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PREOPERATIVELY USE OF BACTERIOPHAGES FOR DECOLONISATION OF STAPHYLOCOCCUS AUREUS FROM NARES IN ORTHOPEDIC IMPLANT SURGERY

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ABSTRACT

Carriers of Staphylococcus aureus (S. aureus) have a higher likelihood of having surgical site infection (SSI). Several eradication regimens have been used. Topical agents alone or in combination with chlorhexidine gluconate have been used for the elimination of both nasal and extra nasal MRSA carriage. Our study focuses on the use of broad spectrum lytic phages as decolonising agent. We retrospectively studied nasal colonisation of St.aureus in 59 outpatients who visited TSMU the first university clinic traumatology department for screening purposes before orthopedic implant surgery. In patients with positive MSSA (15/59) we performed antibiotic ans phage (PYO, STAPHYLOCOCCAL, PHAGIO) susceptibility testing. MRSA was not detected. Phage given alone was able to effectively eradicate Staphylococcus aureus from the nares of 10patients (66,6%) after 5 days of treatment(3 times dayly intranasally) and in 5(33,3%)cases significantly reduced colonisation of MSSA in nose from 10^8CFU/ml to 10^3CFU/ml.
Keywords: Staphylococcus aureus; decolonisation; surgical site infection; phage.

INTRODUCTION

Approximately 20% of healthy adults are persistent nasal carriers of *S. aureus* and 60% harbour it intermittently. Such carriers have been shown to participate in the epidemiology and pathogenesis of *S. aureus* infections and are a potential source of outbreaks especially in hospital settings (1,2). Nasal carriers are at an increased risk of acquiring surgical site infections, foreign body infections and bacteremias (3,4). Although nasal colonisation with MRSA is low but such carriers are a major threat factor for themselves (through auto-infection/endogenous source) as well as can disseminate these highly resistant strains that pose serious difficulty in treatment thereafter. In addition, genotyping studies reveal that as high as 80% of *S. aureus* infections are caused by the patient's own nasal flora (5). Furthermore, there is an association between the presence of nasal *S. aureus* and an increased risk of surgical site infection (SSI), which has been extensively demonstrated in orthopedic surgery . The increasing incidence of antibioticresistant staphylococci (MRSA) threatens the outcome of implant. The ecologic niche of S. aureus is the anterior nares, and 25 to 30 percent of the population is colonized at a given time(8).Patients who are colonized are at 2 to 9 times higher risk to develop SSI than those who are non colonized, most patients who develop a S. aureus surgical site infection are carriers of the strains causing the infection. It has been shown that 85% of SSIs can be traced to endogenous colonization of the patients(6).
The current treatment strategies for nasal decolonisation rely on the use of topical antibiotics such as bacitracin, fusidic acid, ciprofloxacin, rifampicin (7). However, emergence of resistant strains has led to treatment failures. The rapid emergence of resistance to mupirocin therefore calls for search for alternative options. Phage therapy has been shown to be a potential alternative treatment for treating various *S. aureus* infections . Hence, an alternative or supplement to antibiotic therapy, is the use of bacterial viruses (phage/bacteriophage) to target MRSA colonisation in the anterior nares of the affected population. However, there is comparatively limited work published on the use of phages as nasal decolonising agents as compared to their proven therapeutic potential in other infections. (8)Bacteriophage therapy offers a possible alternative to classic antibiotic (antimicrobial chemotherapeutic) treatment to reduce bacterial colonization (9). Bacteriophages are able to infect bacteria and enter either a lysogenic or a lytic cycle, with infection by constitutively lytic bacteriophages generally resulting in rapid cell death. Also, if the phage-to-cell ratio is high enough, lysis from without may cause the cell to burst before infection is initiated (10). A main advantage of bacteriophages is their specificity. Whereas classic therapeutic treatment with chemical antimicrobials affects many different organisms in the body (e.g. the gut microbiota), causing a change in the microbial composition and inducing antimicrobial resistance in a spectrum of bacterial species, bacteriophages are able to specifically target the organism or even only the strain that is causing the infection. The *in vitro* lytic effect of bacteriophages can be easily tested. However, the use of bacteriophages for therapy (e.g. to treat infections or reduce colonization) poses additional challenges like the accessibility of bacteria and *in vivo* inactivation of bacteriophages (11).
The aim of this study was to determine Staphylococcus aureus susceptibility to commercially available phages for successful decolonization.

Material and methods: We retrospectively studied nasal colonisation of St.aureus in 59 outpatients who visited TSMU the first university clinic traumatology department for screening purposes before orthopedic implant surgery. In patients with positive MSSA (15/59) we performed antibiotic ans phage susceptibility testing -Pyo and Staphylococcal(Eliava BioPreparations, Tbilisi, Georgia), Phagyo(JSC "Biochimpharm", Tbilisi, Georgia).PYO and PHAGYO contain phages specific for Staphylococcus spp., Streptococcus spp., E. coli, Pseudomonas aeruginosa, and Proteus spp. STAPHYLOCOCCAL contains phages for *Staphylococcus* spp.
One swab was taken from the nostrils which was rotated gently in both nostrils. Specimens were inoculated onto manitol-salt and 5% sheep blood agar palate. (Biomerieux, France), which were incubated for 20 to 28 hours at 35°C to 37°C. After 24 hours negative plates were incubated for an additional 24 hours. The absolute S. aureus carriage was 25.42%, as 15 out of the 59 patients had S. aureus in the nose. Screening does not yielded positive nasal cultures MRSA. We also performed phage susceptibility testing: A bacterial inoculum standardized at 0.5 McFarland will be prepared and plated in horizontal lines on Mueller-Hinton solid agar (BIO-RAD) and then 20 µL of each phage mixture will be placed on each bacterial line. The plates will be incubated at 35±2°C in normal atmosphere for 24 hours.
The results will be read the following day, quantifying the aspect of lysis: Positive result: confluent lysis(++++), semi-confluent lysis(+++),opaque lysis ++ (>50 plaques), + (20–50 lysis plaques), ± (<20) or Negative result: absence of lysis.

RESULTS

The overall susceptibility of Staphylococcus aureus to STAPHYLOCOCCAL phages was 60%(of wich 9/15 were +++ or ++++). The overall susceptibility to Phagyo and Pyo phage was 26,6%(of wich 4/15 were +++ or++++) and 13.3% (of wich 2/15 were+++ or ++++) respectively. Negative results was not observed.Phage given alone was able to effectively eradicate the MSSA from the nares of 10 patients after 5 days of treatment(3 times dayly intranasally) and in 5(33,3%)cases significantly reduced colonisation of MSSA in nose from 10^8CFU/ml to 10^3CFU/ml
Conclusion:Our results Our study showed that use of single phage for nasal decolonisation was susscesfully reached by use of comertially available phages depnd on phage susceptobility testing results. This results seem promising, especially for the STAPHYLOCOCCAL phage.

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THE FREQUENCY AND DISTRIBUTION OF SISTER CHROMATID EXCHANGES (SCES) IN THE INDIVIDUAL CHROMOSOMES OF HUMAN KARYOTYPE

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INTRODUCTION

The aim of this study was to determine the frequency and distribution of sister chromatid exchanges (SCEs) in the individual chromosomes of human karyotype, Chromosomes were studied in (i) healthy subjects, (ii) subjects with rearrangements of X chromosome, (iii) in lymphoblastoid cell lines isolated from the peripheral blood of patients with acute leukaemias. 5-bromo- 2-deoxyuridine (BUdR) was added for 48, 72 and 96 hours, respectively, in a concentration of 30 µg per ml. The slides were stained according to the technique of Perry and Wolff (4).
The average number of SCEs was 9,2 with no statistically significant differences between the individual groups. Out of the total number of SCEs 20% was found in the centromeric region with no difference between the cells in the 2nd and 3rd divisions. The observed distribution of breakpoints was approximately proportional to the relative length of individual chromosomes with a higher number in long chromosomes and a lower number in the small ones. Non-random distribution of SCEs was only found in the B group of chromosomes of lymphoblastoid cell lines, which showed an excess compared with the SCEs of both the controls and the expected frequency based on the relative length of chromosomes. Neither in the late replicating i(Xq) nor in the early replicating Xq— did the number of SCEs significantly exceed the expected value.

Material and methods

The evaluation of the number and distribution of SCEs was performed in.
Seventy-five mitoses with karyotype 46,X,I (Xq) derived from 4 patients. In one of these subjects a double centromere in the i(Xq) was identified by means of C banding.
Twenty mitoses of one patient with reciprocal translocation 46,XX ,t(X,4) (Xqter→Xq22: :4pl6→4qter). Autoradiography proved that both the deleted X and the B4 chromosome with translocation were early replicating.
Fifty-six mitoses obtained from three lymphoblastoid cell lines, derived from the peripheral blood of patients suffering from acute leukaemias. These were classified as dedifferentiated (Epstein-Barr virus positive), lymphatic and myeloid (both EBV-negative).
One hundred and seventy mitoses from the control group consisting of 3 males with karyotype 46,XY and 4 females with 46,XX karyotype. The mean age of controls was 29.5 years.
The peripheral blood leukocytes were cultivated for 48, 72 and 96 hours in EPL or Parker medium (Usol, Prague), enriched by 20% calf serum, with PHA (Wellcome) and protected by streptomycin and penicillin. Bromodeoxyuridine (Sigma) was added in a concentration of 30 µg/ml since the beginning of cultivation. The tubes were protected from light to avoid photolysis. Colcemid (Ciba) in a concentration of 10 µg/ml was added two hours before harvesting. The cells were hypotonized by 0.075 M KC1 and methanol: acetic acid (3:1) were used for fixation (1).
The lymphoblastoid cell lines were grown as permanent suspension cultures from peripheral blood. They were established and subcultured in RPMI 1,640 medium enriched by 20% fetal calf serum, protected by streptomycin and penicillin, without PHA. BUdR was added in a concentration of 30 µg/ml for 48 or 72 hours following 48 hours of subcultivation. The examination of SCEs was carried out in the 15th, 3rd, and 38th, and in the 16th passages in three individual lines. The harvesting of chromosomes was the same as with the above-mentioned short-term peripheral blood cultures (2).
Chromosomal preparations were stained according to the FPG staining technique of Perry and Wolff (4). Intact mitoses with harlequin chromosomes were photographed and SCEs evaluated first directly under the microscope, then from enlarged negatives or karyotypes. Exchanges occurring in short arms, long arms and in centromeric regions were counted separately. The number of SCEs was examined independently by two experienced observers and expressed as the number of breakpoints. Statistical evaluation was performed by means of t test and χ^2 test.

