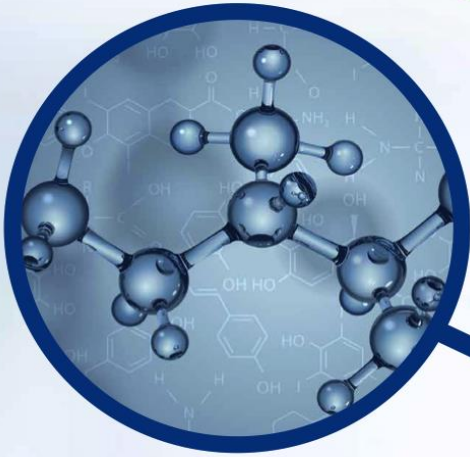# VII.

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Dear Colleagues,

On behalf of the Organizing Committee I am delighted to invite you to the 7th International Congress of Molecular Medicine that will be held in Istanbul, Turkey on 5th – 7th of September 2019 under the auspices of the Turkish Society of Molecular Medicine.

Congress program will consist of outstanding lectures, including keynote talks, plenary sessions, applied workshop, oral & poster presentations & exhibition.

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We are looking forwards to meet you in Istanbul on September 2019 for this outstanding congress and we hope you will enjoy scientific sessions, as well as Turkish hospitality and all the beauty of the Istanbul.

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OP1

Effects of eucalyptus oil in human lung cancer (A549) and mouse fibroblast (L929) cell lines

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Introduction

Eucalyptus species for the time being occur in most tropical and subtropical regions became an important and world widely planted genus containing more than 700 species. Eucalyptus spp. are a rich resource of essential oils of medicinal and commercial importance. There is only a limited number of reports on the possible physiological effects of eucalyptus in cell culture models. The probable lethal dose of pure essential oil of *Eucalyptus* spp. for an adult is in the range of 0.05 mL to 0.5 mL/kg, and severe poisoning has occurred in children after ingestion of 4 to 5 mL. As proposed by previous studies (3) that performed the cytotoxic effect of essential oils, IC50 values between 10–50 µg/mL represent a strong cytotoxic activity on different cell lines.

Malignant tumors are a life-threatening form of cancer that causes serious human health concerns worldwide. Chemotherapy is one of the major approaches to treating cancers, but its clinical use has been limited due to severe side effects. Therefore, the studies aimed at the identication of natural medicines with high efciencies but low toxicities have become the hot eld in cancer research.

It has been shown that many plant essential oils play an important role in the induction of apoptosis in cancer cells. Eucalyptus spp. essential oils are widely used in medicine, pharmaceutics, cosmetics and food industries. Many species of the Eucalyptus have been used widely in folk medicine for a variety of medicinal applications. Moreover, essential oil from *E. camaldulensis* has been reported to have a variety of beneficial efficacies and contains different bioactive ingredients capable to display antibacterial activity, antifungal activity, larvicidal activity, antioxidative and antiradical activities. In addition there are many reports on the cytotoxic effects of essential oils belong to Myrtaceae family plants as described by Ashour.,2008.(1).

However, systemic administration of eucalyptus can have effects on many other different cell types. Although, in vitro cell culture tests suggests that use of eucalyptus is safe for J774A.1 and HeLa cells lines (3), the possible effect of this oil on other cell types such as the lung cancer or fibroblast cell lines has not been extensively studied. Therefore we aimed to investigate the effect of eucalyptus oil on L929 fibroblast, A549 lung cancer cell lines. For this purpose viability, the changes in the ratio of apoptotic cells and cell migration rates were compared with simple and inexpensive methods.

Material and Methods

Cell culture and chemicals

Pulmonary adenocarcinoma human alveolar epithelial (A549 ATCC® CCL-185™) and murine fibroblast (L929 Sigma-aldrich) cell lines were were cultured in high glucose DMEM (Sigma, 5546) supplemeted with P/S (50 U/ml penicillin and 50 µg/ml streptomycin; Biological Industries, 03-031-1B), 1% 2mM L-glutamin

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(Biological Industries, BI03-020-1B), %10 FBS (Biowest, S1810-500). 1,5x106 cells from each cell line were seeded into 10cm plates and split after 72 hours. Commercially available Eucalyptus oil (W246611-Sigma-Aldrich) were dissolved in 10% aqueous dimethylsulfoxide (DMSO), sterilized by filtration through a 0.45 μm membrane filter and prepared fresh for every test.

MTT assay

Approximately 10^4 cells were seeded into each well of a 96-well plate at a volume of 100 μL. The MTT assay was carried out as follows: 10 μL of the 12 mM MTT (Neofrox 3580 MTT) stock solution was added to each well. and incubated at 37°C for 4 hours. 100 μL of medium alone was included as a negative control. After 4 hours of incubation with MTT 75 μL of medium was removed from the wells and then formazan crystals were dissolved with 50 μL of DMSO by mixing thoroughly with the pipette. After an additinal incubation at 37°C for 10 minutes samples were mixed again briefly and absorbance at 540 nm was recorded. The cytotoxicity was expressed as the concentration of sample that inhibited 50% of cell growth (IC50).

Acrydine Orange/Ethidium Bromide double staining

Cells were seeded in a 96-well plate at a density of approximately 10^4 cells/well. Following incubation with eucalyptus for 24 and 48 hours, cells were trypsinized and 10-25 μl cell suspensions were transferred onto glass slides. 1 μl of AO/EtBr staining solution (a mixture of dyes containing 100 μg/ml AO and 100 μg/ml EtBr) was added on cell suspensions and then the samples were covered with a coverslip. The morphology of cells was examined under a fluorescent microscope (Carl-Zeiss/Axio observer 3., Zen 2.3 Blue Edition software) within 20 minutes after adding Ao/EtBr stain. For statistical analysis st least 200 cells were counted and the results were expressed as mean values obtained from at least three independent experiments. Both live and dead cells are stained with AO while, ethidium bromide stains only dead cells which have lost membrane integrity. Live cells appear uniformly green whereas early apoptotic cells show green green dots in their nuclei. Late apoptotic cells stain orange and show condensed and/or often fragmented nuclei. Necrotic cells stain orange, with a nuclear morphology resembling that of viable cells, but without condensed chromatin.

In vitro scratch assay

A scratch on the surface of the well was made with a 10 μl sterile pipette tip in 6-well plates. Following gentle washing with culture medium, photos of the scratch were taken at different time points (0-24, 48 hours) under a microscope at a magnification of 10X (Carl-Zeiss/Axio observer 3) The gap size was analyzed using Image-J software and the rate of cell migration was calculated by comparing the cell-free areas of the scratches at 24 and 48 hour post-wounding and the area of the scratches at 0 hour. The results were expressed as mean of triplicate experiments.

Statistical analysis

Statistical analysis was performed using GraphPad (Prism 5) software. Multiple comparisons were made using Tukey's procedure. P<0.05 was considered statistically significant. Analysis of variance was used for statistical analysis of the apoptotic index among the groups.

Results

Determination of the half-maximal (50%) inhibitory concentration (IC50) is essential for understanding the pharmacological and biological characteristics of an agent. Firstly, we investigated the IC50 values and effects of eucalyptus oil on cell viability by comparing

the changes in proliferation rates of A549 (human pulmonary adenocarcinoma basal epithelial) and L929 (murine fibroblast) cell lines by MTT assay. For this purpose five different concentrations (0.0025%, 0.005%, 0.01%, 0.025% , 0.05% for 24 and 48 hours) according to Miriam F. et. al.,2006.(2) were tested.

IC50 values of A549 and L929 were 0.01% and 0.025% for 24H and 0.005% and 0.01% for 48H respectively. Analysis of data obtained from poliferation assay studies showed that all levels of eucalyptus oil treatment (0.0025%, 0.005%, 0.01%, 0.025% , 0.05% for 24 and 48 hours) inhibited proliferation rates in both of the cell lines tested **(Figure 1a-d).** Eucalyptus oil 0.0025% concentration inhibited proliferation rate by 21% and 32%, 0.005% concentration inhibited by 41% and 50%, 0.01% concentration inhibited by 50% and 67%, 0.025% concentration inhibited by 62% and 67%, 0.05% concentration inhibited by 62% and 74% in the A549 cell line at 24 hour and 48 hour **(Figue 1 a and b)**, while L929 cell line seemed to be more resistant to eucalyptus oil treatment. Fibroblast cell line needed to expose two times higher doses of EO than A549 for inhibitory effect.

Eucalyptus oil treatment resulted in significant inhibition in L929 cell line proliferation rate by 11% and 24% at the concentration of 0.005%, by 26% and 50% at the concentration of 0.01%, by 50% and 68% at the concentration of 0.025% and by 95% and 83% at the concentration of 0.05%, for 24 hour and 48 hour **(Figue 1c and d)**.

As seen in **Figure 1c**, L929 cell line seemed to be resistant to 0.0025% concentration when compared to nontreated L929 cell line at 24 hour. On the other hand, 0.0025% concentration inhibited by 16% in the L929 cell line at 48 hour. Viabilities of both cells were shown to be reduced significantly in the presence of EO (p<0.05). Eucalyptus essential oil exhibited an

inhibitory effect on the growth of both cell lines and the effects of essential oils could be variable according to incubation periods or concentrations tested.
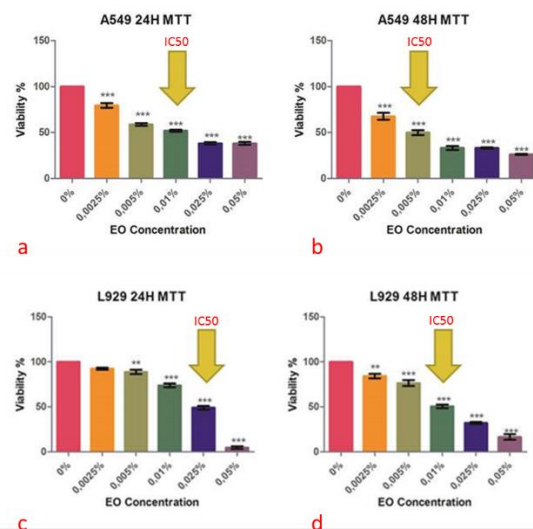

Figure 1. The effect of eucalyptus oil on cell proliferationb. a-b. A549 lung cancer,and **c-d.** L929 fibroblast were treated with eucalyptus oil for 24 and 48 hours in an incubator. MTT assays were performed 24 and 48 hours after treatment with the indicated doses of eucalyptus oil relative % changes in proliferation rates were compared against the non-treated control group (NT) and statistical significance was tested using one-way ANOVA followed by Tukey's multiple-comparisons test (* P<0.05, n=3).

Next, we compared the changes in the ratio of apoptotic cells using "acrydine orange / ethidium bromide" double staining protocol. For this purpose, the changes in the ratio of live, apoptotic and necrotic cells upon exposure to IC50 values of eucalyptus oil for A549 and L929 were evaluated as 0.01%/0.025% for 24 hour and 0.005%/0.01% for 48 hour. Apoptotic cell numbers were significantly (p<0.001) higher on both cells in both incubation time (24h 57±5%, 48h 50±2% for A549 and 24h 48±5%, 48h 49±4% for L929). However, as seen in Figure 2a-b, 0.005% concentration of eucalyptus oil significantly increased the number of necrotic cells of A549 cell line (necrotic cells: 7,66%) for 48 hour. A549 cells were more sensitive cell line (24

hour incubation A549: apoptotic cells 57%, 48 hour incubation A549: apoptotic cells 50,33%) while L929 cells seemed to be more resistant to eucalyptus oil (24 hour incubation L929: apoptotic cells 48,33%, 48 hour incubation L929: apoptotic cells 49%) **(Figure 2a-b)**. In general, the obtained results support an experimental basis for reporting that the eucalyptus oil lead to cell death mostly by apoptotic process. Representative microscope images from AO/EtBr stained samples are presented in **Figure 3**.

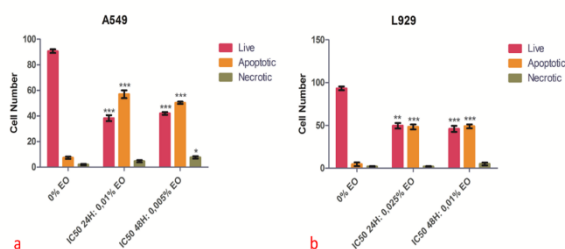Then, we investigated whether or not eucalyptus oil

Figure 2. Eucalyptus induces apoptosis at IC50 values. a. A549 lung cancer, b. L929 fibroblast, cell lines were treated with IC50 values of eucalyptus oil were evaluated as 0.01%/0.025% for 24 hour and 0.005%/0.01% for 48 hours in an incubator. AO/EtBr double staining was performed 24 and 48 hours after treatment with the indicated concentrations of eucalyptus oil. Percentage changes in the ratio of apoptotic cells were compared against the non-treated control group (NT) and statistical significance was tested using one-way ANOVA followed by Tukey's multiple-comparisons test (*,**,*** P<0.05, n=3).

treatment could induce changes in cell migration rates. For this purpose, we tested the effect of IC50 values of eucalyptus oil as 0.01%/0.025% for 24 hour and 0.005%/0.01% for 48 hours.on cell migration rates in A549 and L929 cell lines using *in vitro* scratch assay technique **(Figure 4-5)**. Our findings indicated that eucalyptus oil treatment decreased significantly (p<0.001) cell migration rates both in A549 (24 hour by 62±3%, 48 hour 67±4%) **(Figure 4a-b)** and L929 cell

lines (24 hour by 49±6%, 48 hour 70±4%) **(Figure 4c-d)**. Representative microscope images from scratch assay experiments are presented in **Figure 5**.
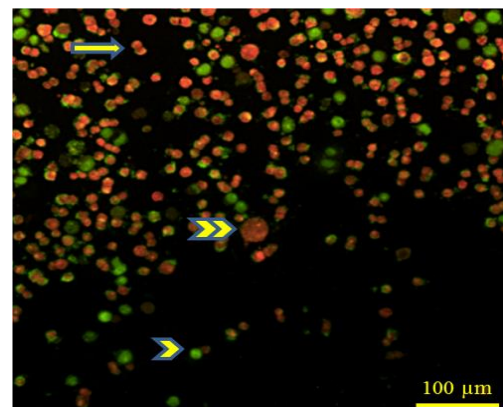

Figure 3. **Representative microscope images from AO/EtBr double staining.** Magnification: 10x A549 cells (0.005% for 48 hours). Arrows point to apoptotic cells, arrow heads point to live cells and double arrow heads point to necrotic cells.
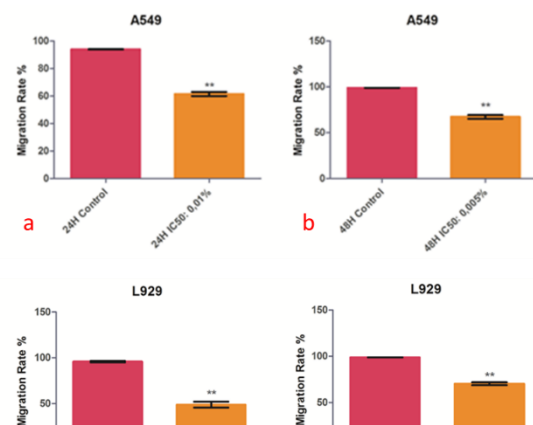

Figure 4. Migration rates were decreased significantly. a. A549 lung cancer (24h 62±3%, 48h 67±4% for A549, **b.** L929 (24h 49±6%, 48h 70±4% for L929) cell lines were treated with IC50 concentration of eucalyptus oil for 24 and 48 hours in an incubator. Scratch assay was performed 24 and 48 hours after treatment with the indicated concentrations of eucalyptus oil. The rate of migration (how soon the gap has been closed) in 24 and 48 hours was calculated by measuring the gap at 0. and 24/48hours after scratcing the plates. % changes in the migration rates were compared against the non-treated control group (NT) and statistical significance was tested using one-way ANOVA followed by Tukey's multiple-comparisons test (** P<0.001, n=3).
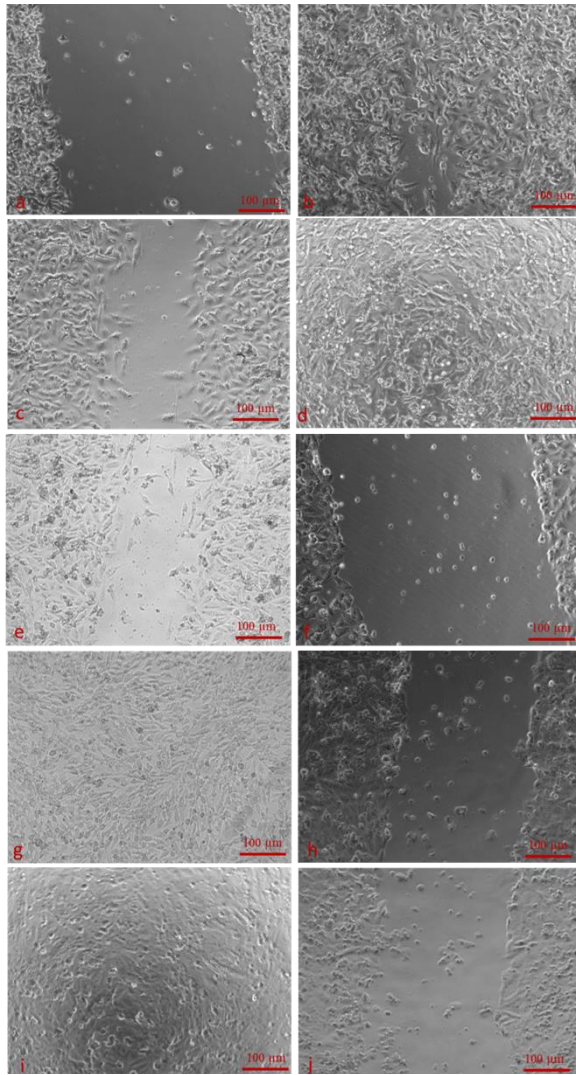
Figure 5. **Representative microscope images from** *in vitro* **scratch assay (Magnification 10X). a.** 0 hour A549 cells **b.** 24 hour 0% eucalyptus oil A549 cells (control for 24h) **c.** 24 hour 0.01% eucalyptus oil A549 cells **d.** 48 hour 0% eucalyptus oil A549 cells (control for 48h) **e.** 48 hour 0.005% eucalyptus oil A549 cells **f.** 0 hour L929 fibroblast cells **g.** 24 hour 0% eucalyptus oil L929 fibroblast cells (control for 24h) **h.** 24 hour 0.025% eucalyptus oil A549 cells **i.** 48 hour 0% eucalyptus oil A549 cells (control for 48h) **j.** 48 hour 0.01% eucalyptus oil A549 cells

Conclusions

The action of drugs on cancer cells can be realized through the induction of apoptosis or necrosis, or by the cell morphological changes caused. In this study, it was found that with the increase of treatment duration and eucalyptus oil concentration, changed cells's growth conditions and exhibited different degrees of inhibitory effects on cell growth and apoptosis. Effects of essential oils could be variable according to incubation periods or concentrations tested (by a specific component or several components present in the complex mix of eucalyptus oil). According to these results, eucalyptus oil is shown to have promising anticancer effects. The observed cytotoxic effect of eucalyptus oil may be attributed to either a specific component or several components present in the complex mix of eucalyptus oil. These findings can be the prerequisite basis for the potential development of these bioactive substances as potent antitumor drugs. However, further studies are still needed to evaluate their toxicity and safety. Furthermore, these data can also pave the way for future development of therapeutic opportunities against cancer. This pilot study will be extended with performing different cell lines. Even so, in vivo studies are needed to go one step further for a better understanding of eucalyptus oil effects.

Key Messages

Eucalyptus oil is shown to have promising anticancer effects. The obtained results support an experimental basis for reporting that the eucalyptus oil lead to cell death mostly by apoptotic process. The observed cytotoxic effect of eucalyptus oil may be attributed to either a specific component or several components present in the complex mix of eucalyptus oil.

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OP2

Role of acacetin on HT-29 colon cancer cell lines

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Introduction

Cancer is a serious health problem and one of the most common cause of death. Cancer development is a complex and multifactorial process which takes place in various stages and grades, which is nearly all organs and tissues are sensitive to it. Colorectal cancer (CRC) is one of the most common malignancies diagnosed in men and women, and also major public health issue in worldwide. Up to 50% of patients with CRC have metastatic form of the disease (1). The epithelial hyperproliferation that leads to adenomas formation is especially the characteristics of early stage of human colon cancer progression. The formation of impaired epithelial is a impact of aberrant activation of cell cycle and unsuccessful cell division with the dysregulation of apoptosis (2).

The choice of lifestyle significantly affects the chances of developing cancer. Dietary habits such as lack of fiber, fruit and vegetable in diet, consumption of high-fat food, overweight, and also sedentary lifestyle, abuse of alcohol and tobacco increases the risk of several cancer, especially CRC (3).

The incidence of a wide range of cancers has been decreased by fruit and vegetables that are sufficient in flavonoids (4). Acacetin is one of these flavonoid compound that has anti-proliferative, anti-inflammatory, anti-peroxidative effects (5) by directing the cell to arrest, triggering apoptosis and suppressing metastasis or angiogenesis (6).

Since conventional methods of treatment cannot completely manage the incidence and outcome of many cancers, new mechanisms for cancer prevention and treatment must be developed urgently (7). Recently, focused attempts have been made to create new nutritional substances as preventive or treatment agents of cancer. We considered that chemoprevention and chemotherapy impacts against colon cancer may be provided by acacetin.

In this study, we intented to investigate inhibitory effect of acacetin in human colon cancer HT-29 cell line. Here, we reported data including that acacetin may stimulate apoptosis. The outcomes proposed that acacetin may be a new therapeutic compound for colon cancer treatment and prevention.

Materials and Methods

HT- 29 cell line was obtained from ATCC. The HT- 29 cell line was cultured in McCoy's 5A medium. After

acacetin treatment on HT- 29, WST-1 was performed to find its role on cell viability. Multiscan ELISA reader (Thermo Fisher Scientific▯, Germany) was used at 450 nm wavelengths for color development. The changes of caspase- 3 enzyme activity in cancer cells were examined which is one other important apoptosis sign by using a caspase- 3 colorimetric assay kit (BioVision▯ Research Products, USA). The level of PS which is apoptotic marker for cell death, was distinguished using by Annexin V-FITC. Cells were analyzed using flow cytometry.

Results

In order to the detection of inhibitory effects for acacetin on HT- 29, the cells were incubated with concentrations (5, 10, 25, 50 and 100 µM) of acacetin for 24, 48 and 72 h using WST- 1.

With regard to the results of WST- 1, changes were found decreasingly in cell proliferation level in the CRC cell line.

Treatment of HT-29 cells with concentrations of acacetin for 48h and analysis of changes in caspase-3 enzyme activities were made to find the relation for apoptosis. Compared with the untreated cells, there was a 1,02 fold increase in caspase-3 activity in response to 48 h treatment with 5 µM, and a 1,12 fold decrease in response to 48h treatment with 10 µM on HT- 29 cells

FITC Annexin V/ PI double staining was applied to HT-29 which had been exposed to 5 µM and 10 µM acacetin for 48 hours on HT- 29 cells. The results demonstrated that 48 h incubation of HT- 29 cells with acacetin increased apoptotic cell death.

Discussion/Conclusion

According to our cytotoxicity test, 5 µM and 10 µM doses were the most effective doses and 48 hours was determined the optimum time for the HT- 29 cells. We showed acacetin-triggered caspase-3 activation in HT-

29 cells with at 5 ▯M. In addition to these data our results showed decrease effect of caspase activation with the incubation 10 ▯M and 25 ▯M acacetin over 48 hours in HT- 29 cells. Therefore, we suggested acacetin stimulated apoptosis in a concentration and time dependent manner, which could be associated with other caspase activities such as caspase-1, caspase-9 or caspase-7.

Pan et al. also investigated that caspases played any role in cell death mechanism stimulated by acacetin. They found acacetin activated apoptosis in a time and dosage- dependent manner in AGS cells via caspase-3 activation but not caspase- 1. Similarly, we found out acacetin treatment to HT- 29 cells promoted caspase-3 activity.

Data that we obtain from this study about apoptotic activities of acacetin, provide an alternative way for the next research designs. Our next purpose is to examine apoptotic activities of other colon cancer cell lines because of the different characterization of the cells. We also intend to analyse the expression of the other apoptotic factors such as caspase-7, caspase-8 or subcellular location of Apaf-1 for the HT-29 cell lines and the other different colon cancer cell line types.

Acknowledgements

Key messages

In present investigation, we examined the effects of flavonoid compound acacetin. According to our findings, acacetin could be a potential chemopreventive agent and can be used to treat tumorigenesis in the future. Our study contributes obtaining data about anticancerogenic effects of acacetin in colon cancer cells. There still needs to

elucidate the mechanism and molecular pathway of acacetin with further extensive experiments.

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OP3

Might the betulinic acid be effective for renal cancer in apoptotic way?

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Introductıon

Renal cancers are 9th in terms of incidence for all cancer types. Recent developed techniques and widespread treatment options were helped to diagnosis renal cancers [1]. Nevertheless the options are getting developed in medical science, herbal extracts or herbal related products have become remarkably an option for all cancer types because of the less side effects for humans [1,2]. Betulinic acid, which is a new candidate for this popular area, is a lupane-type of pentacyclic triterpenoid saponine that obtained from various natural plants. In addition to betulinic acid's anti-inflammatory, antiviral effects, in recent years researhers are focusing on it's anticancer capability [3-5]. There are some literature about significant outcome of betulinic acid's anticancer activity such as on melanoma cells, glioblastoma, lung carcinoma, colorectal carcinoma, breast carcinoma and prostate carcinoma [6-8]. It is believed that the betulinic acid is a member of a new class of potential anticancer drug which show it's activity to induce apoptosis by directly affecting mitochondria [9,10]. Plus, Karna et al. reported that betulinic acid is a amino peptidase N inhibitor [11] that the enzyme is associated with metastatic activity during the tumor development. Also betulinic acid has the ability to inhibiting of growth factor mediated angiogenesis because of modulation of mitochondrial function in endothelial cells [12]. In a single array study about betulinic acid that studied with 60 drug resistant cell line includes ACHN and CAKI-1 have promising results with its remarkable cytotoxic results despite the MDR cell lines [13]. Despite the all results there could not find any enlighten information about molecular

mechanism of betulinic acid and renal cancer cells. Capacity of betulinic acid on renal cancer is the main roof our study for it's interaction with apoptotic way.

Materıals and Methods

Cell Culture and Cell Viability Assay WST-1

ACHN, CAKI-2 and MRC-5 as a healthy cell line were purchased from ATCC (American Type Culture Collection, Manassas, VA). All cells were cultured a with specific media contents according to ATCC instruction in a condition of 37°C in 5% CO2. The cells were treated with betulinic acid in time (24 h, 48 h and 72 h) and dose (1-100 uM; 1 uM, 2,5 uM, 5 uM, 7,5 uM, 10 uM, 25 uM and 50 uM) depended manner. At the end of the all incubation period of each plate, WST-1 solution was added 10 uL and plates were incubated for 2 h in a condition of 37°C in 5% CO2 to measure the color development. The absorbance were measured at 450 nm wavelength by using a Multiscan ELISA reader (Thermo Fisher Scientific, Germany).

Phosphatidylserine measurement to detect apoptosis by Annexin V

During the apoptosis, there are several biochemical changes such as inside the cells and surface the cell membrane. Phosphatidylserine is countable one of the marker for apoptosis with its movement to outer membrane surface of apoptotic cells, which a kind of phospholipid is normally found in the inner surface of cells. To measure of the apoptotic percentage, Annexin V was studied by using flow cytometer.

Statistical Analysis

Results were analyzed by comparing the significance level of p <0.05 with Mann-Whitney U SPSS program.

Results

Betulinic acid's cytotoxic effect on renal cancer cells

According to cytotoxicity results, 25 uM and 50 uM betulinic acid concentrations and 24 h time manner were choosen to continue. CAKI-2 cell line and 25 uM betulinic acid have showed the 42,6% reduction cell viability in 24 h incubation period, reduction cell viability was determined 84,3% for 50 uM concentration (p<0,05). For ACHN cell line results are significant too. 25 uM betulinic acid ended up 53,47% of viability for 24 h and 50 uM betulinic acid ended up 70,6% (p<0,05). On the other hand, we have not observed any important change for viability on MRC-5 cell line.

Betulinic acid triggered apoptosis

ACHN and CAKI-2 cell lines were treated with 25 uM and 50 uM betulinic acid to determine apoptosis. Furthermore, 25 uM betulinic acid was effected for early apoptosis; 13,68% for ACHN and 7,5% for CAKI-2, 50 uM betulinic acid was effected for early apoptosis 22,66% for ACHN and 14,25% for CAKI-2 (p<0,05). Also, In healthy cell line MRC-5, betulinic acid has showed no any apoptotic effect with the 25 uM and 50 uM betulinic acid concentration.

Discussion

Considering the inflammation process and renal cancer relation in our research, betulinic acid, which is a product of plant origin and was investigated in many cancer type based on its apoptotic ability, have investigated on ACHN and CAKI-2 renal cancer lines. Renal cancers were almost an empty spot for betulinic acid and its anticancer impact. Several studies can be found that nominated betulinic acid as a candidate such as on colorectal cancer, pancreatic cancer, and breast cancer [6-8, 13-15]. They have all reported the similar point that there is a big potential for an option for cancer treatment with herbal related product [13-15]. Nevertheless these studies have claimed the cytotoxic activity of betulinic acid on apoptotic way

with several experiments; there are unclear impacts and relation with some cancers such as renal cancers. We believe that our results will be the preliminary for the researchers to focus on betulinic acid by using renal cancer models either in-vitro or in-vivo.

Competing Interests: None.

Ethics Committee Approval: Due to the fact that the study was no human or animal study, no ethical approval has been taken.

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OP4

What are the cytotoxic effects of amoclavin and cisplatin combination on head and neck cancer cell lines?

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Introduction

The increased cancer dependent death rate in years, still remains a big problem for all cancer types. Each year 650,000 new head and neck cancer (HNC) cases are diagnosed and HNC related deaths are reported to be 350,000 worldwide[1,2]. Among all cancer types, HNC is the sixth common type[3,4]. HNC includes mouth, lip, tongue, oral cavities, nose, nasopharynx and larynx tumours. Hence, life threatining locations on such respiratory system, HNC shows very poor prognosis in patients[2,5].

HNC is usually called "head and neck squamous cell carcinoma" (HNSC), because the squamous cells play a pivot role in head and neck carcinogenesis. Almost 90% off all HNC cases are diagnosed as HNCS[4]. HNSC is severly increased with tobacco and alcohol consumption. Another important factor, human papilloma virus (HPV) is also found to be triggering HNSC[6,7]. HNSC's prevelance is more common in males, than it is in females[1,8].

Tumour stages of HNSC in patients is highly important for treatment and surviving rates. However, most of HNSC patients are diagnosed in late stages of disease, so patient's survival rates are found to be decreased. For the early stages of HNSC, surgery or chemotherapy is being usually enough for patients to be cured. Following surgery/chemotherapy, survival rate is not satisfying advanced of HNSC[8–11]. General treatment method depends on chemotherapy implementation with radiotherapy which is known chemoradiotherapy. Cisplatin is widely used chemotherapy agent in HNSC[12–14]. Cisplatin enhances radiotherapy efficacy and inhibits cancer progression. Treatment regimen is cisplatin based chemoradiotherapy and patients are received high dose cisplatin with radiotherapy implementation usually for every three-week[13,14]. Besides the positive effect of cisplatin against carcinogenesis, toxic effects on healthy tissues and cells are controversial[14].

Toxic nature of anticancer agents on healthy cells have always been disconcerting issue[15]. Reducing the toxic effects of anticancer agents on healthy cells is the main factor to increase survival rate for patients. The need for new anticancer agents which are less toxic in healthy but more effective in cancer cells have become increasingly important[15,16].

In this context, we aimed to investigate amoxicilin-clavulanic acid (will be shortly defined as amoclavin) effect on HNSC cells whether if it induces cisplatin or not. Amoclavin is a widely used antibiotic in patients with bacterial infection diseases including otitis media, sinusitis, obstetric, gynaecological and urinary tract infections[17]. Anti-inflammatory effect of amoclavin was shown in ulcerative colitis patients[17].

Materials and Methods

CRL-1623 (SCC-15) and HTB-41 (A-253) HNC cell lines were used to see how amoclavin-cisplatin combination effects treatment comparing to only cisplatin treatment. Only cisplatin, cisplatin-amoclavin combination and only amoclavin treatment groups were included to the experiment. Also MRC-5 cells were treated with same treatment groups to determine how drugs effect on healthy cells. According to WST-1 cell viability assay, 25 uM for CRL-1623cells and 100 uM for HTB-41cell lines and were determined as effective dosages of amoclavin-cisplatin combination for 48 hours incubation period. Our results suggest that as a new combination agent, amoclavin could be used with cisplatin in HNC treatment.

Cell lines and culturing steps

CRL-1623 squamous cell carcinoma and HTB-41epidermoid carcinoma cell lines were used as head and neck cancer cell lines. MRC-5 fibroblastic normal lung cell line was used as control study group. For each cell line, appropriate mediums were prepared. The cell lines were seeded with growth medium in 75 cm^2 flasks and incubated in 5% CO_2 and 37 ºC incubator then growth medium was changed for following two days. After cells reached confluent phase, subculturing steps were done.

Wst-1 cell proliferation assay

CRL-1623, HTB-41 and MRC-5 cell lines were seeded in 96 well plates for each. For each cell line, 10,000 cells were seeded in one well of 96-well plate. Following 24 hours incubation step, growth medium was changed and fresh medium was added to the wells. Three study groups as only amoclavin (Group-1), only cisplatin (Group-2), and amoclavin-cisplatin combination (Group-3) were determined. Incubation periods as 24h, 48h and 72h for each three groups were studied. Different dosages of amoclavin and cisplatin were 5 uM, 10 uM, 25 uM, 50 uM and 100 uM. After the incubation periods, all the wells were added 10 µl WST-1 reagent to determine cell viability. WST-1 added 96-well plates were put in incubator for 4 hours incubation. Cell viability was determined by using multi-well ELISA reader at an 440 nm absorbance.

Results

According to the WST-1 results, amoclavin-cisplatin treatment was found to be more effective than only cisplatin treatment in both cancer cell lines. In HTB-41cell line, only 100 uM cisplatin treatment resulted in a survival rate of 54,5% while 100 uM amoclavin-cisplatin combination therapy reduced the survival rate to 33,7%. In CRL-1623 cell line, only 25 uM cisplatin treatment was resulted as 82,2% survival rate, but 25 uM amoclavin added cisplatin treatment

was found reducing the survival rate to 67,42%. For MRC-5 cell line, 25 uM treatment of amoclavin and cisplatin didn't effect the viability alone or together. Cisplatin treatment with 100 uM dose was decreased the cell viability in MRC-5 to 63,3%. Only 100 uM amoclavin treatment in MRC-5 cell line was resulted 120,02% cell viability. Amoclavin-cisplatin combined treatment in MRC-5 cell line was resulted 94,29% cell viability.

Discussion

In this context, there is no literature in which the relationship between head and neck cancers (HNC) and amoclavin has been investigated and its effect on cell culture has not been found, constitutes the original value of the proposed project. Cisplatin is a strong chemotherapeutic agent for many cancer types. Because of at high dose usage of cisplatin in cancer, so toxic effects also harm the healthy cells[14]. For this reason less toxic agents on cancer treatments have always been looking for[16]. Recently, antibiotics and their combination in cancer treatments have been studied extensively[18]. Because anti-bacterial and anti-inflammatory effects of antibiotics have raised their usage in such cancer treatment experiments[18,19]. In this study our results show that amoclavin and cisplatin combination treatment in HNC cell lines reducing toxic effect of cisplatin while it was alone, and also combination therapy was found less toxic in MRC-5 control cell group than only cisplatin treatment. Our results indicate that, amoclavin and cisplatin combination at low dosages was effective in HNC cell lines and also no toxicity in normal cells was found for 25 uM and 100uM drug combinations.

Recently, the presence of anticancer activities has been investigated by showing anticancer effects of antibiotics in mammalian cells. Bleomycin was used to treat lymphangiomas by local treatment in patients and showed anticarcinogenic effect by inhibiting cell

proliferation[20]. In pancreatic cancers, minocycline and sabutoclax combination induced apoptosis by intrinsic pathway and showed anticarcinogenic effect[21]. Metronidazole usage in colon cancer treatment inhibited cell proliferation[22].

According to our result, it is important that cytotoxic effect of amoclavin with cisplatin combination in HNC cells showed similarity to antibiotic drug combination experiment datas in literature.

Key Messages

Why head and neck cancer patients have poor survival rates?

As it contains many parts of the body's respiratory system, fatal complications related to the respiratory tract may occur.

Why only cisplatin treatment is not effective?

Because high dose cisplatin takes much time between two cure administirations.

What was our intention to do?

By using amoklavin-cisplatin combination with low dosages, we aimed to reduce cisplatin toxicity and induce it's efficacy in treatment.

Competing Interests: No competing interests was declared by the authors.

Ethics Committee Approval: Due to the fact that the study was no human or animal study, no ethical approval has been taken.

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OP5

Investigation of succinic acid effect on renal cancer cell lines

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Introduction

Renal cancers are one of the most common cancers with the highest mortality rate among genitourinary cancers, constitute 2-4% of all cancers[1]. According to GLOBOCAN data of 2018, over 400 000 new renal carcinoma cases were observed in the world and at least 175 000 of them resulted in death[2]. Renal cancers are classified into different subtypes according to their morphological and cytological features. The most common subtype of renal cancer is renal cell carcinomas (RCCs) and constitutes 80% of all renal cancers. Since there are many subtypes of different features in RCC, the clinical course of the disease and response to treatment vary. RCCs are best treated by surgery; however, it has the risk of recurrence[3]. In addition, also considering the possibility of cell resistance after radiotherapy and chemotherapy, it is important to investigate alternative treatment

methods in order to increase the quality and duration of the patients' life after diagnosis.

In recently, studies on the effects of substances of plant or animal origin that do not show toxic effects on healthy cells, on cancer prophylaxis are gaining value. One of these substances, succinic acid, has recently been shown to have apoptotic activity on various types of cancer[4,5]. Succinic acid ($C_4H_6O_4$), obtained by fermentation of agricultural carbohydrates, is widely used in food, pharmaceutical and plastics production industries and also is readily available for daily use[6]. In cellular processes, succinic acid is an intermediate metabolite which is converted to fumarate by succinate dehydrogenase in the tricarboxylic acid (TCA) cycle involved in ATP production. Considering all these properties, there are no known side effects on the physiological state of the cells.

There are many studies using different forms of succinic acid to increase anticancer and apoptotic activity on different cancer cells. Patacsil et al. reported that vitamin E-succinate compound, an analog of alpha tocopherol, reduces cell viability by inducing apoptosis on the PANC-1 pancreatic cancer cell line[7]. Various studies have shown that different forms of succinic acid suppress cell proliferation by triggering apoptosis in human breast cancer, head and neck cancer and mouse breast cancer cell lines.

In this study, we aimed to investigate of succinic acid role on renal cancer cell lines and tried to clarify its molecular mechanism. We suppose that our research could provide a reference for further alternative anticancer therapies.

Materials and Methods

Reagents and chemicals

In the current study, the following cells, drug and chemical reagents were used. Renal cell carcinoma cell line CAKI-2 and ACHN, and MRC-5 cell line as a healthy control was obtained from ATCC (American Type Culture Collection, USA). Succinic acid (S9512) standardized to suit cell culture studies was obtained from Sigma Aldrich (Germany). Cell culture media and supplementary material were obtained from ATCC (USA). The WST-1 cell proliferation assay was purchased from Roche Life Sciences (Germany) and Annexin V-FITC Apoptosis/Necrosis Analysis kit was purchased from Merck Millipore (USA).

Cell culture conditions

Human renal cell carcinoma cell lines CAKI-2 and ACHN, and human lung fibroblast MRC-5 cell line were maintained in McCoy's 5A (modified) and Dulbecco's Modified Eagle's Medium (DMEM), respectively. All mediums were prepared with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin. Cells were incubated in a condition of 37°C in 5% CO_2.

Cell viability assay

WST-1 cell proliferation assay was used to determine the viability of cell lines after succinic acid treatment. The cells were treated with succinic acid at increasing doses (0, 5, 10, 25, 50 and 100 µM) and times (24, 48, and 72 h). After each incubation period, 10 uL of WST-1 solution was added each well and the cells were again incubated for approximately 4 hours. Finally, the absorbance was measured 440 nm with the use of Multiscan ELISA reader (Thermo Fisher Scientific, Germany).

Apoptosis determine by annexin v

Cellular death after treatment with succinic acid, was detected using an Annexin V-FITC/PI apoptosis detection kit (Muse, USA). For this assay, FITC-

conjugated Annexin V and propidium iodide (PI) were added to 1×10^5 cells, after which cells were incubated for 15 min at room temperature in the dark. Following incubation, cells were analyzed by flow cytometry. According to this method, early apoptotic cells stained with Annexin V alone, whereas late apoptotic cells stained with both Annexin V and PI.

Statistical Analysis

Results were analyzed by comparing the significance level of $p < 0.05$ with Mann-Whitney U SPSS 16.0 program.

Results

Succinic acid exhibits cytotoxic effect on renal cancer cells

The cytotoxic effect of succinic acid was observed using WST-1 cell viability assay which is based on enzymatic activity of mitochondrial dehydrogenases present in viable cells. In this experiment renal cancer cell lines CAKI-2, ACHN and healthy control cell line MRC-5 treated with different concentrations of succinic acid (0, 5, 10, 25, 50, and 100 µM) for 24h, 48h and 72h. According to results from WST-1, the cytotoxic activity of succinic acid was found to be dose- and time- dependent on renal cancer cell lines. After 24 hours incubation of succinic acid at 25 and 50 µM concentrations, viability in cells was 10.5% and 9.23% for CAKI-2, 58.43% and 54.54% for ACHN, respectively. Nonetheless, no notable cytotoxic effect was observed after treatment of succinic acid in MRC-5 cells.

Succinic acid induces apoptotic cell death in renal cancer cells

To further evaluation the effect of succinic acid on cell death Annexin V-FITC assay was performed. According to our findings, it was obvious that under 25 and 50 µM concentrations of succinic acid for 24h, apoptosis rates of cells were increased compared to control cells

without succinic acid treatment. The percentage of early apoptotic cells, for CAKI-2, 25 µM 19,40% and 50 µM 28,52% and for ACHN, 25 µM 5,79% and 50 µM 11,1% were observed. In both cell lines, it was seen that succinic acid incubation at these doses did not trigger necrotic cell death. In addition to this, late apoptotic cells also could not be detected statistically significant in both cell lines when compared with early apoptosis.

