	NS
Body fat (%)	40.8 ± 9.3	42.5 ± 9.3	.014
Body fat mass (kg)	31.3 ± 13.4	33.1 ± 13.9	.003
Fasting glucose (mg/dL)	92.9 ± 12.3	91.5 ± 11.4	NS
Fasting insulin (μIU/mL)	12.2 ± 11.3	13.6 ± 13.2	NS
Cholesterol (mg/dL)			
Total	188.9 ± 36.1	258.4 ± 58.1	< .001
High-density lipoprotein	51.4 ± 10.5	57.1 ± 11.3	.004
Low-density lipoprotein	116.3 ± 31.5	168.8 ± 49.9	< .001
Triglycerides (mg/dL)	105.9 ± 52.3	164.8 ± 92.0	< .001
Homeostasis model assessment of insulin resistance	2.9 ± 2.9	3.2 ± 3.5	NS
Free triiodothyronine (pg/mL)	3.2 ± 0.4	1.2 ± 0.5	< .001
Free thyroxine (ng/dL)	1.3 ± 0.2	0.44 ± 0.21	< .001
Thyroid-stimulating hormone (mIU/L)	1.0 ± 0.7	64.2 ± 28.4	< .001
High-sensitivity C-reactive protein (mg/L)	2.1 ± 3.0	2.5 ± 3.1	NS
Leptin (ng/mL)	17.1 ± 9.9	17.7 ± 10.5	NS
Adiponectin (ng/mL)	11.2 ± 6.6	10.5 ± 3.5	NS
Resistin (ng/mL)	18.2 ± 9.9	17.2 ± 7.7	NS
Leptin/body mass index	1.1 ± 0.3	0.6 ± 0.3	< .001
Adiponectin/body mass index	0.4 ± 0.2	0.4 ± 0.2	NS
Resistin/body mass index	0.7 ± 0.3	0.6 ± 0.4	NS
Leptin/fat mass in kg	0.5 ± 0.2	0.5 ± 0.2	NS
Adiponectin/fat mass in kg	0.5 ± 0.4	0.4 ± 0.3	NS
Resistin/fat mass in kg	0.7 ± 0.4	0.6 ± 0.4	NS

Abbreviation: NS = not significant.

^a The data are shown as mean ± standard deviation.

Erbil Y et al are found that baseline measurements in patients (stage 1) and controls were similar (6). Effect of thyroidectomy on lipid profile and endothelial function (stage 2) Serum levels of TSH, FT3 and FT4 during the hypothyroid phase 3 weeks after thyroidectomy (stage 2). Serum TSH concentrations were significantly higher at stage 2 than at stage 1, as were serum levels of total cholesterol and LDL cholesterol, and total cholesterol/HDL cholesterol measurements. There were no significant differences between stages 1 and 2 in serum levels of triglycerides, HDL cholesterol or VLDL cholesterol, hs-CRP concentrations, blood pressure, baseline diameter or BMI. Pulse rate, baseline flow and FMD (flow-mediated arterial dilatation) measurements were significantly lower at stage 1 than at stage 2. Effect of thyroxine treatment on lipid profile and endothelial function (stages 3 and 4). Serum levels of TSH, FT3 and FT4 in the euthyroid phase, 3 months after the beginning of L-thyroxine replacement therapy (stage 3). There were no significant differences in serum thyroid hormone concentrations between stages 1 and 3. Although all patients were euthyroid at stage 3, serum levels of total cholesterol and LDL cholesterol and the total cholesterol/HDL cholesterol ratio were significantly higher than those at stage 1. Blood flow and FMD measurements were significantly lower at stage 3 than at stage 1. However, the impairment of lipid profile and endothelial function at stage 2 was more marked than at stage 3. Six months after the beginning of thyroxine treatment (stage 4), measurements of thyroid function, lipid profile and vascular parameters were similar to those at stage 1 and comparable to values in the control group. ICC values for blood flow and FMD were 0.98 (95 per cent confidence interval 0.991 to

0.996) and 0.99 (0.992 to 0.996) respectively (both $P < 0.001$). Effect of postoperative hypocalcaemia on endothelial function Persistent hypoparathyroidism was not encountered, but three of 22 patients developed transient hypoparathyroidism. These three patients developed asymptomatic hypocalcaemia and they were discharged while on oral calcium, at least 24 h after operation. The treatment for hypocalcaemia was discontinued within 7 days. There was no difference in FMD measurements between the three patients who developed transient hypocalcaemia and 19 normocalcaemic patients (6).

In another study of Chu CH et al in South Korea a total of 1203 patients with thyroid cancer who underwent surgery were enrolled in the study.(7) They excluded patients who received dyslipidemia-related medication, whose lipid profiles were not evaluated pre- and post-operatively, and who were aged under 19 years. From them only on 157 patients were performed bilateral total thyroidectomy. Additionally, the TG levels were significantly decreased in total thyroidectomy groups with levothyroxine supplementation, whereas cholesterol and LDL-C levels showed no significant change. In this study, they confirmed that there were no differences in the levels of cholesterol, LDL-C, TG, and HDL-C before and after surgery (7). Study of Korean population revealed that TSH showed significantly positive correlations with serum total cholesterol, TG, and LDL-C regardless of sex, age, season, obesity, or menopausal status (8, 9, 10).

In prospective study of Zihni İ et al in Turkey fifty-nine patients who underwent total thyroidectomy for multinodular goitre were included. (11). Patients height, weight, and waist circumference were measured, and the body mass index was calculated.

Peripheral blood samples were obtained preoperatively and at the 12th and 24th month after total thyroidectomy to examine the lipid profile, glucose homeostasis, and thyroid function tests. The lipid profile and blood pressure parameters deteriorated, and the mean body mass index and waist circumference with the metabolic syndrome rates significantly increased at the 12th and 24th months follow-up. Preoperative body mass index was independently associated with metabolic syndrome at the 2nd year after total thyroidectomy in a multivariate regression analysis. They conclude that the frequency and severity of metabolic syndrome was increased in patients with a high body mass index after total thyroidectomy (11).

Conclusion

The results are contradictory. Short term effects of hypothyroidism induce significant alterations in the lipid profile. Levothyroxin replacement therapy turn out to have multiple effects on lipid profile and endothelial function. It mostly depends from the starting point of replacement therapy after total thyroidectomy. Many unanswered questions indicates the need of further studies to reveal effects of hypothyroidism and replacement therapy on body mas index, lipid profile in correlation with adipokines, and endothelial function.

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Subjective assessment of depression and anxiety, cognitive functions, daytime sleepiness, insomnia, adverse events from treatment, sleep quality and quality of life in patients with epilepsy on Levetiracetam monotherapy

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Introduction: The information in literature about the effect of Levetiracetam (LEV) monotherapy on depression and anxiety, daytime sleepiness, insomnia, adverse events, sleep quality and quality of life in patients with epilepsy is insufficient. **Methods:** We performed an open, prospective 3-month study with 2000 mg LEV in 25 patients (18 (72%) women and 7 (28%) men, average age 35.08 ± 16.83 years) with epilepsy. The subjective assessment was done at baseline and after therapy through the following questionnaires: Hospital Anxiety and Depression Scale, Epitrack, Epworth Sleepiness Scale, Athens Insomnia Scale, Liverpool Adverse Event Profile, QOLIE-10-P, Pittsburgh Sleep Quality Index. **Results:** In 16 (64%) patients we observed no worsening of depression and anxiety and in 7 (28%) – improvement. According to Epitrack results there was no cognitive dynamics in 18 (72%) participants and improvement in 4 (16%). The daytime sleepiness of 22 (88%) patients was not aggravated, in the rest – was improved. There was no insomnia aggravation in any patients. We found no adverse events worsening in 19 (76%) patients and improvement in 2 (8%). The sleep quality of 17 (68%) patients remained unchanged and in 4 (16%) – was improved. The quality of life of 15 (60%) patients remained unchanged and was improved in 7 (28%). **Conclusion:** The 2000 mg LEV monotherapy in a 3-month period does not worsen the subjective assessment of daytime sleepiness and insomnia in all patients with epilepsy, as well as cognitive functions, depression and anxiety, adverse events, sleep quality and quality of life in most of them.

Introduction

Levetiracetam (LEV) is a newer generation antiepileptic drug with a unique anti-convulsive mechanism of action – binding to ubiquitous administered SV2A protein in the presynaptic neuron terminals [1]. It possesses a broad antiepileptic spectrum and optimal pharmacokinetics. Adverse events from LEV treatment have been reported in 7–55.4% of patients, the most common being: dizziness (5.8–33.3%), asthenia (5.6–35.9%), sleepiness (5–40.4%), headache (3.2–22%), behavior disorders (7.4–25%), insomnia (2–4.8%) [2]. No Bulgarian studies about the effects of LEV on sleep, depression and anxiety, cognitive functions, and quality of life in patients with epilepsy, have been performed.

Purpose

The purpose of our study is to make a subjective assessment of the effects of 2000 mg LEV monotherapy over a 3-month peri-

od on daytime sleepiness, depression and anxiety, cognitive functions, insomnia, adverse events from treatment, sleep quality, and quality of life in patients with epilepsy.

Patients and methods

The study is open, prospective, with the participation of 25 patients with epilepsy. They attended the Clinic of Neurology at the University Hospital in Plovdiv, Bulgaria after one or more seizures to be diagnosed or treated adequately.

All study procedures were performed after approval of the Local Ethics Commission at the Medical University, Plovdiv, Bulgaria. Every patient signed an informed consent form before participating in all study procedures.

The following inclusion criteria were used: a signed informed consent form; patients with newly diagnosed epilepsy, already diagnosed but untreated epilepsy or patients with ceased AEDs for at least a

3-month period prior to study onset; age between 18 and 75 years; pre-existing diagnosis of a primary sleep disorder; absence of other drug therapy affecting daytime sleepiness; absence of poor sleep hygiene; absence of moderate to severe cognitive impairment. The diagnosis of epilepsy is in conformity with the ILAE definition from 2014 [3].

All participants underwent subjective assessment through 1. Epworth sleepiness scale (ESS) – score ≥ 10 indicates excessive daytime sleepiness; 2. Hospital depression and anxiety scale (HADS) – score ≥ 11 indicates depression and anxiety; 3. Pittsburgh sleep quality index (PSQI) – score ≥ 5 indicates poor sleep quality; 4. EpiTrack – score ≤ 31 cognitive dysfunction; 5. Athens insomnia scale (AIS) – score ≥ 6 indicates insomnia; 6. QOLIE-10-P – score 1.4–2.59 indicates excellent and 2.6–4.8 average quality of life; 7. Liverpool adverse events profile (LAEP) – score 19–38 mild, 39–57 moderate and 58–76 severe adverse events. All assessments were performed twice – at study onset and after a 3-month period of LEV treatment. The dynamics in each questionnaire were measured as a shift from one category to another after a 3-month LEV treatment defined as “no change”, “improved” and “worsened”.

All medical history, was collected by a trained neurologist specialized in epilepsy. A detailed physical and neurological examination, electroencephalography (EEG), and a neuroimaging study (CT and/or MRT), as well as blood sampling (full blood count, biochemistry, measurement of serum LEV level following a 3-month LEV treatment to verify compliance), were performed.

Descriptive statistics were used to analyze the frequency, mean and standard deviation for the demographic characteristics as well as for baseline and after therapy scores of MSLT. An exact McNemar's test

was run to compare dichotomous variables before and after LEV therapy (subjective measures – present or absent). The Mann-Whitney U test was used to determine if there were differences in scores between the groups defined by clinical characteristics. The effect size (r) for Mann-Whitney test was calculated as $r = z/(\sqrt{N})$, where z is the value of the test statistic and N is the number of observations. The interpretation of r is: small effect ($0.10–0.3$), moderate effect ($0.30–0.5$) and large effect ($r \geq 0.5$). All statistical analyses were conducted using SPSS version 23. All statistical tests were 2-tailed and conducted at a 5% significance level.

Results

The mean age of the participants was 35.20 ± 16.69 years. The demographic and clinical characteristics of the study participants at the study onset are presented in Table 1.

All patients had LEV serum levels within the therapeutic range (10–40 mg/l) (22.52 ± 8.85) at the end of the 3-month period, which guaranteed compliance. The therapeutic dose of 2000 mg LEV was titrated in a 1-week period and was well-tolerated. Only four patients had a seizure in the 3-month period of observation and none of them had aggravation in seizure severity.

The EEG characteristics of the study participants at the study onset and following a 3-month period with LEV treatment are presented in Table 2.

The percentage of patients with normal and abnormal scores of all questionnaires at study onset and after a 3-month period with LEV treatment are presented in Table 3.

The dynamics after a 3-month period with LEV treatment defined as “no change”, “improved” and “worsened” for all questionnaires is presented in Table 4.

Table 1. Demographic and clinical characteristics of the study participants at the study onset

Demographic and clinical characteristic		N (P%)
Sex	- Female - Male	17 (68%) 8 (32%)
Age	- 18–35 years - 36–50 years - > 50 years	16 (64%) 4 (16%) 5 (20%)
Age of epilepsy onset	- ≤ 18 years - > 18 years	7 (28%) 18 (72%)
Epilepsy diagnosis	- Newly diagnosed - Already diagnosed	15 (60%) 10 (40%)
Epilepsy type	- Generalized - Focal - Generalized and focal	20 (80%) 2 (8%) 3 (12%)
Seizure type	- Generalized - Focal - Generalized and focal	18 (72%) 2 (8%) 5 (20%)
Seizure frequency	- ≤ 1 seizure a year - > 1 seizure a year	10 (40%) 15 (60%)
Seizure severity	- Mild - Severe	6 (24%) 19 (76%)
Etiology	- Unknown - Metabolic/structural	15 (60%) 10 (40%)
Focal neurological symptoms	- Present - Absent	14 (56%) 11 (44%)
Neuroimaging findings	- Normal - Unrelated to epilepsy findings - Related to epilepsy findings	13 (52%) 1 (4%) 11 (44%)
Comorbidities*	- Present - Absent	20 (80%) 5 (20%)

N – number of patients, P (%) – percentage of patients.

*Comorbidities: arterial hypertension in 4 patients and hypothyroidism in one.

Table 2. EEG characteristic of the study participants

EEG finding	At study onset N (P%)	Following LEV treatment N (P%)
Background activity		
- Normal - Depressed and disorganized	17 (68%) 8 (32%)	21 (84%) 4 (16%)
Pathological activity		
- focal slow wave - focal epileptiform - generalized epileptiform - none	5 (20%) 9 (36%) 2 (8%) 9 (36%)	2 (8%) 7 (28%) 2 (8%) 14 (56%)

N – number of patients, P (%) – percentage of patients.

Table 3. Percentage of patients with a normal and an abnormal score of all questionnaires at study onset and after a 3-month period with LEV treatment

Questionnaire/Subjective measures	At study onset		Following LEV treatment		p
	Present	Absent	Present	Absent	
ESS/Excessive daytime sleepiness	7 (28%)	18 (72%)	4 (16%)	21 (84%)	0.25
HADS/Depression and anxiety	10 (40%)	15 (60%)	4 (16%)	21 (84%)	0.11
PSQI/Poor quality of sleep	9 (36%)	16 (64%)	9 (36%)	16 (64%)	1
EpiTrack/Cognitive impairment	8 (32%)	17 (68%)	5 (20%)	20 (80%)	0.25
AIS/Insomnia	2 (8%)	23 (92%)	2 (8%)	23 (92%)	1
QOLI-10-P/Quality of life					
Excellent	13 (52%)		17 (68%)		0.42
Average	12 (48%)		8 (32%)		

Table 4. Dynamics in all questionnaires after a 3-month period with LEV treatment

Questionnaire	Dynamics following LEV treatment		
	No change	Improved	Worsened
ESS	22 (88%)	3 (12%)	0 (0%)
HADS	15 (60%)	8 (32%)	2 (8%)
PSQI	17 (68%)	4 (16%)	4 (16%)
EpiTrack	20 (0%)	4 (16%)	1 (4%)
AIS	25 (100%)	0 (0%)	0 (0%)
QOLIE-10-P	11 (44%)	9 (36%)	5 (20%)
Adverse events	18 (72%)	3 (12%)	4 (16%)

The results showed no statistically significant change in daytime sleepiness depression and anxiety, quality of sleep, cognitive functions, insomnia, quality of life, adverse events before and after LEV treatment.

There was a statistically significant median increase in EpiTrack score when compared pretest and posttest scores in patients with ≤ 1 seizure a year, $z = -2.207$, $p = 0.02$; in patients with severe seizures, $z = -2.401$, $p = 0.016$ and in patients with no focal neurological symptoms $z = -2.365$, $p = 0.018$. The effect sizes were $r = -0.49$, $r = -0.39$, and $r = -0.50$ respectively that represent moderate effects.

We found a statistically significant median decrease in HADS scores before and after LEV-therapy in patients with no pathological changes in EEG, $z = -2.073$, $p = 0.038$ with moderate effect size $r = -0.49$.

Discussion

The results from our study show that 2000 mg LEV monotherapy does not change the subjective assessment of depression and anxiety, cognitive functions, daytime sleepiness, insomnia, adverse events from treatment, sleep quality, and quality of life in patients with epilepsy.

Regarding the subjective assessment of daytime sleepiness, our results are consistent with the data from two studies by Cicilin et al. (14 healthy volunteers treated with 2000 mg/placebo for 3 weeks) and Cho et al. (16 patients with newly diagnosed focal epilepsy treated with 1000 mg LEV) [4, 5]. Another study by Zhou et al. however, observed a significant increase in the ESS score (10 patients with focal epilepsy after 3 weeks of LEV treatment) [6]. The smaller number of participants and the lower daily dose of LEV (1000 mg/day)

and the participation of only patients with focal epilepsy could explain the difference from our study results. However, as our results showed, no significant difference in AIS score was observed before and after LEV treatment in the same study [6]. The results from the study of Cho et al., confirmed no significant difference in PSQI score between baseline and post-treatment with LEV [5].

A literature review of randomized, placebo-controlled trials of add-on LEV treatment in 1595 adult patients with drug-resistant focal epilepsy confirms somnolence as the most common adverse effect of add-on LEV therapy in 14%, rare behavioral disturbances, lack of cognitive impairment, and positive impact on quality of life [7]. We observed a trend toward an increase in the proportion of patients with excellent quality of life, lack of changes in cognitive functions, and no change or improvement in depression and anxiety however, it did not reach statistical significance.

According to our study results patients with ≤ 1 seizure a year, severe seizures, or no focal neurological symptoms have shown improvement in cognitive functions and patients with no pathological EEG changes have fewer symptoms of depression and anxiety after therapy. No similar results and conclusions have been found in the literature.

Conclusion

Although daytime sleepiness and behavior changes are commonly reported side effects from LEV therapy, our results prove that LEV has no such negative impact, which may contribute to its choice in the clinical practice.

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Subacute thyroiditis after asymptomatic SARS-CoV-2 infection

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Introduction: There are many initial or sequel extrapulmonary clinical features of COVID-19. The virus uses ACE2 combined with the TMPRSS2 to infect the host cells, the expression of both is higher in the thyroid in comparison to the lungs. We report a patient with subacute thyroiditis after asymptomatic COVID-19. **Objectives:** Subacute thyroiditis (SAT) is a viral inflammatory disorder of the thyroid that causes destructive thyrotoxicosis. The objective is to report a case of SAT related to asymptomatic SARS-CoV-2 infection. **Methods:** We present the clinical findings, ultrasound features, thyroid function and antibodies tests of a woman with no history of thyroid disorder who presented with clinical features of thyroiditis after asymptomatic COVID-19 infection. **Case presentation:** A 36-year-old woman with neck pain, odynophagia, palpitations, without fever or respiratory symptoms treated with ibuprofen with satisfactory effect. Thyroid function tests showed destructive thyrotoxicosis with negative thyroid antibodies, elevated inflammatory markers and leukocytosis. The neck ultrasound concluded subacute thyroiditis with involvement of the left thyroid lobe. After a gradual resolution, the symptoms worsened and further immunological testing suggested a past asymptomatic COVID-19. Follow up tests revealed worsening of the thyrotoxicosis with bilateral thyroid involvement. After prednisolone treatment symptoms resolved within 1 week with restoration of normal thyroid volume and hypothyroid phase registered after 45 days. **Conclusions:** A relevant target of damage by SARS-CoV-2 could be the thyroid gland in the form of subacute thyroiditis (SAT).

Keywords: *Subacute thyroiditis, SARS-CoV-2, COVID-19, Destructive thyrotoxicosis, Thyroid Ultrasound*

Introduction

The ongoing Coronavirus disease 2019 (COVID-19) pandemic is caused by the severe-acute-respiratory-syndrome-coronavirus-2 (SARS-CoV-2). The disease has a wide spectrum of clinical severity, ranging from asymptomatic cases and the common cold to much more severe and even fatal respiratory damage. (1) Many initial or sequel extrapulmonary clinical features of COVID-19 are emerging in the medical reports. COVID-19 causes pulmonary and systemic inflammation, and there is a known complex connection between the thyroid gland and the occurring inflammatory-immune response. (2). Two plausible mechanisms could be responsible for the changes in the thyroid gland and its hypothalamic-pituitary axis – an indirect effect through abnormal systemic inflammatory-immune responses caused by SARS-CoV-2 infection or a direct viral effect. The virus uses Angiotensin-converting enzyme 2 (ACE2) combined with the transmembrane protease serine 2

(TMPRSS2) to infect the host cells and it is important to note that the expression of both is higher in the thyroid gland in comparison to the lung tissue (Figure 1). (3, 4, 5, 6) Computer simulated models demonstrate that ACE2 expression levels in the thyroid are positively and negatively linked to immune signatures [CD8+ T cells, interferon response, B cells, and natural killer (NK) cells] in males and females (6), which could explain the different immune responses and the distinct thyroid manifestations as a result. It is believed that the uptake of SARS-CoV-2 by the host cells involves other cellular molecules and proteases as a secondary mechanism. (3, 4) Newly emerging data implicates a group of structural proteins of the plasma membrane – the integrins in the cell invasion of SARS-CoV-2. (7) ACE2 binds to integrin to modulate downstream signal transduction. (7) Thyroxin (T4) regulates expression of the genes for the monomeric protein that makes up integrins thus thyroid hormones

could promote the internalization of the integrins (8, 9) and therefore could positively influence the SARS-CoV2 uptake. (9) We report a patient diagnosed with subacute thyroiditis precipitated by asymptomatic COVID-19 infection. A relevant target of damage by SARS-CoV-2 could be the thyroid gland in the form of subacute thyroiditis (SAT).

Objectives

The objective of this work is to report a case of subacute thyroiditis (SAT) related to asymptomatic SARS-CoV-2 infection. SAT is an inflammatory disorder of the thyroid gland that causes destructive thyrotoxicosis and is attributed to a viral or post-viral response. (10) The incidence of the disease is 4.9 cases/100.000/year with a mean age of 46 years and a female-to-male ratio of 3.5:1. (11) SAT presents as unilateral or bilateral neck pain radiating to the jaw or ears with painful and tender thyroid on palpation (12) in combination with systemic symptoms (fever, malaise and anorexia). (13) The exact cause of subacute thyroiditis is not known but multiple viruses (measles, mumps, rubella, coxsackie, adenovirus, chickenpox, cytomegalovirus, Epstein Barr

virus, HIV, Hepatitis E, and influenza) have been implicated to trigger the onset of the disease, either through direct injury or indirectly through circulating viral genome or virus-specific antibodies. (14, 15) SAT is primarily a clinical diagnosis supported by laboratory testing and imaging. (16, 17) The clinical features correlate with a combination of test results – elevated erythrocyte sedimentation rate and C-reactive protein; low TSH; elevated thyroid hormone levels (T4 and T3) and thyroglobulin concentrations; with an absent or low positive titer of circulating thyroperoxidase and thyroglobulin antibodies. (14) SAT is a self-limiting disease with 3 distinct phases: an initial thyrotoxic phase, followed by hypothyroid and euthyroid phases. (14) Acute inflammatory responses in the thyrotoxic phase results in suppressed TSH with elevated thyroglobulin and thyroxine, which may persist up to 6 weeks. The depletion of the thyroid stores presents with elevated TSH to hypothyroid levels. With resolution of SAT, ninety percent of patients regain thyroid function and become euthyroid within 6 to 12 months. (18) Anti-inflammatory agents are the first line of therapy, with corticosteroids used in more severe cases. (14)

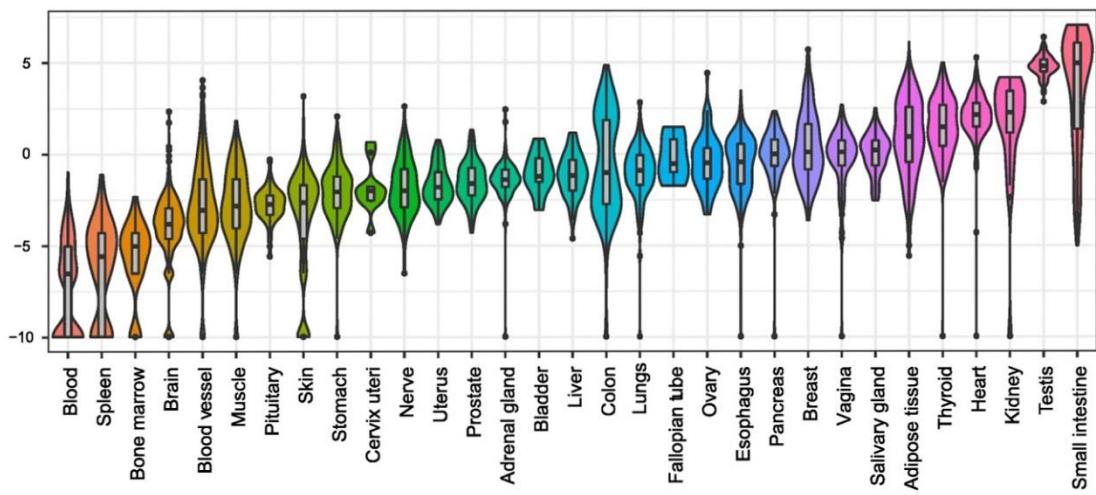


Figure 1. Comparison of ACE2 expression levels across 31 human tissues in genotype-tissue expression (6, 19).

Methods

We describe the clinical findings, thyroid function and antibodies tests, and neck ultrasound features of a 36-year-old female patient with no significant personal or family history of thyroid disorder who presented with clinical features of thyroiditis that began after asymptomatic COVID-19 infection.

Case presentation

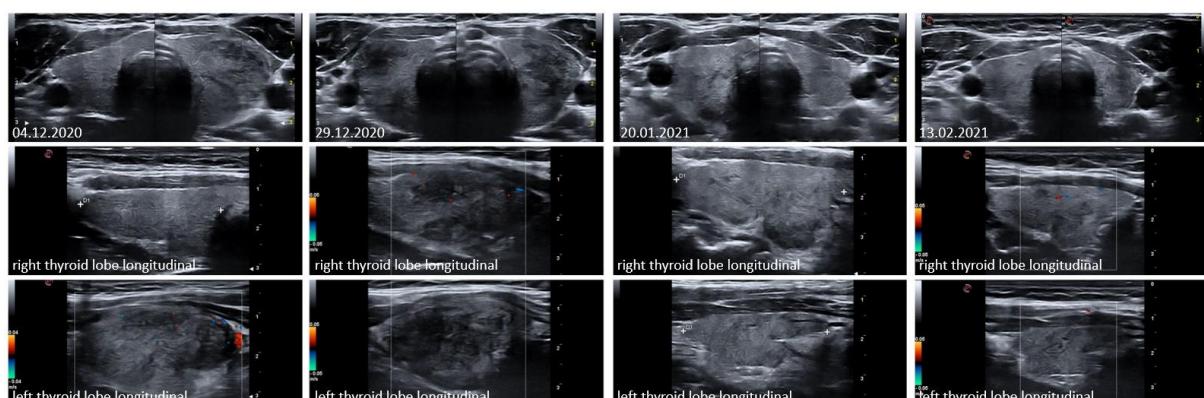
A 36-year-old woman presented with slight left anterior neck pain with irradiation to the jaw associated with odynophagia, palpitations, with no fever or respiratory symptoms. Her primary care physician had already prescribed non-steroidal anti-inflammatory drug (Ibuprofen 400 mg daily) that provided satisfactory relief of her symptoms. It is important to note that the patient refused any treatment with prednisone. Thyroid function tests were consistent with destructive thyrotoxicosis with negative thyroid antibodies and elevated inflammatory markers and white blood cell count (Table 1). The ultrasound of the thyroid gland revealed a hypoechoic, heterogeneous thyroid gland with increased thyroid volume of 15.82 ml with a diffuse, patchy, ill-defined, hypoechoic, non-vascularized area with dimensions of 16/18/24 mm in the lower portion of the left thyroid lobe, suggestive of subacute thyroiditis with predominant involvement of the left lobe (Figure 2). Three weeks after gradual symptom resolution, the patient noted worsening pain

and swelling of her anterior neck in combination with a low-grade fever and moderate odynophagia. A nasopharyngeal reverse transcriptase-PCR (rtPCR) was performed and was negative for SARS-CoV-2, whilst ELISA evaluated SARS-CoV-2 IgG antibodies were greatly increased with negative SARS-CoV-2 IgM antibodies – suggesting a past asymptomatic COVID-19 infection. Follow up tests revealed further elevation of free thyroxine and free triiodothyronine, suppressed thyrotropin with high inflammatory markers and white blood cell count (Table 1). The thyroid ultrasound detected bilateral and diffuse hypoechoic areas characteristic for subacute thyroiditis, presenting newly found diffuse, patchy, ill-defined, hypoechoic, non-vascularized area with dimensions of 19/22/29 mm representing the involvement of the right thyroid lobe, with increased thyroid volume of 20.16 ml. Cervical lymphadenopathy in VI neck quadrant was noted. (Figure 2) The patient was started on 20 mg prednisone daily with a reduction of 5 mg every 2 weeks. Neck pain and fever recovered within one week, inflammatory markers normalized in 20 days (Table 1) and a follow up neck ultrasound showed a hypoechoic, bilaterally heterogeneous thyroid gland with normalized volume of 10.07 ml and relative resolution of the affected thyroid zones in comparison to the previous evaluation (Figure 2). After 45 days we registered hypothyroid phase of the SAT. The patient was scheduled for a follow up visit.

Table 1. Clinical laboratory results

Date	04.12.2020	29.12.2020	20.01.2021	13.02.2021	Reference Range
TSH	0.225	0.009	0.72	5.514	0.27–4.20 mIU/ml
fT3	4.3	6.7	N/A	N/A	3.10–6.8 pmol/l
fT4	21.51	35.96	12.26	10.63	12.0–22.0 pmol/l
Anti-TPO	12.6	N/A	N/A	N/A	< 34 IU/ml
Anti-Tg	82.4	N/A	N/A	N/A	< 115 IU/ml
TSH-R-Ab	0/2	N/A	N/A	N/A	< 1.58 IU/L
Tg	96.1	104.5	N/A	N/A	1.4–78.0 ng/ml
CRP	28.25	59.6	<0.6	<0.6	< 6.0 mg/L
ESR	50.0	77.1	10.1	5.4	0–13 mm/h
WBC	11.2	11.9	8.7	7.4	3.5–10.5 10^9/L
rtPCR	N/A	Neg. /-/	N/A	N/A	Neg /-/
SARS-CoV-2 Ig G ELISA	N/A	1077.4	N/A	N/A	< 50 AU/ml (potentially protective titter > 1050)
SARS-CoV-2 Ig M Qual.	N/A	Neg. /-/	N/A	N/A	Neg /-/

Abbreviations: TSH, thyrotropin; fT3, free triiodothyronine; fT4, free thyroxine; Tg, thyroglobuline; anti-Tg, thyroglobulin antibodies; anti-TPO, thyroperoxidase antibodies; TSH-R-Ab, TSH receptor antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; rtPCR, reverse transcriptase-PCR.

**Figure 2.** Evolution of the ultrasound changes.

Conclusions

We report a case of SAT after an asymptomatic SARS-CoV-2 infection pointing out that SAT may be a presenting symptom or a sequel of COVID-19. For early recognition and timely anti-inflammatory therapy clinicians must be aware of the possibility of thyroid dysfunction after COVID-19 infection.

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Acute pancreatitis as complication of COVID-19 infection – case report

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Introduction: The COVID-19 infection is characterized by a variety of symptoms and even in asymptomatic cases late complications such as muscle weakness, fatigue, arrhythmia and pericarditis can be found. In this article we want to present a patient with acute pancreatitis, as a result of late rare complication, 25 days after treatment and a negative PCR test. **Case report:** A 60-year-old female with moderate clinical symptoms, including astheno-adynamia, subfebrile fever, positive antigen and PCR test. Upon detection of the disease, the patient underwent high resolution computed tomography (HRCT) of the thorax and initial specific changes of the disease were identified. In this HRCT, the images of the abdominal cavity, no changes are found in the area of the pancreas. 10 days after a 14-days-long drug treatment the patient develops symptoms of acute pancreatitis – initially mild, gradually increasing, including pain in the epigastrium with irradiation to the back, nausea, vomiting. The performed laboratory and diagnostic procedures as ultrasound and computed tomography (CT) of the abdomen, verify acute pancreatitis as a result of COVID-19 infection, after all other causes were rejected. **Conclusion:** Although the acute pancreatitis is rare in patients with COVID-19 infection, it should be considered in the differential diagnosis in patients with severe epigastric pain, nausea, and respiratory symptoms.

Keywords: COVID19, acute pancreatitis, abdominal pain

Introduction

Coronavirus is infectious disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and typically presents with pulmonary symptoms like cough, shortness of breath, and fever. However, gastrointestinal manifestations of COVID-19 are increasingly being recognized. Angiotensin-converting enzyme-2 (ACE2) has an important role in the pathophysiology and presentation of this disease. In pancreas, this receptor is expressed in both exocrine glands and islets, being a potential target for the virus.

In this article we want to present a patient with acute pancreatitis, as a result of late rare complication, 10 days after a 14-days-long drug treatment.

Patient

A 60-year old woman presented to the Department of Gastroenterology in University Hospital Kaspela with a 2-day history

of epigastric pain, vomiting, nausea and diarrhea. The patient denied any history of alcoholic abuse or smoking. Her only comorbidity was well-controlled hypertension. She has had a positive COVID-19 test 24 days prior but the disease presented only with a mild cough and a high temperature for 3 days. On physical examination in the day of admission the patient was subfebrile and had tachycardia (110 bpm), blood pressure of 140/90 and temperature of 37°C. Her abdominal examination showed severe epigastric tenderness with a soft abdomen, irradiating towards her back. The rest of the physical exam was nonsignificant. Her laboratory investigations showed elevated serum amylase 250 U/L (40–100 U/L) and lipase 150 U/L (< 70 U/L) and CA19-9 in upper limit: 38 U/ml (< 27 U/ml) (tabl. 1). The liver enzymes were in normal ranges. Other causes of acute pancreatitis such as drugs, trauma and hypotension were excluded.

Table 1. Laboratory analysis on the first day of admission

Parameter	Lab value	Reference range
Amylase	250	40–100 U/L
Lipase	150	< 70 U/L
Alkaline phosphatase	75	35–90 mg/dL
Aspartate aminotransferase, ASAT	39	8–40 IU/L
Alanine aminotransferase, ALAT	46	7–55 IU/L
C-reactive protein	29.6	< 8 mg/L
CA 19-9	38	< 27 U/ml

Diagnostic imaging

Standart abdominal ultrasound (US) was performed to evaluate pancreas. It was performed in B-mode with ultrasound machine Esaote MyLab™ 9 eXP. Pancreatic body and tail were enlarged, visible pancreatic duct and we suspected peripancreatic effusion. The US did not detect any gallstones. These imaging findings and increased tu-

mor marker CA19-9 necessitated the implementation to perform abdominal MRI and the patient was sent to the Department of Diagnostic Imaging in the same hospital. The data of the MRI acquisitions confirmed US findings (fig. 1). The patient denied contrast enhanced MRI with gadolinium.

Contrast enhanced CT (CECT) has higher sensitivity and specificity in diagnostics of pancreatic diseases and differentiation benign of malignant tumors. CECT scan was performed for diagnostic clarity with SIEMENS SOMATOM go.Up. CECT showed increased volume of the pancreas, especially in body and tail, enlarged pancreatic duct, hypodense parenchyma, result of the edema of the tissues and inflammation of peripancreatic fat tissue. The diagnosis was confirmed – acute edematous pancreatitis (fig. 2).

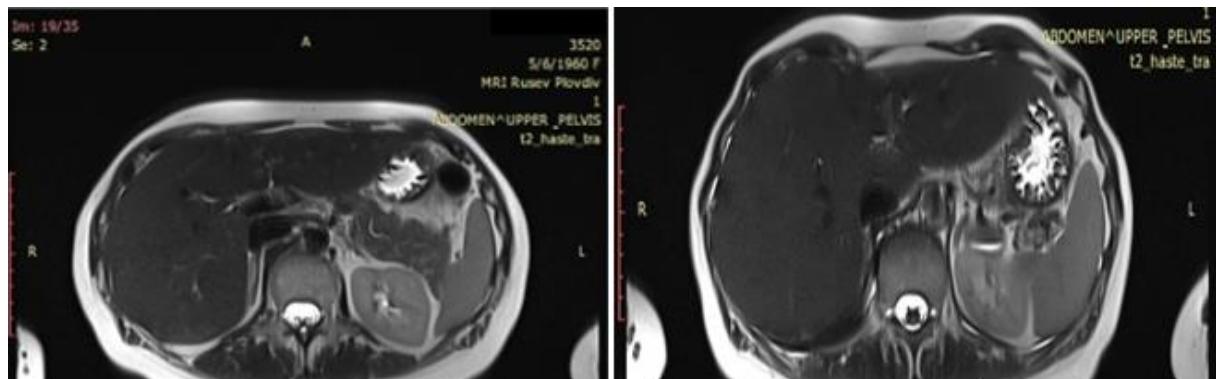


Figure 1. Abdominal MRI: Enlarged pancreatic body and tail, enlargement of the pancreatic duct. A hyperintense lesion in T2 is seen under the pancreatic tail.

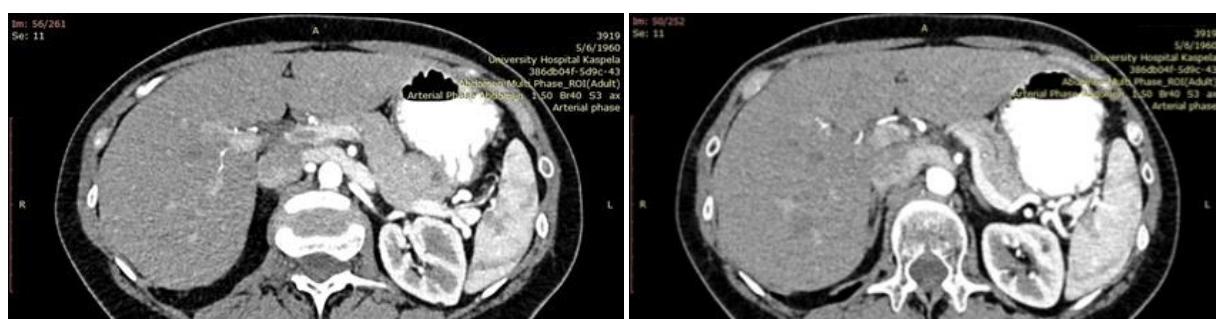


Figure 2. Abdominal CECT: Enlarged pancreatic body and tail, enlargement of the pancreatic duct. No evince of nodular pancreatic lesions. No evidence of stone disease. Decreased density of pancreatic parenchyma. No peripancreatic effusion. The hypointense lesion in MRT examination was confirmed as part of the stomach.

According to the revised Atlanta classification, the diagnosis of acute pancreatitis requires two of the following three features: (a) abdominal pain consistent with acute pancreatitis, (b) serum lipase activity (or amylase activity) at least three times greater than the upper limit of normal and (c) characteristic findings of acute pancreatitis on CECT and, less commonly, MRI or trans-abdominal US. [1]

The patient was treated symptomatically with fluid replacement, correction of electrolyte balance and empiric antibiotics. She had an eventful recovery with resolution of her abdominal symptoms and was discharged after 7 days of hospitalization.

Control CECT was performed three months after treatment. The pancreas was with normal size and shape. The pancreatic duct was not seen. Normal density of the pancreatic parenchyma. There were no nodular pancreatic lesions. The laboratory blood analysis was in normal ranges.

Discussion

Different etiological factors have been described for acute pancreatitis. The most common cause is gallstones and heavy alcohol consummation [2]. Our patient denied alcohol consummation and the US did not show any gallstones, so we suspect relationship between acute pancreatitis and COVID-19 due their temporal association.

The level of ACE2 receptors in pancreas was shown to be higher than in lung and is expressed in both exocrine glands and islets, being potential targets of SARS-CoV-2 virus [3]. Glycosylated-spike (S) protein is one of the structural proteins encoded by the coronavirus genome, and it is a prime inducer of the host immune response. This protein binds to ACE2 receptor protein located on the host cell surface membrane and mediates the host cell invasion [4–6].

Direct cytopathic effects of COVID-19 or immune-mediated and indirect systemic inflammatory response could be the mechanism of pancreatic injury [7, 8]. The study of Wang et al. showed that in a case series of 52 patients with COVID-19 there was 17% of pancreatic injury [9]. Most of the published reports on acute pancreatitis in the context of COVID-19 have diagnosed the pancreatic injury attributing to the typical abdominal pain and elevated pancreatic enzymes [10, 11].

Conclusion

COVID-19 has shown broad spectrum of severity and clinical manifestations. Acute pancreatitis result of COVID-19 is rare. For that reason, clinicians must pay attention to the SARS-CoV-2 infections and related pancreatic complications.

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Serum concentration of chemerin in patients with rheumatoid arthritis

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Aim: To examine the association between the serum concentration of chemerin and markers of inflammation and disease activity in patients with rheumatoid arthritis. **Patients and methods:** This pilot study included 20 patients with rheumatoid arthritis (3 males and 17 females, mean age 59.9 ± 13.1 years). Another 31 age and sex matched cases with no history of joint diseases were recruited as healthy controls (10 males and 21 females, mean age 65.0 ± 9.3 years). The level of chemerin in the serum were measured by enzyme-linked immunosorbent assay (ELISA). Rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum level of uric acid (UA) were evaluated in the subgroup of 20 patients. Disease activity score was determined using the Disease Activity Score-28 for Rheumatoid Arthritis with ESR (DAS28-ESR). **Results:** No significant difference was found in the serum chemerin level between the patient and the control groups [247.0 (226.5–314.6) ng/mL vs. 228.8 (203.1–276.0) ng/mL, $p > 0.05$, respectively]. Nonetheless, positive correlation between serum chemerin concentration and the markers of inflammation was established – ESR ($r = 0.649$, $p = 0.002$), CRP ($r = 0.546$, $p = 0.013$), RF ($r = 0.465$, $p = 0.039$). The chemerin level also correlated positively with the indicator of disease activity – DAS28-ESR ($r = 0.807$, $p < 0.001$). No significant correlation was established between serum chemerin and UA. **Conclusion:** Our results suggest that serum chemerin concentration might reflect the proinflammatory changes associated with RA. Further studies are needed to elucidate its potential role in the pathogenesis of the disease.

Introduction

White adipose tissue (WAT) used to be considered as a fat depot until 1994, when leptin was discovered [1]. Luckily, since then a great variety of adipose-derived factors – named adipokines, have been studied as potential participants not only in metabolic processes, but in inflammation and immunity as well. [2–6] The term adipokine is associated with molecules secreted only by the adipose tissue, while adipocytokines (from Greek *adipo-*, fat; *cytos-*, cell; and *-kinos*, movement) are cell signaling proteins secreted mainly, but not only from WAT. In the present article “adipokine” will be used as a general term for bioactive product produced by the adipose tissue.

Chemerin (also known as tazarotene-induced gene 2 protein (TIG2), retinoic acid receptor responder gene 2 (RARRES2) or

RAR-responsive protein TIG2) was first examined in the context of a psoriatic skin lesion. [7] However, multiple studies revealed this adipokine acts not only as a chemoattractant molecule at the site of inflammation to dendritic cells and macrophages [8], but as one regulating lipid metabolism and adipocyte growth as well [9]. Apart from its metabolic activities, the role of chemerin as a growth factor in osteogenesis and tumor progression is also being investigated. [10–13]

Involvement of this adipokine in rheumatoid arthritis (RA) is based on the discovery that chemerin and its G protein-coupled receptor – Chemokine like receptor 1 (CMKLR1), also known as ChemR23, are expressed mainly, but not only in adipose tissue. For example, dendritic cells and macrophages express the chemerin recep-

tor. Chondrocytes is another type of cells that express both chemerin and its receptor in joints. It is found that pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6 increase the expression of ChemR23 in both endothelial cells and chondrocytes.

On the other hand, chemerin increases production of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-8) and metalloproteinases – (MMPs-2,3,13) in human articular chondrocytes. [14–18]

This new data, showing this molecule affects joint structures, urged a need for conducting studies that deal with chemerin's potential role as a future biomarker of rheumatic arthritis.

Most clinical and experimental studies support the role of chemerin as a non-mechanical link between white adipose tissue and inflammatory and immunity processes in development and progression of rheumatoid arthritis. Despite chemerin was found in inflamed tissues and biological fluids of RA patients, there is still few data regarding its association with markers of inflammation.

Aim

The aim of this pilot study was to examine the association between serum concentration of chemerin and markers of inflammation and disease activity in patients with rheumatoid arthritis.

Patients and methods

Participants: This case-control study included 20 patients with RA recruited from the Outpatient and Inpatient Rheumatology department of Kaspela University Hospital, Plovdiv for the period of January 2020 – January 2021. Another 31 age and sex matched individuals with no history of rheumatic disease were included as controls. Rheumatoid arthritis patients were

evaluated according to the 2010 American College of Rheumatology and the European League Against Rheumatism Criteria [19]. Anamnesis of another rheumatic disease or malignant disease, history of trauma, infection, surgical intervention in the joints, as well as any uncontrolled clinically significant chronic disease were part of the exclusion criteria for study. Written informed consent was taken from all participants in accordance with the declaration of Helsinki.

Data collection: All clinical and demographic data including age (years), gender (male/female), Body Mass Index (BMI – kg/m²), disease duration, treatment methods, data of co-morbidities, such as hypertension and diabetes mellitus type 2, was collected. In the subgroup of 20 patients with RA rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), uric acid (UA) were also evaluated.

Disease activity: The disease activity in patients with RA was determined calculating the Disease Activity Score-28 for Rheumatoid Arthritis with ESR (DAS28-ESR). The formula we used was: DAS28(4) = 0.56*sqrt(t28) + 0.28*sqrt(sw28) + 0.70*Ln(ESR) + 0.014*GH. The twenty-eight tender and swollen joint scores included the ones present in a patient from the following joint areas: shoulders, elbows, wrists, metacarpophalangeal joints, proximal interphalangeal joints and knees. Decimal places in the ESR result were taken into account by the calculation. The calculator is available online on: <http://www.4s-dawn.com/DAS28/>.

Collection and preservation of serum specimens: Four-milliliter blood specimens after eight-hour fasting pause were collected through the ulnar vein in a gel plus clot activator vacuum tubes. They were centrifuged at 3000 rpm for 15 min. Afterwards,

serum was retained and dispensed into Eppendorf tubes immediately. Each tube was labeled and stored at -20°C.

ELISA assay of chemerin in serum:

Quantitative analysis of chemerin in serum was performed by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Biovendor, Modrice, Czech Republic). Blank control, standard and sample wells were set according to the manufacturer's instructions. The test samples were first thawed to room temperature and 100- μ l aliquots of the samples were added into polyclonal anti-human chemerin antibody pre-coated 96-well plates. After a 60 minute incubation followed by washing, 100 μ l biotin labelled polyclonal anti-human chemerin antibody was added and incubated with the captured chemerin for another 60 minutes. Then, after washing, 100 μ l streptavidin-HRP conjugate was added into each well. Next, another 30 minutes of incubation with washing, allowing reaction between the remaining conjugate and 100 μ l of substrate solution. Finally, the reaction was stopped in 15 min by addition of 100 μ l acidic solution and absorbance of the resulting yellow product was measured after 5 min. The absorbance is proportional to the concentration of chemerin. Therefore, a standard curve was constructed by plotting absorbance values against chemerin concentrations of standards, and concentrations of unknown samples were determined using this standard curve.

Statistical analysis: Statistical analysis of the collected data was performed using IBM SPSS Statistics v. 23.0.

For the purposes of statistical analysis BMI, serum chemerin concentration, ESR,

CRP, RF, Uric acid, DAS28-ESR were expressed as median with interquartile range in the descriptive statistics, while age as mean \pm standard deviation (SD) depending on the variable type and its distribution. Testing for normality was performed using the Shapiro-Wilk test due to the sample size. The difference in gender between RA patients and controls were assessed using Chi-square test, and the difference in terms of age and BMI – using t-test. Serum chemerin level between RA patients and controls was analyzed using the Mann-Whitney U test. Correlation analysis was used as a bivariate analysis that measures the strength of association between two variables and the direction of the relationship. When interpreting the strength of correlation, Cohen's convention for small, medium and large effect was used:

- ✓ very strong correlation: \pm (0.7–1);
- ✓ strong correlation: \pm (0.50–0.69);
- ✓ medium correlation: \pm (0.30–0.49);
- ✓ small correlation: \pm (0.10–0.29).

The correlation coefficients used in the present study included determining Pearson correlation coefficient or Spearman rank correlation coefficient, where appropriate. $P < 0.05$ was considered significant.

Results

Basic Clinical Data: Characteristics of the study population are demonstrated in Table 1. There are no statistically significant differences between RA patients and controls in terms of age (59.9 ± 13.1 years vs. 65.0 ± 9.3 years, $p = 0.137$), gender (10/21 vs. 3/17, M/F, Chi-square $p = 0.123$) or BMI (25.5 (24–28) kg/m^2 vs. 27.0 (26–28.5) kg/m^2 , $p = 0.099$).

Table 1. Basic clinical data

	Control group (n=31)	Patients with RA (n=20)	P value
Age (years)	65.0 ± 9.3	59.9 ± 13.1	p = 0.137
Gender (M/F)	3/17	10/21	p=0.123
BMI (kg/m ²)	27.0 (26- 28.5)	25.5 (24- 28)	p= 0.099
Serum chemerin (ng/ml)	228.8 (203.1 – 276.0)	247.0 (226.5–314.6)	p> 0.05
ESR (mm/h)	-	39 (6- 84)	
CRP mg/dL	-	4.9 (2.5- 13.4)	
RF U/ml	-	128 (10-165)	
Uric Acid	-	295 (222- 325)	
DAS28- ESR	-	5.89 (5.30- 6.35)	

Serum level of chemerin

Serum level of chemerin in the two studied groups is shown in Table 1. Compared with healthy controls, patients with RA had slightly higher chemerin concentration in serum that didn't reach statistical significance [247.0 (226.5–314.6) ng/mL и 228.8 (203.1–276.0) ng/mL, p > 0.05, respectively].

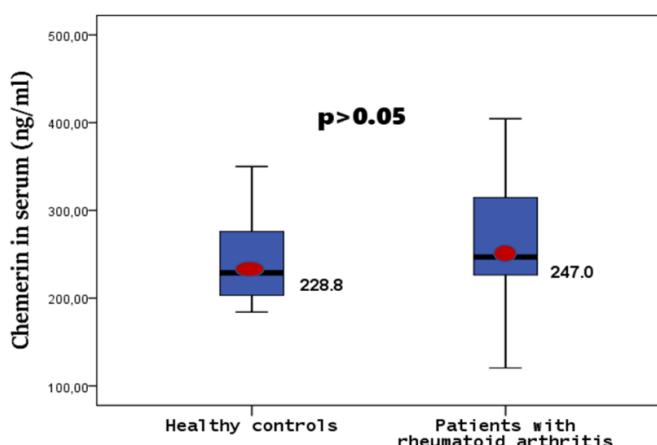


Fig. 1. Serum chemerin in the group of patients with rheumatoid arthritis and the control group: the minimum, the maximum, the median, and the first and third quartiles.

Association between serum chemerin and markers of inflammation and RA disease activity

From our results, it is vivid that there is a strong positive correlation between serum chemerin concentration and ESR ($r = 0.649$, $p = 0.002$), CRP ($r = 0.546$, $p = 0.013$), and medium significant positive correlation with RF ($r = 0.465$, $p = 0.039$). The chemerin level also correlated positively with the indicator of disease activity – DAS28-ESR ($r = 0.807$, $p < 0.001$). No significant correlation was found between serum chemerin and UA ($r = 0.202$, $p = 0.392$).

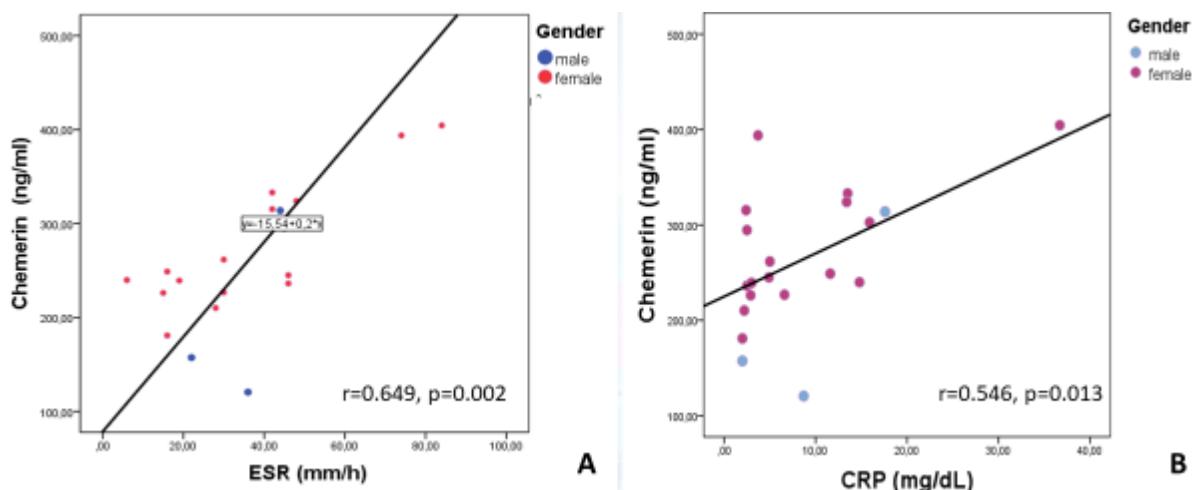


Fig. 2. Correlation of serum chemerin with erythrocyte sedimentation rate (ESR) – A and C-reactive protein (CRP) – B.

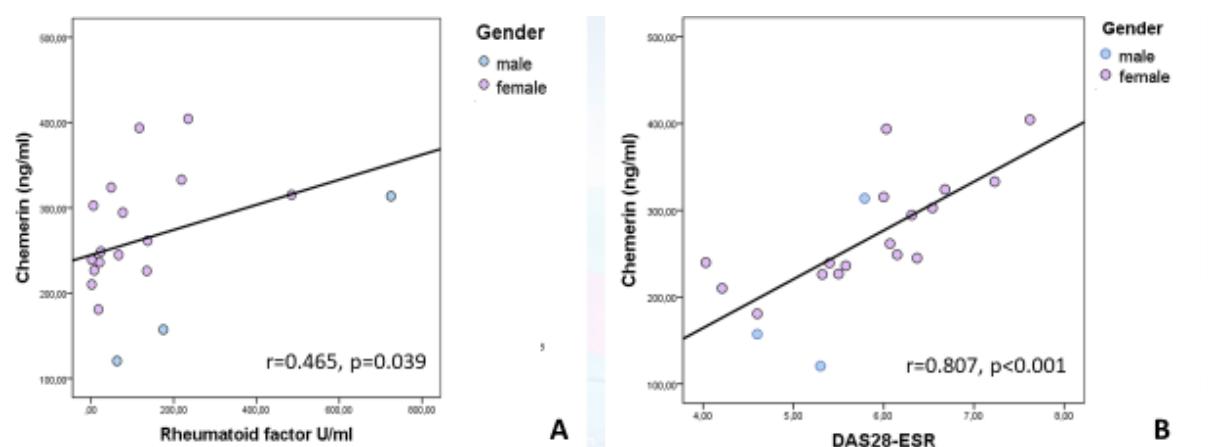


Fig. 3. Correlation of serum chemerin with Rheumatoid factor (RF) – A and Disease Activity Score-28 for Rheumatoid Arthritis with ESR (DAS28-ESR) – B.

Discussion

Rheumatoid arthritis is a chronic systemic autoimmune disease, leading to symmetrical polyarthritis and progressive joint destruction. All these factors of pathogenesis and its clinical course are at the root of both the disability and mortality it causes. Advanced RA patients struggle with limited joint mobility, joint deformities, pain and even disability. Prior to the observation of the typical late-stage manifestations, RA patients have undergone significant joint remodeling. Therefore, establishing the diagnosis as early as possible and predicting its course are of particular importance.

Therefore, using the innovations in the field of molecular biology, researchers have suggested that testing various biomarkers in serum may be very useful. Adipokines as protein mediators secreted by the adipose tissue have been linked to play an essential role in the disease pathogenesis, regulating processes of metabolism, inflammation and immunity. Chemerin, a novel adipokine prompted investigation into the possibility of being implemented as a biomarker of the disease.

In this pilot study, we investigated 20 RA patients and 31 age and sex matched healthy individuals to evaluate the relation-

ship between RA and markers of inflammation and disease activity. Intriguingly, no significant difference was found between the two groups. However, this pilot study includes a small sample size, which might be one of the reasons for this finding. Although similar studies showed higher plasma chemerin levels in the patients group compared to healthy controls, it is worth mentioning that differences in RA severity might also be explanatory for this discrepancy. [20] Moreover, Choi et al. assessed adjusted plasma chemerin levels according to BMI and found them to be significantly higher in the active group (including patients with DAS28 \geq 2.6). Consequently, additional variables related to weight and its distribution (e.g. waist circumference, hip to waist ratio) shall also be taken in consideration in future studies. On the other hand, there is data reporting different isoforms of the chemerin precursor. For example, a study conducted by Zhao et al. examines different isoforms from the process of activating the chemerin molecule in several body fluids – synovial, cerebrospinal fluid and plasma. [21] The team found Chemerin158K protein as the dominant isoform in synovial and cerebrospinal fluids but not in plasma. For this reason, the importance of measuring individual isoforms might be considered as future methodology. Indeed, in our study the difference in serum chemerin between patients with rheumatoid arthritis and healthy controls did not reach statistical significance, probably indicating that the pathological process with chemerin involvement in RA is local. Probably the innate chemotactic mechanisms are taken place in the joints themselves. In fact, both chemerin and its receptor ChemR23 are highly expressed in RA synovial fluid and chemerin activates fibroblast-like synoviocytes. [22] This implicates that chemerin

might be locally released and may participate in the inflammation process merely within the articular joint.

In this study serum chemerin concentrations were positively related to markers of inflammation (CRP, ESR, RF) in RA patients. In addition, positive correlation was established between serum chemerin and disease activity. Indeed, one of the first things one is taught in introductory statistics is that correlation is not causation. Nonetheless, these results are concordant with previous studies [23–25].

One of the recent studies in this area was conducted by Tolusso et al focusing on pigment epithelium derived factor (PEDF) and chemerin in plasma as markers related to low-grade inflammation due to fat accumulation in RA. The team investigated their association with disease activity. Results indicated that plasma levels of chemerin correlate positively with baseline disease activity score in patients with early rheumatoid arthritis. Moreover, in patients with low-moderate disease activity plasma levels of PEDF and chemerin decreased significantly at 6 months after low-calories diet and BMI reduction \geq 5%. In this group there was an improvement in disease activity as well. Authors concluded that both adipokines may be the link mediators between excessive body weight and chronic inflammation in RA. [24]

Another research was designed to focus on the diagnostic ability of serum levels of chemerin, visfatin and their ratio. [25] The team sought an association between these adipokines and other related parameters including body mass index (BMI), lipid profile components, (CRP), and uric acid levels. The aim of the study was to examine the diagnostic ability of the adipokines. According to the results, chemerin showed good sensitivity as a diagnostic marker.

Nonetheless, there is a great variety of other pathologies that could increase serum chemerin, causing its relatively low specificity. Having in mind this discovery and the data from our pilot study, we suggest that if serum chemerin is being tested in a panel with other diagnostic parameters, this disadvantage might be dealt with. In conclusion, the use of serum chemerin as a biomarker for RA is doubtful. But chemerin role beyond the synovial fluid may also be linked with increased risk of atherosclerosis and cardiovascular disease, further debilitating patients with RA. [26]

Several limitations should be taken into account. First, the small sample size in the present study. Second, we only examined chemerin, which is only one of the adipokines involved in inflammatory and immune response. Other possible biomarkers like resistin, leptin, adiponectin etc. as well as their correlations with RA parameters deserve further intensive study. Last, synovial fluid concentration of chemerin in RA patients should also be examined as it might be more sensitive to the noise that results from all complex dynamics that take place within the joints.

In summary, the results of our study suggest that serum chemerin concentration might reflect the proinflammatory changes associated with RA. Further studies are needed to elucidate its potential role as a biomarker of the disease as well as its role in progression of the disease.

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Comparison between body mass index, serum lipids and atherogenic indices in premenopausal women on levothyroxine and thyroid-stimulating hormone below or above 2.5 mIU/l

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Introduction: It is generally recommended that TSH levels should be below 2.5 mIU/l in healthy adult patients on levothyroxine treatment. **Aim:** To compare BMI, lipid levels and atherogenic indices in premenopausal women with autoimmune thyroiditis on levothyroxine replacement therapy according to TSH values and evaluate their association with thyroid function tests. **Patients and methods:** 85 premenopausal women with AIT (mean age 34.8 ± 7.6 years) were included in the study. Serum levels of TSH, free thyroxine (FT4), total cholesterol, HDL and triglycerides were measured; LDL values were calculated using Friedewald formula. Castelli risk index I (CRI) and II (CRII), atherogenic coefficient (AC) and atherogenic index of plasma (AIP) were calculated. All women were euthyroid with TSH levels between 0.4–4.2 mIU/l. **Results:** 43 women had TSH 0.05). No differences between BMI, lipid parameters and atherogenic indices were found between women with TSH below or above 2.5 mIU/l. After adjustment for age and BMI there were no significant associations between TSH and studied parameters. FT4 however showed significant negative correlation with total cholesterol ($p = 0.005$), LDL ($p = 0.002$), CRI ($p = 0.013$), CRII ($p = 0.09$) and AC ($p = 0.013$). **Conclusion:** Our results indicate that there are no differences in major metabolic risk parameters in premenopausal women on levothyroxine provided that TSH is within reference range.

Introduction

Serum thyroid stimulating hormone (TSH) levels provide a sensitive measure of thyroid function. The current approach to primary hypothyroidism treatment is based on the administration of levothyroxine (LT4) at doses that normalize serum TSH levels¹. However, the debate over the optimal TSH target values continues and many authorities recommend that TSH levels should preferably be below 2.5 mIU/l in healthy adult patients on levothyroxine replacement treatment². The uncertainty arises from evidence that a significant proportion of patients treated with LT4 continue to experience residual symptoms suggestive of hypothyroidism, including psychological and metabolic effects despite normal TSH levels³. Therefore, the usefulness of several markers reflecting the effects of thyroid hormones on target organs and tissues such as sex-hormone binding globulin, osteocalcin, creatine kinase, ferritin, total cholesterol, LDL cholesterol, etc. has been widely examined⁴. The relation between thyroid

function, lipid parameters and cardiovascular risk has been a matter of hot discussion for the past decades.

Purpose and tasks

The purpose of the study is to compare body mass index (BMI), lipid levels and atherogenic indices in premenopausal women with autoimmune thyroiditis (AIT) on levothyroxine replacement therapy according to TSH values and evaluate their association with thyroid function tests.

Material and methods

85 premenopausal women with primary autoimmune hypothyroidism were included in this cross-sectional study. Serum fasting morning levels of TSH, free thyroxine (FT4), total cholesterol (t chol), high-density lipoprotein (HDL) and triglycerides (Tg) were measured; low-density lipoprotein (LDL) values were calculated using Friedewald equation: $LDL = T\ chol - HDL - (Tg/2.2)$ where all concentrations are given in mmol/l.

Castelli risk index I (CRI) and II (CRII), atherogenic coefficient (AC) and atherogenic index of plasma (AIP) were calculated using the following formulas:

$$\text{CRI} = \text{total chol}/\text{HDL};$$

$$\text{CRII} = \text{LDL}/\text{HDL};$$

$$\text{AC} = (\text{t chol} - \text{HDL})/\text{HDL};$$

$$\text{AIP} = \log_{10}(\text{Tg}/\text{HDL}).$$

Height and weight were obtained and body mass index (weight/height²) calculated.

All women were euthyroid with TSH levels within the recommended target range between 0.4 and 4.2 mIU/l. The women included had no significant medical conditions, incl. diabetes mellitus and were not taking steroids, metformin, lipid lowering therapy or estrogen containing drugs. They were on a stable levothyroxine dose for at least 6 months prior to inclusion in the study. Pregnant women, women within the first postpartum year and those with history of thyroid surgery or radioiodine therapy were excluded from the study.

Women were divided into two groups according to TSH levels – women with TSH < 2.5 mIU/l and women with TSH ≥ 2.5 mIU/l.

The main characteristics of the women included in the study are presented in tab. 1.

Comparison between values was performed with the Student's paired t-test. Correlations were evaluated with Spearman's coefficient. All tests were done two-sided, and p < 0.05 was considered statistically significant. Statistical analyses were performed with SPSS version 21.0.

Results and discussion

43 women had TSH < 2.5 mIU/l (1.62 ± 0.08) and 42 had TSH ≥ 2.5 mIU/l (3.45 ± 0.08) with similar levels of FT4 in both groups (11.70 ± 0.02 vs 11.17 ± 0.02 pmol/l, p > 0.05). No differences between BMI, lipid parameters and atherogenic in-

dices were found between women with TSH below or above 2.5 mIU/l (tab. 2).

Table 1. Characteristics of the studied women.

	mean ± SD
Age (years)	34.8 ± 7.6
BMI (kg/m ²)	26.6 ± 6.9
TSH (mIU/l)	2.52 ± 1.05
FT4 (pmol/l)	11.44 ± 1.46
T chol (mmol/l)	4.80 ± 0.81
HDL (mmol/l)	1.40 ± 0.39
Tg (mmol/l)	0.98 ± 0.66
LDL (mmol/l)	2.95 ± 0.68
Duration of AIT (months)	53.6 ± 43.3
LT4 dose (µg/daily)	69.3 ± 30.6

Table 2. Comparison between age, BMI, lipids and atherogenic indices between women with TSH < and ≥ 2.5 mIU/l.

	TSH < 2.5 (n = 43)	TSH ≥ 2.5 (n = 42)	p
Age	34.4 ± 0.9	35.3 ± 1.3	0.622
BMI	25.6 ± 0.9	27.7 ± 1.1	0.154
T chol	4.71 ± 0.1	4.91 ± 0.1	0.260
HDL	1.38 ± 0.1	1.42 ± 0.1	0.618
Tg	0.93 ± 0.1	1.03 ± 0.1	0.507
LDL	2.90 ± 0.1	3.01 ± 0.1	0.442
CRI	3.60 ± 1.2	3.70 ± 0.2	0.677
CRII	2.26 ± 0.1	2.29 ± 0.1	0.867
AC	2.60 ± 0.1	2.70 ± 0.2	0.677
AIP	-0.20 ± 0.1	-0.19 ± 0.1	0.884

After adjustment for age and BMI there were no significant associations between TSH and the studied parameteres. FT4 showed significant negative correlation with total cholesterol ($r = -0.306$, $p = 0.005$), LDL ($r = -0.338$, $p = 0.002$) (fig. 1), CRI ($r = -0.272$, $p = 0.013$), CRII ($r = -0.283$, $p = 0.09$) and AC ($r = -0.272$, $p = 0.013$) (fig. 2).