RESULTS

The difference in the number of SCEs found per cell in 50 mitoses of control subjects when evaluated from photomicrographs or karyotypes is small but significant ($p< 0.01$). Therefore, the data described have been obtained from karyotypes only.
The mean value of 9.5 SCEs per mitosis found in the control group was in no way significantly different from mean values of the other groups, i.e. those consisting of pathological karyotypes and lymphoblastoid cell lines ($p >0.05$).

High frequency of SCEs (20%) was found in the centromeric region of chromosomes after the 2nd and 3rd divisions in BUdR medium (6).

The distribution of SCEs both observed and expected on the basis of the relative length of chromosomal groups in the karyotype with results of $\chi 2$ test is shown in Table II. The changes were similar in all observed groups. The only exception were **B** group chromosomes of the lymphoblastoid ceil lines, where the number of breakpoints increased significantly ($p<0.01$). In this particular chromosomal group the breakpoints leading to exchanges were distributed proportionally along the whole length of all chromosomes. Significant difference was found not only as against the expected number of SCEs but also as against the control group. The increased number of breakpoints on the long chromosomes was naturally matched by their decrease in small chromosomes (5).

We preferred to evaluate the non-banded chromosomes (G-banding considerably interferes with the accuracy of SCE calculation), the regions of break points are only roughly delineated. Even so it is clear that some regions are more often involved in SCEs than others. This is the case especially with regions 1q1, 3q2, 4q2, 8c and 16c. SCEs seem to be preferentially located on G-negative bands, as mentioned also by Morad, Jonasson and Lindsten (3)

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CHARACTERIZATION OF HUMAN CHROMOSOMAL CONSTITUTIVE HETEROCHROMATIN

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Keywords: centromeric heterochromatin, chromosome pairs 1, 9 and 16,congenital malformations

Heterochromatin of centromeric chromosome regions contains late replicating, largely repetitive DNA. It is suggested that heterochromatin participates in chromosome pairing, crossing-over and in chromosome disjunction control (1,3).
Centromeric heterochromatin, a variety of heterochromatin, is a tightly packed form of DNA.Centromeric heterochromatin is a constituent in the formation ofactive centromeres in most higher-order organisms; the domain exists on both mitotic and interphase chromosomes. (4,5,6,8)
Centromeric heterochromatin is usually formed on alpha satellite DNA in humans; however, there have been cases where centric heterochromatin and centromeres have formed on originally euchromatin domains lacking alpha satellite DNA; this usually happens as a result of a chromosome breakage event and the formed centromere is called a neocentromere. Centromeric heterochromatin domains are flanked by pericentric heterochromatin.(Fig. 1).

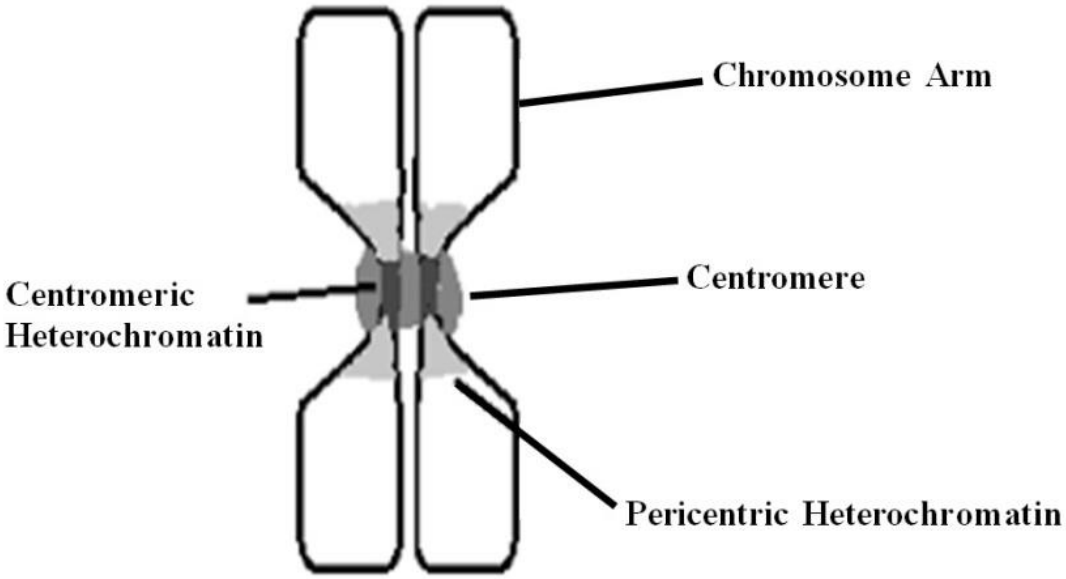

Figure 1. Centromeric heterochromatin

Human chromosomes 1, 9 and 16 possess relatively higher amounts of centric heterochromatin varying in size. Individuals with extremely polymorphous heterochromatic regions may show a decreased relative reproductive fitness and there may be an increased risk of chromosome abnormalities for the progeny (2,7,9,10).
Our study of varying centromeric heterochromatin of the chromosome pairs 1, 9 and 16 was based on data provided by the Cytogenetic Counselling Centre of the AFGEN Genetik Laboratoryin Baku. Preliminary results of this study will be presented below.

Material and methods
The short-term cultivation of human peripheral lymphocytes (60 hours) and trypsin-banding technique were used to prepare thecells for cytogeneticanalysis. The bands on chromosomes were marked according to the International System for Human Cytogenomic Nomenclature (ISCN2016). The size of 1q12, 9q12 and 16q11 bands under study (Fig. 2)were classified from the photographs,using the classic Smarttype Karyotyper method. Two cytogeneticists classified the size of each band independently, and in case of disagreement a third one decided the final classification.

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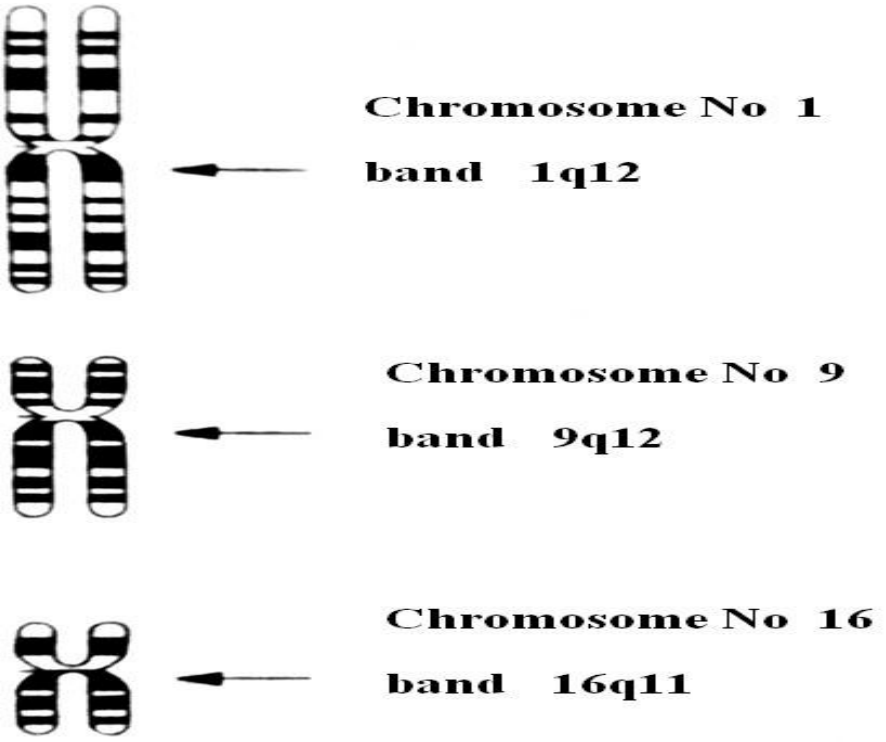

Figure 2. Human chromosomes Nos 1, 9 and 16 according to the International System for Human Cytogenomic Nomenclature (ISCN 2016)

The bands were classified as: normal (+), larger (+ +), very large (+ + +), narrow (±) and pericentric inversions (p.c.i.) The karyotypes were divided into four groups: (*I*) from persons with abnormal phenotype and abnormal karyotype, (*II*) from persons with abnormal phenotype (multiple congenital malformations) and normal karyotype, (*III)* from healthy nearest relatives (parents and sibs) of persons with abnormal phenotype and karyotype, *(IV)* from normal healthy persons with normal phenotype and karyotype without congenital malformations in family history. Abnormal karyotypes here mean inborn chromosomal aberrations (trisomy, deletion, unbalanced translocation), multiple congenital malformations mean abnormalities of at least two organs combined with mental deficiency.

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TABLE I
Variability of bands 1q12, 9q12, 16q11

Group No,of subjects		1q12					9q12					16q11				
		+	++	+++	±	p.c.i	+	++	+++	±	p.c.i	+	++	+++	±	p.c.i p.c.i
(I) 24	No. %	45 93.5	2 4.5	1 2.0	0 0	0 0	44 92.0	2 4.0	1 2.0	1 2.0	0 0 0	48 100.00	0 0	0 0	0 0	0 0
(II) 37	No. %	65 88.0	6 8.0	0 0 0	3 4.0 8	0 0	62 84.0	9 12.0	3 4.0	0 0	0 0	71 94.5	1 1.5	1 1.5	1 1.5	0 0
(III) 26	No. %	41 79.0	3 6.0	0 0	8 15.0	0 0	42 80.5	7 13.5	2 4.0	0 0	1 2.0	52 100.0	0 0	0 0	0 0	0 0
(IV) 81	No. %	152 94.0	7 4.0	0 0	3 2.0	0 0	132 81.5	27 17.0	0 0	2 1.0	1 0.5	159 98.0	2 1.5	0 0 1	1 0.5	0 0
∑	No. %	303 90.2	18 5.4	1 0.3	14 4.1	0 0	280 83.3	45 13.4	6 1.8	3 0.9	2 0.6	330 99.2	3 0.9	1 0.3	1 0.3	0 0

Persons with abnormal phenotype and abnormal karyotype

Persons with abnormal phenotype (multiple congenital malformations) and normal karyotype

(III) Healthy nearest relatives (parents and sibs) of persons with abnormal phenotype and karyotype

(IV) Normal healthy persons with normal phenotype and karyotype without congenital malformations in family history

Classification of centromeric heterochromatin of chromosomes Nos 1, 9 and 16:"+" normal, "++" larger, "+++" very large,"±" narrow, "p.c.i." pericentric inversion

Results and discussion

Our results are presented in Table I. A different variability of centromeric heterochromatin of chromosomes 1, 9 and 16 was observed. Quite a low variability was found in chromosome 16, while chromosomes 9 and 1 showed a high degree of variability, which was more accentuated in chromosome 9 than in chromosome 1.