Discussion

Renal cancer is a type of cancer that does not show any symptoms in the early stages of the disease and in the advanced stages it is very likely to metastasize to surrounding tissues[8]. In addition, there is also a high probability of developing resistance to chemotherapy and radiotherapy methods. When all these reasons are taken into consideration, the necessity of natural substances that do not create toxic effects on healthy cells comes to the fore for the treatment of renal cancer.

In this study, we aimed to investigate the apoptotic activity of succinic acid on renal cancer cell lines which has been shown to have apoptotic and anticancer activity in various cancer types[4,5]. Its widespread usage and production area are advantageous in terms of the availability of succinic acid for therapeutic use.

Apart from the daily use of succinic acid, there are studies of the use of various forms, either directly or in combination with anticancer drugs, as a therapeutic agent in different types of cancer to inhibit tumor cell proliferation, induce apoptosis or enhance the efficacy of anticancer drugs[9–11]. According to a study in the head and neck cancer cell line, α-TOS (Vitamin E-succinate) based polymeric particles not only exhibited anticancer activity but also increased anti-angiogenic activity by suppressing VEGF. In addition, it

has been shown that α-TOS based particles increase anticancer and anti-angiogenic activities at a higher rate compared to the use of polymeric nanoparticles alone[10]. Moreover, Hossain et al. demonstrated that succinate-containing nanoparticles are more successful in enhancing the cytotoxic and targeted efficacy of chemotherapeutic drugs with high side effects such as doxorubicin[11].

There are also studies on various cell lines for the direct anti-cancer activity of succinic acid in cell culture. Iplik et al. studied the cytotoxic and apoptotic efficacy of succinic acid on the endometrial cancer cell line[4]. According to the results of the study, 48 hours incubation of succinic acid at a concentration of 10 µM increased apoptotic death in endometrial cancer cells by 68.65%. The results obtained from our study showed correlation with the apoptotic activity shown on the endometrium[4] and T-cell acute lymphoblastic leukemia cell lines[5]. Besides, in our study, succinic acid did not show any cytotoxic activity in MRC-5 cells used as healthy control, which is consistent with previous studies.

In conclusion, according to the results of all these studies, direct application of succinic acid induces apoptotic cell death in cancer cells is an important finding. Based on this information, we think that the apoptotic efficacy of succinic acid on different types of cancer can be investigated and improved for treatment.

Key Messages:

- Renal cancer is a type of cancer that is highly likely to develop resistance to chemotherapy and radiotherapy.

- Succinic acid has been shown to have high cytotoxic and apoptotic activity on CAKI-2 and ACHN renal cancer cell lines.

- Succinic acid, which has no toxic effect on healthy cells, may be recommended as an alternative treatment for renal cancer.

Ethics Committee Approval: Due to the fact that the study was no human or animal study, no ethical approval has been taken.

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OP6

The relationship between MMP 9 and TIMP 1 genetic variants and serum levels in nasal polyps

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Aims

According to European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012 nasal polyposis is classified as a subgroup of chronic rhinosinusitis rather than a disease. (1) It is an inflammatory condition in which the nasal cavity and one or more sinuses are affected by the nasal mucosa. Many factors are responsible for the etiology of nasal polyposis. They can be grouped as enviromental, atopy and genetic factros. Different theories have been proposed in the pathophysiology of nasal polyposis. There is not yet a descriptive theory to cover all patients. (2) Matrix metalloproteinase (MMP) is an endopeptidase that plays important role in zinc and calcium-dependent extracellular matrix remodelling. TIMP 1 is the natural inhibitor of MMP 7 and MMP 9, which has been shown in many tissues, especially in nasal mucosa. MMP and TIMP play an important role in extracellular matrix hemostasis. The imbalance of between MMP and TIMP causes extracellular matrix deposition. (3)

There are no studies evaluating the effect of genetic polymorphism and serum levels of MMP 9 and its natural inhibtor TIMP 1 which has been shown to play a role in the etiopathogenesis of nasal polyposis. In our study, we aimed to investigate the correlation between DNA and serum levels. Blood samples are taken from patients who is diagnosed nasal polyposis.

Materials and Methods

99 patients with chronic rhinosinusitis with nasal polyposis accompanied with nasal obstruction, rhinorrhea, anosmi and altered taste perception were included in the study between August 2018 and April 2019 at Istanbul University, Istanbul Medical Faculty Otorhinolaryngology Head and Neck Surgery Department. 4 mm 0 and 30 degree rigid endoscopes were used in endoscopic examination. Endoscopic evaluation was performed according to Meltzer's endoscopic nasal polyp grading system. Blood was drawn into EDTA and gel serum tubes for DNA and

serum levels. SNOT-22 questionnaire was used to evaluate quality of life.

DNA isolation was made from blood and polymorphism analysis was made by RT-PCR. TIMP 1 (rs 4898) and Matrix metalloproteinase (MMP 9) (rs 17576) polymorphism analyzes were performed by RT-PCR.

Results

41 patients (41,4%) in Samter (+) group and 58 patients (58,6%) in Samter (-) group were included in this study. Eosinophil ratio was significantly higher in Samter (+) group. The presence of atopy was 82,9 % in Samter (+) group, and 10,3 % in Samter (-) group. The presence of atopy was increased 42- fold in Samter (+) group. When the goups were compared in terms of smoking, Smoking was significantly higher in Samter (-) group. (p:0,033) (Table 1).

Table 1: Demographic and clinical characteristics in Samter (+) and Samter (-) group

	Samter (-)	Samter (+)	P
Age (year) (mean±SD)	46,31±14,06	45,27±12,93	0,708
Gender (Female/Male)	16/42	22/19	0,009
BMI (kg/m²) (mean±SD)	26,80±4,93	26,72±4,0	0,927
Smoking (No/Yes) (%)	60,3/39,7	80,5/19,5	0,033
Alcohol (No/Yes) (%)	94,8/5,2	100/0	0,197
Right polyp grade (mean±SD)	2,64±0,85	2,32±1,15	0,114
Left polyp grade (mean±SD)	2,60±0,81	2,49±1,05	0,557
Eosinophil ratio (mean±SD)	3,75±2,93	5,41±3,53	0,012
Atopy (No/Yes) (%)	89,7/10,3	17,1/82,9	0,000*
Family history(No/Yes) (%)	84,5/15,5	78,0/22,0	0,414

No significant difference was observed between the groups in terms of MMP 9 and TIMP 1 genotype and allelic relationship (Table 2 and 3).

Table 2: MMP 9 genotypes and A/G alleles relationships in Samter (+) and Samter (-) group

	Samter (-) n:58	Samter (+) n:41	P
MMP9 Genotypes			
AA	24 (%41,4)	18 (%43,9)	
GG	8 (%13,8)	6 (%14,6)	
AG	26 (%44,8)	17 (%41,5)	0,946
Alels			
A	74 (%63,7)	53 (%64,6)	
G	42 (%36,2)	29 (%35,3)	0,903

Table 3: TIMP 1 genotypes and C/T alleles relationships in Samter (+) and Samter (-) group

	Samter (-) n:58	Samter (+) n:41	P
TIMP1 Genotypes			
CC	23 (%39,7)	13 (%31,7)	
TT	28 (%48,3)	17 (%41,5)	
CT	7 (%12,1)	11 (%26,8)	0,170
Alels			
C	53 (%45,68)	37 (% 45,12)	
T	63 (%54,31)	45 (%54,87)	0,93

MMP 9 serum levels were significantly higher in GG genotype than AG genotype in Samter (+) group. Serum levels of MMP 9 were significantly higher A (+) allele in Samter (+) group than G (+) allele (Table 4).

Table 4: MMP 9 serum levels according to AA, AG and GG genotypes and A/G alleles

	Samter (-)	P	Samter (+)	P
MMP 9 Genotypes	MMP 9 serum levels		MMP 9 serum levels	
AA	858,39±382,46	ns	795,56±239,75	
GG	565,32±283,60	ns	908,12±241,03	0,012 *vs AG
AG	713,66±409,74	ns	605,00±250,72	
A-	760,18±400,00		605,00±250,72	
A+	565,32±283,60	ns	853,55±243,42	0,028
G-	858,39±382,46		795,56±239,75	
G+	669,70±377,69	ns	829,05±273,97	ns

The mean of SNOT 22 scores were significantly higher in patients who were operated more than two (Table 5).

Table 5: The relationship between the number of surgery and the mean of SNOT 22 score in the whole group

	Number (n)	SNOT 22 (Mean± sd)	P
Number of surgery <2	67	41,57±19,57	
Number of surgery ≥2	30	50,40±15,35	0,019

Vitamin D serum levels were found statistically lower in TIMP 1 CT genotype compared to CC and TT genotypes (Table 6).

Table 6: The mean of SNOT 22 scores and Vitamin D serum levels in MMP 9 and TIMP 1 genotypes in the whole group

	TIMP1			MMP9		
	CC	CT	TT	AA	AG	GG
SNOT 22	47.34 ± 16,30	46,17 ±17,96	43,98 ±18,71	46,49 ±18,68	45,05 ±18,61	43,38 ±10,85
D Vitamini(ng/ml)	19,51 ±6,61	15,40 ±6,44	19,19 ±6,35	18,40 ±6,28	18,67 ±7,26	19,09 ±5,53

Conclusion

We evaluated patients with nasal polyposis, a subgroup of chronic rhinosinusitis, causing to decrease the quality of life and productivity-working capacity in this study. (4) Previous studies have shown that MMP 9 and TIMP 1 endopeptidase which play an important role in lower and upper respiratory tract remodelling and the pathophysiology of nasal polyposis. (5) At the same time, Vitamine D deficiency is associated with the severity of the disease in chronic rhinosinusitis but the mechanism of MMP secretion is not clear. (6)

Stevens et al found that atopy rate was 66 % in chronic rhinosinusitis patients with only nasal polyposis and 85 % in chronic rhinosinusitis patients with nasal polyposis and asthma. Atopy rate was found statistically difference between two groups. (7) In this study, atopy rate was 82,9 % in Samter (+) group, 10,3 % in Samter (-) group. The frequency of atopy was found to be 42-fold higher in Samter (+) group.

No study found that patients with Samter triad had fewer cigarettes. The respiratory system diseases which are associated with asthma are also present in Samter (+) group and they are thought to avoid smoking.

Wang et al also reported that MMP 9 single nucleotide polymorphism (SNP) rs 2664538 (including rs 17576), rs 3787268, rs 2274756 (including rs 17577) and rs 3918242 regions were selected in patients with the chronic rhinosinusitis with nasal polyposis and

controls. In subset analysis, none of the SNPs in the recurrent patients had a significant p value rs 2664538 under recessive model. There was no significant difference between non-recurrent and recurrent cases in other genetic polymorphisms. AA genotype was 10,9%, AG genotype was 28.1%, GG genotype was 56,3% in recurrent nasal polyposis. (8) In our study genotype findings differed from this study. When MMP 9 rs 17576 polymorphism was compared in Samter (+) group and Samter (-) group, No significant difference was found in terms of distribution of AA, AG and GG genotypes. AA genotype was 43,9%, AG genotype was 41,5% and GG genotype was 14,6% in Samter (+) group. As genotype distribution differences between populations, genotypes differed from the studies. In Samter (+) group the risk of having more than two surgeries was found approximately 3- fold in MMP 9 A allele, whereas it was found approximately 2-fold in MMP 9 AA genotype. The patients with Samter triad is more resistant to treatment and requires repeated surgeries.

The effect of these polymorphisms on serum levels has not been investigated. In this study MMP 9 serum levels were examined according to AA, AG and GG genotypes and A/G alleles, TIMP 1 serum levels were analyzed according to CC, CT and TT genotypes and C/T alleles. No significant difference was found in the comparison of TIMP 1 serum levels according to genotypes and alleles. MMP 9 serum levels were significantly higher in GG genotypes compared to AG genotypes. In addition MMP 9 serum levels were higher with A allele compared to G allele.

There is no study which is analyzed genetic polymorphism of TIMP 1in patiens with chronic rhinosinusitis with nasal polyposis. In this study TIMP 1 rs 4898 C/T polymorphism was evaluated in all chronic rhinosinusitis patients wih nasal polyposis. According to CC, CT and TT genotypes and C/T alleles

no significant difference was found between two groups including patients with and without Samter triad.

We didn't know that how Vitamine D effected the secretion of MMP 9. Intracellular production, transcription or translation may be effected. (9) According to MMP 9 and TIMP 1 genotypes Vitamine D levels were analyzed and Vitamine D levels were significantly lower in patients who had TIMP 1 CT genotype. The relationship between TIMP 1 CT genotype and Vitamine D levels has not been shown in the literature.

SNOT 22 is a questionnaire that evaluates nasal and otologic symptoms, sleep and emotional status of patients. The mean SNOT 22 score was 50,4 for the patients who had two and more than two surgeries. It was 41,5 for the patients who had less than two surgeries. The difference between two groups was significant. This suggests that patients who had repeated surgeries will have more severe symptoms.

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OP7

Association of *LOX-1* rs1800449 (G473A) mutation with osteoporosis risk in Turkish postmenopausal women

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Introduction

Osteoporosis is one of the most prevalent systemic skeletal disease affecting millions of people around the world from different ethnic groups and characterized by reduced bone density and deterioration of microarchitectural bone tissue which cause fragility fractures [1, 2]. Osteoporosis occurs when the key components of bone mineral (Ca) and matrix (collagen) decrease from the normal ratio (2:1) of the bone constitution [3]. Bone mineral density (BMD) is an important risk factor and multiple mechanisms affect the regulation of bone remodeling such as hormones, cytokines, age related factors, menopause, drugs *etc.* were identified so far [2, 4].

Lysyl oxidase (LOX) is a copper-dependent amine oxidase enzyme which plays critical roles in osteoblast differentiation, preservation of connective tissue integrity and extracellular matrix (ECM) homeostasis via providing cross-linking of collagen/elastin into insoluble mature forms with its capacity of converting lysine and hydroxylysine residues into highly reactive aldehydes which condense spontaneously with adjacent oxidized groups [5-6].

Five potential LOX family members encoding LOX and LOX-like proteins (LOXL1-4) were identified in mammals up to now [5, 6]. LOXL2/L3/L4 members were proposed to be involved preferentially in collagen IV-based basement membrane regulation, whereas LOX/L1/L5 forms contributed to cross-linkage of elastin [6].

Several studies have been conducted to investigate the effect of the LOX G473A polymorphism in cancers

such as lung, ovary, breast, *etc*. [7-9] and diseases such as coronary artery disease [10], however, any study has not been found in the literature investigating the effect of this polymorphism with the risk of osteoporosis yet.

Smoking is one of the risk factors for osteoporosis development and several studies shows that smoking causes BMD loss via different pathways [11-14]. *LOX-1* rs1800449 (G473A) polymorphism leads to a change in amino acid sequence at the residue 158 in the LOX preproenzyme and cause to a change of Arg to Gln. The effect of *LOX-1* rs1800449 (G473A) polymorphism and smoking were shown as susceptibility factors to lung and colon cancers in previous studies [8]. So, we aimed to investigate the effect of *LOX-1* (G473A) polymorphism and smoking on BMD values in Turkish postmenopausal women.

Material and Methods

Patient samples

Twenty smoking and 202 non-smoking postmenopausal women who were included in our study. Exclusion criteria were conditions, diseases, and/or treatments known to affect bone metabolism, such as malignancies, endocrinologic disorders, severe liver, gastroenteral, skeletal diseases, and current pharmacological treatment with corticosteroids, estrogens, etc. Menopause was defined as amenorrhea of at least 1 year duration. The study was approved by the Local Ethical Committee of Istanbul University Medical Faculty (Protocol No. 2006/2145) and a written, informed consent was obtained from each participant.

BMD measurement

BMD was measured at the level of lumbar spine (L1–L4) and hip (femoral neck and total hip) by dual-energy X-ray absorptiometry (DXA; Lunar DPX). All the measurements were analyzed according to software (Encore 2005) provided by the manufacturer.

Genotyping

Total DNA from blood samples were isolated with salting out procedure [15]. Genotyping for *LOX-1* rs1800449 (G473A) polymorphism was performed using TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific) at Step-One Plus Real-Time PCR Instrument **(**Thermo Fisher Scientific) with the following genotyping method conditions of the instrument: 30 seconds at 60 °C, 10 minutes at 95 °C, 15 seconds at 95 °C, 1 minutes at 60 °C (40 cycles). Genotypes were determined by the instrument in accordance with florescence label (FAM/VIC).

Statistical analysis

Statistical analyses were performed using the SPSS 20 package program. Data were tested for normality of distribution by Kolmogorov Smirnov test. Gene counting methods were performed for the estimation of allele frequencies. Chi-square test was used for the distribution of genotypes and alleles. Mann-Whitney U test was used for the comparison of mean values between study groups. The statistical significance limit was accepted as *p*<0.05.

Results

The frequencies of C and T alleles of *LOX-1* rs1800449 among the postmenopausal women were 83.56% and 16.44%, respectively. The genotype distributions of *LOX-1* rs1800449 (G473A) polymorphism and their consistencies to Hardy-Weinberg Equilibrium (HWE) in the study groups were shown in Table 1. The *LOX-1* rs1800449 (G473A) genotypes and alleles were found consistent to HWE (Table 1).

Table 1: Allele and genotype frequencies of study groups

LOX-1 rs1800449 C>T (G473A)	Study Groups	
	Smoking (n=20)	Non-smoking (n=202)
Genotypes		
CC	15 (75%)	139 (68.8%)
CT	5 (25%)	58 (28.7%)
TT	0 (0%)	5 (2.5%)
HWE	p>0.05	p>0.05
Alleles		
C	20 (100%)	197 (97.5%)
T	5 (25%)	63 (31.2%)

Values are given as number of samples and percentage (%) in the table. Inter-group comparison of importance level was analyzed by Chi-square test. n: number of samples, HWE: Hardy-Weinberg Equilibrium

Comparison of BMD values and biochemical features of the study groups between T allele and CC genotype carriers were given at Table 2. No significant differences between the smoking and non-smoking groups were found in terms of mean age, age of menopause and BMI ($p>0.05$).

Table 2. Comparison of BMD values and biochemical features of the study population between T allele and CC genotype carriers in the study groups

	Smoking (n=20)	Non-smoking (n=202)
Age		
CC genotype	52.87±6.55 (n=15)	59.42±7.54 (n=139)
T allele	55.80±5.21 (n=5)	59.41±8.10 (n=63)
Age of menopause		
CC genotype	46.33±2.01	46.69±5.62
T allele	41.5±7.93	47.09±4.24
BMI (kg/m²)		
CC genotype	27.37±5.73	30.67±4.89
T allele	22.59±2.46	30.37±4.72
Neck BMD (gr/cm²)		
CC genotype	0.83±0.09	0.85±0.11
T allele	0.73±0.07	0.85±0.10
Upper Femoral Neck BMD (gr/cm²)		
CC genotype	0.68±0.09	0.70±0.11
T allele	**0.57±0.08** [1]	0.70±0.10
Lower Femoral Neck BMD (gr/cm²)		
CC genotype	1.01±0.09	0.99±0.12
T allele	**0.89±0.07** [2]	0.99±0.11
Thoracanter BMD (gr/cm²)		
CC genotype	0.70±0.10	0.74±0.10
T allele	**0.59±0.09** [3]	0.75±0.09
Ward's triangle (gr/cm²)		
CC genotype	0.66±0.09	0.68±0.13
T allele	**0.54±0.07** [4]	0.68±0.12
Total BMD (gr/cm²)		
CC genotype	0.88±0.11	0.91±0.12
T allele	**0.77±0.06** [5]	0.91±0.11

Statistical analysis were performed by Mann-Whitney U test. Age, BMI, age of menopause values are given as mean±standart deviation (X±SD). Bold values of p indicates statistical significance. BMI, body mass index; BMD, bone mineral density; n, number of samples. 1 p=0.046; 2 p=0.046; 3 p=0.019; 4 p=0.008; 5 p=0.042

When we assessed whether smoking and having T allele of *LOX-1* rs1800449 (G473A) polymorphism have any effect on BMD values in Turkish postmenopausal women, upper femoral neck (T allele: 0.57±0.08 vs. CC genotype: 0.68±0.09, p=0.046), lower femoral neck (T allele: 0.89±0.07 vs. CC genotype: 1.01±0.09, p=0.046), thoracanter (T allele: 0.59±0.09 vs. CC genotype: 0.70±0.10, p=0.019), Ward's triangle (T allele:0.54±0.07 vs. CC genotype: 0.66±0.09, p=0.008) and total BMD (T allele: 0.77±0.06 vs. CC genotype: 0.88±0.11, p=0.042) values were found lower in T allele carriers of *LOX-1* rs1800449 polymorphism comparing with CC genotype in smoking postmenopausal women. However, this association was not observed in nonsmoking postmenopausal women ($p>0.05$).

Discussion

Several studies have been conducted to investigate the effect of the LOX G473A polymorphism in cancers

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such as lung, ovary, breast, *etc*. [7-9] and diseases such as coronary artery disease [10]. Wang *et al.* demonstrated that individuals with LOX473AA exhibited a higher susceptibility to lung, colon-rectum, colon, and rectum cancers in comparison to GG genotype carriers [8]. In the study of Yang *et al.*, *LOX-1* G473A polymorphism was observed significantly associated with ovarian cancer risk (AA>GG>GA, *p*=0.0062) [9]. Ma *et al.* reported that LOX473AA genotype and A allele were significantly higher in patients with coronary artery disease (CAD) than controls (*p*= 0.002 vs *p*=0.001) [10].

Krall and Dawson-Hughes showed that smokers had lower BMD values than nonsmokers [11]. Smoking leads to changes in Parathyroid hormone (PTH) [12] and vitamin D [13] levels which are key factors in Ca homeostasis and bone metabolism. Smoking was found to be associated with menopause and decreased levels of BMD [14]. In a study of Ko *et al.*, smoking sera given cells showed inhibition of osteo-blast differentiation and bone formation whereas demonstrated an increase in osteoclast differentiation [17].

In our study, upper femoral neck (*p*=0.046), lower femoral neck (*p*=0.046), thoracanter (*p*=0.019), Ward's triangle (*p*=0.008) and total BMD (*p*=0.042) values were found lower in *LOX-1* rs1800449 T allele carriers than smoking postmenopausal women with CC genotype whereas this association was not observed in nonsmoking group (*p*>0.05). So, we propose that smoking *LOX-1* rs1800449 T allele carriers are more susceptible to osteoporosis than postmenopausal women with CC genotype in Turkish population.

The main limitation of our study is that it includes relatively small study groups. So, further studies are required to demonstrate the association of smoking and *LOX-1* rs1800449 (G473A) polymorphism with osteoporosis risk.

Conflict of Interest

Authors declare that no competing interests exist.

Acknowledgments

Key Messages

➢ Smoking is one of the risk factors for osteoporosis development and found to have effects on BMD levels in previous studies.

➢ Smoking in conjunction with *LOX-1* (G473A) polymorphism was shown to have a negative effect on bone volume in mice studies.

➢ In our study, we found that smoking was associated with lower BMD values and a risk factor of osteoporosis development in T-allele carriers of *LOX-1* (G473A) polymorphism comparing to individuals with CC genotype in Turkish postmenopausal women.

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OP8

Long non-coding RNAs and potential lncRNA-mediated molecular mechanisms in pancreatic cancer: A study based on the cancer genome atlas and bioinformatics analyzes

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Introduction

Pancreatic cancer is the most destructive type of malignancy among all types of cancers. According to recent cancer statistics, the 5-year survival rate of pancreatic cancer is 7 %. Unlike other cancers, the survival rate of pancreatic cancer is very low despite the advancing technology and new diagnostic and therapeutic approaches. Ductal adenocarcinoma, representing the most common type of exocrine carcinoma, accounts for approximately 85 % of pancreatic tumors, while tumors derived from endocrine pancreas originating from islet cells represent 5 % [1]. The prevalence of pancreatic ductal cell adenocarcinoma (PDAC) is increasing in developed countries. A number of risk factors associated with increased incidence of PDAC have been identified. Risk factors include age, sex, smoking, alcohol consumption, unbalanced diet, high meat and fat diets, low serum folate levels, obesity, long-standing diabetes, and chronic pancreatitis. In addition, about 10 % of pancreatic cancer is hereditary. The majority of patients with pancreatic cancer harbor germline mutations in KRAS, p16/CDKN2A, SMAD4, TP53, or DNA repair genes [1]. However, in the vast majority of patients with familial risk of having pancreatic cancer, the underlying genetic predisposition is unknown.

Pancreatic cancer is rarely diagnosed until serious clinical symptoms and signs appear, and this is a clinical problem for early diagnosis and treatment of the disease. However, despite the aggressive treatment of the diagnosed disease, its therapeutic efficacy is not satisfactory [1]. Although many fragile sites for pancreatic cancer have been identified in studies to date, the underlying mechanism of transcriptome regulation has not been fully

elucidated. Whole genome sequencing, RNA sequencing and exon sequencing methods have shown that lncRNAs may be a critical factor in the pathogenesis of pancreatic cancer. Moreover, some lncRNA molecules have been reported to be potential biomarkers and / or drug targets for pancreatic cancer [2].

Previously, The Human Genome Project revealed that protein-encoding genes represent less than 2 % of the whole human genome. Accordingly, the remainder of the genome was considered to be "Junk DNA" because they do not encode for proteins. Later studies have revealed that most of the genome encodes for functional RNA molecules, rather than transcriptional noise. These RNA molecules were then called as non-coding RNAs (ncRNAs). NcRNAs are RNA transcripts with no protein coding capacity which have important roles in many biological processes such as regulation of gene expression, chromatin remodeling, DNA methylation as well as histone modification [3]. NcRNAs were generally divided into two main groups as small non-coding RNAs (less than 200 nucleotides in length) and long non-coding RNAs (more than 200 nucleotides in length).

Furthermore, expression levels of (long non-coding RNAs) lncRNAs have shown to be altered in human cancers. Therefore, these functional transcripts have gained special attention in human cancer because of their broad significance in the regulation of vital cellular processes such as cell proliferation, development, differentiation and death. For instance; HULC is a lncRNA molecule which is involved in the progression of pancreas cancer and reported to be candidate biomarker for the early diagnosis of pancreas cancer [4]. Accordingly, identification and validation of these transcripts relevant for the diagnosis, prognosis and treatment of human malignancies is of great interest in these days [3].

Although genomic analyzes have found an increasing list of lncRNAs, the role and functional importance of lncRNAs in the development of pancreatic cancer is mysterious. Therefore, in this study, by using TCGA database, we sought to determine the diagnostic and prognostic significance of lncRNAs altered in pancreatic cancer.

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Search strategy and inclusion criteria

Pancreatic cancer experiments were examined in the TCGA database (https://www.cbioportal.org/) and the experiments showing lncRNA expression were determined. LncRNAs were selected from other genes included in the study, and patient samples with clinical and demographic data were included in the study. A total of 179 patients with pancreatic cancer were included in the study.

Demographic and clinical characteristics and lncRNA dataset

In the TCGA database, data of pancreatic cancer patients were extracted according to the above mentioned criteria and the demographic and clinical characteristics of the patients are summarized in Table 1. Demographic characteristics of the patients included age, sex and alcohol consumption. Clinical features include pancreas cancer subtype, type of sample being studied, postoperative targeted adjuvant treatment receiving state, AJCC metastasis and tumor stage code, and chronic pancreatitis.

Overall analysis was achieved by using expression data of 20 different lncRNAs presented in TCGA database. The expression levels of the selected lncRNAs were analyzed by clinical and demographic characteristics of the patients. Co-expression analyzes were performed to demonstrate the correlation between lncRNAs and KRAS gene, which was frequently deleted in pancreatic cancer. Also, validation of prognostic significance of lncRNAs with TCGA data was achieved by analyzing the

correlations between lncRNA expressions and overall survival and disease-free survival. Kaplan Meier analysis was used in survival analysis.

Statistical Analysis

For the paired comparisons of demographic characteristics and lncRNA expression levels of the patients, t-test was used for samples with normal distribution, and non-parametric Mann Whitney U test was used for samples without normal distribution. ANOVA test was applied for multiple comparisons. Kruskal-Wallis test was used for multiple comparisons without normal distribution. SPSS and GraphPad Prizm softwares were used for statistical analysis and $p < 0.05$ was accepted as statistically significant.

Results

Identification of pancreatic cancer-associated lncRNAs in the TCGA database

Expression data of lncRNAs in TCGA database were obtained from cBioPortal (https://www.cbioportal.org/) "Pancreatic adenocarcinoma (TCGA, Provisional)".Obtained data were analyzed by heatmapper database

http://www.heatmapper.ca/expression/) and corresponding heat-map charts was created (Figure 1A). Also, total number of 20 lncRNA was identified to be differentially expressed in pancreas cancer tissues and relative expression levels of lncRNAs were presented in Figure 1B.
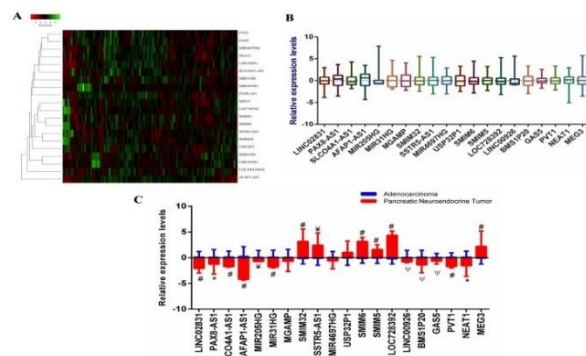

Figure 1. Expression levels of lncRNAs. **(A)** Heatmap graph showing sample-based expression levels **(B)** Expression levels of lncRNAs in patients with pancreatic cancer. **(C)**. Representation of lncRNA expression levels according to adenocarcinoma and Pancreatic Neuroendocrine Tumor subtypes. *****$p < 0.05$, ψ $p < 0.01$, ¥ $p < 0.001$, # $p < 0.0001$.

Table 1. Frequency of clinical features of pancreatic cancer patients according to demographic characteristics

Clinical Features		Total		Age	Gender		Alcohol Consumption		
		n=179	(%)	Mean±SD	Female (n=80)	Male (n=99)	YES (n=102)	NO (n=65)	NA (n=12)
Pancreatic Cancer Sub Type	Adenocarcinoma	170	94,97	64,97±10,93	75 (93,75)	95 (95,96)	96 (94,12)	62 (95,38)	12 (100)
	Pancreatic Neuroendocrine Tumor	8	4,47	57,88±9,17	4 (5,00)	4 (4,04)	5 (4,90)	3 (4,62)	0 (0,00)
	Undifferentiated Pancreatic Cancer	1	0,56	58,00±00	1 81,25)	0 (0,00)	1 (0,98)	0 (0,00)	0 (0,00)
Sample Type	Primer	178	99,44	64,58±10,94	80 (100)	98 (98,99)	102 (100)	64 (98,46)	12 (100)
	Metastatic	1	0,56	70,0±00	0 (0,00)	1 (1,01)	0 (0,00)	1 (1,54)	0 (0,00)
Adjuvant Postoperative Targeted Therapy	Yes	91	50,84	62,90±11,22	40 (50,0)	51 (51,52)	62 (60,78)	24 (36,92)	5 (41,67)
	No	45	25,14	64,91±10,96	17 (21,25)	28 (28,28)	30 (29,41)	13 (20,00)	2 (16,67)
	NA	43	24,02	67,93±9,56	23 (28,75)	20 (20,20)	10 (9,80)	28 (43,08)	5 (41,67)
AJCC Metastasis Stage Code	M0	80	44,69	63,96±10,91	36 (45,00)	44 (44,44)	50 (49,02)	26 (40,00)	4 (33,33)
	M1	5	2,79	65,80±4,91	3 (3,75)	2 (2,02)	2 (1,96)	3 (4,62)	0 (0,00)
	Mx	94	52,51	65,11±11,19	41 (51,25)	53 (53,54)	50 (49,02)	36 (55,38)	8 (66,67)
AJCC Tumor Stage Code	T1	7	3,91	61,43±20,50	3 (3,75)	4 (4,04)	4 (3,92)	3 (4,62)	0 (0,00)
	T2	24	13,41	66,33±9,02	11 (13,75)	13 (13,13)	11 (10,78)	13 (20,00)	0 (0,00)
	T3	144	80,45	64,53±10,61	64 (80,00)	80 (80,81)	84 (82,35)	49 (75,38)	11 (91,67)
	T4	3	1,68	65,67±15,28	2 (2,50)	1 (1,01)	2 (1,96)	0 (0,00)	1 (8,33)
	Tx	1	0,56	55,00±00	0 (0,00)	1 (1,01)	1 (0,98)	0 (0,00)	0 (0,00)
Chronic Pancreatitis	Yes	13	7,26	64,47±11,38	7 (8,75)	6 (6,06)	10 (9,80)	3 (4,62)	0 (0,00)
	No	129	72,07	61,08±10,37	54 (67,50)	75 (75,76)	86 (84,31)	40 (61,54)	3 (25,00)
	NA	37	20,67	66,38±9,2	19 (23,75)	18 (18,18)	6 (5,88)	22 (33,85)	9 (75,00)
Total	n=179			64,61±10,91	80	99	102	65	12
	(%)				44,69	55,31	56,98	36,31	6,7

Table 2. Comparison of lncRNA expression levels with demographic and clinical characteristics of pancreas cancer patients.

LncRNA	Age (<55 vs ≥55)	Gender (female vs male)	Alcohol consumption (yes vs no)	Pancreatic Cancer Sub Type (Adenocarcinoma vs Pancreatic Neuroendocrine Tumor)*	Adjuvant Postoperative Targeted Therapy (yes vs no)	AJCC Metastasis Stage Code*	AJCC Tumor Stage Code**	Chronic Pancreatitis (yes vs no)
LINC02831	0,5124	0,9879	0,5148	<0,0001	**0,0213**	0,1436	0,3126	0,2201
PAX8-AS1	0,6932	**0,0315**	0,3796	**0,0411**	0,1174	0,7340	0,4248	**0,0053**
SLCO4A1-AS1	0,2965	0,1929	0,9289	< 0,0001	0,4033	0,9000	0,0809	0,2866
AFAP1-AS1	0,9759	0,7181	0,6090	< 0,0001	0,2156	0,3619	0,0561	**0,0158**
MIR205HG	0,9740	0,1079	**0,0490**	**0,0008**	0,3006	**0,0342**	0,0864	0,9999
MIR31HG	0,0728	0,6480	0,6379	< 0,0001	0,6507	0,2479	0,4782	0,1289
MGAMP	0,0987	0,7596	0,5355	0,1210	0,1169	0,6707	0,6465	0,9568
SMIM32	0,3336	0,4246	0,3181	< 0,0001	0,9220	**0,0240**	0,0548	0,3023
SSTR5-AS1	0,3542	0,4238	0,4509	0,0002	0,6590	0,4646	0,4542	0,0777
MIR4697HG	0,8687	0,6033	0,8651	0,1337	0,6547	0,1646	0,4677	0,2500
USP32P1	0,9876	0,2088	0,0976	0,1354	0,3241	0,4019	0,7644	0,5719
SMIM6	0,3059	0,9107	0,7995	< 0,0001	0,1044	**0,0007**	0,1663	0,9484
SMIM5	0,1376	0,8823	0,3994	< 0,0001	0,7849	**0,0034**	0,6066	0,1002
LOC728392	0,3896	0,9371	0,8939	< 0,0001	0,6910	0,4894	**0,0042**	0,7279
LINC00926	0,1866	0,1836	**0,0230**	0,0105	**0,0090**	0,8306	0,6209	0,3874
BMS1P20	0,9318	0,2795	**0,0004**	0,0013	**0,0410**	0,5544	0,4517	0,7184
GAS5	0,0657	**0,0124**	0,3157	0,0072	0,9458	0,1106	0,3282	0,3554
PVT1	0,5216	0,9383	0,0759	< 0,0001	0,3781	0,7872	**0,0327**	0,1751
NEAT1	0,5487	0,2486	0,1389	**0,0227**	0,7074	**0,0116**	0,1746	**0,0082**
MEG3	0,9118	0,5621	0,9706	< 0,0001	0,9917	0,2121	0,2587	0,6632

* Undifferentiated Pancreatic Cancer is not included because the sample number is small.
** M1 is not included because the sample number is small.

Diagnostic Roles of LncRNAs in Pancreatic Cancer

To reveal the diagnostic potentials of lncRNAs involved in pancreatic cancer development, correlation analysis were performed to reveal the association of lncRNAs with demographic and clinical data of 179 pancreatic cancer patients (Table 2). Patients were evaluated in two groups in terms of age but there was no statistically significant difference between patients older than 55 years of age and younger than 55 years of age. In addition, when lncRNA expressions were evaluated in terms of gender, the expression levels of PAX8-AS1 and GAS5 lncRNA transcripts were correlated with pancreatic cancer. PAX8-AS1 and GAS5 expression levels were lower in females than males. When lncRNA expression was evaluated in terms of alcohol consumption, MIR205HG, LINC00926 and BMS1P20 lncRNA genes were shown to be markedly correlated with pancreas cancer. MIR205HG expression was higher in alcohol-consuming pancreatic cancer patients compared to non-alcohol consuming patients whereas LINC00926 and BMS1P20 expressions were lower in alcohol-consuming patients than non-alcohol consuming patients.

When lncRNA expressions were evaluated in terms of clinical features, it was found that many lncRNA expressions showed significant changes according to the pancreatic cancer subtype (Figure 1C). When lncRNA expression was evaluated for Adjuvant Postoperative Targeted Therapy, LINC02831, LINC00926 and BMS1P20 expression levels were higher in patients receiving therapy than those not receiving therapy. Given the chronic pancreatitis status, PAX8-AS1, AFAP1-AS1 and NEAT1 lncRNA changes were higher in patients with chronic pancreatitis than in those without chronic pancreatitis. MIR205HG, SMIM32, SMIM5, SMIM6 and NEAT1 genes were found to be markedly altered according to the AJCC metastasis stage. In addition, expression levels of LOC728392 and PVT1 lncRNA transcripts were found to be significantly changed when evaluated according to tumor stage. Sample-based co-expression analyzes were performed between the KRAS gene, which is frequently mutated and differentially expressed in pancreatic cancer, and lncRNAs obtained from TCGA data (Figure 2).
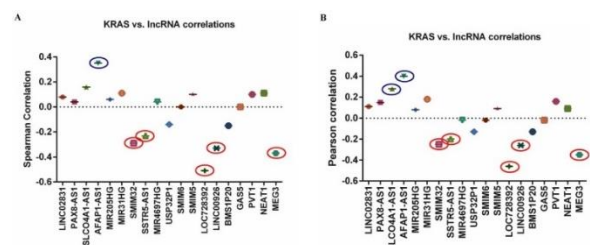

Figure 2. Correlations between LncRNAs and KRAS. **(A)** Spearman correlation between KRAS gene and lncRNAs. **(B)** Pearson correlation between KRAS gene and lncRNAs.

Accordingly, a strong positive correlation was found between KRAS gene and MIR205HG, AFAP1-AS1, PVT1 and SLCO4A1-AS1 genes. In contrast, strong negative correlation was found between KRAS and BMS1P20, LINC00926, MEG3, SMIM32 and SSTR5-AS1 genes. There was a non-significant weak correlation between the other lncRNAs and the KRAS gene.

Prognostic Roles of LncRNAs in Pancreatic Cancer

The lncRNA expression levels and disease-free and overall survival analyzes in pancreatic cancer were analyzed by the Kaplan Meier test. As a result, lncRNA expression was found to be not well-correlated with disease-free and overall survival (p_{OS}=0,379, p_{DFS}=0,738). Overall survival was correlated with MEG3, SMIM6, MIR31HG and disease-free survival was associated with MEG3, MIR31HG, PVT1, SMIM6 and USP32P1 lncRNAs.

Discussion

Herein, we performed overall analysis of lncRNA expression in pancreas cancer by using data obtained from TCGA database and revealed significant correlations between lncRNA expressions and demographic and clinical features of patients. In particular, a significant relationship was found between LINC00926 and alcohol consumption, adjuvant treatment status, and pancreatic cancer subtype. However, more comprehensive in vivo and in vitro studies are demanded to fully elucidate the driving molecular mechanism behind this disease.

MiR-205 expression was significantly higher in cancer tissue and serum of PDAC patients than in age-matched healthy controls and together with the serum CA.19-9 levels, miR-205 increases prognostic accuracy in pancreas cancer [5].

In our study, the findings obtained from TCGA database suggested that MIR205HG is significantly correlated with the demographic features such as alcohol consumption in patients with pancreatic cancer, as well as clinical features such as pancreatic cancer subtype and metastasis. Accordingly, the accumulating evidence increases the potential of MIR205HG and thus in turn miR-205 to be a diagnostic and prognostic biomarker for pancreatic cancer. However, these evidences should be supported by more detailed studies in pancreatic cancer experimental models.

NEAT1 was shown to be associated with many different types of cancer and high NEAT1 expression was well-correlated with poor prognosis. In addition, silencing of NEAT1 in pancreatic was shown to suppress the growth of pancreatic cancer cells by inducing cell cycle arrest and stimulating apoptosis [6]. In our findings, the association of NEAT1 expression with clinical features such as pancreatic cancer subtype, metastasis and chronic pancreatitis status indicates that this lncRNA molecule might be a chief biomarker for pancreatic cancer. Although NEAT1 has been extensively studied in common cancers such as lung, breast, colorectal cancer, studies in pancreatic cancer is very limited. Consequently, these findings suggest that this lncRNA molecule, which is associated with the clinical features of pancreatic cancer, might be important to illuminate the molecular mechanism of pancreatic cancer and further comprehensive studies are needed in this area.

As a result, in this bioinformatics-based study, lncRNAs which have important roles in the molecular mechanism of pancreatic cancer have been revealed. Detailed examinations of these lncRNAs in vitro and in vivo studies might enable the identification of novel diagnostic and prognostic biomarkers for pancreatic cancer.

Key messages

- LncRNAs play fundamental roles in the molecular mechanism of pancreatic cancer.

- MIR205HG expression has been associated with demographic characteristics of pancreatic cancer patients such as alcohol consumption and clinical characteristics such as pancreatic cancer subtype and metastasis code.

- The association of NEAT1 expression with clinical features such as pancreatic cancer subtype, metastasis and chronic pancreatitis status indicates that this lncRNA molecule might be a key biomarker for pancreatic cancer.

