1st INTERNATIONAL CONGRESS ON BIOMEDICAL APPLICATIONS OF NATURAL AND SYNTHETIC MATERIALS

1. Uluslararası Doğal ve Sentetik Materyallerin Biyomedikal Uygulamaları Kongresi

ABSTRACT and PROCEEDING BOOK

23-24 MAY 2024 / KONYA

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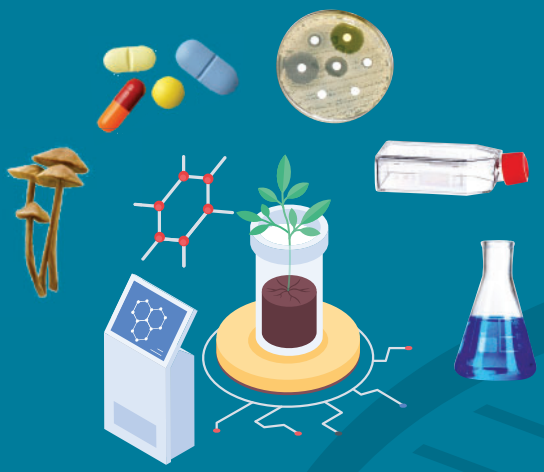

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Editor

Prof. Dr. Emine ARSLAN

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ABSTRACT BOOK

Oral Presentations

OPPORTUNITIES FOR ADVANCEMENTS IN NOVEL NANOBIOMATERIALS IN FORENSIC MEDICINE

Aziz Eftekhari [1]*, Irada Huseynova [2], Rovshan Khalilov [3]
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ABSTRACT

Background of the Study: The field of biomaterial science is a multidisciplinary area that spans various technological and scientific disciplines. It contributes advanced materials that are utilized in the development of increasingly intricate technologies for society. Emerging nanomaterials represent innovative polymers that hold promise for addressing contemporary challenges with durable and effective solutions.

Aim: The primary emphasis in forensic investigations lies in the presentation of evidence collected from the scene of the crime

Material and Method: We performed google scholar and pubmed search on the keywords including *Nanomaterials, Forensic medicine,* nanosensors, nanocodes, nanofilms, nanocoatings from 2018 till 2024.

Results: A deeper understanding of material synthesis and characterization is essential in the field of forensics. The development of novel polymers and nanomaterials, along with advanced equipment, has provided sustainable solutions to various forensic challenges. Due to their distinctive characteristics, the production of nanomaterials like nanosensors, nanocodes, nanofilms, nanocoatings, quantum dots, and composites has seen a rise.

Conclusion: This development has led to expedited analysis, enhanced nano-level identification, and immediate detection, thereby diminishing error potential and significantly reducing the time required for analysis at crime scenes. This work concludes the discussion on the concept of cutting-edge nanomaterials and nanopolymers within the realm of material science. It elucidates the specific areas within forensics that have experienced

Keywords: *Nanomaterials, forensic medicine,* nanosensors, nanocodes, nanofilms, nanocoatings

EFFECTS ON ACE2 GENE EXPRESSION OF DIFFERENT FRACTIONS EXTRACTED FROM *GLYCYRRHIZA GLABRA L*.

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ABSTRACT

Background of the Study: Angiotensin-converting enzyme 2 (ACE2) plays a key role in the entry of coronavirus disease into cells and in the regulation of the renin-angiotensin system (RAS). Modulation of ACE2 mRNA level is an important approach to control other processes in which ACE2 participates and to reduce viral invasion.

Aim: Based on this hypothesis, the roles of various fractions of *Glycyrrhiza glabra* L. roots on ACE2 mRNA expression in A549 cell lines were studied.

Material and Method: Different fractions were obtained by applying water, n-butanol, ethyl acetate and dichloromethane solvents to the methanol:chloroform (1:1) extract of *Glycyrrhiza glabra* L. root by liquid-liquid method. Then, concentrations of 15.6, 31.25, 62.5, 125 and 250 µg/mL of all fractions, respectively, were applied to A549 cell lines by using the MTT method, and non-toxic doses were determined in the range of 15.6-250 µg/mL.

Results: According to the qPCR results of non-toxic doses of the fractions, it was determined that n-butanol fraction decreased the mRNA expression level of the ACE2 gene the most among the Glycyrrhiza glabra (-8.29-fold change). Moreover, dichloromethane and ethylacetate fractions decreased ACE2 mRNA expression by -1.78- and -1.16-fold change, respectively. While, 1.45 fold increase in mRNA expression level was observed after Gg-W application.

Conclusion: Accordingly, fractions of the plant that reduced the ACE2 mRNA level in the A549 cell line may have regulation of RAS and widespread antiviral activity against SARS-CoV viruses. Therefore, further clinical studies are needed to expose the potential components of the effective fractions.

Keywords: ACE2, mRNA expression, *Glycyrrhiza glabra* L.

EFFECTS OF THE PHYTOCHEMICAL COMPOUND CURCUMIN ON THE ENERGY METABOLISM OF BREAST CANCER CELLS

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ABSTRACT

Background of the study: Breast cancer (BC) is one of the main causes of cancer-related deaths in women. Developing new therapeutic agents with fewer side effects for this quite common cancer type has great importance. Curcumin is an important bio-effective extract of the *Curcuma longa* plant and is known to have chemotherapeutic functions as well as many different effects. The antiproliferative, antioxidant and apoptotic effects of curcumin in various cancer types have been demonstrated in different experimental studies.