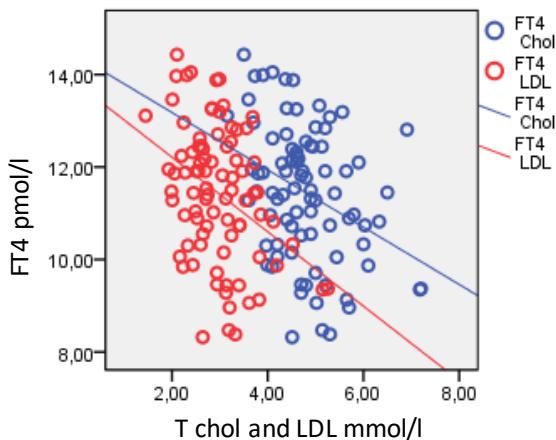


Figure 1. Correlation between FT4 levels and total cholesterol and LDL.

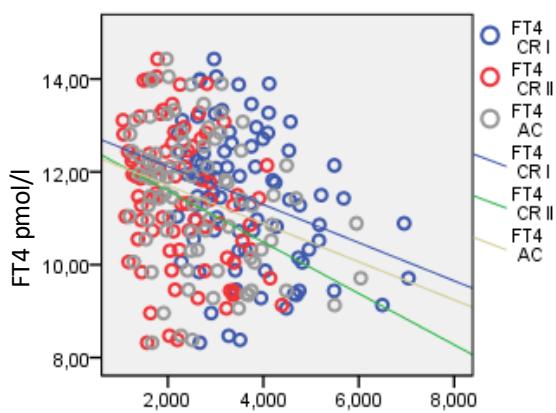


Figure 2. Correlation between FT4 level and CRI, CRII and AC.

The results of the present study indicate that there are no differences in BMI, lipids and atherogenic indices in premenopausal women on levothyroxine provided that TSH is within reference range. However, there is evidence showing that some patients with hypothyroidism on levothyroxine therapy feel considerably better if the levothyroxine dose is increased to lower TSH levels below 2 mIU/l⁵. Thus many authorities have recently recommended lower target TSH values for the treatment of hypothyroidism⁵. Moreover, it has been found that considerable part of hypothyroid treated patients report weight gain despite normal TSH levels, leading to requests for higher L-T4 doses or alternate therapies⁶. Howev-

er, there is no consistent data proving that varying L-T4 dosages within the TSH reference range are actually related to weight or body composition in these patients^{7,8}.

A large population study in the USA showed that hypothyroid patients taking levothyroxine who had a normal TSH level had lower serum T₃ and higher T₄ levels, and consequently lower T₃:T₄ ratios than matched healthy individuals without thyroid problems³. In addition, there were differences noted between the levothyroxine-treated and healthy matched subjects in BMI and cholesterol levels³.

Despite recent trends toward lowering the upper limit of normal TSH range the results of a 12-month study provided no substantial clinical evidence that treatment of primary hypothyroidism should aim at maintaining TSH levels in a low-normal range⁷. No differences in a number of metabolic parameters, incl. lipid profile were found in patients on LT4 with low normal (0.4–2.0 mIU/l) or high normal (2.0–4.0 mIU/l) TSH during the follow-up⁷. In another study on LT4-treated patients who had different TSH levels within the laboratory reference range, no significant differences in BMI, body composition, energy expenditure, or diet intake between subjects with low-normal and high-normal TSH levels were established⁵. Regarding subjective assessment of well-being, it has been shown that LT4 increase to achieve TSH in the lower half of reference range does not lead to improved quality of life.⁵ Some of that equivocal data can be at least partially explained by the finding that impaired deiodinase activity during LT4 treatment alters TSH-FT4 relationship⁹ and consequently serum TSH may not always accurately reflect thyroid hormone levels in all target tissues¹⁰.

Conclusion

There are no differences in major metabolic risk parameters in young premenopausal women on LT4 provided that TSH is within reference range. Increase of LT4 dose in asymptomatic non-pregnant patients with TSH in the upper half of the normal range is not recommended.

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Oral contraception and anterior ocular surface homeostasis

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Objective: To determine the possible relationship between oral contraceptive use and symptoms of anterior ocular surface discomfort – the subjective symptoms of lacrimal dysfunction syndrome.

Material and methodology: A pilot study was conducted – a clinical experiment. Sixty eyes were examined in thirty women, taking oral contraceptive medication (OCPs – oral contraceptive pills) for more than 1 year, with a mean age of 34 ± 2 years, with no systemic or ocular disease. For the control group, 30 women of similar age (35 ± 3 years), non-pregnant, without complaints and eye diseases were studied. Each patient completed an adapted Mc Monnies dry eye questionnaire. A complete ophthalmological examination was performed, including a Schirmer test, tear film break-up time, LIPCOF test (lid parallel conjunctival folds). The eye protection index is calculated. Optical coherence tomography (OCT) was performed on the lower tear meniscus. **Results:** The results of completing the questionnaire show a mild dry eye in all respondents. The Schirmer test at 40% is less than 10 mm. The tear film break up time at 70% is less than 15 seconds, and the eye protection index is above 1. The LIPCOF test in all is zero. OCT-meniscometry shows a slight decrease in the measured parameters. **Conclusion:** There is a relationship between the use of oral contraceptives (and related hormonal changes) and the subjective symptoms of lacrimal dysfunction syndrome.

Keywords: oral contraceptives, dry eye, eye discomfort

Introduction

According to WHO 2009, 60–70% women use one of the method of contraception. Estimated 100 million women use oral contraceptive pills (OCPs) worldwide (1). These are hormonal medication tablets, which contain combination of estrogen and progesterone. Ophthalmic side effects of OCPs are known to be blurring of vision and rarely occlusion of retinal vessels (2). It is proven that many women using oral contraception have ocular complaints such as dry eyes (2). There are relatively many studies on dry eye after menopause conducted, but only few studies were done on effect of oral contraception in age bearing age group.

The symptom complex of dry eye includes a wide range of clinical signs and subjective symptoms, which make its definition and classification diverse. This is one of the reasons why there are many terms associated with this condition – chronic dry eye, tear film dysfunction, dry keratoconjunctivitis. Dry eye syndrome is a common problem in ophthalmic clinical practice. Dry eye syndrome is thought to be the re-

sult of tear film abnormalities, watery tear deficiency or increased evaporation, although it may also result from incomplete eyelid closure or be associated with environmental conditions, or with the patient's hormonal status (2, 8). It has been found that up to 25% of patients who consult an ophthalmologist have dryness to varying degrees (1, 7). Dry eye syndrome can be divided into two types: evaporative dry eye (increased evaporation) or deficiency of the aqueous phase of tears (1). Evaporative dry eye may be due to deficiency of the meibum (lipid component of the tear film) or due to eyelid causes – incomplete or infrequent blinking. Meibomian glands (MG) are holocrine sebaceous glands located in the tarsal plates of the eyelids. The meibum excreted by them forms the surface layer of the tear film, which slows the evaporation and functions as a lubricant in the blinking movements. The secretion of the Meibomian glands is directly affected by the intake of various drugs, as well as by hormonal imbalances of various etiologies.

Many studies had suggested that androgens play a significant role on the structural

characteristics, functional, and pathologic features of the lacrimal gland. There have been some suggestions that oral contraceptives might lead to qualitative and quantitative changes in tear film (11, 14). Androgen receptors are present on various ocular tissues, and sex hormones regulate the secretory functions of lacrimal and meibomian glands, so they might play etiological role in pathogenesis of dry eye syndrome (12, 15). There are few studies those done in reproductive age group of women regarding effect of OCPs on dry eye. OCPs may be a cause of androgen deficiency in child bearing age group women. It is also suggested that oral contraceptive might decrease mucus production, reduce contact lens tolerance, increase foreign body sensation and the risk of dry eye in women using oral contraception (7).

Optical coherence tomography (OCT) is a leading imaging technique with the ability to display live biological tissues at high resolution (3). In recent years, OCT has established itself as an indispensable technique for the examination of the anterior and posterior segment of the eye, an important part of the diagnosis and follow-up of a number of socially significant eye diseases, including tear film and LIPCOF (3-6).

Aim

Objective of present study is to determine the possible relationship between oral contraceptive use and symptoms of anterior ocular surface discomfort – the subjective symptoms of lacrimal dysfunction syndrome, using anterior segment OCT and standard dry eye tests.

Material and methodology

A pilot study was conducted – a clinical experiment. Sixty eyes were examined in thirty women, taking oral contraceptive

medication (OCPs-oral contraceptive pills) for more than 1 year, with a mean age of 34 ± 2 years, with no systemic or ocular disease. For the control group, 30 women of similar age (35 ± 3 years), non-pregnant, without complaints and eye diseases were studied. Each patient completed an adapted Mc Monnies dry eye questionnaire. A complete ophthalmological examination was performed, including a Schirmer test, tear film break-up time, LIPCOF test (lid parallel conjunctival folds). The eye protection index is calculated. Optical coherence tomography (OCT) was performed on the lower tear meniscus.

Optical coherence tomography (OCT) was performed with a 3D Topcon 2000 FA + OCT with a scanning speed of 50,000 A-scans / sec, a resolution of 5 μm , a penetration of up to 2.3 mm and a 16.5 MP camera (Fig. 1). All patients underwent anterior segment optical coherence tomography, Anterior Linear (V) protocol, 6 mm scan length. The centering is in the middle of the lower eyelid edge, at the last straight line, in the light fixation target of the device, immediately after blinking (Fig. 2). The height (TMH-distance from the corneal / tear meniscus border to the tear meniscus/eyelid border) and the depth (TMD-from the middle of the air/tear meniscus interface to the corneal/eyelid border) of the lower lacrimal meniscus were measured with a caliper with the software of the device.



Figure 1. 3D Topcon OCT.



Figure 2. Left: OCT – 6 mm vertical linear scan, centered in the middle of the lower eyelid edge; Right: lacrimal meniscus of the lower eyelid.

Results and discussion

The results of all participants in completing the questionnaire show a slight degree of dry eyes. The Schirmer test at 40% is less than 10 mm. The tear film rupture time

at 70% is less than 15 seconds and the ocular protection index is above 1. The LIPCOF test is zero for all. OCT meniscometry shows a slight decrease in the measured parameters (Table 1, Figure 3, 4).

Table 1. Comparison of the measured indicators in the two groups of patients.

Parameter	Study group	Control group
TMH (μm)	212.4 ± 9	239.6 ± 37
TMD (μm)	133.2 ± 3	151 ± 27
TBUT (sec)	14 ± 1	16 ± 2
Schirmer test (mm)	14 ± 2	16 ± 4
LIPCOF	0	0

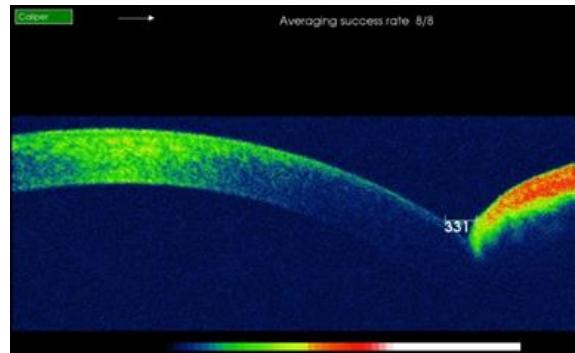
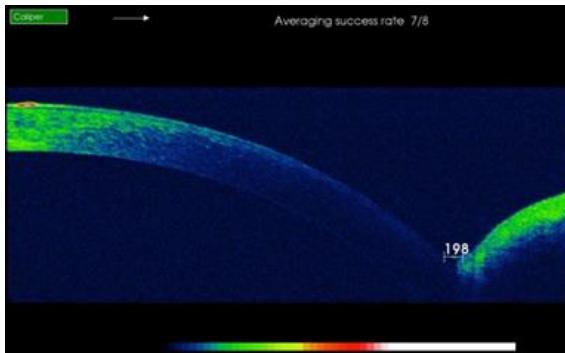


Figure 3. Height of the lacrimal meniscus: Left – in a woman on OCPs (198 μm), right – in control eye (331 μm).

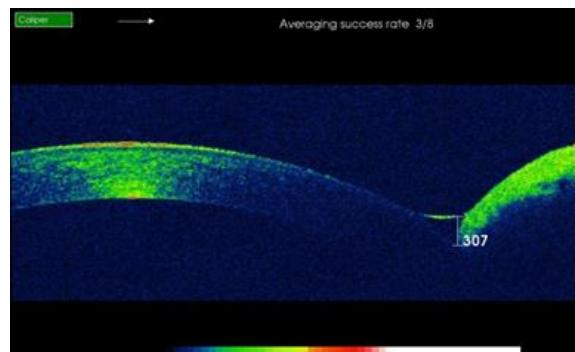
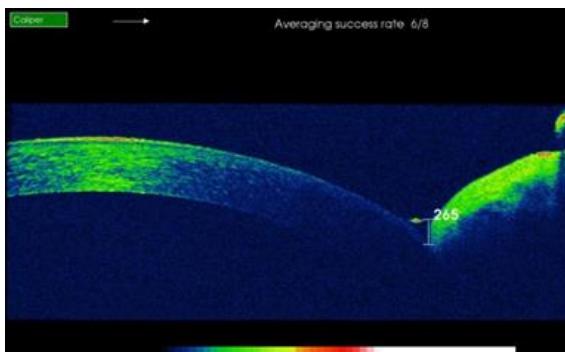


Figure 4. Depth of lacrimal meniscus (TMD): Left – in woman on OCPs (265 μm), right – in control eye (307 μm).

Sex hormones appear to influence functional and structural aspects of the eye and contribute to ocular surface disorders such as dry eye disease. Our results partly support the results of Sharma et al, who revealed that there is significant difference in tear secretion, but not in tear stability between women on oral contraceptives and women not using hormonal contraceptives (10).

Results of present study are consistent with the observation of other authors who carried out a similar study on the correlation in androgen profile in pre-menopausal women with dry eye syndrome (9,12). Chen et al. reported that the oral contraceptives in pre-menopausal women may contribute to dry eye syndrome. Versura et al also inferred that oral contraceptive medication may be an important cause of androgen deficiency in this age group (9, 12).

Nevertheless, there are controversial reports on the effects of oral contraceptives in dry eye pathogenesis. According to Sullivan et al. androgen deficiency may be an important etiological factor in pathogenesis of evaporative dry eye in women (11). On the other hand, Versura et al suggest that oral contraceptives may be an important cause of androgen deficiency in child bearing age group women.

Our observations contrast with that of Burkman et al, who found that there is no significant correlation between serum progesterone levels and tear secretion and tear stability. They also did not find any correlation between tear film stability, secretion and use of injectable hormonal contraceptives in women of child bearing group (13).

Conclusions

Present study suggest that androgen profile decrease in women taking oral contraceptive pills. Results support that use of

oral contraceptive medication may be an etiological factor in pathogenesis of tear dysfunction syndrome among women in reproductive age. On the other hand, OCT meniscometry is an accurate, fast and non-invasive method for quantitative analysis of the lacrimal meniscus with the potential for widespread use in pathology of the anterior ocular surface. Hormonal changes, including oral contraception intake are closely related to the subjective symptoms of lacrimal dysfunction syndrome.

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A rare coexistence of papillary thyroid carcinoma and toxic thyroid adenoma: a case report

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Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy, known for its favorable prognosis after surgical treatment. Toxic adenomas are autonomously functioning benign nodules, effectively treated surgically or with radioactive iodine. The coexistence of these conditions is very rare and requires careful diagnostic workup. We hereby describe a case of a 41-year-old male, presenting with tachycardia, weakness and shortness of breath who was referred for assessment of the thyroid function. The initial sonographic (US) evaluation revealed a large partially cystic nodule (32 mm) in the right thyroid lobe, with borderline low serum TSH and negative thyroid autoantibody titers. The SPECT/CT scan identified a “hot” nodule in the right lobe. Following a decision for a lobectomy, during the preoperative assessment another small nodule (4 mm) with suspicious ultrasound characteristics was found in the left thyroid lobe. The patient underwent a fine-needle aspiration biopsy of the latter with a cytologic result of a malignant thyroid neoplasm. No suspicious cervical lymph nodes or signs of an autoimmune process were found during the examination. Consequently, the patient underwent a total thyroidectomy with central lymph node dissection. Post-surgically, he was classified as a subject with a low-risk of recurrence of the disease and no radioiodine ablation (RIA) was recommended. Conclusion: nodular thyroid pathology is very common and meticulous separate assessment of the functional and morphological characteristics of the nodules should be made in order to choose the appropriate therapeutic approach.

Introduction

Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy (1), known for its favorable prognosis after surgical treatment. Toxic adenomas are autonomously functioning benign nodules, effectively treated surgically or with radioactive iodine. The coexistence of these conditions is very rare and requires careful diagnostic workup.

Case study

A 41-year-old male, presenting with tachycardia, weakness and shortness of breath was referred for assessment of the thyroid function. During the physical examination a palpable firm nodule with a smooth surface was found in the right thyroid lobe. The initial sonographic (US) examination revealed a large partially cystic nodule with diameter of 32 cm (Fig. 1) in the right thyroid lobe with no characteris-

tics suggestive for malignancy – EU-TIRADS 3 (2). Blood tests displayed borderline low serum TSH – 0.51, fT4 – within reference range and negative thyroid autoantibody titers. Due to the specific clinical and laboratory constellation the patient underwent a SPECT/CT scan in search of a toxic adenoma. The scan identified a “hot” nodule in the right lobe (Fig. 2) concurrent with the partially cystic nodule, described on the primary US examination.

The patient was diagnosed with a toxic adenoma and referred for lobectomy of the right thyroid lobe. During the preoperative assessment another small nodule (4 mm) with suspicious ultrasound characteristics – hypoechoic structure, irregular shape and blurred margins was found in the left thyroid lobe (Fig. 3). The nodule was classified in the high-risk category (EU-TIRADS – 5) (2) which requested a performance of a fine-needle aspiration biopsy. The cytologic

result from the biopsy was suggestive for a malignant thyroid neoplasm in the left lobe – Bethesda VI and a benign nodule in the right lobe – Bethesda II. No suspicious cervical lymph nodes or features of an autoimmune process were found during the examination. Consequently, the patient underwent a total thyroidectomy with central lymph node dissection (Fig. 4 and 5).

The histological result confirmed a capsulated thyroid papillary carcinoma 0.3 cm in the left lobe and follicular adenoma in

the right one. There was no capsular infiltration or metastases in the central lymph nodes. Post-surgically, he was classified as a subject with a low-risk of recurrence of the disease and no radioiodine ablation (RIA) was recommended (3). The follow-up of our patient consisted of suppressive therapy with levothyroxine with target levels of TSH < 2, regular clinical and US assessments, thyroglobulin (Tg) and thyroglobulin-antibodies evaluation (TgAb).

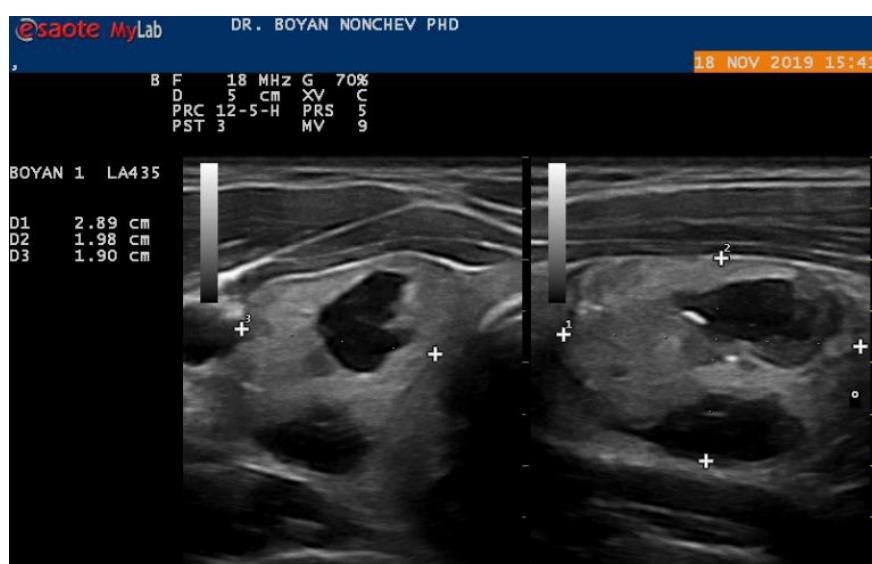


Figure 1. US image of the partially cystic nodule in the right thyroid lobe – EU-TIRADS 3.



Figure 2. SPECT/CT scan of the thyroid gland, revealing increased uptake in an area in the right lobe, concurrent with the partially cystic nodule.

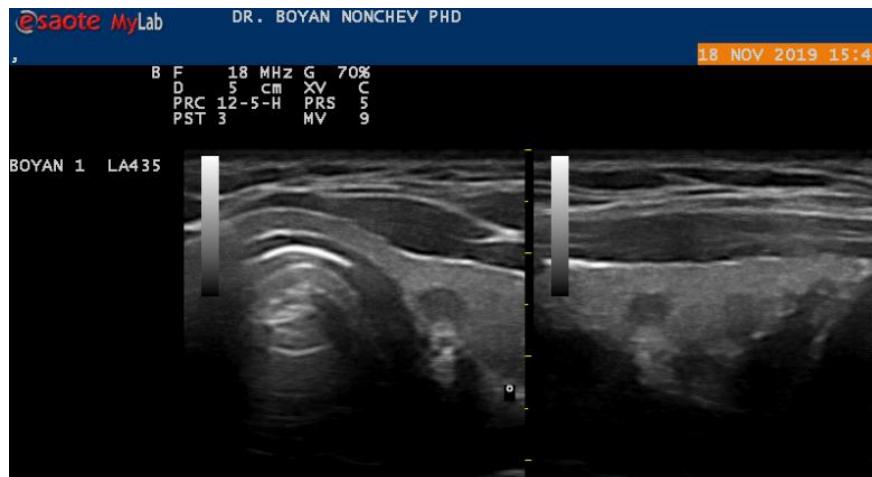


Figure 3. US image of the suspicious nodule in the left thyroid lobe – EU-TIRADS 5.



Figure 4. Macroscopic view of the toxic thyroid adenoma in the right lobe.



Figure 5. Macroscopic view of the malignant nodule in the left thyroid lobe.

Conclusion

Nodular thyroid pathology is very common and meticulous separate assessment of the functional and morphological characteristics of the nodules should be made in order to choose the appropriate therapeutic approach.

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III. Pharmacology and Pharmacotherapy session

Potential of mobile applications in pharmacy in the context of the pandemic – a literature review

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The use of information and communication technologies in pharmacy significantly reduces the spread of the coronavirus infection among patients and pharmacists. The introduction of a multitude of mobile applications aimed at the delivery of pharmaceutical care has contributed to the limitation of the disease. The aim of the following review is to study the potential of information and communication technologies used in pharmacy in the context of the COVID-19 pandemic. **Material and methods:** A search has been conducted using key words in the databases of Scopus, Web of Science, Google Scholar, PubMed, official government sites and sites of software engineers. **Results:** A research has been made on the potential of mobile technologies in pharmacy in the context of COVID-19 in different countries. An analysis of the existing advantages, disadvantages and limitations concerning their use has been made. **Conclusion:** The use of telepharmacy is an example of good practice in the conditions of the pandemic, which came into fruition through the release of a lot of mobile applications aimed at the delivery of pharmaceutical care to patients.

Keywords: mobile applications, telepharmacy, pharmaceutical care, pharmacist, patients

Introduction

The use of information and communication technologies in pharmacy significantly reduces the spread of the coronavirus infection among patients and pharmacists. The introduction of a multitude of mobile applications aimed at the delivery of pharmaceutical care has contributed to its limitation.

The worldwide pandemic has had a significant influence on pharmaceutical care, which forced changes in the administration of pharmaceutical care to patients. Because of the forced quarantine, people often avoid visiting even the pharmacy because they fear that they could get infected. This situation mandated new measures for pharmacists on the front line such as the practice of telepharmacy, which resulted in the introduction of a series of new mobile applications aimed at delivering pharmaceutical cares from a distance.

In January 2019, the population of the earth was of 7,676 billion people. Today, there are 5,11 billion unique mobile phone users and 4,39 billion internet users worldwide. In 2019, 3,48 billion people were using social networks, and their number had risen by 288 million (9%) since January 2018. Out of them, 3,26 billion people use social networks from mobile devices [1].

These numbers give us an idea of the significance of mobile phones today. Mobile health (mHealth) became a new area of medicine and pharmacy aimed at the use of mobile technologies in order to deliver medical care to patients [2]. Mobile applications used in pharmacy are for:

- communication between the pharmacist and the patient for reminders of the timing related to the intake of medicine, as well as information for its side effects;

- distanced reports on the use of medicine for specific groups (children, pregnant women, breastfeeding women, elderly people, people with chronic diseases);
- rules for the intake of different forms of medicine;
- identification of medicine, etc.

A research from K. Margolis et al. indicates that pharmacists working with patients in a telemedical environment could use mobile technologies to influence the outcome of patients. With the help of home telemonitoring for blood pressure, pharmacists can estimate the values of a patient's blood pressure and take correct therapeutic decisions [3]. This is an example of new telepharmaceutical services which use mobile devices to influence the provided care as well as the patient's quality of life, especially for those living in remote rural areas [4].

Aim

The **aim** of the following review is to study the potential of information and communication technologies used in pharmacy in the conditions of the COVID-19 pandemic.

Material and methods

A search has been conducted using key words in the databases of Scopus, Web of Science, Google Scholar, PubMed, official government websites.

Results

A research has been made on the potential of mobile technologies in pharmacy in the conditions of the COVID-19 pandemic in different countries. An analysis has been made of existing advantages, disadvantages and limitations concerning their use.

The COVID-19 pandemic resulted in varied responses around the world, and the most efficient types of the used means were

linked with telecommunication, mainly because of the effectiveness of these means and the nature of the virus which requires distance between patients, pharmacists and doctors in order to reduce the risks of infection.

1. Electronic means for pharmaceutical care employed in France

The healthcare system in France developed multiple electronic tools to be distributed by pharmacists as a response to the COVID-19 pandemic. These tools have been recommended in person upon visitation of a pharmacy and online in public announcements made on social media platforms. These tools include:

- An online interactive map on the "santé.fr" site that indicates all locations in France that provide screening and tests for the virus. This allows citizens to have access to practical information to make the process of getting tested for the virus easier since the site gives information on locations, waiting times, and other details about testing points [5].

- A mobile application called "TousAntiCovid" which is an updated version of "StopCovid" that contains more accessible resources and information about measures to take against the virus. The other function of this application is to alert people in the vicinity of a contact case (someone in contact with the virus but without a confirmed positive test). It is a useful tool for family members as it tells them to avoid contact so that they could avoid spreading a possible infection before it is confirmed. In case of a positive test result for the virus, a pharmacist can use the site pro.tousanticovid.Gouv.fr to generate a code composed of six characters that the patient can use in the application to communicate the event of the infection [6].

- In the same objective of informing citizens as much as possible about what behaviour to adopt in the context of the pandemic, the French Ministry of Health Solidarity (Ministère des Solidarités de la Santé) has created a site with personalized information accessible in three minutes. This site (mesconseils covid.fr) has the advantage of being accessible all while distributing important and relevant information about what safety measures to apply [7]. Pharmacists are also actively encouraged to inform patients of the existence of this site, and to help them use it so that they have access to this information.

Electronic systems designed for healthcare professionals

Healthcare professionals in France are pushing for more availability of resources that have been used by professionals in the mental health field, as the pandemic affects not only the physical health of the population but also their mental health which can lead to health complications. Dr Malina Jordanova of the Bulgarian Academy of Science believes in the prevention of such complications as the consequences of this type of anxiogenic situation are easier to treat if they are confronted early instead of in a later, more developed stage [8]. Thus, she raises her voice for the availability of software for frontline healthcare workers as well as for everyday citizens since this software is already used to great effect by mental health professionals.

2. Electronic means used in other European countries

One of the exemplary countries in Europe for this category of response was Germany, with a science community which is keen on communicating with the public in order to debunk myths and share their expertise on the subject. Germany has one of the leading experts in coronaviruses,

Christian Drosten. In order to answer the public's questions and to share the results of his research in an optimal way, he created "Das Coronavirus Update", a relevant podcast which gained popularity very quickly [9]. He believes the format of a podcast is a good way to explain the science of the virus to the public in an authentic and transparent way.

Germans claim that their children have also been educated about the Coronavirus by a popular German television show called "Die Sendung mit der Maus", which is their equivalent to "Sesame Street" [10].

Germany also released an app called "Corona-Warn-App" which allows users to trace, report and verify local exposure levels to the virus [11]. The application can also warn the users of infection risk levels depending on the date and their location. The application is very successful and is even being used outside of Germany, notably in European countries like Italy, Denmark, Latvia, Spain, Austria, and the Netherlands.

In Belgium, the municipality of Aalter approached the company "Zoovu" and commissioned the development of an application called "Ezkoms" [12] which allows local and national governments to keep citizens informed and safe by notifying them of important details like rubbish collection, local services, shop opening times and travel updates. As it is a mobile application, the automated channeling of information permits citizens to be updated with accurate information at any time of the day, which is far more effective than traditional websites or television channels.

The Economic Risk Management Group (ERMG) in Belgium also developed a tool called "Toolbox-Corona" to aid businesses and their employees in their preparation to resume activities in the context of the pan-

demic, with an emphasis on safety [13].

The Italian Ministry of Health and Minister of Technological Innovation developed an application called Immuni, which tracks contacts between citizens and warns them about people who are potentially exposed to the virus [14]. When a patient is infected, their physician sends a message to all the people who have been in contact with that patient in order to warn them.

The Spanish government developed a site for travelers in Spain called “Spain Travel Health” which allows passengers to fill out a health control form and to subsequently obtain a QR code that they can show at control points when they arrive in Spain [15].

3. Electronic means used in Russia

In light of the SARS-CoV-2 virus epidemic, countries around the world have adopted different means to prevent the spread of the disease. Electronic precautions are often preferred as they eliminate the possibility of human contact which further prevents the spread of the virus.

The Russian government was experimenting with AI (Artificial Intelligence) technologies based on facial recognition in Moscow. They used 160,000+ cameras that had already existed in the city to identify citizens that violated their quarantine. In January 2020, their system identified more than 200 citizens that violated their mandated self-isolation after traveling in foreign countries. During the following months, they implemented system identifying vehicles and claimed that they had identified 504,000 vehicles of people breaking their quarantine in the Krasnodar region, all of that during the first day of the system’s operation [16].

Despite the fast start, there were lots of claims of invasion of privacy and unjusti-

fied fines to Russian citizens. Following this, the system was discontinued, revised, and re-introduced as a mobile application in April 2020.

Since then, all people identified as having been in contact with an infected individual have been obligated to install the “Social Monitoring app” or they would face a fine. Doctors had to convince their family members to sign a quarantine notification which included a small print stating that they were required to install the app. People who did not own a cell phone received special devices with a preinstalled Social Monitoring app. [17]

In contrast with the EU, the use of this app is not voluntary.

4. Electronic means used in Sub-Saharan Africa

Countries in Sub-Saharan Africa present a different situation than Western and Eastern countries as Tele-pharmacy could be an answer to more problems than just COVID-19 as these countries have a very high surface area but a very low number of pharmacists. A report by the World Health Organization indicates that the pharmacist-patient ratio in Ghana is 1 per 10,000, which falls short of the WHO’s recommendation of 1 per 2,000. Up to 7 in 10 pharmacies in Ghana’s rural areas do not have a qualified pharmacist as staff and have to resort to less qualified medical assistants. This is a result of the inability of pharmacies to afford full time wages for pharmacists and the reluctance of pharmacists to relocate to rural areas.

Elvin, a pharmacist in Ghana, has experienced assisting patients through phone calls and video calls and explains that it is fully doable and is maybe the best path to take considering the importance of using cost effective methods. He suggests Go-

Pharma, a mobile telepharmacy application that allows pharmacists to assist pharmacies around the country in real time. It permits qualified pharmacists to bring their services to more Ghanians more efficiently and at a lower cost by being an answer to their low numbers in terms of pharmacists [18].

5. Electronic means used in Australia

In the context of COVID-19, the Society of Hospital Pharmacists of Australia (SHPA) has started doing telepharmacy consultations to continue providing medical advice and medical care while protecting the safety of Australians as this move eliminates the requirement of on-site consultations. Pharmacists are also able to conduct a Home Medicines Review (HMR), a Residential Medication Management Review (RMMR), a MedsCheck and a Diabetes MedsCheck for eligible Australians via the means of video or videoconference [19].

The Australian government also launched a mobile phone application called COVIDSafe on April 26, 2020 [20]. The app uses Bluetooth signals to record a user's contacts with other users and saves the encrypted information on their phone. Installing the app is not mandatory and users must grant permission for their data to be uploaded. The most recent figures available show that around 23% of Australians have downloaded the app. The data of a user who is tested positive for COVID-19 can be uploaded to a central storage system and then be accessed by state authorities so that the user can be traced. After the diagnostics, state and territory health officials will:

- use the contacts registered by the app to update their database to trace who has been in contact with the infected user.
- call the parents/guardians of the users in contact with the infected user to let them know that they may have been exposed.

- offer advice on what to look out for, when and how to get tested, and how to protect friends and family from exposure.

- not name the person infected to the contacted users to protect their privacy.

6. Electronic means used in Canada

Canada's public health department developed two applications as solutions to the COVID-19 epidemic: one for travellers called ArriveCAN and one for residents called COVID Alert.

ArriveCAN is a mobile application used to provide mandatory travel information upon and after one's entry into the Canadian territory. Travellers are required to use it as paper forms are perceived as being incomplete or inaccurate. This solution also eliminates all of the processes related to paper documentation that can add days to the whole progress, whereas checking in online is almost instant. This process requests travellers to comply with the Quarantine Act which considers violating the instructions provided to them and giving inaccurate information as offences. These offences can lead to tickets up to \$3,000, admission into a federal designated quarantine facility, 6 months in prison and a maximum of \$750,000 in fines. Travellers are required to register their travel and contact information, quarantine plan and a self-assessment of their COVID-19 symptoms before entering Canada). They are also requested to confirm their arrival at their designated location the day after, and complete daily symptom self-assessments until the completion of their quarantine period. Travellers also receive calls from Canada officials to ensure that they are complying with the law [21].

COVID Alert is Canada's free exposure notification application that works like any of the other exposure apps through Blue-

tooth and randomly generated codes from the app. It registers people who are tested positive for COVID-19 and notifies people that have been exposed to them. The application works with proximity detection and does not track the location, name, address or health information of the users to protect their privacy [22].

7. Lack of electronic means used in the United States of America

In the USA, the government still resorts to personal check-ups by the police to ensure compliance with self-isolation orders. They have not yet employed a generalized electronic tracking system because the concept itself runs against the Fourth Amendment which protects citizens from “unreasonable search and seizure”. The only situation in which they use electronic tracking is as a court-ordered response when someone fails to obey criminally enforceable quarantine orders [23].

Contrary to other countries which either encourage or mandate the use of digital monitoring to ensure their population’s safety, the government of the United States places the value of personal privacy above this crisis. The result of this decision is over 30,000,000 confirmed cases of COVID-19 and over 550,000 deaths across the country which represents the highest death toll in the world related to the pandemic and proves the efficiency of the electronic means employed by other countries [24].

8. Electronic means used in South-East Asia

- Japan

The Constitution of Japan protects the privacy and private information of its citizens. The Constitution treats the location of a person using a mobile device as such private information and thus secures it from

being shared or exposed. According to an international study [25], the Japanese are the least willing out of all the people in the studied countries to share information with online organizations.

In order to keep location and personal information private, the Japanese government ordered the development of an application using Bluetooth to alert users when they are in proximity of infected users having registered themselves in the application. This application is called “COCOA – COVID-19 Contact App” and has been reported to not work effectively, as Japan experienced a surge in infections after the application’s debut which implies that the project was a failure [26].

- South Korea

South Korea also has data protection laws which protect private information as well as location information. However, after the Middle East Respiratory Syndrome outbreak in 2015, their surveillance was strengthened and as a result of the Infectious Disease Prevention and Control Act, the location information law was overruled and people are now required to cooperate with epidemiological investigations [27].

In March 2020, the government launched a new system to track the movements of people infected with COVID-19 and their contact. The system was developed by the Ministry of Land, Infrastructure and Transport, the Ministry of Science and ICT (Information and Communication Technologies). The Korean National Police Agency, the Credit Finance Association, South Korea’s three mobile carriers, and 22 credit card issuers have joined the system [28, 29].

This system is not voluntary as the government tracks the location of infected citizens without their consent and enforces punishment if the instructions are not respected.

Additionally, because the number of cases of people breaching the self-quarantine raised concerns, the government announced the use of electronic wristbands on people who violate self-isolation rules to better limit the spread of COVID-19 on April 11, 2020 [30].

- China

China has deployed digital technologies, including artificial intelligence and 5G, in the fight with the spread of COVID-19, and these technologies “have effectively improved the efficiency of the country’s efforts in epidemic monitoring, virus tracking, prevention, control and treatment, and resource allocation,” according to an article authored by an official of the Cyberspace Administration of China [31].

Since February 2020, Chinese provinces introduced their own colour-based health code system to control the population’s movements and reduce the spread of the virus. This system was integrated into a popular social media app called WeChat and generates QR codes to citizens as an indicator of their health status. The colours used are green (the user can move freely), yellow (the user must go into self-quarantine for up to 7 days), and red (the user will be quarantined for 14 days). The application is not compulsory, but in many cities is required for citizens to be able to leave their residence and enter most public places [32].

9. Digital healthcare in Bulgaria

In Bulgaria some health care activities and services have been digitized so far and local registers and databases have been set up, but there is no complete software solution and unified information system to serve the electronic management of the health sector [33]. The pandemic has accelerated the e-prescription process, making it

easier for patients, doctors and pharmacists and enhancing pharmaceutical and health services. The electronicization of health care in the conditions of a pandemic will contribute to the provision of citizens and medical professionals with systematized and summarized medical information, which is vital in the process of coping with the coronavirus in Bulgaria.

Conclusion

The usage of telepharmacy is an example of good practice in the context of the COVID-19 pandemic, which came into fruition through the release of a multitude of mobile applications aimed at the delivery of pharmaceutical care to patients.

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Thermal response of iron oxide nanoparticles for magnetic hyperthermia

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The great potential of nanotechnologies makes them perspective field of scientific interest. Magnetic properties of some nanomaterials are powerful manipulation and detection tools and have found various applications in cancer research as drug delivery platforms, as sophisticated contrast agents for improved diagnostic imaging, and for magnetically induced hyperthermy. The most widely investigated magnetic nanomaterials are iron oxides. Iron oxide nanoparticles can absorb the energy from an alternating magnetic field and convert it into heat. Nanoparticle-mediated hyperthermy has the potential to achieve localized tumour heating without harming the surrounding healthy tissues. Another potential cancer treatment method is based on the magneto-mechanical effect of the particles. This method involves the local structural deformation of the macromolecules by means of mechanical activation of the magnetic particles using low frequency alternating magnetic field. The resulting mechanical stress may lead to the local damage of the cell membrane, or may cause internal disturbances in the cytoplasm, perturbations of the lysosomes or a cytoskeleton. The aim of this study is to investigate the behaviour of iron oxide particles in alternating magnetic field and their potential in magnetic particle-mediated therapy.

1. Introduction

Nowadays there are a variety of ways nanotechnology can assist in medicine – carriers for targeting drug delivery, fluorescent biological labels, contrast agents in medical imaging, tumour destruction via heating, est. Among of nanomaterials studies, magnetic iron oxide nanoparticles have emerged as one of top candidates for cancer therapy¹ due to their intrinsic magnetism and good biocompatibility. The iron oxide is tolerated by the body – it is a nutrient and is metabolized using the transferrin pathway. Some superparamagnetic iron oxide-based nanomedicines are already clinically available and are approved to serve as magnetic resonance contrast agents (Feridex, Resovist) and iron replacement injection (Feraheme)². Additionally, the magnetic nanoparticles can be used as an alternative treatment modality via hyperthermia therapy and also can induce cancer cell death via mechanical rotations and vibrations.

The destruction of cancer cells by mag-

neto-mechanical effect of particles is a new strategy for cancer treatment that provide a very localized effect, preserving the neighboring healthy cells. The principle of this technique is to produce low-frequency mechanical vibrations of magnetic particles when alternating magnetic field below 100 Hz is applied. The movement of magnetic particles may damage the cell membrane or may cause particular perturbations of the lysosomes or cytoskeleton, leading to cell apoptosis³. Also it is reported that conjugation of the magneto-mechanical effect with chemotherapy producing synergistic effects, making this method an attractive option for new cancer therapy.

Hyperthermia is one of the oldest therapies and can be used in cancer treatment. When tissue is externally heated, the normal vasculature expands and blood flow is increased in order to carry the heat away. In tumors, the morphologically and functionally primitive vasculature is unable to dissipate the heat so that they are selectively

heated compared to normal tissue. An increase in the local temperature to values between 40 and 44°C negatively impacts cancer growth. Temperature over 47°C lead to predominantly induction of necrosis in healthy and cancer cells, which can cause important side effects due to induced inflammatory responses. Hyperthermia via iron oxide magnetic nanoparticles⁴ with superparamagnetic behaviour has been approved for clinical trials on patients with glioblastoma and prostate cancer. For example MagForce, a nanomedicine company in Berlin, uses ferrofluids to achieve hyperthermia via conversion of energy of an alternating magnetic field into heat⁵.

Laser-induced hyperthermia is known as photothermal therapy, and usually uses radiation in near-infrared region and photo-absorbing agent to convert laser energy into heat, inducing thermal damage. Absorption of light is minimal in a near infrared region between approximately 700 nm and 900 nm, as this region is above the hemoglobin absorption bands and below where absorption by water becomes significant. The laser in this range has the least absorption and maximum penetration into the body. If an exogenous absorber such as iron oxide nanoparticles is introduced into the tissues, near-infrared light can cause photothermal destruction at the targeted site with minimal damage to normal tissue.

The aim of our study is to investigate the properties of iron oxide particles in an alternating magnetic field and their potential in magnetic particle-mediated cancer therapy.

2. Materials and methods

2.1. Synthesis of iron oxide nanoparticles

The iron oxide nanoparticles were synthesized in Faculty of Pharmacy, Medical University of Plovdiv, by co-precipitation of iron chloride salts with sodium hydrox-

ide. For a typical synthesis, FeCl₃.6H₂O and FeCl₂.4H₂O (in molar ratio 2:1) were dissolved in distilled water by magnetic stirring at 60°C in a round-bottom flask protected by nitrogen atmosphere flow. Under conditions of vigorous stirring, NaOH (20% solution) was poured dropwise into the vortex of the iron solution. After stirring for 30 min, the precipitate was separated via permanent magnet and washed three times with methanol.

2.2. Construction of a device for alternating magnetic field

Configurations of 4 and 12 permanent magnets that are arranged to one another, such that the north pole of one magnet is next to the south pole of another were realized. The magnets were placed on a mechanical plate that could rotate continuously. The frequency of rotation can be changed with controller. The resulting alternating magnetic field was measured with a Hall probe and an oscilloscope.

2.3. Modeling and simulations of magnetic field

For modeling and simulation of magnetic field we used the Quick Field 6.4 software.

2.4. Thermal measurements of samples on magnetic device

The temperature changes in samples on the constructed magnetic device were monitored by thermosensor. Dual-channel 13bit analogue-to-digital converter and parallel communication with a computer provided a measurements on temperatures with accuracy ± 0,05°C and allowed collecting, recording and processing of laboratory data. Controlling the temperature changes were performed before each measurement and was guaranteed by the calibrated thermometer Hart 1522 Handheld Standards Thermometers (Hart Scientific Utah, USA).

2.5. Measurements of photothermal conversion effect

For a visual heating effect, the dry iron oxide powders were placed on parafilm. Laser with wavelength of 810 nm (power density 3 W/cm²) was used to irradiate the nanoparticle powders at room temperature. As a control, the parafilm alone was irradiated with the same laser as described above.

In order to characterize the photothermal effects of the water-dispersed samples different iron oxide concentrations (10 mL, 100 and 200 µg/mL) were placed in small transparent glass tubes and the samples were irradiated with an 810 nm laser. The temperature of the samples was monitored with a digital thermometer. The sensor was inserted into the solution. The samples were illuminated for a period of 30 min, and changes in the temperature were recorded every 5 min. As a control, the temperature of distilled water (10 mL) irradiated by the same laser as described above was measured. All experiments were conducted in triplicate.

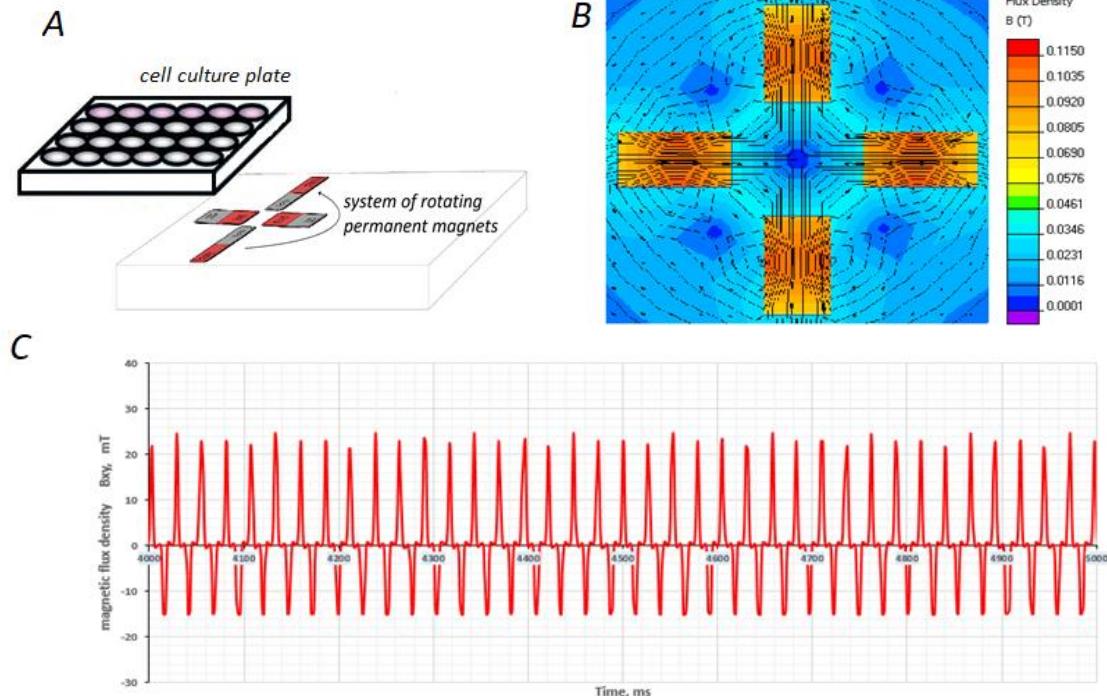
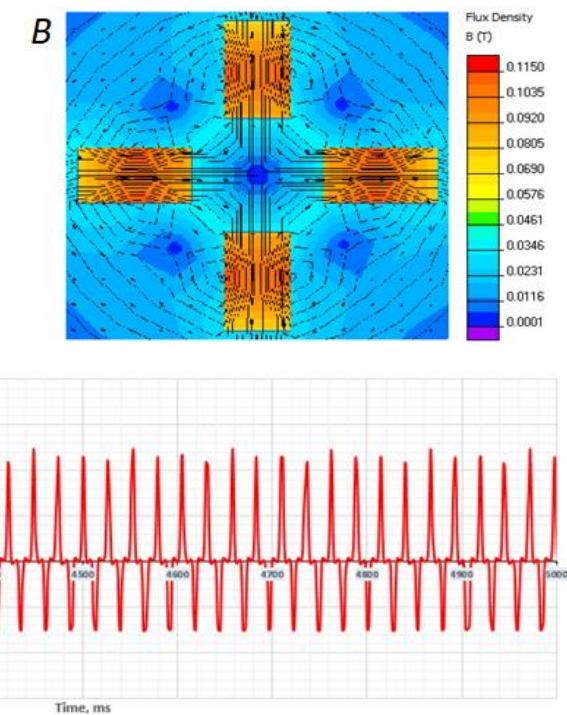


Fig. 1. (A) Schematic representation of the device (B) Magnetic field of quadrupole in the QuickField software (C) The amplitude and shape of the resulting magnetic field.

3. Results and discussion

For investigation of magneto-mechanical effect our supervisor constructed a magnetic device (Fig. 1A) that can actuating nanoparticles. The device uses permanent magnets to generate alternating magnetic field by mechanically rotating them. For modeling magnetic flux density we used QuickField 6.4 software (Fig. 1B).

Various types of magnetic fields can be produced by rotating the permanent magnets and varying their sizes and strength. The amplitude of the resulting magnetic field *can be increased or decreased* simply by changing magnets with stronger or weaker ones respectively. Measurements with a Hall probe and an oscilloscope showed that a quadrupole configuration of four permanent magnets that are arranged perpendicular to one another and rotate at a frequency of about 40 Hz produces homogeneous alternating magnetic field (Fig. 1C).



Studies with distilled water and iron oxide nanoparticles showed that the alternating magnetic field of the device did not lead to increase in the temperature of the samples (Fig. 2). Thus, the device is suitable for future studies of the magneto-mechanical effect on cells without thermal effects.

The visual photothermal conversion effect was studied on dry iron oxide nanoparticle powders, which were placed on parafilm and irradiated with 810 nm laser. Upon laser irradiation, the dry powder generates sufficient heat and burns out a considerable area of the parafilm that is in direct contact with the powder within approximately 60 s, as shown in Fig. 3A and 3B. The parafilm was undamaged without nanopowder when exposed to laser irradiation for the same time period. These results indicated good photothermal effects of iron oxide nanoparticles activated by 810 nm laser irradiation.

In order to characterize the photothermal effect of aqueous suspensions, two iron oxide concentrations (100 and 200 $\mu\text{g/mL}$) in distilled water were irradiated with the same laser for 30 min. Localized heating of the samples was observed via digital thermometer fixed in suspensions near outside the laser beam. The temperature of samples increases was rapid in the first 15 minutes for both nanoparticle concentrations, but then gradually leveled off (Fig. 3C). The iron oxide nanoparticles exhibited a concentration- and time-dependent photothermal effect. For both concentration, increase of temperature (5.7°C for 100 $\mu\text{g/mL}$ and 6.8°C for 200 $\mu\text{g/mL}$) is enough for iron oxide nanoparticles to realize hyperthermia treatment. Our control experiments with aqueous solutions containing no iron oxide nanoparticles showed only up 1°C temperature increase.

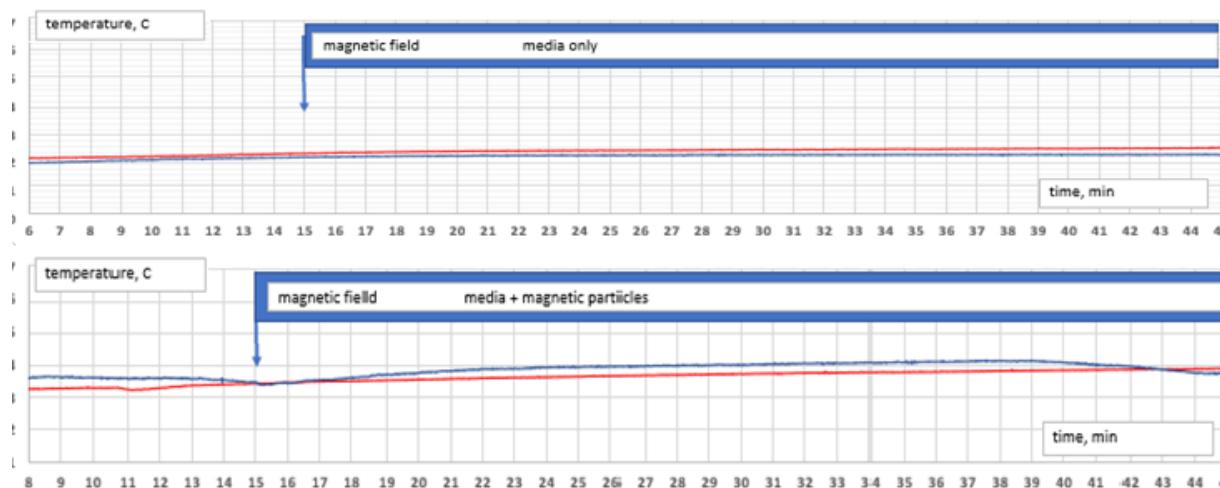


Fig. 2. Thermal response of distilled water (up) and of iron oxide aqueous suspension (down) in low-frequency alternating magnetic field.

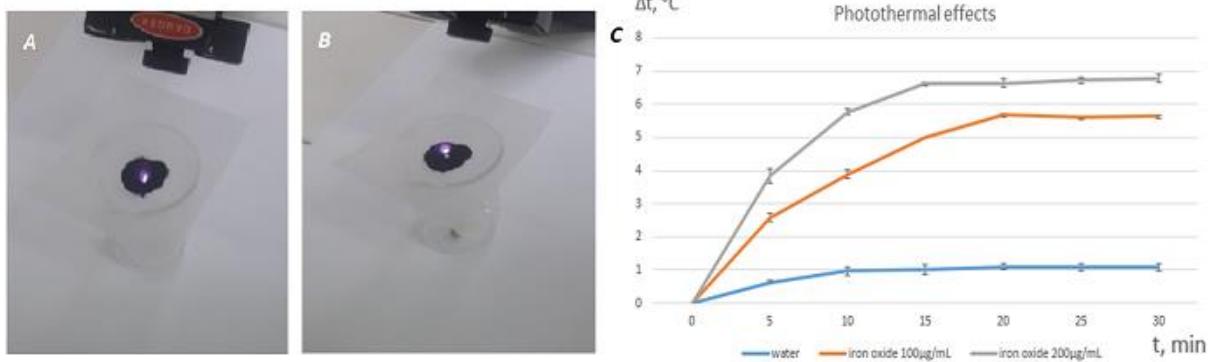


Fig. 3. (A) and (B) Photothermal effect of dry iron oxide nanopowder (C) Photothermal effect of iron oxide at different concentrations (100 µg/mL, orange line and 200 µg/mL, gray line) under 810 nm laser compared with distilled water (blue line).

4. Conclusion

In the current study, measurements showed that the quadrupole configuration of four permanent magnets which rotates with a frequency about 40 Hz produces a homogeneous alternating magnetic field, suitable for future research on the magneto-mechanical effect on cells. Also iron oxide nanoparticles can effectively absorb light energy and convert it to heat under 810 nm laser irradiation and they have a potential in future clinical applications of photothermal therapy.

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Antinociceptive effect of vortioxetine after multiple dose administration in two experimental models of pain

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Introduction: Vortioxetine is antidepressant with potential analgesic activity. Its antinociceptive effect hasn't been studied well yet. **Aim** of the current study was to investigate the possible antinociceptive effect of vortioxetine after a multiple dose administration in rats for 14 days, by using two models of pain.

Materials and methods: 32 male Wistar rats were used, divided in 4 groups ($n = 8$). Group 1 (control) was treated with distilled water 10 ml/kg b.w. per os, group 2 (reference analgesic group) with metamizole 150 mg/kg b.w. per os, group 3 with vortioxetine 3 mg/kg b.w. per os, group 4 with vortioxetine 10 mg/kg b.w. per os. The pain models included – plantar and formalin test. Criteria for analgesic effect was increased latency of time for the plantar test and decreased hind paw licking time for the formalin test. The results were assessed statistically with SPSS 19.0. Each indicator was expressed as Arithmetic mean and Standard error of mean. Groups were compared by using Independent samples T-test. A P value $< 0,05$ was considered statistically significant. **Results:** The vortioxetine group 10 mg/kg b.w. increased significantly the latency of time in the plantar test for the 2nd and 3rd hour of testing, compared to the control. In the formalin test the same group decreased ($p < 0.05$) the hind paw licking time compared to the control for both phases while vortioxetine group 3 mg/kg b.w. has statistically significant effect only in the 1st phase (0–10th minute). **Conclusion:** Vortioxetine shows dose-dependent antinociceptive effect after multiple dose administration.

Introduction

According to the International Association of the Study of Pain (IASP), Pain can be defined as “As an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” Monoamines, such as serotonin (5-HT), norepinephrine and dopamine play important role in the spinal and supraspinal regulation of pain. Because antidepressants increase their levels, they could be used in the treatment of pain. (1)

Vortioxetine is a multimodal antidepressant with a unique mechanism of action that combines inhibition of the high affinity serotonin transporter (SERT) and interaction with multiple serotonin receptor subtypes such as 5-HT_{1A} (full agonist), 5-HT_{1B} (partial agonist), 5-HT₃ (antagonist) and 5-HT₇ (antagonist). (2) Currently, vortioxetine has been studied only for neuropathic pain but it is possible that the drug also has antinociceptive effect. A study by Eide et al.

shows that the tail flick reflex in mice is inhibited by the subarachnoidal administration of 5-HT_{1A} and 5-HT_{1B} agonists therefore the activation of these receptors plays role in the regulation of pain on spinal and supraspinal levels. (3) The antagonism with 5-HT₃ receptors also could be related to antinociception, according to a study of Sagalajev et al. (4)

Aim

The aim of the current study was to investigate the possible antinociceptive effect of vortioxetine after its multiple dose administration for 14 days on rats.

Materials and methods

In this experiment, we used 32 male Wistar rats, divided in 4 groups ($n = 8$). For 14 days, the animals from the different groups received orally by gavage distilled water, metamizole and vortioxetine at two doses – 3 and 10 mg/kg b.w.

The experimental design is shown on Table 1.

Table 1. Experimental design.

Nº	Groups	Oral treatment with:
1	Control group	Distilled water 10 ml/kg b.w
2	Reference control group	Metamizole 150 mg/kg b.w
3	Tested group	Vortioxetine 3 mg/ kg b. w
4	Tested group	Vortioxetine 10 mg/ kg b. w

On Day 15, all animals were tested for antinociception by using two models of pain – Plantar test (Hargreaves' method) and Formalin test.

The Plantar test (Hargreaves' method) uses thermal pain model and consists of a perforated platform, controller and stimulation unit. The animal is placed in an enclosure, over the perforated platform. After some period in which the animal becomes accustomed for the environment, the stimulation unit is placed underneath the platform, just below the animal's front paw. In order to avoid overheating of the paw, the intensity of infrared heating is 50 mW/cm² and the maximum heating time is 30 s. A special filter blocks the visible spectrum of the light so that it doesn't distract the animal's attention. The controller automatically detects the time for which the rat withdraws its paw from the stimulation unit and register it as a time latency that serves as a criteria for antinociceptive effect. Therefore the more the time latency, the more animal is unsusceptible to thermal pain. This test is performed for each animal on the 1st, 2nd and 3rd hour, after they are being treated with the tested substances.

The Formalin test utilizes model of chemical pain. 0,2% 200 µl formalin is administered as an intraplantar injection in one of the animal's hind paws one hour after the rats have been treated with the tested substances. Criteria for antinociception is the decrease in the time of licking of the hind paw compared to the control group.

The test has two phases in which this time is measured: first phase from 0 to 10th minute and second phase from 20th to 30th minute.

The statistical assessment of the results was done with IBM SPSS 19.0. Their distribution was determined with Shapiro-Wilk test. An Arithmetic mean for each group was calculated as well as the Standard error of means (SEM). The groups were compared by using Independent sample T test. A P value < 0,05 was considered statistically significant.

Results and discussion

The plantar test can be classified as a reflexive pain test that triggers specific behavioral response after the application of heat stimuli. It not only activates the nociceptors but also integrates supraspinal structures. (5) These mechanisms are explained in a study of Davies et al. who investigate the relation between the sucrose intake of rats and appearance of thermal analgesia, measured by the plantar test. (6)

In our experiment, the results from the plantar test showed that for the 1st hour of testing only the reference control group, treated with metamizole, significantly increased the time latency, compared to the control treated with distilled water. In the 2nd hour of the test, both the group treated with vortioxetine – 10 mg/kg b.w., as well as the metamizole reference group had statistically significant effect in increasing the time latency indicator, compared to the control group. As for the 3rd hour of testing, the reference control group did not have significant changes in the time latency, compared with control, while the group treated with vortioxetine at a dose of 10 mg, although not so pronounced, still had statistically significant increase in this parameter compared to the control group, treated with dis-

tilled water. The group, treated with vortioxetine at a dose of 3 mg didn't show any significant changes in the time latency, compared to the control group for all 3 hours of testing in the plantar test (fig. 1).

These results suggest that vortioxetine at a dose of 10 mg/kg b.w. has antinociceptive effect which is related to supraspinal mechanisms of action. This effect persists after the effect of the reference substance metamizole has been expired.

The formalin test is well established method that evaluates both peripherals and central mechanisms of antinociceptive effect. The first phase of the test is related to direct stimulation of the nociceptors at peripheral level, provoked by the release of endogenous mediators of pain such as bradykinins and substance P, while the second phase is characterized by inflammation pain caused from the sensibilization by

prostaglandins and histamine and also involves central mechanisms. (7) (8)

In our experiment vortioxetine showed pronounced antinociceptive effect in the formalin test. Both tested groups, compared to the control, decreased the hind paw licking time ($P < 0,05$) for the 1st phase of testing. The same was observed when we compared the reference control group to the control, treated with distilled water. In the 2nd phase of the test the group treated with vortioxetine at a dose of 10 mg, decreased significantly the hind paw licking time, compared to the control while the group treated with vortioxetine at a dose of 3 mg also decreased this indicator, but the difference with the control group wasn't statistically significant ($P > 0,05$). The decrease of the hind paw licking time was significant for the reference control group, compared to the control (fig. 2).

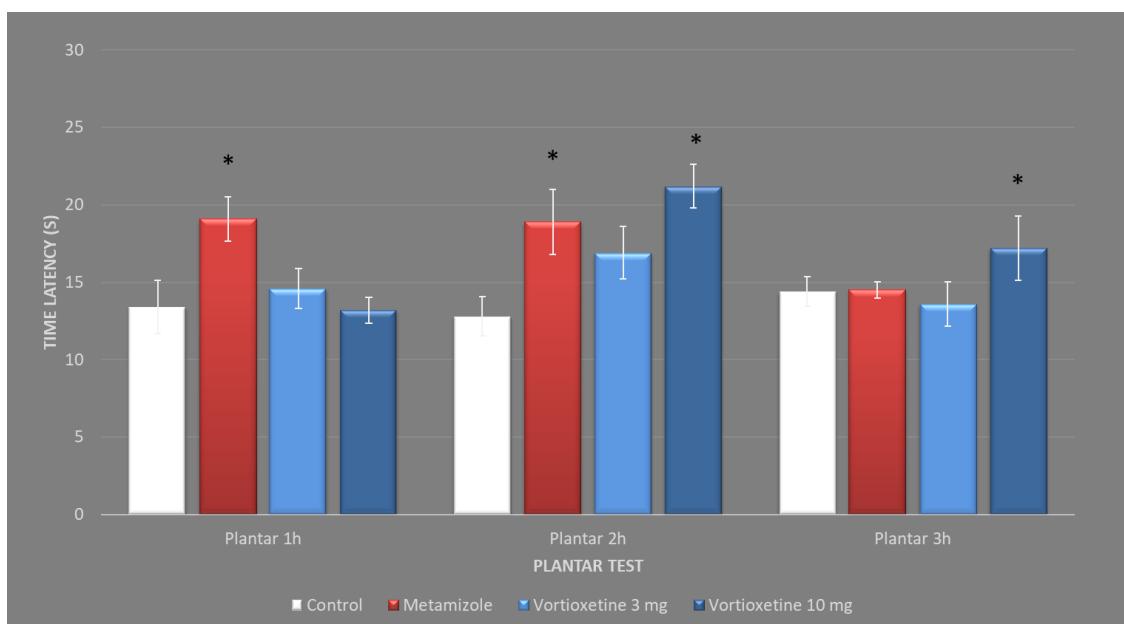


Fig. 1. Time latency, measured in Plantar test for the 1st, 2nd and 3rd hour after the repeated administration for 14 days of vortioxetine in doses of 3 and 10 mg/kg b.w. per os. * $P < 0,05$, compared to the control group.

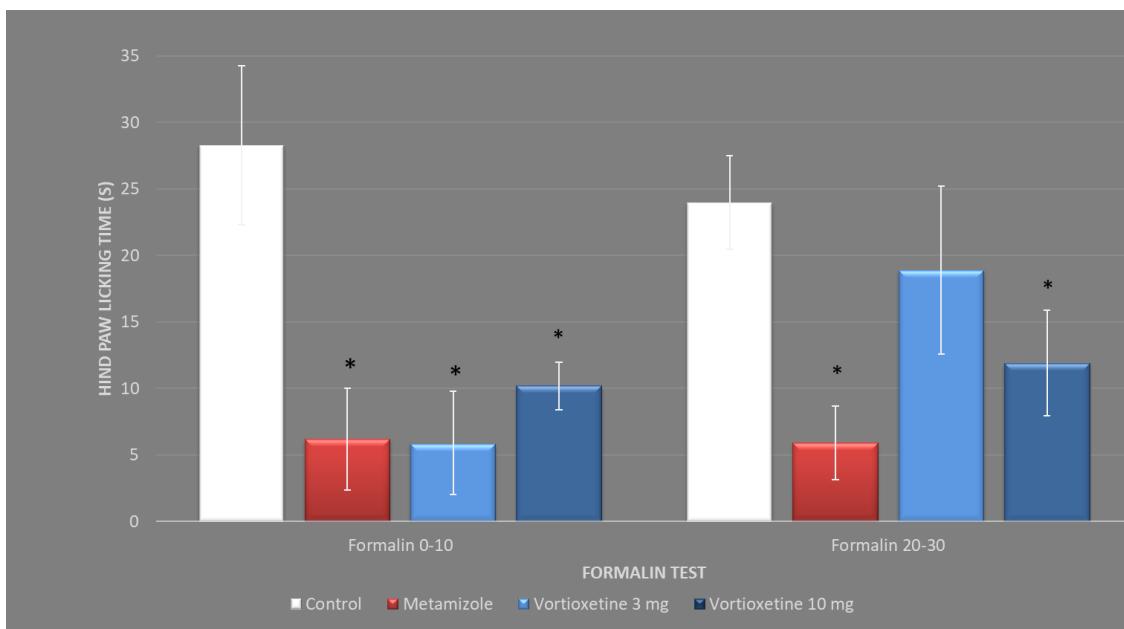


Fig. 2. Hind paw licking time, measured in Formalin test from 0 to 10th minute and from 20th to 30th minute, after the repeated administration for 14 days of vortioxetine in doses of 3 and 10 mg/kg b.w. per os. *P < 0,05, compared to the control group.

The effect of vortioxetine was more pronounced in the first phase of the test, so we suppose that the analgesic effect of the drug is related to peripheral antinociception. The higher dose also demonstrated analgesic effect in the second phase of the test, which is known to be related by acute inflammatory mechanisms and mediated by spinal mechanisms of action. (9) (10) Therefore, it is possible that at a dose of 10 mg/kg b.w. vortioxetine also possesses anti-inflammatory effect.

Conclusion

Vortioxetine showed antinociceptive effect after multiple dose administration for 14 days on rats. The effect was more pronounced at a dose of 10 mg/kg b.w and in the formalin test. More experiments need to be conducted at broader arrange of doses, to see if the antinociceptive effect of vortioxetine is dose-dependent. The drug should also be evaluated for a possible anti-inflammatory effect by using different models of experimental inflammation.

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A novel Celecoxib analog and the role of opioid receptors in its analgesic effect

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Pyrrole-based heterocyclic compounds are a promising perspective in the development of novel molecules with analgesic properties. 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-3-methylpentanoic acid (compound 3d) is a newly synthesized N-pyrrolylcarboxylic acid, based on the structure of the selective COX-2 inhibitor Celecoxib. In previous experiments it demonstrated analgesic activity in animal pain models. The aim of the current study is to assess the involvement of opioid receptors in the analgesic effect of compound 3d. 49 adult male Wistar rats are divided in 7 groups ($n = 7$), treated with saline, compound 3d in doses of 10, 20 and 40 mg/kg b.w. and compound 3d in the same doses with added naloxone 2 mg/kg b.w. intraperitoneally. Antinociception and opioidergic involvement are assessed using paw withdrawal test. Statistical analysis is performed with IBM SPSS 20.0 software, employing One-way ANOVA and Tukey post hoc test. Compound 3d in all experimental doses shows increased latency time for paw withdrawal at 1st, 2nd and 3rd hours of testing, compared to the saline group. The animals treated with naloxone and a following administration of compound 3d demonstrate lack of such increase in the paw withdrawal time compared to the control group. Literary data suggest involvement of the endogenous opioidergic system in the antinociception exerted by NSAIDs. In our study, compound 3d's analgesic effect observed at the paw withdrawal test disappears with concomitant administration of the μ -receptor antagonist naloxone. Opioid receptors seem to play a role in the antinociception induced by the compound.