- Significant correlations were determined between lncRNAs and the KRAS gene, suggesting these lncRNAs are involved in KRAS-mediated oncogenesis.

Conflict of interest

There is no conflict of interest to report.

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OP9

Decreased expression of thioredoxin interacting protein (TXNIP) in colorectal cancer patients

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and which is considered for 10% of new cancer diagnoses. CRC can be gradually developed through an accumulation of different somatic or inherited changes within genome and epigenome[1]. There are three most important molecular pathways leading to CRC development: (1) Somatic or germ line derived genomic instability due to inactivation of several tumor suppressor genes such as APC, TP53 and SMAD4; aberrant DNA methylation, DNA repair defects induced by mutations in mismatch repair genes (MMR); (2) Mutational inactivation of tumor suppressor genes (e.g., APC, TP53, MMR, and TGFb genes); and (3) Over activation of oncogenic pathways including RAS (KRAS and NRAS), BRAF, Phosphatidyl inositol 3-kinase (PIK-3)[2].

TXNIP gene

Thioredoxin (TXN) is a low-molecular-weight redox protein and a putative oncoprotein that provides growth and survival advantages to tumor cells through the activation of redox-sensitive transcription factors, such as nuclear factor kappa B (NF-κB), p53, and activator protein-1 (AP-1). TXN inhibits apoptosis via apoptosis signaling kinase-1 (ASK-1) and phosphatase

and tensin homolog (PTEN)[3]. The overexpression of TXN has been found in several cancers, including lung, pancreatic, cervical, and colorectal cancers as well as hepatomas. The increased expression of TXN in tumors has been associated with decreased patient survival in several cancers and with resistance to anticancer drugs[4]. TXNIP was originally isolated in HL60 cells treated with Vitamin D3. It has been identified as a major redox regulator and a Tumor Suppressor Gene (TSG) in various solid tumors and hematological malignancies. TXNIP overexpression inhibits TXN activity, which in turn inhibits tumor cell proliferation and cell cycle progression[5].

Materıals and Methods

This study included 80 patients with colorectal cancer (45 men and 35 women) and 58 normal control samples(42 men and 16 women) and they were confirmed to be normal on histopathological assessment.

Quantitative real-time polymerase chain reaction

Total RNA was isolated from tissues using commercial RNA extraction kit in accordance with the manufacturer's instructions. A total of 500 ng of total RNA was reverse-transcribed using oligo-dT primers for cDNA synthesis. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was selected as reference genes. The qRT-PCR reaction contained 10 µl 2x RT- HRM Mix, 0,5 µl lc GREEN, 0,6 µl RT-RIN Mix, 0,8 µl forward primer and 0,8 µl reverse primer, 4,8 µl cDNA template, and PCR grade water up to a final volume of 20 µl.

Statistical analysis

Statistical analyses were performed using the SPSS 22.0 (Statistical Package for Social Sciences for Windows) and P ≤ 0.05 were accepted as a significant correlation. The suitability of the variables to normal distribution was evaluated by Kolmogorov-Smirnov-Shapiro Wilk tests. Student's t-test was used to compare the values of individuals with normal distribution.

Results

We found significant downregulation of TXNIP when patients with colorectal cancer (CRC) was compared to normal control samples. RT-PCR assay showed that TXNIP (0.45-fold) expression in colorectal cancer was significantly lower than that in their adjacent normal tissues . TXNIP expression is downregulated in tumor cells but there was no statistically significant difference between patient and healthy control groups (p>0.05). These results suggest a possible role of TXNIP in the pathogenesis of colorectal cancer, as well as its clinical significance.

Table 2: Analysis of thioredoxin-interacting protein mRNA expressing genes

Gene	Fold Change	p value
TXNIP	0.45	0.08

Discussion

It has been argued that the metastatic process, in contrast to conventional wisdom, is a ripe target for therapeutic development, as it widely contributes to cancer patients' deaths, although new clinical trial designs will be needed for validation . Multiple MSGs have been discovered in model systems[6]. Through apparently different pathways, based on the gene-by-gene literature, they lead to the same biological event: suppression of metastasis formation. Since suppressor genes are virtually impossible to directly and uniformly deliver, we and others have focused on downstream pathways, herein transcripts with opposite, presumed pro-metastatic expression patterns. We present the first comprehensive analysis of the downstream transcriptional effects of MSG expression, and identify

several common gene expression pathways of functional and potential therapeutic significance[7]. TXNIP inhibited thioredoxin (TRX), which maintains the cellular redox state [8], suggesting that the TRX-TXNIP interaction may also be important as a redox regulatory mechanism in cellular processes. These observations suggest that redox regulation may be involved in the carcinogenic and inflammatory processes in the colon cancer patients. Various clinical evidence has implicated TXNIP as a novel tumor and metastasis suppressor, of which expression is dramatically reduced in various tumor tissue, including breast, lung and colorectal cancer (CRC)[9].

Key Messages

- TXNIP was shown to significantly reduce gene expression in various human cancers including CRC in previous studies which may be referred as a known potent tumor suppressor.

- TXNIP may be a promising prognostic marker for CRC.

- TXNIP can also be considered as an effective potential treatment for personalized adjuvant CRC based on its expression levels.

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OP10

Association of *LOX-1* rs1800449 (G473A) mutation with osteoporosis risk in Turkish postmenopausal women

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Introduction

Osteoporosis is one of the most prevalent systemic skeletal disease affecting millions of people around the world from different ethnic groups and characterized by reduced bone density and deterioration of microarchitectural bone tissue which cause fragility fractures [1, 2]. Osteoporosis occurs when the key components of bone mineral (Ca) and matrix (collagen) decrease from the normal ratio (2:1) of the bone constitution [3]. Bone mineral density (BMD) is an important risk factor and multiple mechanisms affect the regulation of bone remodeling such as hormones, cytokines, age related factors, menopause, drugs *etc.* were identified so far [2, 4].

Lysyl oxidase (LOX) is a copper-dependent amine oxidase enzyme which plays critical roles in osteoblast differentiation, preservation of connective tissue integrity and extracellular matrix (ECM) homeostasis via providing cross-linking of collagen/elastin into insoluble mature forms with its capacity of converting lysine and hydroxylysine residues into highly reactive aldehydes which condense spontaneously with adjacent oxidized groups [5-6].

Five potential LOX family members encoding LOX and LOX-like proteins (LOXL1-4) were identified in mammals up to now [5, 6]. LOXL2/L3/L4 members were proposed to be involved preferentially in collagen IV-based basement membrane regulation, whereas LOX/L1/L5 forms contributed to cross-linkage of elastin [6].

Several studies have been conducted to investigate the effect of the LOX G473A polymorphism in cancers such as lung, ovary, breast, *etc.* [7-9] and diseases such as coronary artery disease [10], however, any study has not been found in the literature investigating the effect of this polymorphism with the risk of osteoporosis yet.

Smoking is one of the risk factors for osteoporosis development and several studies shows that smoking causes BMD loss via different pathways [11-14]. *LOX-1* rs1800449 (G473A) polymorphism leads to a change in amino acid sequence at the residue 158 in the LOX preproenzyme and cause to a change of Arg to Gln. The effect of *LOX-1* rs1800449 (G473A) polymorphism and smoking were shown as susceptibility factors to lung and colon cancers in previous studies [8]. So, we aimed to investigate the effect of *LOX-1* (G473A) polymorphism and smoking on BMD values in Turkish postmenopausal women.

Material & Methods

Patient samples

Twenty smoking and 202 non-smoking postmenopausal women who were included in our study. Exclusion criteria were conditions, diseases, and/or treatments known to affect bone metabolism, such as malignancies, endocrinologic disorders, severe liver, gastroenteral, skeletal diseases, and current pharmacological treatment with corticosteroids, estrogens, etc. Menopause was defined as amenorrhea of at least 1 year duration. The study was approved by the Local Ethical Committee of Istanbul University Medical Faculty (Protocol No. 2006/2145) and a written, informed consent was obtained from each participant.

BMD measurement

BMD was measured at the level of lumbar spine (L1–L4) and hip (femoral neck and total hip) by dual-energy X-ray absorptiometry (DXA; Lunar DPX). All the measurements were analyzed according to software (Encore 2005) provided by the manufacturer.

Genotyping

Total DNA from blood samples were isolated with salting out procedure [15]. Genotyping for *LOX-1* rs1800449 (G473A) polymorphism was performed

using TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific) at Step-One Plus Real-Time PCR Instrument (Thermo Fisher Scientific) with the following genotyping method conditions of the instrument: 30 seconds at 60 °C, 10 minutes at 95 °C, 15 seconds at 95 °C, 1 minutes at 60 °C (40 cycles). Genotypes were determined by the instrument in accordance with florescence label (FAM/VIC).

Statistical analysis

Statistical analyses were performed using the SPSS 20 package program. Data were tested for normality of distribution by Kolmogorov Smirnov test. Gene counting methods were performed for the estimation of allele frequencies. Chi-square test was used for the distribution of genotypes and alleles. Mann-Whitney U test was used for the comparison of mean values between study groups. The statistical significance limit was accepted as $p<0.05$.

Results

The frequencies of C and T alleles of *LOX-1* rs1800449 among the postmenopausal women were 83.56% and 16.44%, respectively. The genotype distributions of *LOX-1* rs1800449 (G473A) polymorphism and their consistencies to Hardy-Weinberg Equilibrium (HWE) in the study groups were shown in Table 1. The *LOX-1* rs1800449 (G473A) genotypes and alleles were found consistent to HWE (Table 1).

Table 1: Allele and genotype frequencies of study groups

LOX-1 rs1800449 C>T (G473A)	Study Groups	
	Smoking (n=20)	Non-smoking (n=202)
Genotypes		
CC	15 (75%)	139 (68.8%)
CT	5 (25%)	58 (28.7%)
TT	0 (0%)	5 (2.5%)
HWE	*p*>0.05	*p*>0.05
Alleles		
C	20 (100%)	197 (97.5%)
T	5 (25%)	63 (31.2%)

Values are given as number of samples and percentage (%) in the table. Inter-group comparison of importance

level was analyzed by Chi-square test. n: number of samples, HWE: Hardy-Weinberg Equilibrium.

Comparison of BMD values and biochemical features of the study groups between T allele and CC genotype carriers were given at Table 2. No significant differences between the smoking and non-smoking groups were found in terms of mean age, age of menopause and BMI (*p*>0.05).

Table 2. Comparison of BMD values and biochemical features of the study population between T allele and CC genotype carriers in the study groups

	Smoking (n=20)	Non-smoking (n=202)
Age		
CC genotype	52.87±6.55 (n=15)	59.42±7.54 (n=139)
T allele	55.80±5.21 (n=5)	59.41±8.10 (n=63)
Age of menopause		
CC genotype	46.33±2.01	46.69±5.62
T allele	41.5±7.93	47.09±4.24
BMI (kg/m^2)		
CC genotype	27.37±5.73	30.67±4.89
T allele	22.59±2.46	30.37±4.72
Neck BMD (gr/cm^2)		
CC genotype	0.83±0.09	0.85±0.11
T allele	0.73±0.07	0.85±0.10
Upper Femoral Neck BMD (gr/cm^2)		
CC genotype	0.68±0.09	0.70±0.11
T allele	**0.57±0.08** [1]	0.70±0.10
Lower Femoral Neck BMD (gr/cm^2)		
CC genotype	1.01±0.09	0.99±0.12
T allele	**0.89±0.07** [2]	0.99±0.11
Thoracanter BMD (gr/cm^2)		
CC genotype	0.70±0.10	0.74±0.10
T allele	**0.59±0.09** [3]	0.75±0.09
Ward's triangle (gr/cm^2)		
CC genotype	0.66±0.09	0.68±0.13
T allele	**0.54±0.07** [4]	0.68±0.12
Total BMD (gr/cm^2)		
CC genotype	0.88±0.11	0.91±0.12
T allele	**0.77±0.06** [5]	0.91±0.11

Statistical analysis were performed by Mann-Whitney U test. Age, BMI, age of menopause values are given as mean±standart deviation (X±SD). Bold values of *p* indicates statistical significance. BMI, body mass index; BMD, bone mineral density; n, number of samples. 1 *p*=0.046; 2 *p*=0.046; 3 p=0.019; 4 *p*=0.008; 5 *p*=0.042

When we assessed whether smoking and having T allele of *LOX-1* rs1800449 (G473A) polymorphism have any effect on BMD values in Turkish postmenopausal women, upper femoral neck (T allele: 0.57±0.08 vs. CC genotype: 0.68±0.09, *p*=0.046), lower femoral neck (T allele: 0.89±0.07 vs. CC genotype: 1.01±0.09, *p*=0.046), thoracanter (T allele: 0.59±0.09 vs. CC genotype: 0.70±0.10, *p*=0.019), Ward's triangle (T allele:0.54±0.07 vs. CC genotype: 0.66±0.09, *p*=0.008) and total BMD (T allele: 0.77±0.06 vs. CC genotype: 0.88±0.11, *p*=0.042) values were found lower in T allele carriers of *LOX-1* rs1800449 polymorphism comparing with CC genotype in smoking postmenopausal women. However, this association was not observed in nonsmoking postmenopausal women (*p*>0.05).

Discussion

Several studies have been conducted to investigate the effect of the LOX G473A polymorphism in cancers such as lung, ovary, breast, *etc*. [7-9] and diseases such as coronary artery disease [10]. Wang *et al.* demonstrated that individuals with LOX473AA exhibited a higher susceptibility to lung, colon-rectum, colon, and rectum cancers in comparison to GG genotype carriers [8]. In the study of Yang *et al.*, *LOX-1* G473A polymorphism was observed significantly associated with ovarian cancer risk (AA>GG>GA, *p*=0.0062) [9]. Ma *et al.* reported that LOX473AA genotype and A allele were significantly higher in patients with coronary artery disease (CAD) than controls (*p*= 0.002 vs *p*=0.001) [10].

Krall and Dawson-Hughes showed that smokers had lower BMD values than nonsmokers [11]. Smoking leads to changes in Parathyroid hormone (PTH) [12] and vitamin D [13] levels which are key factors in Ca homeostasis and bone metabolism. Smoking was found to be associated with menopause and decreased levels of BMD [14]. In a study of Ko *et al.*,

smoking sera given cells showed inhibition of osteo-blast differentiation and bone formation whereas demonstrated an increase in osteoclast differentiation [17].

In our study, upper femoral neck (*p*=0.046), lower femoral neck (*p*=0.046), thoracanter (*p*=0.019), Ward's triangle (*p*=0.008) and total BMD (*p*=0.042) values were found lower in *LOX-1* rs1800449 T allele carriers than smoking postmenopausal women with CC genotype whereas this association was not observed in nonsmoking group (*p*>0.05). So, we propose that smoking *LOX-1* rs1800449 T allele carriers are more susceptible to osteoporosis than postmenopausal women with CC genotype in Turkish population.

The main limitation of our study is that it includes relatively small study groups. So, further studies are required to demonstrate the association of smoking and *LOX-1* rs1800449 (G473A) polymorphism with osteoporosis risk.

Conflict of Interest

Authors declare that no competing interests exist.

Acknowledgments

Key Messages

➢ Smoking is one of the risk factors for osteoporosis development and found to have effects on BMD levels in previous studies.

➢ Smoking in conjunction with *LOX-1* (G473A) polymorphism was shown to have a negative effect on bone volume in mice studies.

➢ In our study, we found that smoking was associated with lower BMD values and a risk factor of osteoporosis development in T-allele carriers of *LOX-1* (G473A) polymorphism comparing to individuals with

CC genotype in Turkish postmenopausal women.

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OP11

Nutritional disorders and its effects on society

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Introduction

Nutrition is the acquisition and effective use of nutrients from outside in order to carry out all vital functions, especially growth and development in living organisms, and to maintain a healthy life [1]. The aim of this study is to draw attention to the importance and solutions of common nutritional disorders in the community. For this purpose, the previous researches are presented in comparison with the scientific literature. Proper and adequate nutrition is vital for public health. Our researches related to nutritional deficiency and insufficiency were evaluated in the light of related scientific literature.

In human studies, it has been observed that child mortality rate may increase up to ten times in undernourished populations. In Japan, the average height was 138 cm first, fell to 136 cm in 12 years old children when the feeding of the children was disrupted during the war years. After the year 1950, the average height of children increased to 142 cm with the addition of good quality supplements in 12 years old children. In developed countries, data from nutritional science have been utilized and many important and common diseases such as rickets, pallegra, scabies, iron deficiency anemia and iodine deficiency-related thyroid disorders can be prevented. However, in developed countries, as a result of malnutrition, obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular diseases and some types of cancer have occured. The most important preventable cause for a significant number of cardiovascular diseases and cancer types, which are the most common causes of death, are improper and unbalanced nutrition. According to the World Health Organization report in 2006; In developing countries, one third of people are affected by vitamin and mineral deficiencies and therefore the incidence of growth retardation, infection and congenital disorders increases. Vitamin A deficiency is responsible for 18% of maternal deaths, iron deficiency is responsible for 18% of maternal deaths during pregnancy and zinc deficiency is responsible for 5.5% of child deaths. Inadequate and unbalanced nutrition suppresses the immune system and increases the incidence of the disease, leading to more severe diseases. Malnutrition of workers leads to loss of labor and an increase in occupational accidents. Although animal husbandry is widespread in our country, it is difficult to reach animal foods as meat and milk yield is insufficient. As a result of the imbalances in income distribution and economic problems, significant portion of the population prefers the cheapest foods instead of adequate and balanced nutrition. In addition, nutritional disorders increase because of the lack of education, prejudices and wrong information and lack of hygiene about nutrition in our country. As a result of insufficiencies in infant-child nutrition especially in pregnancy and in the first year of child life, mental and physical developmental disabilities are impossible to compensate. For these reasons, it is essential that all individuals are provided with adequate and balanced nutrition in order to achieve normal growth and development, and to reach a society of healthy and mentally competent individuals [1, 2].

Vitamin D levels influence the risk of fracture, rickets, osteomalacia, and osteoporosis. Vitamin D protects the body against muscle weakness, helps regulate the heartbeat, strengthens the immune system and thyroid function, and is necessary for normal blood clotting. Vitamin D increases calcium absorption from the digestive tract, helps the accumulation of calcium in the bones and also accelerates the active transport of calcium. Humans obtain vitamin D from get sunlight and from diet [3].

According to the results of our previous study on vitamin D deficiency (12,920 male and female patients were included in the study); There were significant differences between 25 OH vitamin D levels of patients in the winter season and the spring and summer seasons (<0.05). 25 OH vitamin D levels of men were significantly higher than women (<0.05). 25 OH vitamin D levels were low in 72.48% of all patients (<20 ng/ml). The ratio of 25 OH vitamin D levels less than 10 ng/ml was found in 40.92% of the patients. When assessing 25 OH vitamin D levels, the season of the year and sex of the patients should be taken into consideration. There is a significant deficiency of vitamin D in Turkish society [3].

Zinc is the second most important trace element after iron, which is essential for human health. Daily zinc requirement is up to 15 mg. Nutrition is the most common cause of zinc deficiency worldwide. Zinc deficiency can be seen in old age, pregnancy, breastfeeding and alcoholism. Zinc deficiency slows growth and skeletal development, leads to testicular atrophy and wound healing and deterioration of taste perception. Acrodermatatis enteropatica, bullous / pustular dermatitis, erosive dermatitis, keratosis, atrophy, decubitus ulcer, difficulty in wound healing, recurrent oral aphthae, skin findings including atypical rashes and hair loss can also be observed in zinc deficiency [4].

Oxidative stress increases continuously in human metabolism due to exogenous and endogenous reasons. The body's antioxidant systems should prevent this increase in oxidative stress. If oxidative stress increase cannot be prevented as a result of lack of activity of antioxidant system, more than 100 important diseases occur. Proper and adequate nutrition is one of the most important factors that regulate the antioxidant system and prevent the increase of oxidative stress, preventing the formation of many important diseases in this way. According to our previous study, reducing the frequency of meals and moderately restricting calorie intake increases the activity of the antioxidant system and reduces oxidative stress [5].

Water deficiency: In terms of human biochemistry, many reactions and maintenance of metabolic pathways require water. In terms of nutritional biochemistry, digestion, absorption, transport of blood, metabolism in related organs and organelles and transportation of the products to the required places are possible thanks to water. In addition, water production is provided as a result of reactions that occur during the metabolism of foods. The cell, which is the smallest living unit, depends on water to remain alive, healthy and functional. Water intake more or less than necessary causes various diseases. Therefore, the changing water needs of each individual should be determined correctly and intake should be made accordingly. Another important situation is that if water is contaminated, it can carry many infectious diseases and spread rapidly to the society. Some of these diseases can be fatal. Therefore, the cleaning of drinking water should be paid attention. Body cleaning is possible with water. Another problem that arises in individuals who cannot reach sufficient amount of clean water is serious infectious diseases due to insufficient hygiene. For these reasons, clean and adequate water supply is essential for public health [6].

Conclusion

Malnutrition is one of the biggest hazards to public health. Common nutritional deficiencies should be investigated and prevented. Then, prophylactic replacement should be performed for the general population for common nutritional deficiencies. In addition, education should be organized for the adoption of the right information about adequate and proper nutrition to the society. Proper nutrition, if taken as a lifestyle and made continuous, combined with mobile life and regular, simple exercises will play very important role in the prevention of obesity and all obesity-related chronic diseases. Especially vitamin deficiencies, zinc and iron deficiencies should be followed [7].

Key messages

1. Vitamin D deficiency is common in society and vitamin D protects the body against muscle weakness, helps regulate the heartbeat, strengthens the immune system

and thyroid function, and is necessary for normal blood clotting.

2. Zinc deficiency is common in society and slows growth and skeletal development, leads to testicular atrophy and wound healing and deterioration of taste perception.

3. Contrary to popular opinion, the frequency of meals should be reduced. Reducing the frequency of meals and moderately restricting calorie intake increases the activity of the antioxidant system and reduces oxidative stress.

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OP12

A trial of bone tissue deselurization

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Introduction

In the case of bone tissue loss due to various reasons such as accident, injury, tumor, aging, lack of teeth or congenital deficiency, bone analogs are needed to replace the bone graft taken from the patient. The animal-derived decellularized bone matrix is one of the bone analogues used for this purpose. Synthetic bone analogs are not yet as successful as those of biological origin. Bone analogs of biological origin are used by removing cellular elements to which the transplanted human immune system reacts by accepting them as foreigners by making them antigen free. Today there are many commercial preparations of these products in the world. However, they show quality differences due to differences in preparation protocols. Undoubtedly, the most important factor determining quality is that it is comparable to autogenous bone graft in the formation of bone tissue. In addition, there are some other important factors for quality of bone anologs: similarity to autogenous bone strength, stiffness, easy to cut and shape with saws, can be fixed by methods such as screw wire, durability to the screw, drilling holes, strike wedges to compress. The indispensable condition is that all the pathogenic agents are removed and the product is available in a sterile manner. Musculoskeletal defects have

difficulties for Plastic Surgeons and Orthopedists. It is reported that bone tissue is the most common used tissue of various transplantable tissue types in Plastic Surgery and Orthopedics applications. Bone tissue is shown as the only tissue where restructuring and remodelling events play a role in the healing process rather than scar tissue formation. In bone graft applications, it is reported that the recipient region and graft material are related. Fibroblastic celling in the recipient region and osteogenic quality of the graft are of high importance. The most necessary feature of the ideal graft material is to increase the amount of bone in the grafted area and after that to be replaced with natural bone. In this way the initial stability is increased and resorbs over time. The graft material should be biocompatible, non-toxic, antigenic and non-carcinogenic, easy to obtain, low cost and resistant to infection. Nowadays, autogenous bone graft is the most commonly used and most advantageous bone graft material. It provides optimal harmony in soft tissue and / or bone graft usage in tissue reconstructions in Plastic Surgery and Orthopedics. It should be osteogenic, osteoinductive and osteoconductive properties and it contains a large number of living cells and rich in growth factors and does not cause an immunological reaction. Although autogenous bone grafts are accepted as the gold standard. They cannot be obtained in the desired amount, there is difficulty in shaping, the need for a second operation in the donor area, bleeding, infection, hematoma, loss of sensation of the second surgical procedure, long-term postoperative pain and limitation of motion risks. It is reported that iliac crest morbidity, which is the most common graft site, is encountered frequently and major complications are 8.6% and minor complications are 20.6%. However, as an alternative to autogenous bone grafts in bone graft applications, frozen dried bone allografts bovine bone

grafts (BBG) resorbable or non-resorbable hydroxyapatite (HA) grafts tricalcium phosphate grafts marine algal grafts the use of coral-derived grafts consisting of phosphate and calcium carbonate is increasing. Among all these, allogenic grafts, which organic content and immunological potential have been minimized, have achieved close success to autogenous bone grafts. Resorbable xenogenic graft materials, which are shown as an alternative to autogenous grafts, are frequently used in Plastic Surgery and Orthopedics applications because they allow rapid bone replacement and create a suitable scaffold for new bone formation. It was evaluated the use of animal-derived natural xenogenic graft materials with and without autogenous grafts alone. He reported that at the end of the 6-month waiting period, similar new bone formation values were obtained in both groups in histomorphometric evaluation, and that xenogenic-derived grafts indirectly contributed to new bone formation due to their bioavailability and displacement properties. That xenogenic graft materials could eliminate the difficulty of obtaining autogenous grafts as an alternative to autogenous grafts. It was evaluated the use of 4 different osteoconductive graft materials and reported that BBG is superior to allogenic and synthetic-derived bone grafts and provides high healing potential. Researchers were used for the first time in defects created on sheep tibia BBG and reported that BBG does not support the formation of new bone. John et al. (2004) reported that there is a connection between time and the amount of newly formed bone. The amount of the bone is increased with waiting time and this result was reported to be a common result in the groups used autogenic bone graft and BBG [1-3].

Materials and methods

Decellularization

At the end of preliminary trials; Freeze-thawing with acetone, ethanol mixture (25% acetone, 75% ethanol mixture) on the shaker for 26 hours and at room temperature, then on the shaker for 30 hours and treated with SDS (1% sodium dodecyl sulfate) at room temperature. The cycle was repeated 5 times. After freezing in cold liquid nitrogen, it was allowed to dissolve for 5 minutes in an oven at 56 degrees Celsius and freeze and thaw. Cell-free bone tissue matrix was thought to be obtained. Subsequently, this bone tissue matrix was treated with pH 7.0 Phosphate Buffer Saline (PBS) five times to remove residual chemicals.

There were three groups; 1.control group, 2. experiment 1, 3. experiment 2. Just after the sacrification of the bovine the bone tissue was obtained.

Control group: No treatment was performed.

Experiment 1: Before mentioned acetone, ethanol mixture and SDS processes were performed.

Experiment 2: All the processes before mentioned were performed (acetone, ethanol mixture, SDS and freeze and thaw).

Histological Examinations

For histological examinations bone samples were fixed in %10 neutral buffered formalin. 5% nitric acid was used as a decalcifying solution. Samples were then rinsed in tap water for 24 hours. The decalcifying solution was changed on a daily basis and the decalcification process was ended when the bone was easily penetrated through by a needle without any force. Subsequently, samples were washed in running tap water for 24 hours. And then bone samples were dehydrated through 60% (1h), 70% (1h), 80% (1h) 90% (1h), 95% (2h) and 100% (2h) alcohol. After clearing in two baths of xylene (2h each), the bone samples were embedded in paraffin blocks. 5 µm sections were cut

with using a microtome (Leica Germany) and placed on glass slides.

Hematoxylin and eosin staining was conducted according to routine protocols. All staining procedures were performed at room temperature. The slides were then examined and photographed using a Zeiss Axio Lab A1 microscope. Experimental procedures were shown in figure 1 below.
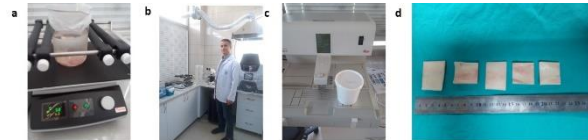

Figure 1. Experimental procedures.
(a) Exposure of bone tissue to chemical processes.
(b) Researcher and implementation of experimental procedures
(c) Histological examination of bone tissues
(d) Macroscopic view of bone tissues obtained at the end of the experiment

Results

Histological investigations indicated the precence of cells. Thus, it was found that decellularization did not ocur completely. As shown in Figure 2 and 3 cell neuclei appear dark blue in hematoxylin and eosin stained sections. Arrows shows the nuclei in these figures. Cell nuclei were examined in hematoxylin and eosin stained sections of treated and control tissues and clearly revealed the presence of osteocytes in all tissues.
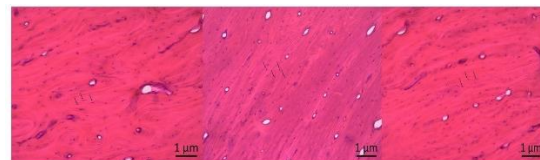

Figure 2. Representative Hematoxylin and eosin staining of bone.
(a) control tissue, (b) experiment 1, (c) experiment 2
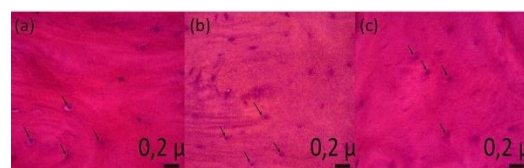(original magnification, X10)

Figure 3. Representative Hematoxylin and eosin staining of bone.
(a) control tissue, (b) experiment 1, (c) experiment 2
(original magnification, X40)

Discussion

Decellularized bone grafts has ideal charecteristics of an allograft because they have natural extracellular matriks and has no cells so they have no toxicity and antigenicity. Decellularization depends on the type of the tissue, selection of decellularizing solution, and decellularization cycle (2, 3). Tissue should allow decellularizing solution to penetrate in all part of the tissue to remove cells. Cancellous bone has large surface for chemicals to penetrate. In compact bone decellularizing solution penetrate from haversian, Volkmann's canal, lacunar and canalicular spaces. This can cause slower decellularization process, and times of using chemicals should be longer than we use, like Dong Joon Lee et al. (2016) did (3). There are a lot of methods to obtain decellularized bone, investigations should continue to have best protocol (4, 5).

In the future investigations if the process time will be short cancellous bone could be used. If the compact bone will be used then the process time should be longer.

Key Messages

1. The animal-derived decellularized bone matrix is one of the bone analogues used as bone grafts.

2. Bovine bone grafts are superior to allogenic and synthetic-derived bone grafts and provides high healing potential.

3. In the future investigations if the process time will be short cancellous bone could be used. If the compact bone will be used then the process time should be longer.

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OP13

B Cell Immunophenotyping and Expression Analysis of B Cell Specific Molecules of Patients with Benign Multiple Sclerosis

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Introduction

Multiple sclerosis is an autoimmune, progressive disease of the CNS with progressive demyelination, inflammation and axonal degeneration [1]. Although the pathogenesis of the disease is not known exactly, it is thought that autoreactive lymphocytes migrate to the CNS through the blood brain barrier and initiate

the inflammation process [2]. Clinical subtypes of MS; relapsing remitting MS (RRMS), primary progressive (PPMS), secondary progressive (SPMS), progressive-recurrent MS (PRMS) and benign MS (BMS) [3]. BMS is a retrospective diagnosis characterized by sparse attacks without serious sequelae and low lesion burden on MRI. Fifteen years after the onset of the disease, people with EDSS scores ≤ 3 are considered BMS [3,4]. Although relatively slow progression is observed in the somatic neurological findings of BMS patients, other nervous system functions may be severely impaired [5]. T lymphocytes have played a major role in MS immunopathogenesis until recently. Autoreactive T lymphocytes and antibodies that develop against CNS elements in MS cases play a role in the formation of tissue lesion and inflammation, and T lymphocytes react to myelin by causing demyelination [6,7]. However, recent studies also have shown the importance of B cells in the pathogenesis of MS. There is evidence that B cells have different effects other than antibody production, such as antigen uptake and presentation, stimulation of T lymphocytes, cytokinin, chemokinin, and neurotropic factors. B cell subtypes are; plasma cells, plasmablast, naive, memory cells, follicular and regulatory B cells. In MS, plasmablasts that mature pass to the periphery and migrate to brain tissue with inflammation. In this case, plasmablast and plasma cell production in lymphoid tissue is increased or memory cells in brain tissue start to produce pathogenic antibodies [6]. The fact that monoclonal antibody-based treatment methods targeting B cells are effective in stopping the progression of the disease suggested that B cells also play a role in the development of disability [8,9]. Cognitive impairment is frequently seen in multiple sclerosis (MS) and affects 70% of patients. Cognitive functions are affected in the late and early stages of the disease (including clinically isolated syndrome),

and impairments in the course of the disease may occur. There are many studies showing that cognitive findings deteriorate in BMS cases [5].

In light of these informations, the aim of our study was to determine the importance of B-cell immunophenotyping in benign MS(BMS) and the importance of B-cell-related genes in expression analysis and the possible relationship of MS subgroups to cognitive processes.

Materials and Methods

Study Groups

The patients diagnosed with Multiple Sclerosis (n=36) according to the McDonald Criteria who were between 18-55 years of age included in this study and 38 age/sex-matched healthy individuals were included as controls. The MS group consisted of 20 patients with BMS and 16 with non-BMS. Individuals with other coexisting neurologic or systemic disorders were excluded.

Immunophenotyping

In order to obtain standard conditions, peripheral blood mononuclear cells (PBMCs) were separated through Ficoll density gradient centrifugation. Cells were resuspended in freezing solution and stored in liquid nitrogen. Frozen PBMCs were thawed and washed in complete medium (enriched with 10% fetal bovin serum, 1% NonEssential amino acids, 1% L-Glutamine, 1% Na-Pyruvate, 1% MEM Vitamin, 1% Penicillin-Streptomycin). Cells were stained with anti-human monoclonal CD19-APC, CD27-FITC, IgD-APC/Cy7, CD138-PE, CD24-PerCP, and CD38-Alexa fluor 700 (Biolegend) conjugates for 30 min at 4 °C, then washed with phosphate-buffered saline (PBS) and resuspended in PBS, and 6 color immunofluorescence staining was performed (BD FACS Aria II). Data were analyzed using the FlowJo software.

RT-PCR Studies

RNA isolation from PBMC was performed according to the instructions of the QIAGEN RNeasy Mini kit (Hilden, Germany). The quality and quantity analysises of the obtained RNA were evaluated spectrophotometrically. For the purity of RNA, the OD value at 260 nm / OD value at 280 nm between 1.9-2.1 were included in the study. Reverse transcription was performed to convert single-stranded and unstable RNA into double-stranded and stable cDNA. All procedures were performed on ice and in accordance with the instructions of the Transcriptor First Strand cDNA Synthesis Kit (Basel, Switzerland).RT-PCR reactions were performed in LightCycler instrument and in accordance with the instructions of the Fast Start DNA Master SYBR Green I kit (Roche, Basel, Switzerland). The primers specific for the genes of B cell subtypes were designed using a compatible programme. Amplification curves and melting peaks of selected dilutions were evaluated to determine the concentration of 600-800 nM (1 µl) reverse and forward primer sequences specific for the gene of each B cell subtype. Samples were studied in duplicate for all genes. As the reference gene for cDNAs, the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, which can be expressed equally in each tissue and responsible for basic cellular functions, was used.

Cognitive Tests

Participants were evaluated by cognitive test contains subtests, which assess MS specific impairments in cognitive functions, such as verbal memory acquisition (Selective Reminding Test, SRT), visual memory acquisition (Spatial Recall Test,SPART), sustained attention and speed of information processing [Paced Auditory Serial Addition Test (PASAT) and Symbol Digit Modalities Test (SDMT)] and verbal fluency and categorical reasoning (Controlled Oral Word Association Test, COWAT) (Table 2). In addition, Beck Depression Inventory (BDI) was administered to evaluate mood status and 9-hole peg and timed 25-foot walk tests were administered to evaluate motor functions.

Statistical Analysis

Demographic and clinical features of the participants were compared using the chi-square test, study t test, analysis of varience (ANOVA). Cell Quest (BD) and FlowJo analysis programs were used for data analysis to determine the peripheral blood cell subgroups. Total cells and B cell subset frequencies were compared using ANOVA and Tukey's post-hoc test in multiple group comparisons and Student's t-test in two-group comparisons. For the relative quantification of target genes, ANOVA and nonparametric Mann Whitney U test were used for analysis of variance between groups. CT values of target genes $2-\Delta\Delta CT$ ($\Delta\Delta CT$ = target gene CT - housekeeping gene CT) formula was used for relative quantification according to GAPDH housekeeping gene. Statistical significance was defined as p value less than 0.05 and analyzes were performed with SPSS 15.0 program. GraphPad Prism 5 program was used for graphics.

Results

Clinical and Demographic Features

The clinical and demographic data of the study group are shown in Table 1.

Table 1: Clinical and demographic data

	BMS (n=20)	Non Benign MS (n=16)	Healty Control (n=28)	P value
Sex (F/M)	17/3	10/6	18/10	0,221
Age (years)	40,5±9,54	47,1±8,4	39,3±8,7	**0,024**
Age at disease onset (years)	25,3±8,39	32,4±9,36		**0,022**
Disease duration (years)	13,5±4,35	14,1±4,12		0,69
EDSS score	2,42±0,52	5,1±0,95		**<0,0001**
Total Attack Scores	7,75±4,33	7,3±4,25		0,76

Immunphenotyping

Distribution of Peripheral Blood B, T and Natural Killer Cells

When PBMCs of all subjects were evaluated; the percentage of CD19 expressing B cells was not found different between the groups but CD3 + T cell (p = 0.0019) and CD3-CD16 + CD56 + natural killer (NK) cell (p = 0.0349) groups were found different. CD3 + T cells were significantly lower in both BMS group (p <0.01) and non-benign MS group (p <0.05) compared to healthy subjects. When NK cells were evaluated, it was determinated that these cells were significantly higher in the non-benign group than the healthy ones (p <0.05).

Distribution of Peripheral Blood B Cell Immunophenotypes Between Groups

In immature (CD19 IgD-CD27-) subgroup of immature B cells that did not encounter antigen, there was no difference between the study groups, whereas the percentage of naïve (CD19 IgD CD27-) cells was higher in the BMS group compared to healthy subjects (p <0.05). In contrast, memory B cells showed significant changes in the opposite direction. Unswitched memory B cells (CD19 IgD CD27) were found to be higher in benign group than healthy subjects (p <0.01). The switched (CD19 IgD-CD27) (p = 0.0525) memory B cells were found to be lower in both patient groups compared to healthy subjects (p <0.05). However, there was no difference between the MS subgroups. Plasmablasts (CD19 CD38 CD138-), which are antibody-producing B cell precursors, had similar percentages in the groups. However, it was observed that antibody-producing B cells, plasma cells,(CD19 CD38 CD138) tended to be lower in the BMS group than non-benign group. In the regulatory B cells (Breg, CD19 CD24 CD38) which had the feature of immune suppressor cell, a significant increase was detected in the BMS group compared to healthy subjects (p <0.05).

Validation of Genes by RT-PCR

Expression analysis of the ATP1B3, BANK1 and BLK genes related to B cell subtypes and TGFB1 and SWAP70 genes related to T cell subtypes were performed. Among the cases, the most significant expression change was determined only in the BANK 1 gene (p = 0.0304). However, no difference was observed when binary comparisons were made.

Cognitive Analysis

It was observed that motor processes in BMS were better protected than non-benign MS. When the cognitive parameters were evaluated, it was found that benign MS cases performed worse in all cognitive functions than the healthy subjects and there was no significant difference between the benign and non-benign MS groups. Beck depression inventory scores were also lower in benign and non-benign MS groups compared to healthy subjects.

Correlation Analysis

There was no correlation between gene expression levels and demographic data, B cell subtype rates and gene expression levels. But inverse correlations were determined between BANK1 and executive functions tests(respectively p=0,028 R=-0,439 ve p=0,0086 R=-0,51).

Discussion

Multiple sclerosis is an autoimmune, progressive disease of the CNS with progressive demyelination, inflammation and axonal degeneration [1]. In addition, B-cells, antibody-producing plasma cells and anti-neuronal antibodies have been reported to play an important role, but B-cells have been reported to be effective in the pathogenesis of the disease [6]. The most common B cell subtype found in MS plaques is a long-lasting plasma cell [10].

As a result of immunophenotyping studies, unswitched and switched memory cells of effector B cells- which have an important role in the pathogenesis of autoimmune diseases due to their pro-inflammatory properties- were suppressed in MS groups. Among these, unswitched memory B cells were lower in benign MS patients than non-benign ones. Plasma cells, another effector B cell group, likewise tended to be low in benign MS cases. Regulatory B lymphocytes with immunosuppressive properties were significantly higher in the benign MS group compared to the other two study groups. These findings suggest that one of the factors that may cause MS to remain at a low level of disability over a long period of illness may be the change in inflammatory memory B and anti-inflammatory regulatory B cell ratios. In addition, this study showed that mechanisms controlling B cell activity suppress MS progression, thus identifying a mechanism that has not been previously described in the literature. Consistent with the results of our study, another study found that the risk of developing MS was low in patients with clinically isolated syndrome with low memory B lymphocyte ratios in peripheral blood [11]. Thus, B cell subtypes have been shown to play a role in preventing the progression of the disease in both early and advanced stages of MS. As an important finding, suppression of T cells and some B cell subtypes was found in benign and non-benign MS cases compared to healthy volunteers. It is possible that this finding is due to the effect of immunomodulatory therapy, and suppression of memory B cells may be a treatment side effect. In this case, the higher level of suppression of memory B cell observed in benign MS may be due to the stronger and more effective response of immunomodulatory therapy for this MS subtype.

Real-time PCR analysis showed no significant difference between groups due to the low number of cases. However, similar to phenotyping studies, TGFβ which is an anti-inflammatory cytokine, expression was found to tended to increase in BMS. The association of this cytokine with regulatory B lymphocyte levels is also known [12,13]. However, there is no study on the effect of TGFβ in benign MS. It is possible that high regulatory B in benign MS is one of the factors that determine low memory B cell ratios. Another interesting feature of our study is related to cognitive tests. It was shown that somatic neurological findings (motor, sensory, vision, balance) are preserved in BMS, but cognitive and limbic networks are affected. In addition, the lack of correlation between B cell subtype rates and B cell gene expression levels and cognitive test scores suggest that B cells play a role mostly in the progression of physical disability, but different factors are effective in the progression of cognitive findings.

One of the limitations in this study, is the use of total peripheral blood mononuclear cells instead of isolated B cells. In future, during immunophenotyping, addition of surface markers and intracellular cytokines will contribute to the identification of different immunological mechanisms.