In all four groups of persons there was a similar pattern of variability with the only exception mentioned below.

On the whole, band 1q12 was either enlarged or diminished, while band 9q12 was most frequently enlarged. Pericentric inversions were observed very rarely and only on chromosome 9.

The only exception was found in the nearest relatives of children with abnormal phenotype and karyotype: an unusually narrow band 1q12 was frequently detected, often onboth members of the chromosomal pair.

The study is to be continued.

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SPECIFIC AND NON-SPECIFIC VAGINITIS

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ABSTRACT

Vaginitis is the most common gynecologic diagnosis in the primary care setting. In approximately 90 percent of affected women, this condition occurs secondary to vulvovaginal candidiasis ortrichomoniasis. Vaginitis develops when the vaginal flora has been altered by introduction of a pathogen or by changes in the vaginal environment that allow pathogens to proliferate. The evaluation of vaginitis requires a directed history and physical examination, with focus on the site of involvement and the characteristics of the vaginal discharge,but mostly depends on bacteriological examination of vaginal samples .Numerous previous studies of nonspecific vaginitis have yielded contradictory results regarding its cause and clinical manifestations, due to a lack of uniform case definition and laboratory methods. We studed 30 womens who visited TSMU the First University Clinic with compliance of vaginitis caused by Candida albicans (21 cases) and Trichomonas vaginalis(9cases). After 2 month of treatment 7 patients experienced leukohhrea, dispareunia etc. Bacteriological investigation of waginal swabs revealed Enterococcus faecalis -4 cases, Staphylococcus aureus -2 cases and Enterobacter aerogenes -1 case. They were successfully treated depend on susceptibility testing results bacteriological testing results. Our study showed that clinical management of nonspecific vaginitis can be successfully reached by bacteriological monitoring of vaginal swabs.

Keywords: candidiasis, vaginitis, trichomoniasis, treatment, antibiotics.

აბსტრაქტი

ვაგინიტი არის ყველაზე გავრცელებული გინეკოლოგიური დაავადება პირველადი სამედიცინო დახმარების რგოლში. დაავადებულ ქალთა დაახლოებით 90 %-ში ეს ვლინდება ვულვოვაგინალური კანდიდოზის ან ტრიქომონიაზის სახით. ვაგინიტის დროს საშოს ფლორა იცვლება პათოგენის შეღწევით ან ვაგინალურ გარემოში მისი ტრანსფორმაციით , რაც ხელს უწყობს პათოგენების გამრავლებას . ვაგინიტის სწორი შეფასება მოითხოვს ზოგად ანამნეზს და ვაგინალური გამონადენის ხასიათის შეფასებას ,თუმცა დიაგნოსტიკაში ყველაზე მნიშვნელოვანია ვაგინალური ფლორის სწორი ბაქტერიოლოგიური კვლევა. თსსუ-ს I საუნივერსიტეტო კლინიკაში გამოვიკვლიეთ ის 30 ქალი,რომლებმაც მიმართეს მეანობა-გინეკოლოგიის დეპარტამენტს ვაგინოზის ჩივილებით და რომელთა ვაგინალური ნაცხებში ბაქტერიოლოგიური კვლევით დადგინდა Candida albicans-ი(21 შემთხვევაში) და Trichomonas vaginalis-ი(9 შემთხვევაში). მკურნალობიდან 2 თვის შემდეგ 8 მათგანს კვლავ აღენიშნებოდათ ლეიკორეა,დისპარეუნია და ა.შ. . საშოს ნაცხის ბაქტერიოლოგიური კვლევებით ამოითესა Enterococcus faecalis -ი უხვი ზრდით -4 შემთხვევაში, Staphylococcus aureus -ი უხვი ზრდით და გამოხატული ჰემოლიზით- 2 შემთხვევაში და Enterobacter aerogenes-1 შემთხვევაში. ლოკალურ ანტიბიოგრამაზე დაყრდნობით მათ წარმატებით ჩაუტარდათ მკურნალობა. ჩვენმა კვლევამ აჩვენა , რომ არასფეციფიკური ვაგინიტების კლინიკური მართვა შესაძლებელია სწორი ბაქტერიოლოგიური მონიტორინგით .

საკვანძო სიტყვები: კანდიდიაზი, ვაგინიტი, ტრიქომონიაზი, მკურნალობა, ანტიბიოტიკები.

Introduction

The vaginal micro-flora is a complicated environment, composed of varying microbiological species in variable quantities and relative proportions.The development of inflammation contribute to impaired metabolism, changes arising from malnutrition on the grounds of senile involution and degeneration after cessation of ovarian function, hysterectomy, total

obesity, tuberculosis, sometimes mechanical damage, unhealed perineal tears; is secondary to inflammation of the cervix and vulva.(1,2)

The prevalence and causes of vaginitis are uncertain, in part because the condition is so often self-diagnosed and self-treated. In addition, vaginitis is frequently asymptomatic or has more than one cause. Most experts believe that up to 90 percent of vaginitis cases are secondary to vulvovaginal candidiasis and trichomoniasis. Noninfectious causes include vaginal atrophy, allergies and chemical irritation(3).

Common symptoms is a sharp hyperemia ,vaginal tissues are infiltrated and edematous. In chronic vaginitisepithelium of the vagina almost completely disappears, pus is secreted directly from the walls of the vagina(4). Vaginal mucosa is sealed, for themost part rough and covered with small nodules (colpitisgranularis). The problem is that some forms of abnormal vaginal micro-flora are neither normal, nor can they be called bacterial vaginosis. Such forms of abnormal flora have been termed 'intermediate flora' in some studies, or been included with full-blown bacterial vaginosis in others.

Vaginal infections if untreated are associated with significantly increased risk of pelvic inflammatory diseases (PID), which can cause tubal infertility, ectopic pregnancy, reproductive dysfunction.Not only specific but also non-specific vaginal infections may cause unexpected pregnancy outcome and premature birth(5) .

In vaginal microflora are some pathologic species which cause specific vaginitis which are well defined, such as Trichomonas vaginitis,Candida albicansand others. The diagnosis and clinical manifestation ofspecies which cause non-specificvaginitis are less clearly defined.This type of undefined abnormal non-specific flora may be of high importance in womenshealth and as a result may cause an infertility(6,7). The microbiology of this vaginitis includes many facultative Gram-negative rods and Gram-positive cocci. Gardner and Dukes' described a specific 'vaginitis' (5) characterized by a grey homogenous and odorous vaginal discharge of more alkaline pH than normal. They attributed the infection to a bacterium they called Haemophilus vaginalis and found a strong correlation with the presence in the vagina of 'clue' cells, described as degenerating epithelial cells with a granular appearance owing to bacteria adherant to their surface(8,9). Numerous previous studies of nonspecific vaginitis have yielded contradictory results regarding its cause and clinical manifestations, due to a lack of uniform case definition and laboratory methods(10,11).

Material and Methods

In our study vaginal swabs were taken from 30 women visited TSMU the First University Clinic Obstetrics and gynecology department with compliance of vaginities caused by Trichomonas vaginitis(9cases) and Candida albicans(21cases) .They have a check-up after treatment(after 2month)with symptoms of leycorrea ,also with complaints of discharge and dyspareunia. Womans age ranges from 21 to32 years.We examined bacteriological swabs after 2 month of treatment of specific vaginities and found non-specific pathogens in the vagina, after the treatment of specific vaginities. Bacteriological research covered: streaking on selective and differential agar(Bio-Rad Laboratores), isolation of poor culture,Gram stain, identification of microbes with rapid identification system (API20E, API Staph, API Strep20, bioMerieux). Antibiotic susceptibility was eveluated by EUCAST standards(2019). Following antibiotics were tested: Ciprofloxacine, levofloxacine, Moxifloxacine, Fosfomycine, Nitrofurantoine, Co-trimoxazole, Ampicilline+sulbactam, Amoxacilline+clavulanic acid and Doxycycline, Ceftriaxone, Amikacine,Nitrofurantoin, Azithromycine.

Results:

After 2 month of treatment (30 patients) 8 patients still experienced leukohhrea , dispareunia etc. Bacteriological investigation of waginal swabs revealed Enterococcus faecalis -4 cases, Staphylococcus aureus -2 cases and Enterobacter aerogenes -1 case . They were successfully treated depend on susceptibility testing results bacteriological testing results. Enterococcus faecalis -4 cases, Staphylococcus aureus -2 cases , Esherichia coli -1 case and Enterobacter aerogenes -1 case .

Both gram -positive and gram-negative organisms were sensitive to Ampicillin-sulbactam, Amoxicillin-clavulanic acid, Amikacin, Fosfomycine,There was a total of 82% resistance to Ciprofloxacin and Levofloxacin and less 15 % was resistant to Moxifloxacin, 10%were resistant to co-trimoxazole. 25% of gram-positive bacteria were shown resistance to Doxycycline and Azithromycine.

This non-specific chronic bacterial vaginitis is successfully treated with appropriate antibiotics that kill the causative organisms mostly in treated cases of specific vaginitis .

CONCLUSION

Our study show current views on effective diagnostic approach and successful treatment of chronic non-specific vaginitis with appropriate antibiotic therapy which depends on local bacterial species and its sensitivity to appropriate antibiotics. Clinical management of nonspecific vaginitis can be successfully reached by bacteriological monitoring of vaginal swabs.

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DIFFICULTY OF MANAGEMENT OF REFRACTORY AND SUPER REFRACTORY STATUS EPILEPTICUS IN PATIENTS WITH COMA

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ABSTRACT

Status epilepticus (SE) is one of the most common neurological conditions, which needs an emergency assistance. In general, the management of SE is more difficult in patients with unknown coma. Under our observation there were 6 patients with different etiologic factors of coma. Three patients from them had immunologic disorder causing status epilepticus. In four of the cases, non-convulsive status epilepticus was identified. In three cases, we have diagnosed progressive forms of main diseases, but we could not find out etiologic factors of manifested immunological disorders. For the treatment, we have decided to use anticonvulsive drugs in combination with hormonotherapy and immunoglobulinotherapy. At the same time, all the patients were under the general anesthesia including Ketamine. Despite of this, in two cases we could not control the SE development for a long period of time. In all of the cases, we have used EEG- monitoring and MRI study in dynamics. These observations showed that the lack of control was related to the exacerbation of the main disease or the processes. All in all, we have arrived at the conclusion that the acute disorder of the central nervous system and its development is very important in the SE management. And the SE management itself defines the solution. It relates to both of the types of statuses: non-convulsive and convulsive. Moreover, timely diagnosis is significant in the management of the refractory SE.
Keywords: Status epilepticus, Immunoglobulin, Coma

Introduction

Nowadays there are many debates about development and managements status epilepticus(SE) in patients with coma. It is especially difficult to treat SE in patients with unknown coma. It is impossible to ascertain final diagnosis in some cases, though we have used different studies. In general the mortality of SE is about 20 %, but it might be more than 40 % in the elderly with acute symptomatic SE [1-5] and many co-morbidities [6].
Treatment of SE, especially of refractory or super-refractory stages, is almost an “evidence-free zone” [7]. So, each clinical case is important and each clinical data must be discussed.