Introduction

The pyrrole heterocycle is included in the chemical structure of many drugs with various activities (1). Some classic and modern non-steroidal anti-inflammatory drugs (NSAIDs) contain a pyrrole ring and its role in suppressing inflammation has been proven in a number of studies (2) (3). Against the background of its diverse biological activity, it has a relatively good tolerability and safety. Pyrrole-based heterocyclic compounds are a promising perspective in the development of novel molecules with analgesic properties. 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-3-methyl-pentanoic acid (compound 3d) is a newly synthesized N-pyrrolylcarboxylic acid, based on the structure of the selective cyclooxygenase-2 (COX-2) inhibitor Celecoxib. The structure of the target molecules is designed to include common elements of the pharmacophore systems of

contemporary NSAIDs with the expectation of possessing their inherent pharmacological activity. However, the COX selectivity of NSAIDs is not limited only to the structural characteristics of the compounds (4). Experimental evidence shows that NSAIDs, besides inhibiting the COX enzyme, also interact with endogenous opioids along the descending pain control system (5) (6). In previous experiments compound 3d demonstrated analgesic activity in animal pain models (7).

Aim

The aim of the current study is to assess the involvement of opioid receptors in the analgesic effect of compound 3d.

Materials and methods

The experiment is approved by the Ethics Committee on Animals of the Bulgarian Food Safety Agency with permit № 128/09.12.2015 and by the decision of the

Ethics Committee at the Medical University of Plovdiv, protocol № 2/31.03.2016.

49 adult male Wistar rats are randomly divided in 7 groups ($n = 7$), treated with saline, compound 3d in doses of 10, 20 and 40 mg/kg b.w. and compound 3d in the same doses with added naloxone 2 mg/kg b.w. intraperitoneally. The animals are maintained on a light/dark cycle of 12/12 h in a temperature-controlled environment with food and water available *ad libitum*.

Antinociception and opioidergic involvement are assessed using paw withdrawal test. Each animal is placed in an individual compartment and left unrestrained. After an acclimation period, an infrared heat source (Ugo Basile, Italy) is positioned under the glass floor directly beneath the hind paw of the animal, and paw withdrawal latency (in seconds) is recorded automatically. Cut-off time is set at 30 seconds to avoid unnecessary overheating of the paw (8). The test is performed 1, 2, and 3 hours after intraperitoneal administration of the tested substances for the respective experimental groups. The criterion for analgesic action is increased reaction time compared to the animals treated with saline.

Statistical analysis of the obtained data was performed with IBM SPSS 20.0 software, using One-way ANOVA and Tukey post hoc test. The normality of distribution was established with Shapiro-Wilk test. Results are expressed as arithmetic mean and standard error of the mean (mean \pm SEM). P value ≤ 0.05 is considered statistically significant.

Results

Compound 3d at dose 10 mg/kg b.w. shows increased latency time for paw withdrawal at 1st, 2nd and 3rd hours of testing, compared to the saline group ($p = 0.001$, $p = 0.003$ and $p = 0.028$, respectively). Similar results are shown by the compound at dose 20 mg/kg b.w. ($p = 0.002$, $p < 0.001$ and $p = 0.009$, respectively). Compound 3d 40 mg/kg b.w. significantly increases paw withdrawal time compared to the control animals at all tested hours ($p < 0.001$, $p < 0.001$ and $p = 0.005$, respectively). The animals treated with naloxone and a following administration of compound 3d in all three experimental doses demonstrate lack of such increase in the paw withdrawal time compared to the control group (Fig. 1).

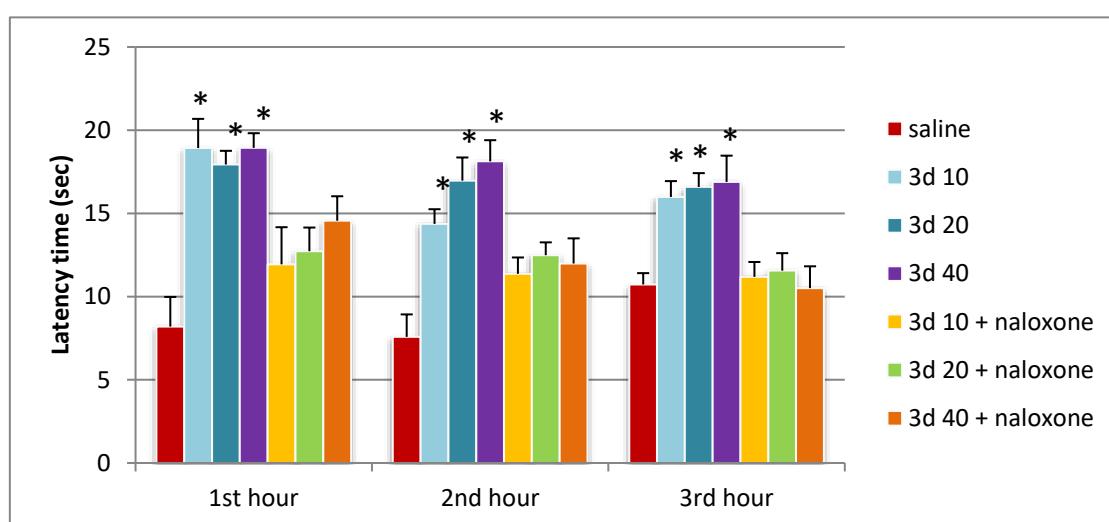


Fig. 1. Effect of compound 3d at doses of 10, 20 and 40 mg/kg b.w. and subsequent administration of naloxone 2 mg/kg b.w. on the nociceptive threshold in paw withdrawal test. * $p < 0.05$

Discussion

Literary data suggest involvement of the endogenous opioidergic system in the antinociception exerted by NSAIDs. In our study, compound 3d's analgesic effect observed at the paw withdrawal test disappears with concomitant administration of the opioid μ -receptor antagonist naloxone.

The paw withdrawal test uses a localized heat source and the thermal stimulus is applied only to the paw of the test animal (8). An advantage of the plantar test is the automatic detection of latency time. Another advantage of this test is the lack of a specific hint for creating a conditioned reflex, i.e. animals cannot learn what behavior interrupts the test and the results are more reliable (9).

Nozadze et al. found that the administration of metamizole, ketorolac and lornoxicam to the central nucleus of the rat amygdala induces tolerance to these drugs, as well as cross-tolerance to morphine in the hot plate test and the tail flick test (10). Tsiklauri et al. confirm these results, and these authors also observed the development of tolerance in the administration of metamizole, ketorolac and lornoxicam intraperitoneally (11). Tortorici et al. found that administration of metamizole to the ventrolateral periaqueductal gray area of rats results in antinociception in the hot plate test. After two days of administration, metamizole induced tolerance, with rats also showing tolerance to morphine and showing withdrawal symptoms with systemic naloxone. These results suggest that metamizole activates endogenous opioid systems and that non-opioid analgesics may lead to opioid tolerance and risk of central nervous system withdrawal syndrome (12). In addition to stimulating the release of endogenous opioids, recent evidence at cellular level suggests that NSAIDs may also

enhance the signaling pathway used by opiates. Co-administration of NSAIDs potentiates the inhibition of gamma-aminobutyric acid (GABA) release by morphine, although NSAIDs have no effect on GABA release in the absence of morphine. A probable explanation for the mechanism of increased analgesia when opioids and NSAIDs are co-administered is that COX blockade diverts arachidonic acid metabolism to lipoxygenase pathways and thus increases the efficacy of opioid receptor agonists (13). The exact molecular mechanism of NSAID tolerance is not yet known, but NSAID tolerance mimics opioid tolerance and administration of naloxone significantly reduces the antinociceptive effects of NSAIDs (14). Ketorolac shows a significant analgesic effect in both hot plate and tail flick and systemic administration of naloxone reduces its analgesic activity (15). The antinociceptive effect of diclofenac is similarly attenuated by systemic administration of naloxone (16).

Conclusions

Compound 3d shows an analgesic effect in experimental conditions as previously established by the authors.

The analgesic effect of the compound disappears after systemic administration of the opioid receptor antagonist naloxone.

Opioid receptors seem to play a role in the antinociception induced by the compound.

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New aspects in the reversal of neuromuscular blockade following general anesthesia: selective relaxant-binding agents – review

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Neuromuscular blockade with non-depolarizing muscle relaxants is an integrative part of the modern concept for balanced general anesthesia, helping to conduct extensive complex surgical interventions. Classical notions of restoring spontaneous movements after muscle relaxation include both waiting for spontaneous recovery after biotransformation of the relaxant, and the use of cholinesterase inhibitors (ChEIs) such as Neostigmine. They produce their effect by inhibition of the serum cholinesterase and thus increase the amount of free acetylcholine at the level of the motor endplate, which displaces the muscle relaxant from the receptor complex and terminates the neuromuscular block. In recent years, a new group of functional antagonists of relaxants, namely selective relaxant-binding agents, has been registered and introduced in the clinical practice. They have a unique mechanism of action and differ significantly from ChEIs in their pharmacodynamics. The first registered drug from this group is Sugammadex – its molecule selectively binds to the steroid relaxants by encapsulation. This leads to a sharp decrease in the free concentration of the muscle relaxant plasma levels and reversal of its clinical effects. Sugammadex has a number of advantages over conventionally used agents such as CHEIs, both due to its high efficiency and rapid onset of action, as well as the absence of side effects. It is for those and other facts that this group of drugs has the potential for a wider application into the clinical practice and will gradually become a tool of choice for restoring neuromuscular transmission following general anesthesia.

Neuromuscular blockade with non-depolarizing muscle relaxants is an integrative part of the modern concept for balanced general anesthesia and used regularly in the clinical practice. Nowadays, non-depolarising muscle relaxants are widely used for maintaining relaxation for the duration of the surgical intervention. They are preferred for maintaining relaxation for a prolonged period of time during surgery, due to their longer duration of action. In addition, their use does not present a risk for a malignant hyperthermia episode in genetically predisposed individuals, and thus makes them a safer option than the depolarising muscle relaxant Suxamethonium.

Restoring spontaneous movements after neuromuscular blockade (NMB) is a key part of ensuring a safe and prompt recovery post-anesthesia. Classical notions of reversing the NMB includes the use of cholinesterase inhibitors (ChEIs). ChEIs produce their effect by inhibition of the serum cholinesterase and thus increase the amount of

free acetylcholine at the level of the motor endplate, which displaces the muscle relaxant from the receptor complex and terminates the neuromuscular block (Fig. 1) This ensures quicker recovery of spontaneous movements and breathing, rather than waiting for the biotrasformation of the muscle relaxant.

The most commonly used ChEI for neuromuscular reversal in Neostigmine. A typical dosage for reversal of the NMB is 0.04–0.08 mg/kg. The effect is apparent in 5–10 min, and lasts for about an hour (halflife of Neostigmine is 45 minutes).

Use of ChEIs, however, has its drawbacks. Due to their indirect mechanism of action, reversal by ChEIs is limited and unpredictable, resulting in a high risk for post-operative residual curarisation [1]. Furthermore, ChEIs interact with the cholinergic system, which causes muscarinic side effects such as bradycardia, hypotension and increased gasintestinal motility. There is a higher risk of spontaneous air-

way obstruction due to bronchospasm, as well as increased bronchial secretion. These effects can be partially counteracted with the simultaneous use of antimuscarinic drugs, such as Atropine. However, the use of the latter comes with the risk of the opposite adverse reactions – tachycardia, altered cardiac conduction, dysrhythmias, urine retention, etc [2]. The use of ChEIs is also associated with numerous drug interactions – agents such as Clindamycin, Propranolol and Procaineamide are known to reverse the effects of Neostigmine in particular [3].

In recent years, a new group of functional antagonists of relaxants, namely selective relaxant-binding agents, has been introduced in the clinical practice. These drugs

have a unique mechanism of action and differ significantly from ChEIs in their pharmacodynamics. The first registered drug from this group is Sugammadex (ORG 25969). It is a gamma-cyclodextrin with a specially modified ring-like structure. It is composed of cyclically arranged oligosaccharides forming a lipophilic core and hydrophilic outer shell. (Fig. 2) Sugammadex has predictable pharmacokinetic properties. The drug is available only in intravenous form. It is given as a single bolus IV injection. The volume of distribution is approximately 18 l. The medication does not bind to the plasma proteins, does not produce any active metabolites and is mostly excreted unchanged through the kidneys. Plasma clearance ranges from 84–138 ml/min [4].

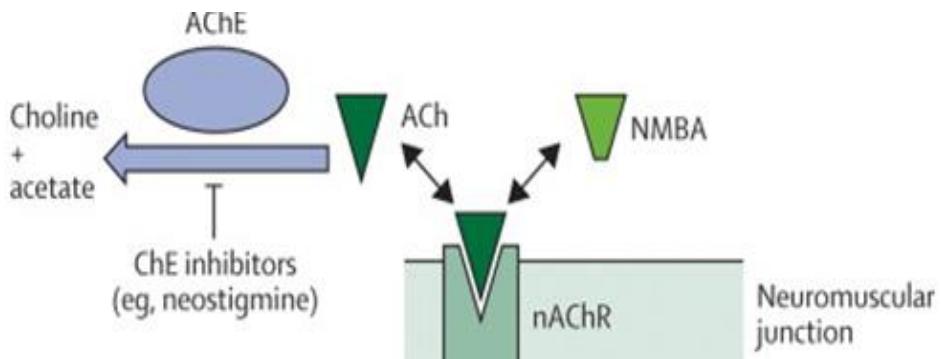


Fig. 1. The mechanism of action of cholinesterase inhibitors.

Smith A. Monitoring of neuromuscular blockade in general anaesthesia. The Lancet. VOLUME 376, ISSUE 9735, P77-79, JULY 10, 2010

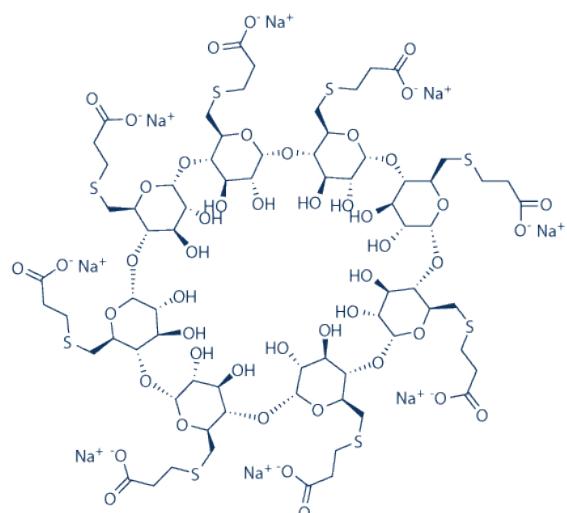


Fig. 2. The chemical structure of Sugammadex.

Naguib, Mohamed MB, BCh, MSc, FFARCSI, MD Sugammadex: Another Milestone in Clinical Neuromuscular Pharmacology. Anesthesia & Analgesia: March 2007 – Volume 104 – Issue 3 – p 575-581 doi: 10.1213/01.ane.0000244594.63318.fc

The mechanism of action of Sugammadex is different to other more conventionally-used reversal drugs. When interacting with steroidal substances (such as the NDMRs Rocuronium and Vecuronium), the latter are incorporated into the lipophilic core of the cyclodextrin molecule. This likens the mechanism of action of Sugammadex to that of a synthetic receptor [5], which binds and encapsulates the muscle relaxant molecule, leading to a rapid decrease in the free plasma concentration of the muscle relaxant and reversal of its clinical effects (Fig. 3).

It has a one-to-one binding capacity, meaning that one molecule of Sugammadex encapsulates one molecule of the aminosteroid muscle relaxant. The optimal dosage for reversal is dependant on the depth of the block, the timing, as well as the dose of relaxant applied (table 1) [6]. Reduced doses (< 2 mg/kg) are associated with prolonged recovery time and incomplete reversal of the block [7]. For reversal of routine and

moderate block with either Rocuronium or Vecuronium, the doses are similar to each other. However, there is not sufficient data present for reversal of a profound Vecuronium-induced block and therefore, the proper dosage has not been formally determined [8]. It is important to note, that Sugammadex has been found most effective in reversing neuromuscular blockade particularly with Rocuronium and Vecuronium. Other aminosteroid relaxants, such as Pancuronium, have been found to have less affinity with it. Reversal of a Pancuronium-induced block would require larger doses of Sugammadex [9]. The drug has no effect on neuromuscular blockade by non-steroidal blockers, such as benzylisoquinolinium compounds (e.g Atracurium, Tubocurarine, etc), or depolarising muscle relaxants (Suxamethonium), because it cannot form inclusion complexes with them. Therefore, it should not be used in conjunction with the latter [10].

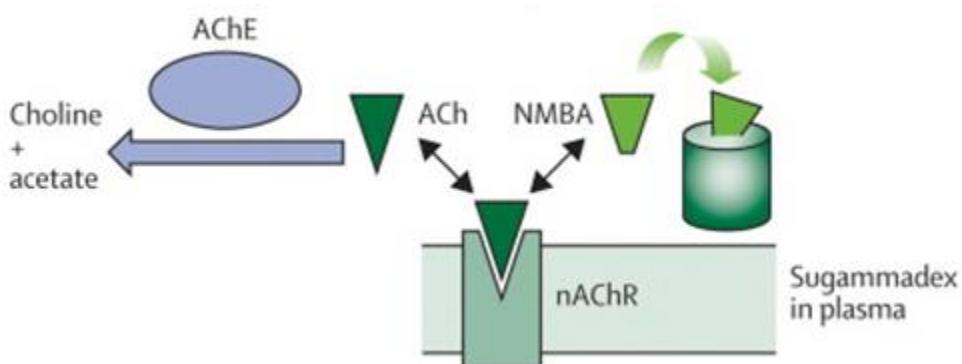


Fig. 3. The mechanism of action of Sugammadex.

Smith A. Monitoring of neuromuscular blockade in general anaesthesia. *The Lancet*. VOLUME 376, ISSUE 9735, P77-79, JULY 10, 2010

Table 1. Doses for reversal of Aminosteroid-induced neuromuscular block

Nag, K.; Singh, D.R; Shetti, A.K.; Kumar, H; Sivashaunmugam, T; Parthasarathy, S. Sugammadex: A revolutionary drug in neuromuscular pharmacology. *Anesthesia Essays Res*. 2013, 7 (3):302-306

Types of Block	Doses for Rocuronium block	Doses for Vecuronium block
Routine	2 mg/kg	2 mg/kg
Moderate	4 mg/kg	4 mg/kg
Deep	16 mg/kg	Not formally established

Sugammadex has a number of advantages over conventionally used agents, such as ChEIs. The Sugammadex molecule itself does not interact with the cholinergic system at any level, thus not causing any of the adverse effects characteristic of ChEIs (bradycardia, hypotension, bronchospasm, etc.) One coherent systematic review found that, compared to Neostigmine, the use of Sugammadex for reversal of the neuromuscular blockade resulted in significantly less instances of bradycardia, postoperative nausea and vomiting, and overall provided a much quicker recovery of spontaneous movements [11]. Moreover, there are benefits in terms of reduced incidence of post-operative residual curarisation [1]. With the use of Sugammadex, the need for applying antimuscarinic agents, such as Atropine, in order to counteract the adverse reaction to the ChEIs becomes absent. Thus, all potential side effects of those drugs (e.g tachycardia, urinary retention, etc) are avoided as well.

So far the use of Sugammadex has been endorsed in all patients ages 2–75 years of age. For children and adolescents, the typical dosage of 2 mg/kg for reversal of a standard block is recommended. The drug has not been approved for use in neonates and infants under 2 years, as well as during pregnancy, as there are no documented studies for those particular populations of patients. Current dosing regimen of the drug is based on ideal bodyweight (IBW). However, in severely obese patients, an increase of the dosage with 40% is recommended, since doses based on IBW in this population have been associated with prolonged recovery time [8].

Sugammadex is generally a well-tolerated drug. Common side effects include dizziness, headaches, fatigue, nausea and vomiting. Some of the more severe

concerning reactions include anaphylactic shock, presenting within the first 5 minutes of exposure [12], as well as a prolongation of the QT interval, correlated with high doses above 32 mg/kg [13]. Earlier studies have suggested an effect on coagulation status, due to early increase of aPTT and prothrombin time (PT), possibly mediated by a temporary inhibition of factor Xa. However, later works suggest that these changes normalise within 1 hour of exposure, and do not correlate with adverse clinical consequences [14].

In conclusion, selective relaxant-binding agents present a step forward in management of muscle relaxation during surgery. With its predictable pharmacodynamics and minimal side effects, the only downside of a drug like Sugammadex remains it's high price. With introduction of more agents with similar mechanism of action, it is likely that the cost will readjust and make this class of drugs ubiquitously available for anesthesiologists worldwide.

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Experimental study of the anxiolytic effect of cannabidiol in a model of acute cold stress

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Cannabidiol (CBD) is a bioactive constituent of Cannabis sativa. In clinical practice its effectiveness has been proven in a narrow range of medical conditions such as epilepsy, chronic pain and vomiting. In recent years interest in CBD as an anxiolytic agent has increased. **The aim** of the present study was to examine the anxiolytic effect of CBD in a model of acute cold stress in rats. **Material and methods:** Male Wistar rats were divided into five groups: control treated with olive oil; control treated with olive oil and exposed to acute cold stress and three experimental groups treated with cannabidiol 2.5, 5 and 10 mg/kg bw. To study the anxiolytic effect, we used elevated plus maze test. The animals were treated with cannabidiol for 14 days. On the 15th day, 60 minutes after dosing, the animals were placed in a refrigerator at 4° for 60 minutes. Behavioural tests were performed immediately thereafter. The following parameters were assessed: number of open arm entries and of enclosed arms entries, time spent in each of them and the ratio open/total arm entries. **Results:** The highest dose of CBD significantly reduced the time spent in the enclosed arms and increased that in the open ones compared to the two control groups ($p < 0.05$). Rats treated with the highest dose of CBD showed increased ratio open/total arm entries compared to the stressed control ($p < 0.05$). **Conclusion:** Cannabidiol at dose 10 mg/kg bw exerts anxiolytic effect in a model of acute cold stress.

Introduction

Cannabis sativa has been used in medicine since ancient times. In traditional Chinese medicine cannabis was applied for the treatment of malaria, asthma and gout and in India for convulsions, neuralgias and migraines [1]. The use of cannabis became very popular in Europe and USA in the nineteenth century. Ethanolic extracts of cannabis (known as cannabis tincture) were used to treat various disorders such as convulsions in infants, cholera, tetanus and rabies [2]. However, in the first half of the twentieth century these practices were abolished owing to an inability to prepare standardised cannabis preparations, which resulted in the risk of producing over- or under-dosed formulations [3].

Cannabis sativa contains over 100 compounds. Among them Δ9-tetrahydrocannabinol (THC) is considered the main component responsible for the psychoactive effects of the plant. Cannabidiol (CBD) is a phytocannabinoid constituent of Cannabis

sativa that lacks the psychoactive effects of THC. CBD has wide therapeutic properties across a range of neuropsychiatric disorders, originating from diverse central nervous system actions – antipsychotic, analgesic, neuroprotective, anticonvulsant, antiemetic, anti-inflammatory activity [3]. In recent years, CBD has engaged increasing interest as a potential anxiolytic treatment [5, 6].

The endocannabinoid (eCB) system plays a crucial role in the regulation of emotional behavior and is essential for synaptic processes that regulate learning and emotional responses, specifically those related to potentially traumatic experiences [7]. The eCB system includes two different cannabinoid receptors (CB1 and CB2), their endogenous ligands and enzymes involved in their synthesis and degradation. eCB signaling is distributed throughout the CNS and peripheral tissues, modifying presynaptic release of both excitatory and inhibitory neurotransmitters. Cannabinoid type 1

(CB1) receptors are expressed by peripheral and central neurons, especially in the central areas that play key roles in anxiety and aversive learning – the amygdala, hippocampus and cerebral cortex [8]. Activation of CB1 receptors produces anxiolytic effects in various models of unconditioned fear, applicable to multiple anxiety disorder symptom domains [5].

CBD has minimal affinity for CB1 and CB2 receptors, but could indirectly activate CB1 receptors by increasing the availability of endogenous endocannabinoids. Anandamide (AEA) is an endogenous cannabinoid that acts as a partial agonist of CB1 receptors and is metabolized by the enzyme fatty acid amide hydrolase (FAAH). CBD has been reported to inhibit FAAH, thus increasing the availability of anandamide and causing activation of the endocannabinoid system. Studies in rodent models have shown that pharmacological activation of the eCB system through CB1-receptor agonists results in decreased behavioral response to aversive memories through the inhibition of memory reconsolidation and enhanced extinction [9].

Another pharmacological mechanism involved in the anxiolytic effect of CBD is facilitation of serotonin 5HT_{1A} receptor mediated neurotransmission in defense-related areas. While in vitro studies suggest CBD acts as a direct 5-HT_{1A} receptor agonist, in vivo studies are more consistent with CBD being an allosteric modulator, or facilitator of 5-HT_{1A} signaling [10]. The 5-HT_{1A} receptor (5-HT1AR) is a well-known anxiolytic target. Data from preclinical studies show that 5-HT1AR agonists possess anxiolytic action in animal models of general anxiety, prevent the adverse effects of stress and could enhance fear extinction [5].

Having broad pharmacological profile

CBD may be a promising approach for the treatment of disorders related to inappropriate retention of aversive memories.

Aim

The aim of the present study was to examine the anxiolytic effect of CBD in a model of acute cold stress in rats.

Material and methods

Ethical statement

The experiment has been approved by Animal Health and Welfare Directorate of the Bulgarian Food Safety Agency, permit № 257/2019 and by the Ethic Committee at Medical University of Plovdiv.

Experimental design

Male Wistar rats (200 ± 20 g bw) were used in the study. Animals were kept under standard laboratory conditions – 12 h light/dark cycle; food and water *ad libitum*. Rats were divided into five groups ($n = 8$): control treated with olive oil; control treated with olive oil and exposed to acute cold stress; three experimental groups treated with cannabidiol 2.5; 5 and 10 mg/kg bw respectively. The animals were treated with cannabidiol (organic CBD, Nature's Way) for 14 days. On the 15th day, 60 minutes after the administration of the substances, the rats were placed in a refrigerator at 4° for 60 minutes. Behavioural testing was performed immediately thereafter.

Elevated plus-maze

Elevated plus-maze is a behavioral method used to anxiolytic effect. Animals are placed in the center of the maze and allowed to move freely in the open or enclosed arms. Rats prefer closed and dark spaces, so avoid the illuminated arms of the maze. Increased residence time in the open arms and an increase in the number of entries correlate with anxiolytic effect. The

following parameters were assessed: number of open arm entries and of enclosed arms entries, time spent in each of them and the ratio open/total arm entries.

Statistics

Statistical analyses were performed using IMB SPSS software 19.0. Mean values \pm SEM were calculated. Results were considered significant at $P < 0.05$. ANOVA was used to compare groups' parameters followed by Tukey's multiple comparison test.

Results

The stressed control group showed increased time spent in the enclosed and decreased time in open arms of the maze compared to the control group not subjected to cold stress. The highest dose of CBD significantly decreased time spent on the enclosed arms and increased that spent on

the open arms compared to the two control groups ($p < 0.05$; $p < 0.015$, respectively) (fig. 1).

The control animals subjected to acute stress showed reduced number of entries in the open and enclosed arms of the maze compared to the control group without stress. Rats treated with the highest dose of CBD showed significant increase in both parameters compared to both control groups and the lower studied doses ($p < 0.01$; $p < 0.0001$; $p < 0.05$, respectively) (fig. 2). Control animals subjected to acute stress showed reduced ratio open/total arm entries compared to the control without stress ($p < 0.5$). The experimental group treated with the highest dose of CBD showed an increase in this ratio compared to the control group, subjected to acute cold stress and compared to the lowest dose of CBD ($p < 0.5$; $p < 0.001$ respectively) (fig. 3).

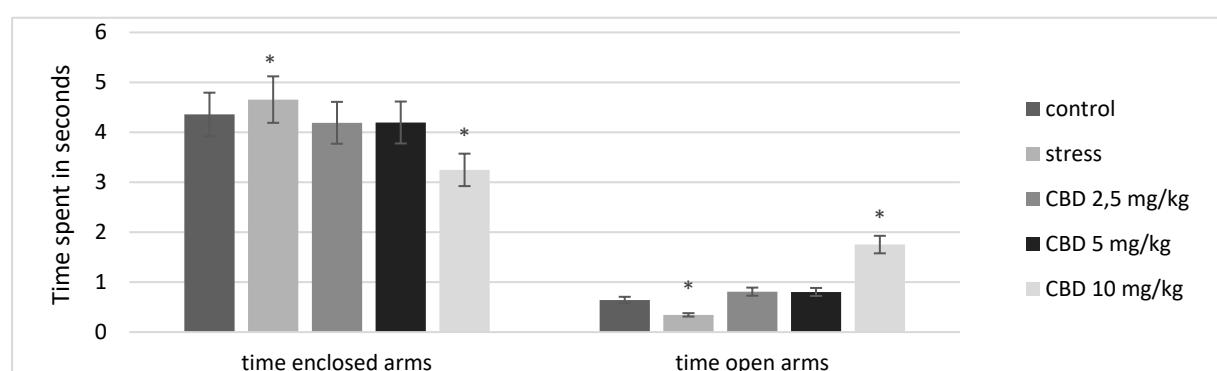


Figure 1. Effect of CBD on the time spent in open and enclosed arms in elevated plus maze.

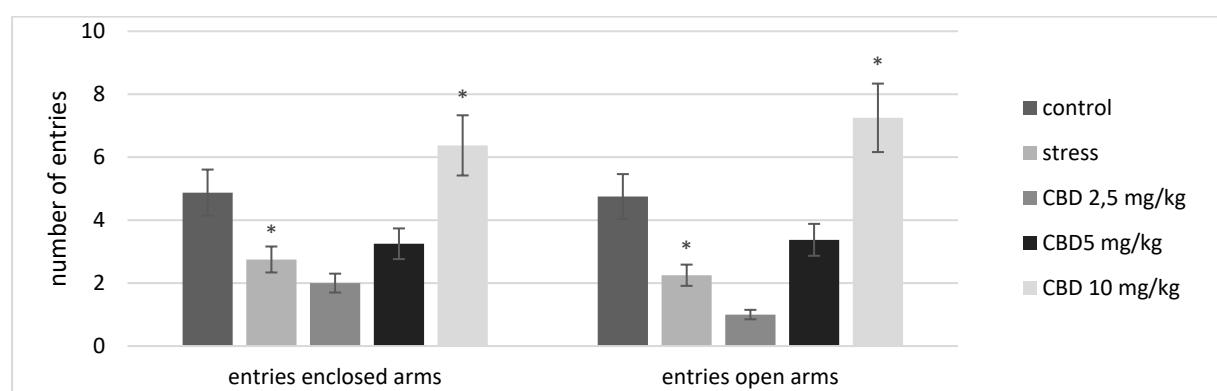


Figure 2. Effect of CBD on the number of entries in open and enclosed arms in the elevated plus maze test.

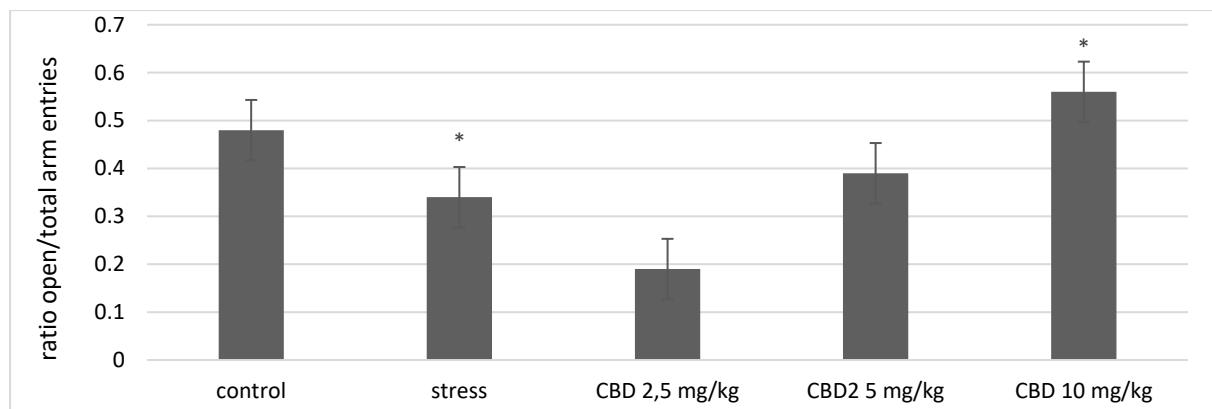


Figure 3. Effect of CBD on the ratio open/total arms in the elevated plus maze test.

Discussion

CBD has been studied in a broad range of animal models of general anxiety, including the elevated plus maze (EPM), the Vogel-conflict test (VCT) and the elevated T maze (ETM) [5]. The elevated plus maze test is based on innate fear of open and elevated spaces and has been usually associated with general anxiety in humans. Increase in time spent and number of entries in open arms can be reported as a manifestation of anxiolytic effect. The results from our study suggest that CBD exerts anxiolytic effects at dose 10 mg/kg bw. Our results confirm the initial findings that low (10 mg/kg) doses of CBD were anxiolytic, while high (100 mg/kg) doses were ineffective [5, 11].

Anxiolytic effects of CBD in models of generalized anxiety have been related to interaction with several receptors and brain regions. The midbrain dorsal periaqueductal gray (DPAG) is integral to anxiety, responsible for autonomic and behavioral responses to threat. DPAG stimulation in humans causes feelings of intense distress. Microinjection of CBD into the DPAG resulted in anxiolytic effects in the EPM, VGC, and ETM that were partially mediated by activation of 5-HT1A receptors [5, 12]. In a chronic study, systemic cannabidiol application prevented increased anxiety produced by chronic unpredictable stress and

additionally increased hippocampal AEA. Campos et al. demonstrated that the anxiolytic effect of CBD in stressed mice is depended upon increased hippocampal neurogenesis by facilitating eCB system [13]. The proneurogenic effect of CBD in the hippocampus was absent in CB1-knockout mice [11]. Fogata et al. reported that repeated administration of CBD could prevent anxiety behaviors caused by chronic stress exposure in mice and furthermore prevented the stress-induced depletion in hippocampal neurogenesis, dendritic remodeling and the expression of synaptic proteins in the hippocampus [14].

Conclusion

The results from our study suggest that cannabidiol exerts anxiolytic effect in an experimental model of acute stress. Data from existing preclinical experiments and our study supports the potential of CBD as a treatment for anxiety disorders.

Acknowledgement

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Management and patient education for gastroesophageal reflux disease

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Introduction: Gastroesophageal reflux disease is a common gastrointestinal diagnosis, a leading reason for endoscopy and cause of potentially serious complications, resulting in significant individual and system-wide health burden and a reason for OTC medication use, also decreases life quality of patients. Pharmacists play an important role in the management of GERD by helping patients to verify their diagnosis, referring them to a doctor for the assessment of alarming symptoms and recommending treatment including medication, diet, and lifestyle advices. **Aim** of this study is to describe the management of GERD patients from the perspective of pharmacists. **Methods:** The literature on the topic was reviewed including the biggest databases and gastroenterological guidelines. **Results:** Pharmacists can contribute to the management of GERD patients, by providing relevant medical information, specific advices for lifestyle modifications and personalized treatment options that take into account the severity and the type of GERD that the patient experiences.

Introduction

Gastroesophageal reflux disease is a common diagnosis and a reason for OTC medication use. This condition results in complications that decrease the life quality of patients. A significant increase in the rate of younger patients with GERD, especially those within the age range of 30–39 years, has been identified.¹ The responsibilities of pharmacists in the management of GERD includes help in diagnosis verification, doctor referral when alarming symptoms are present, recommendation of treatment approach including medication, diet and lifestyle modifications.²

Aim

This study aims to describe the management of GERD patients from the perspective of pharmacists.

Methods

The literature on the topic was reviewed including the biggest databases and gastroenterology guidelines.

Results

GERD is a widespread gastrointestinal diagnosis. A systematic review from 2020 estimates approximately 1.03 billion individuals suffering from the condition globally.³ An increase in time off work and a decrease in work productivity was indicated in patients with disruptive GERD (daily or weekly symptoms), in a recent systematic review on the burden of GERD on quality of life. Low scores on sleep scales were seen compared with patients with less frequent symptoms.⁴

The etiology of GERD is not clearly defined, although multiple risk factors are suggested.

Table 1. Risk factors for reflux symptoms

Risk factors	Description
Hiatus hernia	Hiatus hernia is likely to be caused by the weakening or rupture of the pharyngoesophageal ligament. Patients with a hiatus hernia have more episodes of reflux and more severe reflux esophagitis. ²
Obesity	Obesity is a strong risk factor for gastroesophageal reflux, although the value of weight reduction remains to be proven. ^{5,6}
Tobacco smoking	There was a significant dose-response association between tobacco smoking and reflux symptoms. ⁷
Alcohol	Exposure of the esophagus and stomach to alcohol may cause direct damage to esophageal and gastric mucosae. Also, toxic acetaldehyde metabolized from alcohol could affect the function of the esophagus and stomach. ⁸
Helicobacter pillory infection	Relying upon several population studies is noticeable an inverse relationship between <i>H. pylori</i> and GERD. ⁹
Pregnancy	The prevalence of gastroesophageal reflux disease (GERD) symptoms in pregnancy is very high, up to 80%, with a maximum peak during the third trimester. ¹⁰
Genetics	Twin and family studies have revealed about 31% heritability of the disease. Numerous single-nucleotide polymorphisms in various genes like <i>FOXF1</i> , <i>MHC</i> , <i>CCND1</i> , an anti-inflammatory cytokine, and DNA repair genes have been strongly associated with increased GERD risk. ¹¹
Medications	Several medications can cause GERD symptoms and/or esophageal injury including Nonsteroidal antiinflammatory drugs, antibiotics (eg, tetracyclines and clindamycin), statins, angiotensin-converting enzyme inhibitors, bisphosphonates, vitamin C, potassium, iron, clomipramine, quinidine, anticholinergics, tricyclic antidepressants, corticosteroids (oral and inhaled), β-agonists, nitroglycerines, aminophylline, benzodiazepines, warfarin, cyproterone, ethynodiol, and calcium channel blockers. ^{2,12}
Food	Specific foods can irritate the stomach mucous and worsen the condition of GERD patients.

GERD symptoms

GERD is characterized by pain in the chest area. The presence of heartburn (reflux) and regurgitation is characteristic of most cases of GERD; however, there is significant variability across studies.² Domination of either heartburn or regurgitation is sufficient for a GERD diagnosis.¹³

There are two main types of GERD – erosive reflux disease and non-erosive reflux disease, erosion is found during endoscopy in 25% of the patients. There is no gold standard for diagnosing GERD, although 24-hour pH monitoring (pH probe) is the accepted standard for establishing or excluding its presence.^{13,14} In most cases patients do not experience troublesome

symptoms and endoscopy is not performed, so patients are diagnosed with GERD only symptomatically. The number of patients that search for relief of reflux in the pharmacy is increasing. To help patients with GERD in finding the appropriate OTC treatment pharmacists shall use therapeutic algorithms.

First, the pharmacist should confirm the diagnosis based on the presence of specific symptoms and secondly, suggest a general practitioner referral. The third step is the choice of suitable treatment. The pharmacists should focus on the nature, severity, and frequency of the symptoms-patients with less than three episodes of heartburn and/or acid regurgitation per week might

have the highest benefit from a shortcourse (14 days) of OTC PPIs. Patients who have three or more episodes per week can use the OTC PPIs but should also be encouraged to visit a physician, and those who already have a diagnostic workup can use proton pump inhibitors as rescue treatment if they are known, responders.¹⁵

Questions that should be used by the pharmacists to confirm the diagnose and assure patient's safe and effective treatment

include questions about the age, symptoms, family history, comorbidity, and previous treatment (table 2).

Symptomssuggestive of cardiac-type chest pain, difficulty in or painful swallowing, recurrent bronchial symptoms/cough, hoarseness, signs/symptoms of gastrointestinal bleeding, and progressive unintentional weight loss are alarm features that could be identified in the pharmacy based on the patient's responses.¹⁵

Table 2. Questionnaire for assessment of GERD patients in a pharmacy.

GERD attribute	Question	Key answers
Patient age	What is the age of the patient?	The first appearance of GERD in older people (> 65) is concerning and requires a doctor's referral
Symptoms	What symptoms are you experiencing? Can you show me where the burning occurs? What worsens your symptoms? How severe are your symptoms?	Burning sensation, beginning in the middle of the abdomen and rising toward the throat Rising of food to the throat/ mouth Large meal; Fatty meal; Stooping/ bending Mild/severe
Alarming symptoms	Are you experiencing any other symptoms? (The pharmacists might need to list some of these symptoms as the patient may not associate them with GERD)	Darkened bowel motions, vomiting blood, Crushing chest pain, Frequent anemia, Diagnosed/ suspected anemia, weight loss, difficulty swallowing, severe abdominal pain, exercise-related symptoms, feeling full after a small meal
History	How long have you had these symptoms? How often do you experience these symptoms?	Days/ Months/ Weeks/ Years < 3 times per week > 3 times per week Daily
Comorbidity	Do you have a history of any other health conditions?	Gastric ulcer, Cancer,
Family history	Have any of your parents or siblings experienced such symptoms? What were their diagnoses?	Yes/No GERD, Reflux, Gastrointestinal cancer
Other medicines taken	Do you take any prescription, over-the-counter medicines?	Nonsteroidal anti-inflammatory drugs, antibiotics (eg, tetracyclines and clindamycin), statins, angiotensin-converting enzyme inhibitors, bisphosphonates, vitamin C, potassium, iron, clomipramine, quinidine, anticholinergics, tricyclic antidepressants, etc.
Previous treatment	What treatment have you already tried?	Antacids/ alginates Histamine – H2-receptor antagonists Proton-pump inhibitors

Medical therapy

Antacids and alginate

The mechanism of action of those medicines is related to a decrease in the acidity of the gastric contents. Alginate-based therapies increased the odds of resolution of GERD symptoms when compared to placebo or antacids.¹⁶ Alginate is a bioadhesive allowing it to adhere to the esophageal mucosa and protect it from attack by acidic refluxate. Alginate adhesion lasted up to 1 hour after application.¹⁷

Prokinetic agents

Prokinetics and reflux inhibitors have the potential to control motor abnormalities, but the results of clinical trials are inconsistent. Specific subgroups of GERD patients with visceral hypersensitivity might benefit from antidepressant therapy, but this is to be proved by controlled clinical studies.¹⁸

Risks and benefits of long-term PPI therapy

Effective self-management of gastoesophageal reflux disease with OTC

PPIs could lead to lasting freedom from symptoms and improved quality of life for sufferers.¹⁹ Provided that most PPIs are available as OTC treatment, they are considered safe options for GERD relief. However, they are not a definitive treatment. The mechanism of action of those medicines differs from the actual causes of GERD. Current drugs used for treating GERD are not able to cure the disease, which is substantially due to multiple motor alterations.¹⁹ Negative effects like kidney disease, dementia, fractures, vitamin/mineral deficiencies have been associated with the long-term use of PPIs. However, more evidence supporting these findings is required.¹⁹ Concomitant use of a PPI with clopidogrel should be discouraged due to a reduction of the effectiveness of clopidogrel that may lead to cardiovascular complications.²⁰

Patient education

Advice from pharmacists about over-the-counter treatment for intermittent symptoms of GERD are included in (figure 1).¹⁴

Advise on diet and lifestyle adjustment

PPIs may take up to 3 days to reach their desired effect, with a maximum effect after 5 days

Take PPIs 1 hour before the largest meal (this allows the short half-life drug maximum time to inhibit the active proton pump)

Take acid suppressive therapy consistently at the same time each day, when symptoms are worse (ie, morning or evening)

PPIs are generally well tolerated (common [1%–10%] adverse reactions include diarrhea, headache, nausea, abdominal pain, and constipation)

Take over-the-counter PPIs for a maximum of 14 days

PPIs may not be effective in all people with GERD symptoms

Figure 1. Pharmacists' advice on OTC treatment of GERD.

Pharmacists and patients bring to education and counseling sessions their perceptions of their roles and responsibilities. For the experience to be effective, the pharmacist and the patient need to come to a common understanding about their respective roles and responsibilities. It may be necessary to clarify for patients that pharmacists have an appropriate and important role in providing education and counseling. Patients should be encouraged to be active participants.²¹ (fig. 2)

Patient's role is to adhere to their pharmacotherapeutic regimens, monitor for drug effects, and report their experiences to pharmacists or other members of their health care teams. Optimally, the patient's role should include seeking information and presenting concerns that may make adherence difficult.²²

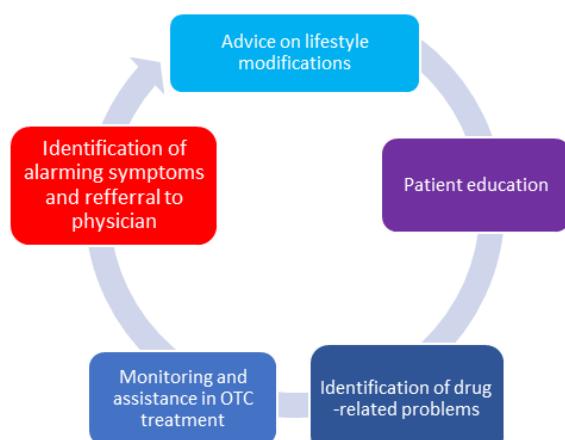


Figure 2. GERD patients counseling process in a pharmacy.

Conclusion

Despite the high prevalence and the numerous treatment options, GERD is a condition that seriously decreases the quality of life of patients. Pharmacists are seen as professionals that can improve the medical care for GERD patients.

Pharmacists can assist patients with accurate self-diagnosis by asking short, simple questions to characterize the nature, se-

verity, and frequency of symptoms. The pharmacist's role is to verify that patients have sufficient understanding, knowledge, and skill to follow their pharmacotherapeutic regimens and monitoring plans. Pharmacists should also seek ways to motivate patients to learn about their treatment and to be active partners in their care. Depending on the health system's policies and procedures, its use of protocols or clinical care plans, and its credentialing of providers, pharmacists may also have disease management roles and responsibilities for specified categories of patients. This expands pharmacists' relationships with patients and the content of education and counseling sessions.

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Anti-inflammatory effect of Satureja montana dry extract

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Introduction: Inflammation and especially low-grade chronic inflammation is associated not only with social diseases like cancer and atherosclerosis but also with many neurological disorders and psychiatric conditions. **Aim:** Our aim was to investigate the anti-inflammatory effect of Satureja montana dry extract after fourteen-day application. **Material and methods:** We used 56 male Wistar rats, divided into 7 groups ($n = 8$), treated with saline, olive oil (negative controls), Indomethacin 25 mg/kg b.w (positive control), dry extract of *S. montana* in doses of 250 mg/kg b.w. and 500 mg/kg b.w, rosmarinic acid – 15 mg/kg b.w., carvacrol – 500 mg/kg b.w. The inflammation was induced by injecting carrageenan into the right hind paw. For investigating the anti-inflammatory activity, we used plethysmometer (Ugo Basile, Italy) before the carrageenan injection and on the 2nd, 3rd, 4th and 24th hour afterwards. **Results:** Compared to saline the dry extract of *Satureja montana* in both doses showed significant anti-inflammatory effect on the 2nd, 3rd and 4th hour. Only the dose of 250 mg/kg b.w as well as the rosmarinic acid reduced significantly the inflammation on the 24th hour. Compared to olive oil carvacrol showed significant anti-inflammatory effect on the 3rd, 4th and 24th hour. None of the tested substances except the dry extract of *Satureja montana* 250 mg/kg b.w. showed anti-inflammatory effect on the 3rd and 4th hour compared to Indomethacin 25 mg/kg b.w., used as positive control. **Conclusion:** Both doses of extract of *S. montana* have anti-inflammatory effect compared to saline after 14-day application, but only the lower dose is comparable to the effect of Indomethacin.

Introduction

There are increasing number of evidences, describing the association between stress and chronic low grade inflammation. Many researchers hypothesize that this uncontrolled inflammation has an important role in the pathogenesis of many chronic conditions like some cardiovascular diseases – hypertension and atherosclerosis, chronic osteoarthritis, cancer, autoimmune disorders, Parkinson's and Alzheimer disease, depression (1–5). This hypothesis underlines possible new target into the control and treatment of the social diseases listed above.

The treatment of chronic low-grade inflammation includes non-pharmacological – mainly changes in the lifestyle and pharmacological methods – some supplements like vitamin D and omega 3 acids, probiotics, angiotensin II receptor blockers (ARBs), metformin. (6, 7) In the recent years the immunomodulatory activity of essential oils and herbal extracts as well as their active ingredients are goal of scientific researches

in the development of new natural anti-inflammatory drugs due to their low toxicity. (8, 9)

Aim

The aim of our study was to investigate the anti-inflammatory effect of *Satureja montana* dry extract after fourteen-day application on rats.

Material and methods

We used 56 male Wistar rats, divided into 7 groups ($n = 8$), treated orally for 14 days with *Satureja montana* in two doses – 250 mg/kg b.w and 500 mg/kg b.w, rosmarinic acid 15 mg/kg b.w and carvacrol 500 mg/kg b.w. Negative control groups were treated orally with saline and olive oil in dose 1 ml for every 100 g b.w. For positive control was used Indomethacin at a dose of 25 mg/kg b.w. The inflammation was induced by injecting 0.1 ml carrageenan into the right hind paw. For investigating the anti-inflammatory activity, we used plethysmometer (Ugo Basile, Italy).

Plethysmometer test. We used ple-

plethysmometer apparatus (Ugo Basile, Italy). It measures the changes in the rodent paw volume by the amount of the displaced water. First measurement is immediately before the carrageenan injection, followed by measurements on the 2nd, 3rd, 4th and 24th hour afterwards. Indicator for the anti-inflammatory activity is the reduced paw edema, compared to negative controls.

Statistical analysis. Statistical data processing was performed with software product IBM SPSS 20.0. A Kolmogorov-Smirnov test was performed to determine the distribution. An arithmetic mean (mean) and a standard error of the arithmetic mean (\pm SEM) were determined for each indicator. Comparison of the results between the groups was performed with Independent Sample T test. Results at significance level $p < 0.05$ were considered statistically significant.

Results

The plethysmometry after inducing paw inflammation with carrageenan showed significant anti-inflammatory effect of the dry extract of *S. montana* 250 mg/kg b.w compared to saline in all measurements.

(Fig. 1.) Compared to Indomethacin the lower dose dry extract reduced paw edema significantly on the 3rd and 4th hour after carrageenan injection – $0.85 \pm 0.11 \text{ cm}^3$ and $0.79 \pm 0.009 \text{ cm}^3$ for *S. montana* and $1.06 \pm 0.03 \text{ cm}^3$ and $0.96 \pm 0.07 \text{ cm}^3$ for Indomethacin respectively. (Fig. 2.) The higher dose of 500 mg/kg b.w had significant anti-inflammatory effect compared only to the negative control group on the 2nd, 3rd and 4th hour after inducing of the inflammation with paw volume $1.11 \pm 0.10 \text{ cm}^3$, $1.17 \pm 0.18 \text{ cm}^3$ and $1.25 \pm 0.13 \text{ cm}^3$ for saline and $0.99 \pm 0.004 \text{ cm}^3$, $0.97 \pm 0.17 \text{ cm}^3$ and $0.87 \pm 0.13 \text{ cm}^3$ for *S. monatana*. (Fig. 1.)

The paw edema in the group, treated with rosmarinic acid on the 24th hour was $1.01 \pm 0.05 \text{ cm}^3$, which was significantly reduced compared to saline – $1.19 \pm 0.16 \text{ cm}^3$. (Fig. 1.)

Compared to olive oil carvacrol showed significant anti-inflammatory effect on the 3rd, 4th and 24th hour with values of paw volume from $1.24 \pm 0.12 \text{ cm}^3$, $1.20 \pm 0.14 \text{ cm}^3$ and $1.12 \pm 0.08 \text{ cm}^3$ in the negative control group and $1.02 \pm 0.14 \text{ cm}^3$, $1.03 \pm 0.14 \text{ cm}^3$ and $1.01 \pm 0.12 \text{ cm}^3$ in the carvacrol group respectively. (Fig. 3)

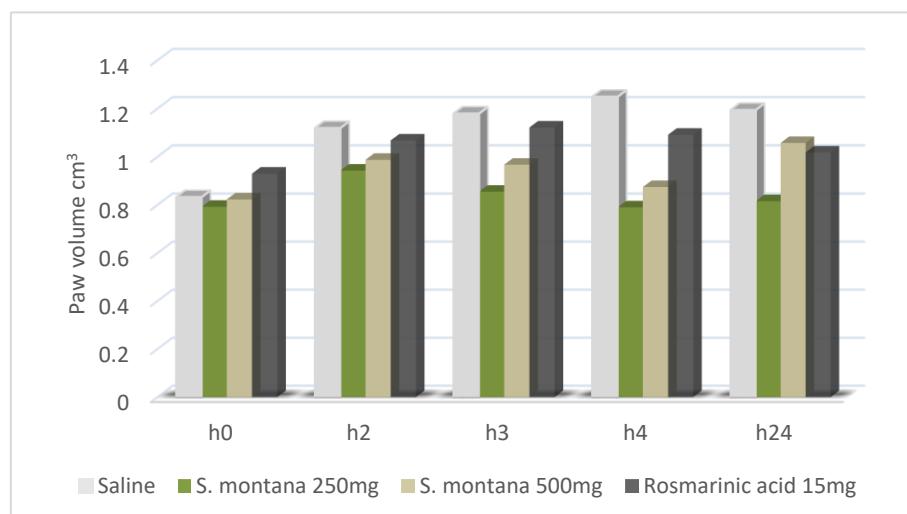


Fig. 1. Antinflammatory effect of dry extract *S. montana* and at doses of 250 mg/kg b.w. and 500 mg/kg b.w. and rosmarinic acid at doses of 15 mg/kg b.w. compared to saline.

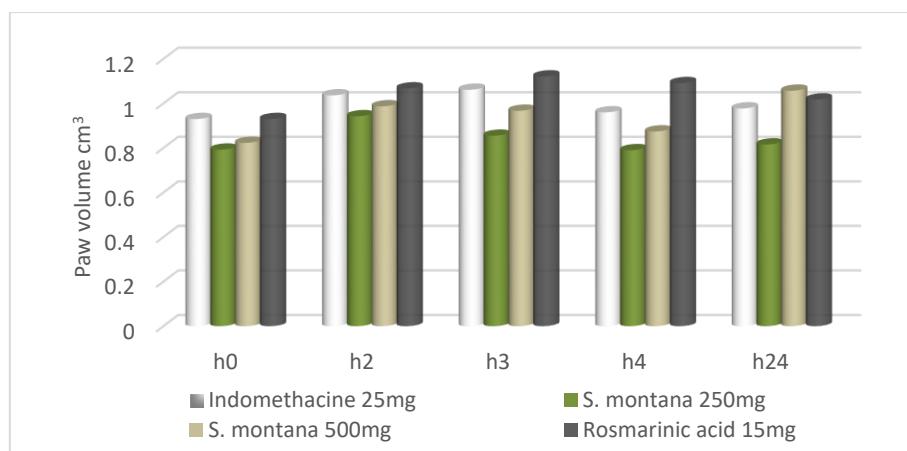


Fig. 2. Antiinflammatory effect of dry extract *S. montana* at doses of 250 mg/kg b.w. and 500 mg/kg b.w. and rosmarinic acid at a dose of 15 mg/kg b.w. compared to Indomethacin.

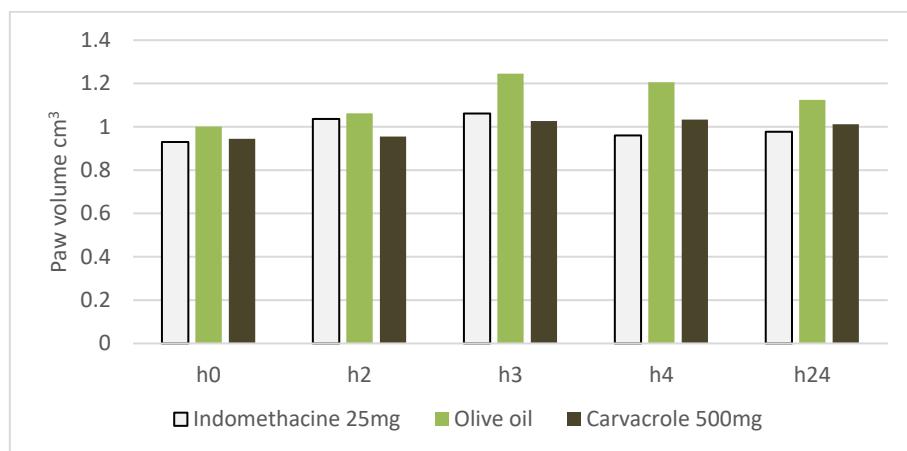


Fig. 3. Anti-inflammatory effect of carvacrol at a dose of 500 mg/kg b.w. compared to saline and Indomethacine.

Discussion

The carrageenan induced inflammation is mediated by a complex of proinflammatory mediators – histamine, 5 hydroxytryptamine in the first phase and bradykinine in the second phase. Prostaglandins, synthesized by COX2, play key role in the last phase of the carrageenan irritation. The increase of the paw volume after carrageenan injection is a marker for the inflammatory response. The quantification of the paw edema is usually evaluated by plethysmometer. (9) The presumed mechanism of action of new molecules and phytoextracts with supposed anti-inflammatory activity is to affect the levels of prostaglandins as well as COX expression and activity.

Despite that other species in the genus *Satureja* except for *S. montana* are known for their anti-inflammatory activity, such pharmacological effect is possible due to the chemical composition of the extract. The alcoholic extracts from *S. montana* contain in significant amount phenolic acids like rosmarinic, caffeic, sinapic, ferulic. (10, 11) The anti-inflammatory effect of rosmarinic acid has been established in many in vitro and in vivo studies. (12) The findings of Rocha J et al. that also show significant decrease of the paw volume in a model of carrageenan-induced paw edema correspond to the results from our study. (13)

Carvacrol is phenolic monoterpene with

many pharmacological effects. In other studies, it shows significant anti-inflammatory effect in different models of inflammation. The exact mechanism of action is not fully understood. (14) The most supposed mechanism is by affecting the synthesis and the release of proinflammatory mediators. (15) Carvacrol is described to reduce the levels of prostaglandins as well as expression of cyclooxygenase 2 (COX2), which are playing a key role in the formation of paw edema. (14, 15).

Conclusion

The dry extract of *Satureja montana* at doses of 250 mg/kg b.w and 500 mg/kg b.w has significant anti-inflammatory effect compared to saline after 14-day application. Rosmarinic acid, one of the active ingredients of the extract, also reduces the paw edema significantly compared only to saline. Carvacrol, a carrier of pharmacological activity of the essential oil of *S. montana*, reduces paw edema significantly compared to negative control. Only the lower dose of the dry extract of *S. montana* is comparable to the effect of Indomethacin, used as positive control. Further research is needed to establish the exact mechanism of anti-inflammatory activity of *S. montana*.

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Development and validation of a simple thin-layer chromatography method for identification of diclofenac sodium and aceclofenac

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Introduction: Diclofenac sodium and aceclofenac are non-steroidal anti-inflammatory drugs with anti-inflammatory and analgesic properties. Nowadays these substances are active compounds of many important medicines. **Aim:** The aim of the study is to develop a simple, sensitive and rapid thin layer chromatography (TLC) method for identification of diclofenac sodium and aceclofenac. **Materials and methods:** Method for identification – TLC. The separation was carried out on Merck aluminium plates precoated with silica gel 60 F254, using mobile phase ammonium: 1-butanol (16:84 v/v). Standard solution preparation and sample solution preparation: solvent ethanol. All reagents have analytical grade purity. The evaluation of the separated bands was carried out at 254 nm. **Results:** We have developed a simple, sensitive and cost effective method. The method was validated according the guidelines of ICH. The proposed method has been successfully applied to the analysis of diclofenac sodium and aceclofenac in pharmaceutical products. **Conclusion:** Compared to some previously reported techniques for identification of diclofenac and aceclofenac this method is simple, sensitive and cost-effective.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are important and commonly prescribed medicines for the treatment of inflammatory and rheumatoid disorders [1]. Diclofenac sodium (fig. 1) and aceclofenac (fig. 2) are non-steroidal anti-inflammatory drugs [2–4] with anti-inflammatory and analgesic properties [1].

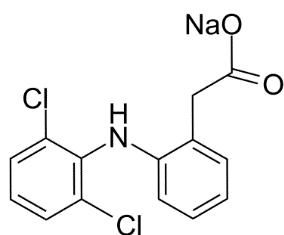


Figure 1. Chemical structure of diclofenac sodium.

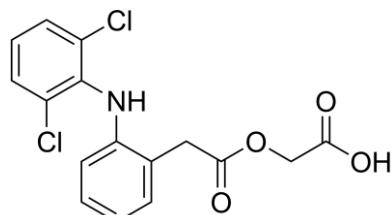


Figure 2. Chemical structure of aceclofenac.

Nowadays these substances are active compounds of many important medicines, widely prescribed for the treatment of musculoskeletal disorders and post-surgery analgesia [1–5].

Diclofenac sodium or sodium 2-[(2,6-dichlorophenyl)amino]phenyl]acetate is white or slightly yellowish, slightly hygroscopic, crystalline powder. It is sparingly soluble in water, freely soluble in methanol, soluble in ethanol and slightly soluble in acetone. Its melting point is about 280°C [6].

For the identification of diclofenac sodium European Pharmacopeia recommends: Infrared absorption spectrophotometry, Thin-layer chromatography (mobile phase-concentrated ammonia R, methanol R, ethyl acetate R (10:10:80 V/V/V); standart solution / sample preparation by dilution with methanol; detection – UV light at 254 nm), reaction of sodium, a specific colour reaction. Related substances for diclofenac sodium should be analysed by liquid chromatography.

Many other approaches for diclofenac sodium quality control are described in scientific data bases: gas chromatography (GC) with mass spectrometry (MS) detection [5], TLC [8,9,11], HPLC [7, 12,13,14], UV-VIS spectrophotometry [10].

According to European Pharmacopeia diclofenac sodium is not only a drug substance but it is also a specified impurity for other drug substance: aceclofenac (fig. 2). According Pharmacopeial quality standards for active substances impurity A (diclofenac) should be not more 0.2 per cent in the total content of aceclofenac. For detection of the related substances of aceclofenac (total 9) EP recommends liquid chromatography [6].

Aceclofenac [[[2-[(2,6-Dichlorophenyl) amino]phenyl]acetyl]oxy]acetic acid has similar physical properties to diclofenac. It is a white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent) [6]. For its identification European Pharmacopeia recommends: Infrared absorption spectrophotometry, measurement of specific absorbance and a colour reaction. No TLC method for identification of aceclofenac is described in the pharmacopeial monograph. Chemically both diclofenac sodium and aceclofenac are acetic acid derivatives and have similar structures and similar physical properties. These similarities facilitate the simultaneous identification of diclofenac sodium and aceclofenac.

Aim

The aim of the study is to develop a simple and rapid and sensitive thin layer chromatography (TLC) method for identification of diclofenac sodium and aceclofenac.

Materials and methods

I. Materials

Reagents:

Reagents of analytical grade purity: ammonium, 1-butanol, ethanol.

Standarts:

Diclofenac sodium salt – Sigma-Aldrich certified reference material.

Aceclofenac – Sigma-Aldrich certified reference material.

II. Method: TLC

1. Instrumentation:

Dark room with UV lamp and scanner – Vilber Lourmat, CN-15.LC.

Sample applicator – 5 µl Micro syringe (Hamilton)

Aluminium TLC plates were pre-coated with silica gel 60 F₂₅₄, dimensions 20 x 20 cm (Sigma Aldrich).

TLC chamber (22 cm x 12 cm x 22 cm).

2. Preparation of standard solutions of diclofenac sodium.

Preparation of stock solution – an accurately weighed quantity of reference standard of diclofenac sodium equivalent to 200 mg was dissolved into a 10 mL 99.98% ethanol to obtain a solution with concentration 20 mg/ml. Working standard solutions were prepared by dilution of stock solution with ethanol. Concentration range of working standard solution was 2000 mkg/ml – 2,5 mkg/ml.

3. Chromatographic conditions: The method was developed using a mobile phase prepared with analytical grade solvents: ammonium:1-butanol (16:84 v/v). The plate was developed about 40 min at 25 ± 1 C in ascending vertical manner in TLC glass chamber (22 cm x 12 cm x 22 cm), previously presaturated for 1 hour with mobile phase. Front – 6 cm. The developed plates were dried on air.

Results and discussion

Thin layer chromatography (TLC) is a separation technique used mainly for identification of compounds or determination of the presence of some impurities and related compounds. Although many new and more analytical approaches have been introduced TLC is still an important technique for the pharmaceutical analysis. A huge array of TLC-based tests for identification is described in European pharmacopeia.

Some of the strengths of this approach are: price (it is cost-effective), simple sample preparation, robustness and easy performance.

Our method was developed using a mobile phase prepared with analytical grade solvents: ammonium: 1-butanol (16:84 v/v). The chromatographic analysis was carried out using aluminium TLC plates, silica gel coated with fluorescent indicator F254 (Silica gel 60 F254 plates).

An accurately weighed quantity of reference standard of diclofenac sodium equivalent to 200 mg was dissolved into a 10 mL 99.98% ethanol to obtain a solution with concentration 20 mg/mL. An accurately weighed quantity of reference standard of aceclofenac equivalent to 200 mg was dissolved into a 10 mL 99.98% ethanol to obtain a solution with concentration 20 mg/mL. Concentration range of working standart solutions: 2000 mkg/mL – 2,5 mkg/mL.

From all solutions 5 µl aliquot parts were spotted onto silica gel plates keeping 10 mm distance between bands. The optimum chamber saturation time for the mobile phase was 40 min at room temperature. The plates were developed up to 6 cm. After the development the plates were dried at room temperature. The scanning was performed in the absorbance mode at 254 nm.

The proposed method allows simultaneous identification of diclofenac sodium and aceclofenac in very low concentrations. The method was applied for identification of diclofenac sodium and aceclofenac in medicinal products.

Method Validation: The method was validated according to the guidelines of ICH [15,16].

Specificity: The results obtained during method development showed that the method was specific for the assay and selective, there were no interferences from the excipients or solvents. A placebo solution was prepared. On the chromatogram with placebo wasn't observed a spot with R_f, corresponding to R_f of diclofenac sodium and aceclofenac. The method was found to be acceptable to ensure the identity of diclofenac sodium and aceclofenac. The R_f values are presented in table 1.

Table 1. R_f values of aceclofenac and diclofenac sodium

Substance	R _f value
aceclofenac	0,33
diclofenac sodium	0,45

Method robustness: The robustness of the assay method was tested by varying the mobile phase solvent composition ratio +/-2% and the saturation times that were established during method development against critical parameter: R_f. The changes in the R_f were +/-0.02 which showed that the method was robust.

Detection limit (DL): DL represents the lowest amount of an analyte in a sample which can be reliably detected. For determining the DL we have used the based on visual evaluation approach. We have established the following results: aceclofenac – DL = 25 mkg/ml, diclofenac – DL = 5 mkg/ml.

Conclusion

A simple, selective, robust and inexpensive TLC technique was developed for simultaneous identification of diclofenac sodium and aceclofenac. Compared to some previously reported techniques for identification the method is simple, sensitive and cost-effective. It could be applied as an identification test for diclofenac sodium or aceclofenac.