Complience with Ethical Standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethical Committee (Project Number

2018/476) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Key Messages

- In this study, benign MS cases considered to be a good prognostic type of MS because of their low disability despite long disease duration were studied.

- Memory and plasma B cells were found to be low(p<0,05) but regulatory B cells were found to be high(p<0,05) in BMS. This study showed that mechanisms controlling B cell activity suppress MS progression, thus identifying a mechanism that has not been previously described in the literature.

- According to cognitive test results, definition of BMS is a deceptive diagnosis.

- One of the limitations in this study, is the use of total peripheral blood mononuclear cells instead of isolated B cells.

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OP14

Investigation of Changes in Memory Protein Expression Levels of MS Patients Due to Cognitive Rehabilitation

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Introduction

Multiple Sclerosis (MS) is defined as a progressive, inflammatory, demyelinating neurodegenerative and autoimmune disease that occurs in the central nervous system (CNS). It is one of the most common neurological diseases in young adults and it is estimated that at least 2.3 million people worldwide have MS[1].

It occurs more often between the ages of 20-40 and is more common in women[2] and the female/male ratio is 2.6[1]. It is known that genetic and environmental factor interactions play a role and increase the risk of disease. As autoimmune factors; abnormal values observed in immunological parameters have been observed in the majority of individuals with MS. The prevalence of familial MS is approximately 13% for all phenotypes, and the risk of recurrence in the family increases with a percentage of genetic sharing[2]. The degree of inheritance is polygenic and contains polymorphisms in several genes, each of which is associated with a small increase in disease risk, among which the polymorphisms in human leukocyte antigen (HLA) class I and class II genes carry the highest MS risk[2].

Environmental factors include vitamin D and sun exposure, infectious agents and viruses, microbiota and smoking and stress, and are known to play a role. Today, it is known that cognitive impairment is seen in 70% of MS patients[3]. Deficiencies in information processing speed represent the most common cognitive deficit in MS[4]. Other openings are observed in attention, executive functions, working memory and long-term memory[4]. In particular, impaired brain connectivity between the prefrontal lobe and the amygdala brain circuits that are important for regulating emotions has been observed in MS patients[5]. Pharmacological treatments have been investigated as curative treatments for cognitive dysfunction in people with MS. In addition to these treatments, non-pharmacological interventions such as cognitive rehabilitation (CR) also play a role. CR techniques are designed to restore strategies or develop strategies to compensate for cognitive dysfunction. Neuro-behavioral interventions using cognitive rehabilitation have had positive effects on cognitive performance and other related skills of MS patients, and in some cases have managed to extend these positive effects to the ability of an MS individual to function daily life[6]. In the study conducted by Messinis et al. Using RehaCom software, a significant improvement was observed in verbal episodic and visual-spatial memory, semantic fluency, processing speed / working memory, response inhibition, attention / visuomotor scanning rate and group switching ability of MS patients after treatment[6].

The aim of this study was to evaluate the effects of rehabilitation on the disease by determining the memory protein expression in immune system cells before and after neurocognitive rehabilitation in MS

patients with RRMS form and to investigate possible changes in the immune system by comparing them with healthy individuals.

Materıals and Methods

Subjects and Sample Collection

A voluntary consent form was signed and information was given to the both MS group and healthy group individuals.

Peripheral blood samples were collected at Istanbul University, Istanbul Medicine Faculty, from the MS patients and healthy volunteers. Blood samples are taken from MS patients at the base and after the 6-month computer assisted cognitive rehabilitation.

Neuropsychological Assessment and Computer Assisted Cognitive Neurorehabilitation

All volunteers who participated in the study were evaluated by Rao's Test Battery (BRB-N), at baseline and after 6 months of computer-assisted cognitive rehabilitation. In addition to these tests, Stroop Color-Word Test was used to evaluate executive functions, Beck Depression Inventory was used to assess mood, and 9-Hole Test and 25-Foot Walk Test were used to evaluate motor functions.

Computer assisted cognitive rehabilitation (CCR) is based on the NOROSOFT Mental Exercise Program. For weekly follow-up, patients were supervised by the program's institutional interface. It was also evaluated monthly by the doctors who participated in the study. Isolation of Peripheral Blood Mononuclear Cells and

Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll density gradient centrifugation, resuspended in freezing solution and stored in liquid nitrogen.

Frozen PBMCs were thawed, washed and incubated with fluorescently labeled monoclonal antibodies Anti-human CD3-FITC, CD16 / CD56-PE, CD45-PerCP, CD19-APC (BD Mix: 342446BD MultitestTM) antibodies were

used to differentiate general cell groups. Anti-human CD19-APC, CD27-FITC, CD24-PerCP, CD38-Alexa Fluor 700, CD138-PE, IgD-APC / Cy7 (Biolegend) antibodies were used to determine B cell subsets. Cells were evaluated with 6 color flow cytometry (BD FACSAria II).

RNA Isolation and cDNA Synthesis

After immunophenotyping, the remaining cells were stored in buffer (RLT buffer) (350µl for $<5x10^6$ cells and 600µl for $5x10^6$-10^7 cells) at -80°C. RNA isolation is done by the kit's procedure (QIAGEN RNeasy Plus Mini Kit) and cDNA synthesis is done by using Roche (Transcrıptor cDNA Synthesis Kit).

Microarray

In the microarray study, all genome expression profiles were obtained by using Sureprint G3 Human Gene Expression V3 microarray (MA) system for RNA materials obtained from peripheral blood samples belonging to a total of 16 individuals including 5 RRMS, 6 Benign MS patients and 5 healthy individuals. 26083 Entrez gene was evaluated. Comparisons between phenotype groups were made in four different combinations; (1) RRMS / control, (2) benign MS / control, (3) RRMS / benign MS and (4) RRMS / benign / control. In all statistical analyzes, genes with a p value of <0.05 and a relative change in expression greater than 2-fold were filtered. As a result of all the analyzes, target genes that are compatible with our hypothesis were determined (Table 1).

Table 1. Determined target genes.

BLK	BANK1	MSLN	GLRA2	RASAL2
TGFB1	BLNK	PXDN	CCL16	ACTB
EBI3	FCRL2	GPRC5C	KCNS3	HPRT1
ATP1B3	SWAP70	CCL16	DGKB	GAPDH

Validation of Genes by Real-Time PCR

Procedures were performed on the Mx3005P qPCR System (Agilent Technologies) using the program and procedure recommended by the kit (Fast Start DNA Master SYBR Green I kit, Roche). For cDNA samples,

the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, which is expressed in equal amounts in each tissue and responsible for basic cellular functions (house-keeping gene, reference gene), was used.

Results

Clinic and Demographic Data

Clinical and demographic data of the study group are shown in Table 2.

Table 2. Clinic and demographic datas

	MS (n=45)	HC (n=28)
Sex (F/M)	26/15	14/14
Age (Year, SD)	42,64±9,6	39,3±8,7
Age of Onset (Year)	30,42±9,87	
Disease Duration (Year, SD)	11,3±5,33	
Last EDSS Score (Mean, SD)	3,29±1,38	
Total Number of Attacks (Mean, SD)	6,49±3,88	
Number of Attacks per Year (Mean, SD)	0,69±0,46	

The most important data of was that all MS cases included in the study were under an immunomodulatory treatment (inferferon-beta, glatiramer acetate or fingolimod).

Cognitive Evaluation

Generally, cognitive and motor function and verbal and visual memory scores were decreased in MS patients. After rehabilitation, improvement in executive functions (PASAT and COWAT tests) was determined. No alterative effect of rehabilitation on verbal and visual memory and motor functions was observed.

Comparison of Peripheral Blood Mononuclear Cell Phenotypes

When 20 benign and 16 non-benign MS cases and 28 healthy volunteers samples were evaluated for PBMC groups; It was found that the percentage of CD19 expressing B cells was not different between the groups and the percentage of CD3$^+$ T cells was significantly lower in the pre-rehabilitation and post-rehabilitation groups. Natural killer (NK) cell groups were determined to be different and this difference was significantly higher only in the post-rehabilitation group than healthy subjects. Rehabilitation was found

to have an effect on the NK cell percentage, although not significant. NKT cells were found to have similar percentages in the study groups.

In immature subgroup of B cells, there was no difference between the study groups, while the percentage of naïve cells was not significantly affected by rehabilitation and was significantly higher than healthy group. Non-transformed memory B cells (unswitched memory B cells) were found to be lower in the pre- and post-rehabilitation groups compared to healthy subjects. The switched memory B cells were found to be significantly lower only after rehabilitation compared to the healthy group. In the subgroup of regulatory B cells (Breg) which had the feature of immunosuppressive cells, it was found that there was an increase after rehabilitation and this was different from the healthy group.

Validation and Expression Results of Candidate Genes Determined by Microarray Analysis

Expression levels of the genes determined by the evaluation of microarray data were evaluated in total PBMCs in blood samples taken from MS patients before and after computer assisted cognitive rehabilitation.

BLK, BANK1, SWAP70, FCRL2 gene expressions were significantly lower in patient groups than healthy donors. TGFB1 and ATP1B3 gene expression is not different between the study groups, after computer rehabilitation significantly increased expression can be interpreted as the shift of cell activity in the direction of immune suppression. There was no correlation between the percentages of B cell subgroups, neuropsychological test scores and gene expression levels during the paired analyzes.

Dıscussion

In our study, a decrease in motor functions and verbal and visual memory were detected in MS patients in accordance with the literature. After computerized

rehabilitation, improvement in executive functions, PASAT and COWAT tests, were determined, These are methods to evaluate executive functions such as complex attention, information processing speed, verbal fluency, and categorical reasoning. It is possible that the executive functions responsible for cognitive networks including the frontal lobe show improvement in the foreground and these anatomic regions may be associated with higher neuronal plasticity capacity. Alternatively, these regions may be less affected in our study cases and therefore have cognitive reserves that can benefit more easily from rehabilitation[8 ,9].

Studies have shown an increase in proinflammatory cytokine levels in patients with depression[9]. Moreira and colleagues' study of cognitive behavioral therapy (CBT) showed that CBT is effective in the treatment of depressive symptoms and reduces TNF-α and IL-6 concentrations in young adults[10]. These findings suggest that methods that do not include anti-inflammatory drug therapy and physical rehabilitation and affect cognition may also be useful in suppressing the pro-inflammatory phenotype that is dominant in MS patients. As expected, in our study, after neurorehabilitation, pro-inflammatory effector memory B cells decreased, anti-inflammatory regulatory B cells (Breg) increased, FCRL2 gene expression decreased in the direction of B lymphocyte proliferation, and TGFB1 and ATP1B3 genes increased. The increase in gene expression level of TGF-beta cytokine, which is known to be produced by Breg cells and which suppresses effector cells, is also consistent with these results. These findings suggest that TGF-beta is particularly important for controlling autoreactive B cells[11]. In the light of these findings, it is possible that cognitive improvement in MS patients receiving neurorehabilitation therapy is at least partly related to TGF-beta increase.

FCRL2 enhances toll-like receptor-mediated B cell proliferation, activation, and survival via the NFkB and MAPK pathways[12]. Therefore, any change in FCRL expression and density in B cells is thought to be important to compensate for BCR signaling and then B cell responses[13]. ATP1B3 is another Na-K ATPase beta subunit isoform which has been shown to suppress the active B lymphocytes and the inflammatory NFkB pathway[14]. After neurorehabilitation, it was found that TGF-beta and ATP1B3, which suppress B cells, were increased and FCRL, which activated B cells, decreased in MS cases. It is possible to suggest that the B cell changes observed in the anti-inflammatory direction and the associated cognitive improvement in immunophenotyping studies are due to the expression of these B cell genes. In conclusion, in a group of MS patients whose clinical and demographic characteristics were similar, there were no changes in their disease and treatment during the study, as a result; after cognitive rehabilitation, anti-inflammatory B cells were increased, the levels of genes supporting B cell development were decreased, and the genes suppressing B cells were increased. These findings support the view that non-biological treatment methods such as cognitive rehabilitation may have molecular and cellular effects and may lead to regression in inflammation and clinical progression.

Key Messages

After 6 months of computer assisted cognitive rehabilitation, PASAT and COWAT test scores were increased.

Proinflammatory effector memory cells decreased while anti-inflammatory regulatory Breg cells increased.

In patients; gene expression level of FCRL2 was decreased after the CCR, while the expression levels of TGFB and ATP1BP were increased.

These findings support the view that non-biological treatment methods such as cognitive rehabilitation may have molecular and cellular effects and may lead to regression in inflammation and clinical progression.

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OP15

Immunophenotyping and investigation of antigenic responses of peripheral blood cells in glycine receptor antibody positive Cryptogenic Focal Epilepsy

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INTRODUCTION

Epilepsy is a common neurological disease that affects more than 50 million people worldwide, characterized by seizures, where the brain becomes susceptible to spontaneous seizures[1], [2]. Cryptogenic epilepsy, which does not meet the classification of idiopathic and symptomatic epilepsy, refers to the class of epilepsy which is thought to have a symptomatic origin but whose cause is unknown. Cryptogenic epilepsies constitute more than 40% of adult-onset epilepsies and the pathological mechanism has not yet been elucidated[3].

It is known that glial activation occurs during epileptogenesis, where astrocytes and microglia increase during seizures. Evidence showing that molecules such as immunoglobulin G (IgG) and albumin infiltrate in the brain parenchyma indicate that patients' blood-brain barrier has deteriorated over time[4], [5]. Recent studies have shown that pro-inflammatory pathways such as interleukin-1 beta (IL-

1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and antibody-mediated mechanisms are active in epilepsy patients[6], [7]. In recent studies, the detection of specific neuronal antibodies in the serum and cerebrospinal fluids (CSF) of patients with different forms of encephalitis accompanied by seizures, led scientists to search for autoantibodies in other diseases characterized by seizures.

In studies so far, antibodies against different intracellular and cell surface receptors such as voltage-gated potassium channel (VGKC) complex, glutamate (N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)) receptors, gamma-aminobutyric acid receptors (GABAA and GABAB) and glutamic acid decarboxylase (GAD) have been identified in different forms of autoantibody-related encephalitis[4], [8], [9]. VGKC, GAD and NMDA receptor antibodies were also found in epilepsy patients[4]. Antibodies (Ab) against GAD, have also been detected in patients with progressive encephalomyelitis with rigidity and myoclonus (PERM). T-cell mediated immune response is thought to be present in PERM with stimulant-sensitive spasms, stiffness and myoclonus symptoms[10], [11]. There is evidence that plasma exchange and intravenous immunoglobulin (IVIG) treatments improve the course of the disease[11]. However, Glycine receptor (GlyR) antibodies that bind to the glycine receptor alpha 1 (GLRA1) subunit have been detected in the sera of patients with PERM disease but without GAD antibody at different stages of the disease[11], [12]. It has been shown that the levels of these antibodies decrease in parallel with the clinical improvement observed after treatment and these antibodies have negative effects on neuron functions in vitro experiments. Besides, GlyR antibody was detected in many cases of cryptogenic epilepsy, and in some of these cases, seizures were observed to

regress after immunosuppression[11]. These findings suggest that GlyR antibodies may have important role in disease development. Thus far, there is no information about the immunological features of GlyR antibodies.

This study was planned based on the assumption that the rates of antigen-specific adaptive immune cells in peripheral blood of GlyR antibody-positive subjects will differ from those without antibody. In this context, the aim of our study is to determine the ratio of the groups of cells in the peripheral blood that play a role in antigen-specific adaptive immunity, which is predicted to be important as a diagnostic marker in Cryptogenic Focal Epilepsy of unknown pathophysiology and/or thought to have a pathogenic role in the disease.

Materials and Methods

Seven GlyR-Ab positive and 15 GlyR-Ab negative Cryptogenic Focal Epilepsy patients and 25 age-sex matched healthy individuals were included in this study.

Determination of GlyR Antibody in Sera Samples of Patients

Specific autoantibodies in sera were determined by immunohistochemical analysis in glycine receptor transfected cell lines. Glycine receptor alpha1-alpha3 transfected human embryonic kidney (HEK) 293 cells were used by immunocytochemical method, autoantibodies in patient sera were tested using live cell staining.

Isolation of Peripheral Blood Mononuclear Cells and Labeling with Fluorochrome Antibodies for Flow Cytometry

PBMC were isolated with Ficoll gradient method and frozen. After all samples were collected, frozen PBMCs were thawed and incubated with fluorescent-labeled monoclonal antibodies for specific B, T cells and subgroups. Anti-human CD3-FITC, CD16/CD56-PE,

CD45-PerCP, CD19-APC (BD mix: 342446BD MultitestTM); CD19-APC, CD27-FITC, CD24-PerCP, CD38-Alexa Fluor 700, CD138-PE, IgD-APC/Cy7 (Biolegend); CD3-Alexa Fluor 700, CD4-PerCP, CD8a-PE/Dazzle, CD25-APC, CD127-PE/Cy7 (Biolegend) antibodies were used. After incubation, cells were prepared for reading by 6 color flow cytometry (BD FACSAria II).

Results

Distribution of Peripheral Blood B, T and Natural Killer Cells

PBMC populations of the patients and healthy individuals have shown that there was no significant difference between the percentages of $CD19^+$ B cell, $CD3^+$ T cell, $CD3^-CD16^+CD56^+$ natural killer (NK) cell and $CD3^+CD16^+CD56^+$NKT cell population.

Distribution of Peripheral Blood B Cell Immunophenotypes Between Groups

An insignificant increase was found in B cell subpopulations in epilepsy patients compared to healthy subjects (p=0.0862). Although there was a significant increase (p=0.0431) in naïve B cells ($CD19^+IgD^+CD27^-$), a significant decrease was found in immature B cells ($CD19^+IgD^-CD27^-$) in epilepsy patients compared to healthy donors. (p=0.0100). There was a decrease in plasmablast cells ($CD19^+CD38^+CD138^-$) in epilepsy group compared to healthy individuals (p=0.0205) and plasma cells ($CD19^+CD38^+CD138^+$) were significantly decreased in epilepsy patients (p=0.0173).

In GlyR-Ab positive, negative and healthy control subjects, there was no significant difference in general B cell distribution between the groups (p=0.2121). Immature B cells ($CD19^+IgD^-CD27^-$) showed a decrease in epilepsy patients compared to healthy donors. However, the decrease in these cells was significant in GlyR-Ab negative cases (p=0.0458), but not in the Ab positive cases (p=0.0917).

Distribution of Peripheral Blood T Cell Immunophenotypes Between Groups

When T cell subtypes were evaluated between epilepsy and healthy control groups, only CD3+CD4+CD25+ T cell group showed an increase significantly meaningful (p=0.0452). Significant difference was not found in other T cells.

While there was no difference in GlyR-Ab positive and negative cases in CD4+ T cells, there was a non-significant decrease in GlyR-Ab positive cases in CD8+ T cells. GlyR-Ab positive cases increased significantly in CD3+CD4+CD25+ T cells (p=0.0178), whereas the increase in Ab negative cases did not reach significance level (p=0.0571). CD3+CD4+CD25high Treg cells showed a significant increase in GlyR-Ab positive cases compared to both Ab negative and healthy individuals (GlyR-Ab(+) vs GlyR-Ab(-), p=0.0200) (GlyR-Ab(+) vs healthy control, p=0.0101). There was no significant difference between the other regulator T cell subgroups (Figure 4).

Discussion

The recently discovered autoantibodies (GlyR, CASPR2, GAD65, etc.) suggest that autoimmune mechanisms may be responsible for the seizure pathophysiology of systemic and neurological CNS diseases characterized by seizures[8], [13], [14]. With this study, we examined NK cell, T cell, T cell subgroups and B cell and its subgroups in Cryptogenic Focal Epilepsy patients with/and without antibodies against GlyR and healthy individuals.

In the literature, a study of patients with Limbic Encephalitis with GABA_B receptor antibody, an increase in CD138+CD19+ plasma cells was observed[15]. In our study, the increase in CD19+CD38+CD138+ plasma cells and CD19+CD38+CD138- plasmablast cells is consistent with this literature. In another study conducted in children with epilepsy, CD4+ cells and CD4+CD25+Foxp3+ Treg

cells were found to be low in patients compared to controls and it was suggested that Treg cells may have an effect on pathogenesis[16]. While this was not consistent with the increase in CD3+CD4+CD25high T cells in our study, changes in CD3+CD4+CD25+ cells were similar. In a study with TLE patients, HLA-DR, CTLA-4, CD25, CD69, IL-23R, IFN-γ, TNF-α and IL-17 expressions associated with regulatory T cells were found to be higher in CD4+ T lymphocytes compared to healthy controls[17]. These findings have been associated with the fact that these cells are chronically active[17]. Also, the results of our study support this research.

Activation of suppressively effective cytokines, such as IL-10 and TGF-β, is an important mechanism in the development of autoimmunity by Treg cells[17] and is supported by many studies that contribute to the pathogenesis of epilepsy. Differences between specific subgroups of T cells between patients with and without autoantibodies may be important in terms of their contribution to autoimmunity and should be further investigated.

In this study, immunophenotyping of immune cells and their contribution to autoantibody development were investigated. However, although the effect of cytokines produced by these cells functionally on autoantibody production, the lack of intracellular cytokine levels and supernatant cytokine levels after cell culture seems to be the limitations of the study. Although there are very few studies on immunophenotyping in antibody positive patients in the literature, our study is the first and extensive immunophenotyping study in epileptic patients with GlyR antibody. These data support that GlyR antibodies may play an important role in the pathogenesis of epilepsy by contributing to T cell-mediated active inflammation in the development of

autoimmunity and provide guidance for further studies.

Key Messages

- Regulatory T cells showed an increase in $CD3^+CD4^+CD25^+$ and $CD3^+CD4^+CD25^{high}$ T cells in GlyR-Ab positive patients.

- Plasma cells ($CD19^+CD38^+CD138^+$) and plasmablast cells ($CD19^+CD38^{++}CD138^-$) in GlyR-Ab positive cases showed a statistically insignificant decrease.

- There was no significant difference between the groups in the general population of B, T, NK and NKT cells.

- Treg cells were found to be associated with epilepsy activity.

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OP16

Long-term follow-up of the gastric adenocarcinoma patients in terms of post-operative outcomes associated with notch signal pathways and target proteins

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Introduction

Gastric cancer (GC) is currently the fourth most common malignancy in the worldwide scale [1–3]. About 95% of gastric cancers are caused by adenocarcinoma originating from the glandular cells of the stomach lining [4]. Most components of Notch signaling are strongly expressed at different levels in gastric carcinoma tissue samples and are associated with a considerable number of clinical parameters [5]. The Notch signaling presents novel therapeutic targets for therapeutic intervention in gastric adenocarcinoma (GAC); however, there is still lack of understanding of the association between molecular mechanisms of Notch signaling and the prognosis of GAC. In this long-term follow-up study, we aimed to investigate the association between Notch signaling

with its receptors and the post-operative outcomes of gastrectomy patients.

Materials and Methods

Gastric tissue samples from gastric mucosa and submucosa of 77 routine patients undergoing gastrectomy surgery in the Surgical Clinic were collected. For the control group, samples were obtained from the surrounding tissue of tumor and distant tissues which included non-tumor gastric tissues. Gastric tissues that were routinely removed by gastric surgery were used and after removal of the gastric tissue, the tumor site was identified and noted, photographed, and 1 cm^3 of tissue from three sites were placed in the fixative solutions for the following different procedures:

Light Microscopy

For light microscopic analysis, tissues fixed and fixed in 4% buffered neutral formaldehyde for 1-7 days and routine light microscopic analysis were performed for 4 days. Sections of 3-5 μm thickness were cut from the paraffin blocks and stained with Hematoxylin + Eosin and Masson. Histopathological examination and evaluation were performed in order to examine the tissue morphology, to determine the tumor site and to evaluate the diagnostic pathology of disease.

Immunohistochemistry

The expressions of four Notch receptors (Notch1, Notch2, Notch3, Notch4), two DSL ligands DLL1 and DLL3 were evaluated by immunohistochemistry (IHC). IHC staining with Streptavidin-Biotin-Peroxidase method was performed using monoclonal and polyclonal antibodies on 3-5 μm thick sections collected from paraffin blocks of three different regions of the stomach. Tissues were labeled with AEC (3-Amino-9-ethylcarbazole) Chromogen Kit for peroxidase activity. The gastric localization of positive immune labeling with the relevant antigens were determined, and changes in the expression of the

proteins and regional differences were be evaluated semi-quantitatively.

Follow-up

Four patients were lost during the follow-up for five years. Thus, remaining total number of 73 patients (54 male, 19 female) undergoing total / subtotal gastrectomy for GAC were evaluated for their postoperative histopathological findings, clinical course, metastatic status, and mortality rates. All parameters were recorded and correlated with IHC results.

Statistical Methods

Histopathological and IHC data, and follow-up results were evaluated by Kolmogorov-Smirnov test for normality of distribution and homogeneity of variance. The Kappa coefficient for compliance was targeted as 0.80. In similar studies in the literature, it was observed that the positive frequency was in the range of 0.50-0.70. The minimum number of patients for 80% Power and 95% confidence level was n = 77 (statstodo.com).

In the comparison of cut off values for quantitative values, the number of patients was calculated as 77 in the confidence interval of 95% and for the power of 80%, which could reveal the difference of 0.65 AUC versus 0.80 AUC in measuring the values.

Statistical analysis of findings GraphPad Instat ver. 3.06 (GraphPad Inc, CA, USA). All statistical calculations were evaluated at 95% confidence interval and P <0.05 was considered statistically significant. All analyzes were tested with 0.05 error margin.

Results

Seventy-three out of 77 patients completed the follow-ups. Most of the operations (60.3%) was subtotal gastrectomy (Table 1). The mean of duration of hospitalization was 7.42 days and the rate of comorbidity among patients was 90.4%.

Table 1. Demographic features of the gastric adenocarcinoma patients (n=73)

Features	Values
Age (Mean ± SEM)	61.37 ± 10.49
Gender [%]	
Male	54 [74]
Female	19 [26]
Gastrectomy type [%]	
Total	29 [39.7]
Subtotal	44 [60.3]
Duration of hospitalization (day) (Mean ± SEM)	7.42 ± 2.16
Comorbidity [%]	66 [90.4]

Clinicopathological features of patients showed that most of them (47.9%) had a tumor located in the gastric antrum with a mean size of 6.15 ± 3.47 cm (Table 2). Most of the tumors (38.4%) were invaded up to the gastric serosa (visceral periton) as determined in T4 stage. The number of metastatic lymph nodes was more than 7 in most of patients (45.2%), as determined in N3 stage. There was distance metastasis in 11.0% of the patients. The most frequent macroscopic type of tumors was ulcerovegetan (Bormann II), observed in 78.1% of patients. The most frequent adenocarcinoma type was Signet-ring cell adenocarcinoma (38.4%). 53.4% of patients had diffuse type of gastric cancer while 43.8% had intestinal type. The highest number of patients (63%) had a low-differentiated histological type of cancer (G3).

Table 2. Clinicopathological features of the gastric adenocarcinoma patients (n=73)

Features	Value
Location of tumor [%]	
Antrum	35 [47.9]
Corpus	30 [41.1]
Cardia	15 [20.5]
Fundus	3 [4.1]
Anteropyloric region	3 [4.1]
All	1 [1.4]
Size of tumor (cm) [Mean ± SD]	6.15 ± 3.47
T-stage [%]	
T1	11 [15.1]
T2	12 [16.4]
T3	22 [30.1]
T4	28 [38.4]
N-stage [%]	
N0	19 [26.0]
N1	8 [11.0]
N2	13 [17.8]

N3	33 [45.2]
M1 [%]	8 [11.0]
Macroscopic type [%]	
Bormann I (Polypoid)	3 [4.1]
Bormann II (Ulcerovegetan)	57 [78.1]
Bormann III (Ulcerative)	2 [2.7]
Bormann IV (Diffuse infiltrative)	11 [15.1]
Adenocarcinoma pattern [%]*	
Papillary	2 [2.7]
Tubular	11 [15.1]
Mucinous	18 [24.7]
Signet-ring cell	28 [38.4]
Other poorly cohesive	9 [12.3]
Mixed	1 [1.4]
Adenosquamous	2 [2.7]
Mixed adeno-neuorendocrine	2 [2.7]
Lauren grading [%]	
Intestinal	32 [43.8]
Diffuse	39 [53.4]
Histological grading [%]	
G1	8 [11.0]
G2	19 [26.0]
G3	46 [63.0]

* Hu B, El Hajj N, Sittler S, Lammert N, Barnes R, Meloni-Ehrig A. Gastric cancer: Classification, histology and application of molecular pathology. *J Gastrointest Oncol*. 2012;3(3):251–261. doi:10.3978/j.issn.2078-6891.2012.021

The immunoreactivities of Notch1, Notch2, Notch3, Notch4 increased significantly with increasing depth of invasion and lymph node metastasis (Table 3), especially in T4 stage (P<0.0001), while DLL1 and DLL3 decreased dramatically in T4 stage (P<0.0001). Only Notch4 increased significantly in the tumors with known distant metastasis while only DLL1 decreased in those tumors (P<0.05).

Table 3. Immunohistochemical scores of the gastric adenocarcinoma tissues compared with T stage

Antibody	T1 (n=11)	T2 (n=12)	T3 (n=22)	T4 (n=28)	TOTAL (n=73)	P value
Notch1	0 ± 0	16.7 ± 32.6	131.8 ± 47.7[h]	264.3 ± 55.9[a]	143.1 ± 117.0	<0.0001
Notch2	54.5 ± 121.4	104.2 ± 125.2	154.5 ± 89.9	250.0 ± 70.7[b]	167.9 ± 119.2	<0.0001
Notch3	36.4 ± 80.9	50.0 ± 36.9	106.8 ± 84.9	264.4 ± 99.9[b]	140.4 ± 121.2	<0.0001
Notch4	0 ± 0	62.5 ± 31.1	79.5 ± 54.9[d]	191.1 ± 63.9[c]	107.5 ± 87.3	<0.0001
DLL1	54.5 ± 121.4[f]	183.3 ± 38.9	100.0 ± 69.0	12.5 ± 57.1[a,f]	73.3 ± 92.8	<0.0001
DLL3	109.1 ± 151.4	200.0 ± 30.2[b]	79.5 ± 73.5	1.8 ± 9.4[s]	74.0 ± 99.0	<0.0001

[a]P<0.05, [b]P<0.01, [c]p<0.001 vs all groups; [d]P<0.05, [g]P<0.01, [e]P<0.001 vs T3 group; hP<0.05, f P<0.001 vs T2 group All values are given as Mean ± Standard deviation, compared with Kruskal-Wallis with post-test + Dunn multiple comparison test.

Follow-up results showed that 57.5% of the patients had morbidity, and 47.9% of them were rehospitalized due to post-operative complications or metastasis

(Table 4). The metastatic rate among patients was 54.8%, correlated significantly with the increasing immunoreactivities of Notch receptors and their ligands, DLL1 and DLL3. One patient died at post-operative 7th month due to sepsis. Another patient died due to multiple metastasis in both lungs and lymph nodes. The morality rate was 2.7%, correlated significantly with the increasing immunoreactivities of Notch receptors and their ligands.

Table 4. Post-operative findings of the gastric adenocarcinoma patients (n=73)

Outcome	Value n [%]
Metastasis	40 [54.8]
Morbidity	42 [57.5]
Rehospitalization	35 [47.9]
Mortality	2 [2.7]

Discussion

The expression pattern of Notch components and their distribution in different parts of the stomach is likely to have diverse roles in gastric cancer. The Notch pathway participates in the process of transformation from gastric epithelial cells to gastric pits. Additionally, Notch1, Notch3, Jagged1, Jagged2 and Hes1 are expressed primarily at the isthmus of gastric mucosa, based on immunohistochemical analysis, and are expressed significantly less in normal gastric tissue compared to gastric cancer tissues [6]. Thus, we observed the immunostainings for Notch1, Notch2, Notch3 and Notch4 mostly in the epithelial regions of tumors, compared with the surrounding tissues and distant parts.

The role of Notch1 in gastric carcinoma compared to other components of Notch signaling. Compared to the expression in healthy stomach mucosal tissue, Notch1 expression is significantly higher in gastric carcinoma and is also intimately associated with tumor

volume, differentiation grade, depth of invasion and vessel invasion, as confirmed by a tissue microarray [7], as in our study in which we also observed the Notch1 staining was correlated with depth of invasion. More importantly, the 3-year survival rate is dramatically lower in patients with higher expression of Notch1 [7]. We compared 5-year mortality rate with Notch1 severity and showed the similar results.

A study by Piazzi et al. assessed the role of Notch1 and the corresponding ligand DLL1 in gastric carcinoma shows that DLL1 is not detected in eight different types of gastric cancer cell lines [8]. In addition, upregulated expression of DLL1 has a high correlation with the initiation of Notch1 signaling, with an increased expression of Notch1 intracellular domain (NICD) and the downstream target gene Hes1. With samples from 52 patients with gastric carcinoma and 21 healthy controls, it was revealed that the expression of DLL1 is correlated with Hes1 expression. On the other hand, from an ING-GAS mouse model infected with H. pylori, it was revealed that methylation silencing of DLL1 has the ability to control Notch1 activity in gastric cancer [8]. Collectively, the DLL1-Notch1 signaling axis and the target gene Hes1 are suggested to have important roles in gastric cancer. We determined that DLL1 immunoreactivity was reduced while Notch1 immunoreactivity was elevated by increasing depth of invasion of gastric tumor, suggesting a modulatory role of DLL1 on Notch1 activity in GAC.

Another study reported that Notch1, Notch2 and Notch3 were expressed in all of the eight gastric cancer cell lines [9]. In a review by Katoh, they concluded that these above-mentioned facts indicate that canonical Notch signaling pathway to inhibit chief cell differentiation is frequently activated in gastric cancer [10]. More studies are needed to show the effect of Notch signaling pathway on the differentiation of gastric cells and even gastric cancer

stem cells. This is the first study on Notch receptors with their ligands DLL1 and DLL3 in human GAC, reporting the correlation between the metastatic and mortality rate, and Notch signaling pathways. In future, genetic alteration of genes encoding Notch signaling-associated molecules may be used as biomarkers for diagnosis and treatment of GAC. γ - Secretase inhibitors, functioning as Notch signaling inhibitors, are suggested to be applied as anti-cancer drugs for gastric cancer and colorectal cancer [10].

Key Messages

- This is the first study on Notch receptors with their ligands DLL1 and DLL3 in human GAC.

- The Notch1, Notch2, Notch3 and Notch4 expressions were mostly observed in the epithelial regions of tumors, compared with the surrounding tissues and distant parts.

- The metastatic and morality rates of GAC patients was correlated significantly with the increasing expression of Notch receptors and their ligands.

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OP17

Identification and validation of narcolepsy-spesific autoantibodies with immunoprecipation and mass spectrophotometry

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Introduction

Narcolepsy is a chronic sleep disorder characterized by abnormal transition to rapid eye movements (REM) sleep phase, increased daytime sleepiness, disturbed nighttime sleep, sleep paralysis and hypnogogic/hypnopompic hallucinations. Cataplexy, which is a sudden loss of muscle tone, may occur in most of the cases[1]. According to the 3rd edition of the International Classification of Sleep Disorders (ICSD), narcolepsy is defined as narkolepsy type 1 and type 2. In narcolepsy type 1 (NT1), cataplexy and low levels of hypocretin in cerebral spinal fluid (CSF) must be seen; whereas for narcolepsy type 2 (NT2) diagnosis, cataplexy must not exist[2]. Although the prevalence of narcolepsy with cataplexy varies in different populations, it is estimated to be 25-50 per 100,000[1].

The studies on narcolepsy have shown that hypocretin/orexin deficiency due to loss of hypocretinergic neurons is the main etiology of the disease. The hypocretin hormone produced by these neurons is thought to be responsible for regulating the sleep-wake cycle, ensuring waking and suppressing the transition to the REM sleep phase[3–7]. Genetic studies over the past 20 years have shown that narcolepsy is associated with human leukocyte antigens (HLA)-DR2 and HLA-DQ1 alleles. In particular, the presence of the DQB1*06:02 allele has been emphasized in the literature for the differential diagnosis of narcolepsy[8]. These findings, suggest that narcolepsy may have an autoimmune basis. Additionally, the detection of increased hypocretin-1 reactive total immunoglobulin (Ig) G and IgM

autoantibodies in NT1 patient sera increased the suspicion that narcolepsy was evaluated as an autoimmune disease[9, 10].

In order to have more information about the immunopathogenesis of narcolepsy which has not been fully elucidated, the aim of this study was to identify the candidate molecules specific to narcolepsy by immunoprecipitation (IP) and Liquid Chromatography Mass Spectrophotometry (LC-MS/MS) and validate the identified candidates. With the identification of possible molecules, it was aimed to make important inferences about narcolepsy as an autoimmune disease and to evaluate new therapeutic options.

Materıals and methods

Subjects

The voluntaries diagnosed with narcolepsy according to the ICSD criteria, who were between 18-55 years of age were selected[11]. Patients with concomitant neurological disease or other primary sleep disorder were excluded from the study. For healthy control group, age and gender matched volunteers were selected.

Immunoprecipitation

The human neuroblastoma cell line SH-SY5Y (ATCC, USA) was used for immunoprecipitation (IP). "P-3296 Protein G on Sepharose 4B fast flow" beads (Sigma-Aldrich, Steinheim, Germany) were used. The pre-clear procedure was applied. Then, the patient group sera were added to the cell lysates and the sera were incubated overnight at +4 °C. The pellet was saved for later in -80 °C.

Liquid Chromatography-Mass /Mass Spectrometry (LC-MS/MS)

FASP (Filter Aided Sample Preparation) protocol was applied for the extraction of proteins as the first step of the of "bottom-up shotgun" mass spectrometer process. Nano-LC pump (Thermo Scientific Dionex UltiMate 3000 RSLC, USA) as hardware for peptide separation by high performance liquid chromatography (HPLC). The eluted peptides were analyzed on a Q Exactive model mass spectrometer orbitrap device (Thermo Fisher Scientific, USA). The raw data were evaluated using the Proteome Discoverer 2.2 (Thermo Scientific, USA) program.

Candidate Protein Validation Studies

As a result of IP and LC-MS/MS analyzes, complement component 4 binding protein alpha (C4BPA) was detected as a candidate antigen in narcolepsy. The determined C4BPA protein level in serum was determined using the commercial C4BPA ELISA kit (KTE63068, Abbkine, China). 41 narcolepsy sera and 26 healthy control sera were studied. Homemade ELISA method was used for the measurement using recombinant C4BPA antigen (0.1 ml, 1.5 mg / mL, Novus NPB1-88263PEP, Novus Biologicals, USA). Serial dilutions of 1: 10000 and 1: 20000 were used for 41 sera from 19 narcolepsy cases and 19 healthy controls. Plates were coated with 0.4 µg C4BPA antigen per well. HRP-conjugated secondary antibody was used in a 1: 5000 ratio. Reading was performed at 450 nm.

Results

Demographic and Clinical Data

In this study, 42 narcolepsy cases were studied. A total of 26 healthy controls matched to the narcolepsy group in terms of age and sex were also evaluated. No statistically significant difference was found between the patients and healthy control subjects in terms of age and gender distribution. Narcolepsy subtypes; sex, sleep scores (REM sleep latency, wakefulness, N1, N2, N3, REM mean sleep latency, SOREM, ESS, PSQI), C4BPA protein and Anti-C4BPA levels were not significantly different between NT1 and NT2.

Validation of Candidate Molecule in Patients and Healthy Controls

42 narcolepsy and 26 healthy controls were evaluated in order to determine C4BPA protein level in narcolepsy patients and to compare the level of candidate protein with healthy group. C4BPA protein levels were found significantly lower in narcolepsy patients compared to healthy controls ($p = 0.0296$). For the 1: 10000 dilution ratio, the anti-C4BPA level of narcolepsy patients was found to be higher than healthy control samples ($p = 0.0615$). The difference in antibody levels of narcolepsy and healthy control groups for the 1: 20000 dilution ratio ($p = 0.0903$) was shown. The difference between the two dilution values in terms of antibody levels $p < 0.05$ was not statistically significant. Correlation analysis between C4BPA protein and C4BPA antibody levels in the study groups for 1: 10000 and 1: 20000 dilutions did not reveal a significant correlation.

Discussion

Narcolepsy is defined as a neurological sleep disorder characterized by hypocretinergic neuron degeneration in the lateral hypothalamus, characterized by clinically increased daytime sleepiness, abnormal transition to the REM sleep phase and associated with cataplexy in most cases[2, 12]. Although recent neurological investigations have increasingly focused on the role of immunity, there is not yet sufficient data to meet Witebsky's criteria for autoimmune disease for narcolepsy[13, 14].

In this study, anti-neuronal autoantibodies were first investigated in narcolepsy cases and then antigen-antibody complexes were separated. When the anti-neuronal antibody detected narcolepsy patient samples were compared with the protein-negative patients at the protein level, the alpha subunit of the complement binding protein 4 (C4BPA), known as the lectin pathway inhibitor of the complement system, was identified as candidate antigen for narcolepsy. When the levels of this candidate protein and antibody levels against this protein were compared with validation studies in healthy and narcolepsy cases, protein levels were significantly lower in narcolepsy patients compared to healthy controls, and C4BPA antibody levels in narcolepsy were relatively high, although not statistically significant.

According to these results; whether the possible antibodies in the subjects specifically bind to the immune complexes or to a specific antibody is not clear. However, the study is important to show that complement-mediated immune mechanisms play a role in the pathogenesis of narcolepsy, and that IgGs conjugated to C4BPA protein may be present in narcolepsy patients.

Although C4BP deficiency is very rare in general terms, decreased C4BPA expression in patients with obstructive sleep apnea syndrome, schizophrenia, and Neuro-Behcet have been reported[15, 16, 17, 18]. The level of C3 protein that interacts with C4BPA in the complement cascade can be further evaluated to confirm the consumption of C4BPA in narcolepsy patients.

Our study is important in the literature to show C4BPA antigen - anti-C4BPA antibody levels for narcolepsy, to perform relevant optimization studies and to investigate possible complement mediated natural immunity mechanisms in narcolepsy by IP and LC-MS/MS methods for the first time. The data provided to consider narcolepsy as an autoimmune disease need to be evaluated with further validation studies which most importantly may confirm the C4BPA protein's presence in neurons, so that new therapeutic options may be available.

Key Messages

- Complement component 4 binding protein alpha (C4BPA), which is an inhibitory protein in complement system, was detected as a candidate antigen in narcolepsy by immunoprecipitation and LC-MS/MS methods.

- C4BPA protein levels in sera were found significantly lower in narcolepsy patients compared to healthy controls (p = 0.0296).