Methods: There were 22 patients with coma under our observation. All patients were divided in two clinical groups: Patients with different etiological factors (autoimmune, unknown) causing coma- 9 cases were included in the first group. We diagnosed non-convulsive status epilepticus (NCSE) in 4 cases from the first group. 13 patient with traumatic brain injury were included in the second group. In 3 cases of this group NCSE was found. The patients with oncological diseases and secondary brain damages were excluded.
All patient underwent the following studies:

- Long-term EEG monitoring in dynamics
- Brain CT or MRT in dynamics
- Objective neurological status (by GCS)
- Other basic clinical and paraclinical studies.

Results: In 3 cases of I group NCSE with refractory and super refractory developing was observed and in 2 cases of II group refractory SE was mentioned.

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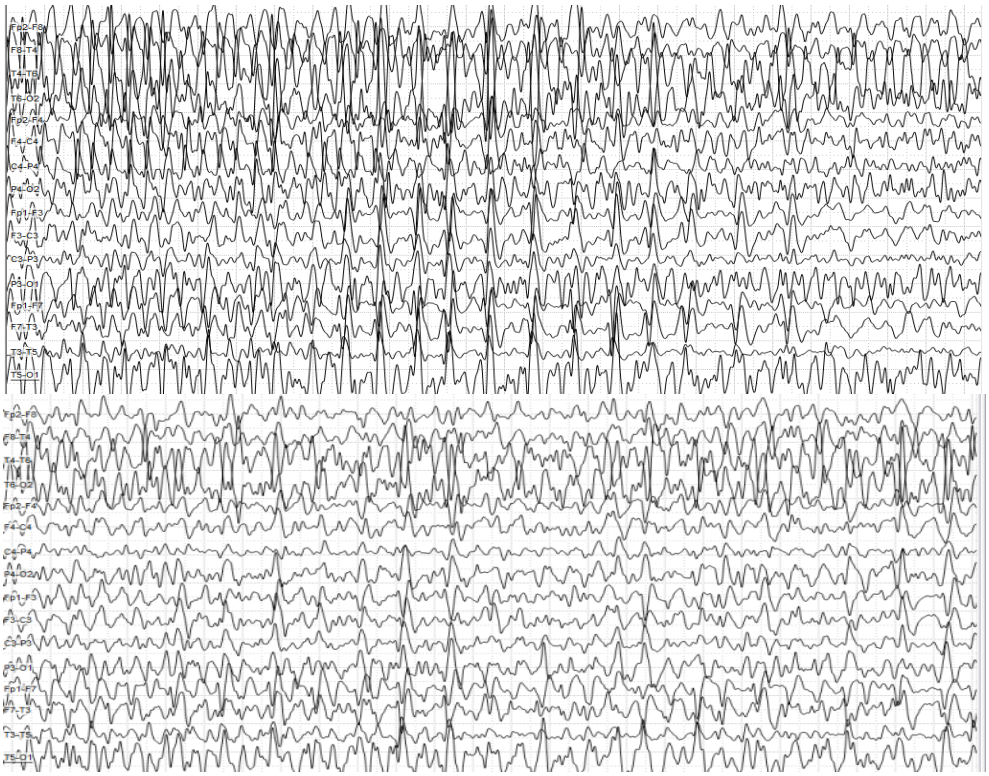
Table 1 shows EEG patterns in all cases of NCSE from both groups.

Number of patient	GCS	EEG patterns
2	4-6	Lateralized periodic discharges (LPDs)
2	4-5	Bilateral periodic discharges (BPDs)
3	3-4	Generalized periodic discharges (PDs)

Long-term EEG monitoring shows development NSCE in all cases in dynamic.



Picture 1. Precious EEG data super-refractory SE.

Picture 1 shows super-refractory SE EEG monitoring findings- Bilateral periodic discharges (BPDs).

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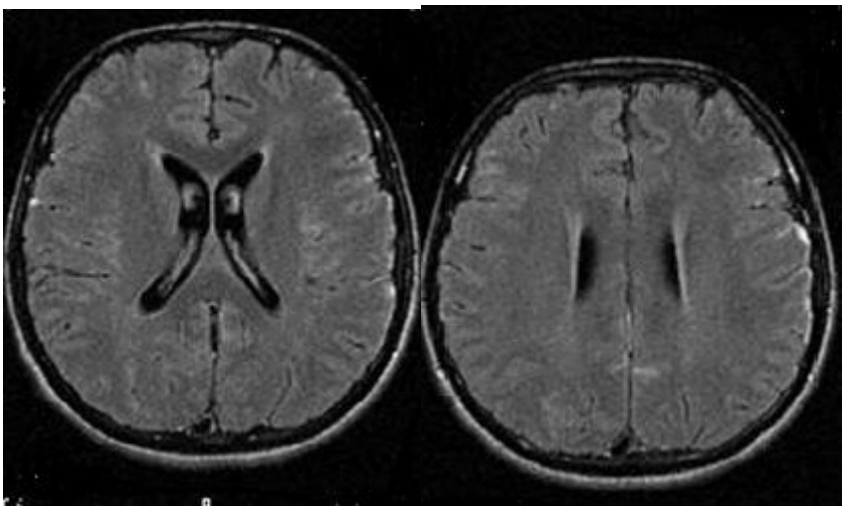
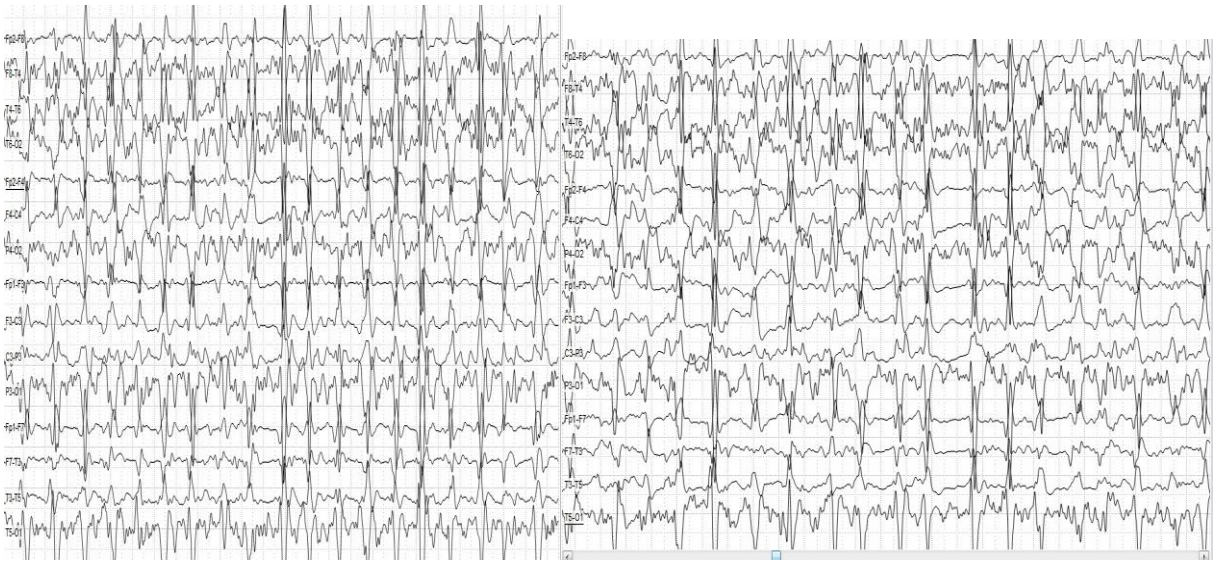


Picture 2. MRI data of patients with autoimmune encephalitis.

EEG data of super-refractory NCSE in patients with autoimmune encephalitis (In dynamics). Picture 3



Picture 3

Picture 3 shows super-refractory SE. In this cases we used VPA, LEV and CBZ together with intravenous Propofol infusion (4 mg/kg/day), pulse corticosteroid therapy with Methylprednizolone, Tiopental and Ketamine infusion –dosage 2.75 mg/kg.n (3 days). Super refractory SE was continued. EEG monitoring shows generalized periodic discharges (PDs)-negative dynamics.

In additional we used plasma exchange (PE), intravenous immunoglobulin (IVIG).Regardless of all these EEG dynamics was negative and MRI study also revealed negative radiological changes. We've got depressing of brain activity while used Thiopental infusion.

We've received maximal depression of brain activity and full control under convulsion. Of course we have positive MRI dynamics in development of main disease (picture 4).