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IV. Surgical session

Therapy of pathological cicatrices and keloids using high-intensity focused ultrasound (HIFU)

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Pathological scars and keloids can form during wound healing when imbalance of anabolic and catabolic processes occurs. The proliferation of fibrous tissue comprises an abnormal response to healing. Both hypertrophic scars and keloids result from this overzealous inflammatory response. Pathological scars are common after surgery, trauma or significant burns. Effective treatment of keloid scars is important because patients are often confronted with major aesthetic, psychological, and physiological consequences. The selection of patients and the treatment were performed in the Clinic of Plastic, Reconstructive and Aesthetic Surgery, University Hospital “St. George”, MU Plovdiv. More than 50 patients underwent thermotherapy with a different number of procedures. All patients showed a decrease in the amount of scar tissue, scar volume and its side effects. A modern treatment method for pathological scars is High-Intensity Focused Ultrasound (HIFU). HIFU is non-invasive method for treating pathological cicatrices. The therapy improves the characteristics of the skin. HIFU works at different depths and reaches high temperatures targeting the deep layers of the skin. Anticatarrhal unguents, silicone plaques and corticosteroids are used as adjuvant therapy. They are integrated into the scar tissue using thermotherapy. The transformation of connective tissue into gelatin occurs upon reaching temperature of 56°C. This reduces the volume of the scar tissue, discomfort, pain and itching. Evaluation of the performed therapy can be conducted with an ultrasound examination, which shows a decrease in the volume of the altered tissue. HIFU is an innovative and effective method for diagnosis and treatment of pathological cicatrices and keloids.

Introduction

The skin is composed of three layers: the epidermis, the dermis, and the hypodermis. When the integrity of the skin is compromised a process of wound healing is activated. Wound healing is a complex process that begins immediately following tissue injury and includes the phases of inflammation, proliferation, and remodeling. (1)

Scars form when an imbalance of anabolic and catabolic processes occurs. Cicatrices are usually classified into atrophic scars, hypertrophic scars, or keloids.

Atrophic scars are a result of a lack of collagen, usually caused by a destructive process. Keloids and hypertrophic scars are caused by trauma, insect bites, severe burns, surgery procedures, vaccination, skin

piercing, acne, folliculitis, chickenpox, and herpes zoster infection. Superficial injuries that do not reach the reticular dermis never cause keloidal and hypertrophic scarring. (2)

A new and innovative method for treating tissue defects is high-intensity focused ultrasound (HIFU). HIFU gained popularity at the end of the 20th century. This treatment method uses a concentrated beam of ultrasound waves that are generated by a focused acoustic transducer. Using HIFU, we are targeting a specific point in space, called focal point.

Depending on the power and pulse duration settings of the treatment, we could achieve effects through mild localized overheating of the focal zone to full coagu-

lative necrosis and irreversible cell death. (3)

Purpose

We aimed to establish the effectiveness of HIFU for treating pathological scars. We are of the opinion that HIFU could be used not only for aesthetic purposes but also for reducing the accompanying symptoms of the scars.

Tasks

Our main objectives are using HIFU as a treatment method for scarring and analysis of the results using ultrasound scans.

Materials and methods

For one year 53 patients participated in our study at the University Hospital "St George". The study is conducted in the Department of Plastic, Reconstructive and Aesthetic surgery, Medical University of Plovdiv, together with the Department of Diagnostic Imaging, Medical University of Plovdiv.

The procedures were performed on an outpatient basis after photographing and ultrasound measurement of the scar. Thermotherapy (above 56°C) was performed locally after the application of ultrasound gel and anti-cicatricial unguents. The thermotherapy is performed at different depths, depending on the location and thickness of the scar. Between 15 and 30 pulses are applied to each area. Most of the patients underwent 2 procedures – 41, 7 people had 3 procedures, and 5 of the patients had 4 procedures.

Results and discussion

The results were evaluated by taking photographs of the patients and performing an ultrasound scan before and after the procedures with HIFU. (fig. 1)

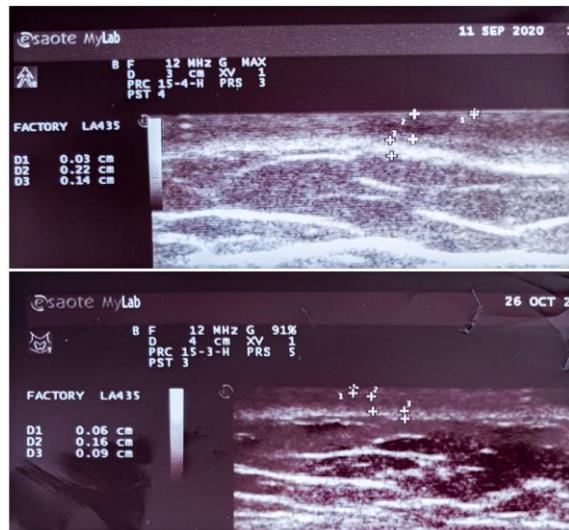


Figure 1. Ultrasound measurement before and after HIFU treatment.

A decrease of the thickness of the D2 – the dermal layer of the skin, rearrangement of the collagen fibers, is detected. As a result, after the procedures, we observe a substantial decrease in the pathological tissue.

In all patients, the scar maturation is completed before the treatment with HIFU. After the procedures, there is a significant softening of the scar and evidently smoother and less pigmented skin (fig. 2).

Apart from the high-intensity focused ultrasound, also adjuvant therapy was applied. Patients were given silicone gel sheets, corticosteroids, and anti-cicatricial unguents.

It is important to be noticed that compared to hypertrophic cicatrices, keloids extend beyond the original borders of the damaged tissue. They can be associated with abnormalities such as pain, pruritus, or burning sensation. The most common locations for keloids are the earlobes, anterior chest wall, shoulders, and upper back. (4) They are not known to spontaneously regress and have high recurrence rates with most therapies. A complete resolution of a keloid scar is rare.

In contrast to keloid scars, hypertrophic scars do not extend beyond the original cutaneous injury's boundaries. They tend to be raised above the surrounding cutaneous topography and firm in consistency. (5)

Studying the histopathology of keloids and hypertrophic scars, keloids have a hap-

hazard array, tongue-like edges. In addition, keloidal collagen is found, horizontal fascicles and inflammation in the papillary dermis are present. On the contrary, in the hypertrophic cicatrices little or no keloidal collagen is found, vertical vessels and vertical fascicles can be examined. (fig. 3, 4)



Figure 2. 4-year-old child with post-burn scarring before and after HIFU-treatment.

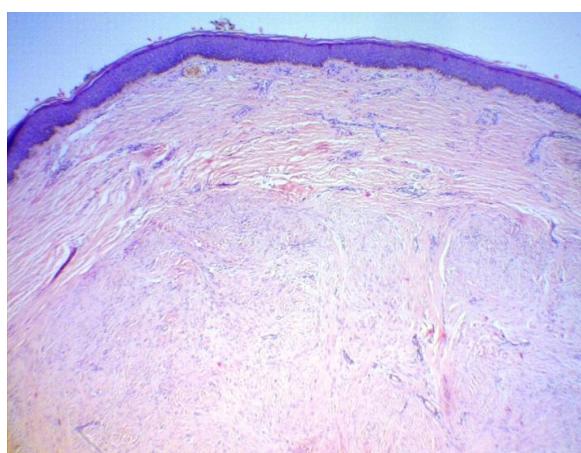


Figure 3. Histopathology of a hypertrophic.

(Анастасова В., Хирургично поведение при пациенти с последствия от изгаряния, дисертационен труд за присъждане на ОНС „Доктор“, МУ – Пловдив 2017)

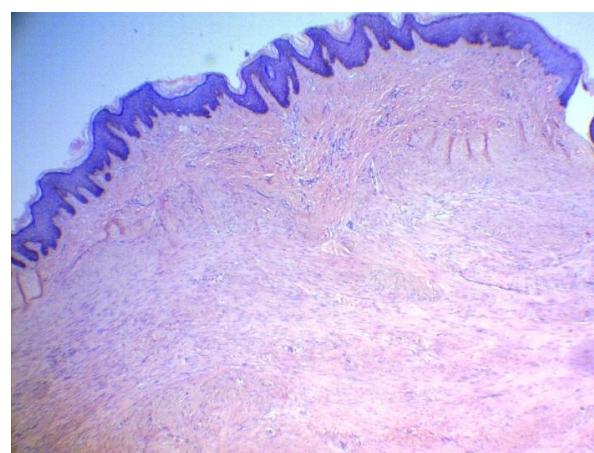


Figure 4. Histopathology of a keloid cicatrix.

HIFU allows non-invasive mild heating of deep-seated tissues or total tissue ablation.

Using the effect of tissue ablation it can be utilized for malignant tissue removal. It's applicable to prostate cancer therapy. Using HIFU lowers the risk of harming the rectum wall and neurovascular bundle in the far-field and there are fewer side effects in the near field. Furthermore, HIFU is being used as a treatment method in early-stage breast cancer and benign and malignant tumors in the abdominal organs with good success rates. (6)

HIFU is a good alternative to hysterectomy for women with uterine fibroids. This allows the preservation of the uterus. (7) Another effective usage of HIFU is performing thalamotomy in order to treat tremor. (8)

For aesthetic purposes, HIFU shows great results in skin rejuvenation and tightening, especially face and neck rejuvenation. This method is frequently combined with other procedures to be most effective. (9) The thermal effect of HIFU helps to achieve greater results in treating acne scars with the use of additional therapeutic methods. (10)

Conclusion

To conclude, our study did not show any side effects during the procedures. In all patients, we observed a decrease in the size of the pathological tissue in the dermis, a decrease in the pigmentation of the scar, and itching reduction.

In closing, we can say that HIFU (high-intensity focused ultrasound) is an innovative method for treating scar tissue. So far in Bulgaria, it is used only for aesthetic purposes, but we believe we could change that, after the results, we have obtained.

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Factors influencing the results of unicompartmental knee arthroplasty

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The purpose of this report is to identify the factors influencing and leading to unsatisfactory results in unicompartmental knee prosthetics. For a ten-year period at the Clinic of Orthopedics and Traumatology at the University Hospital "St. Georgi" we have made 450 unicompartmental knee prostheses. In 17 patients we prosthetized the lateral part of the knee, and in the remaining 433 patients we replaced the medial compartment. The main indication for this type of surgery is isolated osteoarthritis, involving only one part of the joint. We evaluated the factors influencing the results after this type of surgery. In our opinion, these are non-compliance with the indications and contraindications for this type of intervention; poor surgical and / or cementation technique of the components; inappropriately selected prosthesis design; excessive bone removal from the tibial plateau; incorrect positioning of the components (malalignment), as well as damage to a certain prosthetic component. All of the above factors lead to poor results and patient dissatisfaction. Therefore, it is important to know and follow in order to achieve the desired end result after this type of knee surgery.

Introduction

Over the past twenty years, the application of unicompartmental knee arthroplasty (UKA) has significantly increased. (1) There has been an almost threefold increase in cases of UKA compared to total knee arthroplasty (TKA). UKA can be carried out by a minimally invasive approach and is associated with a relatively low incidence of complications. (2) More than 90% prosthetic survival has been reported in ten years of follow-up of the patients concerned. (3) Despite these results, a number of arthroplasty registers indicate a relatively high rate of revision and failure in the UKA, especially when compared with TKA. (4) The frequency and type of complications of UKA vary depending on the type and design of the implant, as well as the follow-up period by the surgeon who performed the surgery. (5)

Purpose

The purpose of this report is to identify the factors influencing and leading respectively to unsatisfactory results in unicompartmental knee prosthetics.

Material and methods

For a ten-year period, we've done 450 unicompartmental knee prostheses. In 17 patients, we replaced the lateral compartment of the knee, and in the remaining 433 patients we replaced the internal compartment. The main indication for this type of prosthetic is isolated osteoarthritis, engaging only one compartment of the joint. Throughout this period, we evaluated the factors influencing and leading accordingly to unsatisfactory results after this type of surgery. This, in our opinion, is a non-compliance with the indications and contraindications to this type of intervention; poor surgical and/or component cementing technique; inappropriately selected prosthesis design; excessive bone removal from the tibial plateau; incorrect positioning of the components (malalignment) as well as the damage to a particular prosthetic component.

Results

Incorrect and inaccurate selection of patients is the main reason for obtaining unwanted results after UKA. These are patients in whom osteoarthritis (OA) affects

more than one section of the knee joint, as well as those in which the pain covers the entire knee. (fig. 1) We had prosthetic patients, in which we had an OA in the femoro patellar joint (fig. 2) as well as obese patients. Unsatisfactory results were obtained after the operational procedure. (fig. 3, 4) Surgical technique is crucial when performing UKA. Lateralization of the femoral component should be performed when a medial UKA is performed to take into account the external rotation in the case of knee extension. When using an entirely polyethylene component, it is important to implant a sufficiently wide tibial implant starting possibly from the medial tibial cortex reaching the anterior cruciate ligament. When cementing the components, drilling, using jet wash and drying of the surfaces before placing the bone cement on them is carried out. Cement should be applied in a

doughy form. This ensures better fixation of femoral and tibial components. During the intervention itself, it is very important to avoid removing too much bone from the tibia in order to prevent fracture and / or failure of the tibial component. Allination and proper positioning of the components is an important condition and relevant for the final result after the UKA. (fig. 5, 6) Sizing of the femoral and tibial condyl is very important, as is that the tibial component must be placed on a healthy medial tibial cortex in order not to lead to failure of the implant, which is a common failure. This complication is also observed with too much withdrawal from the tibial bone or when attaching a small-sized tibial component. Lateral positioning of the femoral component to the femoral cobdyle is correct to avoid medial lateral divergence.



Fig. 1. Female 70 years severe OA.



Fig. 2. Advanced OA and in the PFJ.



Fig. 3. High resection of the tibia.



Fig. 4. OA of the lateral department.



Fig. 5. Valgus position of the femoral component.

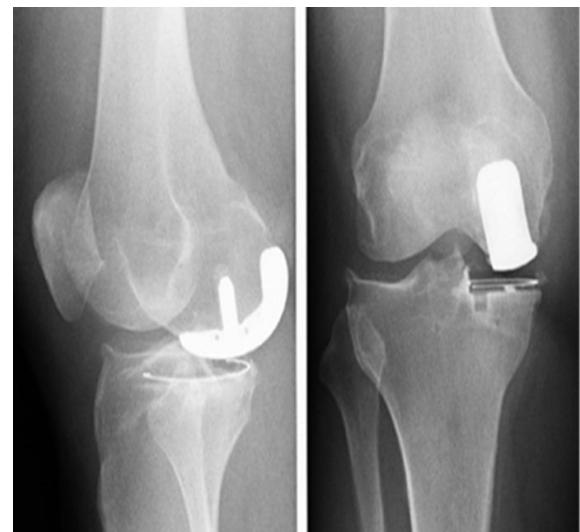


Fig. 6. Varus position of the femoral component.

Discussion

Observance of the classic indications and contraindications of Kozin and Scott to perform UKA are the key to success in this type of intervention. (6) In recent years, the indications introduced by Oxford have significantly expanded the target group for prosthetic knee joint. However, there are contradictions regarding the degree of involvement of the patello-femoral joint by the osteoarthritis process, which can be ignored during a UKA. Some authors claim and believe that there are no contraindications to the carrying out of UKA in the

presence of OA in the PFJ, unless there is significant loss of bone mass and involvement of the lateral facet of the trochlear groove. (7) Performing UKA prosthetics in active patients is also not recommended, as premature loosening and wear of components is possible.

Obesity can also be cited as the cause of failure in a partial knee prosthesis. Behrend and Col. in 2005, they pointed out that obesity was a major factor causing failure in the medium and long-term survival of UKA. (8)

In the literature there are reports that

fractures of the tibial plateau obtained during the insertion of the pins or other fixing devices are described when processing it before implantation of the tibial component. Cases of early occurrence of medial collapse have been reported with significant medial removal of bone mass. In medial implanted entirely polyethylene tibial components, which were tracked over a period of 2.5 years, an 11% sinking of the component was reported. After performed radiographies in these patients, it is established that the tibial component lacks peripheral support and there is significant removal from the bone. (9)

It is important to know that in the rear-medial and lateral part of the resected proximal tibia there is up to 20% more bone substance as opposed to the rest of the articular surface. Therefore, implantation of the tibial component on the more resected antero-medial and a antero-lateral part of the tibial surface can be a problem and tighten up until it sinks. Also to this complication can lead to the placement of a smaller-sized tibial component without support and contact with the medial tibial cortex.

Poor post-UKA results can also occur with poor cement fixation technique when implanted as a result of ignoring important moments such as drilling, jet lavage, and good drying of the cut bone surfaces before cementing the components.

Poor design of prosthetic components can contribute to medial lateral mismatch, mismatch in articulation between component surfaces and dislocation in mobile-bearing model UKA.

Allination and proper positioning of components is crucial for the success of the UKA. Squire and Stake. Initially reported the allination as early as 1999, observing a series of patients with UKA and concluding

that in 46% of the operations there was a loss of lateral joint space of the knee joint afteroperative. (10) The cause of involvement of lateral compartment after medial UKA is usually overcorrection of the pre-operative varus deformation available and after operatively this patient already has valgus deformation of the lower limb. In another series of 100 patients, 83% develop a progressive collapse of the lateral department of the knee, due to the gradual wear and tear of this compartment. (11) According to Berger only 18% develop a collapse of the joint gap after the UKA, in which postoperatively the restoration of the mechanical axis of the lower limb to a neutral position or a slight varus is achieved. (12)

Conclusion

UKA is a successful minimally invasive procedure on the knee joint. However, there are factors that lead to poor results and frustration in the patient after it is carried out. Therefore, it is extremely important to know and observe in order to achieve the desired end result after this type of knee surgery.

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Treatment of medial osteoarthritis of the knee joint with partial prosthetic replacement – postoperative complications

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Objective: To indicate the possible postoperative complications in medial unicompartmental replacement of the knee joint. **Material and methods:** For a ten year period (from 2008–2018) 433 patients with osteoarthritis of the knee joint, affecting only the medial compartment, were operated. In 377 patients we prosthetized the medial compartment of the joint due to primary antero-medial osteoarthritis, and in the remaining 56 patients osteonecrosis of the medial femoral condyle was diagnosed, which necessitated its prosthetic replacement. **Results:** Patients were followed for a period of six years. For the indicated period we reported the following postoperative complications: aseptic loosening of the femoral and tibial part of the prosthesis (in 4 patients); polyethylene wear (in 5 patients); impingement of the components with the adjacent bone (in 3 patients); progression of osteoarthritis in the lateral joint (in 10 patients); severe periprosthetic infection (in one patient) and arthrofibrosis (in 2 patients). **Conclusion:** UKA is a minimally invasive intervention in isolated osteoarthritis of the knee joint. Complications that occur after this procedure can be reduced by careful selection of patients, selection of an appropriate implant and good surgical technique.

Introduction

UKA has become a surgical intervention for the treatment of isolated osteoarthritis in patients of middle and old age. Initially, this procedure was associated with poor end results, but improvements made in the design of the implants used and surgical technique contributed to its development in a positive aspect. (1, 2) It is currently carried out with increasing frequency and gives good to excellent results. Mortality and the possibility of early complications after this type of prosthetics are relatively low. In the long term, however, the procedure has higher levels of revision due to complications compared to the complete replacement of the knee joint. (3-7)

Purpose

The purpose of this article is to indicate possible postoperative complications of medial unicompartmental replacement of the knee joint.

Material and methods

For a period of 10 years (2008–2018) at the Clinic of Orthopedics and Traumatolo-

gy at St. George University Hospital EAD, Plovdiv, Bulgaria had 433 patients with osteoarthritis of the knee joint, affecting only the medial department of the joint. In 377 patients, the medial compartment of the joint is prosthetic due to primary antero-medial osteoarthritis, and in the remaining 56 patients osteonecrosis of the medial hip condyl is diagnosed, which imposed its prosthetic replacement. In all patients, all-poly unicondyl endoprosthesis with a fixed tibial platform was used.

Results

Patients were tracked for a period of six years. For the specified period, we reported the following postoperative complications: aseptic loosening of the femoral and tibial part of the prosthesis (in 4 patients) (fig. 1, 2); polyethylene wear (in 5 patients) (fig. 3), impingement of components with the adjacent bone (in 3 patients); progression of osteoarthritis in the lateral department of the joint (in 10 patients) (fig. 4); severe periprosthetic infection (in one patient) (fig. 5, 6) and arthrofibrosis (in 2 patients).



Fig. 1. 69y F 6 years after UKA.



Fig. 4. 72y F 5 years after UKA.



Fig. 2. 74y F 5 years after UKA.



Fig. 5. 82y M 6 years – infection.



Fig. 3. 77y F 3 years after UKA.



Fig. 6. Resection of components.

Discussion

The complications observed by us after the partial knee prosthetics of the medial department completely colorate with those described in the literature and a number of world arthroplastic registers. Aseptic mechanical loosening of the components is reported as the most common postoperative complication after UKA (8). Young age, excess weight and varus deformation are indicated as possible risk factors causing mechanical failure of the protez components. In fixed-platform UKA, there is a greater contact load on the polyethylene insert, resulting in damage related to loosening of the tibial component (9). Loosening of the tibial component is more common in UKA, where the tibial part is entirely polyethylene (in all-poly design), as opposed to metal-backed models.

The progression of osteoarthritis in the opposite department of the knee joint is also a frequent complication, both in fixed models and those with a removable platform UKA. Overcorrection of the mechanical axis of the lower limb causes degenerative changes in the opposite department of the knee joint. This complication is observed in patients with rheumatoid or psoriatic arthritis. There is a narrowing of the articular space and the formation of osteophytes in the initial stage, which eventually leads to the development of pain, subchondral sclerosis and obliteration of the joint space in the unproctized compartment (11).

Polyethylene wear is a complication that is observed more often in fixed platform UKA. Occurs with incorrect positioning of the prosthesis components (valgus or varus); insufficient correction of the initial preoperative deformation of the knee joint; use of polyethylene less than 6 mm thick; the manufacturing process and methods of sterilisation of polyethylene (12).

The rate of infections after this type of prosthetics ranges from 0.2 to 1% (13). The low frequency is due to the fact that this procedure can be carried out by minimally invasive access, with less damage to adjacent soft tissues, minimal bone resections and a spare of ligamentous structures.

With available infection, debridement is performed with subsequent lavage. In case of non-resection of all components and subsequent one-stage or two-stage revision with primary or revision total knee protesis.

Atrophybrosis after UKA is also a rare complication compared to post-TKA, since UKA is a minimally invasive procedure with minimal damage to the extensor apparatus of the KJ and suprapatellar bursa. Postoperative arthrobrosis in UKA can be treated by regression of the KJ under anesthesia, but arthroscopic removal of intraarticular fibrous tissues and available adhesions is also often required.

Conclusion

UKA is a minimally invasive intervention in isolated osteoarthritis of the knee joint. Complications that occur after this procedure can be reduced, with accurate selection of patients, selection of the appropriate implant and good surgical technique.

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Current aspects in the pathogenesis of endometrial polyps – a literature review

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Endometrial polyps are the most common benign neoplasms in the uterine cavity, which can be diagnosed in reproductive and perimenopausal age, most often between 40 and 49 years. They are the leading cause of infertility in 35% of cases and of irregular uterine bleeding in 50% of women. They belong to the group of preneoplastic diseases of the uterine body. The incidence of their malignant potential is 10–15% in postmenopausal women with genital bleeding. An in-depth understanding of the etiology and pathogenesis of endometrial polyps will open new horizons in their diagnosis, treatment and prevention. The literature review presents contemporary theories about the origin, development and malignant transformation of endometrial polyps.

Keywords: *endometrial polyp, pathogenesis, theories*

Endometrial polyps are benign neoplasms composed of cystically dilated glands with fibrous stroma containing thick walled blood vessels. The endometrium covering the stroma can vary from a normal functional endometrium to one with simple or complex hyperplasia, atrophic or proliferative with or without atypia. Polyps can occur anywhere in the uterine cavity, including the cervical canal and PVCU. Most often they are localized along the uterine fundus and posterior uterine wall followed by the front one and lateral walls.^{1,11}

Endometrial polyps can be single or multiple in 10–20% of cases, range in size from microscopic – 5 mm to macroscopic – 12 cm, filling the entire uterine cavity and cervical canal. They can be pedunculated or sessile and have a spherical or cylindrical shape. Tamoxifen associated endometrial polyps are characteristically multiple, large and fibrous. Their cut surface is smooth, soft and cystic or hard and fibrous, sometimes with a multicystic structure.

The histopathological examination of endometrial polyps can prove foci of endometrial hyperplasia and carcinoma limited to the polyp or part of it.^{1,11}

Endometrial polyps are mostly asymptomatic. Abnormal uterine bleeding is the most common symptom in 68% of women in reproductive and perimenopausal age. Bleeding may be due to stromal congestion in the polyp leading to venous stagnation and apical necrosis.^{2,3,4} Driesler and co-authors further have noted that contrary to what might be expected, the polyp size, the number of polyps and the anatomical location of polyp (polyps) does not appear to correlate with the symptoms of bleeding.⁵ In patients with abnormal uterine bleeding, polyps are 13–50% in premenopausal women and 30% in postmenopausal women. Multiple endometrial polyposis occurs in 26% of postmenopausal women and in 15% premenopausal women.^{6,7,8}

Endometrial polyps are one of the leading causes of infertility. The incidence of this disease in primary infertility is 3.8–38.5% and it is 1.8–17% in secondary infertility.

A small part of the polyps, about 1.0%, can develop hyperplasia or undergo a malignant transformation. The most common subtypes of cancer are endometrioid adenocarcinoma and serous adenocarcinoma. The risk of malignant transformation is associated with age, obesity, hypertension, the

polyp size, the use of tamoxifen and HRT. The frequency of it increases with age. A small percentage of polyps can spontaneously regress.^{9,10}

Endometrial polyps are diagnosed by transvaginal ultrasonography, the use of color Doppler, sonohysterography, hysteroscopy and histopathological examination making the final diagnosis.¹¹

In 1953 Scott published an article entitled "The Elusive Endometrial Polyp" and concluded that "the endometrial polyp remains a mystery in terms of frequency, bleeding potential and possibility of malignant transformation".

To this day, the exact causes of appearance of endometrial polyps are not known, but there are several modern theories related to the etiology and pathogenesis of these benign neoplasms.^{12,11,49}

The altered expression of steroid receptors and altered sensitivity to estrogens and progesterone are of key significance to formation of endometrial polyps.

It is believed that they are associated with estrogen stimulation, the result of increased concentration of estrogen receptors (ER), mainly ER-alpha in the cells of glands covering the polyp stroma compared to the normal endometrium and reduced expression of progesterone receptors (PR) A and B in polyps compared to the normal endometrium. The decreased expression of progesterone could make the polyp less responsive to differentiation and decidualization. As a result of persistence, polyps can increase their sensitivity to mutations and their potential for malignant transformation.

Endometrial polyps contain both ER and PR, and the concentration of these receptors is much higher in the glandular epithelium of the polyp compared to the normal epithelium.

The concentration of ER and PRs decreases in the stromal cells of endometrial polyps, which can prevent the polyp stroma from undergoing decidual changes and menstrual discharge, which is observed in the rest of endometrium.

Some authors point out that, unlike the alpha estrogen receptor, the beta receptor is expressed mainly in the stroma of endometrial polyps and that the degree of this expression is directly related to serum and local estradiol levels. In conclusion it is noted that hyperactivation of the beta estrogen receptor compared to the alpha one during menstrual cycles is a key reason for development of endometrial polyps.^{12,17,15,16,13,14}

Origin from the basal layer

There are modern theories for the development of endometrial polyps from the basal layer with premenopausal women. In 1954 Schroeder noted the presence of basal adenomas in the uterine cavity confirming that the functioning basal layer forms polyps. Because of the fact that they are composed of refractory basal endometrium, their architecture does not correspond to the phase of menstrual cycle. Later Peterson and Novak examined 1,100 polyps and found a histological pattern that differed from the menstrual cycle in 60% of cases. Reslova et al. discovered the existence of a "functional" polyp that resembles the surrounding endometrium and a "hyperplastic" polyp having derived from the basal endometrium, which is dependent on estrogens but resistant to progesterone action. The theory that the endometrial polyps originate from the basal endometrium is supported also by the higher expression of basal and reserve cell marker P63, membrane protein in polyps that appear in postmenopausal women compared to the surrounding endometrium. Since P63 is used as a marker of

the uterine “stem cells”, from which the polyp and the possibility of growth of clonal cells originate.^{12,21,18,19,20}

A subset of 3,272 polyp genes has been identified. Endometrial polyps arise as a result of chromosomal translocations in stromal cells. Dal Cin et al. distinguish three main abnormal subgroups, including 6p21-22, 12q13-15 or 7q22 areas. The expression of p63, aromatase P450 and steroidogenic factor-1 (SF-1) might play a role in the formation of endometrial polyps. Su and Sui. Stewart et al. have established that the immune reactivity of stromal p16 is characteristic of endometrial polyps and relevant to the pathogenesis of endometrial polyps.

Aromatase (CYP19A1) is an enzyme responsible for converting C19 steroids into estrogens, a major factor for circulating estrogen concentrations in postmenopausal women. The role of impaired expression of aromatase as a pathogenic mechanism in estrogen-dependent neoplasms such as endometrial cancer, adenomyosis, endometriosis and uterine myoma as well as endometrial stromal cells throughout the menstrual cycle. Maia and etc. compare the expression of aromatase in polyps and surrounding endometrium. A significantly higher proportion of endometrial polyps express aromatase compared with disease-free endometrium. Pal et al. have biopsied the endometrium during the follicular phase in three different places – the endometrial polyp, adjacent endometrium and distant endometrium in premenopausal women and have proved heterogeneity in the expression of mRNA of cyp19A1. This is the basis to suggest that the pathogenesis of certain endometrial polyps may be independent of the aromatase-associated excess of estrogens.^{12,25,26,27,28,29,30,31,32,33}

Impaired balance between apoptosis and mitotic activity

To regulate endometrial development, there is a balance between mitotic activity and apoptosis during the menstrual cycle. An important role in these processes has been played by the B-cell lymphoma-2 (Bcl-2) marker, which is an inhibitor of apoptosis, and the Ki67 protein, which is a cellular marker for proliferation and cellular mitotic activity.^{11,34} Proliferation and apoptosis are part of the development and growth of endometrial polyps. In estrogen-responsive tissue, these processes are mediated in part by steroid hormones through their receptors. Risberg et al. reported that endometrial polyps had lower Ki-67 results than proliferative, hyperplasia, or malignant endometrium, but not from the secretory endometrium. In this study, the expression in polyps of the antiapoptotic protein B-cell lymphoma 2 (Bcl-2) in polyps was intense in both the glands and the stroma. Maia et al. demonstrated that Ki-67 and Bcl-2 expression in both polyps and the normal endometrium varied steadily throughout the menstrual cycle.¹²

The study by Taylor et al. supports the study partially. Taylor et al. observed a significant increase of the expression of Bcl-2 in the glandular epithelium and stroma of polyps in the proliferative phase compared to the proliferative endometrium, but this increase has not been observed in any of the polyps in the secretory phase. The local increase in Bcl-2 expression in endometrial polyps may explain the failure of polyps to undergo normal cyclic apoptosis and therefore not be secreted during the menstrual cycle. Ki67 expression has been observed in the glandular epithelium of polyps mainly in the proliferative phase. The stromal staining of Ki67 is more obvious in the secretory phase, but it has been found to be

lower than that of the endometrial glands in the proliferative phase. Miranda et al. reported that Ki-67 expression was significantly higher in samples of polyps from tamoxifen-treated women compared to those from women who did not use the hormone.^{35,36,12,11}

The role of local chronic inflammation/mast cells, cytokines and growth factors

The formation of endometrial polyps may be the result of localized inflammation in the endometrium. Mast cells are cells that initiate and control inflammation through the secretion of cytokines and growth factors.^{11,37} Cyclooxygenase-2 (COX-2) is a key enzyme involved in the production of prostaglandins in mast cells. Higher levels have been found in polyps compared to the normal endometrium. Inflammation leads to the formation of new blood vessels and tissue growth.^{38,39} The number of mast cells is seven times higher in endometrial polyps than in the normal endometrium, and it has been found that most of these mast cells are activated. Secretory mast cells are able to induce or enhance angiogenesis and as a result, an increase in blood vessel density is expected. Angiogenic factors such as VEGF and major fibroblast growth factor (bFGF) stimulate mast cell migration.^{40,41,42}

VEGF and transforming growth factor beta-1 (TGF beta-1) were found to be significantly higher in endometrial polyps compared to the normal endometrium. VEGF is angiogenic, while TGF beta-1 is associated with the formation of fibrous tissue, both of which are characteristic of endometrial polyps. This is supported by a higher concentration of Ki67 (tissue proliferative factor) in endometrial polyps than in the normal endometrium. Inflammation can

lead to an overreaction leading to tissue damage. The inflammatory process leads to a change in growth factors, and this may be important for the existence of two different types of endometrial polyps, one of which is hormone-dependent and the other of an inflammatory nature. These variations can cause various symptoms, increase or recurrence, effects on reproduction and malignant transformation.^{11,43,44,45}

The mechanism of malignant transformation in endometrial polyps is thought to be due to COX-2, an enzyme responsible for prostaglandin synthesis. Giordano et al. Also found that the phosphoprotein p53 (P53) and Ki67, both of which are associated with abnormal cell proliferation are significantly higher in tumor cells and are associated with more advanced stage, gradation, subtype, and deep invasion of the myometrium.^{11,9}

Prevention of polyps – levonorgestrel intrauterine system (LNG-IUS) in women treated with tamoxifen. A randomized controlled trial showed that in postmenopausal women already on tamoxifen, 12 months of LNG-IUS treatment resulted in an endometrial decidual response and an inhibitory effect on the polyp. Long-term follow-up (mean 26 months) from the same trial cohort ($n = 122$) showed that there were 8 new polyps developing in the control group and 3 in the intervention group, but the difference was not statistically significant. A recently published systematic review of 2015 using data from Gardner et al. and other authors confirmed that LNG-IUS inhibited polyp formation in women using tamoxifen.^{12,46,47,48}

The mechanism by which progestins interact with endometrial cells to inhibit tamoxifen-induced polyp formation is the subject of current research. Decidualization can play a major role. Is it possible for

LNG-IUS to prevent the formation of polyps in women who do not take tamoxifen remains unanswered.^{12,47,18}

Endometrial polyps are common in gynecological practice. They may be diagnosed accidentally or present with symptoms of abnormal uterine bleeding. Their role as preneoplastic neoplasms makes them a risk factor for endometrial cancer. Treatment of endometrial polyps by hysteroscopic resection is effective and leads to relief of symptoms.

The authors present the opinion of the international medical literature on the etiology, pathogenesis and mechanisms leading to the formation of polyps and express a common opinion on the need for more research in this direction in order to improve their diagnosis, treatment and prevention.

Over the last 50 years, the understanding of endometrial polyps has advanced, but to paraphrase Scott, we can rightly say that the endometrial polyp still remains elusive in many ways.

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Arterial and venous thrombosis in COVID-19 patients – series of clinical cases

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In March, 2020 the WHO announced a global pandemic imposed by the worldwide spread of the SARS CoV-2 virus. The newly emerged pathogen was first described in Wuhan, China in December 2019. Data taken from February 2021 shows that the total number of reported cases is estimated at approximately 109 million, with a calculated fatality rate of over 2 million. The COVID-19 disease has the characteristics of an acute respiratory viral infection and is associated with a wide range of complications, including gastrointestinal events, neurological symptoms, pericarditis, vascular and cardiac insufficiencies, depressive disorders, pulmonary embolism, deep venous and arterial thrombosis. We present a total of five clinical cases – three of lower extremity and two of upper extremity thrombosis. We examine both conditions as a serious complication resulting from a SARS-CoV-2 infection. The patients were treated in succession by the Department of Vascular Surgery and the Department of Burns, Plastic, Reconstructive and Aesthetic Surgery of the “St. George” University Hospital, Plovdiv, Bulgaria. Amputation of the affected limb was required in two of the lower extremity thrombosis cases. Severe soft tissue defects are described in the third case. The upper extremity thrombosis cases received conservative treatment. COVID-19 induced thrombosis of the limbs is a severe condition which seriously deprives the patients’ quality of life if left untreated. Monitoring the coagulation status is crucial in order to successfully avoid the complications of the viral infection. Early treatment and thrombotic prophylaxis both drastically increase the success rate of the COVID-19 induced thrombosis therapy.

Keywords: COVID-19, complications, thrombosis, thrombolytic therapy

Introduction

In March, 2020 the WHO announced a global pandemic imposed by the worldwide spread of the new SARS CoV-2 virus. Data taken from February 2021 shows that the total number of reported cases is estimated at approximately 109 million, with a calculated fatality rate of over 2 million. COVID-19 disease has the characteristics of an acute respiratory viral infection and is associated with a wide range of complications such as gastrointestinal events, neurological symptoms, pericarditis, vascular and cardiac insufficiencies, depressive disorders. Among the non-respiratory manifestations are also venous and arterial thrombotic events. COVID-19 induced coagulopathy affects the vasculature of the lower and upper limbs, lungs, spleen, heart and brain (1), GIT (2) the aorta (3). We hereby present five clinical cases – three of lower extremity and two of upper extremity thrombosis. We examine COVID-19 disease as a possi-

ble factor contributing to the progression of these complications. The patients were treated in succession by the Department of Vascular Surgery and Department of Burns, Plastic, Reconstructive and Aesthetic Surgery of the “St. George” University Hospital, Medical University – Plovdiv, Plovdiv, Bulgaria.

Case 1

47 year-old male with medical history significant for diabetes mellitus type II (DMTII), diabetic polyneuropathy and heart failure presents to the Department of Vascular Surgery of “St. George” University Hospital with a 15 day-history of symptoms of right lower limb ischaemia. The patient has been tested positive for COVID-19 via PCR-test. On physical examination cyanosis of the right foot was reported accompanied by hardly palpable pulse on a. femoralis communis dextra and missing pulsations distally. Ultrasound imaging

supports the physical examination. The patient was diagnosed with thrombosis of a. iliaca externa, a. femoralis superficialis, a. poplitea dextra. Thrombendarterectomy (TEA) was performed of the occluded arteries. The patient underwent necrectomy twice after the surgery due to necrotic lesions in the anterolateral aspect of the right crus. Postoperatively pulsations and warmth of the lower right limb returned to normal and the patient was stable. The patient was given Fraxiparin 0.4 and antibiotics. He was directed to the Department of Burns, Plastic, Reconstructive and Aesthetic Surgery due to soft tissue necrosis and exposed bone on the right calf (Fig. 1). The patient underwent daily necrectomies of bone and soft tissue followed by vacuum assisted closure (VAC) therapy and autoplastica cutis libera (ACL) of the right crural area. (Fig. 2) His therapy included antithrombotic medication. On a follow-up examination the patient seemed stable with visible tissue regeneration on the operated area.



Fig. 1.



Fig. 2.

Case 2

53 year-old female patient with a background of arterial hypertension, ischaemic heart disease and stroke presents to the Department of Vascular Surgery with signs of ischaemia of the left lower limb. The patient reported pain, numbness and cold sensations in the affected area. She was treated for COVID-19 disease before admission. On physical examination there was no palpable pulse on the left ileo-femoral axis and the skin was cold. Ultrasound diagnostic procedures revealed stenotic changes of the left iliac artery and obstruction of the left superficial femoral artery. On CT angiography occlusion of the proximal left superficial femoral artery is visible (Fig. 3) The patient was diagnosed with thrombosis of a. iliaca sinistra. TEA was performed followed by a lower left limb amputation on the level of the thigh. Three weeks later the patient underwent reamputation the reason being infection of the stump. After exact antiseptic debridement VAC drainage was placed on the amputated area. Five days later thrombosis of the left femoral vein occurred which was treated by thrombectomy. The amputation stump was revised and drained. The patient was directed to the Department of Burns, Plastic, Reconstructive and Aesthetic Surgery after necrosis of the amputation stump of the left limb. (Fig. 4) Surgical treatment was applied consisting of staged necrectomy and ACL (meshgraft 1:1,5). (Fig. 5) Medication included Clexane 0.6 s.c. 2 times per day and antibiotics. The wound was closed and the patient was stable at discharge.



Fig. 3.



Fig. 4.



Fig. 5.

Case 3

41 year-old male patient with DMTI has a history of peripheral vascular disease, ischaemic heart disease and records of acute coronary syndrome. The patient was diagnosed with COVID-19 and was then transferred to the Department of Vascular Surgery of St. George Hospital with signs of ischaemia of the right lower limb and reports of cold sensations and pale blue col-

oration. Upon examination there was no palpable pulse in the entire right iliofemoral axis. Ultrasound confirmed thrombosis of a. iliaca dextra. TEA of a. iliaca dex. and a. femoralis dextra was performed. The patient reported bleeding in the right inguinal area. The suture of a. femoralis dextra was revised and a thrombectomy was performed. During a surgical revision a. femoralis superficialis dextra was ligated because of excess bleeding. After the procedure, gangrene developed in the area which led to the amputation of the limb. Necrotic tissue was removed. Seven days later the same limb was amputated again due to necrosis and ultrasound confirmed the presence of deep vein thrombosis in v. femoralis dextra. The patient was put on low molecular weight heparin (LMWH) but his status declined and a thrombectomy was performed. The vein was then ligated. VAC drainage was put in the amputated area. The patient was then directed to the Burns, Plastic, Reconstructive and Aesthetic Surgery Department where graft surgery was performed and the wound was closed. The patient was treated with antibiotics.

Cases 4 and 5

Case 4 is a 62-year-old male treated for a SARS-CoV-2 infection who noticed blue coloration in his fingers and coldness and pain in his left arm after discharge. Physical examination revealed upper-limb ischemia with sensory motor deficit. (Fig. 6) Ultrasound examination found lack of blood flow in a. axillaris, a. brachialis and the arteries of the wrist. TEA was performed the first and second day. Despite the treatment with Heparin 1000E/h with a perfusor, no pulse could be felt in the wrist arteries, which led to a thrombectomy. The next day no pulse was felt in a. radialis. Conservative treatment included Prostavasin 2 amp. x20

mg infusion. The limb was salvaged after the treatment. Antithrombotic prophylaxis was prescribed consisting of Aspirin 1x100 mg and Cilostazol 2x100 mg. Case 5 is a 43-year-old male treated for a SARS-CoV-2 infection who also noticed coldness, numbness, and pain in his upper limb after discharge. Examination revealed upper-limb ischemia. Ultrasound examination found a lack of blood flow in a. brachialis. Thrombectomy was performed. The limb was salvaged after the treatment. Patient underwent thromboprophylaxis.



Fig. 6.

Discussion

The pathophysiology of the arterial and venous thrombosis observed in COVID-19 patients is still not fully understood. However there are some theories that give a viable explanation to the pathogenesis of these complications. The SARS-CoV-2 virus enters and infects the cells via the ACE-2 receptor which is expressed in the vascular endothelium. The hypercoagulable observed in SARS-CoV-2 infection is believed to be caused by disrupting the normal antagonism between the Renin-Angiotensin-Aldosterone System and the ACE-2 enzyme which degrades Angiotensin II. As the virus blocks (4) and causes downregulation (5) of the ACE-2 receptors

Angiotensin II builds up and affects the function of the coagulation system, fibrinolysis system, and platelets. (4) The SARS-CoV2 induced increase in Angiotensin II stimulates the production of IL-6 as well and promotes a cytokine storm. (6) It was established that the spike protein of the virus can directly activate platelets thus promoting aggregation and clot formation with thrombosis as an end result. (7) Furthermore, the activated complement system and NETosis which accompany the viral infection act as prothrombotic factors. (1) The inflammation and cytokine storm that are often found in COVID-19 patients may act as direct cause for the activation of endothelial cells, platelets, and leukocytes, the release of tissue factor (TF), and subsequently triggering the external pathway of coagulation. (5) Inflammatory state is also associated with increased levels of fibrinogen. (4) Events attributable to thrombosis in COVID-19 patients include acute limb ischemia (ALI), abdominal and thoracic aortic thrombosis, mesenteric ischemia, myocardial infarction and acute coronary syndrome, VTE, pulmonary embolism (PE), acute cerebrovascular accident. Such events may occur even among patients already receiving thromboprophylaxis. (5)

Typical laboratory findings in patients with thrombotic events due to COVID-19 include elevated D-dimers, normal or slightly prolonged levels of prothrombin time (PT) and activated partial thromboplastin time (aPTT), normal or increased platelet counts, increased fibrinogen, increased activity of factor 8 and VWF antigen. (8) Recent guidances recommend the use of D-dimer levels, PT and platelet count in all COVID-19 patients, with D-dimer levels considered as the most important given that they are associated with poor outcome if elevated. (8)

When managing the coagulopathy in SARS-CoV-19 patients, subcutaneous LMWH is recommended as part of the thromboprophylaxis. (9) The absence of risk factors for bleeding and contraindications need to be ascertained before starting any form of therapeutic anticoagulation. The diagnosis should be confirmed with CT angiography and/or ultrasound of the limbs. (10) Even though LMWH is generally recommended to be included in the first-line thrombolytic therapy for all patients with COVID-19, unfractionated heparin (UFH) is known to be used as well. (11) Interactions with antiviral medications need to be considered, thus direct oral anticoagulants or vitamin K antagonists are not recommended. (11) LMWH has a more predictable anticoagulant effect response and a longer duration of action which allows it to be administered subcutaneously. UFH has a shorter half-life, has to be IV administered, requires aPTT monitoring, and has protective effects on the endothelium and anti-inflammatory properties which are due to its ability to bind and neutralize inflammatory cytokines. (12) UFH should be used as part of the thrombolytic therapy over LMWH in patients with underlying renal dysfunction and low creatinine clearance (< 15 mL/min). (6) The risk of heparin-induced thrombocytopenia (HIT) is higher with UFH compared to LMWH. (11) In the case of HIT, fondaparinux is one of the recommended substitute anticoagulants. (12)

Conclusion

The described cases show that arterial and venous thrombosis are among the most serious complications in SARS-CoV-19 patients. Upper and lower limb ischemia of unusual severity have been noticed to occur since the beginning of the pandemic. The pathogenesis underlying the SARS-CoV-2

infection related coagulopathy is multifactorial including disrupted RAAS/ACE-2 balance, hyperinflammatory state and platelet hyperactivity. The prevalence of thrombotic cases seems to also be dependent on the severity of the SARS-CoV-19 infection, the presence of other underlying causes and the chosen course of treatment. This is why we believe in the importance of thromboprophylaxis and that it should be done for a period of time after the infection has subsided. More data is needed in order to find the optimal therapeutic approach to prevent such vascular complications of COVID-19 infections.

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Comparison between laparoscopic and abdominal hysterectomy

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The uterus is a dynamic female reproductive organ that is responsible for several functions, including menstruation, implantation, gestation, labour, and delivery. It is responsive to the hormonal milieu within the body, which allows adaptation to the different stages of a woman's reproductive life. The uterus adjusts to reflect changes in ovarian steroid production during the menstrual cycle. It displays rapid growth and contractile activity throughout pregnancy and childbirth. Conditions such as uterine cancer, fibroids, endometriosis or prolapse cause high levels of pain, discomfort, uterine bleeding, and emotional stress. Hysterectomy is a common non-pregnancy-related surgery that is highly successful in curing the disease of concern. The procedure involves removal of the uterus and cervix, and in some cases, the fallopian tubes and ovaries. In this material we are analyzing several cases of hysterectomy. Our focus is on the patients' condition before and after the procedure, the surgical technique and patients' recovery.

Introduction

Hysterectomy is a surgical procedure that involves removal of the uterus and cervix, and for some conditions, the fallopian tubes and ovaries. Reasons for choosing this operation are treatment of uterine cancer and various common noncancerous uterine conditions such as fibroids, endometriosis or prolapse that lead to disabling levels of pain, discomfort, uterine bleeding, and emotional stress. Uterine cancer is defined as any invasive neoplasm of the uterine corpus. No screening tests for cancer of the uterus are currently recommended for asymptomatic women.

The mainstay of primary treatment in endometrial cancer and uterine sarcomas is hysterectomy. Traditionally, hysterectomy is performed using either an abdominal or vaginal approach. More recently, laparoscopic techniques have been used. The decision about the technique used is often related to the surgeon's training and expertise, as the indications for each technique overlap. Although this procedure is highly successful, it is a surgical alternative with the accompanying risks, morbidity, and mortality and it leads to sterility in women who are premenopausal.

Object

In this aspect we overlook two patients that underwent radical hysterectomy – total abdominal and laparoscopic. Our aim is to compare the preoperative and postoperative period of the patients taking into consideration the differences between the two methods.

Methods and materials

Patients

S.A.N. is a 67-year-old pensioner. She has been in menopause for 16 years and has given birth. She was previously diagnosed with diabetes type 2 with diabetic neuropathy, arterial hypertension and obesity. S.A.N. visited her gynecologist because of irregular postmenopausal bleeding. After trial abrasion, the histological results showed endometrial carcinoma. She was diagnosed with a neoplasm of the uterine corpus stage IIa T2NoMo. Her condition was suitable for radical laparoscopic hysterectomy.

S.R.K. is a 45-year-old accountant. By the time of the procedure she was 35 years old. She is premenopausal and has given birth. She visited her gynecologist because of heavy menstruation and sharp pelvic

pain. The histological results revealed ovarian endometriosis stage III N80.1 with infiltrations of the rectum. After a consultation with an anesthesiologist and an internal medicine specialist the surgeon decided that the best approach was total abdominal hysterectomy.

Methods

Laparoscopic hysterectomy

Total Laparoscopic Hysterectomy is defined by the laparoscopic ligation of the ovarian arteries and veins with the removal of the uterus vaginally or abdominally, along with laparoscopic closure of the vaginal cuff. This is in contrast to other methods of removing the uterus, fallopian tubes, and ovaries. A laparoscopic supracervical hysterectomy is completed in a similar fashion, with the exception that the cervix is amputated after occluding the ascending vascular pedicles.

A laparoscopic-assisted hysterectomy (LAVH), a precursor to the TLH, is a technique to secure the ovarian and uterine vasculature via laparoscopy; the remainder of the procedure is completed vaginally. The laparoscope is often reinserted after closure of the vaginal cuff to inspect the abdomen and vaginal cuff for adequate hemostasis at the end of the procedure. This procedure requires adequate uterine descent to safely complete the vaginal portion of the procedure.

Laparoscopic radical hysterectomy has emerged as an alternative to abdominal radical hysterectomy for patients with stage I cervical cancer. Emerging advancements include robotic-assisted laparoscopic hysterectomy, single-incision laparoscopy, and laparoscopic pelvic reconstruction surgery.

Indications for a total laparoscopic hysterectomy (TLH) include leiomyomata,

pelvic organ prolapse, and abnormal uterine bleeding. TLH may also be indicated for resection and debulking of both malignant and premalignant disease, as noted by extensive case series in the gynecologic oncologic literature. Frequently, access to the uterus can be complicated by adhesions, a narrow subpubic arch, a nulliparous pelvis, or leiomyomas, whether they are multiple, large, or found in the lower uterine segment. In these cases, laparoscopy can be used to free adhesions and divide the upper pedicles, allowing for a laparoscopic-assisted vaginal hysterectomy. Oophorectomies can be challenging when approached vaginally, whereas laparoscopic oophorectomies are a natural extension of TLH.

Abdominal hysterectomy

Abdominal hysterectomy was first performed in 1843. Prior attempts at removal of the uterus date back to ancient times, when vaginal hysterectomy was performed to treat uterine prolapse or inversion. Laparoscopic assistance was used to facilitate minimally invasive hysterectomy in 1989 and further advanced in 2005 with the approval of the robotic-assisted technique. Today, abdominal, vaginal, laparoscopic, robot-assisted, and a combination of vaginal and laparoscopic techniques are utilized for hysterectomy.

The surgical approach to hysterectomy depends on the clinical indication, the technical experience of the surgeon, the resources available, the general health condition of the patient, and patient preference. An abdominal hysterectomy involves the removal of the uterus through an incision in the abdominal wall. Large uterine size has been cited as a common reason for choosing the abdominal approach to hysterectomy, as it has been thought that an enlarged uterus may require better visualization and

exposure due to higher risks of blood loss, injury to neighboring viscera, and prolonged operating times. However, there are no specific recommendations up to which uterine weight or size should qualify a patient for abdominal hysterectomy, and studies have shown that minimally invasive techniques, such as laparoscopy, can safely remove larger uteri. [6][7]

Despite these findings, abdominal hysterectomy remains a common route of surgery being most commonly indicated for uterine fibroids, followed by abnormal uterine bleeding, prolapse, and endometriosis. [8] The most common indications for abdominal hysterectomy are the following: [8] abnormal uterine bleeding, malignant and premalignant disease, uterine leiomyoma, endometriosis pelvic organ prolapse, chronic pelvic pain, pelvic infection. Abdominal hysterectomy is often performed in patients with enlarged, bulky uterus or past history of abdominal surgery and in the presence of extrauterine disease, severe adhesions or endometriosis, and gynecological malignancies in whom a minimally invasive route is considered technically challenging. [2] Uterine size larger than 12 weeks' gestation considered a reasonable qualification for an abdominal approach to hysterectomy. The surgical route of hysterectomy should be individualized to each patient. Uterine characteristics include size, mobility, and location, as well as the extent of gynecologic pathology. Prior history of abdominal surgery is important to consider since the anticipated extensive adhesive disease can increase the risk of complications. Other factors to consider include vaginal caliber, potential complication risks based on patient comorbid medical conditions, presence of concomitant pathology, patient preference, and surgeon experience.

Discussion

Preoperative period

Complete blood count showed no deviations in neither of the two patients.

Both of them were in good condition without any complaints. Preoperative ultrasound was used to measure uterine length and width in relation to the pelvis.

Postoperative period

S.A.N. reports that her postoperative stay in the hospital was 2 days long, there were not any intraoperative complications. After her discharge from the hospital, she was taking analgesics for approximately a month. Her recovery period continued three months, during which she had problems with her wound that healed slowly and was inflamed several times. Her doctors told her that obesity was the reason for this problem. She did not have any difficulties with her everyday routine and needed the help of her relatives for only two weeks after the procedure. She experienced incontinence but it had not resulted in surgical reopening. She was able to move and sit without feeling pain after the three months of her full recovery.

S.R.K. claims that her postoperative stay was 5 days long, there were not any intraoperative complications. After her discharge from the hospital, she was taking Femovita in order to reduce the hot flashes and analgesics because of the sharp and consistent pain in the lower abdomen. Her recovery period continued six months during which she found it really hard to sit and move. She needed lots of help for her everyday activities, her professional abilities were affected and she needed a great amount of emotional support since she had significant mood swings. She experienced nocturia and pain during urination and defecation. The analgesics did not relieve the

pain. She reports that even one year after the operation she felt great discomfort in terms of her everyday activities and even her sexual life.

Taking into consideration the gathered information from the two patients, laparoscopic hysterectomy has many advantages compared to the abdominal one. It requires only a few small incisions, compared to a traditional abdominal hysterectomy which is done through a 3–6 inch incision. As a result, there is less blood loss, less scarring and less post-operative pain. A laparoscopic hysterectomy is usually done as an outpatient procedure whereas an abdominal hysterectomy usually requires a 2–5 day hospital stay. The recovery period for this laparoscopic procedure varies and depends on the patients' condition, but it is documented to be significantly shorter than the abdominal one. Laparoscopic radical hysterectomy is introduced to decrease the morbidity of the operative procedure. Numerous studies have compared laparoscopic and abdominal radical hysterectomy for cervical cancer, and showed that the laparoscopic approach is oncologically safe and associated with fewer postoperative complications and earlier recovery [9-16].

However, there have been concerns about the oncologic safety of laparoscopic radical hysterectomy due to difficulties in achieving a sufficient resection margin from the complex procedure itself. Abdominal hysterectomy still remains a common surgical route for benign disease for a wide range of clinical indications. Optimal healthcare outcomes are achieved when there is a collaboration among the healthcare team involved in the care of the patient, from the time the patient checks into the facility at pre-op until the patient is

discharged from the hospital, whether from the post-op recovery unit or after an inpatient stay. An interprofessional team may be made up of nurses, surgical assistants, residents and medical students among other personnel.

Conclusion

In conclusion, the advantages of total laparoscopic hysterectomy compared to abdominal hysterectomy have been well documented and discussed. Visualisation of pelvic anatomy and the ability to minimize blood loss is superior with total laparoscopic hysterectomy. Substantial and dynamic access to the uterine vessels, vagina, and rectum is possible from many angles, especially after introduction of the uterine manipulator in 1995. The advantages of TLH have been firmly established to include reduced short-term morbidity (less blood loss, wound infections, and postoperative pain), shorter hospital stay, and faster resumption of normal activities when compared with abdominal hysterectomy.

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Phantom pain

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Pain takes a major role in our sensory repertoire as an alarm system designed to protect the body from harm. Unlike normal pain, phantom pain has no adaptive function and does not aid in the survival of the organism. It persists despite the lack of any tissue damage. Phantom pain is a type of neuropathic pain which seems to be coming from parts of the body which are not physically present anymore: it usually occurs following an amputation of a limb or another peripheral part of the body – tongue, eyes, mammary glands, penis. In the current material, we are analyzing several cases of phantom pain after amputation of lower or upper limbs. Our attention is on diagnosing the disease and looking at the challenges faced by medical care when dealing with the feeling of a phantom limb.

Keywords: *Phantom pain, Phantom limb*

Introduction

Pain takes a major role in our sensory repertoire as an alarm system designed to protect the body from damage. Unlike normal pain, phantom pain has no adaptive function and does not aid in the survival of the organism. It persists despite the lack of any tissue damage. Phantom pain is a type of neuropathic pain which seems to be coming from parts of the body which are not physically present anymore: it usually occurs following an amputation of a limb or another peripheral part of the body – tongue, eyes, mammary glands, penis. The characteristics of phantom limb pain (PLP) are sharp or shooting, aching, burning, and cramping pain. Pruritus and twitch may also be accompanying symptoms. PLP usually disappears or decreases over time; however, the improvement is poor when it continues for more than 6 months (1). In the current material, we are analyzing several cases of phantom pain after amputation of lower or upper limbs. Our attention is on diagnosing the disease and looking at the challenges faced by medical care when dealing with the feeling of a phantom limb.

Purpose and task

Phantom limb sensation was first described by the French military surgeon Ambroise Pare in the 1500s. Since then, the

number of studies surrounding phantom limb pain has steadily increased. Although there have been many hypotheses on the subject and many treatments have been tried, scientists still do not have a complete understanding of why phantom limbs and phantom pain appear and how to treat it. The purpose of this article is to bring attention to the phenomenon of phantom limb pain and to describe its diagnosis and treatment.

Prevalence of amputation and phantom limb pain

Approximately 1.9 million amputees live in the US alone, out of a population of 328.2 million, this means one in 190 Americans is living with the loss of a limb. That number is expected to double by the year 2050 (2). Amputations are commonly a consequence of diabetes mellitus, trauma, and malignant neoplasms (2). Estimates of PLP incidence differ considerably, depending in part on the sampled population as well as the methods of reporting and data collection. In one large, frequently cited survey of amputees, 78% reported experiencing PLP (3). These numbers mean that a sizeable amount of the population is suffering from PLP while treatment is lacking, and understanding of the subject is limited.

Cause of phantom pain

Phantom pain is caused by elimination or interruption of sensory nerve impulses by destroying or injuring the sensory nerve fibers after amputation or deafferentation. The reported incidence of phantom limb pain after trauma, injury or peripheral vascular diseases is 60% to 80%. Over half the patients with phantom pain have residual limb pain as well. Pain and sensory alterations at the site of amputation predominate in the initial postoperative and posttraumatic period. Phantom limb sensations and pain generally develop within 1 month of the amputation with a second peak of development approximately 12 months postamputation (5). Those who experience it indicate that the pain is real and that it is the body part that is "phantom". Although this phenomenon is widely recognized, it is not well understood. The seeming incongruence of pain in a missing body part, with intervals that range from 1 day to several weeks. Even intervals of over a year have been reported, combined with the difficulty of successfully treating this pain, result in severe chronic pain in a majority of people who have had an amputation. Treatment with drugs that reduce the number of functional sodium channels has been tried with success, as have various neurophysiological manipulations (4).

Clinical cases

We examined 3 patients from the department of plastic, reconstructive, aesthetic surgery and burns of university hospital "Sv. Georgi" in Plovdiv who underwent an amputation and reported experiencing the feeling of phantom limb pain.

Patient №1 is a 10-year-old boy who suffered high voltage burns to both of his arms. The electrical burns were extensive and the arms could not be saved. He un-

derwent a shoulder disarticulation of his left arm and a transhumeral amputation of the right arm. He reports feeling pain from the entirety of both his missing arms, which is a clear example of phantom pain.

Patient №2 is a 58-year-old man who suffered a work accident with high-voltage electrical burns to both of his arms. He was working at a building site and got shocked by a 20-voltage current and therefore underwent Bilateral above-elbow amputation on the distal 1/3 of the humerus. 8 months after surgery, he is now using a prosthetic on his right arm. The patient reports the feeling of having fingernails on his right hand and a constant pain from his right arm. He feels the presence of his left arm and sometimes feels like he is clenching both of his fists.

Patient №3 is a 71-year-old woman who suffered from diabetic gangrene on her right foot. Due to the necrosis she had the distal 2/3s of her right lower leg amputated. 3 months after surgery, she sometimes feels the presence of her amputated lower leg. She feels shivering in her amputated leg when she massages her remaining left leg.

Treatment

Treatment of phantom limb pain may require a multimodal therapeutic approach. First-line therapies include transcutaneous electrical nerve stimulation (TENS), Transcranial direct current stimulation and biofeedback also known as Artificial Reality (AR). During the AR-based therapy sessions patients wear a virtual reality headset and play a set of games. The games require them to actually move their amputated limb and navigate around virtual worlds. The position of the residual leg is tracked with motion sensors and the computer fills in any image of where the missing limb is. Through the headset it looks to the patient

as though their leg or arm is still present. Another therapy method is mirror therapy, during which patients move their healthy arm in front of a mirror in order to produce a perception of movements of the missing limb. Transcranial direct current stimulation (tDCS) is an emerging approach believed to affect the membrane potential and activity threshold of cortical neurons. A study published in the Pain Medicine magazine by N. Segal et al. (6) aimed to assess if a combination of mirror therapy with tDCS results in a superior analgesic effect as compared with mirror therapy alone in patients suffering from phantom pain due to recent amputation. Thirty students were divided into three groups which were: 1) mirror therapy; 2) mirror therapy and sham tDCS; or 3) mirror therapy and tDCS. Three months following treatment, pain intensity was significantly ($P < 0.001$) reduced in the combined treatment group (reduction of 5.4 ± 3.3 points) compared with the other study arms (mirror therapy, 1.2 ± 1.1 ; mirror therapy and sham tDCS, 2.7 ± 3.2) (6). Medication options include anticonvulsants (gabapentin), opioids, and NSAIDs.

Conclusion

In conclusion, phantom limb pain has been documented around 500 years ago and an overwhelming majority of amputees experience it. Amputations are not a rare oc-

currence and millions of people worldwide suffer from PLP after receiving one. Despite this fact, there is still very little known about the true mechanism behind PLP, with many theories highly overlapping. No treatment has yet been clearly proven in terms of its effect and sufferers of PLP do not have a clear path to follow.

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Cavernous internal carotid artery aneurysms – etiology, types and treatment

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Cavernous artery aneurisms are benign vascular lesions, which etiology is connected with some risk factors of the lifestyle, inherited disorders, trauma etc. The types of cavernous internal carotid artery aneurysms can be divided depending on their size and placement and whether they have complications or not. The mentioned factors play a main role in the choice of the following treatment type and are determinant for the pathological progression. Some microsurgical, endovascular and deconstructive strategies find place in the successful treatment of this type of aneurysms. The prognosis is rarely related with life-threatening complications but sometimes the surgical interventions may be unsuccessful, which can only offer conservative medication methods.

Introduction

Cavernous internal carotid artery aneurysms are benign vascular lesions. Like the other types of aneurysms, they are caused by dilatation of the artery's wall, because of the shear stress, which burdens the endothelial functions and is constituted of migrated smooth-muscle cells from tunica media in tunica intima. [1] There are several types of aneurysms: fusiform, saccular, and dissecting aneurism. Some of the risk factors include polycystic kidney disease, inherited connective tissue disorders like Marfan's syndrome, and smoking. [1, 2] The cavernous segment of the internal carotid artery is divided into three subsegments. The first one is the posterior ascending portion, the next one is the horizontal segment and the last one is the anterior vertical portion. [3] The risk of rupture of this type of aneurysms is low, especially if its size is under 13 mm, but complications are sometimes available. They could be the result of the mass effect, which manifests with compression or obstruction of nearby structures, further widening of the aneurism with over 1 mm per year, or rupture. [2, 3] Useful imaging methods for investigating an aneurism are the CT-angiography scan, the MRI angiography, and the DSA. [3, 4] Microsurgical, endovascular, and deconstructive strat-

egies find a place in the successful treatment of this type of aneurysms [2, 3, 4, 5], but conservative therapy can also be helpful in most cases. When the aneurysm doesn't have any signs, which require necessary treatment, it's important to regularly observe its progress and evaluate possible complications. [3]

Clinical case

The patient is a woman at the age of 61. The final diagnoses is a giant partially thrombosed aneurism on the right Internal Carotid Artery (ICA). She has also arterial hypertension. Her complaints date back months and are connected with visual disturbances, headache, which doesn't influence the conservative therapy. Due to her complaints, she had performed an MRI scan and a saccular aneurysm in the cavernous segment of ICA was discovered (fig. 1). Her neurological status showed sixth- and third cranial nerves palsy and trigeminal neuralgia. [1] After neurosurgical consultation, the patient had been evaluated as a good surgical candidate for endovascular embolization of the aneurism. The endovascular treatment was unsuccessful, due to unfavourable anatomy – extreme stenosis of the ICA proximal to the aneurysm neck (fig. 2). Due to the risk of dissection, the

operation was terminated and conservative therapy was prescribed.

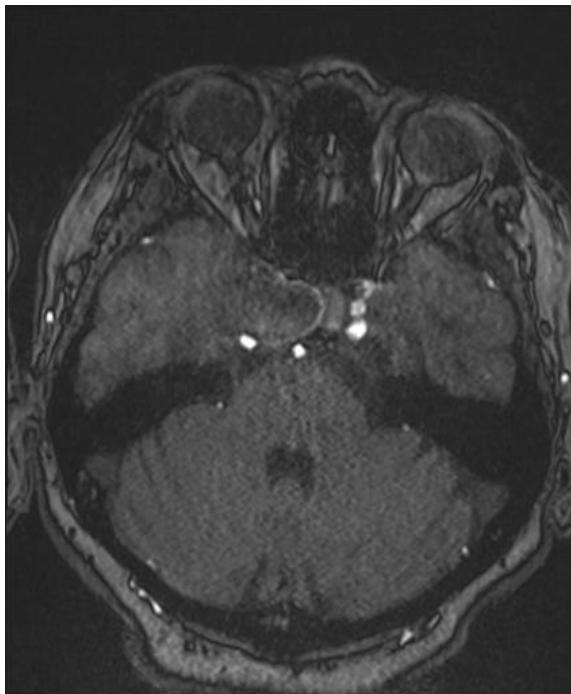


Fig. 1. An MRI image of a right partially thrombosed cavernous carotid aneurysm with compression of cranial nerves.

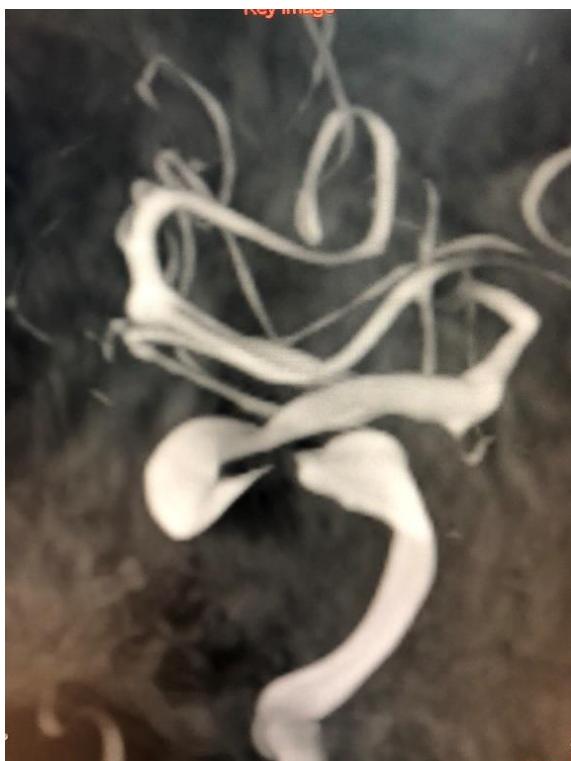


Fig. 2. DSA image of the right ICA. Severe stenosis distal to the cavernous carotid aneurysm.