- Narcolepsy cases seemed to have higher levels of anti-C4BPA compared to healthy subjects, but the difference was not statistically significant.

- Complement mediated autoimmune mechanisms might have an effective role in the etiopathogenesis of narcolepsy. Further validation studies are required for the detection of disease specific autoantibodies.

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OP18

Visualization of Molecular Medicine Data

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Introduction

Cancer can appear in various organs in the body. In 140 out of 184 countries, breast cancer is the most frequently diagnosed cancer of women in the world (1,2). The fifth cause of death from total cancer was breast cancer in 2012 (3).
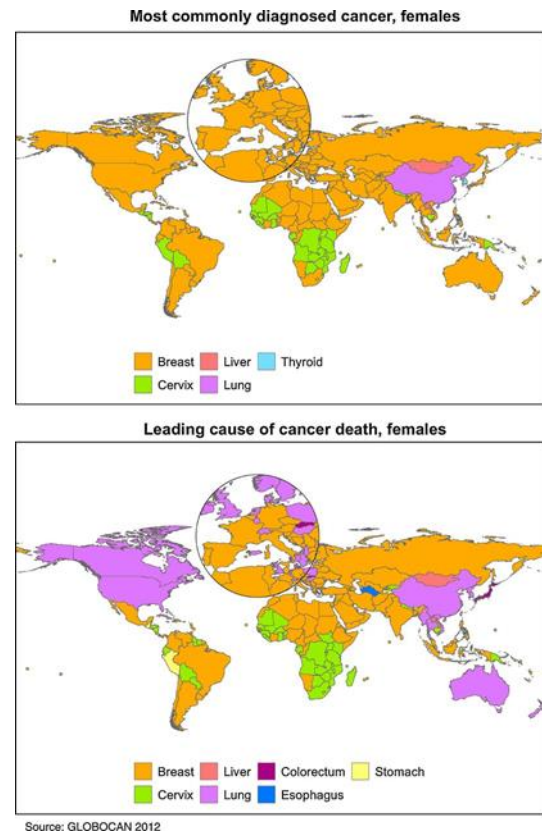

Figure 1: The major causes of cancer death among women were most frequently diagnosed in 2012.

The main purpose of this study is to visualize the breast cancer data and show that doctors (decision makers) can diagnose breast cancer with less data. By this way, we can reduce time and cost and improve quality at the point of diagnosis. The data sets are gathered from the [www.ics.uci.edu] publicly accessible machine learning database of UCI. Breast Cancer Wisconsin (Diagnostic) has 32 features, 569 instance, 2 classes with 357 benign, 212 malignant distribution (4).

I have used numpy, scikit-learn, pandas, seaborn and matplotlib library which are used with Python programming language. PyCharm IDE (Integrated development environment is used to run and debug the program.

First, I begin fundamental data analysis before creating anything like the visualization, features selection, feature extraction or classification. I used the data.head() method to see the first 5 rows. By looking the first 5 rows, we can easily see that id (identity document) cannot be a feature for classification, diagnosis is label for condition. I used:

```
drop_columns        =        ['id',        'diagnosis']
x = data.drop(drop_columns, axis=1)
```

code to drop 'id' and 'diagnosis' from dataframe.

I used:
col1=data.columns
print(col1)
code to see the columns names in dataset.
Then, I print the number of benign (357) and malignant (212).

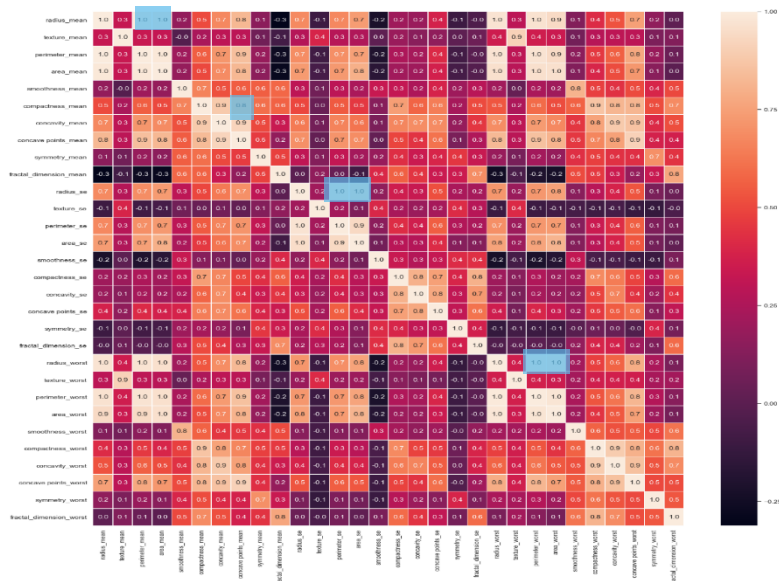The data distribution and its probability density viewed with a Violin Plot.

Figure 3: Heatmap

As we are trying to make a classification, the variables shown in blue can be used, and the ones in orange indicate that these variables cannot be used for classification. Because orange variables have similar characteristics.

Figure 2: Violin Plot

If the correlation coefficient is high between the two variables, these variables cannot be used for classification. We can easily create a heatmap using the Seaborn library in Python, a plot shows the correlation of all features.

As we are trying to make a classification, the variables shown in blue cannot be used. We have to drop these features from dataframe. 14 features dropped from dataf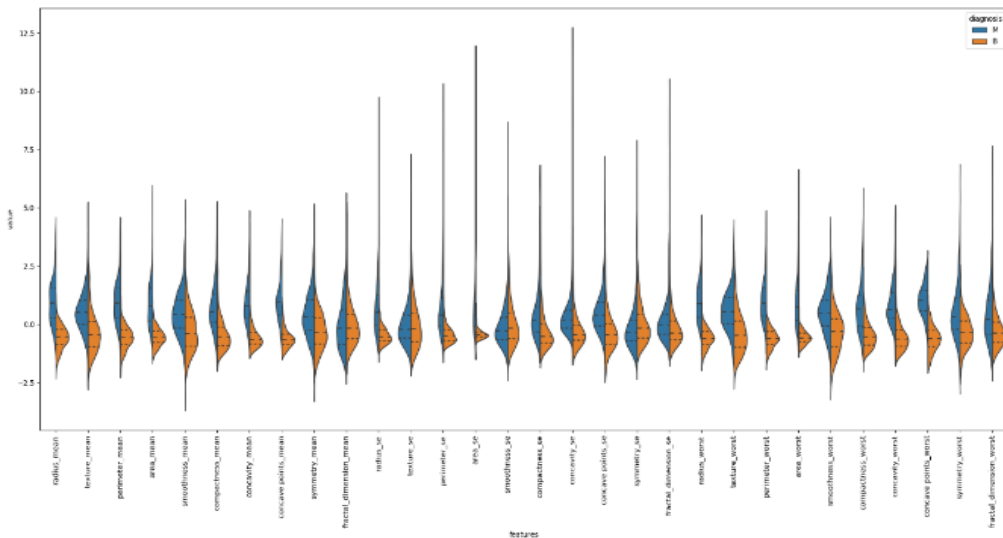rame by analyzing heatmap. After drop correlated features, we create a new heatmap, there are not more correlated features. To verify chosen features are correctly selected, I use random forest algorithm to calculate accuracy. The accuracy is calculated 0.95.

Conclusion

In the future, doctors and engineers should work together to determine whether the number of the features in diseases such as cancer can be reduced or not. This study shows that this cooperation can have valuable contributions.

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OP19

MicroRNA analysis of desidua derived mesenchymal stem cells from preeclampsia patient by droplet digital polymerase chain reaction.

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Introduction

Preeclampsia (PE) is a pregnancy specific disorder. Although it is characterised by hypertension (blood pressure $\geq$ 140/90 mm-Hg) and proteinuria (300 mg/24h) that develops after 20 weeks of gestation, The American College of Obstetricians and Gynecologists (ACOG) reported that preeclampsia may develop without proteinuria. PE, which effects approximately 5% of pregnant women worldwide, causes maternal-fetal morbidity and mortality[1,2]. Although the mechanism of the disease is still not fully understood, it is thought that immune system imbalances at the maternal-fetal interface may be among the causes of the pathogenesis of PE[3–6]. Maternal immune disorders in the feto-placental region are a cause of the development of defective trophoblasts and related maternal-placental pathological abnormalities such as PE[7,8]. Other studies have also reported that impairments in angiogenesis and anti-angiogenic factors result in placental circulatory disorders in patients with PE[9,10]. These findings suggest that abnormalities in placental vascular formation may be one of the possible causes of PE pathogenesis.

Mesenchymal stem cells (MSCs) are multipotent precursor cells that can be easily isolated and can differentiate into various cells such as osteoblasts, adipocytes and chondroblasts[11,12]. MSCs are found mainly in the bone marrow but have also been shown to have similar but non-identical properties in a variety of host tissues including cord blood and umbilical cord, adult peripheral blood, adipose tissue, trabecular bone, and dental pulp[13–18]. In addition to differentiation, they can renew themselves. Thus, they are used in the treatment of many diseases[19]. The maternal-fetal interface is an important source of MSCs[20–22]. Castrechini et al. reported that the vascular niche contains placenta-derived

mesenchymal stem cells that contribute to vascular maturation and stabilization[23]. In another study, abnormal cytokine levels were observed in decidua derived MSCs (dMSCs) from PE patients and high levels of MSC negative markers were found in PE placentas[24,25]. These findings suggest that MSCs may contribute to the pathogenesis of PE.

MicroRNAs (miRNAs) are short (19-25 nucleotides), single-stranded, non-coding RNAs. They regulate gene expression by binding to 3' UTR of target mRNAs[26]. They are known to play an important role in many biological events such as development, differentiation, apoptosis and oncogenesis[27]. MiRNAs also have important effects on differentiation, maturation and function of stem cells[28,29]. Variations in miRNA expression levels were observed in placentas from PE patients[30,31]. This suggests that miRNAs may play a role in the pathogenesis of PE by regulating MSCs.

In the present study, 6 miRNAs are analyzed by droplet digital PCR method using dMSCs from PE patients and control (healthy pregnancy) groups to compare expression levels of miRNAs between the two groups.

Methods

Sample collection

Whole sample collection and analysis processes are ethically approved by Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine Clinical Researches Ethics Committee (Permission no:219819) on 09.06.2017.

In this study, desidua samples were collected from 7 PE and 7 control. The sample collection has been performed by Department of Obstetrics and Gynecology, Cerrahpasa Faculty of Medicine in Istanbul University-Cerrahpasa and samples were delivered in HBSS 1X solution (GIBCO®) containing 1% antibiotic-antimycotic directly to Koç University, KUTTAM. Clinical characteristics of PE and healthy pregnant women were shown in Table 1.

Table 1: Clinical characteristics of PE and healthy pregnant women.

	PREECLAMPSIA (n=7)	CONTROL (n=7)	P VALUE
Age (years)	$31,3 \pm 5,02$	$28,2 \pm 4,7$	Not significant
Gestational age (weeks)	$37,6 \pm 1,53$	$38 \pm 0,92$	Not significant
BMI (Body mass index) (kg.m^{-2})	$36,1 \pm 5,33$	$31,1 \pm 7,1$	Not significant
Systolic blood pressure (SBP) (mmHg)	$143,5 \pm 4,25$	$114,2 \pm 5,07$	$p<0,05$
Diastolic blood pressure (DBP) (mmHg)	$93,3 \pm 3,7$	$78,3 \pm 6,1$	$p<0,05$
Protein in urine (mg/24h)	>3000	No	$p<0,05$

MSC isolation, culture and sorting

Decidua samples were washed with PBS several times. Then tissues were cut into small fragments and incubated in enzyme containing solution (DMEM/F12, 10% FBS, 1% PS, 2% L-glu and 0,4% collagenase) for 2 h with gentle agitation at 37 ˚C. The mixture was centrifuged at 350 g for 25 min, washed with DPBS and resuspended in fresh medium containing DMEM/F12, 10% FBS, 1% PS, 2% L-glu and transferred to six well plates. Cells were incubated at 37 ˚C in an incubator with 5% CO_2 saturating humidity. When cells reached 80% confluency, the cells were detached using 0.25% trypsin/EDTA. DMEM/F12 was used to inactivate trypsin. The culture medium replacement was done every 3-4 days. After 3-6 passages, dMSCs were sorted using specific cell surface antigens (PerCP CD105, FITC CD73, APC CD45, PE CD34).

Droplet digital PCR analysis

Total RNA was extracted using Nucleospin® miRNA (Macherey-Nagel GmbH & Co. KG, Germany) and reverse transcribed into cDNA using miRCURY LNA™ RT Kit (Qiagen, Germany). Companies's guidelines were followed in all steps. The expression level of candidate miRNAs was determined by Eva Green based ddPCR and U6 snRNA was used for normalization. Following miRCURY LNA PCR primer sets were used for the analysis: hsa-let-7b-3p (Cat no:

YP00205653), hsa-let-7f-1-3p (Cat no: YP00204323), hsa-miR-191-3p (Cat no: YP00204196), hsa-miR-33b-3p (Cat no: YP00204462), hsa-miR-425-3p (Cat no: YP00204038), hsa-miR-550a-5p (Cat no: YP00204638), U6 snRNA (Cat no: YP00203907).

Statistical analysis

Copies/µL values of the samples were determined automatically on Quantasoft version 1.7.4. Mann Whitney Test (non-parametric, unpaired) and ROC analysis were performed using Graphpad Prism 8.1.2 with. P values less than 0.05 were considered statistically significant. Target gene analysis using miRGator v3.0 (http://mirgator.kobic.re.kr) and pathway analysis using miRO v2.0 (http://microrna.osumc.edu) were performed.

Results and Discussion

As s result of the analysis, only miR-33b-3p was found statistically significant (p< 0.05, fold change: 4.5) (Figure 1). In addition, ROC analysis has demonstrated that miR-33b-3p seem to be successful in distinguishing PE patients from healthy controls (Figure 1). Bioinformatic analysis have shown that TGFB1 was one of the target gene of miR-33b-3p. Common pathways of PE and miR-33b-3p were analysed and TGFB1 was found to be involved in syndecan-2 mediated signalling pathway, VEGF and VEGFR signalling network, MAPK signalling pathway, mTOR signalling pathway, Akt mediated class I PI3K signaling and focal adhesion kinase mediated signalling.

Human placenta tissue has been demonstrated to be a source of TGFB and express high TGFB mRNA activity[32]. TGFB has been shown to play a role in paracrine regulation of trophoblast-endometrial interaction and trophoblast differentiation[33]. Therefore, it is predicted that members of the TGFB superfamily of growth factors may play a role in the pathogenesis of PE by preventing differentiation of trophoblasts into an invasive phenotype.

This study demonstrated that miR-33b-3p is significantly downregulated in PE patients and is a potential biomarker to distinguish PE from healthy pregnant. However, further studies need to be done.

Key Messages

•We aimed to find putative biomarkers for early preeclampsia detection and to make contribution of PE pathogenesis.

•Our results showed that miR-33b-3p downragulated in preeclamptic dMSC samples.

•Bioinformatical analysis showed that TGFB1 is one of the target gens of miR-33b-3p.

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OP20

Expressıon analysis of mır-16-5p mırna ın polymorphıc helıcobacter pylorı genotypes ın gastrıc cancer

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Introduction

Gastric cancer (GC) is the fourth most common cancer type around the world[1]. Addition to *Helicobacter pylori* infection being in the first place; salt, tobacco products, alcohol consumption, dietary fiber intake, low socio-economic status, obesity and family history are the main risk factors of gastric cancer[2].

H. pylori carries two polymorphic genes called Vacuolating cytotoxin A (VacA) and Cytoxin-Associated Gene A (CagA) which play role in gastric cancer development.

VacA

The *H. pylori* VacA gene is named for its ability to induce vacuolation in infected cells [3].These large, intracellular vacuoles produced by the protein coded by VacA gene [4].The amino acid sequence and structure of VacA differs from the sequence or structure of other known bacterial toxins [5].VacA toxin is produced as 140 kDa and subsequent to proteolytic prosesses, a 88 kDa toxin generated[6].It is considered that VacA gene is located in *H. pylori* strains in order to colonize in stomach cells of bacteria[7-9].

Furthermore, it has also been detected that this gene shows different effects in different cells. Apoptosis triggering by the induction of cytochrome c release from mitochondria [10], urea permease [11], suppression on the activation of T and B lymphocytes[12, 13], induction of autophagy [14] and necrosis triggerring [15] can be ranked among these effects.

VacA gene carries 6 polymorphic regions (S1,S2,M1,M2,i1,i2) [16]. It has been indicated that VacA carrying S1-i1 and S1-i2 forms is more inclined to failure than the ones carrying S2-i2 [16]. In consequence of the comparison between the bacterial strains with VacA gene carrying S1 region and the strains carrying S2 region, it has been determined that the strains carrying S1 region increase the risks for peptic ulcer and gastric cancer [17]. It has also been inferred that the bacteria carrying M1 type VacA raises the risk of developing gastric epithelial damage and gastric cancer, compared to *H. pylori* carrying M2 type VacA. Moreover, the strains carrying i1 poses more risk of gastric cancer than the strains carrying i2 [18].

As a result of VacA causing epithelial cell death, cell proliferation occurs, which is a factor that increases the risk of cancer. It has been reported that VacA disrupts the monolayer epitel layer either by causing cell death or effecting cell to cell connection [19]. Consequently, VacA can increase increase the penetration or invasiveness of carcinogens into the gastric mucosa and the spreadof malignant cells.

CagA

CagPAI is encoded in CagA Cag patogenity island. It's a 40 kb DNA segment integrated into *H. pylori* DNA and the only known effector protein that can be injected into the host cell CagA is the last gene in Cag patogenity island and encodes approximately 130 kDa CagA protein [20].

It has been demonstrated that the infection of *H. pylori* strains carrying positive CagA gene increase the risk of peptic ulser, atrophic gastritis, intestinal metaplasia and gastric cancer; compared to the infection of *H. pylori* strains carrying negative CagA gene [21]. Also, the individuals infected with *H. pylori* carrying the CagA gene are at a greater risk of gastric cancer than the individuals infected with *H. pylori* without the CagA gene [22]. An increase in hydrogen peroxide level and an oxidative DNA damage are seen in the cells with CagA positive strains' infections [23]. Besides, levels of tumor-necrosis factor-α and interleukin-8 (IL8), which are the markers of inflammatory and oxidative stress, also increase [24] and CagA is also used as a marker of gastric cancer [25].

Following the *H. pylori* infection, CagA positive strains inject Cag protein into the host cells by Type IV secretion apparatus. This protein can be phosphorylated here by the Abl kinase and Src kinase family, soon after the localization to the inner surface of plasma membrane. These kinases phosphorylate tyrosine residues with a five amino acid repeat [Glu-

Pro-Ile-Tyr-Ala (EPIYA)] in the carboxy-terminal end of CagA. These repeats can be categorized based on amino acid sequences at the sites adjacent to the EPIYA sequence [EPIYA-A, EPIYA-B, EPIYA-C and EPIYA-D] so as to produce four different EPIYA motifs. These motifs can be seen in various combinations from one region to another [26].For instance, while the people in western countries carry CagA EPIYA-A, EPIYA-B and EPIYA-C motifs; CagA EPIYA-A, EPIYA-B and EPIYA-D motifs are observed in East Asian people[27].

miR-16

miR-16 is a miRNA, which is a member of miR-15 family. It matures from miR-15a/16-1 and miR-15b/16-2. While miR-15a/16-1 localizes on 13th chromosome in humans, miR-15a/16-2 localizes on 3rd chromosome [28]. It is one of the most prominent miRNAs, which play important roles for the biological prossesses in eucaryotic cells. However, how miR-16 performs its physiological functions still remains unclear [29]. The evidences show that miR-16 has significant roles such as suppressing proliferation, promoting apoptosis, cell cycle regulation and suppressing tumorigenicity [30].

It has been observed that miR16 is partially deleted or suppressed in many cancer types such as chronic lymphocytic leukemia [31], prostate cancer [32] and lung cancer [33].

Materıal and Method

Collection of Tissues

Six tumor tissue samples included in this study were obtained from patients who were diagnosed with gastric cancer and underwent gastric surgery in TC Istanbul Training and Research Hospital between 2016-2017 under the responsibility of NPO HIPEC.

AGS (Human Caucasian gastric adenocarcinoma) cell line from ATCC was used for control purposes.

This study was approved by the Ethics Committee of T.C. Istanbul University, Istanbul Faculty of Medicine (no. 1362), and all patients provided written informed consent prior to enrolment.

DNA isolation

To determine CagA and VacA variations, DNA extraction from stomach tissues was performed with the PureLink® Genomic DNA Mini Kit (Thermo Fisher Scientific, USA), following the manufacturer's recommendations. PCR was applied using specific CagA and VacA primers. The concentration and purity of DNA samples was analyzed withMulti Skan GO µDrop spectrophotometer (Thermo Fisher Scientific, USA). The260/280 absorbance ratio of DNA samples was between 1.8 and 2. In addition, DNA samples were loaded in a 1% (w/v) agarose gel then stained with 0.5 µg/mL ethidium bromide solution and visualized under UV light.

Total mRNA isolation and cDNA Synthesis

Nucleospin® RNA kit (Macherey-Nagel, Germany) was used for RNA isolation and kit procedure was applied. PCR was applied. The concentration and purity of RNA samples, was analyzed in a MultiSkan GO µDrop spectrophotometer (Thermo Fisher Scientific). The 260/280 absorbance ratio of RNA samples was between 1,8 and 2.

cDNA synthesized from 400 ng total mRNA with MystiCq™ microRNA cDNA Synthesis Mix according to the manufacturer's protocol.

miRNA Expression Analysis with SYBR Green qRT-PCR

qRT-PCR was performed using SensiFAST™ SYBR® No-ROX Kit (Bioline, UK) following the manufacturer's recommendations. Each PCR was performed in duplicate and non-infected AGS cell line was used as the internal control. RNU6-1 were used for normalisation. qRT-PCR method was conducted

according to following conditions: 2 min. 95 °C, followed by 40 cycles of 5 sec denaturation at 95°C and 60 °C for 30 sec.

Cell Culture

AGS Cell line was incubated in 37 °C %5 CO_2 in DMEM (Gibco; Thermo Fisher Scientific, USA) containing 10% FBS (Gibco; Thermo Fisher Scientific, USA), 100 mg/ml streptomycin and 100 U/ml penicillin (Gibco; Thermo Fisher Scientific, USA).

Result

Determination of VacA Polymorphic Regions by Agarose Gel Electrophoresis

Polymorphic VacA sites were detected in all 6 tissues. M1 region (290 bp), M2 region (352-400 bp), S1 region (259 bp), S2 region (286 bp) and i1 region (426 bp)were detected. S1, M1, M2 and i1 regions were detected in T2 and T3 tissues. S2 and M2 were seen in T1 and T5 tissues and S1 and M2 were seen in T4 and T6 tissues. VacA agarose gel images of all tissues were shown in Figure 1 and Figure 2.
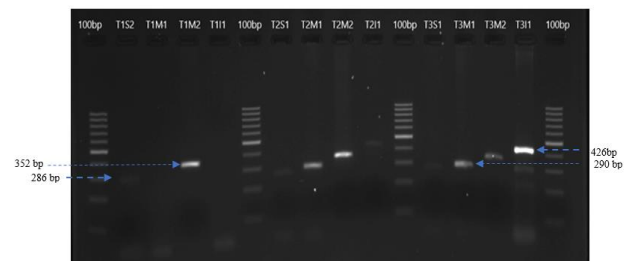

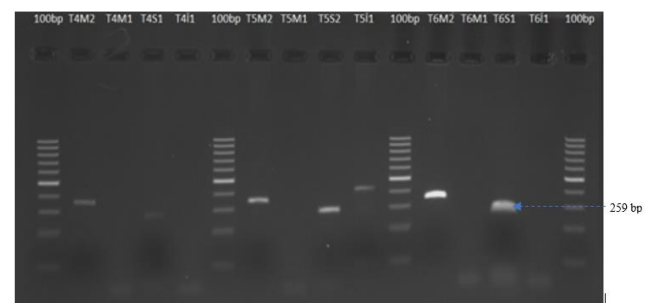**Figure 1.** Agarose gel image of M, S and i VacA polymorphic regions in T1, T2 and T3 tissues

Figure 2. Agarose gel image of M, S and i VacA polymorphic regions in T4, T5 and T6 tissues

Determination of CagA Polymorphic Regions by Agarose Gel Electrophoresis

In 3 of 6 tissues, all CagA variation regions were found. In other words, 3 tissues were found to be CagA positive and other 3 tissues were found to be CagA negative. The 3'region yielded a single band of 544 bp in two tissues (T2 and T5) and two bands of both 544 and 745 bp in one tissue (T3). It is thought that there are two different strains in T3 tissue.

The length of bands allows the EPIYA pattern to be estimated. According to this, it is thought that ABC EPIYA pattern (554 bp)and ABCCC EPIYA pattern (745 bp) were found in the tissues.

In addition, with PCR and agarose gel electrophoresis; R1, R2 and R3 regions were detected in T2, T3 and T5 tissues. As in the 3' region, the R region in the T2 and T5 tissues are yielded 651 bp, while the T3 tissue yielded bands in the region of 651 and 810 bp. According to these results, T2 and T5 tissues which is 651 bp are A type, and T5 which is 651 and 810 bp, were found to contain two types, strain A and C type. 3' variable region and R' region agarose gel results are given in Figure 3.
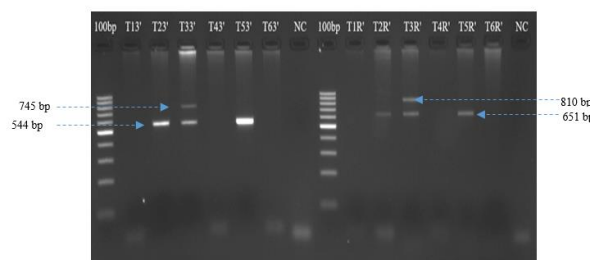

Figure 3. Agarose gel image of Polymorphic CagA 3' and R' regions (NC: negative control)

CagA P1 (264 bp and 291 bp), P2 (309 bp) and P3 (468 bp and 672 bp) motifs were determined by PCR and agarose gel electrophoresis for detection of EPIYA motifs. The results are given in Figure 4 and Figure 5.
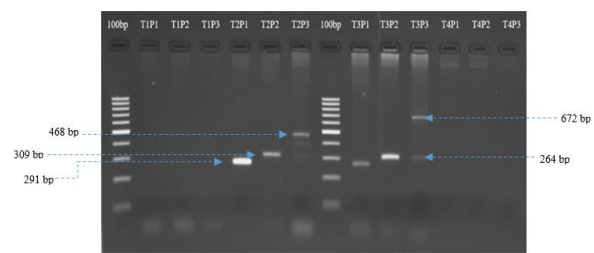

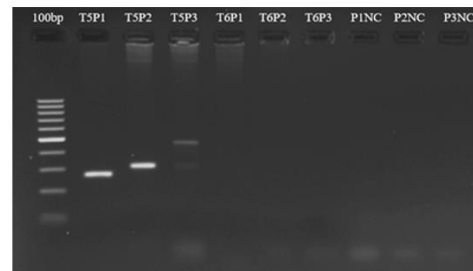**Figure 4.** Agarose gel image of CagA polymorphic P region in T1, T2, T3 and T4 tissues

Figure 5. Agarose gel image of the CagA polymorphic P region in T5 and T6 tissues (NC: negative control)

Expression Analysis of miR-16-5p via qRT-PZR

By using real-time PCR method and SYBR Green fluorescent dye, miR-16-5p expression analysis were performed. AGS gastric cell line was used as control. The results of expression analysis were shown in Table 1. Also these expression levels were given as graphic in figure 6.

When the expression levels of miR-16-5p were examined, it was seen that the most suppression occurs in T3 with 0.022 fold expression. T6, T2, T5, T4 and T1 follows T3 respectively. T3 and T2 carries all polimorphisms in terms of CagA and VacA.

Table 1. miR-16-5p Expression Analysis Values

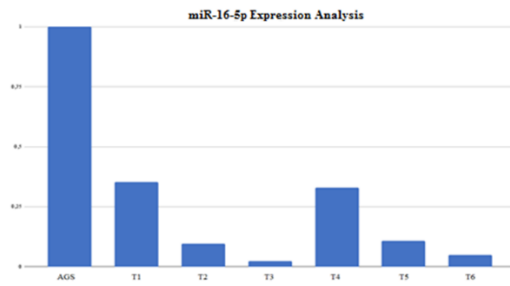| Tissue Samples	ΔCT	ΔΔCT	$2^{-\Delta\Delta CT}$	Standard Error
AGS	4,69	0,00	1	-4,89
T1	6,15	1,46	0,352	-6,25
T2	8,08	3,39	0,095	-8,08
T3	10,21	5,52	0,022	-10,18
T4	2,12	-2,57	0,329	-6,39
T5	7,89	3,20	0,108	-7,92
T6	9,61	4,92	0,048	-9,12

Figure 6. miR-16-5p Expression Levels

Conclusion

In *H. pylori* positive samples, it was observed that miR-16-5p was suppressed compared to AGS control cells, so it can be said that *H. pylori* had an effect on miR-16-5p. The supression of miR-16-5p observed in T3 tissue at most. miR-16-5p is a pro-apoptotic miRNA and its suppression in this tissue carrying all the VacA and CagA polymorphisms suggests that the polymorphic VacA and CagA genes may inhibit apoptosis and cause tumor development. T2 has all the polymorphic regions just like T3. But the suppression was not as much as T3. The reason for this is thought that the suppression of miRNAs may have been caused by different mechanisms other than the CagA and VacA genes.

T6 was the most supressed tissue after T3. This tissue, which does not carry the polymorphic CagA gene, carries VacA S1 and M1 regions. In tissues carrying VacA S1 and M1 regions together, for example T2, T3 and T6 tissues, miR-16-5p appears to be suppressed more than other tissues. In T4, which carries the S1 and M2 polymorphisms together, the suppression level is the lowest. Previous studies have shown that M1 polymorphism increases the risk of cancer more than M2 polymorphism. Thus, in tissues carrying the M1 region, further suppression of a pro-apoptotic miRNA is expected [17].

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OP21

The investigation of serum levels of Vitamin D in gastric cancer development

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Introduction

Gastric cancer (GC) is fourth most common cancer also the second most common cause of cancer-related deaths worldwide [1]. In Turkey, GC is the fifth most common type of cancer in males (14.2%), and the seventh most common type in females (6.3%) [2]. The common etiological factors includes helicobacter pylori infection, Epstein Barr virus, intestinal metaplasia, pernicious anemia, smoking consumption, radiation, dietary factors (a high intake of salt-preserved foods and salt, low intake of fruits and vegatables) and genetic factors. However, the increased standards of hygiene, Helicobacter pylori eradication and conscious nutrition have leading to decline in GC incidence rates within last 10 years [1].

Vitamin D, a steroid hormone, has various biological functions through genomic and non-genomic effect [3–5]. The previous studies suggested the antitumoral role of the active form of vitamin D, 1,25-dihydroxy Vitamin D, treatment on gastric cancer cell line by inducing apoptosis and the regulatory role of vitamin D effect on hedgehog signaling (Hh) by decreasing the mRNA expression of target Hh genes [6,7]. Moreover, the study reported vitamin D regulated *PTEN* expression as a nuclear transcription factor in the HGC-27 gastric cell line [8].

In the literature, the results of studies considering the role of vitamin D metabolism in GC are quite conflicting [3–8], therefore, in the present study, we aimed to determine the serum levels of vitamin D in GC patients and its role in the risk of GC development.

Materials and Methods

Participants

This case-control study includes 161 individuals (%48 GC and %52 healthy subjects). The control group was selected from healthy individuals and the patient group was consisted of patients diagnosed with GC followed by Istanbul University Faculty of Medicine Department of Internal Sciences and Istanbul Training and Research Hospital General Surgery Clinic.

All participants in the study provided their written consent prior to the study. This study approved with the Helsinki Declaration. The study protocol was confirmed by both the Research Fund of Istanbul University (Project No: TYL-2018- 29205) and the Ethical Committee (Decree No. 476413).

Serum Vitamin D Level Measurement by HPLC

Serum vitamin D concentrations were obtained with high-pressure liquid chromatography (HPLC) system. The separation of the analytes was carried out RP C18 analytical column 250x4.6 mm, 5 μm particle size. The detection was performed on commercially available vitamin D analysis kit and bi-level controls. The HPLC protocol was applied as follows : flow rate = 0.7 ml/min; Wavelength = 265 nm; column temperature = approximately 25^0.

Statistical Analysis

All the statistical analyses were carried out using the SPSS21.0 software (Chicago, IL, USA). Estimation of relative risk was determined by calculating confidence intervals and odds ratio (OR). Student's t-test was used to determine the difference in quantitative biochemical parameters as mean±standard deviation. Chi-Square (χ2) test was used to compare the qualitative data as percentage (%). $p < 0.05$ was accepted as statistically significant.

Results

Accordingly, the control and patient groups have similar distribution of age and sex (*p>0.05*). Among the patient groups in early-stage tumor grade (I+II) was less than advanced tumor grade (III+IV).

The means of serum levels of vitamin D in GC patients versus controls were 11.29 ± 5.51 → 16.10 ± 5.66 ng/ml (*p<0.0018*), respectively. Moreover, in male patients, serum levels of vitamin D was found higher than female patients (14.35 ± 5.95 → 8.01 ± 2.20 ng/ml *(p=0.001)*. However, according to clinical parameters and gender when we examine vitamin D levels, no significant diference was obtained *(p> 0.05).*

In the present study, serum levels of vitamin D were found below 30 ng/ml, the border line of normal serum level, in both patient and control groups. The severe vitamin D deficiency which was determined as <10 ng/mL among the study groups. Accordingly, the rate of severe vitamin D deficiency for the development of GC was about 4.107.

Conclusions

Cancer is common public health problem and is the second cancer-related of worldwide [1]. Among various cancer types GC has significant priority with high mortality and morbidity rates [9]. In addition to common risk factors vitamin D status has drawn great interest within last decades [10]. Moreover, in gastric cancer cell line studies it was shown that vitamin D plays an important role in apoptosis, tumorigenesis and inflammation [6].

Vyas et al. (2016) showed a higher prevalence of vitamin D insufficiency (20-29 ng/mL) in gastic adenocarcinoma patients [11]. Similarly, in our study, the serum levels of vitamin D was significantly higher in the control group than the patient group which suggested the association of lower serum levels of vitamin D with GC as a potential risk factor. However, in our both study group, healthy subjects and patients, the serum vitamin D concerations were between 20-10 ng/mL which shows vitamin D insufficiency in the Turkish population. Moreover, the distribution of severe vitamin D deficiency was shown in patients group and the risk of the development of gastric cancer was estimated as approximately 4.107 fold.

As it was well known that life style activations including exposure to sunlight, low intake of vitamin D, malnouishment, body mass index (BMI), insufficient physical exercise, common use of sunscreen were important factors that affect vitamin D status [6]. Accordingly in our study at least, the blood samples of the patiens and control group were taken at the same seasonal periods in order to eleminate the effect of sunlight exposure.

In conclusion, the results of our study support the hypothesis that significant relationship exist between low serum vitamin D concentration and increased risk of GC in the Turkish population. There is a need for further studies that larger groups and wider clinical data should be developed to reach a definite conclusion.

Acknowledgements

Key Messenger

- There is relationship between low serum levels of vitamin D and risk of GC.

- The serum levels of vitamin D in the study groups were vary between 20-10 ng/mL which shows vitamin D defficiency in Turkish population.

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OP22

Effects of fluoxetine on NEGR1 expression in major depressive disorder patients

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Introduction

Major depressive disorder (MDD) is a psychiatric disorder which is characterized by sadness, loss of interest or pleasure [1]. In 2017, the prevalence of depression was 4.4% in Turkey[2].

According to a study conducted in European individuals with MDD, one of 15 genetic loci associated with MDD was *NEGR1*[3].

NEGR1 regulates neurite outgrowth and participate in synapse formation[4]. In a study, *NEGR1*, which was in 1p31.1 to 1p31.3 deletion, was associated with hyperactivity disorder, learning disability, speech and language development[5], dyslexia[6]. *NEGR1* gene polymorphisms have also been associated with white matter integrity[7], obesity[8], eating disorder[9] and depression[3].

Therefore, we aimed to investigate expression levels of NEGR1 gene and protein in MDD patients, and also effects of fluoxetine on NEGR1 expression levels.

Materials and Methods

We studied with 80 participants, comprising 40 MDD patients and 40 healthy controls. MDD patients never received medication treatment during the collection of the samples. The healthy control group was recruited via personal communication. Diagnostic assessments using the Turkish versions of the Structured Clinical Interview for DSM axis I and II disorders (SCID) were performed by trained raters. Depression severity was evaluated using the Beck Depression Inventory (BDI)[10] and Hamilton Rating Scale for Depression (HAMD)[11].

The peripheral blood mononuclear cells (PBMCs) were isolated from blood samples. For cell culture, the cells were suspended in LymphoPrime (Capricorn) and were cultured for 72 hours at 37°C. Samples were divided into 4 different groups; dH_2O treated control samples, fluxetine-treated control samples, dH_2O treated MDD samples, and fluoxetine-treated MDD samples. For preparing gene expression experiments, total RNA from PBMC cell suspension was obtained.

Following RNA extraction, total RNA was reverse-transcribed into cDNA with random hexamers as primers using a commercial kit (Roche Diagnostics). *NEGR1* expression in PBMCs was determined by RT-qPCR with TaqMan probe technology. *NEGR1* mRNA levels were normalized to ACTB, utilizing the $2^{-\Delta Ct}$ method.

The cell homogenates of cell culture suspensions were used for NEGR1 ELISA assay (Elabscience® Human NEGR1, Houston, Texas, USA) following the manufacturer's instructions. Statistical analysis were performed by using SPSS 21.0 program.

Results

Patients with MDD exhibited higher *NEGR1* mRNA levels when compared with healthy controls. However, this difference was not statistically significant ($p > 0.05$). MDD patients exhibited significantly higher NEGR1 protein levels when compared with healthy controls ($p = 0.01$). Fluoxetine-treated cells of MDD group presented elevated *NEGR1* mRNA levels however this result did not reach a statistical significance ($p = 0.10$). NEGR1 protein expression was significantly elevated in fluoxetine treated cells of MDD patients when compared with healthy control cells treated with fluoxetine ($p = 0.01$). Furthermore, positive correlation was found between NEGR1 protein levels and the psychiatric scale Beck scores

in fluoxetine treated cells of MDD group (r = 0.33, *p* = 0.036).

Discussion

Many studies have been conducted on the genetic predisposition factors of MDD[12]. One of the genes related to neurogenesis was indicated as *NEGR1*[13]. A report has showed that, overexpression of *NEGR1* decreased number of synapses formed on dendrites of hippocampal neurons[4]. A study of rat cortical neuron culture showed a significant decrease in the number and length of mature cortical neurons when NEGR1 expression decreased[13]. A previous study reported elevated NEGR1 protein levels in the cerebrospinal fluid of MDD patients[14].

In our study, *NEGR1* gene expression was higher in patients with MDD when compared with controls. However this result did not reach a statistical significance. We also showed that protein levels of NEGR1 were significantly increased in patients with MDD than in controls. Interestingly, the protein levels of NEGR1 were corralated with Beck score (BDI) in fluoxetine-treated MDD group. The protein levels of NEGR1 did not differ when dH$_2$O treated MDD group and fluoxetine-treated MDD group were compared. So, we demonstrated that fluoxetine had no effect on the protein levels of NEGR1 directly but there was a correlation between the protein levels of NEGR1 and Beck scores.

In conclusion, in the present study, when MDD and control groups were compared in terms of NEGR1 protein levels, high levels of NEGR1 protein in MDD group were thought to affect the pathophysiology of MDD.

Acknowledgements

Competing interests

None

Key Messages

- NEGR1 protein levels were significantly elevated in MDD patients compared with controls.

- NEGR1 protein expression was significantly increased in fluoxetine treated cells of MDD patients when compared with healthy control cells treated with fluoxetine.

- Positive correlation was found between NEGR1 protein levels and Beck scores in fluoxetine treated cells of MDD patients.

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OP23

Paradigm of macrophage polarization: the role of cancer associated fibroblasts

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Introduction

Monocytes differentiate into M1/M2 macrophages [1]. There are different types of markers for human macrophages. Beside them, CD14, CD16, CD64, CD86 and HLA-DRα are highly related markers to human M1 macrophages [1]. On the contrary, CD163 and CD206 markers are used for M2 macrophages. [2]. As it is known, macrophages account for up to 50% of the tumor mass in breast cancer . In connection with this, poor prognosis is associated with the number of tumor-associated macrophages (TAM) . When all stages of tumor progression are examined, it is seen that macrophages play a role in all stages [3]. For example, macrophages stimulate angiogenesis in the primary tumor and thus facilitate invasion. The effects of macrophages during metastasis are that they prepare the pre-metastatic region and support the spread and growth of tumor cells. In addition, they exert an immunosuppressive micro-environment by altering natural killer (NK) and T cell functions [3].

Most macrophages in tumor-microenvironment display M2-like-features. Identification of mechanisms affecting macrophage-alterations in tumour-milieu may provide crucial information concerning diagnostic/therapeutic strategies against cancer. Current study aims to shed light on the effects of cancer/tumour associated fibroblasts (CAFs) on macrophage differentiation in breast cancer.

Fibroblasts, which are among the most common cells in tumor stroma, turn into cancer/tumour associated fibroblasts (CAFs) in the cancer milieu. There are similarities between CAFs and myofibroblasts (spindle shaped activated fibroblasts) [4]. As it is well known, there is not a specific marker expressed only by CAFs. However, α smooth muscle actin (α-SMA) is frequently

utilized as a CAF marker in the literature [5]. CAFs undertake an importatnt role in shaping the tumour micro-environment, initiating as well as progressing the tumor [6]. Recent studies have shown that CAFs regulate immune cell uptake and function of these cells. Finally, there are few studies showing the influence of stromal fibroblasts on monocyte / macrophage in breast cancer.

Materials and Methods

Fibroblast Isolation

Collagenase / Hyaluronidase were used to isolate normal fibroblasts (NFs) and CAFs; from patients undergoing reduction mammoplasty and total mastectomy, respectively.

Immunocytochemical characterizations of NFs and CAFs

Surface expressions of markers (α-smooth-muscle-actin [α-SMA], vimentin), which differentiate CAFs from NFs, were analyzed with immunocytochemistry.