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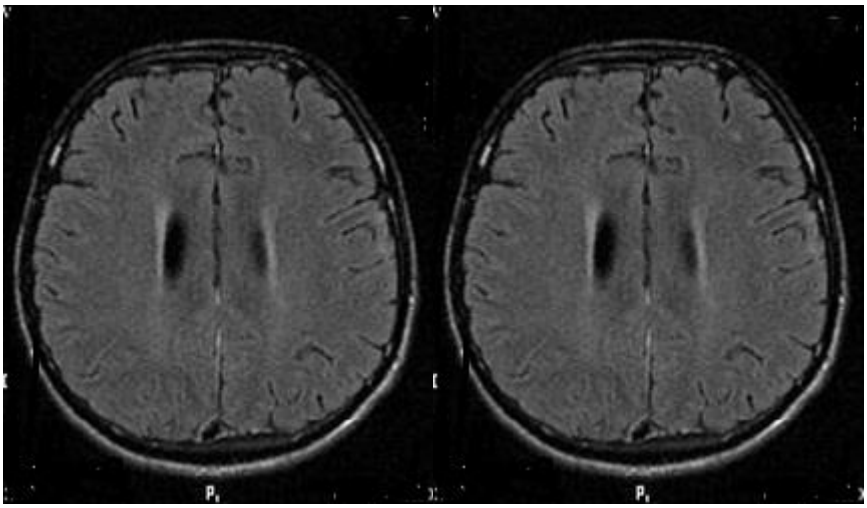
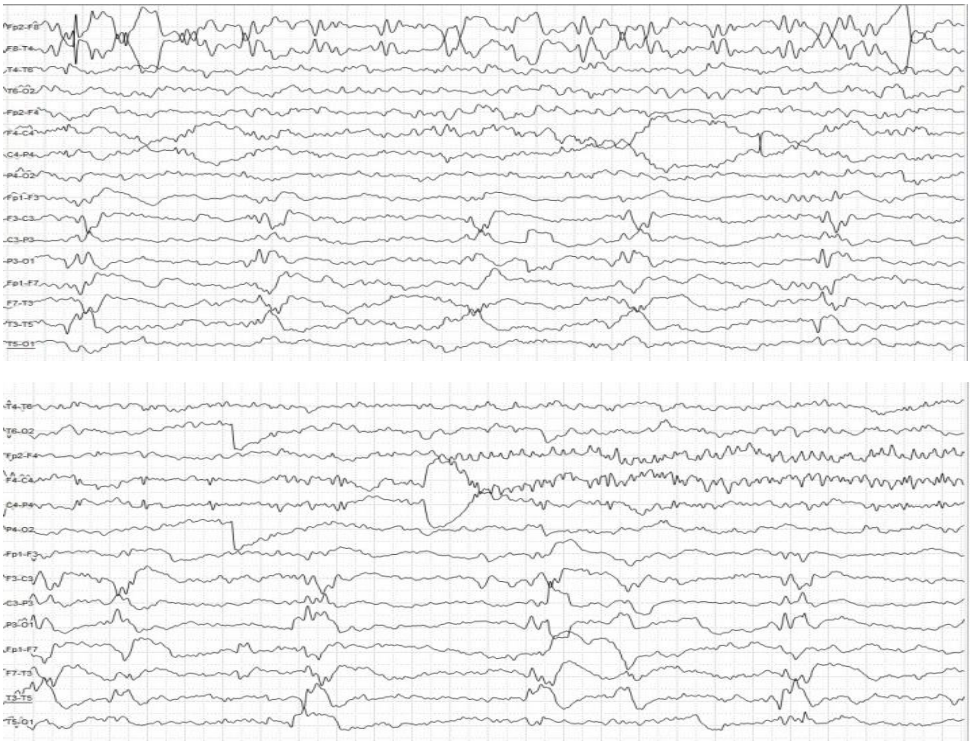


Picture 4. MRI data in dynamics.

Patient with refractory NCSE with lateralized periodic discharges (LPDs). Picture 5



Picture 5

MRI finding in this cases

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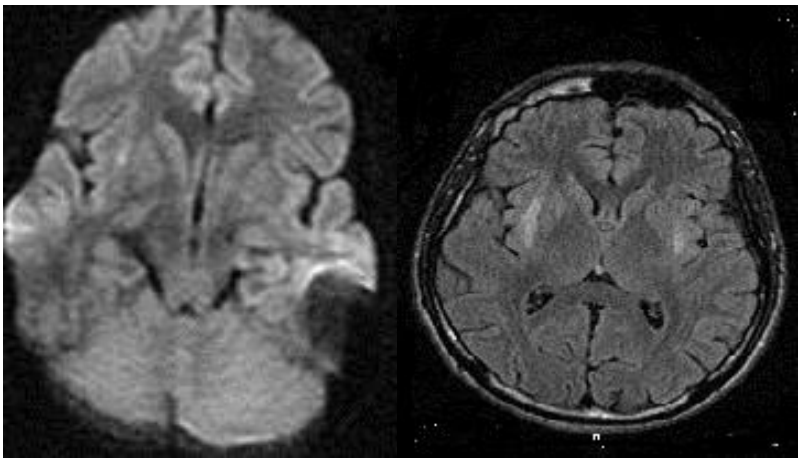
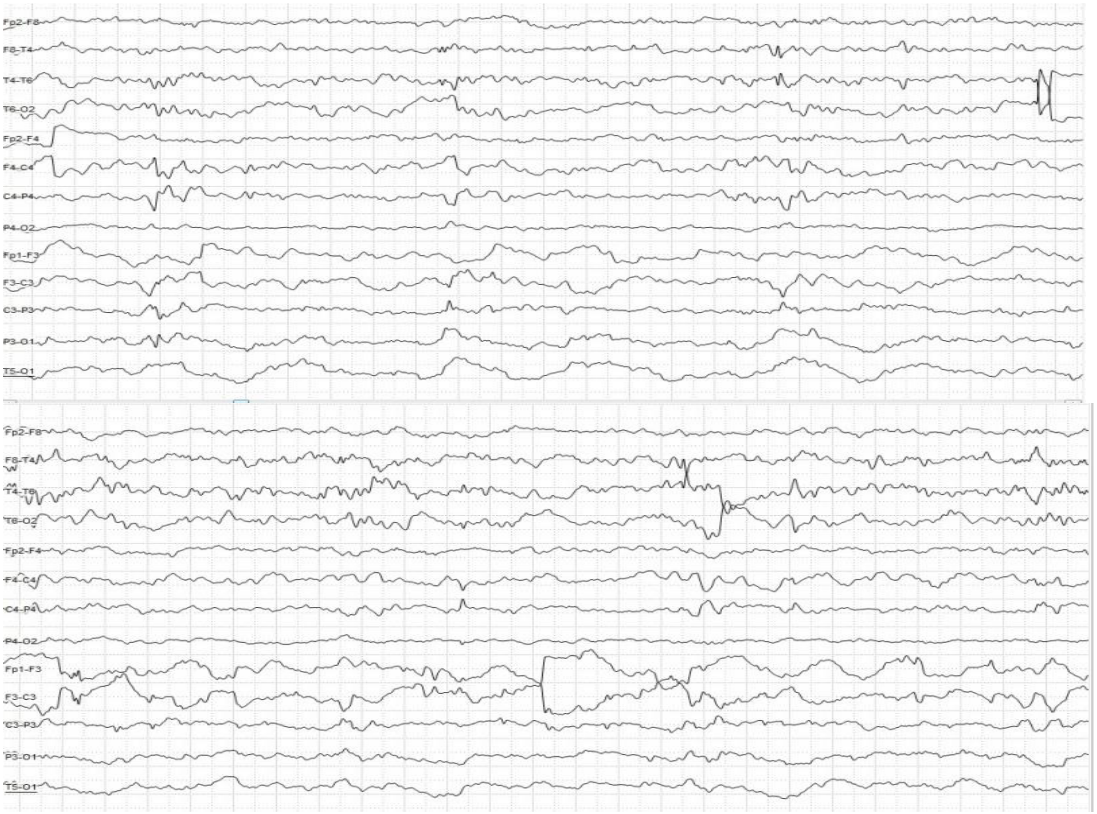


Picture 6

EEG changes in dynamics –without LPDs .

Picture 7



Picture 7.

MRI changes in dynamics.

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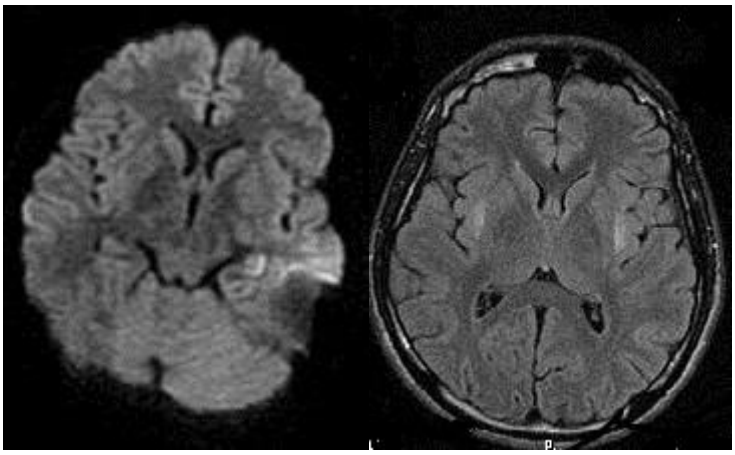

Picture 8

For management of SE we've used treatment with Valprotid Acid (VPA), Levetiracetam (LEV) and Carbamazepin (CBZ) in high doses.
All cases of refractory and super-refractory SE were hard to be managed and defended from developing main brain damages. Picture 5, 6, 7, 8 shows EEG and MRI data dynamics of refractory NCSE.

Conclusion: Refractory and super refractory NCSE have severe clinical developments. It's difficult to manage each case and it needs treated individually. Outcome of these cases depends on what is the cause of initial disease and its severness. super refractory SE (SRSE) is defined as SE that continues for 24 hours or more after the use of anesthetic therapy, including cases that recur on weaning of the anesthestic agent. RSE occurs in 23%-48% of the patients and SRSE in approximately 22% of the patients with SE. SE etiology is a potentially modifiable outcome predictor that should always be specifically addressed. Aggressive management of underlying etiology and prevention of systemic complications may improve outcome in adult RSE patients.

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COMPARISON EEG PATTERNS OF PATIENTS TRAUMATIC AND POSTANOXIC BRAIN INJURY

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INTRODUCTION

Anoxic brain damage after cardiac arrest is one of the most common causes of coma worldwide. Only n Europe, approximately 176,000 patients are admitted, yearly. Of all comatose patients after cardiac arrest surviving to hospital admission, 40–66% never regain consciousness as a result of severe post anoxic encephalopathy (1.2). The various EEG patterns in coma correlate with degree of impairment of consciousness and depth of coma (3.4) and have been used for several decades to prognosticate the outcome of coma. However, the characteristic EEG patterns are not in all cases specific for the etiology of coma (5-7). The recent studies highlight the relevance of postanoxic brain damage and it's important to conduct more research on this topic, in order to correctly determine the management of patients' and their survival. Some authors concerned on the residual myoclonus after survival from the acute episode. EEG patterns are the most important parameters in the diagnosis, as well as the prognosis of coma stages. After cardiac arrest, the most common EEG patterns are suppression-burst patterns in association with seizures.

Keywords: Coma,burst-syndrom, convulsive syndromes.

OBJECTIVES: The aim of our study is to signify convulsive syndrome in patients with postanoxic coma and to differentiate it from convulsive syndrome in patients with posttraumatic and other genesis comatose conditions, as well as management and prognosis of outcomes.

METHODS: We observed 69 patients (24 female, 45 male) aged from 20 to 72 years with coma caused by postanoxic and traumatic brain injury. These patients have been investigated over the period of time between 2012 and 2017 at Central University Clinic after Academic N.Kipshidze.
We have divided all patients in two separate clinical groups: 31 patients with post anoxic brain injuries and 38 patients with traumatic brain injury. All patients underwent following studies:
1.Neurological status assessment with GCS 2. Continue EEG-monitoring in dynamics 3. CT and MRI observation in dynamics

RESULTS: Different types of convulsion were observed in 9 patients out of 31 in the first group.

Table 1 shows different EEG patterns in postanoxic brain injury.