Discussion

The aneurysms in the cavernous segment of the ICA are about 4% of all intracranial aneurysms. They can have a traumatic, infectious, or idiopathic origin. The most common ones are those connected with traumas and especially on the skull base. Infectious agents are very rare – by bacterial endocarditis for example. [3] The idiopathic cavernous carotid aneurysms (CCAs) are associated with risk factors such as smoking, hypertension, connective tissue diseases, family history, and even sexual intercourse. [1, 2, 3]

As mentioned above the aneurism is formed by systematic hemodynamic stress, endothelial dysfunction, and arterial inflammation, which is caused by the migration of smooth muscle cells from tunica media to tunica intima, a following myointimal hyperplasia and invasion of macrophages in the artery's wall. They release different enzymes like matrixmetallproteases, which break down the components of the extracellular matrix and contribute to the further development of the aneurism. [1]

The CCA ruptures rarely, but it can cause not only subarachnoid haemorrhage like other types of intracranial aneurysms but also a carotid-cavernous fistula [6] and haemorrhage in the sphenoidal sinus when a bone erosion is available. Epistaxis is also possible but is a rare symptom of a ruptured CCA. [3, 6]

The most used imaging methods for investigating the CCA are CT-angiography, MRI-angiography, and the DSA. Using the CT-angiography the anatomy of the aneurism can be more precisely defined – dimensions of its dome, the width of the neck, connections with other vascular lesions, or erosion of bones. The MRI angiography is the imaging technique, where the signal emitted from surrounding osse-

ous structures is significantly reduced and 3D reconstruction of the images is possible to receive more information about the relations between the vessels and the aneurism's characteristics. [3]

The treatment methods for CCAs could be microsurgical, endovascular, and some deconstructive strategies. The microsurgical methods have a purpose to exclude the aneurism from the main blood circulation without affecting the lumen of the parent artery. This kind of treatment is possible for lesions, situated on the anterior portion of the internal carotid artery, the anterior genu, and the horizontal part. It's inappropriate for aneurysms in the posterior ascending segment, because of its intimate connections to the nearby passing cranial nerves and the risk of morbidity. The main microsurgical method is clipping. The goal is to put the clips around the aneurism's neck to return the normal circulation of the blood and perfusion of the brain parenchyma. Clipping is known for its durability. [2] Other strategies are the endovascular ones. Coil embolization is one of them. The main nature of the embolization is to make a thrombus in the aneurism and to exclude it from the blood flow. That can be reached by detachable coils, which are inserted in the aneurism and the loops should be very dense for achieving the maximal effect. Another endovascular strategy is the stand placement, which purpose is to decrease the blood flow in the aneurism. By the endovascular treatment, the access to the internal carotid artery is through the common carotid artery or the femoral artery. Despite the good surgical results of microsurgical constructive strategies, due to the relationship of the aneurysms with the cranial nerves and the cavernous sinus, the endovascular treatment of CCA has been favored over the microsurgical techniques. Another type of

managing the complications of the aneurysm's nature is the deconstructive strategies. They include occlusion of the parent artery and eventually an extra- or intracranial bypass if the collateral circulation is not enough for the normal function of the brain. The occlusion can be done through a microsurgical or endovascular procedure, but when we juxtapose the risk of complications, the endovascular treatments are the preferred ones with mortality rate 0–1,7% and morbidity 2.7–6.6% in comparison to the microsurgical ones with morbidity and mortality rates 9–22%. The deconstructive strategies are used, when it is impossible to undertake another surgical approach.

Conclusion

Depending on the type, location, size, the natural history of the CCA, and the clinical symptoms, the medical professionals should choose the most appropriate imaging and treatment methods for reducing the symptoms and manage successfully the aneurismal lesion. [3, 5, 6] The clinical case above is here to show that not always the CCA can be treated surgically with success and this is one of the circumstances when conservative therapy is indicated.

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Behavioral changes caused by colloid cyst of the third ventricle – case report

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Colloid cysts are rare small, gelatinous neoplasms lined by a single layer of mucin-secreting columnar epithelium that are thought to arise from errors in folding of the primitive neuroepithelium. They usually occupy the anterior third ventricle at or near the foramen of Monroe in 99% of cases. Despite their benign histology carry a poor prognosis, with a mortality greater than 10% in symptomatic cases. We present a case of 64-year-old female who presented with frequent paroxysmal episodes of severe headache and nausea. Gradually, she developed behavioral changes with bradypsychia and disorientation. Magnetic resonance imaging (MRI) of the brain was performed because of the symptoms suggestive of increased intracranial pressure and intracranial pathology. It demonstrated a cystic lesion located in the anterior part of the third ventricle which blocked the Foramina of Monroe and caused biventricular obstructive hydrocephalus. The patient was operated via left precoronal interhemispheric-transcallosal approach and the lesion was totally removed. The postoperative period was uneventful, preoperative symptoms completely resolved. At 3rd year follow-up, the patient demonstrated complete neurological recovery and MRI of the brain confirmed total removal with no signs of recurrence. The current paper discusses the clinical presentation, anatomical and surgical considerations of the treatment of these rare lesions.

Keywords: *colloid cyst, third ventricle, surgery, hydrocephalus, symptoms*

Introduction

The colloid cyst was first described by Wallmann in 1858 in a patient presenting with gait ataxia and incontinence. It was 64 years later that Walter Dandy performed the first successful surgical resection of such a lesion, located in the 3rd ventricle. Since then, there has been great development in our understanding of the pathophysiological mechanisms of colloid cysts. According to the findings of Dandy and Wallmann, colloid cysts are now considered to be primary brain tumors that arise in the ventricular system [1]. In most cases (99%) they develop at the dorsal aspect of the foramen of Monro, with peak incidence between 3rd and 5th decade of life. This position within the ventricular system means the cysts are liable to compress the foramen of Monro and disrupt the normal circulation of cerebrospinal fluid (CSF) as they enlarge, causing hydrocephalus [1, 2]. In addition to this mechanism, colloid cysts may also directly

compress periventricular structures resulting in a separate set of neurological sequelae, with psychiatric symptoms dominating [2]. The symptoms of hydrocephalus are well recognized by physicians as being synonymous with intracranial pathology, and result in prompt referral to neuroimaging services. By contrast, psychiatric symptoms are not specific to one type of pathology, therefore, investigation and treatment are more likely to take a protracted course, with referral to psychiatric services commonly taking place prior to a full diagnostic workup for organic causes of the symptoms [8]. Colloid cysts carry a poor prognosis once symptomatic and as such prompt diagnosis, facilitated by a clear understanding of their histology, neuroanatomy and variety of clinical presentation is crucial [8]. In the current paper, we report a case of a female patient who suffered from obstructive internal hydrocephalus caused by a colloid cyst that presented with sudden behavioral changes.

Case report

A 64-year-old female developed sudden behavioral changes. She became slow, moody, and confused. This condition gradually deteriorated over time. Initially, she was hospitalized in Neurology ward and computed tomography (CT) scan was performed which detected an ovoid third ventricle lesion that obstructed the foramina of Monroe and caused biventricular hydrocephalus (Fig. 1). For this reason, she was relocated to our department for further diagnostic evaluation and treatment. Upon admission to the Neurosurgery clinic, the neurological status revealed symptoms of increased intracranial pressure.

Additionally, preoperative MRI was performed that demonstrated well-defined focal intraventricular lesion with dimensions 15/12 mm, located in the third ventricle, obstructing the Monroe foramina of the lat-

eral ventricles with resulting occlusive asymmetric biventricular hydrocephalus. The lesion showed discrete peripheral ring enhancement after gadolinium administration (Fig. 2). The MRI characteristics were consistent with colloid cyst of the third ventricle.

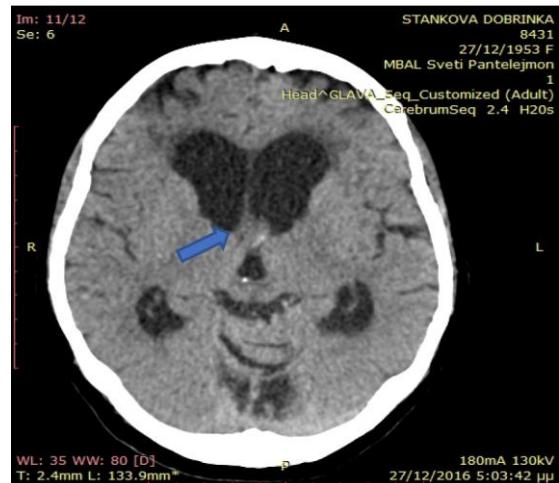


Figure 1. Axial CT scan demonstrating an ovoid third ventricular lesion (arrow).

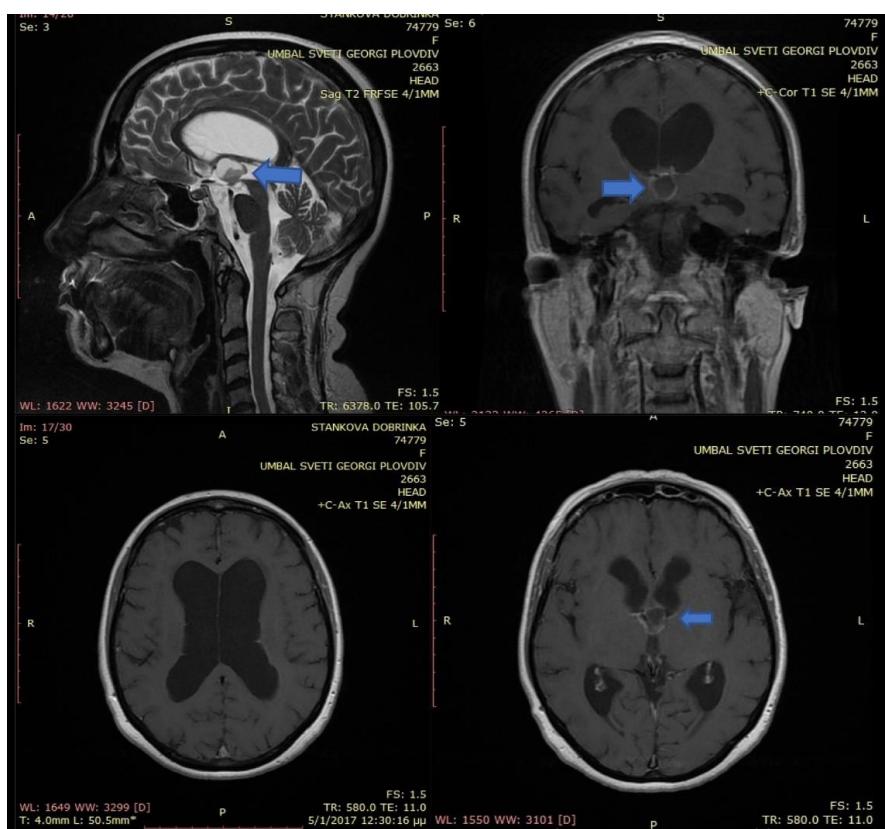


Fig. 2. The T2-saggital view of the MRI (left upper image – arrow) presenting a mass closely attached to the roof of the anterior third ventricle; the T1-coronal view presents a well circumscribed formation (right upper image – arrow); the axial MRI (both lower images) showed the cyst obstructing the ventricles and causing obstructive hydrocephalus.

Based on the clinical and neuroimaging data, the patient was scheduled for surgical treatment. The tumor was accessed via left interhemispheric-transcallosal access and using meticulous microsurgical technique the tumor was debulked and detached from the roof of the third ventricle just posterior to the foramina of Monroe. Total removal of the mass was achieved. (Fig. 3)

The histological examination confirmed the colloid cyst with focal chronic inflammatory infiltration of its wall.

The postoperative period was uneventful. After discharge she did excellent long-term recovery but bradypsychia was persistent for several months.

Discussion

The most common symptoms of colloid cyst tend to be headache, nausea, vomiting, visual disturbance, and behavioral changes (apathy, dementia, memory defects, and confabulation etc). The mental status of the patient can be affected by the disturbance of the normal flow of CSF which is a key factor for causing all those symptoms by increasing the ICP (intracranial pressure)

gradually and suppressing structures of the brain that cause those symptoms. Compression of the hippocampal formation and fornix by the dilated lateral ventricles may explain the development of progressive dementia. These medial temporal lobe structures are particularly important in the encoding of new memory, especially episodic. Impaired anterograde semantic memory (memory for facts) and a variable temporal gradient of retrograde amnesia can also happen due to damage to hippocampus and fornix [3].

There have been cases of patients presenting with psychiatric symptoms caused by colloid cysts without evidence of hydrocephalus. According to a recent research, there are cases of patients presenting with symptoms including hypomania, paranoid delusions, memory loss and anxiety attacks (with tachycardia, tachypnea, and diaphoresis) that improved after operative removal of the colloid cyst. There is a suggestion that these symptoms could be caused by direct pressure on the structures surrounding the third ventricle [9].



Fig. 3. A, B. Macroscopic view from the operative approach and resection of the tumor. The cyst was adjacent to the choroid plexus and obstructed the Monroe foramina – on the left picture (pre-operatively) the cyst is visible while in the right one the cyst is totally removed and detached from the Chpl. (T – tumor; CHPL – choroid plexus; TSV – thalamostriate vein).

The third ventricle lies between the diencephalon and the structures that surround it are the medial thalamus and hypothalamus laterally, the fornix and corpus callosum superiorly and the infundibulum inferiorly, which attaches mammillary bodies to the upper end of the midbrain. These structures are important components of the putative limbic system, an interconnected network of cortical and subcortical areas involved in visceral and autonomic processes. Cortical structures including the hippocampal formation and cingulate gyrus are connected by pathways (fimbria-fornices and mammillary bodies) to subcortical regions such amygdala, nucleus accumbens, hypothalamus and anterior thalamic nucleus [7].

In terms of the symptoms experienced by patients, the hypomania and delusions could be linked to dysfunction of both the nucleus accumbens, which forms part of the dopaminergic pathway associated with pleasurable stimuli and delusions and hallucinations, and the amygdala, which plays a key role defining a stimulus and how to respond appropriately. It also has been shown that lesions affecting the periventricular and cortical limbic areas can cause emotional disturbances, as most of the patient presented with colloid cyst. Damage to the hypothalamus could result in anxiety attacks with physiological abnormalities as it provides connections to the anterior, and posterior pituitary gland, the autonomic nervous system (which can cause sudden death due to diffuse myocardial injury that leads to cardiac arrest) [10].

Therefore, early radiological evaluation is essential for the early diagnosis of symptomatic patients. Computerized Tomography (CT) and Magnetic Resonance (MR) imaging provide vital information to the surgeon in order to evaluate the exact localization of the cyst and to plan the proper

method of treatment by also considering the size and the content of the cyst, clues that are crucial for the successful operation. Cyst density varies from hypo to hyperdense on CT and hypo to hyperintense on MR images, as their radiological density depends to cholesterol content of the cyst. The more viscous hyperdense cysts are difficult to drain and often require further neurosurgical intervention in contrast with the iso/ hypodense cysts, which are associated with successful needle aspiration [9].

We compared our case with those reported in a study by S.B. Buklina, in which 52 patients with colloid cyst were evaluated about their results in removal of the tumor [11], and with a study of by John E. Lawrence where a summary of 43 patients were studied about neuropsychological changes during the course of the disease and the effectiveness of different approaches to the tumor [9].

Compared to our case, the localization of the cyst has an important role as it affects a variety of mechanisms that are related to different parts of the normal brain function of the individual. We evaluated the data for the patients that had similar preoperative symptoms such as bradypycchia, identical dimensions of approximately 15 mm and localization of the cyst that was posterior to Monroe foramina adherent to the roof of the third ventricle, as it was in our case report. In most of those patients the tumor was accessed via interhemispheric-transcallosal access that we also utilized in our case. In most cases, only memory changes were observed after the operation, “fragmented brain” symptoms have never been observed after the transcallosal approach, as in our case. If adhesions of the cyst capsule to adjacent parts of the fornix are found, it must be considered that even if there was no visible intraoperative injury to the fornix, alt-

ough micro-traumatization during cyst removal cannot be ruled out [9]. There is a consensus that injury to the fornix causes memory impairment in patients after colloid cyst removal. It has been shown that the most severe and persistent memory disorders were detected in patients with bilateral damage to the fornix [11]. However, even in the case of unilateral injury, especially on the left, memory disorders can be very significant. It should be emphasized also that there was not a correlation of the different dimensions of the cyst, as there was a variety of outcomes in similar dimensioned cysts in contrast with the content of the cyst which had a dominant role in debulking and removal from the third ventricle [9,11]. The most critical part that has to be emphasized is that the patients with severe postoperative memory impairment belonged to the oldest age group, something that is seen in our case too. According to the authors, patient's age may affect two different mechanisms which are the plasticity of brain structures, and characteristics of microcirculation in the brain areas related to the surgical site [11].

Conclusion

Despite the presence of hydrocephalus, the surgical team should always be aware of development of psychiatric symptoms in patients with colloid cyst of the third ventricle as the tumor itself could damage important structures of the brain that support the normal mental health. It is important to choose the correct surgical approach by profound analysis of the preoperative imaging CT and MRI data. Adequate imaging should be performed early after initial

symptoms occur in order to avoid delayed diagnosis, resulting in further damaging of structures that are vital and could worsen the mentality of the individual but also lead to demise.

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Kasabach–Merritt syndrome rare condition of abdominal pain and thrombocytopenia

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Introduction: Kasabach–Merritt syndrome is characterized with giant hemangioma with different localizations. Most common localizations of giant hemangioma is liver parenchyma. Aim of this report is to present rare case of Kasabach–Merritt syndrome with abdominal pain and border-line thrombocytopenia and clinical difficulties with therapeutic choice. **Materials and methods:** We collect full data of the patient from hospital patient history documentation. **Case report:** Thirty years patient with unclear abdominal pain and heaviness after consuming food. Without family history, co-morbidity and no social risk factors like smoking and drinking alcohol. Patient was in excellent general condition and hemodynamic stable. Nothing pathological reports from any physical examination from cardio-vascular, pulmonary, digestive and urinary systems. Report for tenderness and pain in right upper abdominal region. In Abdominal Ultrasound was detected multiple focal liver lesions with different localization and size with ultrasound characteristics of liver hemangioma. Patient was acquainted with his condition and life-time risk of rupture of liver hemangioma and other complications from disease. We called the patient for next visit after 6 months later and give him some advices for live-style changes. **Discussion:** Kasabach–Merritt syndrome with liver localization may be result of congenital or acquired hemangioma and thrombocytopenia as a result of liver type hypersplenism. Unclear abdominal pain with migrating localization may be cause of benign or malign tumor in liver. Every giant hemangioma with subcapsular localization have life-time risk for rupture and massive hemorrhage and death after trauma.. Spontaneously obliteration of these giant hemangioma is rarely and inductive sclerotherapy with different methods like radiofrequent – ablation (RFA), alcohol – ablation etc. have a high operative risk for uncontrollable bleeding and insufficient results. Alternative treatment of giant hemangioma is surgical liver resection, but this is extremely aggressive therapeutic method. In reported patient hemangioma is not indicated for liver resection in this condition we recommended sit and stay algorithm with long life-time abdominal ultrasound follow up on every 6 months. **Conclusion:** Unclear abdominal pain and thrombocytopenia may be result of giant liver hemangioma. We recommended most common treatment in liver hemangioma that is not indicated for active treatment is stay and sit with life-time follow up.

Keywords: *Kasabach-Merrit syndrome, thrombocytopenia, abdominal pain*

Introduction

Kasabach–Merritt syndrome is characterized with giant hemangioma with different localizations. Most common localizations of giant hemangioma is liver parenchyma. The syndrome constellate with borderline thrombocytopenia (consummative type), local compressive symptoms and giant hemangioma. Giant liver hemangioma may consume like hypersplenism thrombocytes and induce border line thrombocytopenia¹. There are some classifications of liver hemangiomas most common and useable is by the size of the lesion that classify giant hemangioma above 5 to 6 centimeters. Kasabach–Merritt syndrome is asymptomatic disease, but in some cases are present

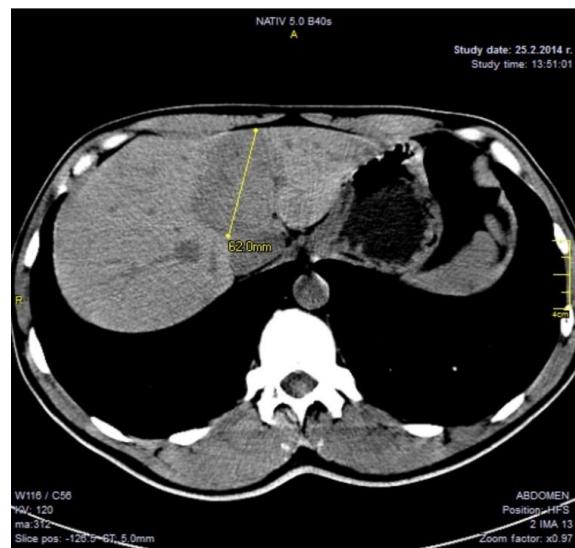
with local abdominal pain, cava syndrome, dyspeptic symptoms etc. Aim of this report is to present rare case of Kasabach–Merritt syndrome with abdominal pain and border-line thrombocytopenia and clinical difficulties with therapeutic choice. Exclude other serious malignant disease is basic point of diagnostic algorithm and patient life-quality and survival².

Materials and methods

We collect full data of the patient from hospital patient history documentation. Were used all available imaging and laboratory examination to present this case as a patient case report (PCR).

Case report

Thirty years patient with unclear abdominal pain and heaviness after consuming food. Without family history, comorbidity and no social risk factors like smoking and drinking alcohol. Patient was in excellent general condition and hemodynamic stable. Nothing pathological reports from any physical examination from cardiovascular, pulmonary, digestive and urinary systems. Report for tenderness and pain in right upper abdominal region. From laboratory examination only that need report is border thrombocytopenia $130 \text{ } 10^9/\text{l}$ with reference hemostasiological markers like (APTT, INR etc.). X-ray from chest and abdomen didn't show any pathological changes. Next step of diagnostic algorithm is other image examinations like computer tomography (CT) and abdominal ultrasound (AUS). In AUS was detected multiple focal liver lesions with different localization and size with ultrasound characteristics of liver hemangioma. For exclude malignancy we made additional laboratory test for tumor markers (CEA, CA19.9), but they also been negative. For accurate the diagnoses and treatment options we make CT that reported analogical information like AUS but in detailed localization and better visualization. Lesions were between 7 and 32 millimeters and one giant hemangioma above 6 centimeters in VI liver segment that across whole liver parenchyma to the Glisone capsule. This giant hemangioma is extremely near to right hepatic vein sinus and inferior caval vein. After deeply discussion we make decision for only follow up during 6 months with control AUS. Patient was acquainted with his condition and life-time risk of rupture of liver hemangioma and other complications from disease. We called the patient for next visit after 6 months later and give him some advices for live-style changes.



Pic. 1. AUS liver hemangioma.

Discussion

Kasabach–Merritt syndrome with liver localization may be result of congenital or acquired hemangioma and thrombocytopenia as a result of liver type hypersplenism³. Unclear abdominal pain with migrating localization may be cause of benign or malign tumor in liver. This symptoms with combination with vomiting and heaviness after nutrition may be result of tumor compression on nearby structures (Glison capsule, duodenum, pylorus etc.). Every giant hemangioma with subcapsular localization have life-time risk for rupture and massive hemorrhage and death after trauma¹. Border line thrombocytopenia is risk factor for prolonged bleeding and may worse abdominal hemorrhage. Spontaneously obliteration of these giant hemangioma is rarely and inductive sclerotherapy with different methods like radio-frequent-ablation (RFA), alcohol-ablation etc. have a high operative risk for uncontrollable bleeding and insufficient results². Alternative treatment of giant hemangioma is surgical liver resection, but this is extremely aggressive therapeutic method. Liver resection needs selection of patient with lateral formations in II, III and IV liver sector. Laparoscopic liver resec-

tion in lateral segments for hemangioma report good results^{3,4}. In reported patient hemangioma is not indicated for liver resection or other alternative therapeutics methods, he stands near to right hepatic vein sinus and large liver outflow vessels. In this condition we recommended sit and stay algorithm with long life-time AUS follow up on every 6 months^{5,6}.

Conclusion

1. Unclear abdominal pain and thrombocytopenia may be result of giant liver hemangioma.

2. We recommended most common treatment in liver hemangioma that is not indicated for active treatment is stay and sit with life-time follow up

3. Most dramatically complication of liver hemangioma is rupture and abdominal hemorrhage.

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Rare case of cervical haemangioma

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Haemangioma of the uterine cervix is a rare benign tumour, and there are only 55 cases currently described in the literature. In most cases, cervical haemangiomas show asymptomatic behaviour. To date, there are no recurrences or adverse outcomes that have been reported after surgical excision, and that is the reason why it is the main curative method. Microscopically, cervical haemangiomas are composed of numerous dilated thin-walled vascular channels, which consists of variable sizes and configurations, and some are filled with erythrocytes. These blood vessels are separated by connective tissue septa and they are lined by a single layer of flattened endothelium without atypical features. We present a case report of a 47-year-old woman with cervical haemangioma which was diagnosed incidentally in abrasion of the uterine cervix for persistent menorrhagia.

Keywords: *hemangioma, uterine cervix, cervical hemangioma*

Introduction

Haemangioma are benign vascular tumour found in the human body (Ahern JK et al., 1978; Bharti P et al., 2012). This type of tumour can be described and identified as tumours consists of vessels that are filled with blood. Anatomically, haemangiomas are localised and confined around the head and neck region. They may also be seen in various visceral organs, and in those cases they are commonly found in the liver.

The most common type of haemangiomas is capillary. Clinically, they appear as small or large, flat or slightly elevated, with colours ranging from red to purple, soft and lobulated lesions, varying in sizes that ranges from millimetres to centimetres in its diameter.

Chou WY et al., noted that uterine haemangioma was first described in 1897 during an autopsy of young woman who developed dyspnoea and anaemia, and eventually died 24 hours after giving birth to twins. The study mentioned that due to low numbers of historical case reports, it was concluded that its incidence cannot be fully and accurately

determined (Chou WY et al., 2012).

Mulliken and Glowacki, published a seminal study which laid the foundation regarding vascular lesions and its identification. Lesions can develop into two distinct phases, namely proliferative phase that occurs between 0 to 12 months, and involution that occurs between 1 to 5 years during the child’s development (Mulliken JB et al., 1982). Endothelial markers such as CD31, CD34, and Willebrand factor which tends to react with vascular spaces found in individual cells (Chou WY et al., 2012). On the other hand, mesothelial markers such as cytokeratin and Calretinin generally produces negative results (Chou WY et al., 2012). Notably, these markers can be identified in both lesions, but in fully involuted phase they get lost (Vijayakumar et al., 2013). Haemangiomas are commonly associated with various obstetric and gynaecological complications, ranging from intermenstrual spotting, menometrorrhagia and infertility to maternal and fetal demise from pronounced bleeding of the gravid uterus (Benjamin, M.A et al., 2010).

Case report

We present a case concerning a 47-year-old woman, who is admitted to the Clinic of Obstetrics and Gynecology with persistent menorrhagia.

Histological analysis of the tissues was performed using automatic tissue processor “DIAPATHEN ISO 9001:2000” and 4–5 µm formalin-fixed paraffin-embedded tissue sections underwent routine staining with hematoxylin-eosin (HE).

Complete morphological examination of the material revealed numerous dilated thin-walled vascular channels, which consists of variable sizes and configurations, and some are filled with erythrocytes (Fig. 1A, B). These blood vessels are separated by connective tissue septa and they are lined by a single layer of flattened endothelium without atypical features (Fig. 1B).

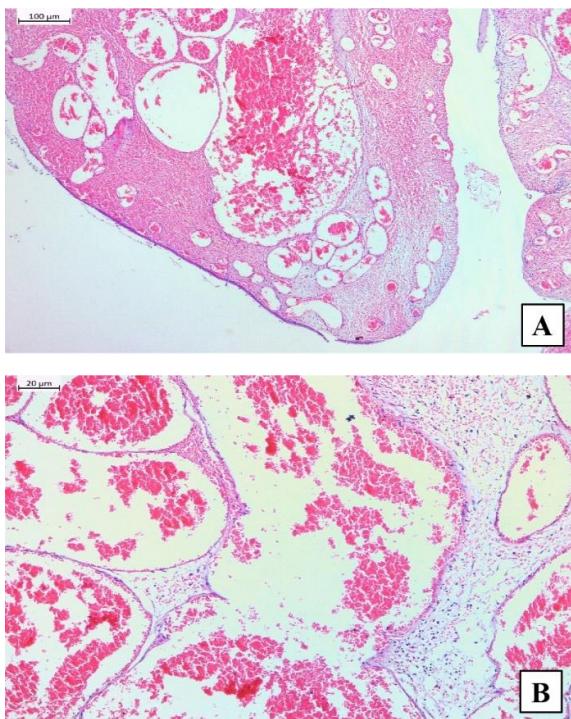


Figure 1A and B. There are numerous dilated thin-walled vascular channels, of variable size and configuration some of which were filled with erythrocytes. These blood vessels are separated by connective tissue septa with area of hemorrhages.

Discussion

Hemangioma is a common benign tumor, but the detection of this vascular lesion in the female genital tract, especially in the cervix, is rare (Ahern JK et al., 1978; Bharti P et al., 2012). Due to their small size and asymptomatic nature, most hemangiomas are accidental findings, but they may present with abnormal vaginal bleeding in the form of menometrorrhagia and postcoital spotting (Mahapatra S et al., 2013; Ahern JK et al., 1978; Bharti P et al., 2012; Ozyer S et al., 2006; Riggs J et al., 2003). Benjamin et al., stated that early detection and intervention could have resulted in the retention of a patient’s uterus, thus reducing her morbidity (Benjamin, M.A et al., 2010). They noted that the possibility of ramifying hemangiomas should be considered during differential diagnosis pertaining to persistent menorrhagia, taking these into account it could then be used as a preventative measure that can mitigate possible delays for both treatment and the appropriate intervention for the patient. Several methods of investigation were mentioned in various literatures ideally during patient examinations as part of differential diagnosis e.g. vaginal examination, ultrasonography, hysteroscopy, uterine curettage specimens. Despite a plethora of options, these methods were deemed to be less informative and inconclusive. Chiu et al., stated that the most appropriate as a definite method is a final histological examination (Chou WY et al., 2012).

Current literature states that there is still uncertainty with regards to treating haemangiomas. Despite this, multiple studies have shown that certain conservative treatments can be utilised e.g. carbon dioxide laser excision, knife excision, cryotherapy, radiotherapy, electrocautery, internal artery ligation, uterine artery embolization,

local excision, and laser ablation. (Benjamin, M.A. et al., 2010; Busca A et al., 2016; Reggiani Bonetti L et al., 2009). In situations where conservative method of treatment does not work, a more invasive approach is recommended such as hysterectomy especially in cases where there's an abnormal vaginal bleeding (Aka KE et al., 2017).

Conclusion

We believe that the rarity of this clinical case report and the discrepancy of published articles relating to this topic will be an important addition to the current medical literature.

Conflict of interests

The authors state no conflict of interest.

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Pneumothorax associated with COVID-19 – the first three cases

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Introduction: Cases of pneumothorax have been reported by various authors in patients with COVID-19. The association between these two diseases, as well as its frequency, have not yet been well studied. **Aim:** To present the first three cases of spontaneous pneumothorax associated with COVID-19 registered in the University Hospital “Sveti Georgi” Plovdiv. **Clinical cases:** Three cases of pneumothorax associated with COVID-19 were presented in two men aged 76 and 33 years and one woman aged 72 years. All three patients were on mechanical ventilation. They underwent thoracentesis with the placement of a chest drain. Due to the worsening of the underlying disease, all three patients died. **Discussion:** Pneumothorax associated with COVID-19 has been reported in 1% of patients requiring hospitalization. Association between barotrauma and pneumothorax is observed in the intubated patients in ICU. Another pathogenetic mechanism is the diffuse alveolar damage caused by the virus with the formation of interstitial emphysema and pneumatocele. The surgical method of choice is thoracentesis. The outcome in patients with COVID-19 and pneumothorax depends on the severity of the underlying lung injury. **Conclusion:** Pneumothorax is a rare but serious complication of COVID-19. It is often associated with poor outcome, especially in patients on mechanical ventilation.

Introduction

At the end of 2019, a new coronavirus (SARS-CoV-2) associated with clusters of severe pneumonia was identified in Wuhan, China. On 11th February 2020, the World Health Organization (WHO) named the new coronavirus disease COVID-19. On 11th March 2020, with the rapid and sustained increase in the number of the infected and with new outbreaks in various parts of the world, COVID-19 was declared pandemic. Since its beginning to the end of 2020, there were approximately 79 million confirmed cases worldwide with over 1.5 million deaths. [1, 2]

The clinical course of COVID-19 varies from mildly symptomatic or even asymptomatic to severe disease. The most common symptoms are fever, cough, fatigue, sore throat, myalgia, shortness of breath, loss of sense of smell and taste, gastrointestinal symptoms. Approximately 80% of laboratory-confirmed patients have mild to moderate disease, and 6% have severe disease. People over the age of 60 and those with comorbidities such as hypertension, diabetes, cardiovascular disease, chronic

respiratory disease and cancer are at greater risk of death. [3, 4]

Spontaneous pneumothorax as a complication of coronavirus infection was first reported in severe acute respiratory syndrome (SARS) caused by SARS-coronavirus (CoV-1). Its incidence was 1.7% of cases, and it was considered as a negative predictive marker of survival. [70] Cases of pneumothorax have also been reported by various authors in patients with COVID-19. The association between these two diseases, as well as its frequency, has not yet been well studied. [5, 6]

Aim

To present the first three cases of spontaneous pneumothorax associated with COVID-19 pneumonia at University Hospital “Sveti Georgi” Plovdiv, Bulgaria. They were registered in November 2020.

Clinical case 1

A 76-years-old male with positive PCR for COVID-19 and bilateral pneumonia was admitted to the Department of Infectious diseases. He had arterial hypertension, ischemic heart disease and benign prostate

hypertrophy as comorbidities. The laboratory tests at admission were following: HGB – 140.0 g/l; WBC – 34.13 10⁹/l; PLT – 232.0 10⁹/l; SaO₂ – 90% pCO₂ – 29.0 mmHg; pO₂ – 56.0 mmHg; pH (T) – 7.44; glucose – 6.4 mmol/l; CRP – 44.0 mg/l; Prothrombin time % – 34.4 %; INR – 1.57; fibrinogen – 3.46 g/l; D-Dimer – 9.16 mg/l; The treatment regimen was following: Ceftriaxone 2x2 g i.v., Levofloxacin 1x500 mg i.v., Dexamethasone 2x8 mg i.v., Pantoprazole 20 mg 2x1; Clexane 0.4 2x1 s.c. Due to the severe course of the pneumonia with decreasing the SaO₂ below 50% the patient was intubated 11 days after the hospitalization. Two days later the treating physician noticed an increasing in size subcutaneous emphysema. Chest X-ray was performed

and revealed right-sided pneumothorax. A thoracocentesis in 5th intercostal space (Büllau point) was performed by thoracic surgeon. The control X-ray 24 hours later showed left-sided pneumothorax with pneumomediastinum. (Fig. 1) Another thoracocentesis was performed placing a chest tube on the left. The control chest x-rays on the 24th and 72nd hour registered expanded lungs with decreased subcutaneous emphysema. Five days after the onset of the first pneumothorax the patient's condition deteriorated severely with aggravation of the respiratory failure, tachypnoea and hypotension. Despite the resuscitation measures the patient died 5 days after the onset of pneumothorax and 18 days after the admission.



Fig. 1. Left-sided pneumothorax and subcutaneous emphysema.

Clinical case 2

A 33-years-old male with clinical, serological and imaging evidence of COVID-19 pneumonia and no other comorbidities was hospitalized in the Department of Infectious diseases. The laboratory tests at the admission were following: HGB – 132.0 g/l; WBC – 27.65 10⁹/l; PLT – 389.0 10⁹/l; SaO₂ – 86.3 %; pH – 7.401; pCO₂ – 27.4 mmHg; pO₂ – 51.8 mmHg; glucose – 10.7 mmol/l; AST – 38.0 U/l; ALT – 17.0 U/l; Protrombin time, % – 85.9%; INR – 1.02; fibrinogen – 7.74 g/l; D-Dimer – 35,2 mg/l. The patient was intubated and placed on mechanical ventilation 24 hours after the admission due to severe worsening of the

condition with SaO₂ below 40%. 48 hours later, due to deterioration in respiratory status, a chest radiograph was performed and revealed partial bilateral pneumothorax. (Fig. 2) Two chest drains were placed on the left and right. Control radiographs at 24th and 72nd hours registered expanded lungs with infiltrative changes bilaterally. (Fig. 3) Despite the aggressive ventilation regimen and conservative therapy with Remdesivir, Colistin, Meronem, Dexamethasone, Clexane, with a tendency for desaturation and progressive deterioration, the patient died 10 days after the onset of pneumothorax and 11 days after hospitalization.



Fig. 2. Partial bilateral pneumothorax with subcutaneous emphysema.



Fig. 3. Control with expanded lungs and reduction of the subcutaneous emphysema.

Clinical case 3

A 72-year-old female with concomitant arterial hypertension and obesity was admitted in a very serious condition after ineffective home treatment for COVID-19. Due to the poor indicators ($\text{SaO}_2 - 40\%$) the patient was intubated and placed on mechanical ventilation in the intensive care unit. Four days after hospitalization, the patient had deteriorating respiratory status and bilateral cervical subcutaneous emphysema. Chest x-ray was performed and revealed right-sided pneumothorax. (Fig. 4) Right-

sided thoracocentesis with placing a chest drain was performed. The control radiography 24 hours later registered expanded lungs and diffuse infiltrative changes bilaterally. A reduction in subcutaneous emphysema was observed. The patient had a progressive deterioration in general condition, with poor oxygenation and a tendency to hypertension, despite the aggressive ventilation. Lethal outcome was registered in the patient 5 days after the onset of the pneumothorax and 9 days after the hospitalization.

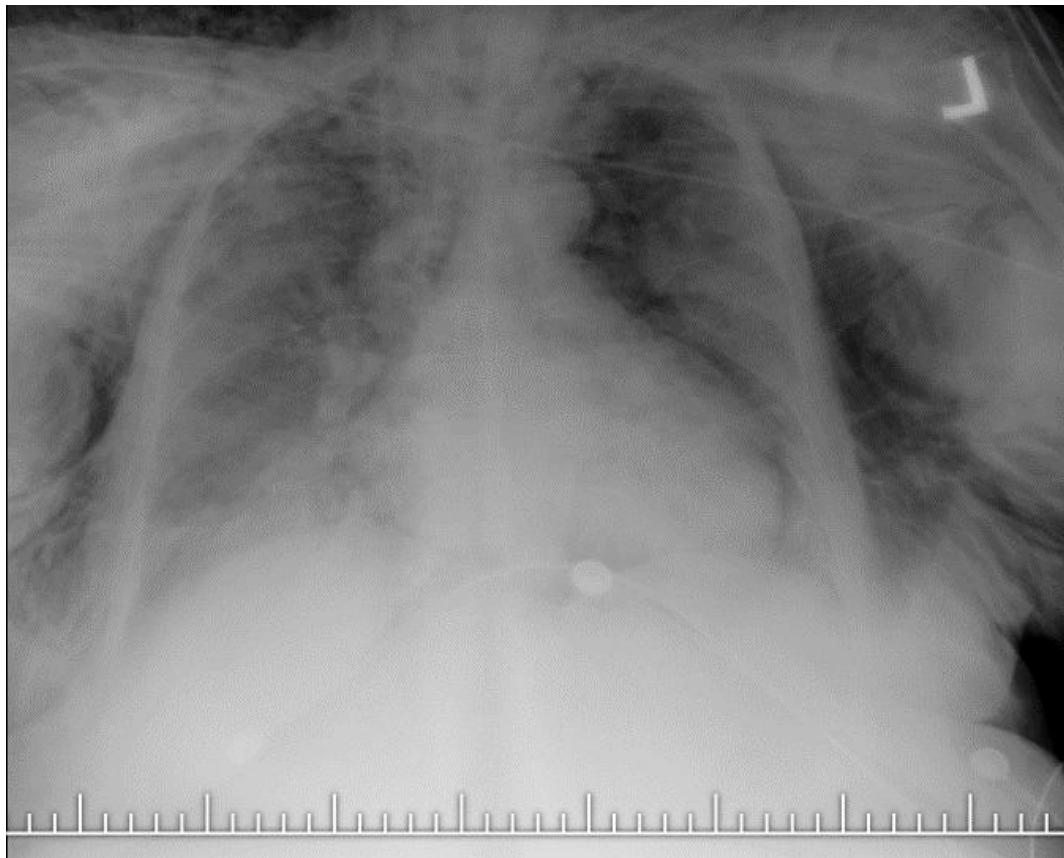


Fig. 4. Massive subcutaneous emphysema.

Discussion

Pneumothorax associated with COVID-19 has been reported in 1% of the hospitalized patients, in 2% of those in the ICU, and in 1% of those who have died from the infection. [7] An association between the barotrauma from the mechanical ventilation and the occurrence of pneumothorax and/or pneumomediastinum was found in the intubated patients, as such was observed in 15% of the cases. [8]

The pathogenetic mechanism of pneumothorax in patients with COVID-19 is explained with the diffuse alveolar damage caused by the virus itself. It leads to alveolar rupture followed by interstitial emphysema and formation of pneumatocele. Multinucleated syncytial cells and atypically enlarged pneumocytes are observed in the alveolar spaces, as a result of the viral cytopathic effect. Pneumocyte desquamation and formation of hyaline membranes that

are associated with acute respiratory distress syndrome may also be detected. Rupture of the pneumocele leads to pneumothorax. This can occur in both non-intubated patients (due to severe cough) and those on mechanical ventilation (as a result of the positive pressure barotrauma).[9]

Pneumothorax is characterized as a serious but relatively rare complication in patients with COVID-19. Chen et al. described only 1 case out of 99 confirmed patients. Yang et al., found only one with pneumothorax out of 92 autopsied patients. [7,10]

A detailed study about the problem was performed by Martinelli et al. They followed up 60 patients with COVID-19 and pneumothorax. They found that it was more common in men and was more often right-sided. It was more common after the age of 50. Both patients on mechanical ventilation and those with spontaneous breathing were

almost equally affected, but the survival rate in the first group was lower. As a therapeutic method of choice, the authors suggested placement of a chest drain. [11] The outcome in patients with COVID-19 pneumonia and pneumothorax depends on the severity of the underlying lung injury and the impaired gas exchange. High-volume surgical interventions are recommended to be avoided. For this reason, the placement of a chest drain, even for a longer period of time (15–20 days), provides very good results. [12]

This article presented the first three cases of pneumothorax associated with COVID-19 in the University Hospital “Sveti Georgi” Plovdiv. All of them were registered in November 2020, when was the peak of the contagion in Bulgaria. Two of the patients were over 70 years old, which corresponds to the most affected age group by the disease. All three cases were observed in patients placed on mechanical ventilation due to the severe course of the underlying disease. The probable mechanism of pneumothorax in them was the barotrauma from the positive respiratory pressure, as well as the diffuse alveolar damage from the virus, leading to the formation of interstitial emphysema and pneumatocele. The surgical method of choice in all three patients was thoracentesis with placement of a chest drain. The same procedure was proposed by the other authors cited in this article. The severe course of the underlying disease combined with pneumothorax as a complication led to lethal outcome in all the three cases.

Conclusion

Pneumothorax is a rare but serious complication of COVID-19. It is often associat-

ed with poor outcome of the disease, especially in patients placed on mechanical ventilation.

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Impaired interhemispheric connectivity associated with long-term neurocognitive disfunctions of a patient after traumatic brain injury

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Diffusion tensor tractography (DTT) and resting state functional magnetic resonance imaging (rsfMRI) are diffusion tensor imaging (DTI) and blood oxygenation level dependent (BOLD) based methods for evaluation of the structural and functional integrity white and grey brain matter, respectively. The brain regions commonly affected by traumatic brain injury include the corpus callosum, internal capsule, mesencephalon and extensive areas of the cortex and cortico-subcortical areas. We present a case of a 56-year-old man who presented a severe traumatic brain injury and was evaluated in his fifth-year follow-up after the trauma. Diverse persistent focal neurological symptoms were detected, including mild cognitive impairments and reduced quality of life on his self-evaluation. In order to examine the underlying functional and morphological substrate of the associated neurocognitive impairments, an automated DTT and rsfMRI were performed. Significant decrease of the number of commissural fibers of the corpus callosum and association fibers of cerebral hemispheres was observed, as well as a decrease in dynamics of the activation of neural networks in different cortical areas on the rsfMRI, compared to a healthy control subject. The social importance of traumatic brain injury sequelae define the need to determine the morphological and functional substrate of the associated neurological and cognitive impairments. The results from imaging studies such as rsfMRI and DTT provide scientific evidence for the mechanisms of development of such long-term dysfunctions. Thus, physicians will be empowered to introduce more efficient and targeted therapeutic and rehabilitation protocols that aim to reduce the neurocognitive deterioration, improving the quality of life and increasing the social restoration of traumatic brain injury patients.

Keywords: Traumatic brain injury, DTI, rsfMRI, quality of life, cognition

Introduction

Traumatic brain injury (TBI) is a major neurosurgical and public health problem [1]. Computed Tomography (CT) is the leading neuroimaging method of choice during the first 24 h after the injury due to its accessibility, cost-effectiveness and shorter imaging time [1-3]. However, conventional CT has limitations caused by beam-hardening effects or artifacts near metal objects which can worsen the image quality and prevent accurate assessment. CT can also miss small hemorrhages [3] [4]. In these cases, the lack of neuroradiological evidence related to TBI led to the use of more sensitive methods for neuroim-

aging such as Diffusion Tensor Imaging (DTI), Diffusion Tensor Tractography (DTT) and resting state functional Magnetic Resonance Imaging (rsfMRI) [5-7].

Case report

In order to demonstrate the clinical use of the contemporary MRI sequences, we present a case of a man, 56-years, who suffered from a severe TBI due to a fall from height. At hospital admission, the patient was evaluated as being 7 point according to the Glasgow Coma Scale (GCS). The initial CT scan of the head revealed intraventricular hemorrhage and contusion of the left cerebellar hemisphere (Fig. 1).

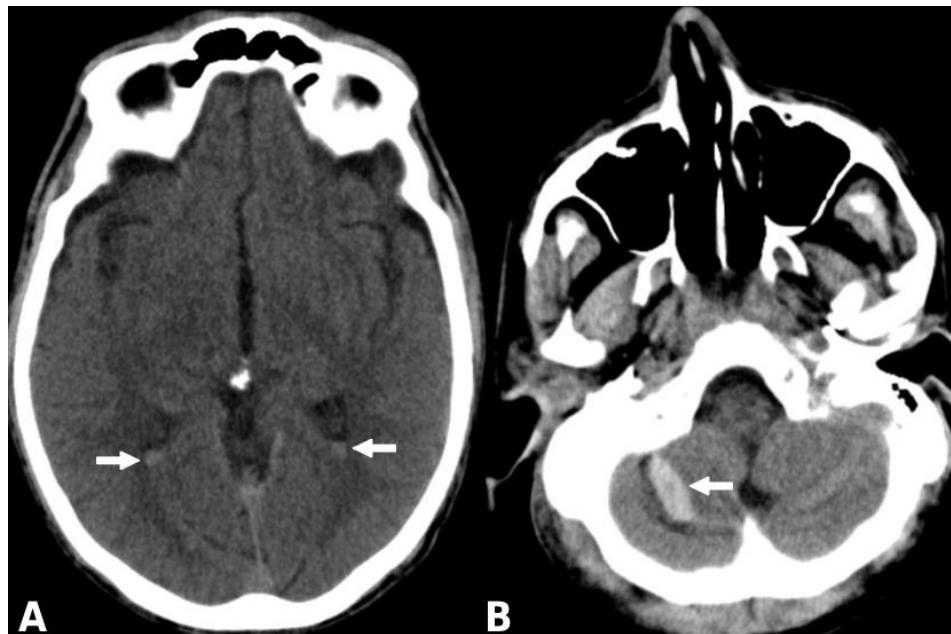


Fig. 1. The initial CT of the patient showing A/ Intraventricular hemorrhage (arrows); B/ Contusion of the right cerebellar hemisphere (arrow).

The first four days of his hospital stay the patient was in the intensive care unit (ICU) for active surveillance of the vital functions. After a recovery of consciousness, he then spent eighteen more days in the Clinic of Neurosurgery before he was discharged from the hospital. At discharge the patient was evaluated as Grade 4 according to the Glasgow Outcome Scale-Extended (GOSE), which is considered as poor outcome from the TBI.

A follow-up at the fifth year after the trauma was performed. The patient was evaluated as Grade 6 according to GOSE. Diverse persistent focal neurological symptoms were detected such as ataxia, dyscoordination, dysphagia, dysarthria, dysphonia and residual left hemiparesis. The cognitive functions were examined using the Mini-Mental State Examination (MMSE) scale and mild cognitive impairments were established, affecting the attention, memory and cognitive domains. The QOL self-

evaluation was assessed with the Quality of Life After Brain Injury (QOLIBRI) scale which revealed physical problems, problems in the everyday functioning and cognitive problems.

Structural and functional MRI were also performed. The structural sequences showed residual post-hemorrhagic zone in the left cerebellar hemisphere (Fig. 2).

The DTT and subsequent 3D reconstruction of fibers showed substantial global reduction in the number of fibers throughout the brain and corpus callosum. The results were compared to a healthy control subject (Fig. 3).

The rsfMRI was used to examine eight regions of interest, including the Default Brain Network (DBN). There was a substantial decrease in dynamics of the activation of neural networks in different cortical areas, compared to a healthy control subject.

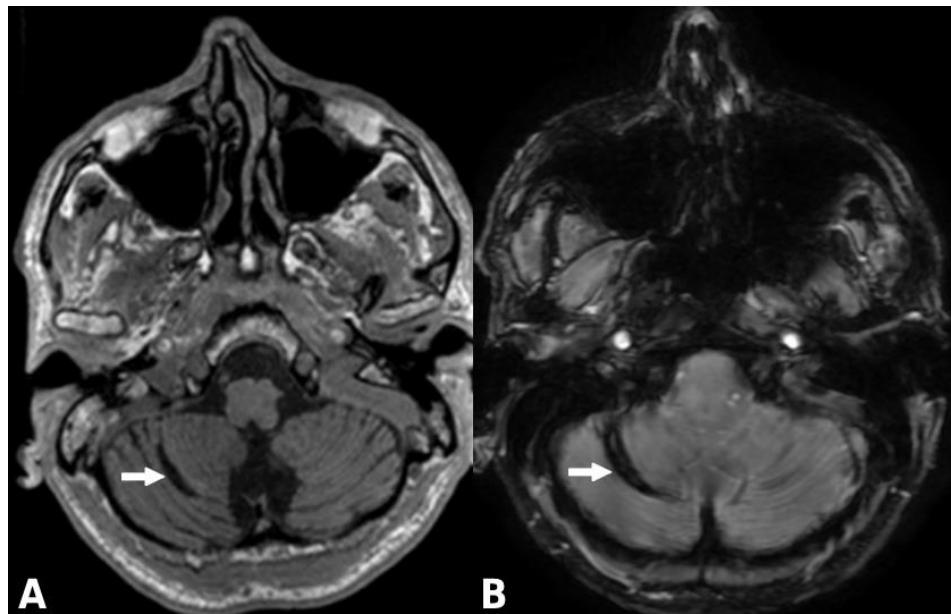


Fig. 2. A/ Axial T1 MRI; B/ Susceptibility Weighted Imaging (SWI) MRI showed residual hemosiderin accumulation (arrow).

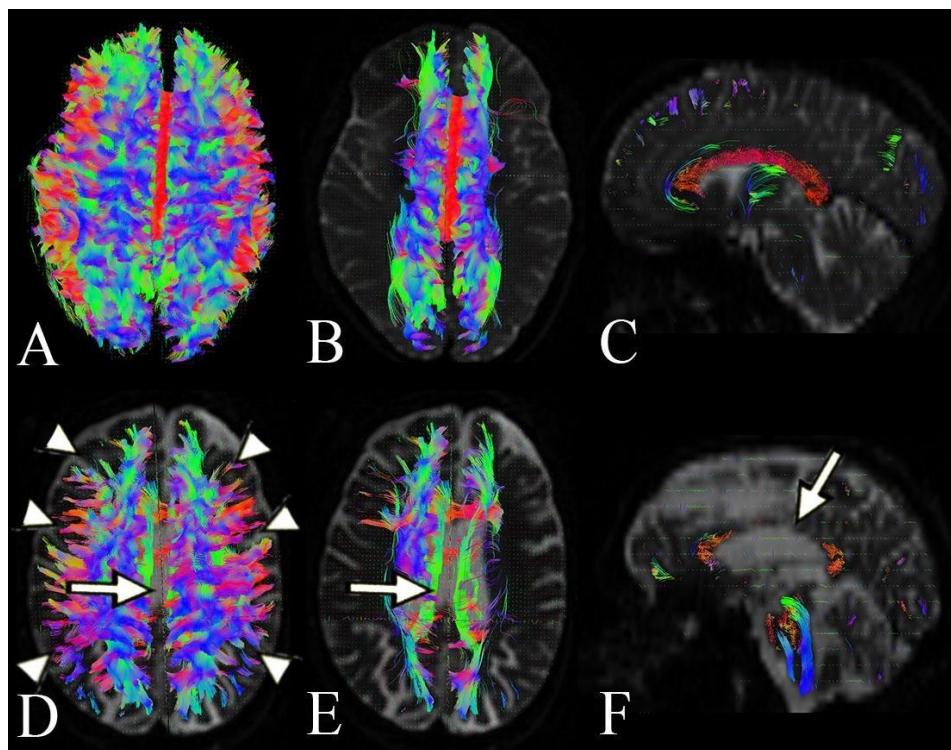


Fig. 3. 3D reconstruction of fibers of a healthy control subject (A-C) compared to a patient after severe TBI (D-F): A/ Superior view of the whole brain tractography; B/ Superior view of the corpus callosum; C/ Lateral view of the corpus callosum; D/ Substantial reduction in the number of fibers in all the brain (arrowheads) and disruption in the fibers of the body of corpus callosum (arrow); E/ Superior view of the corpus callosum; F/ Lateral view of the corpus callosum.

Discussion

The brain regions commonly affected by traumatic brain injury (TBI) include the corpus callosum (CC), internal capsule, mesencephalon and extensive areas of the cortex and cortico-subcortical areas [8]. In many cases CT is unable to detect minor non-hemorrhagic lesions or microscopic hemorrhages, thus, advanced MRI studies are required in such cases [1].

DTI is an advanced specific MRI sequence which allows visualization of white matter tracts that connect different parts of the brain by measuring the anisotropy of water diffusion [9]. DTT allows for 3D visualization of white matter fibers reconstructions derived from the DTI data [10]. Therefore, these methods are used to sensitively detect microstructural white matter changes in patients with TBI that cannot be visualized by conventional CT and MRI protocols [10, 11]. As presented in our case, the initial CT showed no information about a CC injury. However, a substantial reduction in the number of fibers throughout the brain and corpus callosum was established by the DTT.

RsfMRI studies the changes of the levels of oxyhemoglobin and deoxyhemoglobin in the brain cells. This approach is called blood oxygen level-dependent (BOLD) imaging and provides precise analysis of brain networks and their connectivity with other networks and remote cortical brain areas [12]. The most commonly examined network with rsfMRI is the DMN: a set of brain regions that are active in resting subjects [13-15]. The main reason for using rsfMRI in TBI patients is to search for abnormal functional connectivity in comparison with a healthy subject [16]. In our case subsequent group level analysis of rsfMRI will be performed to achieve statistically significant results.

Conclusion

Proper neuroimaging studies are essential for patients after TBI because in the majority of cases they may lead to progressive cerebral impairments such as postconcussive syndrome, traumatic encephalopathy, sleeping disorders and neurodegenerative diseases. The social importance of TBI sequelae define the need to determine the morphological and functional substrate of the associated neurological and cognitive impairments. The results from imaging studies such as rsfMRI and DTI provide scientific evidence for the mechanisms of development of such long-term dysfunctions. Thus, physicians will be empowered to perform accurate diagnosis and introduce more efficient and targeted therapeutic and rehabilitation protocols that aim to reduce the neurocognitive deterioration, improving the QOL and increasing the social reintegration of TBI patients.

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Reconstruction of soft tissue defects of the head with rotational flaps

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The scalp region of the head and neck, in particular, is a common site involved with skin cancer. Most common tumors of the scalp include squamous cell cancer, basal cell cancer, and melanoma. Risk factors for their appearance are age and sun exposure. Often, the tumors progressively advance, with tumor disintegration, severe infection, and engagement of the periosteum or even dura mater. The treatment in these cases includes surgical excision of the tumor with subsequent reconstruction of the defect. Scalp reconstruction includes vascularized soft tissue coverage, acceptable cosmetic appearance, and minimal donor site morbidity. Reconstructive options for scalp defects include local and regional flaps or free tissue transfer. Local flaps have the advantage of good reliability and blood supply, low donor site morbidity, good color match. The aim of this report is to present 5 clinical cases that involve scalp reconstruction with rotation flaps and discuss the results after the surgical interventions. These five patients had advanced squamous and basal cell carcinoma that caused severe defects on the scalp, some of which involved the cranium and dura mater, as well.

Introduction

There are many reasons for scalp defects, such as: injuries, burns, advanced tumors – basal cell or squamous cell carcinoma, and malignant melanoma, difficult recovery due to previous surgical interventions.⁽¹⁾

The clinical cases that we present are related to soft tissue defects of the scalp caused by advanced carcinoma, with tumor decay, superimposed infection, involvement of the periosteum, skull, dura mater, as well.

The treatment involves a clear margin excision of the tumor and subsequent reconstruction of the defect. Reconstruction can be accomplished by using rotational flaps, free flaps, skin graft or tissue expander. The best option for covering large defects is using rotational flaps which provide sufficient blood supply and reliability, low donor site morbidity and good skin color match.^(1,2)

It is necessary to take into account the anatomical features of the scalp, the blood supply, and the size of the defect in order to form a reliable flap. The scalp consists of 5 layers: skin, subcutaneous tissue, galea ap-

oneurotica, loose connective tissue and pericranium, and those layers are supplied by paired arteries that are involved in rich interconnections within the subcutaneous layers. From anterior to posterior, these are the supratrochlear arteries, supraorbital arteries, superficial temporal arteries, the posterior auricular and occipital arteries.^(2,3,4)

The scalp flap is raised below the layer of galea aponeurotica and its length should be substantially longer than the diameter of the defect to prevent excessive tension when closing the wound. Of utmost importance is to preserve the superficial temporal or occipital arteries in order for the flap to survive, as those arteries provide the principal blood supply, and in most cases the entire scalp will survive as long as one of these vessels is present.^(5,6,7)

Clinical case 1:

The first clinical case is a 60-year-old man with an advanced basal cell carcinoma, affecting the pericranium, the skull and the dura mater. (pic. 1) The scanner shows a round hyperdense shadow on the left frontal lobe, which was suspicious of tumor infiltration, as well. (pic. 2) Intraoperatively, it

appeared to be an abscess due to the tumor decay and subsequent inflammation. Tumor resection was made, the infiltrated part of the skull, dura mater and brain were also removed. The resected part of the dura was

substituted for fascia lata of the thigh. Temporoparietal flap was used to close the defect. For the donor site skin graft from the inguinal area was harvested. (pics 3, 4) Pictures 6 and 7: One week after the surgery.



Picture 1.



Picture 2.



Picture 3.



Picture 4.



Picture 5.



Picture 6.

Clinical case 2:

The second patient is a 78-years old man with a history of an advanced basal cell carcinoma, which caused a large defect of the parietal area of the scalp, affecting the peri-cranium, bone and meninges, as well.



Picture 7.

Clear-margin resection of the tumor was performed. Afterwards, rotational temporo-parietal flap along with skin graft from the suprapubic area were used to cover the defect area. (pics. 9, 10).



Picture 8.



Picture 10.



Picture 10.



Picture 11.



Picture 12.

Clinical case 3:

The third clinical case is about a man with a history of an advanced squamous cell carcinoma originating from the lower eyelid and conjunctiva. Exenteration of the orbital content was performed along with ligation of

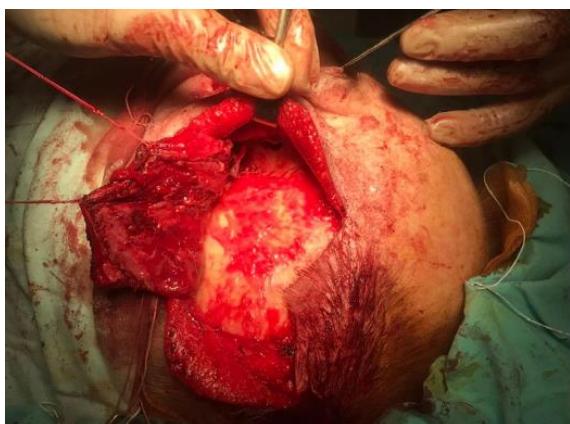
the ophthalmic artery and optic nerve. Temporal muscle flap (*pic. 15*) was used to fill the orbit through an access opening of the lateral orbital wall. (*pic. 16*) This muscle flap provides blood and nutrient supply to the walls and prevents skull deformities.



Picture 13.



Picture 14.



Picture 15.



Picture 16.



Picture 17.



Picture 18.

Clinical case 4:

The fourth clinical case is about a patient with a squamous cell carcinoma affecting the left medial canthus and conjunctiva and spreading between the medial orbital wall and the eyeball. Exenteration was per-

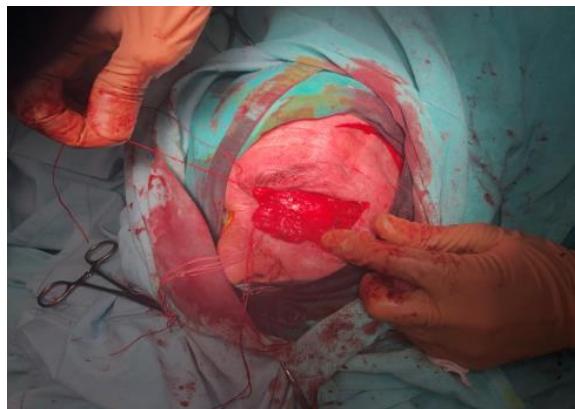


Picture 19.



Picture 21.

formed with a temporal muscle flap reconstruction to substitute the removed orbital content. Access opening of the lateral wall of the orbit was made for the temporal muscle. (pic. 20) The residual eyelids were sutured together.



Picture 20.



Picture 22.

Clinical case 5:

The last case is about a 65-year-old patient with a history of parasagittal meningioma. A surgery, 2 years prior to the reconstructive one, was successfully performed with a removal of the tumor. The access opening of the skull was covered with a titanium plate and a skin flap. Due to complications, the wound did not heal properly

and the edges retracted with time. As a result, the titanium plate was exposed, which put the patient into risk of a serious infection. The surgical intervention included removal of the plate, debridement, closure of smaller openings, and covering the defect with a temporoparietal flap and additional skin graft from the inguinal area. (pic. 24)



Picture 23.



Picture 24.



Picture 25.



Picture 26.

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Retrograde intrarenal surgery – opportunities and future

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Retrograde intrarenal surgery /RIRS/ gained considerable popularity throughout the last decade thanks to the progress of cutting-edge technologies and the perfection of instruments used for that purpose. From a rarely used method in the past, this surgery has currently turned into an essential therapeutic opportunity regarding the treatment of urolithiasis, as well as for the diagnosis and the treatment of low-risk urothelial carcinomas of the upper urinary tract. The objective of this synopsis is to follow through and summarize the opportunities and the contemporary trends of the method. The presented data are based on publications for over a period of five years /2016–2020/, and personal experience. The profound analysis outlines the following fundamental trends: constant improvement of the effectiveness profile and safety of the used technics throughout production of new generations of fiberoptic and digital utereroscopes. It is assumed that in the future they will be refined by expanding the indications and the scope of application, for instance, for patients with anatomic abnormalities, obesity, hemorrhagic diathesis, pregnant women and others. However, for lithotripsy the correlation between effectiveness and price, compared to other treatment methods, still remains a matter of debate. In conclusion, RIRS has established as a procedure with constant advance in the applied technique. It became broadly popular by virtue of its minimal invasiveness, its short healing time and good results. In the next stages of its development this method needs to reach a better correlation priceand effectiveness, and to expand its usage in wider borders of indications.

Introduction

Retrograde intrarenal surgery (RIRS) is a multistep procedure which is primarily used to treat urolithiasis and low-risk urothelial carcinomas of the upper urinary tract. The current evolution of the flexible ureteroscope and auxiliary equipment has facilitated the procedure and the success rates have risen. From a rarely used method in the past, this type of surgery has currently turned into an essential therapeutic opportunity for patients with renal stones smaller than 2 cm. It is also a choice for treatment in cases with unsuccessful Shock-wave lithotripsy (SWL), Percutaneous nephrolithotomy (PCNL) or for those who have contraindications regarding the latter (pregnancy, obesity).

Evidence acquisition

The presented data are thoroughly based on scientific publications for over a period of five years /2016–2020/, along with personal experience.

Objective

The objective of this synopsis is to follow through and summarize the opportunities, indications and contemporary trends of the method.

Historical evolution

The historical evolution of endoscopic access to the renal collecting system for the diagnosis and treatment of upper urinary tract diseases via flexible ureteroscopy (fURS) has been closely related to the advent of technology. Initially described by Marshall in 1964 – it had only diagnostic, not therapeutic properties. In 1971 Takayasu reported the first successful fURS in humans, but the technique did not gain popularity at that time. After 16 years, in 1987, Bagley described the technique and its indications and implemented the use of flexible ureteroscopes in the clinical practice not only as a diagnostic, but also as a therapeutic procedure. Later G.J. Fuchs and A. M. Fuchs systematized it in the way as it is known nowadays.

From past to present

Between the discovery of RIRS to it becoming a well-established procedure and standard for treatment of renal stones 1–2 cm in size, lies a long technological progress. The use of fURS brought a significant advance in terms of efficacy and satisfactory outcomes, regarding RIRS. Improvements have been made including miniaturization of the scopes and the ancillary devices, image quality, durability and visibility. Development of the endourologic armamentarium has always integrated the necessity of reducing the caliber of the latest generation of fURS, with a particular dedication to the design of the tip of URS. Several companies have provided their URS with a tapered tip, allowing a size reduction at the distal tip less or equal to 9 Fr, which remarkably corresponds with the CT-scan measurements of the ureteral diameter [1]. The durability of the fURSs has always been an important matter of discussion. A randomized comparative trial evaluated the life span of different URSs and concluded that the mean device durability ranged from 5,3 to 18 cases before major repairs were needed. The introduction of the digital scopes led to the improvement of the surgical results due to the better visibility, manoeuvrability and durability [2]. Recent studies show that digital scopes allowed a 20% reduction of the operative time, which was considered as a result of their better visibility and maneuver ability [3]. Despite their similar construct design, the weight of the URS also varies. The digital URS, weighing less than the fiberoptic ones, are said to decrease the muscle activity during operation, thus reducing the muscle strain and preventing the operator of fatigue [4][5]. Another milestone in the ureteroscopy in the current decade are the single-use fURS. Disposable scopes have been devel-

oped to overcome some of the limitations of the reusable fURS, such as the ability to keep optimal 270° deflection in both directions all the time, the lack of sterilization, which may potentially save time and enable a reduction of the costs. Single-use fURS are always readily available and sterile and do not require a dedicated sterilization process. However, fURS do hide some hazards which should be considered. Technical weakness when operating a challenging case due to low built quality and unexpected instrument deficiencies such a spontaneous loss of vision or deflection mechanism failure may appear [6].