CD14+ monocyte and CD4+ T cell isolation from peripheral blood and culture

Magnetic-bead-based-selection-protocols were utilized to isolate CD14+ monocytes and CD4+ T-cells from PBMCs.

Flow cytometry investigations for phenotypical features

Surface expressions of CD206, CD163 were analyzed with flow cytometry.

Carboxyfluorescein Diacetate Succinimidyl Ester assay to determine proliferation

Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) tagged CD4+ T-lymphocytes (CD3/CD28-magnetic bead activated) were utilized to analyze the functional influence of CAF / NF-trained monocytes on CD4+ T lymphocytes.

Cell migration analyses

Trans-well-migration-assays were used to demonstrate the alterations in monocyte-recruitment due to CAFs/NFs.

EMT protein expression analysis (Western Blot)

E-cadherin, vimentin protein expressions were determined by Western blot.

Cell invasion analyses

Trans-well-inserts were used to analyze alterations in MDA-MB-231 breast-cancer-cell-invasion mediated by CAF/NF-educated-monocytes.

Results

CAFs expressed α-SMA unlike NFs. CAFs were able to potently recruit monocytes. This recruitment may be achieved through Monocyte-chemotactic-protein-1 (MCP-1) or stromal-cell-derived-factor-1 (SDF-1) cytokines, since monocyte-migration was significantly reduced due to MCP-1 or SDF-1 inhibition via MCP-1 or CXCR4(a-chemokine-receptor-specific for SDF-1) blocking-antibodies. CAF-trained cells' expressions of CD163, CD206 (related-with M2 macrophages) were higher than NF-trained cells. T-cell-mediated immune-responses were shown to be suppressed by CAF-trained-monocytes. CAF-trained-monocytes augmented breast-cancer cell-invasion, unlike NF-trained-monocytes. CAF-trained-monocytes augmented vimentin expression, suppressed E-cadherin-expression in cancer-cells. Furthermore, CAFs trans-differentiated M1-macrophages to M2-like-macrophages, since CD163-expression significantly increased in M1-macrophages due to CAFs.

Discussion

CAFs polarized monocytes to M2 like pro-tumoral macrophages both phenotypically as well as functionally; unlike NFs. CAFs were highly potetnt in recruiting monocytes. MCP-1, SDF-1, which are

secreted from stromal-cells, may prove to be vital monocyte chemotactic cytokines.

Conflicts of interest

Author declares no conflicts of interest.

Key messages:

Cancer associated fibroblasts polarize monocytes to M2 macrophages via MCP-1 and SDF-1 and; therefore, modify the tumour microenvironment into a immune suppressive milieu.

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OP24

Investigation of the relation of Leptin G-2548A and LeptinR-Q223R gene variants with serum leptin and leptin receptor levels in ovarian cancer patients

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Introduction.

Gynecologic cancers cover a significant proportion of morbidity and mortality in women after breast cancer (1). Despite the developments in recent years, ovarian cancer still has the highest mortality among gynecological cancers (2). Leptin is a peptide hormone, usually produced by adipocytes (3), containing 167 amino acids, weighing 16kDa (4). In addition, small amounts of leptin are synthesized in placenta, gastric mucosa, bone marrow, mammalian epithelium, skeletal muscle, pituitary gland, hypothalamus, testicular bone (5) and ovary (6). Leptin performs its physiological activity through the leptin receptor which is a member of class 1 cytokine receptor family (7). The major signaling pathways are JAK / STAT, MAPK / ERK and P13K / Akt (8). The leptin receptor was first expressed in the brain and regulates food intake and energy metabolism with negative feedback effect on the brain (4). In addition to providing metabolic energy regulation, leptin play an important role in the

regulation of many systems such as gastrointestinal system, sympathetic nerve activation, sexual development and reproduction, hematopoiesis, immune system regulation, skeletal system development, and cardiovascular system (4, 9). Several polymorphisms have been identified in the leptin and leptin receptor genes. However, a few of these polymorphisms are functional and have been preferred in studies on cancer (10). The leptin G-2548A polymorphism refers to the single nucleotide change that occurs when the leptin gene is transformed into Guaninin-Adenine at nucleotide 2548 at the 5 'end of the promoter region. LeptinR-Q223R polymorphism of the leptin receptor gene is expressed with 6 exon 223 start codon, 668 nucleotides Adeninin-Guanine conversion (CAG-CGG) (11). Leptin and leptin receptors have been found to be closely related to the promotion of angiogenesis and increase in tumor proliferation in human and animal cell cultures. These effects of leptin and leptin receptors have been proven in embryonic cells, adipocytes, glia, endothelial cells, hematopoietic cells, bening and maling epithelial cells, kidney, colon, liver and pancreas (4).Recent studies have shown that leptin stimulates growth, migration, invasion and angiogenesis. This supports the aggressive cancer phenotype. Although increasing evidence indicates that leptin may be effective in the development of ovarian cancer, information on its molecular mechanism and its effect on cell cycle and apoptosis regulation is still limited (12). In this study, we aimed to determine the prevalence of Leptin G-2548A and LeptinR-Q223R polymorphisms in ovarian cancer patients and to evaluate the possible relationship with ovarian cancer by comparing patient leptin and leptin receptor levels with healthy controls.

Materials and Methods.

The study included 61 ovarian cancer patients and 51 healthy individuals. The ovarian cancer patients who

histologically confirmed were selected from the Yeditepe University Hospital and Istanbul University, Department of Obstetrics and Gynecology Clinics and the control group were selected in the same Clinics. For this study, approval was obtained from Yeditepe University Clinical Research Ethics Committee (numbered 37068608-6100-15-1025 and dated 08.04.2015). All blood samples were taken into tube containing EDTA and stored at +4 ^{0}C until DNA isolation. Genomic DNA of blood samples were isolated by Invitrogen iPrep Purification Instrument and iPrep PureLink gDNA Blood Kits (Invitrogen, Life Technologies, Carlsbad, CA, USA). NanoDrop 2000 instrument (Thermo Scientific, Waltham, MA, USA) was used to determine DNA levels. Serum samples of individuals were used for ELISA analysis. Serum samples were stored at -20 ° C until ELISA analysis. Genotyping of samples was performed using PCR-RFLP method. Leptin G-2548A and LeptinR-Q223R polymorphisms were determined using sense 5' - TTT CCT GTA ATT TTC CCG TGA - 3' and antisense 5' - AAA GCA AAG ACA GGC ATA AAA - 3' primer sets for Leptin G-2548A and sense 5' - ACC CTT TAA GCT GGG TGT CCC AAA TAG - 3' and antisense 5' - AGC TAG CAA ATA TTT TTG TAA GCA ATT - 3' primer sets for LeptinR-Q223R. After observing PCR product for Leptin G-2548A and LeptinR-Q223R on agarose gel (2%), PCR products were cut with the restriction enzyme HinP1I (37°C, 2 hour) and MspI (37°C, 2 hour), respectively. All the digested products were separated by 2% agarose gel electrophoresis. Leptin and soluble leptin receptor levels in serum samples were determined by Sandwich ELISA method using BOSTER brand Human Leptin PicoKine ™ ELISA (EK0437, Boster / USA) Kit and Human Leptin receptor PicoKine ™ ELISA (EK0439, Boster / USA) Kit, respectively. SPSS 22 package program was used for statistical analysis.

Results

In our study, no statistically significant difference was observed between the healthy control group and the ovarian cancer patients in terms of age (p=0,104). Furthermore, body mass index, body surface area and fasting blood glucose levels of the patients were significantly higher than healthy controls (p <0.001). In addition, a statistically significant difference was found between the patient and the control groups in terms of alcohol consumption (p <0.001). There were 24 (47.1%) smoking and 27 (52.9%) non-smoking individuals in the control group, while 10 (16.4%) smoking and 51 (83.6%) non-smoking individuals in the patient group and it was found that smoking factor decreased the risk of ovarian cancer approximately 4.5 times (x^2:12,355, p<0,001, OR:0,221, %95 CI 0,092-0,528). While 41.2% of the postmenopausal were in the control group, 83.6% were in the patient group and postmenopausal period were increased the risk of ovarian cancer. Serum leptin levels (p <0.001, 95% CI 3.86 ± 6.64) were found to be significantly higher in the patient group, but there was no significant difference between soluble leptin receptor levels between the groups (p = 0.199, 95% CI 0, 25-0.54).

The genotype and allele distributions of the Leptin G-2548A and Leptin-Q223R polymorphisms were compared in control and ovarian cancer patient groups; no statistically significant difference was observe between the groups (respectively x^2 = 2.097, p = 0.350 and x^2=3,842, p=0,146). When the distribution of serum leptin and soluble leptin receptor levels according to the genotypes of Leptin G-2548A and LeptinR-Q223R polymorphism was examined, no statistically significant difference was found in the patient and control groups.

Discussion and Conclusion

In order to understand the effect of genetic factors on ovarian cancer, the relationship between Leptin G-2548A and LeptinR-Q223R gene variants, serum leptin and soluble leptin receptor levels and ovarian cancer was investigated. Body mass index (BMI) is commonly used to determine obesity based on World Health Organization's classification of obesity. (13). Engeland et al. suggested that body mass index and height increase the risk of ovarian cancer in young women (14). Although postmenopausal obesity is associated with high estrogen and androgen levels, there is no consistency in its association with ovarian cancer. The relationship between ovarian cancer and BMI varies with postmenopausal estrogen usage. In a study conducted in women who did not have menopausal hormone therapy, a positive correlation was found between BMI and increased risk of ovarian cancer (15). According to the results of our study, when we compared the body mass index of the ovarian cancer patient and control group, it was found that the BMI of the patients (29.15 ± 5.83) increased significantly compared to the control group (22.91 ± 3.61) (p <0.001) and increased body mass index increase ovarian cancer risk. It is thought that the increased levels of fatty tissue-derived estrogen and androgen may play a role in the risk of ovarian cancer due to the fact that the majority of our patient group is in postmenopausal period and most of their BMI values are above 25 (73.6%). In vitro studies have shown that estrodiol and estrone stimulate proliferation in normal and superficial epithelial ovarian cancer cell cultures (16). Fasting blood glucose values of the patient group (95.98 ± 11.79 mg / dl) were higher than the control group (86.51 ± 7.46 mg / dl). Lamkin et al. suggest that glucose levels may be an important prognostic factor in cancer patients. At the same time, it was found that the expression of the transmembrane protein GLUT1, which is responsible for glucose uptake, increased in ovarian tumors and this may have a negative effect on survival for ovarian cancer (17). On the other hand,

Lambe et al. could not determine a clear relationship between glucose levels and ovarian cancer risk (18). While previous studies have shown that smoking is not associated with ovarian cancer risk (19, 20), a systematic literature review and Meta-Analysis of Jordan et al. showed that smoking is a risk factor for mucinous ovarian cancer (21). It is thought that the genotoxic and mitogenic effects of estrogen are effective in the neoplastic transformation of normal ovarian surface epithelial cells and play a role in stimulating cell growth and inhibiting apoptosis, and nicotine and other tobacco compounds may have protective effects on cancer formation (22). In our study, it was determined that smoking have a protective effect of 4.5 times on ovarian cancer. In order to determine the accuracy of the protective effect we found, we think that the reliability of the information can be increased by working in larger population sections and collecting more detailed data with usage amounts of tobacco compounds. In the study to understand the molecular mechanism of leptin effect on apoptosis and cell cycle control, Ptak et al. observed that both the long and short form of the leptin receptor was expressed in epithelial ovarian cancer cell culture (OVCAR-3). In addition, by exposing the cells to different doses of leptin, leptin has a stimulating effect on the cell cycle with increasing the cell population and inhibits the expression of proteins and pro-apoptotic genes in the apoptotic pathway (12). Jin et al, were reported that leptin levels of ovarian cancer patients are lower than healthy individuals (23). In our study, serum leptin levels of patient group were higher compared to the control group (p <0.001). Mor et al. prepared a panel of biomarkers including leptin, prolactin, osteopontin and IGF-II for the diagnosis of epithelial ovarian cancer. It has been reported that these four analytes exhibit 95% sensitivity, 95% specificity, 95% positive

predictive value and 94% negative predictive value (24). We think that these single studies on serum leptin levels and the relationships between diseases and these levels may be the source for marker panels and may lead to the establishment of reliable panels that can yield more effective and definitive results in diagnosis. Recently, it is interesting to understand the impact of genetic background and genetic variations on cancer development and progression. However, due to the heterogeneity of diseases, there are difficulties to determine which genes may be more sensitive for studies. With the informations such as the leptin gene is associated with obesity and obesity may be a risk factor for cancer development, researchers have focused their attention on the effect of variations in the leptin receptor gene required for leptin and its biological function on cancer. Yang et al. conducted a meta-analysis to investigate the effect of Leptin G-2548A polymorphism on cancer risk. According to the study data, the homozygous mutant (AA) and homozygous native (GG) genotype have been reported to increase the risk of non Hodgink lenfs lymphoma cancer (OR: 1.28, 95% CI 1.07-1.54). It has also been reported that patients with AA genotype are at higher risk in colorectal cancer patients than those without AG genotype (25). Ünsal et al. investigated the effects of Leptin G-2548A and LeptinR-Q223R polymorphisms on lung cancer and suggested that carrying the GA, AA genotypes and A allele in Leptin G-2548A polymorphism may be a risk factor for the disease. In addition, in terms of LeptinR-Q223R polymorphism could not detect a significant difference in patients and controls (26). In a study conducted on Iranian women, it is reported that Leptin G-2548A and LeptinR-Q223R polymorphisms and serum levels do not increase breast cancer risk (27). In contrast to this study, Anuradha et al. suggested that the RR homozygous mutant genotype may be a risk factor for

the development of breast cancer in terms of LeptinR-Q223R polymorphism (28). Rodrigues et al. examined leptin receptor expression and Gln223Arg polymorphism as a prognostic factor in oral and oropharyngeal cancer and they observed that the RR homozygous mutant genotype reduced the risk of disease 2.5 times in QQ homozygous wild type carriers in cancer development. They also report that weak leptin receptor expression has a similar effect with polymorphism and it may increase tumor leptin levels and help tumor invasion and metastasis. It is thought that there is a change in the level of leptin in the receptor function, signal capacity and circulation as a result of polymorphism. This polymorphism occurs in the extracellular region and the changing amino acid charge causes a weak relationship between leptin and its receptor. Therefore, it is thought to cause a decrease in cell growth signals. Pathways under the control of the leptin receptor are effective on cell survival and differentiation. Therefore, it is thought that this polymorphism may have inhibitory effect on cell growth (29). Due to the small sample size in our study groups, we think that Leptin G-2548A and LeptinR-Q223R gene variations do not fully express whether leptin and soluble leptin receptor serum levels are related to ovarian cancer. We believe that extensive research by using large groups to determine these links will yield more reliable results. In this way; it is thought that future studies will contribute to the genotypic relationship between leptin and disease and to solve the molecular mechanisms in the pathogenesis of ovarian cancer.

Key Messages. Ovarian cancer, Leptin, Leptin receptor, polymorphism

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OP25

Research of the AdipoQ, AdipoR1 and AdipoR2 genetic variation effects at the obese coronary artery disease patients

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Introduction

In recent years, obesity (Body Mass Index between 30 and 40 kg/m^2) has become one of the biggest health

problems on a worldly scale, and its incidence is increasing. It is associated with high blood pressure, heart diseases, diabetes, high cholesterol, joint diseases, respiratory disease, and gall bladder disorders [1]. The prevalence of obesity was found as 22.3% in Turkey. This rate was shown to be 29.9% for women, and 12.9% for men [2]. The risk factors for obesity include alcohol use, decrease in physical activity, number of births, age, gender, nutrition habits, smoking cessation, etc. However, there are more than 200 candidate genes that are considered to cause obesity.

Although atherosclerosis was known as a degenerative event until twenty years ago, it is considered as a multifactorial disease in recent years. Atherosclerotic lesions are caused by the increase in the inflammatory response given to the changes in endothelium and smooth muscle cells in the artery wall. There are a large number of growth factors, cytokines and vasocoregulator molecules in this formation [3]. There are increased plasma cholesterol levels, hypertension, diabetes, obesity, insulin resistance, hyperinsulinemia, smoking, hyperfibrinogenesis, age, male gender among the risk factors involved in the formation of atherosclerosis [4].

In recent years, adipose tissue was considered as an important endocrine organ secreting some factors that are associated with food intake, lipid and carbohydrate metabolism and other processes [5]. Various bioactive molecules that are called adipokines are produced in adipose tissue. Adiponectin is a protein that is synthesized from white adipocytokine tissue, and belongs to adipocytokine family [6]. Adiponectin is an adipocyte playing key roles in obesity-related cardiovascular disorders (insulin resistance, atherosclerosis) [7].

Adiponectin is encoded by a gene called ADIPOQ. Several polymorphic markers were detected that played roles in the correlations between adiponectin levels and phenotype metabolic syndrome and Type 2 Diabetes. The ADIPOQ gene is used in the second part. The Timin-Guanine change in +45 position of the exon is one of these [8]. Adiponectin has two receptor forms, which are AdipoR1 and AdipoR2. AdipoR1 is often expressed in skeletal muscle (with high affinity). However, AdipoR2 is mostly expressed in the liver and shows moderate-level affinity [9]. The expression of adiponectin receptors occurs in pancreatic β cells, skeletal muscle, liver, and adipose tissue [10].

Since adipose tissue is effective in both obesity and atherosclerosis, and since adiponectin takes active roles in adipose tissue, we aimed to examine the relations between T-G polymorphism in the second exon of ADIPOR1 gene, A-G polymorphism in the ADIPOR1 gene 1st intron 106th position, and A-T polymorphism in 219th position in the ADIPOR2 gene and obese-atherosclerosis patients.

Materials and Methods

A total of 71 obese patients and 51 healthy control group patients who had coronary artery disease who referred to Istanbul Private Bahcelievler Medicana Hospital outpatient or inpatient clinics were included in the study. The evaluation of patients according to clinical parameters was carried out by the clinic where their blood samples were provided. After the blood samples were classified, they were sent to the Molecular Medicine Department of I.U. DETAE.

The DNA isolation, Polymerase Chain Reaction, Restriction Fragment Length Polymorphism and Agaronose Gel Electrophoresis techniques were used for polymorphism analysis of AdipoQ, AdipoR1 and AdipoR2 genes in the patient and healthy control samples.

Results

As a result of the Binary Logistic Regression analysis, it was determined that homozygous mutation genotype (GG and TT) in ADIPOQ gene was not a risk factor in the disease alone when evaluated together with the risk factors (p=0.153, p=0.312, respectively). However, it was also determined that the BMI, LDL and HDL were statistically significant as risk factors which were evaluated in the presence of AdipoQ GG and AdipoQ TT homozygous genotype. However, TG alone was found not to be statistically significant in the presence of homozygote GG and homozygote TT genotypes. When the lower and upper values were evaluated, it was determined that HDL, which was significant, is also protective against the disease.

When the homozygous mutation genotype (AA and GG) in the ADIPOR1 gene is evaluated together with the risk factors, it was observed that they were not risk factors in the disease alone (p=0,238, p=0,863, respectively). However, it was observed that the BMI, LDL and HDL, which are among the risk factors, were statistically significant when evaluated in the presence of AdipoR1 AA and AdipoR1 GG homozygous genotype. However, it was determined that TG alone was not statistically significant in the presence of homozygote AA and homozygote GG genotype. When the lower and upper values were evaluated, it was seen that HDL, which was significant, is protective against the disease.

When homozygous mutation genotype (AA and TT) in ADIPOR2 gene was evaluated together with the risk factors, it was determined that it is not a risk factor in the disease alone (p=0, 073, p=0, 611, respectively). However, it was also determined that the BMI, LDL and HDL, which are risk factors evaluated in the presence of AdipoR2 (AA and TT) homozygous genotype, were statistically significant. However, TG was not statistically significant alone in the presence of homozygote AA and homozygous TT genotype. When the lower and upper values were evaluated, it was seen that the HDL, which was significant, is protective against the disease.

Discussion

The ADIPOQ gene polymorphism findings that were obtained in our study correlate with the literature. The AdipoQ gene polymorphism, especially independently from the classical cardiovascular risk formation, was shown to have an effect on metabolic diseases [11].

A study conducted on Type 2 diabetes patients in the Saudi population showed that adiponectin gene mutation is an important factor in the emergence of cardiovascular diseases. When the gene they were working on was ADIPOQ was considered, in our thesis, it was observed that the ADIPOQ GG mutant that has protective features is effective in the formation of CAD in the Saudi population [12]. In another study, the relation between the mutations in various SNP regions of adiponectin and the development of colorectal cancer was investigated. It was observed that the mutation in the ADIPOR1 gene had no effect in the development of colorectal cancer in different study groups they formed. For this reason, it has no triggering activity. In our study, it was determined that the ADIPOR1 gene mutation had no effects on the development of coronary artery diseases; on the contrary, it had a protective role with HDL against the disease [13]. According to the results of a study that examined the relation between adiponectin and coronary artery disease, ADIPOR2 gene polymorphism was shown to have important effects on the development of coronary artery disease, which supports our hypothesis in our study that it provides protection in individuals [14]. In a study conducted on the French population examining the relation between adiponectin and metabolic disease, it was shown that ADIPOR2 gene polymorphism had a moderate effect.

In our study, the low mutation rate of ADIPOR2 gene polymorphism, and therefore high rate of wild type 50-50 gene, supports our hypothesis that it is protective against the disease [15].

In our study, we determined that adiponectin genes have no individual efficacy in the development of the disease. However, when we consider the clinical parameters and risk factors, it was determined that BMI and LDL have effects on disease development in the presence of adiponectin gene polymorphisms. Similarly, when we evaluated the polymorphism results in our genes together with HDL, it was determined that they had protective effects on coronary artery disease.

Conclusion

The importance of the results may increase by including metabolic syndrome groups in the study and by increasing the number of cases. Similarly, more detailed information may be obtained by dividing the BMI scores into classifications. In our study, mutations were detected in our genes in a certain group in the control group that did not have coronary artery disease. If this group is followed-up for longer periods, additional data may be obtained on the development of coronary artery disease in these individuals. Again, the present study of ours, the serum adiponectin levels of the patients and the control group, in which we examined the genetic variations, may be examined to expand the scope of the study and more detailed data can be obtained.

Key Messages

- How do polymorphism results in ADIPOQ, ADIPOR1 and ADIPOR2 genes relate with HDL in Coronary Artery Disease?

It was determined that HDL has protective effects on Coronary Artery Disease.

- What parameters are effective on the development of Coronary Artery Disease in the presence of adiponectin gene polymorphisms?

BMI and LDL

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OP26

TRIM33 Gene Expression Levels in Colorectal Cancer Patients and Analysis of Variants of This Gene by Sequence Analysis

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Introduction

Cancer is one of the leading causes of deaths worldwide and cancer accounted for about 13% of all deaths in 2004. More than 70% of cancer-related deaths occur in low and middle income countries. Cancer-related deaths are estimated to reach 11.5 million deaths worldwide by 2030. [1,2]

Colorectal cancer is the third most common cancer type worldwide. In 2012, approximately 1.4 million cases of colorectal cancer were recorded in the world. In 2015, 1.7 million colon and rectal cancer cases were recorded and caused 832,000 deaths.[3] Survival rate in colorectal cancer depends on the stage of diagnosis. Diagnosis at later stages shows worse survival rate. The 5-year survival rate is 90% for colorectal cancers with early diagnosis and 13% for colorectal cancers with late diagnosis. [4]

Industrialization and urbanization have increased the incidence of this type of cancer. It is common in high income countries but is also increasing in low and middle income countries. It is more common in men than in women. Half of all reported cases are fatal. Colorectal cancer is the fourth most common cause of cancer-related deaths. [5]

Carcinogens taken from foods and beverages can interact directly with the cells that make up the colon and rectum. It may also develop with a history of colorectal cancer, ulcerative colitis and Crohn's disease. [15]

The incidence of colorectal cancer has been identified as 50/100,000 new cases annually and its prevalence is increasing. [14]

Mutations that play a role in sporadic or hereditary colorectal cancer have been investigated for approximately 30 years. Some oncogen and tumor suppressor genes (APC, KRAS, p53) have been identified as mutants in many colorectal cancer types. [10] However, chromosomal instability (CIN) caused by

increased chromosomal abnormalities (aneuploidy) or microsatellite imbalance (MSI) caused by defects in repair of mismatches are important factors. [15]

Another reason that is effective in 40% of colorectal cancers is the gain of protooncogenes as a result of changes in DNA methylation or loss of function of tumor suppressor genes. [7,8]

Colorectal cancer is roughly classified as sporadic (75%), familial but not classified CRC (20%), Hereditary nonpoliposis CRC (Lynch Syndrome) (3%), Familial Adenomatous polyposis (1%) and other rare syndromes (1%).898 [6]

TRIM33 is a human gene, also known as TIF1-γ. The protein encoded by this gene is a transcriptional corepressor. [13] Several different transcription factors have been identified, such as SMAD4, which interact with TRIM33. TRIM33 acts as a tumor suppressor gene. [6] The gene encoded protein E3 acts as ubiquitin-protein ligase. E3 acts as an inhibitor on the SMAD4-dependent TGF-β/BMP signaling pathway. [12] TRIM33 is responsible for the ubiquitination of SMAD4. The addition of ubiquitin to SMAD4 via TRIM33 inhibits the formation of a stable complex with active SMAD2 3, thereby inhibiting the TGF-β/BMP signaling pathway. [11] TRIM33 is also known to act as a tumor suppressor in human myelomonocytic leukemia and pancreatic cancer. [7,8,9]

Conclusion

We investigated the effect of the TRIM33 gene in patients with corolectal cancer, considering the relationship of the Trim33 gene with the Wnt signaling pathway, the TGF-β signaling pathway, and Smad4, which plays a role in these pathways. Colon samples from 80 patients and 60 healthy individuals were used for this purpose. RNA isolation was performed from the samples belonging to the patient and healthy group and the change in gene expression was examined by RT-qPCR. The results were examined by

T-test and a statistically significant difference was found. The gene expression change found by Delta-delta Ct method was determined as 1.78 times and it was found that the patient samples expressed TRIM33 gene approximately 2 times more than the control samples.

In addition, the obtained amplicons were sequenced and SNPs were screened which could lead to discrimination between patient and control groups. High-resolution melting (HRM) analysis was performed to reduce sample numbers prior to sequencing without losing representation potential. The HRM analysis sequence specifically reduced the time and cost of the analysis procedures by grouping samples of the same sequence thanks to the difference graphs that revealed different profiles even at a single base change of amplicons. The samples selected from the groups obtained according to HRM results were sequenced and the sequences examined. it cannot distinguish a particular group from others. The sequences were queried in the NCBI database using the BLAST algorithm and confirmed that the correct regions were analyzed. Then, the dendrogram and the sequences of the control and patient groups were compared with each other. A non-random relationship was sought between the patient and control groups and the sequences were first aligned with each other. Similarly, a similar clustering between the similarity matrix and the control and patient groups was sought, but it was observed that the samples came together randomly. Finally, dendrograms were created and examined whether there was a distinction in this way and it was found that the branches could not distinguish a particular group from the others.

Considering the results obtained, it can be said that the change in gene expression of TRIM33 gene is associated with colorectal cancer. In the context of the

exon of the same gene, no polymorphism causing the disease was found. The human Trim33 gene contains 21 exons. [14] However, in order to indicate that the disease is not related to polymorphisms in this gene, further studies should be performed in other exons of this gene.

Key messages

There are so much gene mutations studied for understand the colorectal cancer. Especially oncogenes and tumor suppressor genes have been studied. TRIM33 is a tumor suppressor gene and plays a role in the TGF-β pathway causing colorectal cancer. This study is the first study to investigate the relationship between TRIM33 gene and colorectal cancer. Unlike other studies, we used HRM analysis, so that we could obtain results using fewer samples.

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OP27

A First Preliminary Report: Potential Role of Soluble CTLA4, GITR, Ox40L, CD40 and CD40L as Inflammatory Biomarkers in Non-Small Cell Lung Cancer Patients

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, of which 85% is non-small cell lung cancer (NSCLC) [1]. Detection of driver oncogene mutations that significantly prolong survival is of great importance in the treatment of NSCLC [2]. Furthermore, the progress of immunotherapy in recent years is known to greatly support NSCLC treatment. Immune control point inhibitors (ICIs) produce improved survival and significant response rates in NSCLC patients, but many do not benefit from ICIs that develop hyper-progressive disease or immune-related adverse events [3]. Therefore, it is important to identify biomarkers for better selection of patients and for more effective use of ICIs [3]. Blood biomarkers are one of the liquid biopsy materials obtained by an economical and easy method and therefore are one of the target materials for the investigation of immune biomarkers [3][4]. Therefore, the ratio and combination of molecules controlling the immune system in the plasma of NSCLC patients will help NSCLC treatment [3].

Immune control points consist of inhibitory and stimulatory pathways that help to limit inflammation [5]. Cancer cells avoid the immune system by having immunosuppressive receptors on their surface or by creating inhibitory effects on T cell activators. ICI enhances the body's immunological activity against tumors by blocking or stimulating target proteins in the immune system [5]. Cytotoxic T-lymphocyte-associated molecule-4 (CTLA-4) is one of the most widely studied and recognized immune system control point pathways, such as programmed cell death receptor-1 (PD-1). ICIs blocking pathways such as CTLA-4 and PD-1 are currently used for a wide variety of malignancies. Furthermore, molecules targeting tumor micro-media components such as agonists of excitatory control point pathways such as OX40L, GITR, CD40 and CD40L, are being investigated [5].

In this study, we aim to investigate sCTLA-4, sOX40L, sGITR, sCD40 and sCD40L levels in lung cancer patients and controls and whether these molecules have more than one effect on triggering carcinogenesis by inflammatory pathways.

Materials and Methods

This study was performed to determine and compare serum levels of CTLA-4, OX40L, GITR, CD40 and CD40L in healthy control group and lung cancer patients. The study design, protocols and informed consent approved by the Ethics Committee of Istanbul Cerrahpaşa University Faculty of Medicine. 20 patients who were admitted to the general surgery polyclinic of the Training and Research Hospital of Istanbul Cerrahpaşa University. After diagnosed as lung cancer were accepted as the patient group and 20 patients without any previous medical intervention were accepted as the control group. Informed consent was obtained from all individuals involved in this study. And then a list was created for all patients, including age, gender, smoking history, and alcohol consumption. CTLA-4, OX40L, GITR, CD40 and CD40L respectively for determination of serum levels of patients and controls, sCTLA-4, sOX40L, sGITR, sCD40 and sCD40L ELISA assay kit (Abbkin, China) was carried out by. 10 µl patient and control serum samples (CTLA-

4; 1/9, OX40L;1/5, GITR;1/80, CD40; 1/9, CD40L; 1/9 dilution) were used for Elisa analysis. Briefly, serum samples and standards were added to the 96-well plate and incubated 45 minutes at 37ºC. After incubation washing 5 times with washing buffer (250 µl), followed by incubation 30 minute at 37ºC with HRP-conjugated streptavidin. After than we washed again 5 times with washing buffer (250 µl), A and B substrate solution (50 µl) was used for color development, plate was incubated for 15 minutes at 37 ºC in dark environment. The detection limit for CTLA-4, OX40L, GITR, CD40 and CD40L proteins were given by the manufacturer as respectively as 30pg/mL-486pg/mL; 1 pg/mL - 16 pg/mL; 1.5 pg/mL - 24 pg/mL; 7.5ng/L and 120ng/L; 150ng/L and 2400ng/L. The optical density was read with a wavelength at 450 nm with a plate reader (Thermo Multiskan Go) after stop solution (50 µl).

Statistical Analysis

Results were analyzed by comparing the significance level of p <0.05 with Mann-Whitney U SPSS 16.0 program.

Results

Table 1; Serum levels of sCTLA-4, sOX40L, sGITR, sCD40 and sCD40L in in lung cancer patients and controls.

VARİA BLES	CTLA-4 SERUM LEVELS (pg/ml)	OX40L SERUM LEVELS (ng/ml)	CD40 SERUM LEVELS (ng/L)	CD40L SERUM LEVELS (ng/L)	GITR SERUM LEVELS (pg/ml)
Patients (SE)	116,8393± 11,00241	8,9282±0,9 7081	10,2757±2, 28531	756,7000±6 1,91731	42,3503± 2,20863
Controls (SE)	83,3541±5, 59681	11,5164±2, 15352	37,2300±1 1,69332	654,4833±3 0,59025	41,5052± 2,40183
P-value	0,004	0,640	0,011	0,377	0,538

The CTLA4 level was 116.839 ± 11.002 pg / ml in the patients and 83.354 ± 5.596 pg / ml in the control group (p = 0.004). The GITR level was 42.350 ± 2.208 pg / ml in the patients and 41.505 ± 2.401 pg / ml in the control group (p = 0.538). OX40L level was 8.928 ± 0.970 ng / m in the patient group but 11.516 ± 2.153

ng / ml in the control group (p = 0.640). CD40 and CD40L were found to be 10.275 ± 2.285 ng /L, 756.700 ± 61.917 ng /L in the patient group and 37.230 ± 11.693 ng /L, 654.483 ± 30.590 ng /L in the control group, respectively (p = 0.004; p = 0.377).

Discussion

In recent years, rapid progression of immunotherapy has been shown to improve prognosis and quality of life for patients with NSCLC. Wei et al studied expression levels of NSCLC immunosuppressive inhibitors (CTLA-4, LAG-3, PD-1, and CD39) and demonstrated that CTLA-4 expression levels were higher than controls. [6]. In another study, CTLA-4 in plasma samples obtained from healthy donors or patients of different tumor types [NSCLC (stage III and IV), RCC (stage III, IV and recurrent), OVC (stage II IV IV)] and the patients were found to be higher than the control [7]. In our study, we found that the CTLA-4 level was higher in patients as correlated with the literature.

According to the results of our study, in contrast to the increased level of co-inhibitor CTLA-4 and co-activator CD40 in NSCLC compared to the control group. In 2015, NSCLC study in China measured the CD40 level and found that it increased in patients compared to the control group. And they suggested that sCD40 secreted by tumor cells could block T cell activation by binding to CD40L on T-cells and increase the ability to escape anti-tumor immune function [8]. In another study on pancreatic cancer, CD40 expression was found to be significantly higher in tumor tissues compared to internal cavity, and when the serum CD40 level of the same patients was examined, it was found to be significantly higher than the control group [9]. Also he et al. proposed CD40 as a potential tool for anti-tumor treatments [9]. In addition to cancer, CD40 was found to be higher in polycystic ovary syndrome (PCOS) and liver diseases than in control [PCOS /

Healthy group; 3.1 ± 2.0. 2.05 ± 1.0, p = 0.002 - Liver patients / healthy group (P <0.001)] [10][11]. CD40 was measured in plasma of NSCLC patients and sCD40 levels were found to be associated with advanced disease and poor prognosis [8]. It can be argued that increased sCD40 may be a useful prognostic marker in other diseases affecting the immune system as well as in modulating antitumor responses.

We investigated of GITR, CD40L and OX40L molecules in the literature which awe could not correlate significantly in our study. İn a study of GITR, they analyzed in role of cytokines to prostate cancer and tumor and inflammatory mechanism by cytokine antibody microarray [12]. GITR was found to be elevated in specimens with significant neutrophilic inflammation of the gland lumen and in specimens with lymphocytic inflammation of the prostate stroma [12]. In another study of prostate cancer, CD40L levels secreted by activated T lymphocytes and platelets were examined. CD40L with SDF-1a and insulin was found in high levels in the serum of prostate cancer patients. And they also suggest those molecules as serum biomarkers for early diagnosis and prognosis [13]. For OX40L, in a study of plasma samples of adult T-cell leukemia (ATL) patients, it found that sOX40 levels were elevated in patients with acute ATL and proposed as an additional diagnostic marker. However, sOX40L levels were not detected in any plasma sample (<2 pg / mL) [14].

Conclusion

Apart from PD-1 blockade, other immune control points were considered important in immunotherapy [15]. They observed long-term clinical outcomes and immune responses by blocking CD40 and CTLA-4 in patients with metastatic melanoma. The results, although promising, have been associated with increased T cell infiltration without therapeutic PD-1 / PD-L1 blockade, And they also have shown that beyond PD-1 there may be other opportunities for immune activation in cancer immunotherapy [15].

In our study, rising CD40 and CTLA-4 levels in peripheral blood may be associated with immune escape, accelerated disease progression, and poor prognosis in NSCLC. Further analyses with larger groups should be done to find out possible correlations between these molecules and the risk for NSCLC.

Key Messages:

- Immune control points consist of inhibitory and stimulatory pathways that help to limit inflammation and cancer cells must avoid these molecules to survive.
- The identification of immune biomarkers that significantly prolong survival is very important in the treatment of NSCLC.
- Increased levels of CTLA-4 and CD40 levels in T cells in peripheral blood may be associated with immune escape, accelerated disease progression, and poor prognosis in NSCLC.

Competing Interests: No competing interests was declared by the authors.

Ethics Committee Approval:" Clinical Investigations Ethics Committee of Istanbul Education and Research Hospital" was approved by ethics committee.

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OP28

Investigation Of Critical Antioxidant Enzyme Genes Variants In Polycystic Ovarian Syndrome Patients

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Introduction

Aim: Polycystic ovary syndrome (PCOS); is a complex, chronic, metabolic disease characterized by anovulation and hyperandrogenism, affecting approximately 5-10 % of reproductive period (1).

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Estrogen and its metabolites play a role in tumor development with direct damage to DNA. At the same time estrogen and its metabolites enter the redox cycle and form oxygen radicals, which cause oxidative stress, lipid peroxidation and cause DNA damage (2-4). Polymorphisms in genes encoding antioxidant enzymes cause various diseases (5). Oxidant and antioxidant system, which are important in te physiological process in organism, have many roles on female reproductivities. Infertility etiopathogenesis play a role in oxidative stress in women. Oxidative stress has been shown to play a role in the development of reproductive diseases such as polycystic ovary syndrome, endometriosis and unexplained infertility (6). Nitric oxide (NO) is a mediator role in reproductive events; NO is one of the many intraovarian agents involved in the ovary. NO as an antioxidant, may play a role in pubertal maturation, ovulation capacity, early embryological development, gestational continuation and menopause timing, as well as relaxation in vascular smooth muscles, as compared to preliminary studies in humans, and also NO has vasodilation effect (7,8). Another antioxidant gene is glutathione peroxidase 1 (GPX1) gene that is expressed in prostate, breast and reproductive tracts cells and protects them against oxidative damage. Low GPX1 activity increases oxidative stress increases susceptibility to various diseases and cancers (9). Superoxide dismutase 2 (SOD2) directly converts superoxide radical to hydrogen peroxide and molecular oxygen. It has been shown that polymorphism inducing genes encoding SOD enzyme are predisposed to Behçet, diabetes and various types of cancer (10). Another antioxidant gene is Catalase. Catalase; in the glycoprotein structure, it is a hemoprotein composed of four subunits and mainly found in the cytoplasm and endoplasmic reticulum of the cell. Particularly when the amount of H_2O_2 is

excessively increased, it enters the circuit and turns this molecule into water with a great specificity (11,12). We investigated the effectiveness of the polymorphism of genes encoding antioxidant enzymes such as SOD2, GPX1, CAT and eNOS in the etiopathogenesis of PCOS.

Methods

The patient groups of our study was composed of 100 female patients aged 15-39 years who applied to Bolu Abant Izzet Baysal University Medical Faculty Obstetrics and Gynecology Department and clinically diagnosed as PCOS. Ethics committee approval for the study was obtained from Istanbul University Clinical Research Ethics Commitee. Informed consent was obtained from all individual participants included in the study. When the control group was established, care was taken to ensure that therewas no evidence of clinical or biochemical hyperandrogenism with a regular mens cycle, and that they did not have diagnostic criteria for PCOS. Peripheral venous blood of the subjects included in the study were taken with the tubes with ethylenediaminetetraacetic acid (EDTA). Subsequently, genomic DNAs were isolated at molecular genetics laboratory of our deparment using appropriate isolation kit. Polymerase chain reactions (PCRs) were performed under appropriate conditions using the isolated DNAs, and primers designed for the gene regions. The PCR, restriction enzyme digestion, and electrophoresis procedures of the study were carried out at Istanbul University, Aziz Sancar Experimental Medical Research Institute, Molecular Medicine Department. In addition, fasting blood sugar (FBS), insulin, thyroid stimulating hormone (TSH), free triiodothyronine (free T3), free thyroxine (free T4), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very-low density lipoprotein (VLDL), luteinizing hormone (LH), estradiol 2 (E2), prolactin hormone, progesterone, testosterone,

dehydroepiandrosterone (DHEAS), follicle-stimulating hormone (FSH) and waist circumferencewere evaluated.

Statsitical analysis of the data was performed using the SPSS 17.0 package program. Chi-square test was used to compare categorical variables. In comparison of continuous variables between groups, it was determined whether they were parametric or non-parametric by shapiro Wilk test. Student's t test was then performed for parametric subjects and Mann Whitney U test for non-parametric subject. The analysis of dependent and independent variables was performed by Binary Logistic Regression analysis. Assessment of risk factors for all genotypes and alleles belonging to the gene was performed using the Kruskall Wallis test, provided that the groups were within the group.

Results

Significant results were found for the weight and waist circumference of patients and control groups (p<0,001 and p <0,001). There was no significant difference between the groups in terms of age and height (p = 0,085 and p = 0,243) . In terms of biochemical parameters, TG (p <0,001), VLDL (p <0,001), free T3 (p=0,027), FSH (p<0,001), DHEAS (p=0,006), testosterone (p<0,001), fasting blood sugar (p=0,027), insülin (p=0,001). As a result of analysis of the polymorphisms in the genes, polymorphisms in GPX1 (p = 0,002), eNOS (p = 0,001), CAT (p = 0,031) genes were found to be significant among the groups. When the genotypes and alleles of the genes were evaluated, SOD2 (TT), GPX11 (TT) and eNOS (TT) homozygous mutation genotypes were statistically significant between the groups (p = 0,024 and p = 0003) while CAT (TT) showed no significant difference between the groups for homozygous genotypes (p =0,262 and p = 0,535). In addition, GPX1 (TT) and eNOS (TT) genotypes alone and in patients and controls were evaluated with

other risk factors. The genotype of homozygous mutation in GPX1 gene was found to be significant among the groups. However, GPX1 homozygote mutation was also found to be significant when compared to PCOS risk factors. In addition, GPX1 homozygote mutation genotype, TG, FSH and DHEAS were found to be significant in PCOS (p=0,001, p = 0,005 and p = 0,026). Significant results were obtained when the presence of the eNOS homozygous mutation genotype and the risk factors of PCOS were evaluated together. TG, FSH and DHEAS were statistically significant in the presence of the eNOS homozygous mutation genotype (p<0,001, p = 0,014 and p = 0,005). When the risk factors for PCOS were evaluated in the presence of heterozygous genotypes of the genes, it was found that GPX1 CT was significant (p =0,038), and SOD2 TC, eNOS GT and CAT AT genotypes were not significant. (p = 0,301, p =0,403 and p = 0,733). TG, FSH and DHEAS are significant risk factors. (p=0,001, p = 0,007 and p = 0,013). The GPX1 heterozygous genotype was found to be significant when assessed by PCOS risk factors and heterozygous genotypes of other genes. In addition, when GPX1 CT genotype was analyzed together with PCOS risk factors, TG, FSH and DHEAS were significantly found in the presence of GPX1 heterozygous genotype (p = 0,001, p = 0,005 and p = 0,014). The analysis PCOS risk factors, which is significant with the disease-associated mutant alleles, was found to be significant among the mutant allele groups in the eNOS gene (p = 0,007).