Table 1

Number of patiens	GCS	Lateralized periodic discharges (LPDs)	Bilateral periodic discharges (BPDs)	Generalized periodic discharges (PDs)	suppression	Suppression Burst	Suppression-burst with identical burses
10	5-6	----	2	2	2	1	1
9	4-5	1	2	1	2	1	2
12	3-4	----	----	2	6	6	-

Picture 1 and 2 show PDs in patient with postanoxic brain injury after cardiac arrest

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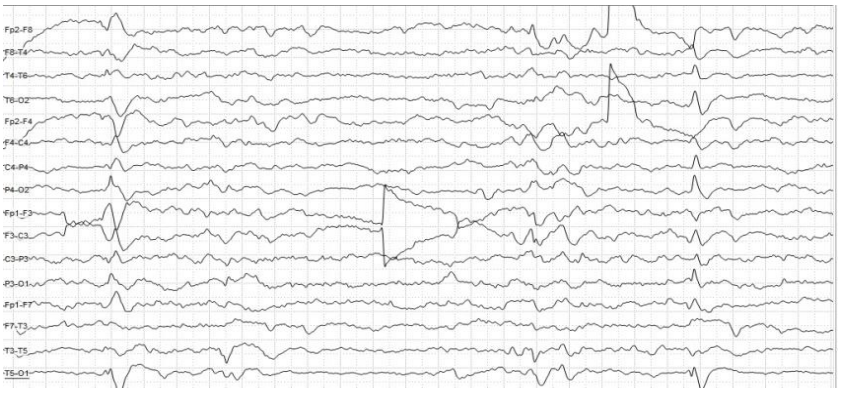
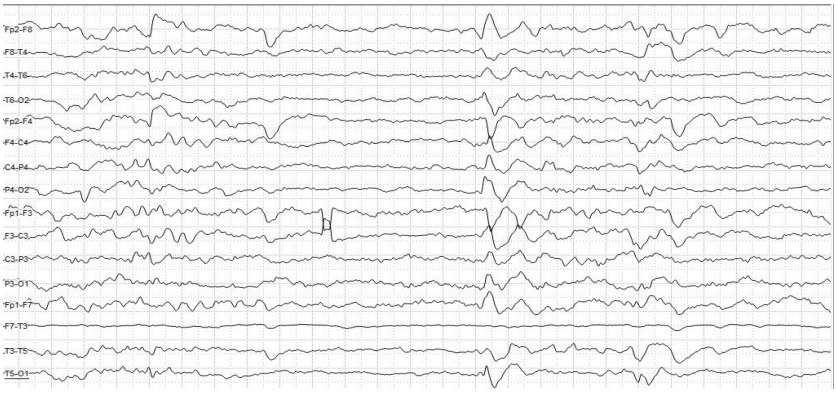
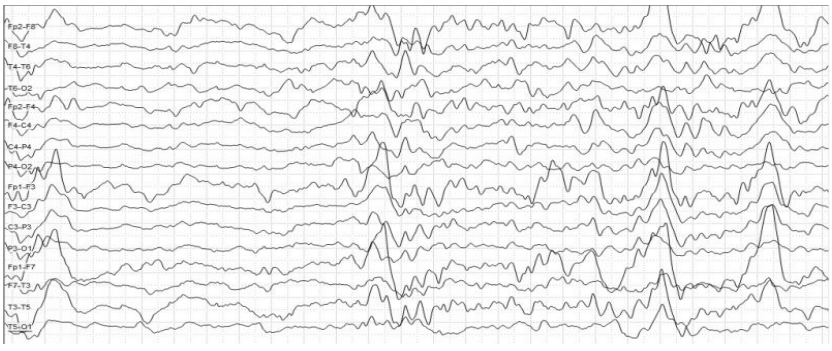


Picture 1



Picture 2

In 15 patients from the second group, we have found out different kinds of EEG patterns, which are the following: lateralized periodic discharges (LPDs) in 9 cases, bilateral periodic discharges (BPDs) in 4 cases and generalized periodic discharges (GPDs) in 2 cases. All patients with convulsive syndrome underwent AED treatment.
Pictures 3 and 4 show suppression-burst with identical burses in 57-year old male patient after cardiac arrest



Picture 3

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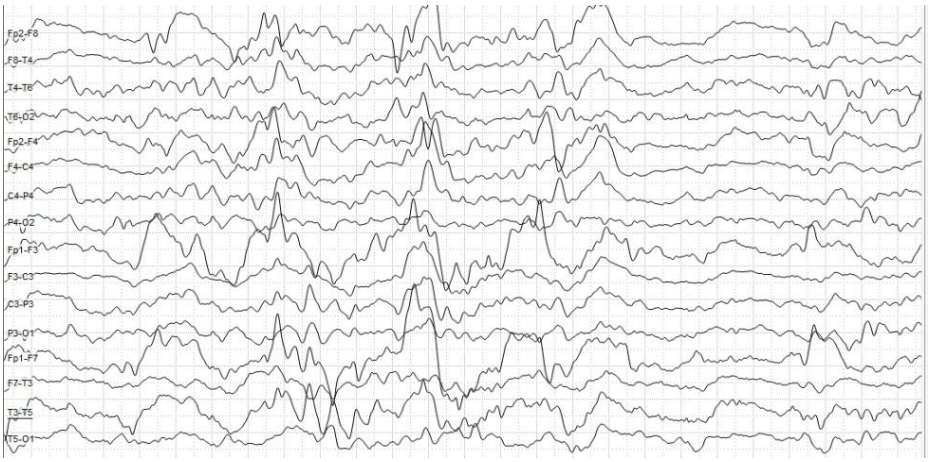
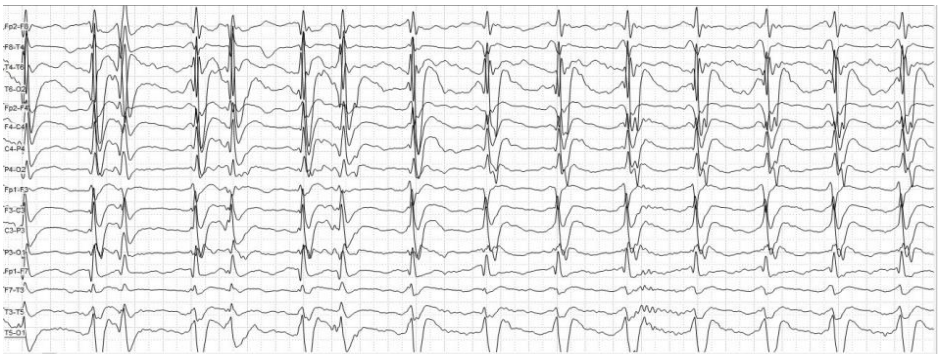
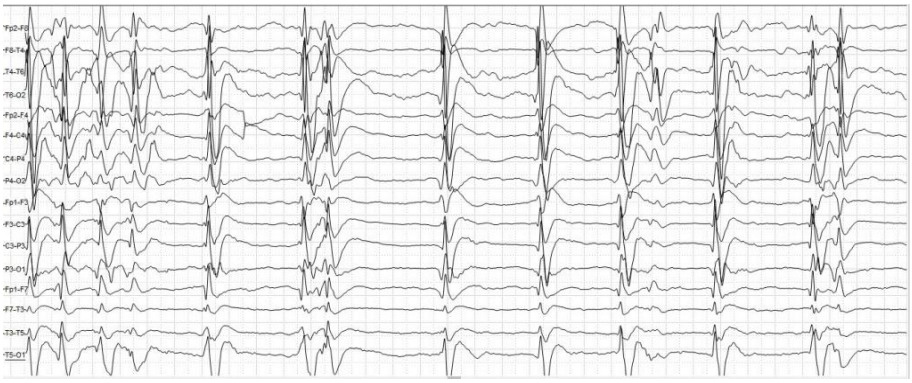


Picture 4

Pictures 5 and 6 show generalized periodic discharges (GPDs) in 61-year old male patient after cardiac arrest



Picture 5



Picture 6

Pictures 7 and 8 show dynamic changes in EEG (suppression)

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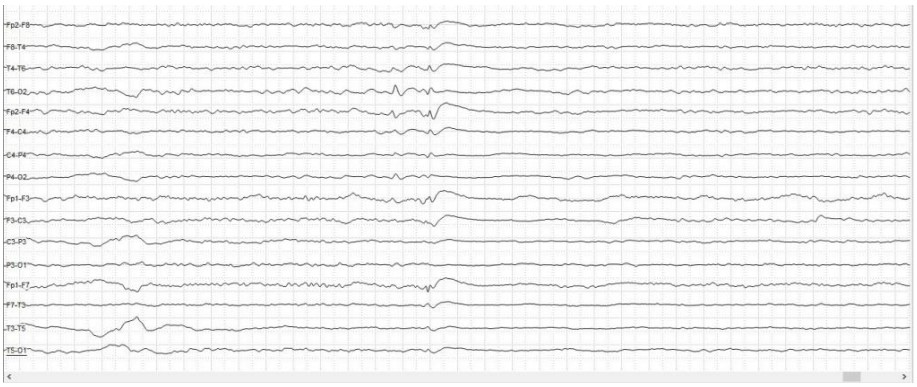
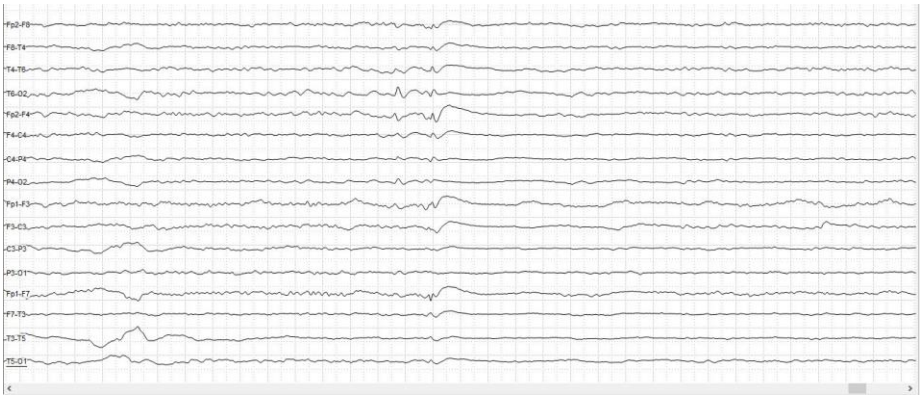
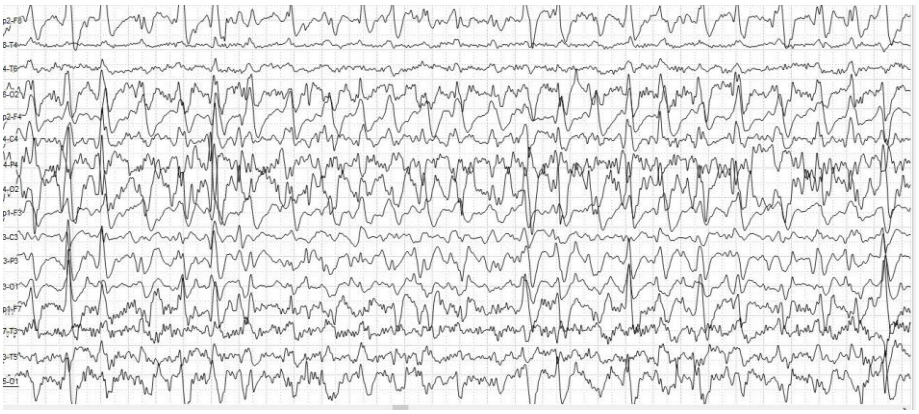