Methodology

Chronologically speaking, the procedure (RIRS) may begin with preoperative stenting and end with postoperative stenting. For this matter the term Double-J stent needs to be introduced. Double-J (D-J stent) is a self-retained stent, commonly made of polyurethane, which is placed in the ureter. It represents a thin tube, usually 5 or 6 Fr in diameter and 26 to 30 cm in length. The name D-J stent refers to the J shape at each end of the stent. One of the ends remains in the collecting part of the kidney (pelvicalyceal system) and the other – in the urinary bladder. Its aim is to ensure passive dilatation of the ureter in order to facilitate the access [7]. It is still a matter of debate whether preoperative stenting is indicated in all patients. Recent studies suggest that it should be used if there are difficulties encountered when accessing the ureter [7]. Jessen et al [8] published a retrospective survey in which 253 patients with renal stones only underwent RIRS with and without a pre-RIRS stent. Higher Stone Free Rate (SFR) and lower complication rate were registered, regarding the patients with a pre-RIRS stent. These outcomes

were attributed to the passive dilatation of the ureter, the sustained low intrarenal pressure and the easier navigation with fURS in the collecting system. No advantages were noticed for what concerns the operative time. Then the procedure continues by the bladder being entered either with a cystoscope or a semi-rigid ureteroscope, in order to visualize its walls and to identify the location of the ureteric orifices. A hydrophilic guidewire is sent to the ureter, under fluoroscopic guidance, when the ureteral orifice is seen [9]. Even though the use of a second hydrophilic guidewire (safety guidewire) is not compulsory, it ensures the access to the collecting system and presuming that an ureteral wall injury occurs, it facilitates the insertion of a stent [10]. After the guidewire is sent, the renal pelvis is reached through the ureter with a semi-rigid ureteroscope. Thus, the ureter can be examined for a possible stricture and if ureteral stones are also present, they can be treated as well. Once the renal pelvis is reached, the semi-rigid ureteroscope is being removed, and the flexible ureteroscope is inserted either via an Ureteral Access Sheath (UAS) or directly through the guidewire under fluoroscopic control. Afterwards, a nelaton catheter can be placed into the bladder to prevent it from overdistention. An appropriate UAS should be chosen according to the flexible ureteroscope used. To choose the right UAS for the certain patient, some more factors should be taken into consideration: stone burden, endoscope size, upper urinary tract anatomy and surgeon's preference. Its major advantages include: easy repetitive access to the kidney for stone fragments removal, less traumatism to the scopes, decreasing the intrarenal pressure by drainage of the irrigation fluid around the scope [2; 9]. It is suggested that the 10/12Fr UAS may be considered the ideal size fitting for

most of the fURSSs, guaranteeing an optimal balance between intrarenal pressure and irrigation flow [2]. Various papers have shown that it decreases the operation time and increases the SFR. The use of UAS is also associated with fewer postoperative infective complications as a result of the lower intrarenal pressure. It should be taken into consideration that when UAS is used, ureteral wall injury is possible. Therefore, the use of UAS is recommended in patients with higher stone burden, requiring more intraureteral passages to retrieve fragments and in those patients who are prestented. Into the already inserted UAS, a flexible ureteroscope (fURS) is entered. The currently used single use digital ureteroscopes are equipped with ancillary devices such as laser fibers, baskets and graspers. The standard for stone lithotripsy during fURS has become the laser lithotripsy using Holmium: YAG laser due to its efficacy and safety. It is effective for any composition of stones. The stones are being ablated via a photothermal effect, which results in the residual water in the stone absorbing the laser radiation, thus creating a vapour pressure that breaks the stone [11; 12]. The current generation of laser lithotripters has enabled us to set three different parameters – pulse energy (J), pulse frequency (Hz), pulse duration, according to the effect we want to produce. Fragmentation: High energy (1–2 J), low frequency (3–5 Hz) and short pulse duration. The stones are being broken into smaller fragments, which need to be removed subsequently with a basket. Dusting: Low energy (0,2–0,5 J), high frequency (10–20 Hz) and long pulse duration. The stones are being reduced to fine dust and fragments smaller than 2 mm in diameter, that are supposed to evacuate spontaneously, which therefore removes the necessity of time-consuming stone fragment retrieval.

The combination of these techniques is possible and should be considered in order to achieve better results. Selecting the most appropriate technique depends on intrarenal anatomy, stone size, its composition and location. Although still under exploration, the thilium fiber laser is said to become an equal alternative to Holminum: YAG laser in the near future due to its smaller fibers, which can contribute to reducing the working channel size [13]. During the operation a relocation of a lower pole stone to a more suitable position within the collecting system by means of a nitinol basket is possible. Studies have shown that the replacement technique had higher SFR for patients with lower pole stones of 1–2 cm, than the fragmentation in situ. After the stones are treated, the procedure can end with postoperative stenting. Its main potential benefit is better control of postoperative pain. However, studies suggest that routine postoperative stenting is not beneficial to all patients and should be performed in selected cases as ureteric injury during fURS, residual fragments, ureteral strictures and pregnancy. Also, an ureteral stent may be placed to prevent the incidence of postoperative ureteric obstruction and renal colic secondary to ureteral edema. Postoperative prophylaxis with broad spectrum antibiotics is recommended by the EAU. In terms of complications, our experience with fURS shows 13,1% incidence, which according to literature data may vary between 6–16%. Regarding the ureteroscope itself, no technical problems or damage were observed.

SFR in RIRS and PCNL

When comparing the SFR in the management of renal stones some predictive factors for stone-free status should be taken into consideration: stone volume, stone number, stone density, hydronephrosis, sur-

geon experience and stone location in the lower calyx. Regarding the latter, when treating lower calyceal stones, multivariate analysis found an infundibulumpelvic angle of $< 30^\circ$, short infundibular length, large infundibular width and low calyceal pelvic height as factors associated with a lower SFR. Some studies have shown similar SFR of RIRS in such cases compared to PCNL [14].

As a result of the growth of expertise and refinement of technology many surgeons try to push indication for RIRS for larger renal stones, which are traditionally managed by PCNL. When comparing the two techniques for renal stones not located in the lower calyx, SFR for RIRS is inversely related to stone size. On the contrary, SFR for PCNL is the least affected by stone size. For treatment of stones > 20 mm, single procedure SFR is in favour of PCNL compared to RIRS, although RIRS is often superior to PCNL in terms of lower complication rates, blood loss and hospital stay. Series with more than 50 patients reported mean SFR between 88% and 93,5%. However, a few reports showed comparable SFR between PCNL and RIRS when retreatment rates were not considered. When treating renal stones under 20 mm RIRS showed a significant greater SFR compared to micro-PCNL.

RIRS in the management of special conditions

When special medical conditions such as bleeding diathesis, obesity, skeletal anomalies and pregnancy are present, initial surgical approach with RIRS for treatment of ureteral and renal stones is recommended. RIRS is also a modality of choice in patients with liver dysfunction and thrombocytopenia. Therapies in which aspirin, clopidogrel and warfarin are present do not

serve as a contraindication for performing RIRS.

Studies have shown that obese patients with renal or ureteral stones up to 20 mm were effectively managed with RIRS. SFRs in these patients ranged between 33% and 97%.

Considering that PCNL and SWL are both contraindicated in the setting of pregnancy, urolithiasis in this cases has been successfully managed via RIRS [15]. Although the majority of the available studies include a small number of RIRS cases, none of them registered an obstetric complication which resulted in any serious foetal or maternal damage.

RIRS showed high effectiveness in treatment of stones in anomalous and horseshoe kidneys. Although in this cases PCNL has been regarded as the golden standard, its invasiveness and complications are superior to RIRS. Stones in transplanted kidneys have also been successfully treated with RIRS. High SFRs and lack of deterioration of the renal function of the kidney were noted.

In the recent years RIRS has also gained popularity in the treatment of pediatric cases with stones bigger than 20 mm. The need of adapting the instruments to a smaller caliber of urinary organs is essential in order to extend RIRSs use to a wider range of patients and indications. Another positive aspect regarding RIRS is associated with its lower radiation exposure and complication rates in comparison with PCNL.

Conclusion

The retrograde intrarenal surgery has established as a diagnostic-therapeutic procedure with constant advance in the applied technique. It became broadly popular by virtue of its minimal invasiveness, short healing time and good results. In the next

stages of its development this method needs to reach a better correlation between price and effectiveness. However, we believe that future upgrades will position this technique as a treatment of choice for a wider range of indications.

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Recurrent laryngeal nerve injury in thyroid surgery

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Introduction: The risk of damage to the recurrent laryngeal nerves in thyroid surgery has been known. Nowadays, the frequency of this complication is significantly reduced due to the use of new surgical methods. Despite the efforts, nerve damages remain high and depends of the volume of the surgery and surgical experience. **Objective:** To analyze causes leading to recurrent laryngeal nerves injury in thyroid surgery, and create a manners for their prevention. **Materials and methods:** For this time interval 2012–2016 in the department of Surgery in UMHAT "Kaspela" – Plovdiv, n = 2104 surgeries of the thyroid gland have been done. The thyroid pathology is as follows the multinodular goiter with Graves diseases 33%. The second place is taken by patients with euthyroid nodular goiter 26%, while thyroid cancer represents 10%, followed by Hashimoto 6% and other diseases 25%. **Results:** Injury to the recurrent laryngeal nerve was observed in n = 21 patients (0.99%). In n = 16 of them (76%) the surgery was performed due to recurrent nodular goiter and five (23.81%) because of advanced thyroid carcinoma. Fortunately there was no permanent damage of the recurrent laryngeal nerve in any patient and all of this cases were fully recovered after 1–3 month. **Conclusion:** The highest rate of postoperative complications of RLN injuries are registered in patients with recurrent nodular goiter and advanced carcinomas. The identification of the nerve, using dissection and/or neural monitoring, decreases the incidence of neural damage and transient and permanent paresis.

Keywords: Thyroidectomy, Recurrent laryngeal nerve, Neuromonitoring

The risk of damage to the recurrent laryngeal nerves in thyroid surgery has been known for a long time. Galenus reports that harming the recurrent laryngeal nerve in monkeys and pigs leads to a serious deterioration of the phonation. Nowadays, the frequency of this complication is significantly reduced due to the use of new surgical methods. Despite the efforts, nerve damages remain high 0.4–7.8% of the cases depending on the volume of the surgery^{1,2}.

The main risk factors for damaging to recurrent laryngeal nerves are:

- ✓ malignancies of the thyroid gland;
- ✓ large nodular or ectopically located thyroid gland;
- ✓ anatomical abnormalities of the nerve;

- ✓ non-identification of the nerve during surgery;
- ✓ high degrees goiters;
- ✓ previous thyroid surgery.

The prevention of injuries to RLN depends on pre- and intraoperative approaches. A preliminary study is recommended for the detection of existing laryngeal dysfunction in the case of a prior neck surgery or a voice pathology study during the clinical examination. Echternach et al., performed systemic pre- and post-operative laryngoscopy to check the vocal chords function of 761 patients. They prove that 20% of patients with postoperative RLN dysfunction had previous surgery, with (18%) recurrent nerve injuries and voice changes⁴. (Fig. 1)

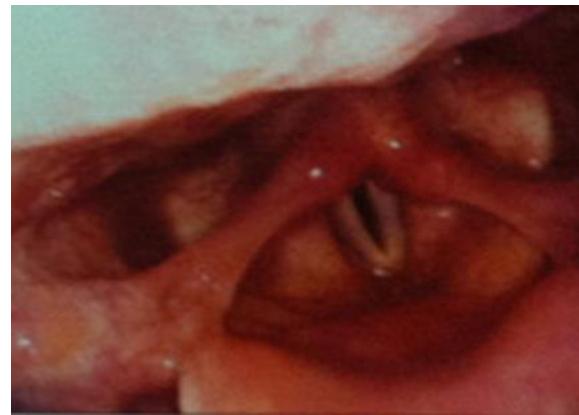


Fig. 1. a/ Normal vocal cords.

Intraoperative prevention is based on gentle dissection techniques, avoiding too much towing, improving of haemostatic techniques, and using methods for detecting and monitoring the recurrent laryngeal nerve.⁵

As for the various techniques of haemostasis, classical clamping and ligation, metal clips, monopolar or bipolar coagulations have been displaced by newer ones such as thermofusion and ultrasound haemostasis. Two meta-analyses comparing hemostasis with thermofusion⁶ and ultrasound did not reveal a significant difference in the incidence of RLN injuries, respectively 2.5% transient and 0.5% permanent nerve damage.

Regarding to methods related to nerve detection, three main approaches are used, each have advantages and disadvantages.



b/ Paresis of the right vocal cord.

Lateral access is most commonly used, while the lower approach (uncovering the RLN under the inferior pole of thyroid gland) may be safer if there is prior surgery in the neck area. Upper access is preferred when there is large goiter or with substernal location. Regardless of the used approach, the nerve must be systematically identified and traced; In 1938 Lahey demonstrated that nerve tracking significantly reduces the risk of his injury^{7,8}.

Neural monitoring is a method used in thyroid surgery for decades in some thyroid centers. It is developed to assist intraoperative nerve recognition and to clarify the mechanisms causing damaging despite their meticulous identifying and preservation.⁹ (Fig. 2)

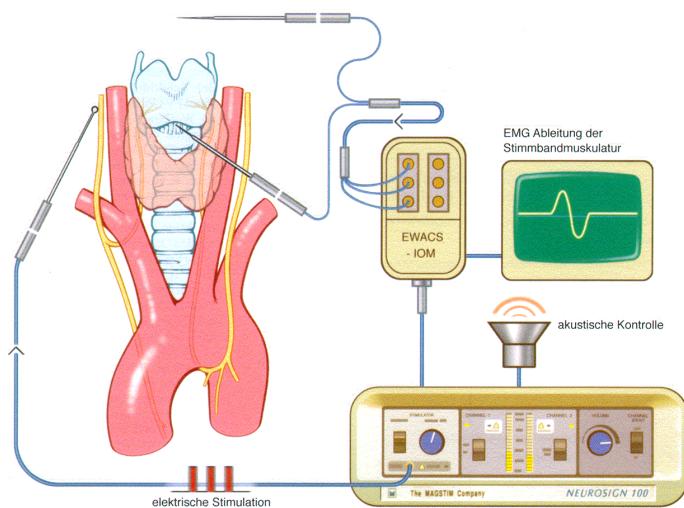


Fig. 2. Intraoperative neuromonitoring (internerve.com).

Objective

To provide study and analysis of causes leading to damage to recurrent laryngeal nerves in thyroid surgery.

Materials and Methods

For the described period 01.01.2011–01.01.2016 there were n = 2104 thyroid surgery were done in the Department of Surgery of the University Hospital “Kaspela” – Plovdiv. The structure of thyroid pathology indicates that the highest relative part 33%, was occupied by patients with Marin-Lenard syndrome. The second place with 26% is borrowed by euthyroid nodular goiter, while thyroid cancer taking third place 10%. Following are patients with Hashimoto and others diseases.

Results

The type of surgery is as follows: total thyroidectomy n = 1231(58.50%) lobectomy n = 812 patients (38.60), near total thyroidectomy n = 12 patients (0.58%), MRLND unilateral n = 42 patients (1.99%), bilateral MRLND n = 7 patients (0.33%). (Fig. 3)

Injury to the recurrent laryngeal nerve was observed in the n = 21 patients (0.99%), In n = 16 of them (76.19%) main pathology was recurrent nodular goiter and in n = 5 (23.81%) advanced thyroid cancer.

In all cases the surgical procedure was total thyroidectomy. We were not observed permanent lesion of RLN its function was fully recovered within 1–3 months. For preservation of the nerve we used visual identification during its cervical route until reaching the laryngeal musculature.

Discussion

The frequency of RLN injury ranges between 0.5–5% according to different authors.¹⁰ Our results indicate injury in 0.99% of the patients, mainly observed in recurrent goiter and advanced carcinomas, which coincides with the literature data and is due to the available dense adhesions and the changed anatomy from previous surgery, or nerve involvement from the tumor process in Berry's ligament. The technique and the surgeon's experience are not irrelevant.¹¹

Typically, nerve damage is unilateral and transient, but rarely it may be bilateral.¹² To avoid these complication it is necessary to tracing the RLN during him cervical route. Visual identification of RLN allows the surgeon to work with a significant level of safety, to assure his anatomical integrity as well as to save additional nerve branches if they persist.¹³ The visual identification of the nerve (Fig. 4) significantly reduces the incidence of neural damage and it ranges between 0–2.1%¹⁴

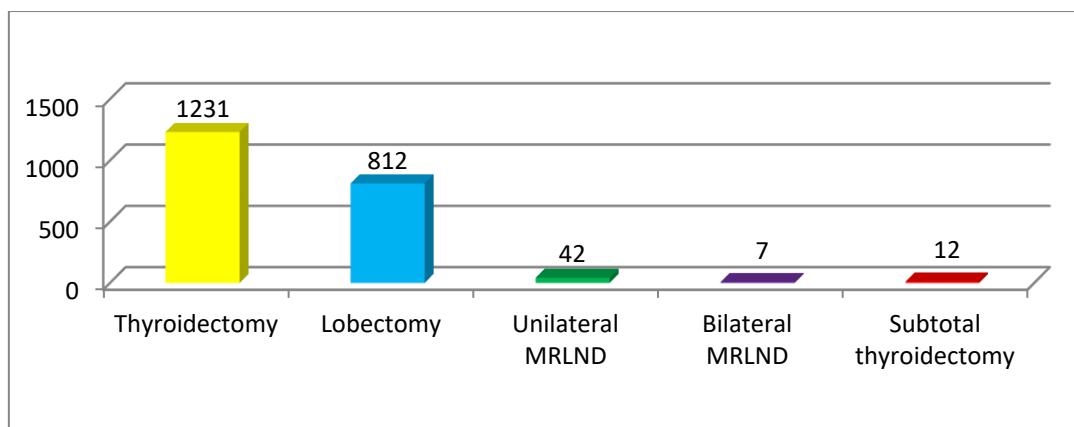


Fig. 3. Types of surgeries.

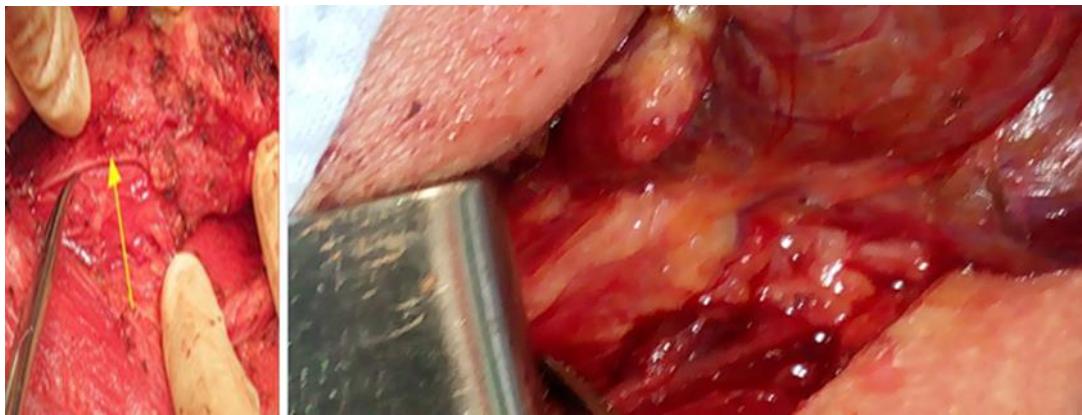


Fig. 4. Intraoperative nerve tracking and its entry into the larynx.

According to a study of 447 patients, neural monitoring has a positive prognostic value of 40% and 100% negative prognostic value for nerve injury with a sensitivity of 63% and a specificity of 97%.¹⁵ Barczynski is comparing the identification of n. laryngeus reccurens by standard dissection and neural monitoring in n = 1,000 patients undergoing total thyroidectomy and concludes that neural monitoring reduces basically the incidence of transient paresis of RLN.¹⁶

Higgins meta-analysis proves that the systemized visualization of the recurrent laryngeal nerve is still the best technique to reduce the risk of its injury. He announces rate of nerve injury of 3.25% using neural stimulation and 3.12% in cases with nerve identification.

Conclusion

According to most thyroid surgeons, the misidentification of the recurrent nerve during surgery increase the risk to be injured. Surgeons disagreeing with visual identification point that the nerve could be injured during the identification, as well as the need for – anatomical knowledge of its normal cervical course and anatomical variations. They recommend subtotal lobectomy or intraparenchymal dissection as a measure for the preservation of RLN. The most ef-

fective method to prevent RLN injury in thyroid surgery is still being sought.

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Laparoscopic splenectomy our experience

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Introduction: First splenectomy was carried out by Andirano Zaccarello in 1549 years of a young woman with an enlarged spleen who survived six years after surgery. After introduction of laparoscopic splenectomy in 1991 by Delaitre and Maignen it goes wide in practice. **Aim:** To present our experience and results in the treatment of the elective spleen diseases, and presenting the advantages of laparoscopic versus conventional splenectomy. **Materials and methods:** This study includes n = 28 patients with conventional or laparoscopic splenectomy in the Department of Surgery of UMHAT "Kapsela" for the period 2015–2019. The information was gathered from a hospital records and post discharge follow up. **Results:** For the described period of time n = 28 patients with splenic diseases had surgery, 21 (75%) underwent laparoscopic and 7 (25%) conventional splenectomy: n = 16 men (57,14%) and n = 12 women (42.86%). The average age of those patients were 51 years (± 12) and the medium hospital stay 6 days (± 2). Postoperative complications occurred in n = 2 (9.52%) patients. **Conclusion:** Laparoscopic splenectomy is safe and reliable procedure having the advantages of laparoscopic surgery including short operating time, lesser operative trauma, low incidence of post-operative complications, less hospital stay. With the accumulation of experience in mini invasive surgery, it becomes the first choice for the treatment of elective spleen diseases.

Keywords: Laparoscopic splenectomy, Spleen surgery

Introduction

First splenectomy was carried out by Andirano Zaccarello in 1549 years of a young woman with an enlarged spleen who survived six years after surgery^{1,2,3}. Quittenbaum performed the first successful splenectomy on haematological disease in 1862⁴. Over the years, removal of the spleen became a main option in traumatic injuries and in some of the haematological diseases. The rapid development of laparoscopic surgery in the 1990s did not pass the spleen as a subject of mini-invasive surgery. After introduction of laparoscopic splenectomy in 1991, by Delaitre and Maignen it goes wide in practice⁵. Despite the difficulties and fears of working on a large parenchymal organ, the need for special technical skills to deal with a possible hemorrhage and the long learning curve, many authors quote studies indicate that laparoscopic splenectomy is feasible, safe, and with significant advantages over conventional surgery⁶.

Aim

To present our experience and results in the treatment of the elective spleen diseases, and evaluating the advantages of laparoscopic versus conventional splenectomy.

Materials and methods

This study includes all patients with open or laparoscopic splenectomy in the Department of Surgery of UMHAT "Kapsela" for the period 2015–2019. The information was gathered from a history of the disease.

Results

During this time interval n = 28 elective splenectomies were performed, n = 26 of them were about for hematological disease at n = 1 for sub capsular hematoma, and n = 1 for abscess.

Of these n = 21 (75%) laparoscopic and n = 7 (25%) conventional. In conventional group we used a median laparotomy, in the laparoscopic we prefer three trocars technique (2x10 mm and 1x5 mm) and a lateral approach. (Fig. 1)

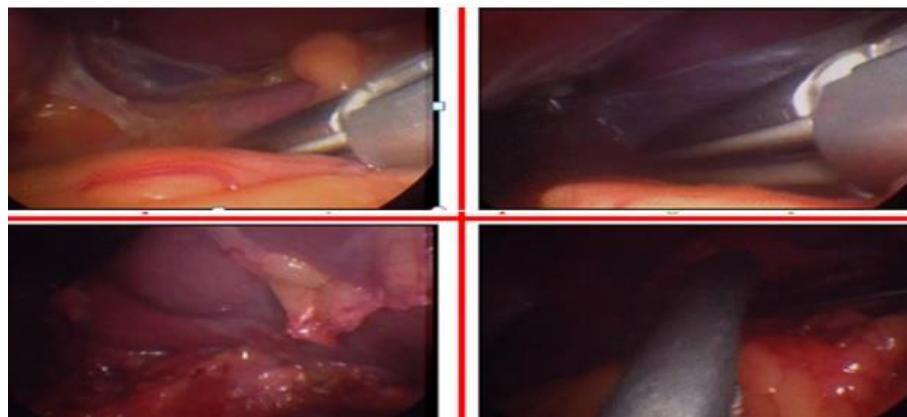


Fig. 1. Stages of laparoscopic splenectomy.

The sex distribution is as follows males n = 16 (57.14%), females n = 12 (42.86%). The mean age of patients was 51 (± 12) years. The average hospital stays is 6 (± 2) days in laparoscopic and 9 (± 2) days in the conventional group. Others comparison between two groups (Table 1).

Table 1. Comparison between conventional and mini invasive group

	Laparoscopic	Conventional
Hospital stay	6 / ± 2 /	9 / ± 2 /
Analgesics stop	after 24 h	after 72 h
Flatus and defecation	24–36 h	36–48 h
Verticalization	after 24 h	after 48 h
Blood loss	30–50 ml	50–70 ml
Duration of surgery	83 min	102 min

Complications were observed in n = 2 (28.57%) of patients in the conventional and also in n = 2 (9.52%) of the mini-invasive group. The complications are as follows (Table 2, Fig. 2):

Table 2. Complications

Complications	Conventional	Mini invasive
Bleeding in early post-operative period	-	1
Post-operative bowel obstruction	1	-
Wound inflammation	1	1

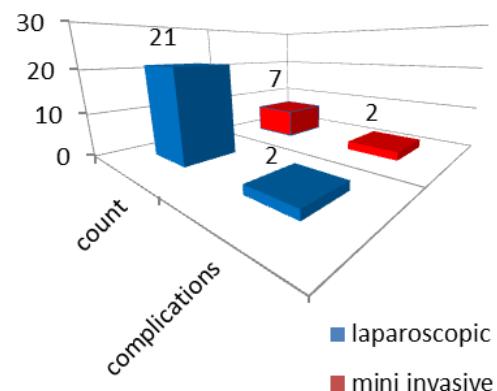


Fig. 2. Complications in both groups.

Bleeding in the early postoperative period in the laparoscopic group originated from the abdominal wall at the site of insertion of the working port. It was controlled by placing hemostatic sutures. Inflammation of the wound was observed at n = 2 patients, respectively, one from each group. In mini invasive group inflammation involved the area where the specimen was removed the same it was improved within 3–4 days with removal of skin sutures and insertion of drains. In conventional group a severe inflammation was observed and it was treated with opening of the entire wound, and repeated debridement of necrotic tissue till the appearance of fresh granulations. Subsequent secondary suture was done.

An early bowel obstruction was observed in conventional group that was most

likely due to fresh adhesions and overcome with conservative treatment within two days.

The extension of hospital stay in patients with complications in both groups is as follows (Fig. 3):

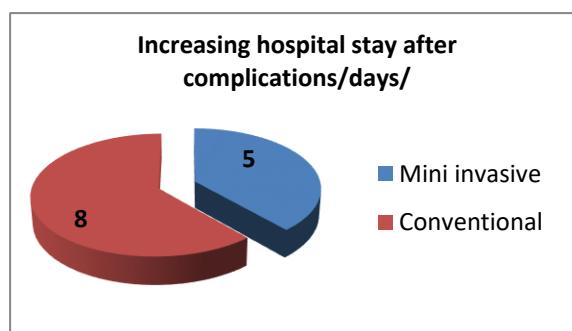


Fig. 3. Increasing hospital stay in complicated cases.

We can compare our results with literature date (Table 3).

The average surgery duration in our laparoscopic group is 83 minutes and 102 minutes in conventional group. This difference is mostly due to the time it takes to close the surgical wound. Other authors in-

dicate a duration of laparoscopic splenectomy in the range 88–170 min, but always shorter than the conventional group, as are our results.^{7,8,9} As a reason for reducing the operating time is the accumulation of experience in laparoscopic surgery called “Learning curve effect”.

The hospital stay, as we have already noted in the mini-invasive group, is 6 (\pm 2) days and in the conventional 8 (\pm 2) days, considerably longer than the literature data on average 2.8–3.3¹⁰ days. This difference is due to compliance with the provisions of the health insurance fund in Bulgaria.

We only made a conversion in one patient (4.76%). The reason for this was the presence of abscess in the spleen, with formation of the abscess cavity by the left flexure, the tail of the pancreas, the stomach, and the accompanying severe adhesions, which complicated the safe dissection of the anatomical structures. The frequency of conversion by literary data is between 4–6%¹⁰.

Table 3. Literature data complications rate. (Br J Surg. 2011)

Series	Year	N	Operation time (minutes)	Hospital stay (days)	Follow-up (months)	Recurrence/non-responders (%)	Conversion rate (%)	Morbidity (%)
Lee <i>et al.</i> ^[12]	1997	15	200	6	12	7	0	20
Meyer <i>et al.</i> ^[13]	1998	16	123	4.6	12	14	0	0
Chung <i>et al.</i> ^[14]	1999	40	128	7	29	10	0	7.5
Stanton ^[15]	1999	30	150	2.3	30	11	7	13.3
Szold <i>et al.</i> ^[16]	2000	60	78	2.3	16	14	0	5
Schwartz <i>et al.</i> ^[17]	2001	8	70	2.5	32	13	0	12.5
Bresler <i>et al.</i> ^[18]	2002	27	90	7	28	0	3	11
Delaitre <i>et al.</i> ^[19]	2002	209	144	6.1	16	8	17	10.5
Keidar <i>et al.</i> ^[20]	2003	12	80	5.5	36	25	0	33
Pace <i>et al.</i> ^[21]	2003	52	160	2	51	13	3.8	5.7
Wu <i>et al.</i> ^[22]	2004	67	150	3.2	23	26	0	5
Berends <i>et al.</i> ^[23]	2004	50	159	5.5	41	14	22	7
Khan and Nixon ^[24]	2007	40	NS	2.9	60	13	4	3.4
Kang <i>et al.</i> ^[25]	2007	59	125	NS	54	11	5	10.9
Prasad <i>et al.</i> ^[26]	2009	29	139	2	19	14	2	24
Current series	2011	20	100	4	7	5	10	5

One of the criticisms of laparoscopic splenectomy is the omission of additional spleens and recurrence of the hematological disease. In a large study carried out by Brunt et al., It was found that additional spleen removal was documented in 15% of the patients, a result that corresponds to the incidence of this anatomical anomaly occurring within 10–20% of patients undergoing splenectomy for hematological disease. Unlike these data, only in 4.8% of the cases in conventional group have been removed additional spleens¹¹. In our case, we have not experienced one and we have no data on the development of the relapse of the disease for this reason.

Conclusion

Laparoscopic splenectomy is safe and feasible procedure having the advantages of laparoscopic surgery including shorter operating time, lesser operative trauma, low incidence of post-operative complications, shorter hospital stay. With the accumulation of sufficient experience in mini invasive surgery, it becomes the first choice for the treatment of elective spleen diseases.

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Ultrasonography as a method of intraoperative navigation in surgery of nonpalpable breast lesions – initial results

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The increasing breast cancer awareness result in the occurrence of more clinically negative breast lesions. As a result of the previously mentioned statement, nowadays more and more breast-conservative surgeries are made. The utilization of ultrasound as a method of intraoperative navigation in breast cancer surgery is a relatively new concept in surgical oncology, which aims not only to obtain cancer-free resection margins, but to spare more healthy breast tissues and structures not infiltrated by tumor cells. The aim of this article is to present the intraoperative ultrasound as a technique of a navigation and the results we obtained while using it. Metrics were presented with Mean and Standard Deviation (SD). Nominal and ordinal data are presented in number and percentage (%). The initial results of our study present a total number of seven patients, who were eligible for intraoperative ultrasound surgery. Mean age of the patients – 54.70 (\pm 10.48). Malignant tumors were found in 4 (57%) and in 3 (43%) of them – benign. The imaging diagnostic size measurement of the tumors was 1.026 cm (\pm 0.23) and the pathological – 1.11 cm (\pm 0.33). The pathological tumor stage was T1b in 3 (75%) women and T1c in 1 (25%) of them. The shortest mean distance from the tumor was 0.35 cm (\pm 0.21) and the mean distant – 1.90 cm (\pm 0.73). Exact localisation and tumor-free resection margins were obtained in all of the patients. The results of the study so far show that intra-operative ultrasound is a reliable and helpful tool for tumor localization and exact oncological excision.

Introduction

Breast carcinoma is now the oncological disease with the highest morbidity and mortality rate in woman in the world. In the United States one in eight women is diagnosed with breast cancer during their life. (1, 2). Most of the developed countries worldwide offer screening programs and awareness initiatives leading to the occurrence of more patients affected by this disease but in an earlier stage.

The concept of the surgical treatment of these patients nowadays is in the direction of de-escalation. Worldwide recommendation are on one hand – the oncological accuracy and on the other – preservation of healthy breast tissue, in order to optimize the aesthetic appearance of the breast. The utilization of intraoperative ultrasound

(IOUS) as a method of navigation in breast cancer surgery is a relatively new concept in surgical oncology, which aims the current goal of breast surgery – not only to obtain cancer-free resection margins, but to spare structures not infiltrated by tumor cells.

Aim

The aim of this article is to present the intraoperative ultrasound as a technique of a navigation and the initial results we obtained while using it.

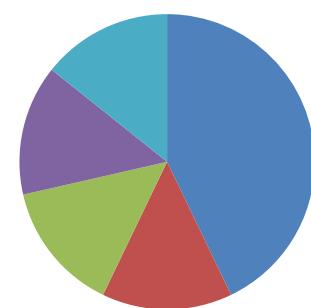
Methods

Metrics were compared by t-test for independent samples and presented with Mean and Standard Deviation (SD). Nominal and ordinal data are presented in number and percentage (%).

Results

The initial results of our study present a total number of seven patients, who were chosen after randomization. Six of them were operated in the Surgical Department of the Complex Oncological Center in Plovdiv and one of them was operated in the Surgical Department of Kaspela University Hospital. The mean age of the patients was 54.70 (± 10.48) years old. The mean body mass index was 31.20 (± 7.25). Nonpalpable lesions were found in the left breast of 5 (71%) patients and in the right of 2 (29%) of them. The imaging diagnostic size measurement of the tumors was 1.026 cm (± 0.23) and the pathological – 1.11 cm (± 0.33). Malignant tumors were found in 4 (57%) of the patients and in 3 (43%) of them – benign. The pathological distribution of the tumors was as follows: 3 (43%) of the tumors were an invasive ductal carcinoma, 1 (14%) invasive lobular cancer, 1 (14%) complex fibroadenoma, 1 (14%) leiomyoma and one mixed-type fibroadenoma (14%) (Fig. 1). The pathological tumor stage was T1b in 3 (75%) women and T1c in 1 (25%) of them (Fig. 2).

Pathological distribution



- Invasive ductal carcinoma
- Invasive lobular carcinoma
- Complex fibroadenoma
- Leiomyoma
- Mixed-type fibroadenoma

Fig. 1.

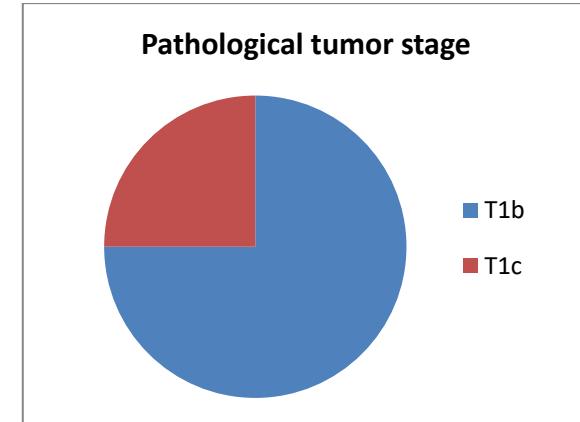


Fig. 2.

The shortest mean distance from the tumor was 0.35 (± 0.21) cm and the mean distant – 1.90 (± 0.73). Exact localization and tumor-free resection margins were obtained in all of the patients. There was no necessity of performing a re-excision or a secondary surgery in all of the patients. There were no compilation occurred in the intra- or post-operative period of every patient.

Discussion

After the results obtained by U. Veronesi, Fisher and Forrest it was clear, that breast-conservative surgery, followed by adjuvant radiotherapy is now a standard treatment of early breast cancer and its effectiveness and safety, do not distinguish from the results of the mastectomy (3, 4, 5). The worldwide guidelines propose that negative resection margins are being achieved when there are no tumor cells on ink or a distance of 2mm in ductal in situ carcinoma (7, 8, 9).

These factors draw attention to different kinds of methods aiming to localize lesions that were small in size and nonpalpable. There are series of studies that demonstrate high rates 90 and 93% of negative resection margins using intraoperative ultrasound technique (10, 11).

The results we obtained so far show a 100% identification rate with 100% nega-

tive resection margins in T1b and T1c tumors. The strongest predictor for local recurrence is the surgical resection margins (11-17). In specialized establishments where a frozen section pathological examination of the resection margins is made, there is a lower risk of leaving cancer cells in the tumor bed and re-shaving of the specific tumor border could be made, but a consecutive surgery may also lead to the excision of more healthy tissue, resulting in a worse aesthetic outcome.

Intraoperative ultrasound was also performed in small benign lesions with full success.

Conclusion

The initial results are part of a bigger prospective, randomized study regarding the intraoperative ultrasound navigation for clinically negative breast lesions.

Despite the rare pathology. so far, the presented data are favorable, but in order to make a valuable statement, more cases are going to be included.

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Retrospective 10 year analysis of breast cancer prevalence in Plovdiv, Smolyan and Pazardzik region

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Breast cancer is the most common cancer in female patients and ranks first as a leading oncological cause of their death worldwide. The aim of our study is to present a retrospective complex analysis of the prevalence of breast cancer in the regions of Plovdiv, Smolyan and Pazarzik for a period of 10 years. The data for this analysis was obtained from the oncological register of the Complex oncological center in Plovdiv, Bulgaria. The results were summarized in tables with appropriate graphics. For comparison of proportions, Fisher's exact test was used. Statistical analysis showed that the widest range ($N = 27$) of morphological types of oncological breast diseases is observed in the Plovdiv region, followed by Pazardzhik and Smolyan. The highest number of new cases of breast cancer were registered in age group between 61 and 80 years. The relative share of new patients in this age group is between 45% (2018) and 49.60% (2017) of the total number of registered cases. The lowest number of new cases of breast cancer were registered in age group ≤ 30 years. The most common morphological type is the invasive ductal type followed by the lobular type. Regarding the degree of differentiation, the highest relative share make up cases of moderately differentiated breast cancer (G2). Stage II breast cancer predominate in the majority of age groups, with few exceptions, most often in the most age group > 81 years, in which stage IV carcinomas are more common.

Keywords: Breast, Breast cancer, breast cancer surgery, surgery

Introduction

Breast cancer is the most common cancer in female patients and ranks first as a leading oncological cause of their death worldwide based on the statistics of the International Agency for research on Cancer" and the "World health organization".

According to the previously mentioned sources, in Bulgaria for 2018, there were more than 4000 newly diagnosed cases of breast cancer. (1)

Aim

The aim of our study is to present a retrospective complex analysis of the prevalence of breast cancer in the regions of Plovdiv, Smolyan and Pazarzik for a period of 10 years.

Materials and methods

The data for this analysis was obtained from the oncological register of the Complex oncological center in Plovdiv, Bulgaria for the period of 2010–2019 year. The results were summarized in tables with appropriate graphics. For comparison of proportions. Fisher's exact test was used.

Results and discussion

For Plovdiv district (Fig. 1) the lowest number of newly diagnosed cases of breast cancer was observed in 2017 ($N = 353$) and the highest in 2011 ($N = 451$).

Over a ten-year period, women with breast cancer accounted for between 98% and 99.80% of the total number of new cases, and the relative proportion of men varied between 0.20% and 2% (Fig. 1).

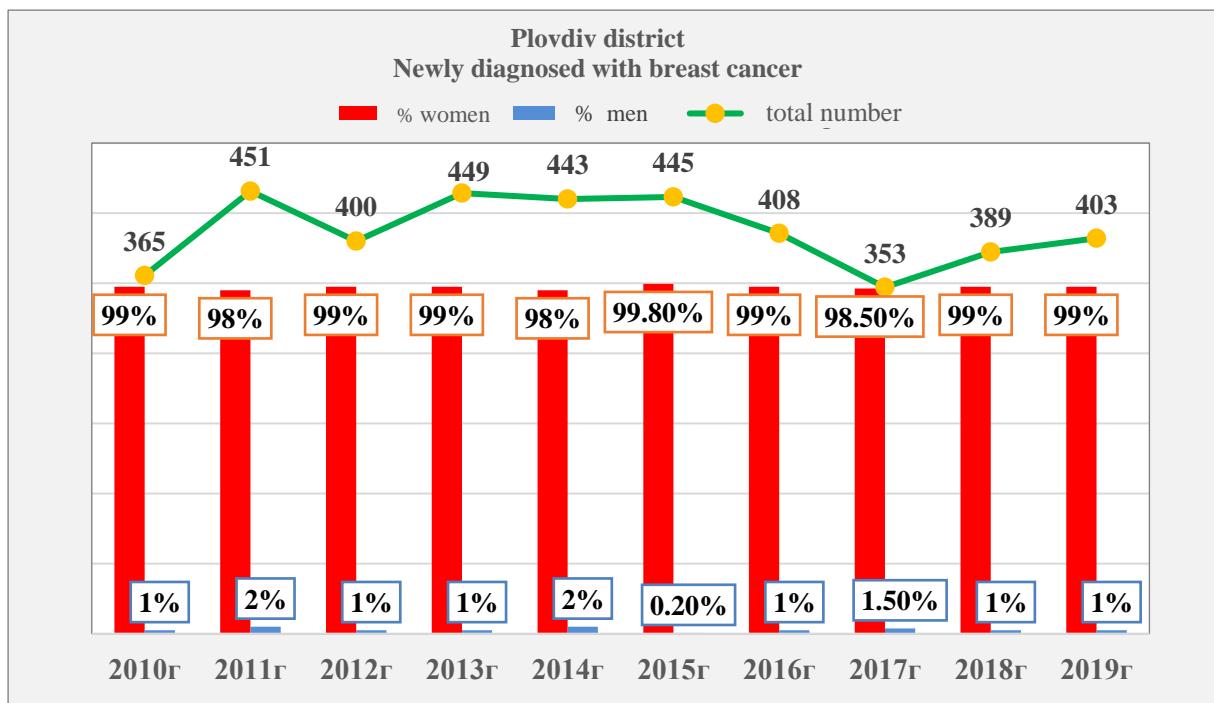


Fig. 1.

The dynamics in the newly diagnosed cases of breast cancer in Pazardzhik district for the period from 2010 to 2019 is illustrated in Fig. 2.

The lowest number was established in 2018 ($N = 99$) and the highest in 2010 ($N = 141$). The distribution of cases by gender,

shows a relative share of women between 100% (2014) and 96% (2018 and 2019). Accordingly, the relative proportion of men with breast cancer varies between 0% and 4%, with the highest percentage in the last two years, 2018 and 2019, of the study period.

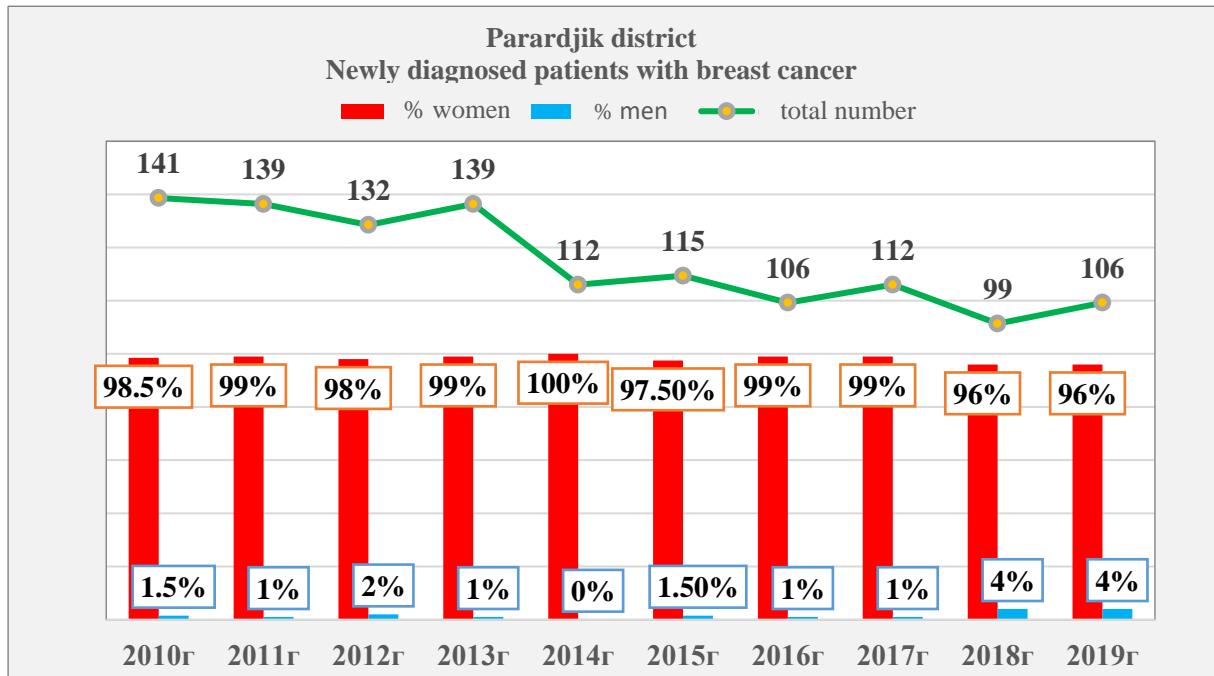


Fig. 2.

For Smolyan district, the dynamics in the incidence of breast cancer can be traced in Fig. 2. The least cases ($N = 41$) were registered in 2019 and the most ($N = 54$) in 2012, 2013 and 2014. Women make up 100% of patients, with the exception of 2011 and 2012, when 2% of registered cases were men and 98% women.

The total number of newly diagnosed cases of breast cancer for the three districts (Plovdiv, Pazardzhik and Smolyan) for each year and the relative share of cases for each quadrant, based on the International Classification of Diseases nomenclature (ICDN) are presented on Table 1.

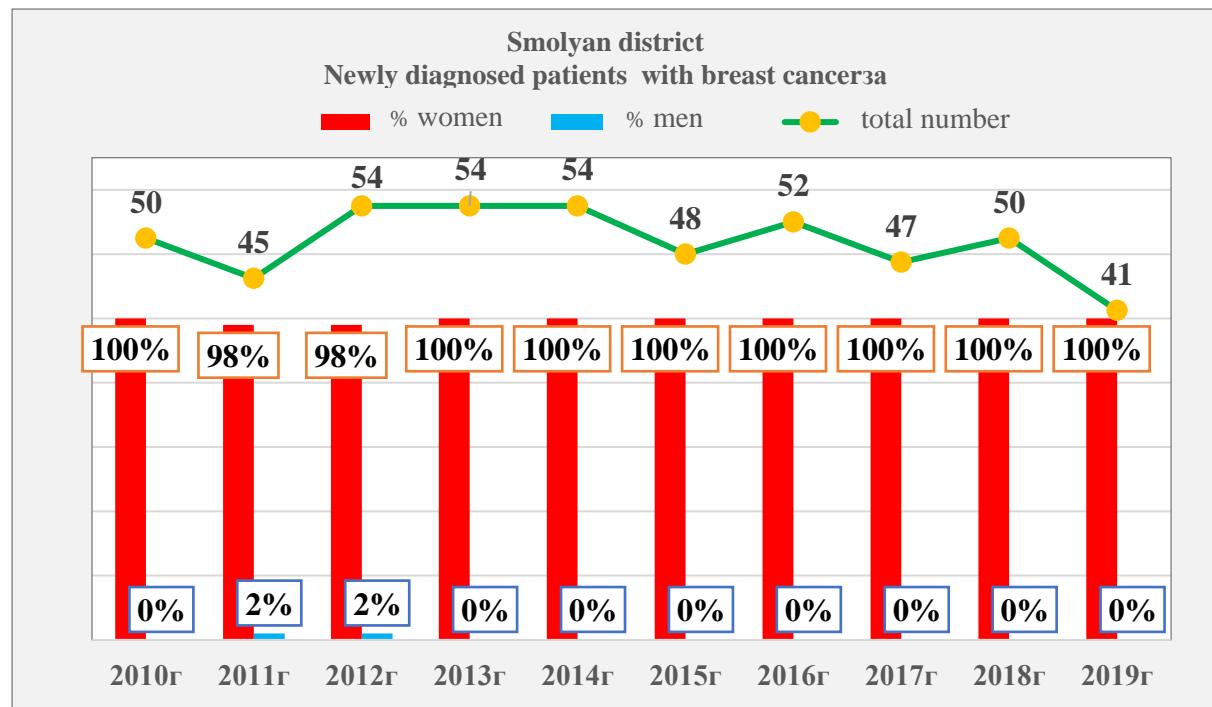


Fig. 3.

Table 1.

ICDN	Year									
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
C50.0	10.7%	15%	9%	11%	11%	10%	3%	2%	1.50%	2%
C50.1	20%	17%	18.5%	15%	16%	11.50%	11%	16%	14%	10.80%
C50.2	10%	7%	10%	10%	12%	15%	13%	14%	12%	15%
C50.3	4%	4%	5%	6%	6%	5%	6%	5%	6%	4%
C50.4	44%	45%	43.5%	46%	42%	46%	50%	47%	46.5%	48%
C50.5	9.7%	11%	11%	9%	10%	8%	9%	10%	9%	9%
C50.6	0.30%	0%	0%	0%	0%	0.20%	0.40%	0.20%	0%	0.20%
C50.8	0.30%	0.60%	2%	1%	1%	1.30%	4%	4%	6%	8%
C50.9	1%	0.40%	1%	2%	2%	3%	3.60%	1.80%	5%	3%
TOTAL %	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Number	556	635	586	642	609	608	566	512	538	545

The analysis, showed that the most common localization was the upper outer quadrant, followed by the central and the least affected was the so called axillary elongation of the gland.

The widest range ($N = 27$) of morphological types of oncological breast diseases were observed in the Plovdiv region, followed by Pazardzhik ($N = 19$) and Smolyan ($N = 11$).

The most common morphological types for the three regions was the invasive ductal cancer followed by the lobular cancer.

The highest number of new cases of breast cancer were registered in age group between 61 and 80 years. The relative share of new patients in this age group was between 45% (2018) and 49.60% (2017) of the total number of registered cases. The lowest number of new cases of breast cancer were registered in age group ≤ 30 years. Regarding the degree of differentiation, the highest relative share is the moderately differentiated breast cancer (G2).

In all age groups, throughout the period from 2010 to 2019, the lowest relative share were cases of undifferentiated breast cancer (G4), with a range of variability between 0% and 2%.

In the period 2010–2019 the highest relative share were cases of stage II cancer, with a range of variability between 34% (2018) and 44% (2018). The second relative share are the cases with stage I cancer, with a range of variability between 26% (2010) and 34% (2019). Cases of stage III cancer account for between 17% (2013) and 25% (2018) of all registered cases. The lowest is the relative share of registered cases with stage IV breast cancer. Their percentage

varies between 6% (2015 and 2018 year) and 11% (2011).

Conclusion

The occurrence of breast cancer vary widely, because of population structure, lifestyle, genetic s and environment (2, 3). Potential reasons for the decreasing numbers of breast cancer cases in these current regions, could be multifactorial. Urbanization, social media influence, alternative medicine and breast cancer awareness programs influence different parts of the society in a delicate and controversial ways. The high relative share of stage one breast cancer patients, shows that even without a working screening program, there is a positive tendency of diagnosing this disease in an early form, with the ability to initiate the complex oncological therapy with the highest chance of definitive treatment, better quality of life and disease free survival rate.

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V. Dental Medicine session

Selective laser melting in CAD/CAM dentistry

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Introduction: Selective laser melting (SLM) is an additive manufacturing technique for production of metal components directly from a 3D CAD model, by fusing fine layers of metal powder using high-power source of a focused laser beam. SLM is a method for creating metal framework of dental restorations when using innovative CAD/CAM technologies. **Purpose:** The aim of this study is to review the advantages and disadvantages of dental restorations made of Co-Cr alloys, produced by SLM, to evaluate their quality and compare it with conventionally fabricated ones. **Materials and methods:** A computerized database search was conducted for peer-reviewed scientific research studies regarding the use of SLM in Co-Cr dental alloys. The online Research Databases used for the purpose of this article were PubMed, Dentistry & Oral Sciences Source (EBSCO), ScienceDirect and Research Gate. **Results:** Dental Co-Cr frameworks made by SLM have more homogeneous microstructure with minimal porosity due to local melting and rapid solidification of metallic powder. Compared to cast technique, mechanical and electrochemical properties of SLM restorations are enhanced, but hardness and stiffness are increased. SLM provides better internal fitting, marginal adaptation and comparative bond strength to porcelain.

Conclusion: Selective laser melting of Co-Cr alloys is a good example of new technologies based on digitization. SLM technique appears promising given the limitations of casting and milling techniques. It provides less expensive and more quickly produced metallic dental restorations without compromising their quality, gaining ground in the everyday production of prosthetic restorations.

Introduction

Selective laser melting (SLM), also known as selective laser sintering, is a specific 3D printing technique, which utilizes high power-density laser to fully melt and fuse metallic powders to produce near net-shape parts with near full density (up to 99.9% relative density) [1]. The technique is developed to melt and fuse metallic powders via high power-density laser. The operating principle of an SLM system is that a 3D file of the model of the desired object, created by a CAD software, is divided into vertical or horizontal layers and sent to the laser-sintering device [2]. The principle of the SLM process starts with a building platform applied with very thin layers of metallic powders, which are completely melted later by the thermal energy induced by one or several laser beams. The cross-section area of the designed 3D part is built by se-

lectively melted and re-solidified metallic powders in each layer. The building platform is then lowered by a small distance and a new layer of powder is deposited and levelled by a re-coater. The laser beam(s) can be directed and focused through a computer-generated pattern by carefully designed scanner optics. Therefore, the powder particles can be selectively melted in the powder bed and form the shape of 3D objects according to the CAD design [3]. SLM is more eco-friendly technology, compared to conventional methods like casting and milling, because it's based on digital technologies (3D imaging, intraoral scans, computer-aided design and computer-aided manufacturing – CAD-CAM, cone-beam computer tomography – CBCT, computer-aided implantology) and unnecessary materials for impressions, gypsum models are not wasted [4].

The most commonly used material is Co-Cr alloy that consists of 53–67% of Co, 25–32% of Cr, 2–6% of Mo, and small quantities of W, Si, Al, and others [5]. Dental alloys on the basis of cobalt and chromium are one of the most preferred for production of metal frameworks of dental restorations because of their high strength, high corrosion and wear resistance, high biocompatibility, and a relatively low cost as well as the accuracy and surface roughness of dental restorations are discussed. These properties are due to the relatively large amount of Cr, dense passive layer of Cr₂O₃ with 1–4 nm thickness on the surface as well as carbides formed in the microstructure of the details. The biological, mechanical and structural properties of dental alloys depend on the manufacturing process and the technological programs [6, 7]. Since the beginning of the last century, lost-wax casting is the most common method for production of metal restorations in dentistry [4]. At this conventional technology, the metal frameworks are fabricated by centrifugal casting using hand-built wax patterns. The technological process is characterized with large amount of manual work, and sometimes it may lead to low accuracy and unsatisfactory quality. Better accuracy can be achieved by using computer-aided design and computer-aided manufacturing (CAD/CAM) and 3D printing systems [8].

Purpose

The aim of this study is to review the advantages and disadvantages of dental restorations made of Co-Cr alloys, produced by SLM, to evaluate their quality and compare it with conventionally fabricated ones.

Materials and methods

A computerized database search was conducted for peer-reviewed scientific re-

search studies regarding the use of SLM in Co-Cr dental alloys. The online Research Databases used for the purpose of this article are PubMed, Dentistry & Oral Sciences Source (EBSCO), ScienceDirect and Research Gate. The following key words were used: “selective laser melting”, “dentistry” “Co-Cr”, “CAD-CAM”. EBSCO provided a total amount of 41 scientific articles; 34 of them were used for the purpose of this review. The search included articles, published in the last 15 years – 2006–2021.

Results and Discussion

SLM devices and the accompanying CAD software are constantly developing along with the worldwide developing of engineering and software technologies, and new products are being launched regularly in the markets [9]. One of the most common used SLM devices are SLM Realizer 2, MCP-HEK (SLM Tech Center, Borch, Germany)[10], PM 100 Dental System (Phenix Systems, Clermont-Ferrard, France), Bego Medifacuring System (Bego Medical, Bremen, Germany), Biomain AB (Helsingborg, Sweden) [11], and EOSINT M250 Xtended (EOS, Munich, Germany) [12]. Each system is being used with a specific metallic powder. But Microstructure, porosity, and mechanical properties of dental frameworks are dependent not only on the alloy but also on the operational parameters of the SLM device, such as laser power and scan spacing [12].

Therefore, a comparison among different studies requires knowledge of the elemental composition of the material and manufacturing parameters as well. Through backscattered electron images from the surfaces of SLM, milled and cast Co-Cr alloys are established significant differences in their microstructure. The microstructure of the sintered Co-Cr alloy is lamellar in na-

ture, with two dominant phases – an indexed X-ray Powder Diffraction (XRD) diagram shows the presence of both the face-centred cubic phase (fcc, or also known as ϵ -phase) of Co or Cr and the hexagonal close-packed phase (hcp, or also known as γ -phase) of Co [13]. The SLM technique revealed no second or intermetallic phase, based on the differences in mean atomic number [14]. Contrary to the structure of SLM, the other techniques show a dispersed heavier second phase in the matrix, which is a common finding for cast [12] and milled surfaces [15]. When a restoration is machined with the milling technique, the Co-Cr alloy block is prefabricated by the manufacturer, without information about the thermomechanical history of the initial block; therefore, great differences in microstructural characteristics can be expected among different manufacturers. The latter has been experimentally verified in a recent study reporting microstructural differences in Co-Cr blanks for dental machining [15]. Regarding the observation of a cast Co-Cr specimen, the microstructure has a less bulky and more dendritic-like morphology [16]. Cast Co-Cr alloys have the characteristic of dendritic microstructure with a dispersed heavier phase in interdendritic positions, while the microstructure of SLM is dependent mainly on operational parameters. On the other hand and contrary to SLM and cast methods, the microstructures revealed by milling depend not on the technique but solely on the characteristics and thermomechanical history of premanufactured blocks [15]. The microstructure of the specimens made with SLM gives high corrosion resistance and could not be etched by immersion in any reagent [17]. Pores with different sizes, elongated along the direction of the layers melting, as well as unmelted powder can be observed in the

whole volume. Distribution of the chemical elements in the dense areas of the SLM sample is observed, which provides more homogeneous microstructure [18].

Porosity is undesirable, as it causes the mechanical properties to deteriorate [19] and increases susceptibility to corrosion, such as crevice corrosion and pitting corrosion [20]. Basically, porosity is the presence of pores or small holes in a structure. SLM and milling techniques provide structures with limited internal porosity, in contrast to cast Co-Cr dental alloys and these differences are attributed to the characteristics and limitations of the fabrication technique [21]. Theoretically, the SLM technique provides structures with up to 100% nominal density of the sintered alloy [14] but this is strongly dependent on the proper adjustment of operating conditions including laser power, scan spacing, scan rate, and scan thickness [12]. This might explain the contradictory findings for the presence of slight porosity [22] and pore-free structures [14]. A fundamental difference is that the development of the porosity in SLM and casting is dependent on the operational parameters of the technique and material limitations, while that in milling, as in microstructure, is solely dependent on the initial quality of Co-Cr blanks [15].

Mechanical properties of Co-Cr-Mo alloys depend on the microstructure, its morphology and composition, γ - ϵ ratio [18]. Slight changes in the elemental compositions of carbide-forming elements, such as W, Cr, Mo, or elements with a low atomic radius, such as C or N, may subsequently affect mechanical properties [23]. Mechanical properties are affected by many factors, such as phase, grain orientation, grain boundary conditions, defects, etc [18]. When samples with dendrite morphology and carbides with round shape and small

sizes are present, the dendrites are composed of γ phase with lower strength, while the interdendritic regions consist of γ phase/intermetallic eutectic with carbides, defining the higher hardness. The prevailing volume fraction of the lower strength γ phase is the main reason for the lower hardness of the cast samples, while the microstructural inhomogeneity is responsible for the higher fluctuations of the hardness values. The SLM process characterizes with high heating and cooling rates, leading to fine grained microstructure of the solidified layer. As the heat is lead away through the solid body, phase transformations run in the underneath layers heated above the transition temperatures, resulting in more homogeneous microstructure. Increasing the samples volume during the manufacturing process reduces the cooling rates which lead to slight decrease of hardness [17, 18].

In comparison with cast alloys, SLM prepared specimens show increase in yield strength and tensile strength. This improvement is due to SLM specimens' unique microstructure. The higher tensile strength of the SLM specimens may be due to the finer grain size, cellular dendrite, and elongated precipitates. Residual stress in the SLM specimen generates the residual strain and affects the tensile strength. The presence of residual stresses during sintering is possible reason for the increased hardness in the SLM [18, 24]. Components manufactured by SLM undergo deformation because of residual stress [25]. During SLM, the last fused top layer shrinks upon cooling with a magnitude dependent on the underlying (already solidified) material, and between layers, large residual stresses accumulate, resulting in the distortion of the product [25]. Sintered Co-Cr alloy shows higher hardness compared to the same cast alloy. Relevant data found in the

literature indicate that the hardness of sintered dental Co-Cr alloys ranges 440–475 HV10 (HV – Vickers Hardness), i.e. 382 HV10, whereas the hardness of the cast Co-Cr alloy ranges 325–374 HV10 [26]. Higher hardness and more homogeneous microstructure result in increased corrosion and wear resistance [26]. An in vitro study, comparing the mechanical properties of Co-Cr specimens made by three technologies – SLM, casting and milling, shows that there is a statistically significant correlation between hardness and stiffness (-0.4 , $P < 0.005$) [27]. Values, measured for SLM frameworks, show almost two times higher stiffness (4072 ± 13 N/mm) and microhardness (466 ± 13 HV), compared to casted (stiffness 2796 ± 584 N/mm; microhardness 264 ± 11 HV) and milled specimens (stiffness 2490 ± 557 N/mm; microhardness 270 ± 16 HV). Higher hardness and stiffness lead to increased brittleness. The SLM frameworks appear less ductile than the other two and more homogenous in material composition [27].

Heat treatment is being used to reduce and avoid the deformation, especially for fixed partial dentures with a long span and RPDs. Heat treatment can eliminate the crystal defects of metals, such as dislocations, to stabilize the microstructure with the reduction of residual stress [28]. The mechanical properties of alloys are also affected by the heat treatment [28]. An in vitro study comparing two different heat treatments – one on 880°C and second on 1100°C , states that the second one (at 1100°C) is more suitable for relieving the residual stress, considering the toughness of dental prostheses [29]. Recent study [30] reveals (using X-ray diffraction) that γ -face-centered cubic phase dominates in Co-Cr specimens produced by SLM and selective laser melting followed by heat treat-

ment. The selective laser melting followed by heat treatment group specimens exhibits the highest elongation and retentive forces compared with the casting and SLM (without heat treatment) groups.

Relevant data found in the literature indicate that the average surface roughness (the profile roughness parameter) immediately after sintering is about $8 \mu\text{m}$ [31]. After sand-blasting with Al_2O_3 , the roughness is reduced due to surface homogenization and uniformization. The roughness of the sintered Co-Cr alloy surfaces is several times greater than the roughness of the cast alloy surfaces. This may cause a problem when making removable dental restorations. On the other hand, a rough surface increases the wettability and reduces the contact angle, which enhances the bond between the metal and the ceramics [32].

Metal-based dental restorations have been reported to have excellent fitting accuracy. With the development of SLM technology, it is possible to automatize several steps in the manufacturing process, which can eliminate sources of error [33]. For traditional cast restorations, the suitability of the marginal fit can be compromised by shrinkage of the impression material or gypsum, deformation of the wax pattern, and variations in the thickness of the applied adhesion agent. The automated method of the SLM approach prevents such errors, which allows the prosthetic crown to be delivered directly to the mouth without the need for impression or creating a gypsum cast. This method also allows greater design flexibility. By comparison between SLM fabrication and standard casting method, using Co-Cr alloy, a significant difference between the manufactured crowns was observed. The mean marginal gap width of the cast crown group is reported to be significantly wider than that of the

SLM-fabricated crown group. The difference in marginal gap widths between the two groups indicates that SLM technology leads to a superior marginal fit compared with the traditional casting approach and may be considered suitable for clinical applications to improve restoration performance [34]. When comparing SLM-fabricated crowns with crowns made with milling technique, the SLM technique produced poorer internal and marginal fit [35].

Conclusion

The advantages of SLM technology with respect to the conventional technologies of casting and milling of Co-Cr dental frameworks, are homogenous metal structure, good marginal fitting accuracy and eco-friendly technology. The current SLM devices provide metallic restorations made of Co-Cr alloys for removable and fixed partial dentures without compromising the alloy or restoration properties at a fraction of the time and cost, showing great potential to replace the aforementioned fabrication techniques in the long term; however, further clinical studies are essential to increase the acceptance of this technology by the worldwide dental community. The residual stresses and their distribution along with the appropriate post-SLM thermal treatment should be considered in optimizing operational parameters of this technique.

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Difference in preparation surface area – Endocrown vs. Classical crown. A rapid communication

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Introduction: Resistant tooth preparation form and retention are among a variety of factors influencing clinical success, survivability, and longevity of indirect fixed restorations. Retention is proportional to the surface area of the preparation. An alternative preparation for endodontically treated teeth is the endocrown. This design may provide an increase in adhesive area in combination with a more conservative preparation approach. **Aim/Purpose:** The purpose of the current rapid communication is to objectively demonstrate the difference in preparation surface of two designs – classical crown and endocrown. **Material and methods:** A standard typodont first lower right molar (Frasaco^{GmbH}, Germany) was prepared for full coverage crown following standard protocol. The preparation was digitized with an intraoral scanner and subsequently modified for an endocrown design. This design was also scanned, and both were imported in the Blender system for 3D modeling. The Add-on Neuromorph was used to calculate the surface area of the preparation. **Results and discussion:** The measured surface area of the classical crown preparation design is 144.76 mm², whereas in the endocrown design it is 161.79 mm². The difference between them is 11.11%. The surface gain with the endocrown design is comparable to a 9° axial wall inclination change in a molar preparation. **Conclusion:** The increased surface area can partially explain the similar and better published results for endocrown restorations, in relation to the classical full-coverage crown design.

Keywords: Endocrown, Surface area, Preparation design, Bonding area, Adhesive area

Introduction

Endodontic treatment of teeth requires removal of substantial amount of coronal dental structures leading to a compromised dentine core, which often necessitates a restoration with a prosthetic construction. An alternative to the classical full coverage crown is the endocrown. This is a monolithic adhesive restoration with a supragingival preparation margin. The endocrown uses the pulp chamber for accessory retention without entering the root-canal system, which makes it a more conservative approach compared to post and core restorative methods (1). Two main modalities for endocrown preparation are described in the specialized literature – with a butt-joint preparation junction and a chamfer – ¼ elliptical circumferential border (2, 3).

During the past two decades an emerging tendency of increasing number of publications regarding the use of endocrowns can be observed [Fig. 1]. The reported results from different authors suggest a com-

parable and even better clinical performance as well as fracture resistance in relation to full-coverage crowns (3, 4). Assumptions for increased retention were made, due to the specific preparation design and the adhesive type of cementation (5). It is well known that survivability and success of fixed prosthetic restorations is directly related to resistant preparation shape and retention, the latter strongly correlated to the surface area of the preparation (6). In a study on 278 digital replicas of prepared teeth with endocrown and full coverage classical crown designs, Mörmann and Bindl did not find a significant difference in surface area available for cementation between the two modalities. However, the study did not account for the difference between tooth sizes in different patients (7). To our knowledge there are no studies in the specialized literature that aim to make a direct surface area comparison between the classical full-coverage crown design and the endocrown design.

Purpose

The purpose of the current rapid communication is to objectively demonstrate the difference in the preparation surface of two preparation designs – classical crown and endocrown.

Materials and method

A standard typodont first lower right molar (Frasaco^{GmbH}, Germany) was prepared for full coverage crown. The preparation design followed the guidelines and instruments (Bur set 1720 NTI, Germany) included in the minimally invasive system – V-prep (8). An optical impression was taken with an intraoral scanner (TriOs, 3Shape, Denmark) and the virtual model exported as STL file. Subsequently the preparation was modified with the simulation of an endodontic access cavity with a mean depth of 2 mm using the same burs [Fig. 2]. A second virtual model was obtained through scanning and file type conversion. Both digital replicas were imported in the freeware environment for 3D manipulation – Blender version 2.8¹. All faces representing the preparation surface for both designs were selected and their surface area calculated with the use of the Add-on – “Neuromorph”.