The combination of SOD2 TT homozygous mutation with GPX1 TT, eNOS TT and CAT TT homozygous mutations did not show any significance for PCOS in the patient and control group (p = 0,346, p = 0,577 and p = 1,000). In addition, the association of genotype GPX1 TT homozygous mutation with eNOS TT and CAT TT homozygote mutation genotype was found to be significant for PCOS among the groups (p <0,001, p

<0,001). In addition, the combination of the eNOS TT homozygous mutation with the CAT TT genotype was found to be significant (p <0,001). When the combined analysis of SOD2 gene and CATgene was performed, it was found that there was a significant difference between SOD2 heterozygous genotype carriers and CAT wild type genotype carriers. The proportion of patients with SOD2 heterozygote genotype carriers and CAT wild type genotypes was found to be more significant (p = 0,048). When combined with GPX1 and eNOS genotypes, GPX1 mutant genotype carriers and eNOS mutant genotype carriers showed a significant difference in disease-related groups (p = 0,001). In addition, it can be said that the risk of disease may be low even with the GPX1 heterozygote genotype and eNOS wild type genotype. GPX1 heterozygous genotype carriers and CAT wild genotype carriers were found to be significant in terms of protection against disease when combined genetic analysis of GPX1 and CAT gene genotypes were performed (p = 0,001). GPX1 heterozygote genotype and CAT wild type genotype association; It is found in 10% in control group and 1% in patient group. The significance of the combinations of heterozygous genotypes in the genes between the groups in terms of PCOS was evaluated by chi-square test. The combination of the SOD2 TT homozygous mutation with the GPX1 TT, eNOS TT and CAT TT homozygous mutations did not appear to be meaningful in terms of PCOS in the patient and control group. There was no statistically significant difference (p = 0,315, p = 0,440, p = 1,000) as a result of the combination of SOD2 heterozygous genotype with GPX1, eNOS and CAT heterozygote genotype. The combination of the GPX1 CT heterozygous genotype with the eNOS GT genotype and the CAT AT genotype did not yield any conclusive results (p=0,063, p= 0,083). In addition, there was no significant result in the co-transformation of eNOS GT heterozygote

genotype and CAT AT heterozygote genotype (p = 0,050).

Conclusion

When the different genotypes in the genes are analyzed in combination, and when the different genotypes come together, the activity in the disease varies greatly. It will be possible to better understand the role of polymorphisms in genes encoding antioxidant enzymes in the etiopathogenesis of PCOS in future studies with a more comprehensive patient and control group. In our study, statistically significant results have been found in terms of obesity and weight in our patient group. In our study, HDL level in PCOS was found to be lower than control group. TG level was higher in PCOS than control group and a meaningful result was obtained. In this study, dehydroepiandrosterone sulphate and testosterone levels were also found to be significant among the groups. In addition, the amount of free testosterone is increasing. Increased free estradiol and free estradiol lead to suppression of FSH levels and increased LH in women with PCOS. In this study, it can be said that FSH hormone is protective against the disease in PCOS. TG, FSH and DHEAS were found to be significant in the presence of SOD2 homozygous mutation genotype. when we evaluated the mutant allele combination of eNOS and T mutant allele in SOD2 gene, significant results were obtained in terms of PCOS. In addition, when combined analysis of SOD2 gene and CAT gene was found, there was a significant difference between groups carrying SOD2 heterozygote genotype and CAT wild type genotype. Regression analysis of the risk factors in PCOS by homozygous mutation of GPX1 and other genes showed that the presence of the GPX1 homozygote mutation genotype (TT) was significant and pose a risk to the disease. Significant results were obtained in terms of Catalase-21A / T gene polymorphism between control and patient groups in

our study. Together with heterozygous genotype of catalase homozygous mutation genotype and other genes, TG, FSH and DHEAS were found to be statistically significant. The genotype of the eNOS homozygote mutation was also found to be significant when the eNOS gene was evaluated together with PCOS risk factors and homozygous mutations in other genes

Key Messages:

Polymorphisms in genes encoding antioxidant enzymes cause various diseases. Oxidant and antioxidant system, which are important in te physiological process in organism, have many roles on female reproductivities. Infertility etiopathogenesis play a role in oxidative stress in women. Therefore, we investigated the effectiveness of the polymorphism of genes encoding antioxidant enzymes such as SOD2, GPX1, CAT and eNOS in the etiopathogenesis of PCOS.

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OP29

Enhancing sensitivity of qPCR assays targeting *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* by using a mutant Taq DNA polymerase

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İntroduction

The leading causative agents of bacterial meningitis infection are *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae*. If bacterial meningitis is not appropriately treated it can lead to stoke, paralysis and death (1). Mortality rates are as high as 30% in developed countries and up to 50% in developing countries (2). Post-treatment onset of bacterial meningitis sequelae, such as severe neurological or motor impairment, occurs in almost half of all survivors (3).

Although global vaccination programmes have significantly reduced the occurrence, approximately 1.2 million cases are reported annually, with increasing incidents of drug resistance leading to treatment failures (3,4). Also, the vaccination programmes have led to bacterial serotype replacement which has resulted in emergence of non-vaccine or non-typeable bacterial meningitis incidence (2).

Rapid and accurate diagnosis of bacterial meningitis infection supports targeted antimicrobial treatment that prevents antimicrobial-resistance dissemination and lower associated disease-burden (5). The microscopic identification has poor diagnostic sensitivity and specifity (2). Culture methods are time-consuming and unreliable if antimicrobial agents have been administered before samples are collected (6).

Nucleic acid amplification-based methods for rapid, accurate and highthroughput diagnosis of bacterial meningitis such as Loop-mediated isothermal amplification (LAMP) (7), recombinase polymerase amplification (RPA) (8) and real-time polymerase chain reaction (PCR) (9) are capable of detecting non-viable pathogens and rapidly becoming the diagnostic method of choice over conventional methods in clinical settings (2,10,11). Real-time PCR is also known as quantitative real-time PCR (Q-PCR/qPCR) is the current "gold standard" molecular diagnostic method, with clinical sensitivity and specificity of up to 100% (2,9). Although LAMP and RPA based methods offer faster diagnosis using lower-cost equipment, their consumable costs are much higher than the qPCR-based methods. The main cost difference is originated from type, concentration and number of the enzymes used for the each amplification technology (7–9). Relative cost of the enzymes is roughly 1:2:3 for qPCR:LAMP:RPA which implies that qPCR is more suitable for high-throughput central analysis laboratories and LAMP and RPA are more suitable for the point of care diagnostics.

Taq DNA polymerase is the most extensively used DNA polymerase for PCR (12,13). Protein engineering techniques are powerful ways to create mutant enzymes from the known DNA polymerases (14). It was reported that Taq DNA polymerases with mutations at amino acid sequence positions 742 and 743 show improved primer extension ability, DNA affinity and PCR performance (15). Arginine at amino acid sequence position no 536 of the wild type Taq DNA Polymerase is part of the DNA binding site (16). Our research group changed arginine at the position 536 into tryptophan to produce a mutant Taq DNA

Polymerase with enhanced DNA affinity and PCR performance (Patent application; Turkish Patent Institute 2018/10825). In this study, the mutant and the wild type enzymes were integrated into qPCR-based assays that are based on Centers for Disease Control and Prevention (CDC) protocols. Analytical performance of the enzymes were comparatively evaluated for detection and typing of *S.pneumoniae*, *N.meningitidis* and *H.influenzae*.

Conclusion

Our results have shown that it is possible to enhance analytical sensitivity of qPCR assays targeting *S.pneumoniae*, *N.meningitidis*, *H.influenzae*, *N.meningitidis* serogroups A, B, C, W135, X, Y, *H.influenzae* serotypes A, B, C, D, E, F and *S. pneumoniae* serotypes 1, 3, 4, 5, 6, 7, 8, 9VA, 12, 14, 15AF, 15BC, 17, 18, 19A, 19F, 23F, 33AF by using an engineered Taq DNA polymerase that has arginine instead of tryptophan at the amino acid position 536. Our results have also revealed that *S.pneumoniae*, *N.meningitidis* and *H.influenzae* at the lower concentration limit of medical decision point can directly be identified in csf and serum samples using the qPCR assays containing the mutant enzyme.

KEY MESSAGES

Substitution of arginine at the position 536 into tryptophan enhances PCR performance of Taq DNA polymerase.

Bacterial meningitis agents at very low concentrations can directly be identified in csf and serum samples by using the mutant Taq DNA polymerase.

Rapid and accurate diagnosis of bacterial meningitis infection allows medical decisions to be made in a timely manner.

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OP30

Development of a real-time PCR panel targeting 32 DNA polymorphisms related to athletic performance

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Introduction

Humans vary in their success in sports, and this variability mostly depends on genetic factors. More than 155 genetic markers were found to be linked to elite athlete status (1). Importantly, 41 markers were identified within the last years by performing genome-wide association studies (GWASs) that represent a promising and productive way to study sports-related phenotypes. Future research, including multicentre GWASs and whole-genome sequencing in large cohorts of athletes with further validation and replication, will substantially contribute to the discovery of large numbers of the causal genetic variants (mutations and DNA polymorphisms) that would explain the heritability of athlete status and related phenotypes.

Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variation. An SNP is a single base-pair mutation at a specific locus, usually consisting of two alleles. Because SNPs are conserved during evolution, they have been proposed as markers for relating the phenotypic data to the genotypic data. The increase of interest in SNPs has been reflected by the furious development of a diverse range of SNP genotyping methods such as microarray, real-time PCR (qPCR), high resolution melting (HRM), restriction fragment length polymorphism (RFLP), Sanger DNA sequencing and new generation sequencing (NGS) (2–

6). NGS and microarray provide the fastest and the most cost-effective solutions for studies analyzing a large number of SNPs such as GWASs. If the analyzed genome region is limited, Sanger DNA sequencing offers a cost-effective solution. HRM, qPCR and RFLP are the most cost-effective and fastest methods to screen e few or dozens of SNPs. Although the instrumentation costs of qPCR and HRM are higher than that of RFLP, they are relatively much faster technologies.

QPCR-based methods for SNP/mutation detection are broadly categorized into two types (3). The first category is polymorphic or mutant allele-directed specific analysis using primers matched with substituted nucleotide or using oligonucleotides to block or clamp the nontargeted template. The second category is melting curve analysis, which is combined with hydrolysis probes, hybridization probes, or double-stranded DNA-binding fluorescent dyes. Novel approaches have made qPCR-based SNP detection methods increasingly more sophisticated. These advances include simplification of nucleic acid preparation and improvement of the subsequent amplification steps and cost-reduction of qPCR instruments such that testing may be performed with relative ease in various clinical settings (3).

It is possible to amplify DNA directly from various clinical sample types using amplification facilitators, such as betaine, polyethylene glycol (PEG), and bovine serum albumin (BSA) (7). By eliminating the need for nucleic acid extraction using a mixture of PCR facilitators, we developed a new qPCR-based panel to reduce the analysis costs and labor-intensive sample preparation steps for analyzing the well-known 32 different DNA polymorphisms related to athletic performance.

Discussion

Our results showed that qPCR offers as sensitive and reliable variant detection as Sanger DNA sequencing does. It was previously reported that compared with Sanger, NGS and qPCR assays have significantly higher sensitivity, as Sanger failed to detect variants with mutation rates lower than 15% (8). Furthermore, compared with Sanger, qPCR offers easier workflow, lower consumable and instrumentation costs, and shorter assay times.

When comparing NGS vs. qPCR technologies, the key differences are discovery power, sample throughput, and cost-effectiveness. While both offer highly sensitive and reliable variant detection, NGS is time-consuming and less cost-effective for sequencing low numbers of targets (1–30 targets). Furthermore, capital equipment already placed in most labs for qPCR while the high-cost NGS instrumentation placed in limited numbers of labs. On the other hand, qPCR can only detect known sequences. In contrast, NGS is a hypothesis-free approach that does not require prior knowledge of sequence information. NGS provides higher discovery power to detect novel genes and higher sensitivity to quantify rare variants and transcripts (8).

Conclusion

It was previously is estimated that the application of physical, mental, physiological, body composition and genetic tests all together while determining the talent selection in sports is the most effective method to detect talented athletes (9). In this study, the developed qPCR test and the Sanger DNA sequencing were applied to 23 young athletes from ENKA Sports Club (Turkey) whose genetic profiles, physical characteristics, and basic motor skills were evaluated together to improve their athletic training process. In this study, we did not attempt to question the utility of the genetic profiles for talent identification or sports

event selection or training process assistance. We instead questioned the utility of the qPCR assays for correct identification of the genetic profiles. Our results showed that qPCR offers as sensitive and reliable variant detection as Sanger DNA sequencing does. Compared with Sanger, qPCR offers easier workflow, lower consumable and instrumentation costs, and shorter assay times.

The athletic success depends on a combination of genetic, physiological, behavioral, and environmental factors. One should consider genetic testing not only for talent identification or sports event selection but also for possible assistance in the training process itself. The developed method allows the trainers to use genetic profiling more often by lowering the high cost and the labor-intensive laboratory work of DNA sequencing-based technologies.

Key messages

QPCR is as sensitive and reliable as Sanger DNA sequencing is for screening genetic variations related to athletic performance.

Compared with Sanger DNA sequencing, qPCR offers easier workflow, lower consumable and instrumentation costs, and shorter assay times for screening SNPs.

It is possible to eliminate nucleic acid extraction before qPCR using PCR facilitators such as PEG 200, BSA, betaine, tween 20, and sodium azide.

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OP31

Evaluation of four novel multiplex real-time PCR panels for syndromic testing

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Introduction

Development of commercial panel-based molecular diagnostics for the rapid detection of pathogens in blood, respiratory specimens, stool, and cerebrospinal fluid (csf) has resulted in a paradigm shift in clinical microbiology and clinical practice (1). Molecular assays that simultaneously detect and identify multiple pathogens associated with clinical syndromes, such as bloodstream, respiratory, gastrointestinal (GI), or central nervous system (CNS) infections have the clear advantages of rapid turnaround time and the detection of a large number of microorganisms and promise to improve health care.

Molecular syndromic testing technologies have also introduced challenges, including cost and the definition of ideal test utilization strategies and test interpretation (1). Current clinical practice guidelines do not provide guidance as to how results should be interpreted. Clinicians may not be familiar with all organisms and/or resistance genes detected. Panel compositions vary somewhat between manufacturers; their generally fixed panel composition may present challenges in certain circumstances. The design of these multiplex platforms, even those marketed to be closed systems, carries a risk of contamination, which may be challenging to recognize. Additional challenges include determining how multiplex panels should be integrated into laboratory workflows as well as how results should be monitored for accuracy following implementation.

It is anticipated that syndromic testing will likely be utilized more in the future (1). It is important to have a clear understanding of the performance characteristics and limitations when implementing multiplex assays. In this study, we evaluated the analytical and clinical performance of four novel multiplex real-time PCR (qPCR) assays to diagnose pathogens directly in whole blood, csf, respiratory specimens, and stool.

Conclusion

The Bio-Speedy® qPCR syndromic testing platforms have similar advantages and disadvantages of the semi-automated syndromic testing platforms over the fully automated ones. The semi-automated systems offer a cost and time effective solution for clinical microbiology laboratories testing a high number of samples, whereas the fully automated systems are the only options for the urgent clinical cases and the point-of-care diagnostics. Although the fully automated systems have a clear advantage of a much faster and easier run, the semi-automated platforms analyze much more samples per run with much lower costs per sample. The higher testing capacity and lower cost of the semi-automated systems also allow screening of a wider range of pathogens per assay.

All the off-panel targets of the Bio-Speedy® qPCR syndromic tests are the clinically relevant pathogens. When the prevalence and pathogen-specific treatment options of the off-panel microorganisms (2) were evaluated together, it was concluded that the exclusion of *Candida* spp., *Acinetobacter* spp., and *S. maltophilia* in the Bio-Speedy® sepsis panel is a drawback.

Sensitivity and specificity of the Bio-Speedy® qPCR panels for syndromic testing were in the higher ranges of the previously reported clinical performances. The Bio-Speedy® qPCR assay performed directly on whole blood may complement blood culture, by yielding highly sensitive and specific results in less than 3 hours. Rapid detection and identification of a causative pathogen of bacteremia and severe sepsis leads to timely initiation of adequate antibiotic treatment which is associated with decreased morbidity and mortality, limited use of inappropriate antimicrobial drugs, reduced development of antibiotic resistance and reduced healthcare costs (3–6).

Health care providers now have the option of ordering a single test designed to detect a number of organisms associated with an infectious syndrome, rather than ordering a series of individual, pathogen-specific assays. Sensitive and specific analysis of the high number and variety of samples in a single run using the Bio-Speedy® molecular testing panels enables health care providers to rapidly and cost-effectively diagnose certain infections. The timely diagnosis of infectious diseases may influence decisions regarding antimicrobial therapy, antimicrobial stewardship, and infection prevention and control.

Key messages

The evaluated molecular testing panels allow sensitive and specific detection of pathogens directly in blood, stool, cerebrospinal fluid, and respiratory specimens.

Analysis of the high number and variety of samples in a single run using the evaluated molecular testing panels enables health care providers to rapidly and cost-effectively diagnose the infections.

Candida spp., *Acinetobacter* spp., and *S. maltophilia* must be added to the Bio-Speedy® qPCR sepsis panel for better coverage of the relevant pathogens

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OP32

Development of a new multiplex real-time PCR assay for rapid screening of hospital-acquired infection agents

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Introduction

Nosocomial infection is defined as infections that occur in a hospitalized patient for various reasons, which do not occur at the time of admission to the hospital and develop within 48-72 hours after hospitalization or within ten days after discharge (1). Nosocomial infection increases the length of hospital stay, treatment costs, and labor loss and threatens healthcare workers as well as patients. It may cause infections with a high mortality rate in all risky patients, especially immunocompromised patients. These infections cause increasing treatment costs and long hospitalization periods and threaten health care in hospitals.

Carbapenem-resistant *Enterobacteriaceae* (CRE), methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) are the leading causes of nosocomial infections (1). It is the most effective infection control method to detect these pathogens quickly without spreading in the hospital and to prevent them from spreading by contact isolation.

Detection of nosocomial infection agents can be performed effectively by real-time PCR (qPCR), which is known to provide much faster results compared to conventional culture-based methods and more sensitive than rapid immunological tests. Commercial molecular tests for screening nosocomial infection agents (BD MAX™, Xpert®, cobas®, ELITe MGB®, GenoQuick®, eazyplex®) do not require any culture steps before detection and can be completed in hours. Many in-house tests have also been developed for molecular screening of nosocomial infection agents (2–4). With the commercial and in-house tests, the agents can be screened with 90% to 100% specificity and sensitivity. Since these tests require expert and experienced personnel, fully automated systems such as Cepheid Xpert® platform have been widely used all over the world (5,6). The most important reason why these tests do not find widespread use in developing countries is high analysis costs.

It is possible to amplify DNA directly from blood and feces (7), and swab samples (8) using amplification facilitators, such as betaine, polyethylene glycol (PEG) and bovine serum albumin (BSA). To reduce the analysis costs and labor-intensive sample preparation steps, we developed a new qPCR assay to directly detect nosocomial infection agents in nasal and rectal swab samples in 1.5 hours without the need for nucleic acid extraction, by using a mixture of PCR facilitators.

Conclusion

The clinical performance of the developed qPCR assays was found to be as high as that of the gold standard methods. Elimination of the nucleic acid extraction step reduced the analysis cost and duration by at least 30% and resulted in an easier qPCR assay workflow. In this context, the study has achieved all its objectives successfully.

The easy to use, fast and reliable commercial test systems used for molecular analysis of nosocomial infection agents have high unit costs, which is the biggest obstacle to the widespread use of such systems in developing countries. Fast, easy-to-use,

and low-cost qPCR assays developed in this study have the ability to screen antibiotic-resistant hospital-acquired infection agents in infected/colonized patients with an equal clinical performance of the culture-based methods. The greatest success that can be achieved via the wider application of fast molecular screening tests in infection control is to quickly decide which patient should be kept in contact isolation in cases where hospital infection management is performed with limited resources and to prevent the cost of unnecessary isolation applications.

Key messages

Commercial molecular screening assays for nosocomial infection agents have high unit costs, which is the biggest obstacle to the widespread use of such systems.

Elimination of the nucleic acid extraction step significantly reduced the molecular analysis cost and duration and resulted in an easier qPCR assay workflow.

The clinical performance of the developed qPCR assays was found to be as high as that of the gold standard methods.

Clostridium difficile and its toxin genes must be added to the qPCR assay's target list.

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OP33

A cost-effective real-time PCR panel for rapid assessment of genetic obesity risk

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Introduction

Genome-wide association studies (GWAS) for body mass index (BMI), waist-to-hip ratio, and other adiposity traits have identified more than 300 single-nucleotide polymorphisms (SNPs) (1). Genes near loci regulating total body mass are enriched for expression in the central nervous system (CNS), whereas genes for fat distribution are enriched in adipose tissue itself. The studies have revealed that our increasingly obesogenic environment might be amplifying genetic risk for obesity, which is causally associated with diabetes, coronary heart disease, specific cancers, and other conditions.

Many studies constructing genetic risk scores for BMI used the 32 SNPs reported in the 2010 GIANT meta-analysis (2). A BMI genetic risk score based on these 32 loci was generated in more than 8000 individuals (3). A genetic risk score is the sum of BMI-increasing alleles (0, 1, or 2) at each of the SNPs robustly associated with BMI.

The increase of interest in SNPs has been reflected by the development of a diverse range of SNP genotyping methods such as microarray, real-time PCR (qPCR), high resolution melting (HRM), restriction fragment length polymorphism (RFLP), Sanger DNA sequencing and next-generation sequencing (NGS) (4–8). NGS and microarray provide the fastest and the most cost-effective solutions for studies analyzing a large number of SNPs such as GWASs. If the analyzed genome region is limited, Sanger DNA sequencing offers a cost-effective solution. HRM, qPCR and RFLP are the most cost-effective and fastest methods to screen e few or dozens of SNPs. Although the instrumentation costs of qPCR and HRM are higher than that of RFLP, they are relatively much faster technologies.

QPCR-based methods for SNP/mutation detection are broadly categorized into two types (5). The first category is polymorphic or mutant allele-directed specific analysis using primers matched with substituted nucleotide or using oligonucleotides to block or clamp the nontargeted template. The second category is melting curve analysis, which is combined with hydrolysis probes, hybridization probes, or double-stranded DNA-binding fluorescent dyes. Novel approaches have made qPCR-based SNP detection methods increasingly more sophisticated. These advances include simplification of nucleic acid preparation and improvement of the subsequent amplification steps and cost-reduction of qPCR instruments such that testing may be performed with relative ease in various clinical settings (5).

It is possible to amplify DNA directly from various clinical sample types using amplification facilitators, such as betaine, polyethylene glycol (PEG), and bovine serum albumin (BSA) (9). By eliminating the need for nucleic acid extraction using a mixture of PCR facilitators, we developed a new qPCR-based panel to reduce the analysis costs and labor-intensive sample preparation steps for analyzing the well-known 32 different DNA polymorphisms related to obesity risk.

Conclusion

Our results showed that It is possible to eliminate nucleic acid extraction before qPCR using PCR facilitators. This elimination simplified qPCR-based SNP screening workflows. Our results also showed that qPCR offers as sensitive and reliable variant detection as Sanger DNA sequencing does. Compared with Sanger, qPCR offers easier workflow, lower consumable and instrumentation costs, and shorter

assay times, which allows the dietitians and clinicians to use genetic profiling more often.

Most SNPs incorporated into genetic risk scores were discovered in Europeans, possibly limiting their applicability to other populations (1). In this study, we showed that these SNPs can be applied to the Turkish population for the assessment of genetic obesity risk and genetic risk scores are useful tools for elucidating obesity risk.

Key messages

QPCR is as sensitive and reliable as Sanger DNA sequencing is for screening genetic variations related to obesity.

Compared with Sanger DNA sequencing, qPCR offers easier workflow, lower consumable and instrumentation costs, and shorter assay times for screening SNPs.

It is possible to eliminate nucleic acid extraction before qPCR using PCR facilitators such as PEG 200, BSA, betaine, tween 20, and sodium azide.

Genetic risk scoring based on SNPs discovered in Europeans is also a powerful tool for elucidating obesity risk in the Turkish population.

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OP34

Investigation of the Effectiveness of ITRT Method (ATP-Tumor Chemosensitivity) in Colon Cancer Patients for Patient-Specific Drug Selection

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Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide (1) and in many countries the standard treatment for high-risk stage II and stage III colon cancer (CC) is surgery followed by adjuvant chemotherapy (2,3). That is why effective and less toxic treatments are required to prevent recurrence and prolong survival of the CRC patients after surgery. The benefit of adjuvant chemotherapy has been clearly established in the adjuvant setting for node-positive colon cancer. Standard treatment options include fluorouracil (FU) or capecitabine with or without oxaliplatin. Data from randomized clinical trials (RCTs) have demonstrated substantial benefits of chemotherapy in stage III CC patients, with estimates for reduction in all-cause mortality ranging from 24% to 52% (5-7). 5-Fluorouracil (5-FU), as single agent or in combination, has been the mainstay of medical treatment for CRC for over 40 years. This is due to its relatively low toxicity and the inability of newer drugs, used as single agents, to achieve significantly better response rates. The response rates and overall survival achievable with this combination are 18–22% and 12–15 months, respectively (8,9). In combination with 5-FU, oxaliplatin and irinotecan have produced response rates of up to 50%, but this has not translated into better overall survival.

Aim

The aim of our study is to predict clinical chemotherapy sensitivity of colon cancer in the adjuvant setting by using Individualized Tumor Response Test (ITRT) (ATP-Tumor Chemosensitivity, Oncogramme) and provide reference for clinical treatment.

Materials and Metods

In this prospective single center study, we enrolled 47 preoperatively diagnosed locally advanced CC patients between August 2014 and August 2016. The biopsies were taken from the luminal surface of the resection specimens by a pathologist or a surgeon. The solid tumor material was minced into small fragments, dissociated with collagenase overnight, purified to remove excess debris and resuspended in serum-free complete assay medium containing antibiotics. The cells were then counted and assessed for viability. The final cell suspension was made up to a concentration of 20 000 cells/ml for solid tumors. Round-bottomed polypropylene 96-well plates were prepared with CAM and cytotoxic drugs at six dilutions (6.25%, 12,5%, 25%, 50%, 100%, 200%) of the test drug concentration (TDC) in triplicate. The TDC for each drug has been previously calculated from pharmacokinetic and response data (Table 1) (10). After 6 days of incubation at 37°C at 100% humidity in 5% CO_2, the cells were lysed with a detergent-based Tumor Cell Extraction Reagent and the ATP content was measured by using a microplate luminometer.
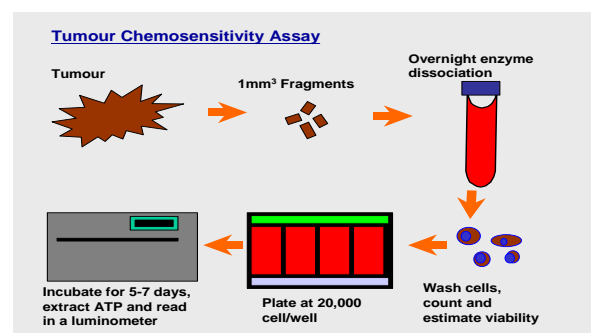
Figure 1. Tumor Chemosensitivity Assay

Table 1 TDC for each drug

Drug	100% TDC (mcg/ml)
5-Fluorouracil (5-FU)	22,5
Irinotecan	14,0
Oxaliplatin	1,8

Results and Conclusion

In the end we have enrolled 23 patients into the study out of 47 patients. Eight patients were excluded,

because they did not receive chemotherapy due to early stage colon cancer, which was revealed by the pathologic report. The other remaining 16 patients were excluded because of plate infection.
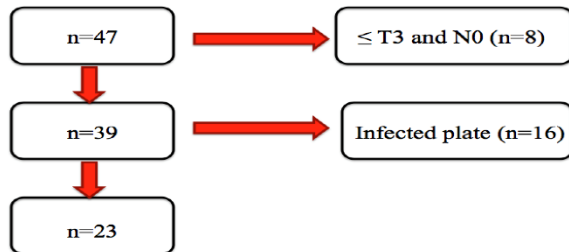

Figure2.

The evaluability rate of colorectal specimens was 59% (23 of 37). The failures were due to infection of the plates and were experienced at the beginning of the study. As a result, additional antibiotics were added to the medium to cover anaerobic and fungal infections. For comparison between drugs and tumors, an IndexSUM < 250, representing an average 50% inhibition across all concentrations tested, was used to indicate sensitivity. All single agents tested were active on the basis of the IndexSUM <250 threshold (Fig. 3). The most active single agent tested was 5-FU, followed by oxaliplatin and irinotecan (Table 2).

Table 2 Median values for each drug and combination in both groups

	Resistant		Sensitive		Z	p
	Mean	Std Dev	Mean	Std Dev		
5-FU Index	407,58	115,415	198,27	114,203	3,323	0,001
Oxaliplatin Index	510,83	106,771	319,64	136,795	2,955	0,003
Irinotecan Index	522,33	128,212	343,36	140,903	2,770	0,006
5-FU + Irinotecan Index	478,83	211,297	263,64	129,924	2,523	0,012
CEA	11,40	20,145	5,41	6,648	0,816	0,414
CA19-9	107,33	272,007	18,44	10,632	0,163	0,870
Tumor size	53,75	20,145	52,82	12,552	0,526	0,599
Lymph node (LN)	2,75	3,980	1,00	1,549	0,546	0,585
Overall Survival	0,73	0,467	0,90	0,316	0,982	0,326
Disease-free Survival (DFS)	32,30	18,139	31,00	11,681	0,076	0,940

The most striking finding was the correlation between the 5-FU index value, which is the standard chemotherapeutic agent in the treatment of colon cancer, and the presence of metastatic or non-metastatic lymph nodes in terms of susceptiblility to 5-FU in both group. This remarkable finding has a relatively high correlation coefficient and a statistically

significant p value (*p=0.042, p=0.003*). The correlation with metastatic lymph node was negative. That means, increased index values (relatively poor effect) are associated with decreased metastatic lymph nodes. Similarly, there was a negative correlation between oxaliplatin index value and lymph nodes in the drug sensitive group *(p=0,027).*

Table 3 Correlation among chemotherapeutical agents and clinical parameters in both gorups

Drug Resistant Group	CEA	TS	LN	OS	DFS
	p	p	p	p	p
5-FU Index	0,488	0,879	**0,042**	0,088	0,283
Oxaliplatin Index	0,356	0,083	0,154	0,250	0,177
Irinotecan Index	0,286	0,315	0,252	0,493	0,455
5-FU + Irinotekan Index	0,356	0,251	0,035	0,813	0,544

Drug Sensitive Group	CEA	TS	LN	OS	DFS
	p	p	p	p	p
5-FU Index	0,934	0,572	**0,003**	0,541	0,688
Oxaliplatin Index	0,276	0,289	**0,027**	0,698	0,920
Irinotecan Index	0,138	0,905	0,809	0,099	0,650
5-FU + Irinotecan Index	0,556	0,198	0,914	0,361	0,868

ATP-TCA may be an ideal method of in vitro drug sensitivity testing, which could effectively predict clinical chemotherapy sensitivity before the application of adjuvant chemotherapeutical agents in the postoperative setting. Due to this method there could be a paradigm shift towards *''tailored targeted therapy''*. However, before clinical application and standardization, the recently discovered method should be validated by more phase I clinical trials.

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**ABSTRACTS OF
ORAL PRESENTATIONS**

OP1

Investigation of miR-34a Target Genes in Multiple Myeloma Cell Lines

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Abstract

Differential expression of miR-34a, a well-known tumor suppressor microRNA, directly or indirectly alters the expression of a large number of oncogenes in cancer cells. Reduced miR-34a expression in tumors was reported to contribute to carcinogenesis processes by targeting various genes including *WNT1*, *CDK4,* and *SOX2*. In this study, we aimed at investigating the interaction between miR-34a and its targets *WNT1, CDK4* and *SOX2* by performing functional studies on RPMI8226 and U266 Multiple Myeloma (MM) cells.

We overexpressed miR-34a in RPMI8226 and U266 cells and evaluated its effects on cell proliferation by WST-8 technique. Differential expression of *WNT1, CDK4* and *SOX2* as well as epithelial mesenchymal transition (EMT) marker genes were measured with quantitative real time PCR in miR-34a overexpressing cells compared to controls.

Overexpression of miR-34a significantly inhibited the proliferative potential of MM cells. Its upregulation caused significant downregulation of *CDK4* expression, although no alterations were detected in the levels of *WNT1*, *SOX2* and EMT markers.

Deregulation and overexpression of **CDK4**, which plays a critical role in the cell cycle, has been reported in many cancers, including MM. Several studies have shown that **CDK4** is targeted by miR-34a, however, the relationship between miR-34a and **CDK4** is reported for the first time in MM in this study.

Since miR-34a-related treatment studies are in phase-1 clinical trials, demonstration of tumor suppressor potential of miR-34a, and deregulation of its target genes in MM cells might suggest as a potential target for those miR-34a-related treatment approaches in MM.

OP2

Effects of Simvastatin on Antioxidant Enzymes Chain And Lung Tissue in Sepsis Induced Rats

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Abstract

In addition to cholesterol-lowering effects, simvastatin has many pleitropic effects such as improving endothelial function, reducing oxidative stress and inflammation. Sepsis is an infection-induced systemic inflammatory response syndrome. There is a relationship between pathogens and host immune

defense mechanisms during infection. It is known that increased production of reactive oxygen species (ROS) is observed as a result of this interaction. The overproduction of reactive oxygen species is believed to directly contribute to permanent tissue damage through membrane lipid peroxidation, inflammatory cascade initiation, and systemic disregulations. It is known that ROS in sepsis is derived from NADPH oxidase- acute inflammatory responses generated by LPS administration in vivo, limiting various reduction reactions, such as changes in cytotoxicity and reduced glutathione (GSH) levels, derived from this enzyme sources. In addition, the ROS levels disrupt the balance between the oxidant-antioxidant system in favor of the oxidant system. In this study, we aimed to investigate the effects of Simvastatin on serum anti-oxidant enzymes chain and morphology of lung tissue, one of the first organs affected by sepsis in LPS.

After obtaining the necessary ethical permissions (Ethics no: 2012/138) *Wistar albino* adult male rats; control (n = 8), LPS (n = 10), Simvastatin (n = 10) Simvastatin+LPS (n=10). LPS applied 20 mg / kg i.p. for 4 hours. Simvastastin 20 mg / kg p.o. was given for 5 days. Four hours after LPS injection, rats were decapitated under anesthesia and blood samples were taken from the heart. Serum glutathione peroxidase (GSH-Px), glutathione reductase (GR) and superoxide dismutase (SOD) levels were analyzed by ELISA. Lung tissues which are taken into 10% formaldehyde are stained with hematoxylin-eosin dyes and examined morphologically under light microscope. IBM SPSS Statistics ver. 21.0 was used to determine the statistical significance of the results, with P < 0.05 indicating statistical significance.Tukey test was performed for pairwise comparisons.

In our findings, serum GR and GSH-Px levels were significantly decreased (p <0.01) in sepsis induced LPS group compared to other experimental groups. SOD levels were significantly decreased in LPS and Simvastatin+LPS groups (p <0.01, p <0.05, respectively) compared to other experimental groups. We observed the levels of GR and GSH-Px closer to controls results in Simvastatin treated LPS group. Histological analysis revealed leukocyte infiltration and bleeding sites in the lung tissue in the LPS group. LPS group was found to be significantly more damaged than the other groups according to tissue injury scoring.

It is observed that LPS decreases the levels of GR, GSH-Px, SOD enzymes in the anti-oxidant chain of sepsis, by distrubing the balance to oxidative stress and anti-oxidant enzymes in favor of the oxidant system.

The present study suggested that simvastatin, is a lipid-lowering medication act against tissue damages introduced following the experimental sepsis induced model, likely caused by free oxygen radicals.

OP3

Role of CYP3A4*1B gene variant in substance use disorder

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Abstract

Aim

Cytochrome P450 (CYP450) enzymes metabolize about 70 % of drugs for clinical use and one of them is

CYP3A4*1B. The aim of this study is to find out possible association between the CYP3A4*1B gene (rs2740574) and substance use disorder susceptibility in Turkish population

Materials and Methods

One hundred fifty-eight patients with substance use disorder and 100 sex, age and ethnic matched controls were enrolled in this study. The genotyping was analyzed by polymerase chain reaction-restriction fragment length polymorphism method by using MboII restriction endonuclease. The association between the variant and substance use disorder was analyzed by using SPSS 21 and de-Finetti program

Results

CYP3A4 gene (MboII gene variant in 5'promoter region) genotype distributions of substance use disorder patients were not significantly different from healthy controls. When the substance use and healthy groups were compared in terms of allel frequency, increasing G allele frequency were observed in CYP3A4 variants in substance use (p: 0,042).

Conclusion

Whether there was an association MboII gene variant in CYP3A4 gene 5'promoter region and with was investigated for the first time in this study in literature and it was demonstrated that increasing G allele existed in Turkish substance use patients. It was planned to research the other variants of CYP3A4 gene in the future.

Disclosure statement: The authors declare no conflicts of interest.

Keywords: CYP3A4,substance, MboII, PCR-RFLP

OP4

Antipsychotics in the treatment of schizophrenia related to substance use disorders

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Abstract

Psychotic disorders and substance use disorders frequently occur concomitantly. Prevalence of substance use disorder is 15% in population however substance use disorder is seen in 50% of psychotic disorder cases. Psychoses caused by substance use are disorders that occur during intoxication or withdrawal and last for at least 48 hours. It has been observed that the use of cannabis and synthetic cannabinoids triggers psychosis in individuals with genetic predisposition, and cannabis-induced psychosis cases become schizophrenia up to 50% over time. However, 30% of amphetamine, 24% of opioids and 5% of alcohol are reported to cause schizophrenia in the period following psychosis.

In order to be successful in the treatment of psychotic disorders caused by substance use, both, substance-related disorder and psychosis should be treated. For this purpose besides pharmacological therapy, a holistic treatment program including psychosocial approaches such as motivational interviewing, cognitive behavioral therapy, operant methods should be preferred.

In the pharmacological treatment of schizophrenia typical and atypical antipsychotics are used. Nowadays, atypical antipsychotics are increasingly replacing typical antipsychotics in the treatment of schizophrenia. At the same time, because of atypical

antipsychotics' effect on the common neural circuit that is thought to play a role in the development of substance addiction, the place of these drugs in the treatment of substance addiction comes to the forefront.

In this presentation, pharmacological treatment with antipsychotics which are used for schizophrenia related of substance use disorders are examined with current data.

Key Words: Substance addiction, Schizophrenia, Atypical antipsychotics

OP5

The effect of *APOA5* -1131T>C polymorphism on plasma lipid levels in Turkish coronary artery disease patients

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Abstract

Aims

Coronary artery disease (CAD) is one of the of utmost public health problem worldwide. It is known that apolipoproteins play crucial role in lipid metabolism which is important for the CAD development. Apolipoprotein A-V modulates plasma triglyceride (TG) levels and in previous studies, it was shown that the *APOA5* -1131T>C polymorphism affects plasma lipid levels. In this study, we investigated whether the rs662799 polymorphism was a risk factor for CAD development and the plasma lipid levels were affected from this variation or not in Turk CAD and non-CAD individuals.

Methods

Unselected 448 CAD (≥1 coronary lesion with ≥50% stenosis) and non-CAD (coronary lesion with ≤30% stenosis) individuals were genotyped for *APOA5* rs662799 T/C polymorphism using LightCycler480 with hydrolysis probes. DNA samples of individuals were obtained with inorganic method from leukocytes. The angiographic severity and extent of the disease were assessed by using Gensini and SYNTAX scoring systems.

Results

No significant difference was found in genotype frequencies between CAD and non-CAD groups. Plasma total cholesterol (TC), LDL-C, and TG levels were found higher only in C allele carriers of non-CAD group (p <0.05). According to gender-specific analysis in rare allele carriers of non-CAD group, plasma TC levels were found higher in both male and female groups, while LDL-C and TG levels were found higher only in female individuals (p <0.05).

Conclusions

As a result, it was concluded that being rare allele carrier in the non-CAD group could lead to an increase in lipid levels, especially in women.

OP6

Identification of *ica*-dependent biofilm production by *Staphylococcus aureus* clinical isolates and antibiofilm effects of ascorbic acid against biofilm production

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Abstract
Aims

Staphylococcus aureus (*S.aureus*) that are life-threatening pathogens having high morbidity and mortality rate causes nosocomial and community-acquired infections. *S.aureus* causes biofilm-related infections such as wound infection, urinary catheter-related urinary tract infections (UC-UTI), endocarditis, artritis, sepsis. Most of invasive *S.aureus* infections are associated with nasal carriage and contamination of indwelling-devices during surgical intervention. It is hard to treat infections caused by biofilm-producing *S.aureus*, due to resisting to antimicrobials. In some cases, treatment can be impossible and recurrent. In this study, after, *ica*-dependent biofilm-producer *S.aureus* isolates were detected, antibiofilm effect of ascorbic acid (AA) against biofilm formation of isolates were investigated.

Material and Methods

21 MSSA clinical isolates stored in our bacterial stock were used, two of which had been isolated on UCs, and the remaining isolated from nasal carriers. Antibiofilm study was proceeded with 3 *ica*-dependent biofilm-producer isolates (MSSA2-4) and ATCC 29213 (MSSA1). Biofilms and antibiofilm effect of AA were detected by MtP method. 16S-rRNA, *nuc, ica*A and *ica*D genes and expression levels of *ica*A and *ica*D of isolates were detected by RT-PCR.