Picture 7



Picture 8

Pictures 9 and 10 show generalized period discharges (GPDs) in 69-year old female patient with anoxic brain injury



Picture 9

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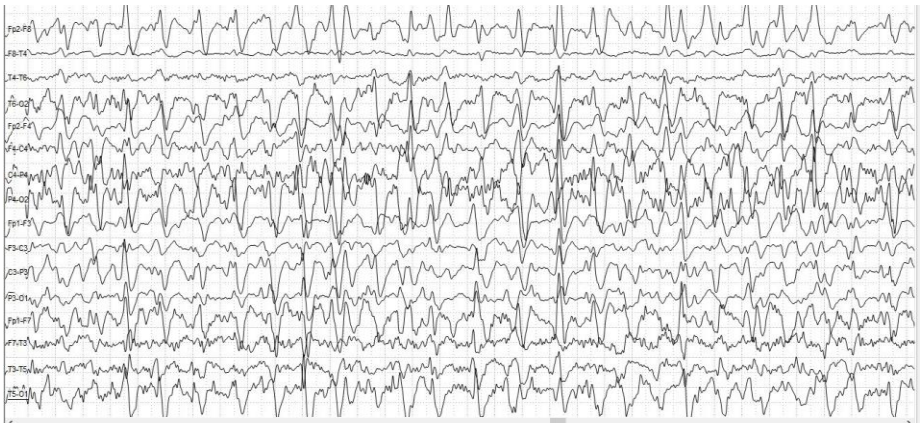
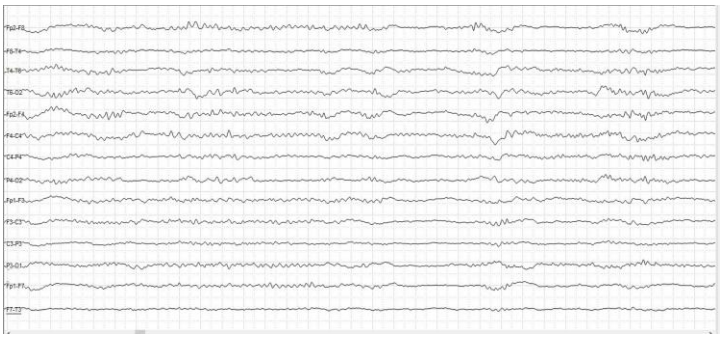
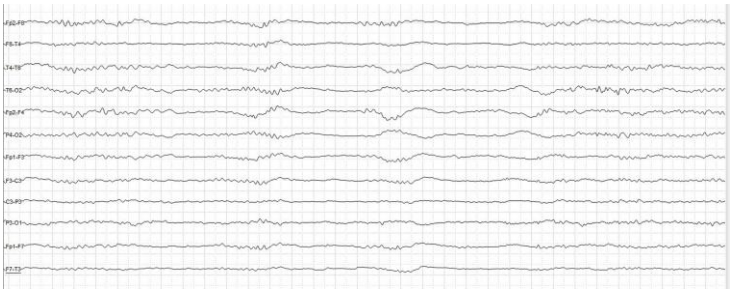


Picture 10

Pictures 11 and 12 show the same patient in dynamic EEG pattern (suppression burst activity)



Picture 11



Picture 12

Pictures 13,14,15 show the EEG of patient after cardiac arrest ,with shows suppression-burst activity with GDs (generalised discharges) in patient with postanoxic brain injury (after CPR)

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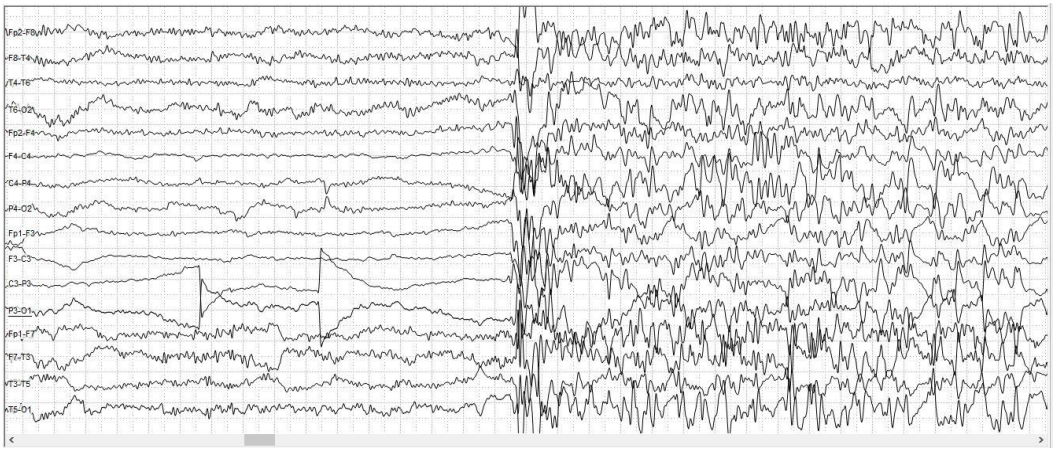
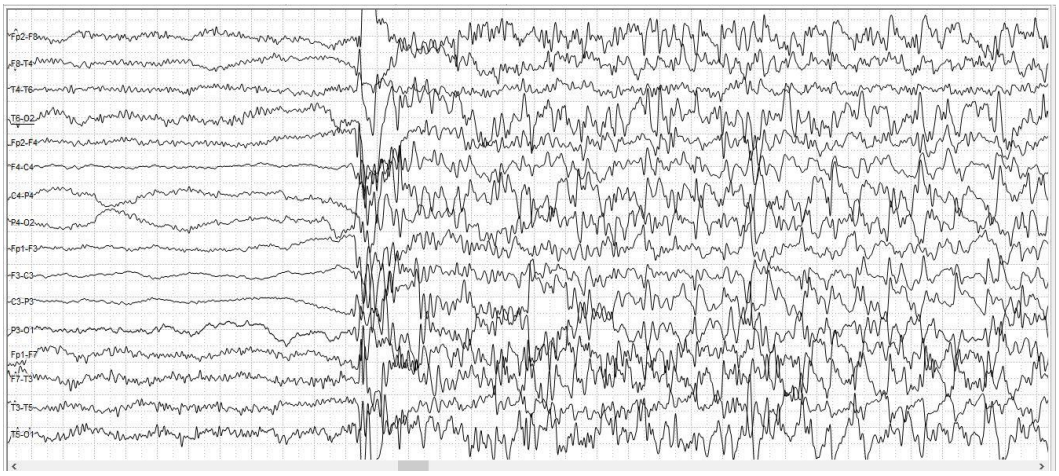
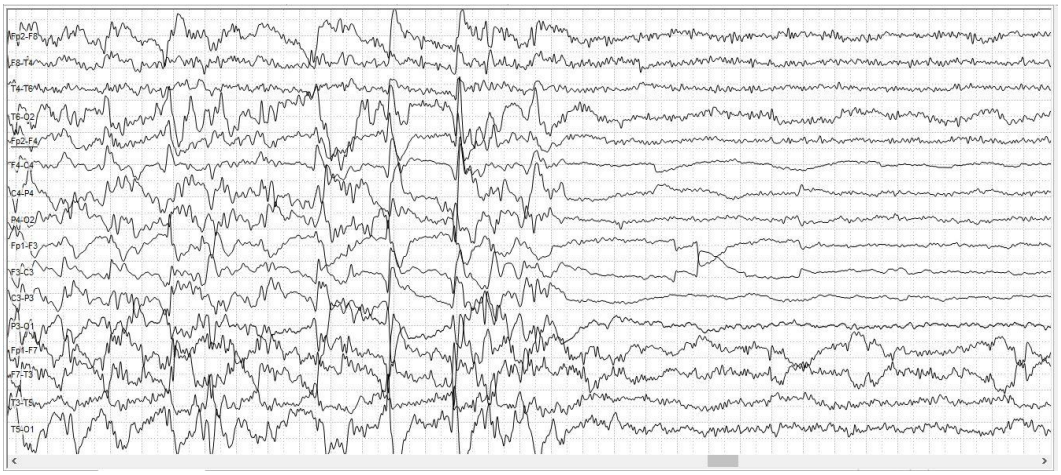


Picture 13



Picture 14



Picture 15

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DISCUSSION: According to our study, the comparison between the two patient groups show that the most harsh development is described in patients with post anoxic brain injury. In our perspective, despite the duration of anoxia, cortex damages can be considered as severe. Burst suppression is an electroencephalography (EEG) pattern that is characterized by periods of high-voltage electrical activity alternating with periods of no activity in the brain. High-voltage bursts of slow, sharp, and spiking activity alternating with a suppressed background have been termed burst suppression .Typical burst suppression pattern reflects severe diffuse cerebral dysfunction and is generally seen with comatose patients that are minimally reactive to stimuli.This pattern can be seen with powerful sedating anesthetics such as Propofol, midazolam, or phenobarbital. It also can be seen after severe insults such as high grade subarachnoid hemorrhage and anoxic brain injury.Generalized periodic discharges (GPDs) are generalized discharges that recur with a relatively uniform morphology and duration.Many disease states can cause GPDs, such as anoxia, toxic/metabolic encephalopathy, infections, nonconvulsive status epilepticus, and hypothermia. Generally, GPDs are morphologically similar regardless of etiology. Otherwise, GPDs occur in up to 20% of patients in coma with severe postanoxic encephalopathy after cardiac arrest, depending on the definition. These are present within the first 12–48h after resuscitation.Other causes are diffuse metabolic encephalopathy, including sepsis associated encephalopathy, and acute brain injury, including stroke.Rare intracranial infections, such as subacute sclerosing panencephalitis and Creutzfeldt–Jakob disease, may be associated with GPD. Generalized periodic discharges result from metabolic derangements or ischemia/hypoxia. The pathophysiology is likely diverse, but selective synaptic failure is a probable common mechanism. Generalized periodic discharges (GPDs) are basically associated with severe brain injuries. The suppression- burst syndrome (after GPDs) requires the accurate management for optimal positive dynamics. EEG shows eneralized period discharges in patient after cardiac arrest and with anoxic brain injury . From the first group , in patient with anoxic brain injury, 5 patient had generalized periodic discharges ,10-supression, 10 –burst suppression. From a second group, in patient with traumatic brain injury, 9 had a LPD_s(lateralized periodic discharges). Periodic lateralized epileptiform discharges have long been associated with acute focal lesions in the central nervous system. lateralized periodic discharges (LPDs) are infrequent electroencephalograph (EEG) findings, and may present in ictal or interictal form. They are regarded as potential electrophysiologic signs of convulsive or nonconvulsive status epilepticus

CONCLUSIONS

Serial myoclonic and tonic-clonic generalized seizures most frequently are in patients with postanoxic coma. The quality of anoxic brain injury is directly correlated to severity of convulsive syndromes. Serial myioclonic and tonic-clonic convulsions are results of severe anoxic damages, but after comparision of two group development of coma (by GSC) isn't directly correlated with types of convulsions, and encephalogram in the first group of patients registered suppressive and Burst- suppressive activities, while in patients with post-traumatic injuries was manifested LPD_s(lateralized periodic discharges) and BLD(bilateral periodic discharges).