Results and discussion

The surface area of the prepared surface for the classical full-coverage crown design was 144.76 mm² and for the endocrown – 161.79 mm². The percent-difference in the adhesive surface between the two was calculated at 11.11%.

The surface area in contact with the cement is considered a factor that influences retention, clinical success, and longevity of indirect restorations (10, 11). M. Darvwniza et al reported a significant linear correlation between adhesive area and retentive forces in full-coverage crowns (12).

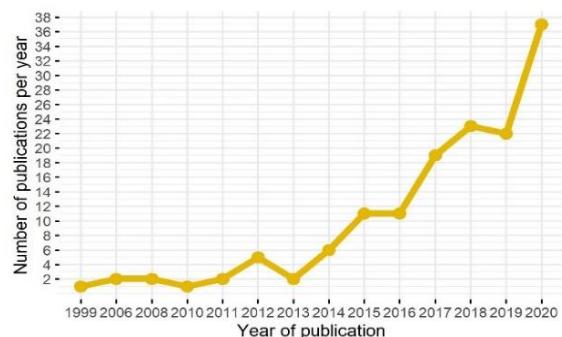


Figure 1. Number of publications per year (source PubMed, key word “endocrown”).

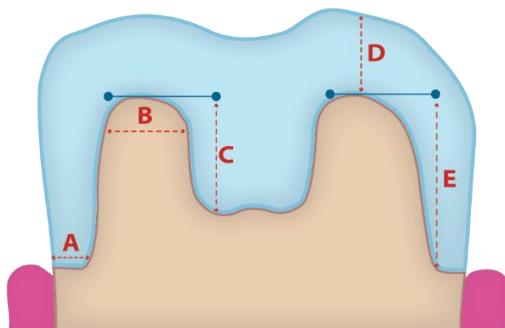


Figure 2. Features of endocrown preparation: A – 0.8 mm, B – ≥ 1.5 mm, C – ≥ 2.00 mm, D – ≥ 1.5 mm, E – ≥ 3 mm.

Calculating the surface of the tooth preparation is a complicated, time-consuming, and technically sensitive process. Different methods have been proposed to measure the adhesive area including – adapting a foil to the tooth preparation and measuring its surface or weight as well as calculating the surface of geometric figures resembling the prepared tooth (6). Recently digital methods are also used (7, 13).

The proposed method in this rapid communication showed high accuracy in the preliminary tests conducted on simple geometric figures, constructed in a modelling software – sphere, cube, cone, and cylinder [Fig. 3]. The accuracy of the results in this study are highly dependent on the system used to digitize the prepared teeth. The intraoral scanner used in this study, has a reported precision of $\pm 20 \mu\text{m}$ (15). The method employed for preparing the study samples – transformation of the preparation design with the addition of a retention cavity

¹ <http://www.blender.org>

at the pulp chamber area creates a test setting, which allows direct comparison and eliminates the different tooth sizes in the sample. The additional adhesive area

achieved modifying the preparation in the current study is comparable to a change in the TOC of the axial walls of 9° in a classical preparation of a molar (16).

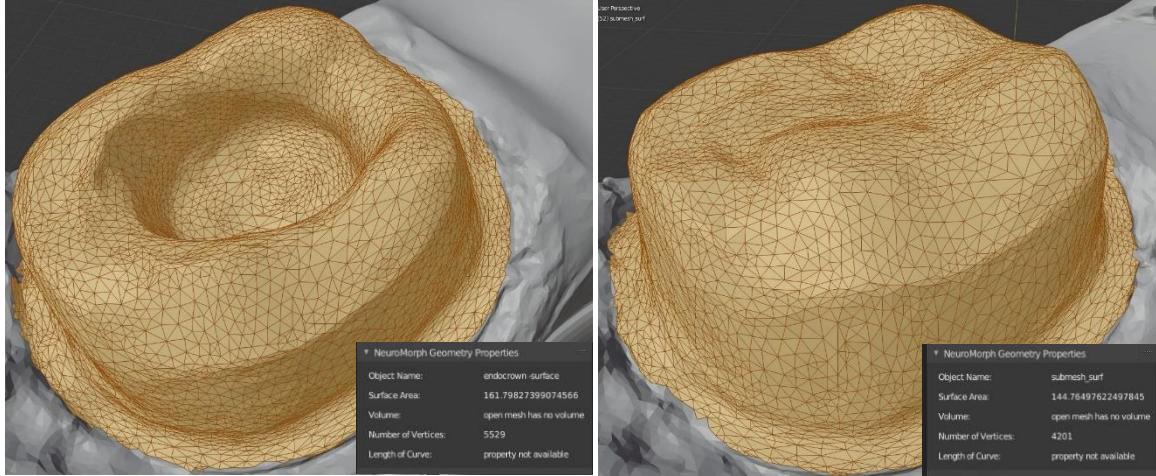


Figure 3. Preparation surface area measurement using Blender and Neuromorph.

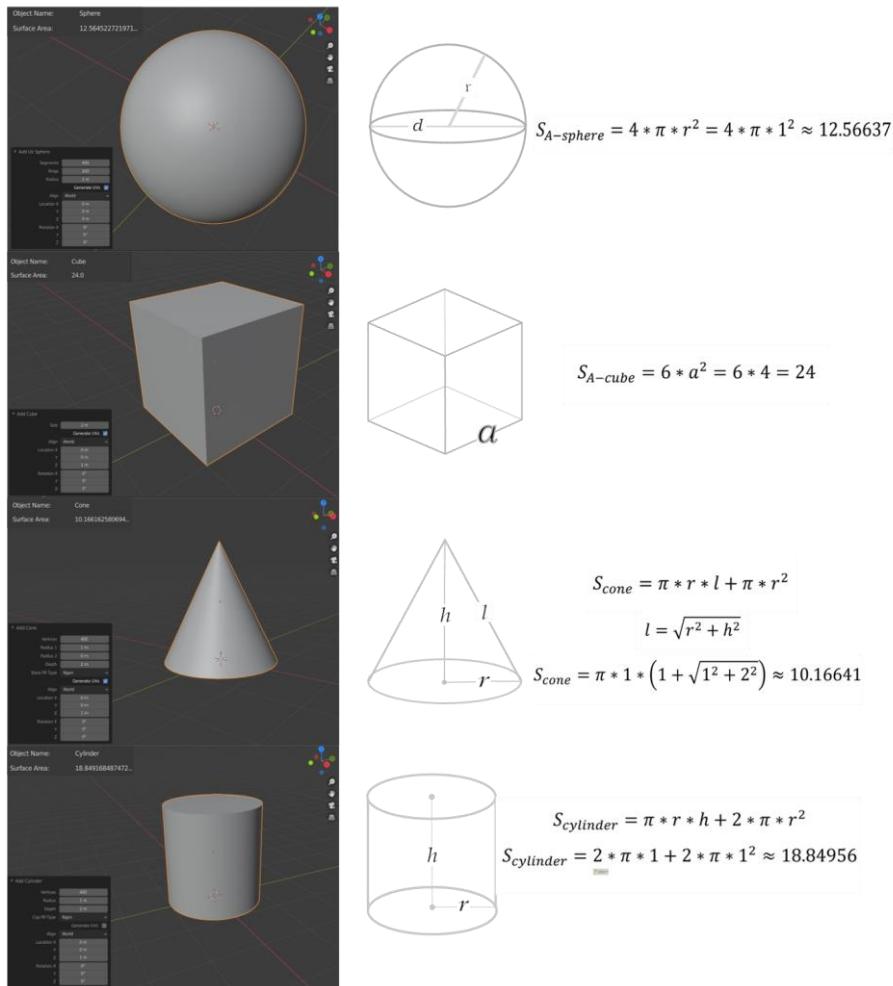


Figure 4. Results obtained in the preliminary test for accuracy of the method used for surface area calculation. Software-constructed geometric figures with predetermined parameters and surface area calculated using the described methodology (left) and surface area calculated with the mathematical formulas for the corresponding objects (right).

Further research with inclusion of a larger study sample and teeth from different groups is necessary to confirm the current results.

Conclusion

The increased adhesive area in the endo-crown preparation design communicated in this study, might explain the comparable and better results for these types of constructions against classical full-coverage crowns in various clinical and laboratory settings.

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In vitro study of root temperature changes during cryotherapy in an extracted human tooth – a pilot study

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Introduction: Intracanal cryotherapy is an innovative method for minimizing “flare-up” after endodontic treatment. **Purpose:** Two-zone *in vitro* study of root temperature changes during cryotherapy with 2 irrigation solutions. **Material and methods:** Extracted single-root tooth is standardized up to 15 mm root length. Three zones are marked on the external root surface. The root canal was instrumented up to 25/08 with WaveOne file. Three final irrigation protocols were applied using Endo Vac system: 1st – with saline at room temperature (control), 2nd – with saline at 2.5°C and 3rd – with 17% EDTA solution at 2.5°C. The initial, lowest and after 5 min irrigation temperatures were recorded on the apical and middle root zones by two thermosensors. **Results and discussion:** In protocol 3, 13.1°C and 10.4°C were recorded for the middle and apical third respectively. After five minutes of irrigation, temperatures change by 6% and 8%, respectively. In protocol 2, 13.3°C was recorded for middle and 10.1°C for apical third, which after five minutes of irrigation changed by 60% and 105%, respectively. The temperature was maintained 10°C lower than the initial for both thermosensors for 4 min at protocol 3 and only in the apical zone for 2 min at protocol 2.

Introduction

Pain control (during and after root canal treatment) is an important aspect of the endodontic practice (1). The main cause of postoperative pain is trauma to the periapical tissues by mechanical, chemical or microbiological origin, which leads to an inflammation known as “flare-up” (2, 3, 4, 5, 6). An innovative approach in managing postoperative pain is the use of intracanal cryotherapy. The method represents irrigation with a solution cooled to 1–4°C and delivered to full working length as the final rinse of the root canals, immediately before obturation.

Purpose

The purpose of the present study is to investigate the *in vitro* method of intracanal cryotherapy and the factors influencing it.

Material and methods

An extracted single-rooted tooth was standardized up to 15 mm root length, di-

vided in 3 zones. The root canal was shaped up to size 25/08 with a WaveOne file. Three final irrigation protocols with Endo Vac (negative pressure irrigation system) were applied:

1st – with a saline solution at room temperature – 20 ml

2nd – with a 2.5°C saline – 5 ml

3rd – with a 2.5°C 17% EDTA – 20 ml

The temperature on the apical and middle root zones was registered by two fixed thermocouples during the whole experiment.

The room temperature was 23.3°C during all irrigations. There were five minutes intervals between each irrigation protocol in order for the root canal to reach the initial temperature again.

Results and discussion

For the middle third of the root surface the lowest measured temperature during the control irrigation was 23.7°C and for the apical third of the root surface – 23.1°C.

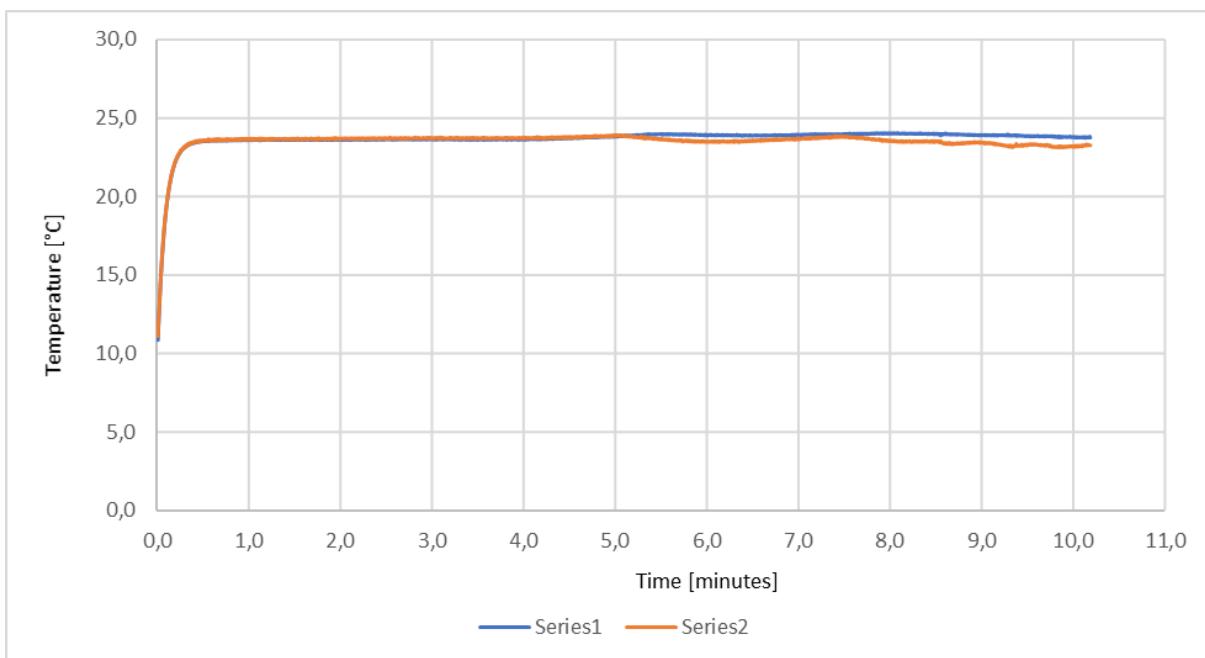


Fig. 1. Control irrigation with saline at room temperature.

First experimental irrigation with 5 ml 2.5°C saline

The middle thermocouple recorded initial temperature – 23.3°C, lowest temperature – 13.3°C and final temperature – 21.2°C. The apical thermocouple recorded

initial temperature – 23.2°C, lowest temperature – 10.1°C and final temperature – 20.7°C. The temperature was kept more than 10°C lower than the initial one only in the apical zone for 2 minutes.

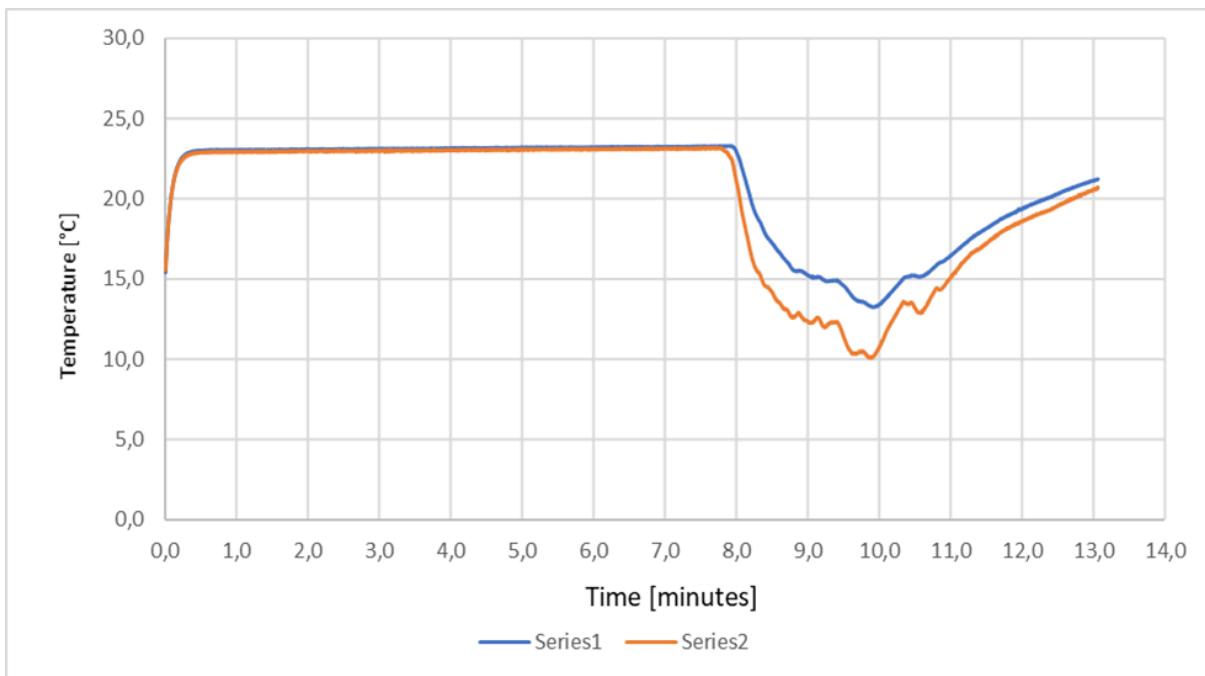


Fig. 2. First experimental irrigation with 5 ml 2.5°C saline.

Second experimental irrigation with 20 ml 2.5°C EDTA

The middle thermocouple recorded initial temperature – 24°C, lowest temperature – 13.1°C and final temperature – 14.3°C. The apical thermocouple recorded initial temperature – 24°C, lowest temperature – 10.4°C and final temperature – 11.3°C. The temperature was kept more than 10°C lower than the initial one for both thermocouples for 4 minutes.

Gade V. et al.(7) define cryotherapy as a new trend in the field of endodontics and its protocol has not yet been clarified. In the *in vivo* studies conducted so far various final irrigants are used – saline, NaOCl, EDTA. We chose to use saline and EDTA as they are some of the most widely used irrigants in endodontics. Different temperatures of the solutions in the range between 1.5°C and 4°C are mentioned in the literature. Our study was conducted with solutions with a temperature of 2.5°C.

According to data from the literature, the amount of irrigant used for final rinse ranges from 5 ml (8, 9), 10 ml (10,11) up to 20 ml (12, 13, 14), as well as the duration of the final irrigation itself ranging from 1min. (9, 11) up to 5 min. (12, 8, 13, 14, 10). We chose to work with 5ml and 20ml, for a time of 5 minutes. Our study used an EndoVac negative pressure system as did some of the other authors (11, 12, 13).

In their *in vitro* study, Vera J. et al.(15) used 20 ml 2.5°C saline for experimental irrigation for 5 minutes. They registered retention between the original temperature and the lowest measured temperature of more than 10°C for 4 minutes. Our results from final irrigation with EDTA are similar to those of Vera J. et al., as they registered a temperature reduction of 14.33°C compared to our registered temperature reduction of 13.6°C. Their lowest temperature reached is 5.98°C and ours is 10.4°C.

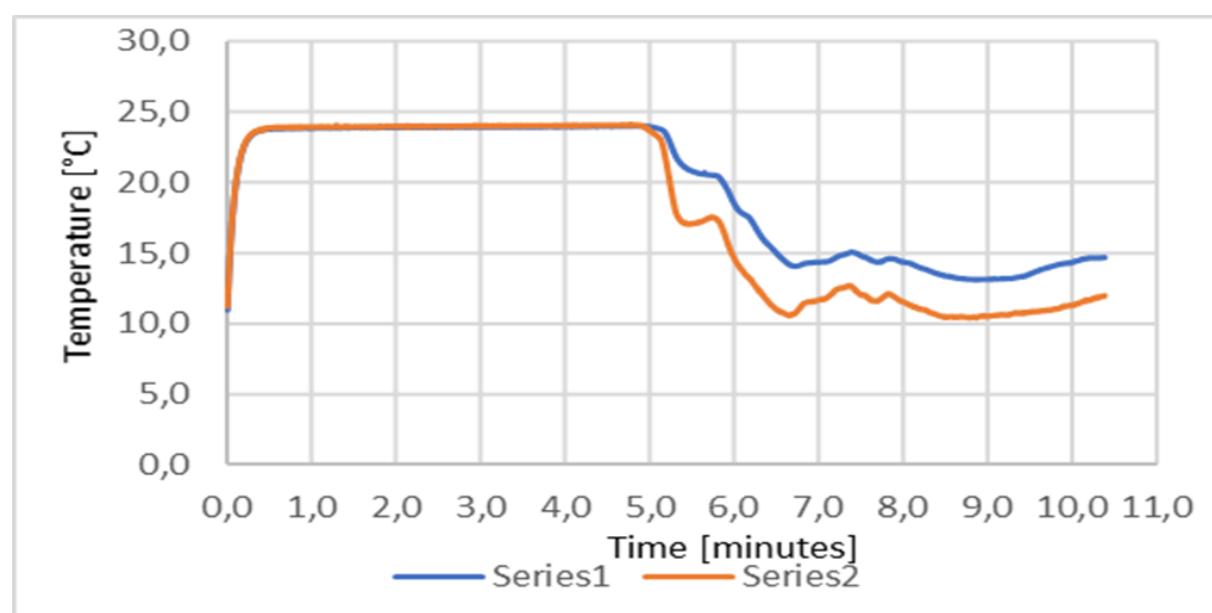


Fig. 3. Second experimental irrigation with 20 ml 2.5°C EDTA.

The differences in the results obtained could be explained by the fact that the temperature of the syringe rises very quickly as soon as it is removed from the refrigerator, even though it is placed in an ice tray.

This highlights the need for a change in the experiments' protocol as follows:

(1) positioning of a thermosensor to measure the temperature of the syringe during the entire irrigation,

(2) wrapping the entire syringe with ice in order to maintain the temperature throughout the entire irrigation.

As in Keskin C et al. (8) research, 5 ml were used for the second experimental irrigation for 5 minutes, unlike Jain A. et al. (9) which worked with 5 ml of cooled saline for 1 min. Reduction and retention of the temperature was achieved by more than 10°C lower than the initial one only in the apical zone for about 2 minutes.

Based on the data obtained from this experiment, it is necessary to conclude that the time of 1–2 minutes is insufficient to reach the benefits of vasoconstriction. Therefore, a quantity of 5 ml and a time of 1 minute are insufficient to achieve a beneficial effect of the procedure.

The analysis of the available literature indicates that in their experiments, the researchers used only one thermocouple. In this experiment, it was chosen to work with two thermocouples located on the middle and apical root surface. The difference in the lowest temperature recorded by the two thermocouples at the same time at the first experimental irrigation was 3.2°C and in the second experimental irrigation – 2.7°C. It is necessary to conclude that a lower temperature is reached in the apical zone of the root surface. This on one hand takes into account the success of the EndoVac system and on the other creates the need to use 2 thermocouples in further experiments.

Conclusions

Based on the results obtained, a change in the experiment's protocol is required as follows:

1. Positioning of a sensor to measure the temperature of the syringe during the entire irrigation

2. Wrapping the syringe with ice in order to maintain the temperature throughout the entire irrigation

3. Measurement at 2 points using 2 thermocouples to take account of the temperature difference between the individual areas of the root surface

4. Use of a sufficient amount of irritant – more than 5 ml and duration of the procedure more than 2 minutes.

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Possibilities of 3D printing in the sphere of dental medicine – literature overview

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In recent years the use of 3D printers for medical and dental applications has drastically increased. The more widespread availability and better quality of the final product are the main contributing factors. The goal of this research is the overview of available literature on the possibilities of 3D printing in the sphere of dental medicine, taking into consideration not only the clinical uses but also the possibilities it provides for education, scientific research, and experimentation. In order to fulfill that goal, all 338 publications in PubMed with key words 3d printing and dental medicine during the period from 2010 to 2020 were examined. The integration of this technology in dental medicine is signified by the growing number of publications on this topic. Their number has significantly increased in the last 10 years. This overview examines the possibilities and limitations of 3D printing in the dental practice and education.

3D printing is a process of fabricating different constructions by utilizing additive manufacturing, through which the object is constructed by layering the building materials. The most commonly used “printing” systems nowadays are:

- SLS (selective laser sintering), SLA (stereolithography) (Figure 1),
- FDM (fused deposition modelling) (Figure 2),

- PP (polyjet printing) (Figure 3),
- bioprinting (Figure 4).

SLS and SLA employ a laser, which fashions the desired item layer-by-layer. SLS utilizes powder-based materials, which fuse into a monolithic object, whilst SLA is liquid resin-based (1). Albeit, having a high printing resolution those types of printers are handicapped by object shrinkage.

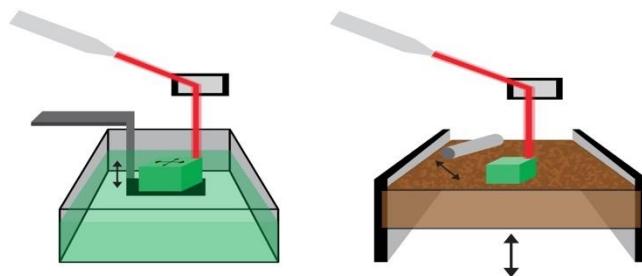


Figure 1. SLS (on the left) and SLA (on the right).

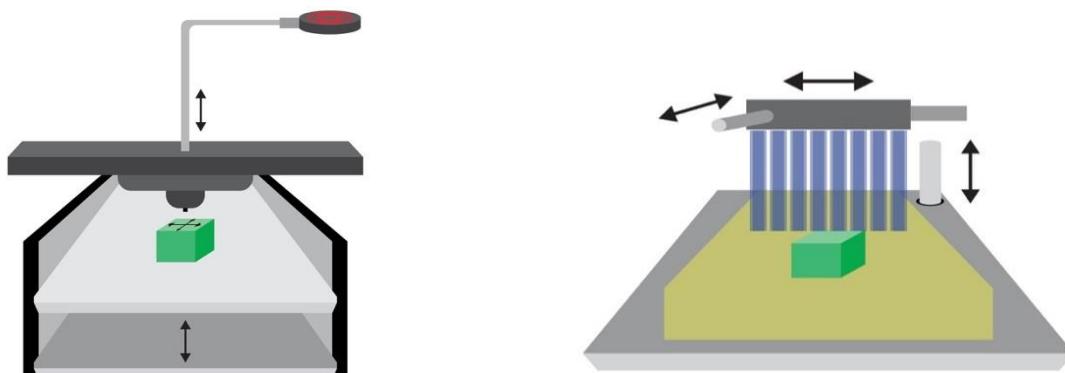


Figure 2. FDM.

Figure 3. Polyjet printer.

FDM is a method of printing by delivering the liquified material onto a printing bed, through a hot nozzle, that moves two-dimensionally on the horizontal plane. Once a single layer has been deposited the printing bed or the nozzle shift vertically, and the production of the subsequent layer commences. This is the most widely applied type of printer, because of its comparatively low price point and availability, however, it has the lowest end product resolution among other limitations (2).

The PP method employs the use of liquid photopolymers, that are deposited by the printer heads jetting layers of it followed by UV light curing. Not only does it provide final products with the highest resolution, but it also has a wide array of available materials to choose from that possess a variety of mechanical properties depending on the needs of the operator/user. Considering the use of liquid materials during the printing process the utilization of support materials is imperative, which increases the post-print model processing, as is with FDM printers (3).

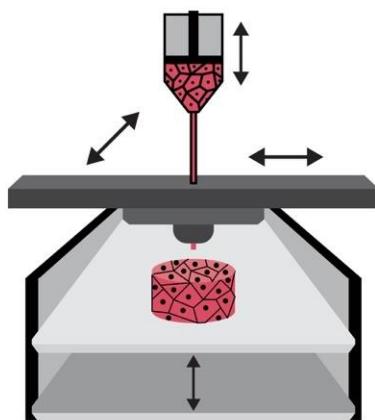


Figure 4. Bioprinter.

Bioprinting is a way of creating various structures out of “living matter”, using cell ink-based or microtissue/spheroid-based systems (4). 3D bioprinting is a revolutionary advancement in the sphere of medicine,

dental medicine and pharmacology. It allows for the replacement of lost or damaged organs and tissues with functioning transplants “raised” in a laboratory.

Regardless of the 3D printing method used in production, the technological protocol can be divided into 4 stages:

- creating a digital 3D model,
- processing and slicing of the 3D model into a multitude of two-dimensional layers,
- three-dimensional printing of the end product layer-by-layer,
- post-processing (5).

Applications of 3D printers in oral and maxillofacial surgery

The introduction of three-dimensional imaging to the field of oral and maxillofacial surgery has significantly improved diagnostics and planning of surgical interventions. As a result of the introduction of 3D printers, it is now feasible to use the information attained through three-dimensional image-diagnostic methods to fabricate patient-specific appliances. They can be separated into a few groups. Those are implants, surgical guides, splints, and contour models. Additive manufacturing gives us the ability to utilize materials possessing a wide variety of physical and biological qualities. An example is a fabrication of a scaffold tailored to the patients' specifications, not only regarding the size and shape but even the material from which it is made, making it more individualized by considering the required mechanical properties of the end product. Moreover, the success rate of the surgical placement of the scaffold can be improved by the incorporation of different factors and compounds, e.g., osteoinductive factors (bone morphogenic protein 2), thus accelerating and enhancing vascularisation and integration of the bone graft (6).

Another possibility of 3D printers is bone bioprinting using bio-inks and hydrogels, including decellularized matrix-based inks (7). In addition to biocompatibility, these hydrogels have been further functionalized as carriers for growth factors. 3D printing also made it conceivable to completely plan and perform the surgical reconstruction of maxillomandibular defects by three-dimensional virtual techniques. Based on the information obtained by CT imaging and a computer software-aided analysis a three-dimensional model can be rendered, enabling the surgeon to prepare an individual treatment plan. Studying the model surgeons are able to plan every step of the procedure in detail followed by the printing of surgical guides, thus simplifying and ameliorating the results of the operation (8; 9). With the help of this software, we can aid the patient in understanding the surgical strategy and visualize the treatment outcome even before the execution of the surgery (10).

In the realm of orthognathic surgery 3D printing finds its application in the fabrication of personalized orthognathic surgery guide (POSG) systems as well as custom titanium plates which can be placed once the bone segments are in an exact position (11).

Applications of 3D printers in periodontics

Periodontics is another area of dental medicine where 3D printing is widely applicable. It is most commonly utilized for patient-specific surgical guides for esthetic gingival correction. Additive manufacturing in regenerative periodontology is generally used for guided tissue regeneration, which aims to prevent the ingrowth of the oral epithelium simultaneously stimulating regeneration of the bone and periodontal liga-

ments (12). It is applicable in instances of tissue loss due to periodontitis. A scaffold enriched with growth factors, genetically modified cells, or bioactive proteins promotes regeneration of the lost bone and tissue pertaining to the supporting structures of the tooth (13). These scaffolds are manufactured by using patient-tailored, printed, wax molds. Moreover, we can remodel bone and soft tissue when placing an implant by adding sufficient amounts of bone tissue and limiting the proliferation of oral epithelium towards the bone defect.

Applications of 3D printers in orthodontics

Orthodontics is a field where the exactitude as well as the potential for customization of 3D printers can be employed not only for manufacturing orthodontic appliances exceedingly more precise than ones made following the conventional methods, but for thorough planning, depicting and predicting treatment results. To date, 3D printing is mainly used for the production of aligners by employing SLA to create patient-specific casting molds from which the silicone splint is casted (14). This method is applicable to the production of other types of splints as well, resulting in a much more precise end result than the one achieved through more orthodox approaches. Another area of application for additive manufacturing is the construction of individual orthodontic brackets. They are manufactured with the patient's tooth surfaces in mind and can be attached using 3D printed guides limiting the possibility for errors. Furthermore, they can be made with case-specific angulation and from a wide range of appropriate materials (15). Last but not least, bioprinting has proven useful in researches aiming to better our understanding of how the jaw bones grow and how forces used in

orthodontics affect them. Some studies are aimed at enhancing the anchorage or retention of teeth and even accelerating orthodontic tooth movement (16).

Application of 3D printers in endodontics

In a similar manner to how the paradigm shift from hand files to rotary instruments for root canal instrumentation significantly increased the possibilities for a successful result from endodontic treatments, the utilization of 3D printers in endodontics as a whole will have the same effect. Endo-access guides are employed for teeth with anomalies in root canal anatomy (curved, calcified, or otherwise impeding the securing of a reproducible glide path), or molars with complex root canal anatomies, where X-rays will possibly give us a misleading image due to the layering of a 3D object on a 2D radiograph, which can result in obliteration of accessory and lateral canals. They are fabricated using a 3D printed model of the tooth, scheduled for treatment, with the inclusion of the root canal system. This approach allows for more conservative endodontic access and easier glide path formation. In addition to endodontic access guide production, that of guides for apicoectomy is possible (17). There are ongoing studies about the implications of bioprinting for pulp-dentin complex regeneration. There is in vitro evidence supporting the claim of increasing osteogenic activity of pulp cells when 3D printed polycaprolactone coated with freeze-dried platelet-rich plasma (PRP) is applied (18). In addition, there have been successful laboratory trials of generating an anatomically shaped tooth-like tissue utilizing poly-epsilon-caprolactone and hydroxyapatite scaffolds (19).

Applications of 3D printers in prosthodontics

Prosthodontics is the dental specialty utilizing additive manufacturing to the fullest extent. Each and every type of prosthetic construction can be easily produced by 3D printers and significantly faster and in fewer steps than with pre-digitalization methods. The increasing availability and technological advancement will make chair-side denture fabrication in one visit possible sooner rather than later. With the integration of intraoral scanning which is replacing the traditional intraoral impression, a logical improvement to the workflow would be the digitalization of manufacturing (20). Regarding that, it is proven that 3D printing yields final products with greater quality than CAD/CAM milling, the exception being ceramic construction, for which more research is required. Employing an additive technique allows for the reproduction of more complex anatomical structures and requires less post-production processing. Nonetheless, milling can weaken the structure of the final product and holds the risk of crack formation. Dental implants can also be manufactured through selective laser melting or electron beam melting (21).

Applications of 3D printing in education

3D printing presents us with extraordinary possibilities for educating dental medicine students, as well as doctors in training, even dental practitioners preparing for a complicated procedure. Due to the fact that additive manufacturing can reproduce complex anatomical structures in great detail. At present, the training of dental students is done on idealistic plastic typodonts, more often than not those are an unrealistic representation of the dentoalveolar apparatus. By utilizing data from CT examinations we can

create models, which include different abnormalities, thus preparing future doctors of dental medicine for the treatment of actual patients (22). In surgery, these models find an even wider application, due to the fact, high-quality printers can create models which are comprised of both hard and soft tissue, although they do not fully recreate the conditions inside the oral cavity they are an enormous upgrade to the previously available options (23).

3D printing is a relatively new technological advancement and as it develops it will find a more widespread role in our everyday practice as dental and medical care providers. It allows for a faster, more precise, and safer workflow. Although it is not yet a commonly-used method, it has tremendous potential for the betterment of currently available dental procedures and training in dental medicine. The prospect of using stem cells and bioprinted scaffolds with the intention of “planting” a tooth germ in place of a missing tooth or to regenerate tissue lost after a large trauma or radical surgical procedure can give all of us hope for a brighter future, where healthcare will flourish hand-in-hand with science advancing our quality of life substantially.

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Aesthetic prosthetic rehabilitation by digital smile design

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Introduction. Aesthetic prosthetic treatment is a part of the rehabilitation of the masticatory system. The visualization of the planned final result in advance is the beginning of the clinical protocol. The prepared prognostic result by the treatment team must be approved by the patient. **Aim.** The aim of the study is to present a protocol for making temporary and definite fixed prostheses by digital planning and 3D printing. **Materials and methods.** For the aim of this study TRIOS intraoral scanner and Canon camera were used. The software for planning was TRIOS Smile Design. This software allows planning and visualizing of the result of prosthetic treatment in the aesthetic zone. This was made by superimposing of 2D picture of the patient and 3D intraoral scans. The final result information was sent to the dental laboratory. In the lab a digital prognostic wax-up was made and printed on models. The planned temporaries could be made by milling or printing. **Results.** Classically, the final visualization is made by wax-up on plaster models. It could be followed by mock-up with temporary material on natural teeth. Entering the new digital technologies, all this can happen virtually. Highly aesthetic temporary and permanent restorations were made. **Conclusion.** Digital Smile Design gives many possibilities for aesthetic rehabilitation. The virtual planning and machine manufacturing of the temporaries saves much team and patient time.

Keywords: *Digital Smile Design, virtual planning, 3D printing, CAD/CAM*

Introduction

Aesthetic prosthetic treatment is a main part of the rehabilitation of the masticatory system [1, 2]. The visualization of the planned result in advance is the beginning of the clinical protocol [3]. The prepared prognostic result by the treatment team obligatory must be approved by the patient [4].

Aim

The aim of the study is to present a fully digital protocol for making temporary and permanent fixed restorations using 2D Digital Smile Design, 3D digital wax-up and mock-up and 3D printing and milling options.

Materials and methods

For the aim of this study TRIOS intraoral scanner and Canon camera were used. The software for planning was TRIOS Smile Design. This software allows planning and visualization of the final result of the prosthetic treatment in the aesthetic

zone (in most of the cases in the area of the upper frontal teeth). This was made by superimposing of 2D photo picture of the patient and 3D digital impressions. The result of the planning was sent to the dental laboratory. In the lab a prognostic virtual wax-up was made in Dental Designer, 3Shape, Denmark and was printed on a model. The planned temporaries could also be made by milling or 3D printing.

The procedure started with intraoral scanning. Using TRIOS Intraoral Scanner, the initial situation of the upper and lower dental arches was scanned (Fig. 1 and 2). Digital bite registrations were taken too. Next, photo pictures of the patient face with wide smile with (Fig. 3) and without retractors (Fig. 4) were made with professional photo camera Canon EOS1200. Both data from the intraoral scanner and the camera were imported in the TRIOS software with activated option for 2D Smile Design. The images were aligned via antropometric points.



Fig. 1. Initial situation (frontal view).



Fig. 2. Initial situation (side view).



Fig. 3. Clinical photo with retractor.



Fig. 4. Clinical photo without retractor.

In the next step different tooth shapes and sizes from the virtual library were tried. We offered to the patient 2D design of her smile with oval (Fig. 5), triangular (Fig. 6), rectangular (Fig. 7) and square shape (Fig. 8) of teeth with different sizes. Discussing the options, triangle shape for the teeth was chosen. This shape matched to the face of the patient. Finishing the smile design the patient could see how she would look like at the end of the treatment and the dental team understand the patient expectations. According to the 2D Smile Design in the dental office 3D digital wax-up was made in Dental Designer, 3Shape, Denmark laboratory software and it was 3D printed on models (Fig. 9).

The clinical work started with removal of the old upper restorations and fabrication of direct prognostic mock-up with compo-

site material Structur, VOCO, Germany (Fig. 10). Some of the teeth needed to be build-up. The crowns of the preserved abutment teeth were prepared and finished and the damaged teeth were restored with digital post-and-core restorations. An optical impression was taken from the upper arch with post-and-core scan bodies (Fig. 11) and was sent to the laboratory. According to the approved tooth design, the dental technician made the metal post-and-core restorations and 3D printed composite provisional bridge restorations (Fig. 12). On the next clinical visit, the post-and-core and the upper provisional bridge restorations were fixed, so the patient received her new upper teeth made upon the virtual plan (Fig. 13). She was with the temporaries for a few weeks. No complaints were reported in this period. After that the final digital impres-

sions for the upper permanent restorations were taken (Fig. 14). The treatment of the upper arch was accomplished with metal ceramic bridges with the same design (Fig. 15).

The lower arch was proceeded in the same succession. The difference between

the two arches was the presence of four implants in the distal areas of the lower jaw. The implant position was digitally scanned with scan bodies fixed to the implants (Fig. 16). The final metal ceramic bridge restorations are waiting to be fabricated in the dental lab.



Fig. 5. 2D Smile Design with oval teeth.



Fig. 6. 2D Smile Design with triangular teeth.



Fig. 7. 2D Smile Design with rectangular teeth.



Fig. 8. 2D Smile Design with square teeth.



Fig. 9. 3D printed prognostic digital wax-up.



Fig. 10. Direct prognostic mock-up.



Fig. 11. Impression for post-and-core restorations.



Fig. 12. Metal post-and-core restorations and 3D printed provisional bridge restorations.



Fig. 13. 3D printed mock-up.

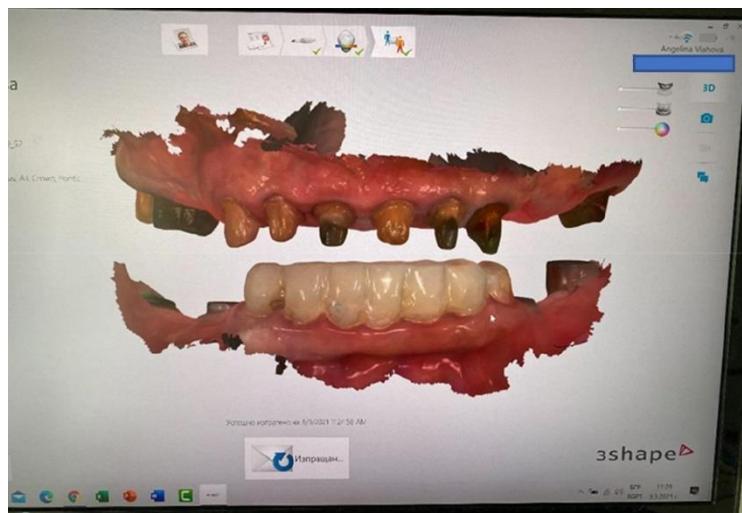


Fig. 14. Digital impressions for the upper permanent bridges.



Fig. 15. Upper metal ceramic restorations.



Fig. 16. Digital impression for the lower bridges.

Results and discussion

Classically, the visualization of the result of the prosthetic treatment was made by analogue wax-up on plaster models. It could be followed by mock-up with temporary material on natural teeth [5]. Entering the new digital technologies, today all this

can be made virtually [6, 7]. Digital Smile Design and 3D printing and milling provide an excellent alternative to the classical analogue protocol [8, 9].

2D Digital Smile Design ensured predictability of the prosthetic treatment. Highly aesthetic direct and indirect temporaries

were made according to the digital wax-up. From the beginning the dental team knew the expectations of the patient and both the patient and the team were prepared for the desired result of the aesthetic prosthetic treatment.

Conclusion

Digital Smile Design gives many possibilities for aesthetic rehabilitations. The virtual planning and machine manufacturing of the temporaries saves much team and patient time.

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Clinical protocol for digital planning of single implants: a pilot study

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Introduction: The planning and positioning of dental implants in an optimal site are very important for the durability and trouble-free maintenance of the implant-prosthetic restorations. Besides their diagnostic value, 3D images give the chance for precise planning and positioning of dental implants and subsequent constructions. **Aim:** The aim of our study was to create a protocol for digital planning and guided implantation of single implants in partially edentulous patients with single tooth missing and control method as well. **Materials and methods:** TRIOS Intraoral Scanner, CBCT Radiograph, Implant Studio Software, 3D Printer and CAD/CAM Equipment were used. The images of intraoral scanning and CBCT were superimposed digitally through benchmark points. The precise matching of both images gives the chance to plan the implant position and temporary crown as well. The correct crown position according to the implant, adjacent and opposite teeth was one of the criteria for implant placement estimation. After planning, the surgical guide was made by 3D printing. The temporary crown was fabricated by CAD/CAM before implantation too. The operation was made by guided surgery. **Results:** The result was a very precise implant positioning. **Conclusion:** Placing digitally planned single implants by guided surgery gives predictable results. The analysis of all stages is meant to create a simplified and reliable protocol for placing single or several implants.

Keywords: *guided surgery, digital implant planning*

Introduction

The planning and positioning of dental implants in an optimal site are very important for the durability and trouble-free maintenance of the implant-supported restorations [1, 2]. In the early years of the implant treatment, the number of diagnostic methods was limited. The implants length was determined on panoramic X-ray with scaled template on it [3]. The implants diameter was chosen on cut plaster model with marked gingiva thickness on it [4]. Nowadays 3D images – cone beam computer topographies (CBCTs) give the best diagnostic value and chance for precise planning of dental implants [5].

Aim

The aim of this study was to present a protocol for digital planning, guided implantation of single implants in partially edentulous patients with single missing tooth and a control method.

Materials and methods

TRIOS Intraoral Scanner, Computer Tomograph ProMax 3D, Planmeca, 3Shape Implant Studio Software, 3D Printer Form-labs and CAM S2-Impression, VHF 5-axis milling machine were used for the clinical protocol. The digital impressions and the CBCT were superimposed digitally through reference points. The precise matching of both gave the chance to plan the implant

position and to design a temporary crown as well. The correct crown position according to the implant, adjacent and antagonist teeth was one of the criteria for implant placement evaluation. After planning, the surgical guide was made by 3D printing. The temporary crown was designed and milled of PMMA before implantation. The operation was made by fully guided surgery.

After the alignment between the intraoral scan images and CBCT an analysis of the whole jaw of interest was made. On the site for implantation, the dimensions of the alveolar bone, the distances to the adjacent teeth and the antagonists and to the maxillary sinus or to the mandibular canal were measured. After analysis of the bone volume and quality, a suitable implant from the virtual library was selected. For upper jaw, where bone was softer and porous, implants with deep threads condensing the bone were preferred. For lower jaw, where the bone was thicker and harder, implants with shallow threads were chosen. The Implant Studio software interpreted data about bone density and this information was taken into consideration as well. The optimal implant size according to the bone qualities and the requirements for minimal distances to adjacent anatomical structures were selected. The graphic picture of the implant was superimposed on the X-ray image on the site for implantation. By the respective points for changing the implant position, the implant was moved virtually to its optimal site, corresponding to all planning principles and to the projection of the prosthetic restoration (Fig. 1).

A planning check-up was made and the surgical guide was designed (Fig. 2). It must lay exactly on the available teeth and must be placed and displaced easily. The surgical guide leads the osteotomy so that the implant to be placed exactly on the 3D

planned position. The information for the planned surgical guide was transformed into STL file and sent to the laboratory for 3D printing of SG Resin, Formlabs, USA (Fig. 3). According to the planned implant position the temporary crown was designed in Dental Designer, 3Shape, Denmark (Fig. 4). This crown was made of PMMA Telio-CAD, Ivoclar Vivadent, Lichtenstein (Fig. 5). After milling of the screw retained temporary restoration a titanium base was cemented extraorally to the crown using Multilink Hybrid Abutment Cement, Ivoclar Vivadent, Lichtenstein (Fig. 6).

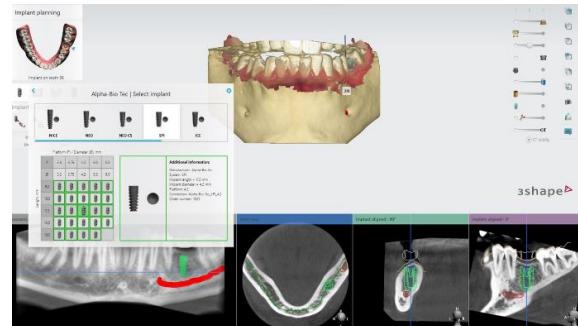


Fig. 1. Digital implant planning.

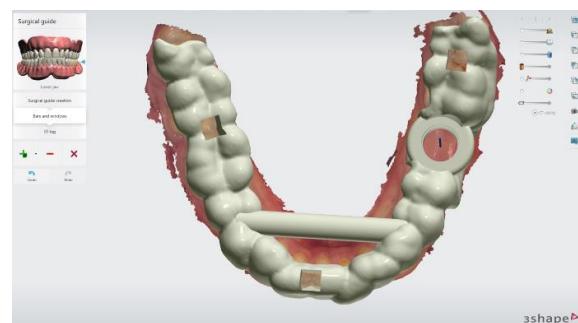


Fig. 2. Surgical guide design.



Fig. 3. Surgical guide 3D printing.



Fig. 4. Design of the screw-retained crown.



Fig. 5. PMMA crown.



Fig. 6. The crown with the cemented Ti-base.

The surgical procedure started with a check-up of the passive fit of the surgical guide. After anaesthetizing the guided surgery protocol was followed. The surgical guide was placed on the occlusal surfaces of the teeth. First, a punch in the gingiva was made using a handpiece tissue punch (Fig. 7). Next, the protocol continued with drilling of implant bed by consecutive use of osteotomy drills, corresponding the implant size (Fig. 8). All these manipulations and the final implant placement were accomplished through the surgical guide (fully guided surgical procedure) (Fig. 9).



Fig. 7. Tissue punch drill.



Fig. 8. Guided surgery protocol.

After implant placement, the implant primary stability was measured with Osstell IDx, W&H, Austria. If buco-lingual and mesio-distal values were over 70, an immediate loading was made. For a check-up of the accuracy of implant planning and the implantation the temporary crown was placed on the implant and its position was evaluated in relation to the implant platform, the gingiva, the adjacent and the opposite teeth.

Results

A precise implant positioning was evaluated. An immediate loading was performed (Fig. 10). Digital implant planning created predictable results.

18 patients were made till now and additional 22 patients will be treated with two different implant systems Alpha Bio Tec and AB-Dental, Israel) and will be investigated according to this clinical protocol

during a scientific project ДПДП-6/2019 of Medical University – Plovdiv. This study was approved by the Ethical Committee of Medical University – Plovdiv (protocol number 5793/18.07.2019).



Fig. 9. Implant placement through the guide.



Fig. 10. Immediate implant loading.

Discussion

The optimal position of the implant was chosen according to the bone anatomy (from the CBCT), the mucosa (from the digital impressions) and the shape of the future virtual crown (created in the 3Shape Implant Studio Software) [6]. The confirmation of the clinical observations analyzing the obtained parallel radiographs revealed an excellent fit between the Ti-base and the implant platforms in all cases.

The pilot results reported in this article support the findings of studies published by Lanis et al. (2015) [7], Pozzi et al. (2016) [8] and Vlahova et al. (2017 and 2018) [9, 10] in that, combination of digital surface scanners and CT's facilitates predictability of the results in guided implant surgery.

Conclusion

The preliminary results of placing digitally planned single implants by guided surgery gave predictable results. The analysis of all stages is meant to create a simplified and reliable protocol for placing single or several implants via fully guided procedure.

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A fully digital approach in aesthetic rehabilitation. Case report

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Background: Recent developments in 3D facial scanning technology allows the adoption of facially driven rehabilitation as part of the standard prosthetic treatment protocol in esthetically challenging cases.

Purpose: The aim of this case report is to present a fully digital approach for case managing, that combines intraoral and face 3D scanning, virtual smile design, followed by computer assisted manufacturing of the final restorations. **Case report:** A 61-year-old woman came to us with an esthetic concern in her upper frontal teeth – a metal-ceramic fixed partial denture on 12, 11 and 21 with unsatisfying appearance. The adjacent teeth were with severe incisal abrasion, disproportionate crown heights and uneven gingival line. Intraoral and facial scans were obtained using Trios and Bellus3D face scanner and superimposed in Dental Designer Studio to create a virtual patient. DSD and virtual wax-up were performed using the patient's facial and intraoral features as guidelines. A diagnostic model was printed, and a vacuum formed matrix produced for motivational mock-up, control of the tooth preparation and guided gingivectomy on 13 and 12. The prostheses prototypes were 3D printed from castable wax and the restorations fabricated from lithium disilicate ceramics. The veneers on teeth 13, 22, 23 and the FPD on 12, 11, 21, were cemented with self-adhesive resin cement. The ability to build a virtual patient in 3 dimensions during routine prosthodontic treatment, creates new opportunities for a patient-centered approach towards esthetics.

Conclusion: 3D facial scans facilitate patient-dentist-lab communication, improve predictability, and reduce chair time by providing valuable guidelines for treatment planning and execution.

Introduction

The use of digital methods in dentistry has increased in recent years due to the progress made in technologies such as intraoral scanning (IOS), face scanning (FS), and improvement of computer-aided design and computer-assisted manufacturing (CAD/CAM) software. As a result, clinical practice and laboratory techniques are shifting towards virtual-based workflows.^{1,2} Integration between 3D surface and radiographic files and the possibility of their accurate superimposition allows the creation of a virtual dental patient. The fusion of these digital data types contributes to the creation of replicable models and enhances anatomical structures analysis, thus treatment planning. It can also be used to simulates prosthetic outcomes in advance and clarify patients' expectations, facilitates effective patient and interdisciplinary team communication and precision documentation.^{1,3}

Digital workflow starts with digitization of the patient – acquisition of 3D data with face and intraoral scanners. Superimposition of the 3D images is done and with Digital Smile Design (DSD) tools a plan for facially driven esthetic rehabilitation is provided. The facial analysis, with all the interrelated anatomic parts involved in the patient smile (lips, cheeks, gums, and teeth) is highly advised to deliver a successful facial and smile rejuvenation for treatment in the aesthetic zone.⁴ Subsequently a computer assisted manufacturing of the digitally planned final restorations yields a precise and predictable outcome.⁵

Aim

This report aims to present a fully digital approach for aesthetically driven, minimally invasive prosthetic treatment, that uses intraoral and facial 3D scanning data for planning, virtual modelling and subsequent manufacturing of the final restorations.

Method

A 61-year-old woman came to us with an esthetic concern in her upper frontal teeth – a metal-ceramic fixed partial denture on 12, 11 and 21 with unsatisfying appearance. (Fig. 1) The adjacent teeth were with severe incisal abrasion, disproportionate crown heights and uneven gingival line. Diagnostic scans were obtained using Trios (3Shape, Denmark). Two conventional impressions were also taken with Speedex (Coltène Whaledent GmbH). A decision was made to use the scans produced from

the working models (Fig. 2). A facial scan with a maximum forced smile and Photo Cheek Retractors were obtained with the Bellus3D face scanner. Both 3D files from the face scan and working models, were superimposed in Dental Designer Studio (3Shape, Denmark) by a 3-point alignment procedure to create a virtual patient (Fig. 3-a). Utilizing the DSD approach a digital wax-up was performed considering the following as design guidelines (Fig. 3-b):

1. the patient's facial features.
2. the patient's intraoral morphology.



Fig. 1. Disproportionate crown heights and uneven gingival line, visible on the retracted photo and on the conventional diagnostic models.

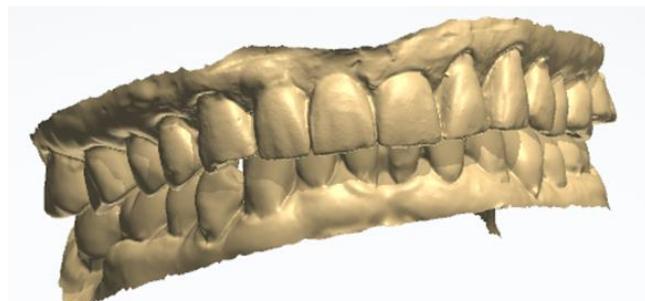


Fig. 2. Digitalized diagnostic models from the laboratory scan.



Fig. 3. Superimposed images of dentition and facial scan – a) and DSD performed – b).

The fused STL files with the proposed design was printer from grey resin (Formlabs, USA) (Fig. 4). It was then used to fabricate a vacuum formed matrix (Fig. 5) for the motivational mock-up, made with TempSpan (Pentron Clinical, USA), for aesthetic and functional evaluation. In the new model, vertical dimension remained unchanged (Fig. 6).

Guided gingivectomy on 13, 12 and 21 was performed using the motivational mock-up as guideline (Fig. 7). After the aesthetic approval of the proposed design (Fig. 8) minimally invasive tooth prepara-



Fig. 4. Printed model with the proposed design.



Fig. 6. Motivational mock-up.



Fig. 7. Motivational mock-up guiding the crown lengthening: a) before crown lengthening; b) After crown lengthening.

tion was performed and a new IOSSs were obtained. 3D master models were printed (Fig. 9). The prostheses prototypes were 3D printed from castable wax and the restorations fabricated from lithium disilicate ceramics (IPS Empress Esthetic of Ivoclar Vivadent Inc.) (Fig. 10).

The veneers on teeth 13, 22, 23 and the FPD on 12, 11, 21, were adhesively luted to the abutments with self-adhesive resin cement (Variolink Esthetic DC of Ivoclar Vivadent) (Fig. 11). A vacuum formed appliance was made for protection of the final restorations.



Fig. 5. The vacuum formed matrix and cartridge with double-cured C and B material.



Discussion

Superimpositions of data from IOS and FS under static conditions, allows creation of a 3D virtual patient. There are some low-cost proposals in the literature for providing FS with mobile phones⁶. 3D software facilitates precise facially guided smile design in aesthetic prosthodontic cases, that can be easily reviewed and remade if changes are necessary. Virtual treatment simulation can create mock-ups before any irreversible procedures are performed (Fig. 6). Pre-op visualization involves the patient in the design process, increases the patient's education and treatment plan and results acceptance.

Digital format of the data enhances interdisciplinary communication. It facilitates treatment decision and the digital execution of the approved rehabilitation.



Fig. 8. Provisionals.



Fig. 9. Printed 3D models.



Fig. 10. Prostheses prototypes from castable wax.



Fig. 11. Patient after the luting of the final restorations.

Conclusion

3D facial scans improve predictability and reduce chair time by providing valuable guidelines for treatment planning and execution. The benefits of facial scans may integrate them into daily practice for treatment planning and visualization as a common technique for all esthetic rehabilitations.

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Sausage technique for horizontal bone augmentation in the anterior mandibular segment: case report

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The development of bone augmentation procedures has allowed placement of dental implants into jaw bone areas lacking an amount of bone sufficient for standard implant placement. Of the different techniques, the best documented and the most widely used method to augment bone in localized alveolar defects is guided bone regeneration. The case report refers to a patient with a partly edentulous mandible (missing central and lateral incisors on both sides) with a knife-edge alveolar ridge. A preoperative CT was done and the anterior ridge width was measured at 3 to 3.5 mm. Placement of implants was impossible without obtaining a proper width of the alveolar bone. A horizontal ridge augmentation was carried out with the help of sticky bone and a collagen membrane, which was stretched out over the graft and fixed with pins to the lingual and buccal cortical plates (sausage technique). The buccal and lingual flaps that were reflected were both released from the periosteum and were sutured together. A preoperative CT before implant placement showed 6 to 6.5 mm gain in the width of the ridge and as a result two 3.3x12 mm implants were placed in the defect. In this case report the sausage technique for horizontal ridge augmentation proved to be relatively predictable and successful procedure.

Introduction

It is well established that tooth extraction is followed by a reduction of the buccolingual as well as apicocoronal dimension of the alveolar ridge at the edentulous site^{1,2}. The mandibular frontal edentulous region in most cases has sufficient bone height, but lacks the buccolingual width to accommodate a proper implant diameter. The development of bone augmentation procedures has allowed placement of dental implants into jaw bone areas lacking an amount of bone sufficient for standard implant placement. Of the different techniques, the best documented and the most widely used method to augment bone in localized alveolar defects is guided bone regeneration.

Case report

A 62-years old female patient walked in our dental office with a missing lateral and central incisors of the mandible. The patients had a knife edge mandibular ridge, which required augmentation, before proper diameter implants could be placed (Figure 1).

The interdental distance was enough for two implants to be placed but the width of the ridge was insufficient. The sausage³ technique was chosen for horizontal bone augmentation. A trapezoid mucoperiosteal flaps were elevated from the buccal and lingual side (Figure 2). Bone scraper was used for collection of cortical bone particles from the mentum (Figure 3).



Figure 1. Preoperative view of the edentulous region.



Figure 2. Flap design.

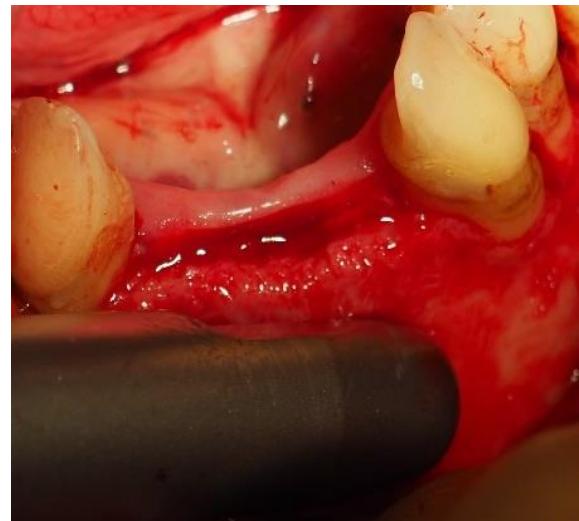


Figure 3. Collection of autograft particles.

Sticky bone was made with 50% autograft and 50% xenograft (Geistlich Bio-Oss granules), together with i-prf (Figure 4).

Decortication holes were made on the buccal cortical plate so that sponge bone bleeding was present (Figure 5). The sticky bone was adjusted on the buccal and then stabilized with a collagen resorbable membrane (Geistlich Bio-Gide collagen membrane), which was stretched and fixed with pins to the buccal and lingual bone plate (Figure 6).

Prf membranes were overlayed over the resorbable collagen membrane. Releasing incisions in the periosteum were carried out and the flaps were sutured together with a 6/0 polypropylene suturing material (Figure 7).

The preoperative CT measured the ridge width at the frontal region between 3 and 3.5 mm, whereas the CT, that was done before implant placement, 4 months after the procedure showed a gain of 6 to 6.5 mm in the width of the ridge. Two implants (BTK safe 3.3x12 mm) were placed in the defect (Figure 8).



Figure 4. Sticky bone.



Figure 5. Decortication holes at 4 mm depth



Figure 6. Placement of the graft and stretching and fixing the membrane with titanium pins.



Figure 7. Postoperative view.

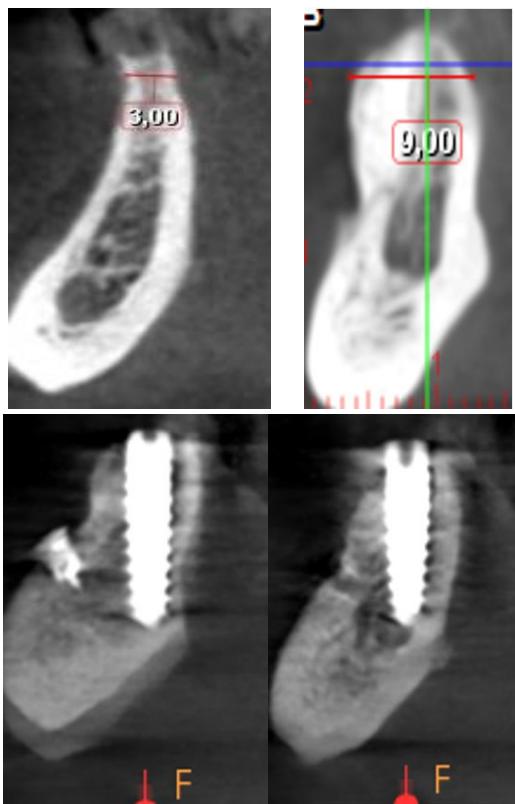


Figure 8. Preoperative and postoperative CT before and after implant placement.

Conclusion

As a conclusion the sausage technique can be used as a predictable way to augment the width of the ridge before implant placement. It can also be a good alternative to GBR with non resorbable membranes (ti-mesh and e-ptfe, reinforced with ti membranes), which are still regarded as the gold standard, but are reported to cause some soft tissue problems and need removing after the graft has matured.

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Alternative methods for caries removal

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Dental caries is the most common chronic dental disease in the world. It is defined as a multifactorial microbial infectious disease characterized by demineralization of the inorganic and destruction of the organic substance of the tooth. Conventional treatments for caries removal are often associated with pain and fear. Minimally invasive treatment offers an attractive alternative to managing carious lesions in a more conservative and effective manner, resulting in enhanced preservation of tooth structure. The aim of this review article is to analyze the scientific literature regarding the alternative therapeutic approach to the caries lesion management. A search was performed to summarize the evidence behind the alternative methods for caries removal, including the use of air abrasion, lasers, ultrasonic devices, polymer burs, At-traumatic restorative technique, chemo-mechanical caries removal, and their advantages and disadvantages to be discussed. The findings of the literature review give grounds to undertake studies investigating the efficiency of the different methods for caries removal. Future explorations will be interesting as the profession begins adopting the alternative caries management strategies that may decrease the use of behaviour management techniques in the treatment of paediatric dental patients.

Introduction

In recent years, the management of dental carious lesions changes from a traditional surgical approach to a “biological” or “medical” approach. Davis and Makinson described a completely new approach for “Minimal Treatment” of dental caries and first termed it “Minimal Intervention Dentistry” (MID) in literature [1]. On one hand, an important concept, that governed the development of MID, is the ability to reduce the need for cutting away healthy tooth tissues by removal of the `infected` dentin as part of the cavity preparation process and preserving the “affected” dentin [1]. The differences between infected and affected dentin are described in Table 1 [2].

On the other hand, the use of alternative and complementary methods reducing the need for local anesthesia and obtaining minimal or no pain during cavity preparation also contributes greatly to attaining the primary aim of MID. According to the concept of MID, the so-called “4S” principle as a minimally invasive approach in behavioral dentistry is developed. It is based on removing four of the major primary sensory triggers for dental anxiety when in dental setting – sight (air turbine drill), sounds (drilling), sensations (high-frequency vibrations), smells [3, 4]. It is used most commonly in conjunction with other alternative methods to mitigate anxious behaviors and their consequences.

Table 1. Difference between infected and affected dentin

CARIES DENTIN DIFFERENTIATION	
Infected dentin	Affected dentin
<ul style="list-style-type: none">• soft (cottage cheese) superficial layer• yellowish to brown• can be deformed by pressing an instrument into it• can be easily removed by gently scraping with minimal force• total removal is required• irreversible destruction of collagen cross-linking• lack of mineral content• high bacterial concentrations	<ul style="list-style-type: none">• firmer and light brown than infected dentin (leatherlike)• no deformation when an instrument is placed into it• can be scraped off with medium pressure• the inner layer, located under the infected dentin• it is preserved after preparation• presence of the collagen network• some mineral content• reversibly denatured• no bacteria
UNABLE TO UNDERGO EMINERALIZATION!	
POTENTIAL FOR REMINERALIZATION!	

A range of approaches can be used and they can be mixed and matched in accordance with the principles of MID – Atraumatic Restorative Treatment (ART), Polymer burs, Chemo-mechanical caries removal, Ultrasonic excavation, Air abrasion, Er:YAG and Er,Cr:YSGG laser therapy [5, 6, 7, 8]. The dental practitioner chooses an alternative method for caries treatment to meet the particular situation.

The aim of this review article is to analyze the scientific literature regarding the alternative methods for caries removal and their advantages and disadvantages to be discussed.

The dentists' choice of method for excavation should be guided by the clinical requirements and outcomes. The characteristics of the method for caries removal include selective removal of infected dentin, being a reliable and effective method, painless and silent, minimal pressure caused during excavation, no vibration and heat generation, convenient and easy to use in the dental office [5]. The common distinct features of these alternative techniques involve a conservative, selective excavation of carious dentin with irreversibly denatured collagen fibers and maximum preservation of the structures with a potential for remineralization. The main distinctions between these techniques are based on the techniques and means for excavation, the amount and type of excavated dentin, different methods for control in the process of excavation [5].

1. POLYMER BURS

The use of the polymer burs is an alternative to the conventional stainless steel burs for the excavation of carious dentin (CeraBur K1SM (KOMET USA) – round bur, made of ceramics [9]. Their main advantage is the selective removal only of the

infected dentin owing to the material hardness which does not allow an excessive preparation. Once all soft, carious dentin has been removed, the instruments automatically blunt on hard healthy dentin [10]. When compared to the diamond and steel burs, the polymer burs remove dental tissues selectively but require two to three times longer for the caries-removal procedure [7, 11]. The main disadvantages are the excessive excavation of healthy dentin when high pressure is applied, breaking of the bur when it comes into direct contact with the enamel, the sensitivity of the technique, the use of polymer burs in combination with steel or diamond burs is required when we work in the enamel. However, in comparison with the alternative methods for caries removal, the mechanical excavation with rotary instruments is a non-selective removal of carious tissue, provokes discomfort and pain, as well as increased vibrations and contact pressure, risk of overheating of the surrounding tissues along with the unpleasant stressful sound of the rotary instruments. MID may involve the use of the conventional rotary instruments – high-speed handpiece, only to get access to the carious tissues [1].

2. AIR ABRASION

Air abrasion is a pseudo-mechanical, non-rotary method of cutting and removing dental hard tissue [11]. It removes tooth structure using a fine stream of aluminium oxide particles generated from compressed air. This method ensures a selective caries removal, until it reaches a surface with dry and hard dentin, as well as preserving healthy and remineralizable tissue. The efficiency of the removal is relative to the hardness of the tissue or material being removed and the operating parameters of the air abrasion devices [12]. Special nozzles

provide exceptional accuracy of particle stream as well as low air pressure (1.5–3 bars) and an adequate suction is required. The quantity of tooth removal and depth of penetration depends on the amount of air pressure, particle size, quantity of particles passing through the nozzle, diameter and angulation of nozzle of the handpiece, distance from the object, time of exposure to the object [6, 8].