Results

MICs of AA prevented biofilm formation of MSSA1 and MSSA3. Also, 1/2 MIC of AA prevented biofilm formation of MSSA3. It was observed that biofilm formation decreased with the concentration increased (Table1).

Table1. The effects of ascorbic acid against biofilm formation of MSSA isolates

Bacteria	Bacterial Control	Optical Density (Mean±Standard Deviation)				
		MIC	1/2 MIC	1/4 MIC	1/8 MIC	1/16 MIC
MSSA1	0.332 [a]±0.06	**0.256 mg/mL**	**0.128 mg/mL**	**0.064 mg/mL**	**0.032 mg/mL**	**0.016 mg/mL**
		ND	0.052 [b]±0.05	0.201 [c]±0.07	0.259 [d]±0.01	0.281 [e]±0.10
MSSA2	0.373 [a]±0.10	**0.032 mg/mL**	**0.016 mg/mL**	**0.008 mg/mL**	**0.004 mg/mL**	**0.002 mg/mL**
		0.087 [b]±0.10	0.112 [c]±0.03	0.134 [d]±0.01	0.171 [e]±0.06	0.192 [f]±0.05
MSSA3	0.165 [a]±0.05	**0.128 mg/mL**	**0.064 mg/mL**	**0.032 mg/mL**	**0.016 mg/mL**	**0.008 mg/mL**
		ND	ND	0.105 [b]±0.01	0.132 [c]±0.07	0.153 [a]±0.03
MSSA4	0.156 [a]±0.08	**0.128 mg/mL**	**0.064 mg/mL**	**0.032 mg/mL**	**0.016 mg/mL**	**0.008 mg/mL**
		0.072 [b]±0.08	0.102 [c]±0.03	0.129 [d]±0.1	0.135 [e]±0.05	0.149 [ae]±0.04

Different letters written in every row indicates significance between treatments. (*$p < 0.05$), MIC: Minimum Inhibitory Concentration.

There were no serious increase in *ica* gene expression of MSSA1 and MSSA2. Expression of *ica*A and *ica*D of MSSA3 decreased 13% and 38%, respectively. Expression of *ica*A in MSSA4 decreased 12%.

Conclusion

According to our study, AA can bu used as antibiofilm agent to prevent biofilm formation of *S.aureus*, consequently, biofilm-related infections.

OP7

Investigation of glycolysis and it's molecular regulation upon glucose limitation in leukemia cells.

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Abstract

Aims

Leukemia cells as other cancer cells multiply rapidly and they have increased nutrient needs. The most commonly used nutrient by cancer cells is glucose and therefore it is hypothesized that glucose is present at a low level in the microenvironment of cancer cells. Metabolic changes in leukemia cells due to nutrient deficiency add liabilities to the cells. In recent studies, increased glycolysis and reprogrammed glucose metabolism have been shown in different tumors. The aim of this study is to investigate the expression of key genes responsible in glucose metabolism in low glucose conditions and to search for oncogenes responsible for the regulation of glucose metabolism in cancer cells.

Methods

In this study, we control expression of rate-limiting glycolytic enzymes' and electron transport chain complex protein coding mRNA expressions in K562, NB-4 and HL-60 cell lines in low glucose (1mM) medium compare to normal (10mM) medium with qRT-PCR assay. Additionally, we controlled STAT3 protein expressions and phosphorylation in all cell lines with immunoblotting.

Results

PKM2 and LDHA mRNA expressions were significantly decreased in low glucose conditions. HK1 and HK2 expressions were increased in K562 cells ($p<0.001$). We also found that PFKL expression was decreased in K562 cells. NDUFB8 gene expression was found lowered in K562 and NB-4 cells in low glucose media. Similarly, STAT3 protein expression was decreased in those cells.

Conclusion

Our results show that targeting glucose metabolism can reduce expression of glycolytic genes and therefore suggest that glucose metabolism may be a target in the treatment of leukemia.

OP8

Successful generation of bispecific antibodies by CrossMAb technology

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Abstract

Monoclonal antibodies have become an important class of protein-based drugs for the treatment of cancer. However, single-target immunotherapy does not seem to destroy cancer cells sufficiently. Bispecific antibodies (BsAbs), which bind two different antigens, have been posited as potential cancer therapeutic agents for decades.

Aim

Our aim is to generate BsAbs to combine specificities of two antibodies by using knobsinto-holes (KiH) technology that enables heavy-chain heterodimerization which was forced by introducing different mutations into CH3 domains, and CrossMAb technology to achieve correct pairing of light chains by exchanging the CH1 domains of heavy chain and light chain.

Methods

Two in-house chimeric antibodies targeting different tumor antigens were used to combine. Both mutations were introduced to the corresponding chains of antibodies by molecular cloning then, each antibody was transfected into mammalian cells to produce homodimer antibodies which contain the same heavy chain mutation. Homodimers were combined in a ratio to obtain heterodimer BsAbs which contain different heavy chain mutations. Antibody behavior was checked by SDS-PAGE, EIF Electrophoresis, ELISA, and FPLC.

Results

We achieved to produce BsAbs and proved their binding capacity to antigens by ELISA. EIF-gel and FPLC purification do not show the exact purity however, they indicated the heterodimer presence.

Conclusions

Both strategies work well to produce BsAbs and remain antibody binding behavior suggesting that introduced mutations did not affect the antibody characteristics and behavior. EIF-gels showed heterodimers ran at the expected pI values indicating successful combination of antibodies. However, exact purity needs to be detected by more precise methods.

OP9

The role of pericytes on the efficacy of Bevacizumab in colorectal cancer

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Abstract

Aims

Bevacizumab is widely used in the treatment of metastatic colorectal cancer. Although, it is no predictive markers have been found to select the patient group who will benefit from Bevacizumab. CSPG4 (Chondroitin Sulfate Proteoglycan-4) released by pericytes helps in guidance of tip cell that yields angiogenic growth. Low pericytic area is usually associated with poor prognosis in patients. In the current study we aimed to identify the effect of the presence of pericyte and CSG4 expression in the tumor tissue of patients on the efficacy of the Bevacizumab.

Methods

Fifty patients with metastatic or recurrent colorectal cancer who had been treated Bevacizumab combined with chemotherapy were included studied. RNA isolation and cDNA synthesis were performed from the paraffin-embedded tumor tissues. The expressions of HBA (Human beta-Actin), VEGFA (Vascular Endothelial Growth Factor-A) and CSPG4 were examined using the qPCR method. The tumor tissues of patients were also immunohistochemically stained with anti-CD31, anti-alpha-SMA and anti-CD34 antibodies.

Results

Though not significant, objective response rate in CSPG4_positive patients, was better to CSPG4_negative ones. Likewise, the overall survival (OS) and PFS (progression-free survival) time of CSPG4_ positive group was longer than that of CSPG4_negative group. PFS was significantly longer in the VEGFA_low group. No significant correlation was found between CD34, SMA positivity and OS and PFS. However, both OS and PFS were better in CD31_low group.

Conclusions

Our results suggest that Bevacizumab may be more effective in patients with less vascular density. The emerging role of pericytes on the efficacy of Bevacizumab may be better evaluated by increasing the number of patients.

OP10

Study of the relationship between hematological values and stress in rats after forced swimming test

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Abstract
Aims

Stress can affect many physiological systems in organism. The purpose of this study is to investiagte the effect of stress on some hematological parameters.

Method

Wistar albino Female rats divided into three groups (n=6),(C: control, SG: short-term stress group, LG: long-term stress group). Forced swimming test (FST) were conducted 24 h after a pretest session for 5 minute, by placing the rats in rectangular shape filled with water 40 cm (RT; 50 cm deep). Subjects were forced to swim on the purpose of inducing short term stress or long term stress for one and seven consecutive days long respectively. Red blood cell (RBC), hemoglobine (HGB), HCT (hematocrite) , mean corpuscular volume (MCV), White blood cell (WBC), lymphocyte and monocytes were analysed via hemograme and cortisol as a stress biomarker was evaluated by ELISA. Behaviors such as immobility, climbing, swimming were recorded by digital camera and analysed by two different researchers.

Results

Moncytes, MCV and RBC (p<0.05); HBG, HCT, WBC and lymphocyte(p<0.01) were increased significantly in the short-term stress group versus control group. Only lymphocytes as hematological parameters increased in the long-term stress group(p<0.01). Although cortisol increased in both stress groups, no statistical difference was found(p>0.05). Immobility time increased gradually seven consecutive days and more than short term stress group(p<0.05). Swimming and climbing behaviour decreased in long term stress group vs short term stress group(p<0.05).

Conclusions: Hematological parameters have been considerably altered in the short term stress. Exposing stress for longer term results no significant change on the parameters, as animals may have adapted to the froced swimming test.

Keywords: Hematological parameters, Forced swimming test, Long term stress, Acute stress

OP11

The impact of genetic manipulation of NRH quinone oxidoreductase-2 levels on growth and proliferation of ovarian cancer cell lines

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Abstract

Aims:

NRH quinone oxidoreductase-2 (NQO2) has been implicated in maintaining cellular redox status and acting as a nanny/chaperone protein in cancer cells. As such, there is a possibility that NQO2 could be a potential therapeutic target. We have therefore evaluated the function of NQO2 in NQO2-overexpressing TOV-112D (NQO2-OE) and NQO2-silencing SKOV-3 genetically modified ovarian cancer cell sub-lines (OVCs).

Methods

Western blot analysis was used to measure protein level of NQO2 and cell cycle markers in the generated cell sub-lines. Cell proliferation was monitored using trypan blue and clonogenic assays. Flow cytometeric analysis was performed to determine distribution of cells through cell cycle phases in addition to measure intracellular ROS levels.

Results

NQO2-OE cells showed more aggressive growth pattern than wild-type cells. This was consistently associated with an enhancement in the progression of cells through cell cycle phases and significant 2.6-fold reduction in Rb expression. A reduction in ROS levels in NQO2-OE cells may also explain this enhancement in cell growth. Overexpressing NQO2 also resulted in

destabilisation of CDK4 and cyclin D1 with 30% and 50% relative reduction in their expression levels respectively, and concomitant increase in p-cyclin D1 (Thr286). The involvement of NQO2 in controlling cyclin D1 turnover is also confirmed in SKOV-3 cells when genetic silencing of NQO2 was accompanied by significant 3-fold reduction in p-cyclin D1 and subsequent stabilisation of cyclin D1 levels.

Conclusion

NQO2 has contributory roles in altering the proliferation characteristics of OVCs which, however, varied among different cell lines of the same type of cancer.

OP12

Association of reelin silencing and topo isomerase IIβ expression in human SH-SY5Y neuroblastoma cell line

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Aim

Reelin signaling controls many pathways in neurodegenerative conditions including neuronal migration, synaptic plasticty, tau phosphorylation and amyloidogenic amyloid precursor protein (APP) processing. Due to its crutial role in brain development and neuronal differentiation and association of the topo IIβ down regulation with PD-like and AD-like pathology through regulation of neuronal axon growth, our aim was to investigate how different

degree of Reelin silencing regulates the expression of topo IIβ in SH-SY5Y cells.

Methods

For this purpose, 10, 15, 20 and 25 nm of specific small interfering RNAs (siRNA) of reelin was transfected by Lipofectamine RNAiMAX agent in SH-SY5Y cells. The silencing of reelin was monitored by mRNA expression via quantitative reverse transcriptase real time polymerase chain reaction (RT-qPCR). Reelin mediated molecular alterations on mRNA and protein levels were analyzed by both RT-qPCR and Western blot methods.

Results

Reelin silencing decreased the number of living cells morphologically and increased the expression of Bax/Bcl-2 activating apoptotic pathway. In line with reeling silencing, topo IIβ, PSEN1 and BACE1 expressions were also downregulated while expressions of pTAU and APP were upregulated.

Discussion

Our results suggest that reelin silencing might be related to AD signaling pathway in an association with topo IIβ. However, further studies should be conducted to elucidate the molecular mechanisms underlying AD pathology due to the downregulation of reelin and topo IIβ expressions.

OP13

Oral TQ administration recovers Aβ induced memory loss by promoting cell survival and mitigating pathophysiological burden in the hippocampus of a sporadic model of AD rats.

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Abstract

Aims

Two pathological hallmarks of Alzheimer's disease (AD); amyloid beta (Aβ) plaques and neurofibrillary tangles initiate a degeneration process ending up with synaptic deficiency, neuronal damage and loss in hippocampus. Currently, there is no therapeutic option for retrieval of cognitive functions rather than symptomatic recovery. Many researchers focus on herbal compounds for their roles as antioxidants, anti-inflammatories, neuroprotectants and neuromodulators. One of those molecules, Thymoquinone (TQ) is the bioactive component of *Nigella sativa* and its protective action on neurons is already known. In the present study, therapeutic role of TQ was investigated by evaluating structural and functional alterations in a rat model of Alzheimer's disease.

Methods

6 months-old rats were infused with aggregated $Aβ_{1-42}$ fragment which was placed into hippocampus via an infusion canula. Animals were orally treated with TQ (20 mg/kg) for 15 days. Then memory and learning skills were evaluated. After removal of brains functional tests were followed by histological stainings for finding out the plaque deposition, neuronal survival, and neuronal degeneration. Also, acetyl choline esterase (AChE) amount was quantified from the hippocampal samples. Lastly, expressional profile of key proteins of Alzheimer's pathology; Aβ, phosphorylated tau, and BACE-1 were analyzed by western blotting.

Results

TQ provided a significant recovery in memory function. Neuronal loss in hippocampus upon Aβ infusion was

found to disappear after TQ treatment. Similarly, plaque formation and degeneration were significantly lower in TQ treated animals. TQ also decreased the level of AChE in hippocampus. Lastly, total amount of Aβ and BACE-1 which were upregulated depending on $A\beta_{1-42}$ infusion were lowered by TQ. Also, TQ caused an apparent reduction in phosphorylation of tau according to both control and $A\beta_{1-42}$ groups.

Conclusions

In conclusion, TQ can be an effective molecular agent for clearance of amyloid beta plaques and reversal of impaired memory functions.

OP14

The role of RASSF4 gene in head and neck cancer patients

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Abstract

Aims

Head and neck cancers are a group of cancers that begin in the oral cavity, nose, pharynx, larynx, salivary glands. The RASSF family consists of 10 proteins and exhibits inhibiting growth of the RAS oncogene. They are thought to be involved in cell cycle control, apoptosis, cell migration and mitosis control.

Methods: Gene expression levels were compared between tumor and healthy tissues of 69 patients diagnosed with head and neck cancer by RT-PCR method. In addition, DNA samples taken from 69 patients with head and neck cancer and 70 healthy individuals and RASSF4 rs7896801 and rs884879 gene variants were determined by RT-PCR method. RASSF4 serum levels were compared with ELISA method in the study groups.

Results

There was no statistically significant difference in gene expression between tumor and healthy tissue of 69 patients. As a result of experimental studies, the frequency of G allele carrying in rs7896801 patients increased 2.4 fold. The frequency of carrying AA genotype increased statistically in the control group. The frequency of carrying CC genotype in rs884879 was 2.2 times higher in healthy group compared to patients. In the patients, it was observed that the frequency of carrying T allele increased significantly. There was no statistically significant difference between study groups according to serum RASSF4 levels.

Conclusions

This is the first study which is examined RASSF4 gene expression, serum levels, and SNP variants in Turkish patients with head and neck cancer. We believe that these preliminary data will lead to further studies for RASSF4 protein.

OP15

The neuroprotective effect of lamotrigine against glutamate excitotoxicity in SH-SY5Y human neuroblastoma cells

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Abstract

Aim

Glutamate-induced excitotoxicity is shown to be involved in the pathophysiology of neurodegenerative disorders. It has been proposed that lamotrigine used as antiepileptic and in bipolar disorder may be protective against excitotoxic insults. There are overlapping results between nervous and immune systems and also the role of proinflammatory cytokines and oxidative stress have been found in pathophysiology of neurodegenerative disorders. Therefore, the present study has been designed to investigate the neuroprotective effects of lamotrigine against the glutamate excitotoxicity and their interactions with proinflammatory cytokines and oxidative stress using human neuroblastoma cell line (SH-SY5Y).

Methods

SH-SY5Y cells were pretreated with lamotrigine (50 - 150 µM) and glutamate (15 mM). The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltertazolium bromide (MTT) assay was performed for determination of cell viability. The proinflammatory cytokines, interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) were determined by using ELISA and also oxidative stress parameters, superoxide dismutase (SOD) and H_2O_2 were evaluated.

Results

Glutamate (15 mM) significantly decreased cell viability in SH-SY5Y cells at 3rd h and 24th h in a time and dose-dependent manner (~60% of control). The cells pre-treated with 100 µM lamotrigine significantly restored cytotoxic effect of glutamate.

IL-1β, IL-6 and TNF-α, which were increased after induction with glutamate, could be attenuated by lamotrigine. H_2O_2 was decreased and SOD activity was increased by lamotrigine.

Conclusions

The results of the present study suggest that lamotrigine could attenuate glutamate induced excitotoxicity through antioxidant and anti-inflammatory activities.

OP16

The role of pericytes in MS pathophysiology

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Abstract

Aims

Multiple sclerosis (MS) is an autoimmune disease of central nervous system (CNS) characterized with demyelination and unpredictible clinical attacks. Studies indicate that inflammation firstly starts in the peripheral immune system and then effects CNS due to the disturbance of blood brain barrier (BBB) system. Pericyte is a cell type that is mostly found in BBB and acts as regulator of blood volume in veins. Recent studies show that pericytes also have immunological

functions. Our aim was to find out the role of pericytes in MS pathogenesis.

Methods

We immunized mice with myelin oligodendrocyte glycoprotein (MOG) to induce experimental autoimmune encephalomyelitis (EAE). We collected sera at early (15 days) and late stage (40 days) experiment groups. We investigated immunopathologic features and pericytes in the brain samples, serum anti-MOG and cytokine levels (IL-6, IFNγ, IL-17a). Moreover human vascular pericyte cells were treated with serum samples of EAE and thereafter IL-6 and IL-17a release and viability of the cells were investigated.

Results

EAE model was verified with clinical features, demonstration of demyelination, infiltrates and serum anti-MOG antibodies. Pericytes had a higher abundance in EAE lesions. Serum levels of IL-6, IFNγ, IL-17a were significantly increased in EAE mice. Pericytes incubated with early and late stage sera produced higher levels of IL-17a and IL-6 and underwent apoptosis.

Conclusion

Our results suggest that proinflammatory cytokines may induce pericytes apoptosis and trigger an inflammatory phenotype of pericytes. Pericytes may have important roles in MS pathogenesis by contributing to inflammation in lesion sites.

Key Words: multiple sclerosis, demyelinization, pericyte, apoptosis, autoimmunity

OP17

Effect Of Lycopene On Oxidative Stress In SHS5Y5 Cells Induced by As2O3

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Abstract
Aims

Arsenic trioxide (As$_2$O$_3$), a toxic substance, may cause neurodegenerative changes as it crosses the blood brain barrier. Lycopene (Lyc), a non-provitamin carotenoid, found in tomatoes and other fruits, is one of the most potent naturally and abundantly occurring antioxidant among all the dietary carotenoids. The aim of this study was to determine the possible protective effects of lycopene on oxidative stress-induced neuronal toxicity induced by arsenic trioxide using human neuroblastoma SH-SY5Y cells, widely used as model of neurons.

Methods

The SH-SY5Y cells were treated with various concentrations of As$_2$O$_3$ alone, Lyc alone (final concentrations in the well were 2–10 µM and 0.5–5 µM, respectively) and a combination of both (As$_2$O$_3$ +Lyc). The cell viability were investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltertazolium bromide (MTT) test and the effect of Lyc on As$_2$O$_3$ induced oxidative stress was examined using total antioxidant–oxidant status (TAS-TOS) kits.

Results

Specific experiments were conducted by treating SHSY5Y cells with 2 µM arsenic, since treatment with 2 µM arsenic for 24 h decreased cell viability (% 29) compared to the control group. Pre-treatment with 8 µM lycopene decreased cytotoxic effect of As$_2$O$_3$. TOS was increased in As$_2$O$_3$ group compared to control and decreased with lycopene pretreatment. TAS was

increased in lycopene pretreated group compared to As2O$_3$.

Conclusion

The results demonstrate that lycopene exerts its neuroprotective activity against arsenic -induced oxidative stress in SH SY5Y cells.

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Keywords: Lycopene, arsenic trioxide, SH- SY5Y, cell viability, neurodegenerative, oxidative stress.

OP18

Impaired expression of some miRNA machninery genes in bone marrow samples of patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome

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Abstract

Aims

Normal differentiation is impaired in myeloid hematological malignancies. microRNAs which play important role in normal differentiation of hematopoietic stem/progenitor cells are key regulators of hematopoiesis. Aberrant miRNA expression is a feature of leukemia and the impaired function of miRNA biogenesis genes can cause aberrant miRNA expression. The aim of the present study was to investigate possible relationship between the expression levels of miRNA machinery genes *Dicer, Drosha, DGCR8, AGO1* and *TARBP2* and AML and MDS patients.

Methods: Using qPCR, the expression of five selected miRNA machinery-related genes was eveluated in the bone marrow samples of 20 patients with AML, 34 patients with MDS and in 7 healthy subjects.

Results

While *Dicer, Drosha, AGO1* mRNA expression levels were significantly lower (p<0,01), only *DGCR8* mRNA expression showed significantly overexpression in AML and MDS patients compared to control (p>0,01). *TARBP2* expression was significantly downregulated in MDS but it was similar found the level in AML. Also, there was significantly difference in *TARBP2* expression level between AML and MDS patients.

Conclusions

Our findings provide evidence for deregulated expression of *Dicer, Drosha, DGCR8, AGO1* and *TARPB2* genes in AML and MDS, suggesting their levels may serve as indicator for AML and MDS development. The roles of these genes in these diseases should be further explored may contribute to the development of new treatment strategies.

Keywords: myeloid haematological malignancies, MDS, AML, miRNA biogenesis, gene expression

OP19

Daily flavonoid nutrition diet model in breast cancer: Investigation of effects of consuming at non-cytotoxic dose of flavonoid components on molecular pathways in the hormone positive breast cancer cell line

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Abstract

Flavonoids, secondary metabolites of plants, have bioactive effects such as antioxidant, antibacterial, antifungal, antiviral and anticancer. They can affect many cellular pathways and selectively lead cancer cells to death using the apoptosis pathway.

Cancer patients are encouraged to have a diet, rich in flavonoid content, in order to eliminate side effects of chemotherapy and radiotherapy. Therefore, it is important to model and evaluate the cellular effects of consuming daily dose of flavonoid on these patients. Our team and other researchers have previously demonstrated the cytotoxic effect of high-dose flavonoid components on hormone positive and negative breast cancer cell lines. On the other hand; there is no study, focusing on modeling the amount of flavonoid taken in consistent daily diet, to investigate the effect of flavonoid components on cancer cells at non-cytotoxic doses.

Therefore, in our study, non-cytotoxic (control and 16 µg/ml) flavonoid mixture was applied to MCF-7 hormone positive breast cancer cell line and the effects of flavonoids on intracellular protein expression at low doses were investigated by peptide analysis on LC-MS/MS platform.

A total of 214 proteins were identified and 36 proteins with significant expression change (decreasing/increasing) with 1.2 fold change and above were identified. Significant changes were observed in the pathways in which carbon metabolism, amino acid biosynthesis, and alternative mRNA splicing mechanism were involved.

In present study, we showed not only protective effects of consuming flavonoids at low dosage but also different mechanisms have role in this issue.

OP20

Effect of high glucose on nocodazole-induced mitotic arrest

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Abstract

Aims

Anti-cancer treatments trigger acute hyperglycemia both in diabetic and non-diabetic cancer patients. Mortality rate is higher in diabetic cancer patients compared to non-diabetic cancer patients. A possible explanation for this may be the reduced efficiency of chemotherapy due to hyperglycemia. Although, the negative effect of hyperglycemia on cancer treatment has been reported previously, the underlying mechanism of this effect is still not known. The aim of this study was to investigate the effect of high glucose concentrations on the mitotic arrest induced by an anti-cancer drug in *Sachharomyces cerevisiae*.

Methods

Effect of high glucose (20%, 40%, and 60%) on the viability was examined by the spotting, colony forming unit (cfu) assays and PI staining. Mitotic arrest was induced by a 3 hour-nocodazole treatment in wild type cells. In *cdc23* conditional mutants mitotic arrest was induced by shifting the temperature to restrictive for 3 hours. Mitotic arrest was examined using DAPI staining in both strains.

Results

20% glucose did not significantly effect viability during the experiment (3 hours). 40% and 60% glucose induced cell death on the second and third hour, respectively. The proportion of mitotic cells were significantly higher at the first hour in all high glucose concentrations in wild type cells. In *cdc23* cells, the proportion of mitotic cells were not significantly different during the experiment (3 hours) in all high glucose concentrations tested.

Conclusions

Sublethal concentrations of high glucose delay mitosis by 1-hour in yeast and this delay is dependent on spindle assembly checkpoint activation.

OP21

Detection of chromosome aberrations by fluorescence in situ hybridization went unnoticed with the use of conventional cytogenetics in acute lymphoblastic leukaemia

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Abstract
Aims:

Conventional cytogenetic analysis (CCA) plays an essential role for acute lymphoblastic leukemia (ALL) patients. However,it is technically challenging and have limitations. The aim of the study was to identify chromosome aberrations that unrecognized or undetected with CCA and show sensitive detection, also significant advances of multiprobe fluorescence in situ hybridization (FISH) panel.

Methods

Ten newly diagnosed ALL patients were included in the study. Karyotyping was performed using G-banding

and FISH assay was carried out using multiprobe panel designed with different genes (cMYC, p16, E2A, MLL, IGH, TEL/AML1, BCR/ABL, centromere 10 and 17, CHIC2).

Results

Chromosome banding analysis showed 70% of the cases had normal karyotypes, 30% had no metaphases. Among the cases evaluated by FISH, cMYC, E2A, CHIC2, and IGH breakapart were detected in 70% of the patients, while MLL breakapart were detected in 80%, centromere 17 were detected in 60%, centromere 10 were detected in 50%, and p16 deletion were detected 30% of the patients. TEL/AML1 and BCR/ABL translocations were observed in none of the cases. cMYC rearrangements were found statistically significant between the patient and control groups (p<0,0423).

Conclusions

While CCA is still a gold standard, for spotting smaller chromosomal rearrangements, FISH considered as a successful method. In our study, we detected rearrangements that cannot be determined by CCA. Being indicative for treatment planning, identification of abnormalities is clinically important. Our study showed prognosis and treatment of ALL should be supported by FISH. Also, for the rapid and more accurate results, critical gene determinants for ALL can be easily examined.

OP22

The Role of T Helper 1 and T Regulatory Cells in Type 1 Diabetes Mellitus

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Abstract

Aims

The aim of this study was to investigate the importance of Th1 and Treg cells in the development and process of Type 1 Diabetes Mellitus.

Methods

Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood samples from newly diagnosed (ND), 1 year (1YD) and 5 year (5YD) type 1 diabetes (T1D) patients (8 patients each) and 8 healthy controls, and cultured for 24 hours under unstimulated (US) and hr-IL 12, hr-IL 2 stimulated conditions. After cell culture, the cell ratios of Th1 and regulator T (Treg) and the intracellular levels of IFN-γ, TNF-α, IL-10 and TGF-β cytokines were evaluated using Flow Cytometry; whereas mRNA expressions of the transcription factors T-bet and FOXP3 of these cells were determined by Real-Time PCR.

Results

CD3$^+$CD8$^-$ Th intracellular IFN-γ cytokine levels were high in all patients under US and stimulated conditions. Significant increase was detected in TNF-α levels of all patients under US conditions. Intracellular TNF-α levels of ND and 1YD were significantly higher than healthy controls under IL-12 cytokine-stimulated conditions. CD4$^+$CD25^{high} intracellular TGF-β levels were detected significantly lower than healthy controls in US conditions. Intracellular IL-10 levels were significantly low in all patients under stimulated conditions. Th1/Treg % ratios were found high in all patient groups. FOXP3 mRNA expressions were observed low in patients, while T-bet mRNA levels were higher than healthy controls under stimulated and unstimulated conditions.

Conclusion

Our results suggested that Th cell subgroups play an important role in the pathogenesis of T1D.

OP23

Identification of differentially expressed genes and pathways between low and high grade ovarian cancer by integrated bioinformatics analysis

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Abstract

Aim

To provide a better understanding of high grade ovarian cancer (HGOC) at the molecular level, this

study aimed to identify the key genes and pathways associated with HGOC by using integrated bioinformatics analysis.

Methods

The microarray datasets (GSE6008 and GSE14764) as the training set and another independent microarray dataset (GSE23603) as the validation set which included low and high grade ovarian tumor samples were downloaded from the ArrayExpress database. Following normalization, differentially expressed genes (DEGs) were obtained using R software. Functional enrichment analysis (gene ontology and pathway analysis) were performed for DEGs using DAVID database. Protein-protein interaction (PPI) network was established by STRING; the network served to find hub genes for HGOC.

Results

A total of 106 common DEGs were screened out from all 3 datasets, among which 66 genes were upregulated and 40 genes were downregulated. The DEGs were classified into three groups by gene ontology terms (21 biological process, 10 molecular function and 12 cellular component). The KEGG pathway analysis showed that DEGs were enriched in metabolic pathways, oxidative phosphorylation, drug metabolism and cell cycle. The top 10 hub genes in the constructed PPI network were GMPS, RFC4, YWHAZ, CHEK1, CYC1, MRPL13, MRPL15, GMPS, SDHA and CLPB.

Conclusions

These hub genes and key pathways could improve our understanding of the process of the underlying molecular events and may be potential biomarkers and therapeutic targets for the diagnosis and treatment of HGOC. Further biological experiments are required to confirm the function of the identified genes.

Key words: ovarian cancer, novel biomarkers, bioinformatics analysis

OP24

Bioinformatic analysis of differentially expressed genes for bladder cancer

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Abstract

Introduction

The bladder cancer (BC) is caused pathology by both exogenous environmental and endogenous molecular factors. Although several genes have been implicated in its pathology, the molecular pathogenesis of BC is still debated. Bioinformatic analysis evaluates high numbers of proteins in a single study, increasing the opportunity to identify possible biomarkers for disorders.

Aim

The aim of this study is to identify biomarkers fort he identification of BC using several bioinformatic analytical tools and methods.

Material and Method:

Bladder tumour samples and normal specimens were compared for each probe-set with T-test in GSE13507

and GSE37817 datasets, statistical datasets were verified with GSE52519 and E-MTAB-1940 data sets. Differential gene expression, hierarchical clustering, gene ontology enrichment analysis and heuristic online phenotype prediction algorithm methods were utilized. Statistical genes were assessed in the human protein atlas database.

Results

GSE13507 (6271 probe-set) and GSE37817 (3267 probe-set) data were significant after the extraction of probe-sets without gene annotation information. Common probe-sets in both datasets (2888) were further narrowed by analysing the first 100 probe-set with increased and decreased expressions in BC. Among the 200 probe-sets, 68 were significant for both datasets with similar fold-change values (Pearson r: 0.995). The human protein atlas database revealed similar protein expression levels for *CKAP2L*, *AURKB*, *CDC25A*, *APIP* and *LGALS3* for control and BC groups.

Discussion

This bioinformatic study revealed five candidate biomarkers for the early diagnosis of BC. It is suggested that these candidate proteins be investigated in wet-lab to identify their roles in different types of BC pathology.

OP25

Might there be a link between VNTR Variants in the eNOS, HPER and XRCC4 Genes and the etiopathogenesis of Bipolar Disorders?

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Background

Bipolar disorder (BD) is one of the major psychiatric disorder, and multiple genes and environmental factors determine its pathogenesis. Recent reports have indicated that some the variable number of tandem repeat (VNTR) sequences may function as transcriptional or translational regulators. This study aimed to evaluate whether VNTR variants in the Endothelial Nitric Oxide Synthase (eNOS), the X-ray repair cross-complementing group 4 (XRCC4) and the Period Circadian Regulator 3 (PER3) gene play any role in BD ethiopathogenesis and to evaluate the association between variants and clinical features.

Material and methods

Present study included 104 individuals with BD and 100 healthy controls. These variants were analyzed using PCR.

Results

The patients with BD had lower eNOS VNTR-II genotype than the healthy control subjects (p=0.026). patients (p=0.009). The DD genotype of eNOS was found to be higher in patients with family substance, alcohol use and nicotine dependence respectively (p=0.027, p=0.016, p=0.009). No difference was observed in the distribution of XRCC4, PER3 and genotypes between patients with BD and healthy controls. The II genotype of XRCC4 was found to be higher in patients with a history of psychotic mania and was statistically significant (p=0.022). No significant differences were observed for the eNOS, XRCC4, PER3 genotype and allele frequencies when female group compared to male group (p>0.05).

Conclusion:

Our results showed that the VNTR variants in eNOS and XRCC4 genes might play a potential role in BD pathophysiology and its association with psychotic mania, substance and alcohol use with BD patients.

Key words: Bipolar Disorders, VNTR Variants, eNOS, HPER, XRCC4, etiopathogenesis.

OP26

Investigation of the eNOS (rs1799983) and IFN-γ (rs6255944) gene variants with susceptibility to Schizophrenia and Bipolar Disorders in a Turkish cohort

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Background

Despite intensive efforts, the molecular mechanisms underlying bipolar disorders (BD) and schizophrenia (SCZ) remain elusive. The aim of this study was to investigate the role eNOS, and IFN-γ variants in SCZ and BD and to evaluate the association between these variants and clinical features.

Material and methods:

We performed two case-control comparisons. The eNOS (rs1799983) and IFN-γ (rs6255944) gene variants were genotyped 104 individuals with BD, 128 patients with SCZ and 100 healthy controls. These variants were analyzed using PCR-SSP/RFLP. The relationships between the genotypes and the clinical parameters in BD and SCZ patients were evaluated.

Results:

No significant differences were observed between groups for the eNOS, IFN-γ genotype and allele frequencies in BD patients. Comparing clinical parameters, the AA genotype (low expression level) of IFN-γ was found to be higher in patients with nicotine use (p=0.021) and the AT genotype (middle expression level) of IFN-γ was found to be lower in patients with nicotine use (p=0.026). Also, the patients with BD had higher eNOS-TT genotype with nicotine use (p=0.002). Also, the patients with SCZ had higher TT/T genotype/allele of eNOS than the healthy control subjects (p=0.032).

Conclusion:

Our results suggest that the eNOS variant may have important effects related to susceptibility to SCZ respectively. Also, the patients with BD and SCZ had higher eNOS-TT and IFN-γ-AT genotype with nicotine use. Our results showed that the IFN-γ and eNOS variants might not play a potential role in BD and SCZ pathophysiology.

Key words: Schizophrenia, Bipolar Disorders, eNOS, IFN-γ, variants, clinical parameters.

OP27

Predicting the Predisposition to Colorectal Cancer based on SNP Profiles of Immune Checkpoints using Supervised Learning Models

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Abstract

Aim

The goal this analysis is to explore the machine learning-based assessment of predisposition to colorectal cancer based on the single nucleotide polymorphisms (SNP). Such a computational approach may be used as a risk indicator and an auxiliary diagnosis method complementary to other diagnosis tools, such as, biopsy, CT scan, and MRI. Moreover, it may be used to develop a low-cost screening test for early detection of colorectal cancers to improve the public health.

Dataset

The dataset includes SNPs observed in particular colorectal-cancer-associated genomic loci that are located within DNA regions of 11 selected genes, namely; p16, MDM2, GAL3, TIM1, TRAIL, PD1, PDL1, CD28, CD27, and CD40. The dataset included 50 healthy individuals (control group) and 65 colorectal cancer patients. The dataset also includes additional information for patients only; such as, the age of the patient, the stage of the cancer, the location of the tumor, perineural invasion of the tumor, and tumor differentiation.

Methods

We employ several supervised classification algorithms, namely, Logistic Regression (LR), Random Forests (RF), and Support Vector Machine (SVM). Besides, we apply knn-based data imputation to fill the missing genotype values. The scripts are written in Python programming language using the Scikit-learn library. To evaluate different approaches and models, we exploit f1-scores and Area Under Curve (AUC) values in ROC curves.

Results

We make the following observations:

(1)   Logistic Regression-based classifier using one-hot encoding for feature representation and knn-based imputation to complete the missing data performs the best among the studied classifiers in terms of both f1-score (88%) and AUC value (0.88).

(2)   Knn-based data imputation increases the f1-scores around 18% (from 70% to 88%).

(3)   Based on the high accuracy of the constructed logistic regression models, the studied 11 genes may be considered as a gene panel candidate for the risk screening of colon cancer.

(4)   Based on the ANOVA analysis, the following genotypes are the statistically significant (i.e., $p\text{-val} < 0.05$) discriminating features between the control group and colon cancer patients. This shows that the colon cancer is associated with multiple genes in complex interactions.

	F values	P values
CD40 C/C	37.5083	1.36e-08
CD28 C/C	18.6100	3.44981e-05
CD40 C/T	14.3344	0.0002
PD1 C/T	10.5136	0.0015
TRAIL C/T	7.2887	0.0080
TRAIL C/C	7.2887	0.0080
CD28 T/T	6.7818	0.0104
CD27 T/T	6.1596	0.0145
PDL1 A/C	5.7026	0.0185
PDL1 A/A	5.6480	0.0191
LGALS3 C/C	4.8505	0.0296

OP27

Expression Analysis of Mir-16-5p Mirna in Polymorphic *Helicobacter Pylori* Genotypes in Gastric Cancer

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Abstract

Aim

Being one of the most common cancer types around the world, gastric cancer (GC) ranks as the second cancer type leading to cancer-related deaths. MicroRNAs are small, non-coding RNA molecules. It is known that they have important roles in cancer mechanisms. They can both trigger and suppress tumourigenesis. In this study, miR-16-5p miRNA levels in *Helicobacter pylori* infected tissues are compared with a control cell line, which is named as AGS.

Method

Polymorphic VacA and CagA were detected in different tissues via PCR. miR-16-5p levels were evaluated by using real-time PCR method. The levels of miR-16-5p in tissues were compared with AGS control cell line. Result: Polymorphic VacA sites were observed in all six tissues and CagA variation sites were found in three of six tissues. It was concluded that miR-16-5p was suppressed at different levels depending on polymorphic sites carried by the tissues. It was found that the tissues carrying VacA M1 polymorphic region has the most suppress levels.

Conclusion

It can be conferred that all CagA and VacA polymorphisms reduces the expression level of miR-16-5p, although the suppressing level of miR-16-5p varies based on the regions, in which polymorphism develops. From this point of view, it can be inferred that *H. pylori* has a suppressing effect on miR-16-5p, a pro-apoptotic miRNA.

Keywords: Gastric cancer, Helicobacter pylori, MiR-98, MiR-16-5p

OP28

SEPTIN12 c.474 G>A Polymorphism as a Risk Factor in Teratozoospermia Patients

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Aim

SEPTIN12 gene is one of the testis-specific genes with a causative role in spermatogenic defects and c.474 G>A (rs759991) polymorphism in the *SEPTIN12* gene creates a novel splice variant and the resulting truncated protein was found to be more prevalent in infertile men compared to fertile controls in the previous studies. In this study, we aimed to investigate the association of *SEPTIN12* c.474 G>A polymorphism with male infertility.

Methods

Forty-eight teratozoospermia patients and 164 fertile controls were genotyped by PCR-RFLP method and the results were statistically evaluated with dominant, recessive, codominant and additive models. The effects of *SEPTIN12* c.474 G>A polymorphism and smoking on sperm parameters of teratozoospermia patients were assessed.

Results

Although no statistical difference was found between teratozoospermia and fertile control patients in terms of genotype distributions, significance was identified between the genotypes of all and non-smoking

teratozoopermia patients in terms of neck defects (p=0.059 and p=0.019, respectively). Significance was higher when dominant model was applied (p=0.019 and p=0.013, respectively). We also found that that smoking affects morphology in teratozoospermia patients in terms of head defects (p=0.012) and is a high risk factor for the A allele carriers in teratozoospermia patients according to dominant model (p=0.014, OR=14.62).

Conclusion

Smoking is a risk factor for the A allele carriers of *SEPTIN12* c.474 G>A polymorphism and affects sperm morphology in teratozoospermia patients.

Keywords: male infertility, *SEPTIN12*, c.474 G>A polymorphism, teratozoospermia.

OP29

The therapeutic effects of viscum album and probiotics on CCl₄-induced chronic liver injury in rats

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Aims

The present study aimed to investigate the therapeutic effects of mistletoe (Viscum album) extract and probiotics in chronic liver injury induced by carbon tetrachloride (CCl₄).

Methods

Thirty-two Wistar male rats were randomly assigned to four groups: Control, CCl₄, CCl₄+Viscum album (VAC) and CCl₄+Viscum album+Probiotics (VAPC). Chronic liver injury was induced by intraperitoneal injection of 1 mg/kg CCl₄ using a single dose every two days for fourteen days. The methanolic mistletoe extract was prepared in Pharmacognosy Laboratory at Anadolu University. During the study, misletoe (300 mg/kg) and probiotics were administered orogastrically daily to the related groups. On the fifteenth day, blood/liver tissue samples were taken from all study groups. Some enzyme activities (ALT,AST,ALP,LDH), and lipid profile (total cholesterol,HDL-c,LDL-c,triglycerides), total protein, albumin, total/direct bilirubin levels were measured in serum samples; catalase activity, MDA and GSH levels were also measured in tissue samples. For histological analysis, Hematoxylin-Eosin staining performed on liver tissue sections.

Results

Statistical findings showed that CCl₄ caused a significant increase in serum enzyme activities and lipid levels compared to the control. These values were significantly closer to control group values in treatment groups, particularly in VAPC group. There was a significant increase in catalase activity and decrease in MDA levels in VAPC compared to CCl₄ group. While necrotic cells with eosinophilic cytoplasm, inflammation and vascular congestion in portal area were observed in CCl₄ group, administration of probiotics ameliorated these changes bringing liver histology to a similar state in control, excluding a few macrovesicular structures.

Conclusions

According to biochemical/histological findings, the use of mistletoe extract and probiotics showed remarkable

therapeutic effect in chronic liver injury induced by CCl_4. These results suggest that the addition of probiotics to diet may be beneficial to enhance intestinal flora in treatments associated with liver injury.

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Key Words: Carbon tetrachloride, chronic liver injury, lipid profile, probiotics, viscum album.