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GRAM-NEGATIVE FOLLICULITES CAUSED BY KLEBSIELLA PNEUMONIAE IN IMMUNOCOMPETENT PATIENT (CASE REPORT)

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ABSTRACT

Bacterial folliculitis is marked by itchy, white, pus-filled bumps. It occurs when hair follicles become infected with bacteria, usually Staphylococcus aureus. This bacteria live on the skin all the time. But they generally cause problems only when they enter your body through a cut or other wound. Gram-negative bacilli are ubiquitous. They are found in 10-15% of the intertriginous bacterial flora. Gram-negative folliculitis is relatively uncommon. Gram-negative folliculitis may be the result of long-term antibacterial treatment in acne patients . It is caused by bacterial interference and replacement of the Gram-positive flora of the facial skin and the mucous membranes of the nose and infestation with Gram-negative bacteria. These Gram-negative bacteria include Escherischia coli, Pseudomonas aeruginosa, Serratia marescens, Klebsiella and Proteus mirabilis. The diagnosis depends of the characteristic clinical features and localization, the patient's history, as well as the result of bacteriological investigation. The significance of the isolation of Gram-negative bacilli from non-specific lesions must be carefully evaluated. The occurrence of Gram-negative folliculitis in acne patients is believed to be generally underestimated, since correct sampling and bacteriology is rarely performed by clinicians. Gram-negative acne is an unusual, distinctive disease that is probably increasing in frequency. It seems directly related to long-term therapy with multiple antibiotics and in one of our cases with the misue of topical corticosteroids.
Keywords: Skin, infection, bacteria, treatment, antibiotics.

INTRODUCTION

Gram-negative folliculitis (GNF) is a hair follicle infection by Gram-negative organisms that can occur as a complication in patients receiving prolonged treatment with broad spectrum antibiotics for the treatment of acne vulgaris and rosacea. It must be suspected in a sudden exacerbation of acne treatment or in patients non-responding to conventional acne treatments (1). There are two clinical variants of GNF; type I, is the most common, about 80% of cases, with the presence of multiple papules and pustules in the middle of the face; Oral isotretinoin is the treatment of choice at doses of 0.5 to 1mg/kg/day for 4-5 months (2). Its mechanism of action is to control the proliferation of Gram-negative bacteria through microenvironmental changes produced in the skin and nasal mucous (5,6). Gram-negative folliculitis was first reported in 1968 by Fulton et al (3), in a group of patients with acne vulgaris resistant to conventional treatments (4). It is a hair follicle infection that occurs mainly in patients with inflammatory acne or rosacea that have long treatments with broad spectrum antibiotics, mainly tetracycline . It should be suspected when there is an increase in pustules with resistance to systemic treatment (5). It is reported a prevalence of 4%. Prolonged treatment alters the normal bacterial flora of the nasal mucous and adjacent skin with reduced Gram-positive bacteria and coagulase positive aerobic diphtheroids, with an increase in Gram-negative bacilli mainly enteric bacteria (6).

Its characteristic features include: predominance in male gender, severe seborrhea, papules, pustules and perinasal and/or perioral involvement, recurrent folliculitis of the scalp, and prolonged antibacterial pretreatment, asymptomatic intervals tend to be shortened, acne and rosacea resistant to conventional treatment and isolation repeatedly of Gram-negative bacteria in cultures of pustules and facial nasal mucous (7). Gram-negative folliculitis has been reported after eradication of recurrent staphylococcal pyodermas and prolonged treatment with topical antibacterials. Bartholow & Maibach, described a patient with acne who had been treated with topical clindamycin, followed by benzoyl peroxide and topical erythromycin and developed GNF due to E. coli. Fulton et al , described patients using antibacterial soaps, which selectively inhibit Gram-positive bacteria(8) . Clinically differentiated two types of GNF. The type I, superficial or pustular, is the most common (80-90%), with presence of multiple papules and pustules in the middle of the face, 3 to 6 mm in diameter with an erythematous halo, mainly caused by E. coli, Klebsiella sp., Enterobacteria sp. and P. aeruginosa. The type II, deep or nodular (10-20%), is characterized by deep and painfully

inflammatory nodules or cysts, on the face, neck and/or chest, caused by Proteus mirabilis. Sebaceous follicles are colonized with these bacteria, mainly in the perioral and perinasal zone, with subsequent follicular and perifollicular inflammation with formation of papules and pustules (9,10). Marples et al (11), confirmed an inverse ratio between nasal carriers of S. aureus and enterobacteria. The proportion of Gram-negative bacteria results in a 1% of the total flora under normal conditions. In the case of nasal carriers this ratio increases 3-4%, being nasal cavity the reservoir for facial cases of GNF .

It is well known that tetracyclines, the most commonly used systemic antibiotic in acne and rosacea, impair protein synthesis and function of lymphocytes and neutrophil chemotaxis, increasing the risk of bacterial infection . The diagnosis should be confirmed with a smear of the pustules. The lesions (pustules) and the anterior nasal mucosa should be sampled for bacteriological studies. Treatment antibiotics must be withdrawn (12).

Case report

A 27-year old female came to the First university Clinic at TSMU dermatologist with a skin disease that affects the perioral zone, characterized by multiple papules and pustules . The patient underwent clinical examination and Using a sterile technique, lesions were sampled, and obtained pus was sent for culture . Two culture media blood agar and MacConkey agar were used. After 24 hours, monomorphous gram-negative bacteria with the mucoid colonies grew on both media. Identification was done by biochemical test Api 20E (Biomerieux, France). Isolate was tested according to EUCAST 2019 guidelines.Antibiotic susceptibility testing (AST) by Kirby-Bauer disk-diffusion method showed good sensitivity to following antibiotics: Ampicillin+Sulbactam, Amoxicillin +clavulanic acid, Amikacin, Gentamicin, Chloramphenicol. Isolate was resistant to all generation of cephalosporins and fluoroquinolones. Treatment was initiated with oral Ciprofloxacine to which the organism was sensitive (500mg twice dayly for one week) and topicaly solution whith antibiotics(10% camphor alcohol, ciprofloxacine and metronidazole) and Epiduo gel. Patient responded to the treatment very well.

Conclusion

The occurrence of Gram-negative folliculitis in acne patients is believed to be generally underestimated, since correct sampling and bacteriology is rarely performed by clinicians. The diagnosis depends of the characteristic clinical features and localization, the patient's history, as well as the result of bacteriological investigation.Treatment should be initiated depend on antibiogram results .

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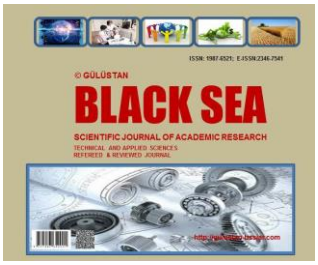
TECHNICAL AND APPLIED SCIENCES



Applied Geometry, Engineering Drawing, Ergonomics and Safety of Life
Machines and Mechanical Engineering
History of Science and Technics
Electrical engineering, Radio Engineering, Telecommunications, and Electronics
Civil Engineering and Architecture
Information, Computing and Automation
Mining and Geodesy Sciences
Metallurgy and Energy
Chemical Technology, Chemistry Sciences
Technology of Food Products
Technology of Materials and Products Textile and Light-load industry
Machinery in Agricultural Production
History of Art
Project and Program Management
Innovative Technologies
Repair and Reconstruction
Materials Science and Engineering
Engineering Physics
Mathematics & Applied Mathematics

REGIONAL DEVELOPMENT, OLIMPIC AND PROFESSIONAL SPORT

History of tourism
Theoretical and methodological foundations of tourism and recreation
Tourist market , its current state and development forecasts
Training and methodological support
Physical training
Olimpic sport
Professional sport
People health

ECONOMIC, MANAGEMENT & MARKETING SCIENCES

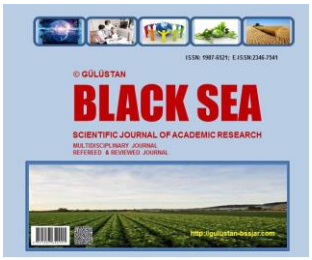
Economics and Management of Enterprises
Economy and Management of a National Economy
Mathematical Methods, Models and Information Technologies in Economics
Accounting, Analysis and Auditing
Money, Finance and Credit
Demography, Labor Conomics
Management and Marketing
Economic Science

LEGAL AND POLITICAL SCIENCE

Theory and History of State and Law
International Law
Branches of Law
Judicial System and Philosophy of Law
Theory and History of Political Science
Political Institutions and Processes
Political Culture and Ideology
Political Problems of International Systems and Global Development

CONFERENCE NEWSLETTER

MULTIDISCIPLINARY JOURNAL



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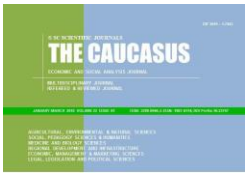
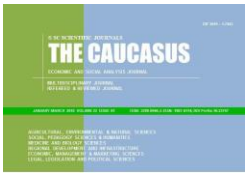


CONFERENCE NEWSLETTER



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Prophylactic Medicine
Theoretical Medicine
Stomatology & Dentistry
Veterinary Medicine and Zoo
Drug Technology and Organization of Pharmaceutical Business
Pharmaceutical Chemistry and Pharmacology
Standardization and Organization of Medicines Production
History of Pharmacy
Innovations in Medicine
Biophysics and Biochemistry
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Botany and Virology
Microbiology and Hydrobiology
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