Air abrasion is used to treat initial dentin caries lesions of the pits and fissures of the occlusal surface or on smooth surfaces [8]. After caries removal by air abrasion, the only choice for restoration is the adhesive materials. To minimize the inhalation of particles by the patient, the use of a rubber dam and special face masks are required. The advantages of air abrasion include the higher level in the cooperation in patient's behavior, elimination of the risk of overheating, vibration and mechanical stimuli. The main disadvantage is the loss of tactile sensation during caries excavation. Nowadays, Sandman Dental (Sweden) is widely used, combining a stream of alumina microparticles with a fine and precisely directed flow of compressed air. It ensures controlled treatment at low pressure with the exact amount of powder needed [12].

3. ULTRASONIC CARIES REMOVAL SYSTEM

The ultrasonic caries removal includes the use of different diamond-coated ultrasonic tips, ensuring selective removal of the decayed tissues and preserving the healthy enamel. The method presents advantages such as production of less vibrations and mechanical trauma as well as elimination of the unpleasant sound of the air turbine [6, 13]. The unilateral use of the diamond-coated tip one side only in proximal cavities ensures the preservation of the

healthy enamel of the adjacent tooth. Piezon Master 600 is a combined ultrasonic system for preventive procedures, periodontal treatment, endodontic treatment, minimally invasive treatment of fissure caries, preparation of the occlusal surface before a sealant application. The Vector system is a new method combining both ultrasonic effects and microabrasive action of quartz crystal suspension [11].

4. ATRAUMATIC RESTORATIVE TREATMENT (ART TECHNIQUE)

Atraumatic Restorative Treatment is a *minimally invasive technique for caries removal with hand instruments followed by restoration of the cavity and sealing pits and fissures with an adhesive material such as glass ionomer cementum [14]*. The soft part of the carious lesion is removed manually by a special set of hand instruments, called excavators, for removal of only the infected dentin (unable to undergo remineralization) until reaching firmer and light brown, affected dentin. Williams et al. point out the 'atraumatic' component of the technique concerning the patient's comfort, as the noise and vibration related to the bur are absent [15]. This effect is further enhanced by the fact that local anesthesia is rarely used [11]. ART is used to treat occlusal and cervical caries lesions without pulp involvement and periapical lesions and ensures self-control during the process of excavation due to the manual tactile sensation of the dentist/operator. It can be combined with a chemical agent which dissolves carious dentin so that caries is easy to remove with hand instruments.

5. CHEMO-MECHANICAL CARIES REMOVAL

Chemo-mechanical caries removal involves the *selective removal of carious tis-*

sue and maximal preservation of *healthy dental tissue*. The mechanism of action includes treatment of the carious dentin with a chemical agent before the caries removal, resulting in changes in its structure and composition. and more simple, fast and selective process of caries excavation. A combination of hand instruments and chemical agent – inorganic or organic, provides tactile feedback to the operator.

5.1. Inorganic agents

The CarisolvTM gel is the *most* successful and *commonly* used agent. It consists of a mixture of *sodium hypochlorite and three amino acids*. When in contact with amino acids, the reaction results in chloramine formation. Active free chlorine is said to remove only the infected dentine where collagen is degraded, maintaining the demineralized portion that is capable of being remineralized and repaired [16]. The authors indicate as disadvantages of the method the extra-time protocol and additional use of conventional rotary instruments for access to the caries dentin [17].

5.2. Organic agents (Enzymes)

Numerous studies have examined the efficiency of certain enzymes in the removal of carious dentin. Among the chemo-mechanical organic agents, the most popular is Papacarie, composed basically of papain, as an active ingredient, chloramine, toluidine blue-colorings [18]. Papain is a proteolytic enzyme having bactericide, bacteriostatic and anti-inflammatory properties [19]. It acts only on the carious dental tissue, due to the lack of an antiprotease α 1-antitrypsin, which inhibits protein digestion in sound collagen-based tissue. Along with debriding the collagen fibers which are partially degraded, it ensures preserving the healthy tissue as it is not demineralized and

it does not have any exposed collagen fibers. Chloramine has bactericide and disinfectant properties. Toluidine blue has an antimicrobial effect on *Streptococcus mutans* [7]. Other gels based on papain are Brix 3000 (Brix SRL Argentina) and *Carie-care™* (*Uni Biotech, Pharmaceuticals Pvt., India*). Several studies evaluated the capabilities of the proteolytic enzyme preparation of pronase (*Streptomyces griseus*), collagenase (*Achromobacter collagenase*) and a new pepsin enzyme in a phosphoric acid solution (SFC-VIII, 3M ESPE) for dentin *caries removal*. Chemo-mechanical excavation is well accepted by pediatric patients and very useful in the treatment of children with uncooperative behavior as it requires no anesthesia [20].

6. LASERS

Lasers usually used for ablation of hard dental tissues are high-power lasers – Er-YAG lasers and Er, Cr:YSGG lasers. The mechanism of action is “thermo-mechanical” ablation. Laser ablation is more effective in structures containing large amounts of water (demineralized dentin). The main principle in laser treatment is the selection of proper parameters for maximal effect in ablation and minimal side effects. Parameters are different for laser ablation in enamel, dentin, for primary and permanent teeth.

The numerous advantages of Er:YAG laser prompt a great increase in the research of its application in pediatric dentistry [21, 22]. The results obtained show that they are an effective technique for dental treatment of children [23]. The most commonly promoted advantages of lasers for hard dental tissue therapy in pediatric patients are minimal intervention and prevention, safety due to the low penetration depth of the laser beam, selective removal of caries lesion,

lack of thermal damage, no pain perception and use of local anesthesia, significant decrease of patient discomfort and dental anxiety and increase of subjective acceptance and tolerance of laser therapy in children [24, 25].

Conclusion

The findings of the literature review give grounds to undertake studies investigating the efficiency of the different methods for caries removal. Future explorations will be interesting as dental practitioners begin adopting alternative caries management strategies that may decrease the use of behavior management techniques in the treatment of pediatric dental patients.

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Subepithelial connective tissue graft in the plasty of gingival recession

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Gingival recession can be defined as a partially exposure of root surface (buccally and orally) of single and multiple teeth due to apical migration of the gingival margin. Etiology is complex – incorrect tooth brushing technique, accumulation of dental plaque and calculus, orthodontic treatment. The aim of present poster is to present the treatment of single maxillary canine gingival recession. Dates from family history showed previously provided orthodontic treatment. Recipient-site preparation includes horizontal incisions at the level of the cement enamel junction, vertical incisions on both sides reach to the alveolar mucosa, partial-thickness flap reflection under local anesthesia. From the palatal aspect of the first molar, second premolar horizontal incision 3–4 mm apically to the free gingival margin and vertical incision medially are made. After exposure of subepithelial connective tissue with a blade through the horizontal incision line the graft is harvested. The root surface is polished, a mixture of tetracycline tablets and saline solution is placed over the root surface followed by suturing for graft fixation. The partial-thickness flap is placed in its initially anatomical position. The suturing material from the donor and recipient sites are removed two weeks later. Clinical examination conducted 6 months later showed 80% root coverage.

Introduction

Gingival recession can be defined as a partial exposure of the root surface (buccally or orally) of one or multiple teeth due to apical migration of the gingival margin. Etiology is complex – incorrect tooth brushing technique, accumulation of dental plaque and calculus, orthodontic treatment, periodontal treatment, occlusal trauma, etc. (1). It can also be related to dentine hypersensitivity, root caries, abrasion, cervical erosion due to exposure of the root surface (2). Predisposing factors of gingival recession development are decreased alveolar bone crest thickness, combined with thinner gingival margin, commonly observed in maxillary canines and mandibular incisors; dehiscence (reduced buccal bone crest thickness) and abnormal frenulum insertion close to cervical region of gingiva (3). Gingival recession is commonly observed in adult patients. According to Mythri S, Arunkumar SM, Hegde S, et al. (4) gingival recessions are more frequently seen in mandibular incisors (43.0%). Kassab MM, Cohen RE. (5) determined that more than

50% of the population has one or more sites with gingival recession of 1 mm or more. Surgical procedures can be divided into the following types: pedicle soft tissue graft procedures (laterally positioned flap, double papilla flap, coronally repositioned flap) and free soft tissue graft procedures (free gingival graft, subepithelial connective tissue graft) (6). A technique with a subepithelial connective tissue graft is indicated for areas of single or multiple root coverage, mainly in the maxilla, and for areas requiring a combination of ridge augmentation and root coverage. The treatment success of the mentioned method is related to the double blood supply at the recipient site from the underlying connective tissue base and the overlying recipient flap. The donor site stays a closed wound, which leads to less postoperative discomfort (7).

Case Report

A 30-year-old female patient, not medically compromised (Amb. № 103/2020.10.21) referred to the Department of Oral Surgery, Faculty of Dental Medi-

cine, Medical University of Plovdiv, Bulgaria. The chief complaints were esthetic disturbances associated with root exposure of upper right canine. Dates from medical history showed traumatic tooth brushing, previously provided orthodontic treatment and sensitivity to cold drinks. On intraoral examination a single maxillary canine gingival recession, Miller's Class II (marginal recession extending to the mucogingival junction) was revealed (Fig. 1). Based on these findings, the surgical protocol included: recipient-site preparation with a horizontal incision at the level of the cementoenamel junction, vertical incisions on both sides reaching to the alveolar mucosa and partial-thickness flap reflection under

local anesthesia (Fig. 2).

From the palatal aspect of the first molar and the second premolar were made first a horizontal incision 3–4 mm apically to the free gingival margin and then medially to it a vertical incision. After exposure of the subepithelial connective tissue the graft was harvested with a blade (Fig. 3).

The root surface was polished and tetracycline solution was placed over it, followed by suturing the graft and placing the partial-thickness flap in its initially anatomical position (Fig. 4). The suturing material from the donor and recipient sites was removed after two weeks (Fig. 5). Clinical examination provided 6 months later showed 80% root coverage (Fig. 6).



Fig. 1. Before procedure.



Fig. 2. Flap design.

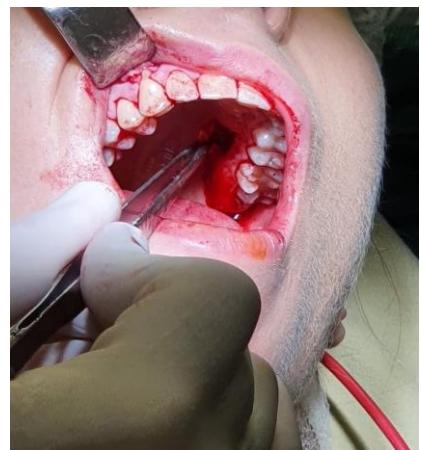


Fig. 3. Donor site.



Fig. 4. Graft fixation and flap reflection.



Fig. 5. Two weeks later.

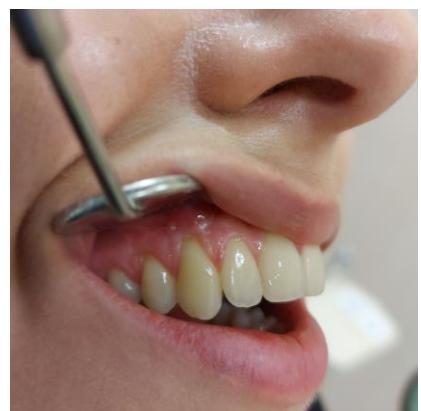


Fig. 6. 6 months later.

Discussion

The main indication for treatment of gingival recessions is patient's demand (8). In recent years different techniques for surgical treatment of gingival recessions have been introduced such as guided tissue regeneration, free gingival graft procedure, enamel matrix derivates, laterally positioned flap, tunnel technique etc. showing different degree of root coverage and long-term clinical outcomes (9). When compared the options for treatment of Miller's Class I and Class II gingival recessions using coronally advanced flap (CAF) the cases treated with coronally advanced flap CAF + connective tissue graft CTG showed better results with a statistically significant difference (10). The bilaminar technique with a subepithelial connective tissue graft is considered to be the gold standard in the treatment (11).

Conclusion

The present clinical case showed that the treatment approach with a subepithelial connective tissue graft is a successful technique for achieving significant root coverage, clinical attachment and keratinized tissue gain for single maxillary canine recession.

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Nonsurgical clinical management of periapical lesions using long term calcium hydroxide paste – report of several cases

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Introduction: Calcium hydroxide is used extensively as an intracanal medicament in endodontics for many years. Various biological properties have been attributed to this substance, such as antimicrobial activity, high alkalinity, inhibition of tooth resorption. It is used in various clinical situations, such as to promote apexification, to enhance healing of periapical lesions and to control exudation in teeth with persistent periapical inflammation. **The aim** is to present cases in which calcium hydroxide was used as an intracanal medicament for long term treatment of teeth with persisting chronological periapical lesions, aiming to avoid further surgical interventions. **Material and methods:** In this study teeth with chronological periapical lesions with the need of orthograde endodontic re-treatment were included. The protocol of the treatment is the same for all patients. The root canals are instrumented with Pro Taper Retreatment files D1, D2, D3, Pro Taper Next, using 2,5% NaOCl and 17% EDTA. The root canals are filled for three months with ex-tempore prepared calcium hydroxide paste, and after that – fully obturated. The healing progress in response to calcium hydroxide paste is assessed for a period of three months, six months and one year, using clinical methods and x-rays. **Results:** All the patients showed lack of clinical symptoms and radiographic data of upcoming healing process in the period of the study. **Conclusion:** Calcium hydroxide enhanced and shortened healing time of periapical lesions. The less invasive procedure avoids possible following endodontic surgery and reduces bone resorption.

Introduction

The focus of endodontics is the prevention and elimination of apical disease. A systemic literature review by Froum 2011 [1] showed that the ideal management of lesions should focus on infection control of the lesion and regeneration of lost support. The treatment options for large periapical lesions range from conventional non-surgical root canal therapy to surgical interventions [2]. The objective of this paper is to report the clinical cases of 4 patients, presenting a chronic apical periodontitis and the success rate of a root canal therapy using long-term calcium hydroxide as root dressing.

Clinical report

Four clinical cases diagnosed with chronic apical periodontitis were treated with long term calcium hydroxide dressing for a period of 1 month. All of the patients had radiographic evidence of apical periodontitis and previous endodontic treatment performed. Some of the teeth had clinical

signs of apical periodontitis, such as sinus tract or acute apical abscess. All of the teeth had sufficient coronal tooth structure for adequate isolation and restoration. The clinical observation did not show any existing coronal fractures that propagate across the pulpal floor or beyond the canal orifice level. All patients signed an informed consent concerning their present status, treatment protocol, possible complications and expected treatment results. The applied treatment protocol was one and the same for all patients. All patients were treated in private clinical practice with the assistance of fifth grade dental student. Local anesthesia, caries removal and rubber dam isolatation were performed. Following endodontic access preparation, canals were treated with Pro Taper Retreatment files D1, D2, D3 (Dentsply Maillefer) and fractured instruments, if needed, were removed under DM magnification. After obtaining patency, canals were instrumented to a minimum size of 30/07 taper (Pro Taper Next, Dentsply, Maillefer), bigger taper and Do diameter

was used were needed. 2,5% sodium hypochlorite irrigation was used copiously throughout the treatment. Final irrigation of 2,5% NaOCL and 17% EDTA solution, was agitated with passive ultrasonic irrigation (PUI). After drying with paper points, the canals were temporary obturated with ex tempore prepared calcium hydroxide paste, by mixing calcium hydroxide powder and distilled water. A layer of Ca(OH)₂ powder was placed over the orifices. Calcium hydroxide paste was loaded in the canals, using a lentulo spiral and replaced three times for a period of 30 days. Each time the access cavity was closed with ster-

ile Teflon tape and a definitive coronal restoration with GIC and composite material. After 1 month the root canal dressing was removed, using copious irrigation with 2.5% NaOCl and 17% EDTA. The irrigants were mechanically activated using passive ultrasonic irrigation (PUI). All canals were filled with gutta-percha and TotalFill BC sealer (FKG, Dentire SA). Control x-rays were taken immediately after definitive obturation of the root canals and 12 months later. During this period of time the patients had two appointments –at the third and the sixth month. They didn't show any clinical symptoms.



Fig. 1. Initial radiograph.



Fig. 2. Root canal filling.



Fig. 3. Radiographic follow up 12 months.



Fig. 1. Initial radiograph.



Fig. 2. Root canal filling.



Fig. 3. Radiographic follow up 12 months.



Fig. 1. Initial radiograph.



Fig. 2. Root canal filling.



Fig. 3. Radiographic follow up 12 months.



Fig. 1. Initial radiograph.



Fig. 2. Root canal filling.



Fig. 3. Radiographic follow up 12 months.

Discussion

In the presented clinical cases with persisting periapical lesions we made a choice for nonsurgical, multiple-visit treatment, combined with long term intracanal calcium hydroxide dressing. The nonsurgical approach was dictated by the statistic high rates of complete or partial healing after non-surgical endodontic therapy of approximately 90% [3]. It is also well known that nonsurgical root canal treatment should always be the first choice in cases of nonvital teeth with infected root canals. Periapical surgery is less recommended. Since it being an invasive procedure, involves risk of damaging the bone support and underlying important anatomic structures which might cause more post-operative pain and discomfort. Role of bacteria in the causation and the persistence of AP are well-established. Mechanical debridement combined with antibacterial irrigation can render 40–60% of the treated teeth bacteria-negative [4]. In addition to mechanical debridement and antibacterial irrigation, use of calcium hydroxide as intracanal medicament has been shown to reduce the bacterial count further by 80–100%. This has been the basis of the multi-visit treatment of AP [5]. Even though modern endodontics state that cleaning and shaping have greater importance than intracanal dressing for the success rate of endodontic treatment, intracanal medicaments are still used when more than one appointment is needed for root canal treatment and most likely in the presence of a large periapical granuloma or cyst. Several authors conducted studies on the time needed for calcium hydroxide-based dressings to completely eradicate pathogens associated with periapical lesions and promote bone healing. While Estrela et al claim a minimum of 60 days necessary for this process, Han et al declare, based on

their in vitro research, that a 3-week period is required for the same effect. According to Sjogren et al, at least 7 days are necessary for the destruction of microorganisms from infected root canals of periapical lesions [6]. Although periapical bone healing for smaller lesions can take up to 6 months, large periapical lesions require 9 to 12 months for complete bone regeneration around the apical region as the average healing rate is about $3 \text{ mm}^2/\text{month}$. The intracanal medicament for extensive bone destroying lesions should be placed for at least 1 to 2 months and refreshed once every few weeks, according to Dixit et al [7]. As the reported clinical cases had well defined, visible on the x-ray periapical lesions we made a choice for long term intracanal dressing, refreshing the calcium hydroxide dressing during a period of 10 days. Moreover in some of the cases, more than one visit was needed to remove the old root canal filling material and some of the fractured instruments. The need to refresh the calcium hydroxide dressing several times dictated our decision for ex tempore prepared paste mixed with distilled water. Ca(OH)_2 should be combined with a liquid vehicle because the delivery of dry Ca(OH)_2 powder alone is difficult, and fluid is required for the release of hydroxyl ions. Sterile water or saline are the most commonly used carriers. The vehicles mixed with Ca(OH)_2 powder play an important role in the overall dissociation process because they determine the velocity of ionic dissociation causing the paste to be solubilized and resorbed at various rates by the periapical tissues and from within the root canal. The lower the viscosity, the higher will be the ionic dissociation. Aqueous solutions promote rapid ion liberation and should be used in clinical situations. Compared with water-soluble agents, vis-

cous and oily vehicles prolong the action of the calcium hydroxide but can have associated negative side effects, mainly difficulty of removing them from the canal walls, which affect the adherence of sealer or other materials used to fill the canal. Because studies have shown that premixed pastes tend to wash out over time, calcium hydroxide powder was placed over the paste as a reservoir [8]. The complete removal of calcium hydroxide is crucial for the tight obturation of the root canal. Remaining calcium hydroxide may prevent the tight adaptation of endodontic sealer to the root canal wall and the penetration of sealers into dentinal tubules. For final irrigation and removal of the calcium hydroxide from the root canal walls we completed passive ultrasonic irrigation with Endo-U-files. PUI is regarded as the gold standard for irrigant activation throughout the literature [9]. Control x-rays for assessment of the healing process were made after 12 months. During this period the teeth didn't show any clinical symptoms. All teeth revealed signs of healing, visible on the control x-rays. After root canal treatment the probability of healing increases over time, however periapical changes are evident by 1 year. Orstavik concluded that 1-year follow-up predicts long-term success [10].

Conclusion

Calcium hydroxide enhanced the healing of the periapical lesions in the reported cases. The less invasive procedure avoided possible following endodontic surgery and reduces bone resorption. The sufficient me-

chanical cleaning of the endodontic system, copious irrigation using sodium hypochlorite and Ca(OH)2 based intracanal medicaments are these three steps that can assure a satisfactory outcome of extensive bone destroying lesions.

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Plasma levels of buffered articaine and metabolite articaine acid compared with non-buffered versus

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Buffered anesthetics have potential advantages. Sodium bicarbonate added to local anesthetics increased absorption of anesthetic related to fastly elimination from the body. **The purpose** of our investigation is to confirmed or rejected a working hypothesis – mean blood level differences of 4% articaine with 1:100,000 epinephrine for each patient are significantly lower after mandibular nerve block with the buffered drug compared with the non-buffered analog. **Materials and methods:** venous blood samples for articaine were taken 5 minutes after a mandibular nerve block with 68 mg of the buffered and non-buffered anesthetic. Outcome variables were injected dose in mg/kg, patients' blood articaine and articainic acid levels in mg/L. Plasma buffered and non-buffered versus levels were analyzed with liquid chromatography-tandem mass spectrometry. **Results:** median injected dose with buffered agent was 0.84 mg/kg (11 subjects) as well as median injected dose with non-buffered analog – 0.86 mg/kg (28 subjects). Median buffered articaine plasma levels (11 blood samples) at 5 minutes were 0.28 mg/L while median non-buffered articaine plasma levels (28 blood samples) were 0,37 mg/L. No difference in blood plasma levels exist between buffered and non-buffered anesthetic ($p > 0.05$). Mean buffered articainic acid plasma levels (11 blood samples) at 5 minutes were 0.09 mg/L while mean non-buffered articainic acid plasma levels (28 blood samples) were 0.38 mg/L. **Conclusion:** preliminary working hypothesis is not confirmed by mean plasma levels of buffered and non-buffered anesthetics. The present project is granted to "National Science Fund", Contract № KP-06-M23/2, 2018.12.18.

Introduction

Fear of injection during dental manipulation remains a main problem requiring knowledges about behavioural psychology before conduction of dental treatment. The main purposes of clinicians are to investigate a new methods of the pain management (1). Local anesthetics solutions consist of 2 forms – tertiary and quaternary. The quaternary form is prevalence in a solutions with a low pH. The tertiary form characterized by lipid soluble and respond to across nerve membrane, followed by conversion into the quaternary form. The main function of quaternary form is to block a nerve conduction. In the presence of local anesthetic solutions in the market with a vasoconstrictor have a pH approximately 3.5. At this level of pH composed of a small percentage of tertiary form which lead to speed of onset of anesthesia reducing. In the literature was described the normal pH of human tissue (7.4). Buffering of local anesthetic solutions with a 8.4% Sodium bicar-

bonate is related to tertiary form increases (2). Buffered anesthetics have undoubted advantages. Buffering local anesthetics with sodium bicarbonate significantly decreased pain during injection on the first place. In case of increase pH of the local anesthetic solution up to 7.4 before injection, the speed of onset of the anesthetic action is increased, as well as the patient comfort. Not in the last place, buffering anesthetic solution should be the 6,000-fold increase in the number of anaesthetic molecules ability to across into the nerve, which associated with more profound anesthetic effect (3). In the literature there are different methods for determination of dental local anesthetics in human plasma. One of them is a liquid chromatography-tandem mass spectrometry. Hoizey G, Lamiable D, Gozalo C., et al. (4) are used a specific liquid chromatography–mass spectrometric (LC–MS) method for the quantitation of articaine in human plasma and revealed that the method have good specificity and sensitivity. The solid-

phase extraction (SPE) is used for preconcentration of articaine and the metabolite articainic acid and high-performance liquid chromatography (HPLC) for the determination of both compounds in human serum (5). There is a fully validated a new method for the simultaneous detection of articaine and mepivacaine in whole blood by liquid chromatography-tandem mass spectrometry in multiple reaction monitoring mode (transitions: articaine, $285 \rightarrow 8658$ m/z; mepivacaine, $247 \rightarrow 9870$ m/z; lidocaine – internal standard –, $235 \rightarrow 8658$ m/z) (6).

Ethical Approval

The Local Ethics Committee for Scientific Research of the Medical University – Plovdiv, Bulgaria approved this study and with the Helsinki Declaration of 1975, as revised in 2000.

Inclusion and exclusion criteria

1. Inclusion Criteria:

- give written, informed consent;
- be 18–80 years of age;
- be a patient who can be expected to keep with the protocol;
- present lower jaw dental disease with indication for extraction;
- be willing to attend the department for 3 or more appointments.

2. Exclusion Criteria:

- a history of allergy, sensitivity, or any other form of adverse reactions to articaine, or epinephrine;
- a history of general disorders that would exclude administration of an articaine or epinephrine (liver, kidney, cardiovascular diseases, uncontrolled diabetes, pheochromocytoma etc.);

- current systemic medication which contraindicates the use of articaine or epinephrine;
- pregnant and lactating women;
- alcohol abuse;
- received an anesthetic, analgesic or sedative within 24 hours prior to the procedure;
- acute odontogenic infections need from immediate treatment;
- inclusion in a clinical study of an investigational drug within the 1 months before.

Patients and methods

The **purpose** of this study was to compared the blood level of 4% articaine with 1:100,000 epinephrine and his metabolite articaine acid with the non-buffered analogs after mandibular nerve block.

Statistical Analysis

dates were analyzed with IBM SPSS statistics software 21.0 Version. Mann-Whitney Test was used to assess the number of mandibular nerve blocks, injected dose in mg/kg, patients' blood articaine and articainic acid levels in mg/L. Levene's test and t-test were used to perform multiple comparisons related to blood plasma articaine and articainic acid levels in mg/L of the buffered and non-buffered anesthetic. Statistical significance was set at a P value less than 0.05 for all outcomes. In this study was involved 39 patients (20 – males, 19 – females) aged 22–78 years. Median age was 47.38 ± 15.62 while median body weight was 81.45 ± 10.86 kg. The median injected dose with buffered articaine was 0.84 ± 0.11 mg/kg (11 subjects) as well as median injected dose with non-buffered articaine – 0.86 ± 0.11 mg/kg (28 subjects) (Fig. 1).

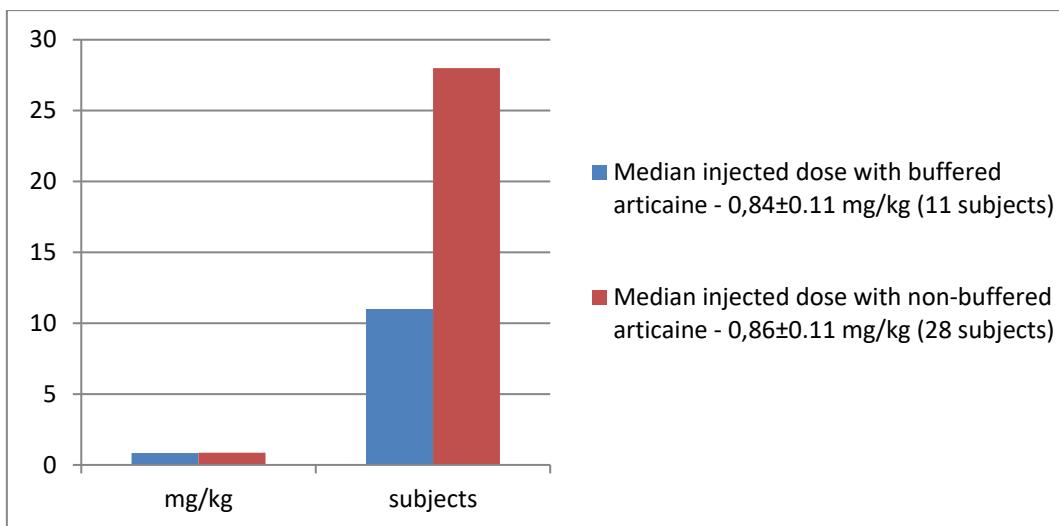


Fig. 1. Median injected dose with buffered and non-buffered articaine.

Venous blood samples were drawn from the antecubital fossa 5 minutes after the injection of the buffered and non-buffered articaine and assayed for plasma articaine and articaine acid levels with a core shell Kinetex® 100x3.0 mm, 1.7 µm particles analytical column (Phenomenex, CA, USA). The time to maximum concentrations of articaine was reached 10 to 15 minutes after submucosal injection while the time to maximum concentrations of his metabolite articaine acid occurs approximately 45 minutes after submucosal administration. The median blood buffered articaine level at 5 minutes after injection for

11 subjects (11 blood samples) was 0.28 ± 0.22 mg/L. The median blood non-buffered articaine level at 5 minutes after injection for 28 subjects (28 blood samples) was 0.37 ± 0.31 mg/L (Fig. 2). The difference between patient preferences for buffered or non-buffered articaine formulation wasn't statistically significant ($P = 0.265$; $p > 0.05$). The median blood buffered articainic acid level at 5 minutes after injection for 11 subjects (11 blood samples) was 0.09 ± 0.04 mg/L. The median blood non-buffered articainic acid level at 5 minutes after injection for 28 subjects (28 blood samples) was 0.38 ± 0.45 mg/L (Fig. 3).

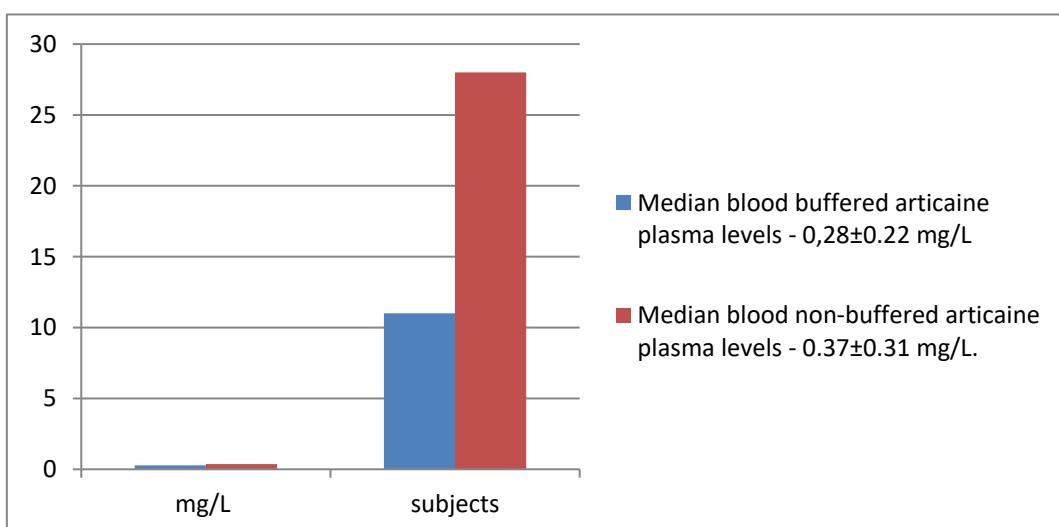


Fig. 2. Median blood buffered and non-buffered articaine plasma levels.

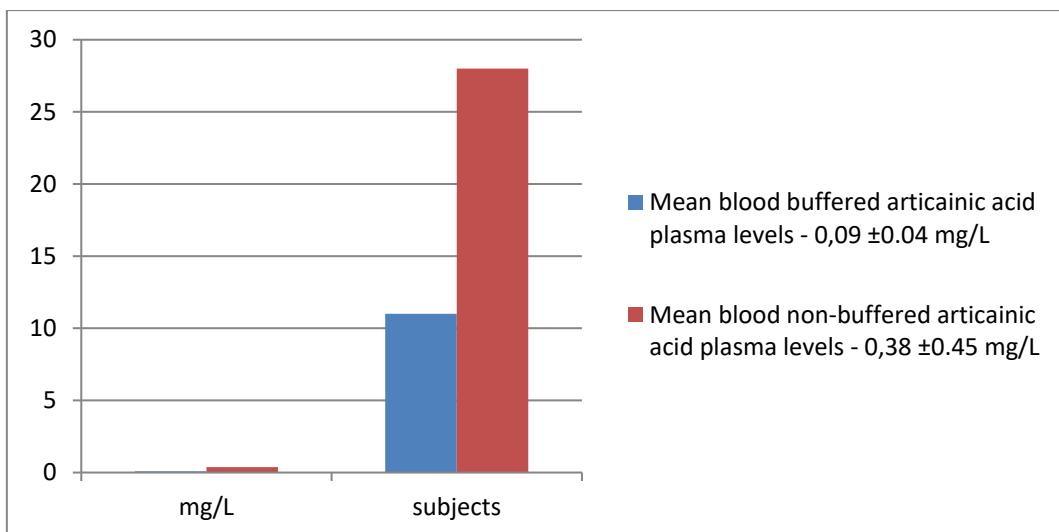


Fig. 3. Median blood buffered and non-buffered articainic acid plasma levels.

Discussion

Pain control is the main factor of patient safety and comfort. In the literature was found that 4 percent articaine with epinephrine 1:100,000 was well-tolerated in 882 subjects. It also ensured pain decrease during most dental interventions and increase to onset and duration of anesthesia (7). Martinez AM. (8) applied conventionally buffered technique of 4% Articaine, 1:100 000 Epinephrine during third molar surgery and concluded that buffered anesthetic with 8.4% Sodium bicarbonate decreased time to achieve latency period and injection pain compare to usage of non-buffered 4% Articaine, 1:100 000 Epinephrine.

In dentistry the pharmacokinetics of local anesthetics has been studied in topical, infiltration and mandibular nerve block anesthesia. Excepts the method of application, the plasma and serum levels of local anesthetics are related to the different factors such as cardiovascular system absorption, distribution rate, drug elimination etc. By the usage of liquid chromatography-tandem mass spectrometry (LC-MS/MS), local anesthetics and especially their metabolites are easily identified than those obtained in gas chromatography by flame ionisation

detectors (GC-FID) and gas chromatography by mass-spectrometry (GC-MS) (9).

The results of our investigation are applicable to people aged from 22 years to 78 years, of both genders, who received mandibular nerve block injection with 4% buffered and non-buffered articaine with epinephrine. There wasn't difference in the concentration of epinephrine – 1:100,000.

Results obtained from us revealed that no difference in blood plasma levels exist between buffered and non-buffered anesthetic ($P = 0.265$; $p > 0.05$). Previous study provided from Phero JA, Nelson B, Davis B., et al (10) concluded that the mean blood level differences of lidocaine for each patient were significantly lower after nerve block with the buffered drug compared with the non-buffered agent ($P < 0.01$). For serum lidocaine levels they assayed with a Sciex TripleTOF liquid chromatography-mass spectrometry (LC-MS; Sciex, Framingham, MA) equipped with a C18 Hypersil (10 2.1 mm, 3.0 mm).

Conclusion

Preliminary working hypothesis was not confirmed by mean plasma levels of buffered and non-buffered anesthetics.

Acknowledgements

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VI. Social Medicine and Public Health session

Oral health self-assessment among haemophilia families

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Introduction: Clinical evaluation of dental diseases can clarify their etiology and prognosis, but it provides a little information about the level of impairment in terms of patients perspective in cases of chronic diseases such as hereditary coagulopathies. **Aim:** The aim of this study is to research and analyse the oral health self-assessment among families with haemophilia. **Materials and methods:** A sociological survey was conducted among families with haemophilia during the period 2019–2020. Direct individual questionnaires were created based on OHIP-14. The data analysis was performed with the software package IBM SPSS Statistics v. 25 and Microsoft Office Excel 2016. **Results:** Questionnaires were completed by 31 children with haemophilia and 33 parents. Participants rated their dental health level on Likert scale as “excellent” (children with haemophilia: n = 16; $51.64 \pm 8.98\%$ and parents: n = 18; $54.55 \pm 8.94\%$), “average” (n = 9; $29.03 \pm 8.15\%$ and n = 11; $33.33 \pm 8.47\%$) and “poor” (n = 2; $6.45 \pm 4.41\%$ and n = 4; $12.12 \pm 5.86\%$; $p > 0.05$). A significant association is observed between oral health evaluation and factors such as the absence of toothache ($r^2 = 15.37$; $p = 0.004$). **Discussion:** In order to eliminate the dilemma regarding the quality of life of hemophiliacs, we should guarantee dental treatment in a multidisciplinary team approach. Moreover, what should be provided is accessible, effective care, provided by every general dentist. **Conclusion:** This study is the first to evaluate the oral health self-assessment among families with haemophilia in Bulgaria. The research elucidates the reasons leading to lower quality of life, as toothache or discomfort, nutritional problems, aesthetic aspects of the smile.

Introduction

The assessment of dental status and quality of life related to oral health is significantly influenced by the presence of chronic diseases, such as hereditary coagulopathy. Bleeding in the oral cavity is a common symptom in people with haemophilia in medical and dental aspects, compromising their well-being. [1] Moreover, in children with haemophilia, there is an increased risk of life-threatening bleeding caused by invasive dental procedures. Clinical evaluation of dental diseases can clarify their etiology and prognosis, but it provides little information about the level of impairment in terms of the patient's perspective. Multiple studies prove that deterioration in children's dental status could be predicted by their parental oral health. [2] Therefore, crucial factors for establishing oral health habits and preventing significant bleeding in children are family culture and health

education of the family environment. Children's behaviour is largely a mirror-reflecting image of their parents, therefore self-assessment of the oral health status of families with haemophilia should be simultaneously performed among their members. Successful dental evaluation of this small population could be realised only through cooperation among children with haemophilia, their family and the haemophilia treating centres to attain higher a oral health-related quality of life in the young generation. [3–5]

Purpose

The purpose of this study is to research and analyse the oral health self-assessment among families with haemophilia.

Materials and methods

A sociological survey was conducted among families with haemophilia during

the period 2017–2020. Direct individual questionnaires were created based on OHIP-14. The data analysis was performed with the software package IBM SPSS Statistics v. 25 and Microsoft Office Excel 2016. Primary sociological data about children with haemophilia and parents was collected through direct individual questionnaires. The questionnaires were original, based on OHIP-14. The use of the sociological approach provides information about the demographic characteristics of the contingent, their knowledge about oral hygiene and their habits, self-assessment of the oral status and an opportunity to compare both groups. Some questions contained options for more than one answer. Comparisons between the groups of children with haemophilia and parents were performed using the Mann–Whitney U test, and values $p < 0.05$ were considered significant. The Chi-square test was used for comparison of categorical variables.

Results

Questionnaires were completed by 31 boys with haemophilia between 7 and 18 years ($\bar{x} = 11.55$, $Sx = 0.58$, $Mo = 14.00$, $Me = 12.00$) and 33 parents of children with haemophilia. Participants rated their dental health level on a Likert scale as “excellent” (children with haemophilia: $n = 16$; $51.64 \pm 8.98\%$ and parents: $n = 18$; $54.55 \pm 8.94\%$), “average” ($n = 9$; $29.03 \pm 8.15\%$ and $n = 11$; $33.33 \pm 8.47\%$) and “poor” ($n = 2$;

$6.45 \pm 4.41\%$ and $n = 4$; $12.12 \pm 5.86\%$; $p > 0.05$). There were similarities between the respondents, in the perception of their oral status, as more than half of the respondents are defining it as better than the others were. There was a small group of children with haemophilia, who were unable to rate their dental status. It could be clarified through a more detailed analysis of the opinion of children with haemophilia, through the sociological approach of the interview, which is recommended for future research.

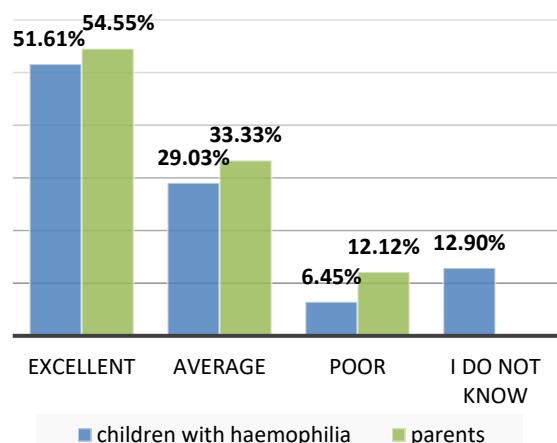


Figure. 1. Oral health self-assessment of children and adults.

A significant association is observed between the oral health evaluation as “excellent” and factors such as the absence of toothache ($\chi^2 = 15,37$; $p = 0,004$). (Table 1) The lower frequency of pain in the tissues of the oral cavity is, the lower self-assessment of parents is.

Table 1. Oral health self-assessment based on the frequency of toothache

		Oral health self-assessment									
		excellent			average			poor			
		n	%	Sp	n	%	Sp	n	%	Sp	Total
Frequency of toothache	sometimes	0	0,00%	0	1	9,09%	8,67%	2	50,00%	25%	3
	rarely	10	55,56%	11,71%	9	81,82%	11,63%	0	0,00%		19
	never	8	44,44%	11,71%	1	9,09%	8,67%	2	50,00%	25%	11
	Total	18	100,00%		11	100,00%		4	100,00%		

Most of the respondents from the adult group had “rarely” or “never” toothache. (Figure 2) They evaluated their oral health status as significantly better than those who had sometimes toothache in the past.

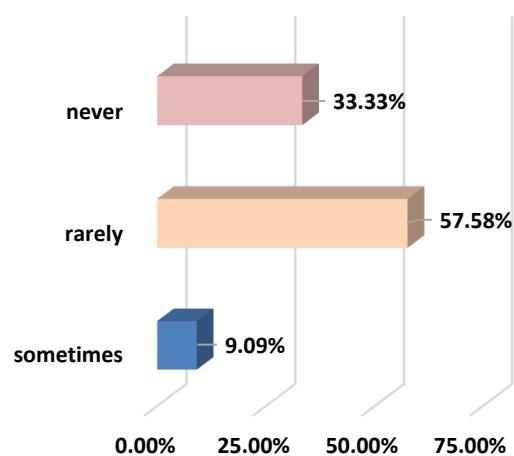


Figure 2. Toothache frequency in the last year.

Parents of children with haemophilia, who visited a dentist one or two times in the last year, described their dental status as better than the others ($\chi^2 = 17,35$; $p = 0,008$). (Figure 3) One or two dental visits a year are the typical recommendations for most patients. The frequency of visits two times per year is accepted as a golden rule and minimum for a healthy oral cavity. During the check-up, dental professionals examine carefully for oral pathology, which could cause damage to the soft tissues aiming to prevent future bleeding. In case of healthy dentition, appointments are scheduled in advance for regular annual prophylaxis and remotivation for oral hygiene improvement. What should be considered in the individual approach, are the intervals between the oral health examinations. Especially for patients with comorbidity and children, visits should be based on the personal needs, starting from an earliest moment when primary dentition begins to erupt. [6]

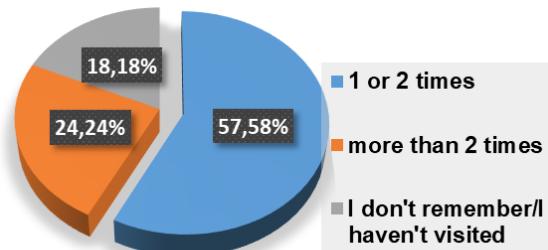


Figure 3. Visits to the dentist in the last year.

Discussion

Most participants self-rated their dental status as excellent with no statistical difference among the groups, which was in contrast to the literature. Only a few studies aim to measure the quality of life related to oral health status, but to clinically evaluate their oral hygiene habits (OHI-S), caries experience (DMFT), periodontal status (gingival inflammation index, periodontal index). [3, 7–11] This research is important for children with haemophilia as it helps to create future actions to improve the self-concept of oral health from individual to community level. Essential measures could be professional support and provision of tailored oral health care. To eliminate the dilemma regarding the quality of life of people with haemophilia, what should be guaranteed is a dental treatment in a multi-disciplinary team approach. In need of referral to a dentist, it should be provided to the nearest ambulatory for the patient. Effective, specialized care is crucial, but nowadays awareness should be raised in the access to primary dental care for people with bleeding disorders, provided by every local general dentist. [12]

Furthermore, what should be considered is the individual approach, where intervals between the oral examinations, especially for patients with comorbidity, should be based on their needs. Bulgarian National Health Insurance Fund covers dental examination once a year, including diagnostics

and dental advice for adults and children, which could be increased to once every six months. [13] The focus of the national policies is the health education of children and parents about proper oral hygiene, fluoride prevention, proper nutrition and control of carbohydrate intake, chewing sugar-free gum, and regular visits to the dentist. [14] More attention should be spread to the increase in the share of families who have the right dental knowledge, adequate dental behaviour and skills to protect their oral health.

Conclusion

This study is the first to evaluate the oral health self-assessment among families with haemophilia in Bulgaria. The research elucidates the reasons leading to lower quality of life, like toothache or discomfort, and visits to the dentist. Future research should be conducted to assess other factors deteriorating quality of life related to oral health, like nutritional habits, aesthetic aspects of the smile and treatment need of children with haemophilia.

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Anxiety of health complications and association with number of body modifications

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For thousands of years, people have changed the appearance of their bodies with cosmetics, jewelry, tattoos, piercings and other surgical procedures. Body piercing and tattooing are practiced in almost every culture. In some cases, they may be associated with ritual ceremonies and religious rites. Very often tattooing and piercing poses significant health risks. This article presents some of the health risks associated with the most common body modifications (tattoos and piercings). A number of studies comparing the adverse events after tattoos and piercings have found that piercing is associated with a significantly higher incidence of complications than tattooing, although it is done in a professional settings. The ever-increasing number of people with tattoos and piercings and the risks they may pose to health is the reason European Commission has taken measures to regulate the use of certain paints for tattooing, due to suspicions they are carcinogenic. The latest changes that Brussels has made to the regulation ban green and blue colors in some cosmetic products. From January 4th, 2022 the introduction of more stringent control over tattoo paints is forthcoming, as experts believe that as a long-term consequence the ink in the skin can lead to cancer, genetic mutations, reproductive problems and allergies.

Introduction

From time immemorial, people have deliberately sought a way to leave lasting marks on their bodies. The reasons for this were different – rituals associated with the transition from one age to another, protection from negative influences, expression of belonging to a particular social group, treatment, beautification, memories and more. This proves that at every stage of human development body modifications have been and continue to be expressive forms. That is why they have always been perceived as one of the means of communication. Despite their great popularity, their placement is often associated with significant health risks, which are described in the scientific literature. In the current article we concern only the complications of tattoos and piercings as body modifications.

The process of tattooing involves damaging the skin barrier, which may result in penetration of infectious agents. These include bacteria, viruses and fungi. Tattooing may be associated with the potential for

transmission of hepatitis B (HBV) and C virus (HCV) and human immunodeficiency virus (HIV) [1]. In present days hygiene standards have improved and the potential for transmission of these severe diseases is almost nonexistent. The tattooing is done in professional tattoo settings and sterile disposable needles and other disposable materials are used [2]. Tattoos which are performed in unsanitary poorly equipped settings, by unlicensed practitioners pose a significant risk for the transmission of these viruses [3]. Even if appropriate measures are taken, contamination still happens at the tattoo site and thus may occur skin infection. The rate of infections is regarded as very low. However, the overall rate of skin infections is still unknown, mostly due to the self-treatment of mild infections in which no medical care is sought. This is not true for the rate of systemic complications, as they are not self-medicated, and require specialized medical treatment [4]. However, many infections are due to insufficient care of tattoo site. The most common complica-

tions of tattooing are bacterial infections. It has been reported that tattoo-related skin infections are occurring in 1–5% of tattoo recipients [5]. Systemic bacterial infection may develop especially when tattooing is not performed in professional setting. This may include – toxic shock, epidural abscesses, endocarditis, septicemia, fungal infections [6, 7]. Other complications include systemic viral infections, cell-mediated tattoo reactions, lichenoid reaction, tuberculoid, sarcoidal, necrobiotic granulomatous reactions, pseudolymphoma and cancer.

Piercing is the practice of puncturing a fistula-like opening with a large-bore needle through the skin or cartilage or with cutting a part of the human body, in which jewelry or implant could be inserted (8, 9, 10). Motives for obtaining piercing are diverse. They include: beautification, expression of individuality, personal history, physical endurance, group affiliations, spirituality, addiction of piercing, sexual signaling and stimulation. Complications of body piercing have been reported at frequencies of 17% to 46% [11]. Most are minor, but rarely do occur major complications. The most common complications of piercing are infections, bleeding, metal allergy and problems relating to tissue trauma [12]. Local infection is the most common piercing complication, occurring in approximately 20% of all piercings. Some risk exists for transmission of HBV, HBC and HIV. Systemic infections like microbial endocarditis also may occur, but very rarely. Those with accompanying medical conditions have the highest risk. During most body piercing procedures some blood loss is normal. Excessive bleeding from highly vascular organs such as the tongue or penis that lasts a few days is not unusual. Serious hemorrhage has been reported, including hypovo-

lemic shock. If the corpus cavernosum is affected, hemorrhage into the spongy tissues may lead to long-term erectile dysfunction. Allergy to metal is also frequent. There are reports stating that 17% of individuals with piercing have a positive reaction to nickel, the most common metal allergen. Besides allergies to cobalt and chromium are also demonstrated. Metal allergy is more likely to develop in individuals with multiple piercings, than those with one piercing. Unintentional wounds caused by the piercer, unwanted tissue trauma may occur at the time of piercing or after during the healing process or unintentional accidents. Individual differences in healing and scarification process can lead to certain complications of genital piercings such as urethral stricture or fistula, avulsion of the clitoral hood or other anatomical anomalies. Somewhat external sites, such as earlobes, are specifically prone to tearing due to catching on outfit or hair, falling on ear, violent sports, assault, or accidents. Some individuals are vulnerable to formation of keloids such as scars with autonomous uncontrolled growth that pervade beyond the immediate area of the wound. Oral piercings, including the tongue, negatively affect dental health condition. Cracks and fissures of the dental enamel, gingival recession and increased dental caries are commonly occurring. Halitosis due to food decay is a condition of many pierced individuals.

The number of individuals having body modifications has significantly increased over the past few decades and there have been no major studies that test pain perception in these subjects [13, 14, 15].

Material and methods

The survey includes 122 respondents. The respondents are consisted of 86 subjects with tattoos and 84 subjects who had

piercings. All subjects were aged between 18–63 years. The mean age of the contingent is 24.20 years (SD – 7.57 and range – 45). The men were 24 (19.7%) and were on mean age of 22.17 years (SD – 2.73 and range 11; 19–30), and women were 98 (80.3%) on mean age – 24.69 years (SD – 8.27 and range – 45; 18–63).

For the purpose of the study, a questionnaire was developed. The questionnaire includes 30 items, that cover socio-demographic characteristics, motives for body modification, preferred type and location, the impact of the social environment, health risks, self-assessment of the psycho-emotional state during body modification and others. It was distributed and completed by subjects online.

Data of health concerns about complications during or following body modification are collected as binary information (0 – none, no anxiety/fear, 1 – there is anxiety/fear, regardless of its strength). The number of tattoos and piercings is divided into the following groups: 1 – one tattoo/piercing, 2 – from 2 to 5 tattoos/piercings, 3 – 6 and more tattoos/ piercings.

The data was analyzed with statistical software package methods SPSS v.26.0. Descriptive statistics and nonparametric analyzes were used – Kruskal-Wallis test and Mann-Whitney U-test. The level of statistical significance was $p < 0.05$, two tailed and 95% confidence interval.

Study aim

The present study aims to examine the relationship between some of the most common health concerns about body modifications and the number of tattoos and piercings. The administered questionnaire examines anxiety/fears about inflammation, pain and contamination during and after tattooing or piercing.

Results

For the purpose of our study, it was important to determine whether participants had physical health concerns prior to body modification. Among the subjects with tattoos 88.4% answered positively to the question: “Did you have any worries before getting a tattoo”. The predominant concerns were related to anxiety of inflammation (39.5%), anxiety of pain (27.9%) and anxiety of contamination (25.6%).

As the data is not continuous and lacks normal distribution, the non-parametric Kruskal-Wallis test for distribution difference between three or more independent groups was used. The test showed a significant statistical difference between the groups of number of tattoos in “Anxiety of pain” $p < .049$ and “Anxiety of infection” $p < .034$. As there are no post hoc tests for the Kruskal-Wallis test, several Mann-Whitney U-tests were performed with a Bonferroni correction. In the item “Anxiety of pain” there is a statistically significant difference between the 1st and 2nd group, $p < .045$. In “Fear of contamination” there is a statistically significant difference between the 2nd and 3rd group, $p < .027$.

Among the subjects with piercings – 83.1% answered positively to the question: “Did you have any worries before getting a piercing”. The subjects answered positively for the predominant health concerns related to anxiety of inflammation – 31.0%, anxiety of pain – 26.2% and anxiety of contamination – 16.7%. The Kruskal-Wallis test showed a significant statistical difference between the groups of number of piercings in “Anxiety of Inflammation” $p < .016$. Several Mann-Whitney U-tests were performed with a Bonferroni correction. In “Anxiety of inflammation” there is a statistically significant difference between the 1st and 3rd group, $p < .036$ and the 2nd and 3rd group, $p < .024$.

Table 1. Overall comparison of Anxiety of inflammation, Anxiety of pain and Anxiety of contamination irrespective of number of tattoos groups

	Anxiety of inflammation	Anxiety of pain	Anxiety of contamination
Kruskal-Wallis H	.437	6.047	6.753
Asymp. Sig.	.804	.049	.034

Table 2. Comparison of Anxiety of pain and Anxiety of contamination across the number of tattoos groups

	n of tattoos	Mean Rank	Comparison	Mann-whitney U	Asymp. sig	Bonferroni adj.
Aniety of pain	1 st group – 1	39.12	1–2	500.0	.015	.045
	2 nd group – 2–5	50.13	1–3	240.0	.765	1.000
	3 rd group – 6 and more	41.17	2–3	152.0	.115	.345
Anxiety of contamination	1 st group – 1	42.21	1–2	546.0	.098	.294
	2 nd group – 2–5	49.13	1–3	198.0	.169	.507
	3 rd group – 6 and more	33.00	2–3	120.0	.009	.027

Table 3. Overall comparison of Anxiety of inflammation, Anxiety of pain and Anxiety of contamination irrespective of number of piercing groups

	Anxiety of inflammation	Anxiety of pain	Anxiety of contamination
Kruskal-Wallis H	8.320	2.145	2.915
Asymp. Sig.	.016	.342	.233

Table 4. Comparison of Anxiety of inflammation across the number of tattoos groups

	n of piercings	Mean Rank	Comparison	Mann-whitney U	Asymp. sig	Bonferroni adj.
Anxiety of inflammation	1 st group – 1	44.45	1–2	628.0	.787	1.000
	2 nd group – 2–5	45.62	1–3	136.0	.012	.036
	3 rd group – 6 and more	27.50	2–3	116.0	.008	.024

Discussion

It is evident that less subjects with piercing have anxiety for complications compared to subjects with tattoos. The anxiety of inflammation, anxiety of pain and anxiety of contamination follow a different frequency pattern in tattooed and pierced subjects. Overall the frequency of positive answers for anxiety of inflammation, pain and contamination is lower in the pierced subsample. It is evident that tattooed subjects differ across groups of number of tattoos in anxiety of pain and anxiety of contamination. Subjects with piercing differ across groups of number of piercings in anxiety of inflammation.

Conclusion

The grown-up popularity of body modifications represents a challenge for health not only of healthcare professionals but also of the obtaining individuals and the legal regulations. The intersection of fashion, expression, psychology and health requires that clinicians be prepared to provide safe and respectful care. Body modification providers must be able to accurately counsel and guide before initiation of procedures. It is important that they know how to recognize and treat minor complications and refer accurately in situations with higher health stakes. Most of individuals who have obtained any kind of modification are typical-

ly enthusiastic about their experiences and are willing to share it with interested healthcare providers. These clinicians who demonstrate a nonjudgmental, discussing and informational attitude to body modifications, can help create a healthy social environment in which complications receive prompt and appropriate health care. It is noteworthy that subjects who are willing to place a tattoo or piercing should seek and receive more health information about body modifications and their complications.

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Covid pandemic: eye comfort and remote education

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Covid-19 pandemic has profound effect on all societies in the world, and has had different health, economic, social, and psychological results. Social isolation measures have been taken to slow the disease; among them is closing all the universities in Bulgaria since October 2020, and switching to distance learning process. Although remote education has the effect of reducing the transmission rate, it may have negative effects on eye health. **Aim:** The purpose of this research is to evaluate the effect of remote education on eye comfort of medical students in Covid-19 pandemic. **Material and methods:** For this aim, 190 students with a mean age of 23.26 from Medical university of Pleven, were asked about their eye health and comfort of anterior ocular surface during distance-learning semester. They were interviewed anonymously, using online questionnaire, partially based on Standardized Patient Evaluation of Eye Dryness (SPEED) Questionnaire, in the period between 1–20 December 2020. **Results:** Findings of the study highlighted that digital and online learning increases screen time at potentially dangerous for eye health levels. 48.9% of students use a monitor more than 8 hours daily, 37.8% – between 5 and 8 hours per day, 11.2% from 3 to 5 hours, only 1.6% reported screen time less than 3 hours. Constant eye fatigue reported 18.1% of students. Almost 1/3 (30.9%) of responders complain of often sensation of eye fatigue, 33.5% reported eye fatigue sometimes. 40.6 percent of responders had complaints of burning or watering sometimes, while 6.4% had these symptoms constantly, and 16% had them often. During online learning process, 7.4% of students had constant feeling of eye soreness and irritation, 25% had it often, and 35.6% felt it sometimes. According to answers, dryness, grittiness, scratchiness in their eyes experienced constantly 9.6% of asked students, often – 19.1%, sometimes – 44.1%. 16.5 percent of responders report their complaints were bothersome-irritating and interfering with their day. 24.5% describe the severity of their symptoms as uncomfortable – irritating; 36.7% described them as tolerable, only 20.7% did not have any problems. **Conclusion:** As a conclusion of this study, it was observed that eye health and eye fatigue among medical university students was negatively affected by the distance learning education during the Covid-19 pandemic social isolation. Increased screen time during online semester causes digital eye strain, which interferes with the quality of life and is an emerging public health issue.

Introduction

Covid-19 pandemic has profound effect on all societies in the world, and has had different health, economic, social, and psychological results. Social isolation measures have been taken to slow the disease; among them is closing all the universities in Bulgaria since October 2020, and switching to distance learning process. Although remote education has the effect of reducing the transmission rate, it may have negative effects on eye health. Digital eye strain, or computer visual syndrome, is an emerging occupational and public health issue. This is a condition characterised by visual disturbance and/or eye discomfort related to the use of digital devices (computers, tablets, smartphones) and resulting from a range of stresses on the ocular environment (1, 2).

It is known that around 90% of electron-

ic device users experience symptoms of digital eye strain. There are studies, which suggest that the following factors are associated with digital eye strain: uncorrected refractive errors, vergence or accommodation anomalies, altered blinking pattern (incomplete blinking, reduced rate of blinking), intense light exposure, not proper working distance (more often closer), and smaller font size (2, 3, 4).

Aim

The purpose of this research is to evaluate the effect of remote education on eye comfort of medical students in Covid-19 pandemic.

Material and methods

For this aim, 190 students with a mean age of 23.26 from Medical university of

Pleven, were asked about their eye health and comfort of anterior ocular surface during distance-learning semester. They were interviewed anonymously, using online questionnaire – Google docs, partially based on Standardized Patient Evaluation of Eye Dryness (SPEED) Questionnaire, in the period between 1–20 December 2020. The eye fatigue questionnaire consisted of 6 questions, focused on sensation of ocular discomfort and its severity and lasting during remote education – usual screen time, complaints of burning or watering, feeling of eye soreness and irritation, dryness, grittiness, scratchiness. Also, survey respondents were asked to describe the severity of their complaints – as bothersome (irritating,

and interfering with their day), uncomfortable (irritating), tolerable, or absence of symptoms.

Results

Findings of the study highlighted that digital and online learning increases screen time at potentially dangerous for eye health levels. 48.9% of students use a monitor more than 8 hours daily, 37.8% – between 5 and 8 hours per day, 11.2% from 3 to 5 hours, only 1.6% reported screen time less than 3 hours (Figure 1).

Feeling of dryness, grittiness, scratchiness in their eyes experienced constantly 9.6% of asked students, often – 19.1%, sometimes – 44.1% (Figure 2).

What is your usual daily screen-time (time spent using a monitor-computer, smartphone, tablet, TV, etc.) ?
188 responses

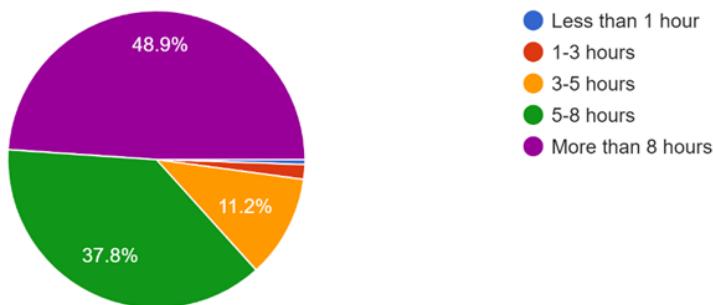


Figure 1. Usual daily screen time of responders.

During online education, do you experience Dryness, Grittiness, Scratchiness in your eyes?
188 responses

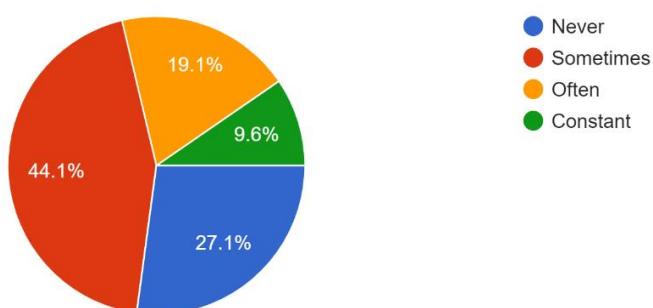


Figure 2. Sensation of dryness, grittiness, scratchiness among responders.

During online learning process, 7.4% of students had constant feeling of eye soreness and irritation, 25% had it often, and 35.6% felt it sometimes (Figure 3).

40.6 percent of responders had complaints of burning or watering sometimes, while 6.4% had these symptoms constantly, and 16% had them often (Figure 4).

Constant eye fatigue reported 18.1% of students. Almost 1/3 (30.9%) of responders

complain of often sensation of eye fatigue, 33.5% reported eye fatigue sometimes (Figure 5). 16.5 percent of responders report their complaints were bothersome – irritating and interfering with their day. 24.5% describe the severity of their symptoms as uncomfortable – irritating; 36.7% described them as tolerable, only 20.7% did not have any problems (Figure 6).

During online education do you experience Soreness or Irritation in your eyes?

188 responses

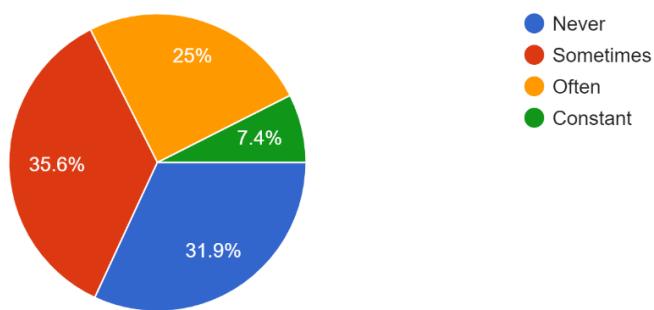


Figure 3. Soreness or irritation during remote learning process – results among students.

Since online education did you experience Burning or Watering in your eyes?

187 responses

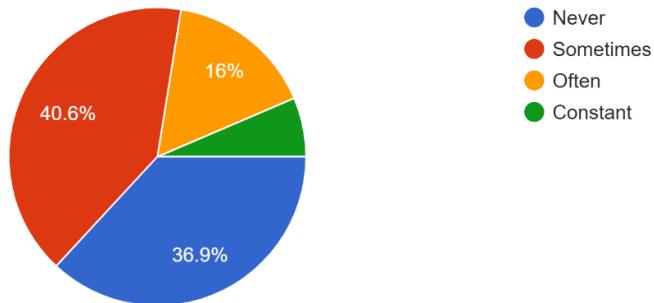


Figure 4. Experienced burning and watering of eyes during remote learning period.

Since online education did you experience Eye Fatigue?

188 responses

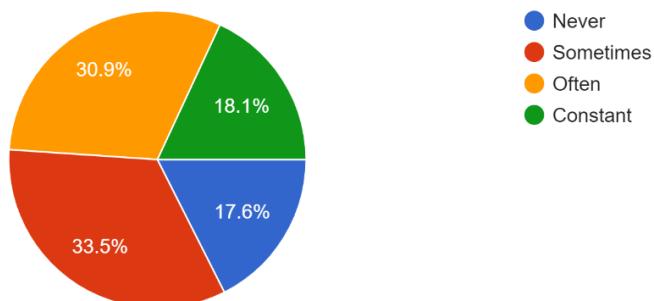


Figure 5. Eye fatigue experienced during online education.

How would you describe the severity of your symptoms?

188 responses

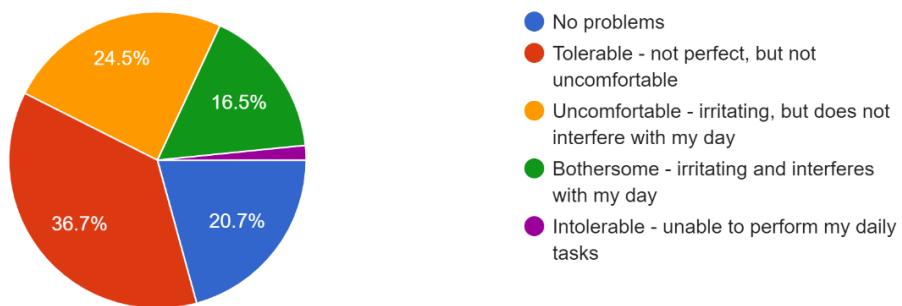


Figure 6. Severity of symptoms, according to answers of interviewed students.

Discussion

In this study, the effect of online education on eye health in Covid-19 period was investigated by online survey and results highlighted that digital and online learning increases screen time at potentially dangerous for eye health levels. The majority of respondents (86.7%) report screen time more than 5 hours daily, over 80% of students have experienced eye fatigue of different severity, and almost 80% report eye discomfort.

Previous studies have also shown that eye health related to screen usage may be seriously affected. Digital screens like computers, tablets and smartphones can cause harm by radiating blue light-short high energy waves, that may penetrate ocular tissues and can contribute to photochemical retinal cells damage (4). In 2020 Huseyin Kaya evaluated the effect of online education on eye health in Covid-19 pandemic and presented a new scale on this subject, using e-mail survey (5). Author interviewed 402 students from Pamukkale University and observed that the eye health of respondents was negatively affected by the online education of the Covid-19 pandemic process – eye fatigue increases as the result of online education deteriorate eye health (5).

Zhao et al investigated homeschooling behaviors and feelings of school-age children, with online surveys, obtained separately from students, parents, and teachers of grades 1–9 in 15 Chinese provinces. In the beginning of 2020 spring semester, due to the COVID-19 pandemic, all school-age children in China were homeschooled via live/recorded broadcasts, using digital devices. Sixty-nine percent of the parents reported their children had more than 3 hours of daily screen time, and 82% of students had less than 2 hours of daily outdoor activity. Ninety-five percent of the parents were concerned about their children's eyesight. Authors concluded that long screen time and insufficient outdoor activities can severely affect children's eyesight, so appropriate eye-protection measures should be implemented (6). Another study of Agarwal et al. also supports our results. Their findings indicated that extended digital device use of more than 6 hours leads to increasing of eye fatigue complaints (7).

Conclusion

As a conclusion of this study, it was observed that eye health and eye fatigue among medical university students was negatively affected by the distance learning education during the Covid-19 pandemic

social isolation. Increased screen time during online semester causes digital eye strain, which interferes with the quality of life and is an emerging public health issue. Students need to limit screen time for their physical and emotional health, to use eye lubricants, to keep strict visual hygiene, proper ergonomic computer work station, and use optimal correction of refractive errors.

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