Aim: In our study, we aimed to investigate the anticancer effects of curcumin especially on the energy metabolism of MDA-MB 231 human BC cells.

Material and Method: MDA-MB 231 cells were cultured appropriately and treated with 1, 5 and 10μM curcumin according to IC_{50} dose calculated after MTT. For the evaluation of energy metabolism, cell ATP, ADP and AMP levels were determined by HPLC and also the biochemical levels of lactate dehydrogenase and catalase, which indicate the biochemical metabolic events of aerobic glycolysis, one of the metabolic pathways used by cancer cells for energy supply, were measured by using ELISA and Aebi 1983 method. Additionally, the expression levels of some genes related to maintain cellular redox homeostasis and cancer energy metabolism were analyzed by qPCR.

Results: According to our results, after curcumin treatment we detected a dose dependent increse of ATP, but decreases of ADP ve AMP levels. Compared to the control group, there were increases in the catalase enzyme levels. LDH enzyme level increased in 1mM curcumin treatment, while no significant change was detected in other doses. It was determined that there was dose-dependent variability in *GLUT1*, *CYP2E1* and *LDHA* gene expressions, however, there was a 2-fold or more decreases in the expression of all 3 genes at 10μM treatment.

Conclusion: Our results show that curcumin may be an effective agent in blocking the energy pathway of BC cells. However, further and comprehensive studies are needed to determine the most effective doses and to increase therapeutic effectiveness by ensuring greater penetration into target cells.

Keywords: Breast cancer, MDA-MB 231 cell line, curcumin, cancer energy metabolism.

MEROPENEM RELEASE PERFORMANCE AND ACTIVITY OF ALGINATE CHITOSAN COATED MICROCAPSULE WITH SPOROPOLLENIN

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ABSTRACT

Background of the Study: Drug delivery systems allow to optimize the treatment process by precisely controlling the release of drugs in the body. In particular, the encapsulation technique contributes to reducing side effects and increasing the stability of the drug by providing a more controlled release of drugs into the body.

Aim: The main objective of this study is to produce microcapsules using sporopollenin, alginate and chitosan obtained from the pollen of the *Cedrus Libani* tree and to realize the controlled release of Meropenem, which is an antibiotic effective, in these microcapsules.

Material and Method: Combining sporopollene with nano-sized additives can add a new dimension to controlled drug release technology. The hydrogel layer formed by alginate with Ca^{2+} cations protects the biological activity of the drug while also controlling the diffusion of the drug out. In this way, a suitable environment is provided for the encapsulation process of sporopollene and alginate. The sporopollene microcapsules were used for the preparation of alginate and chitosan. SEM and FTIR encapsulation efficiency were analyzed for characterization, and *in vitro* drug release study was performed by dialysis method from water.

Results: In the characterization studies, microcapsules exhibited high encapsulation efficiency, SEM and FTIR. Whole amount of meropenem was released from sporopollenin alginate-chitosan microcapsules at the end of 25 h, and a slower and controlled drug release was obtained compared to free drug solution. The efficiency of microcapsules were found higher than 84.38%, and controlling drug release stability remained for 25 h.

Conclusion: Meropenem loaded microcapsules formulations as alginate chitosan agents were successfully prepared. Future studies are continuing to evaluate the antimicrobial and imaging efficacy of the formulations through in vitro cell culture studies.

Keywords: Alginate, *Cedrus libani*, chitosan, drug delivery, meropenem

EFFECTS OF AGED GARLIC EXTRACT AND S-ALLYLSISTEIN ON ALLERGIC RHINITIS AND BLOOD PRESSURE

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ABSTRACT

Background of the Study: Allergic rhinitis, causes hypoxia and sympathetic system activation, resulting in increased blood pressure. Aged Garlic Extract (AGE) is a product of fermentation of garlic at high temperature and humidity. AGE are converted into antioxidant compounds during the ageing process; S-allylcysteine (SAC) is one of them.

Aim: The aim of this study was to determine endothelin-1 (ET-1) levels after treatment with AGE and SAC in ovalbumin (OVA)-sensitised allergic rats and to investigate the histopathological effects of OVA, AGE and SAC on nasal and lung tissues.

Material and Method: AGE and SAC treatments were administered in an appropriate manner to the groups of animals in which OVA-induced allergic rhinitis models were established. At the end of the experiment, ET-1 levels were measured in the serum of the animals using Elisa method. Histopathological evaluation was performed using haematoxylin-eosin staining in lung and nasal tissues.

Results: ET-1 levels showed a significant difference between all groups ($p \leq 0.05$) and were highest in the OVA group. After treatment, AGE decreased ET-1 levels more than SAC. In haematoxylin-eosin specimens, classic allergic rhinitis pathology was observed in lung tissue. In the OVA and SAC groups, a small amount of lung fibrosis was observed. No differences in the olfactory and respiratory epithelium and no increase in goblet cells were observed in nasal tissue. The inflammation score was higher in OVA group than in the controls ($p<0.01$). Compared to OVA group, inflammation score was lower in AGE group ($p<0.05$).

Conclusion: In our study, AGE was found to be more effective than SAC in regulating blood pressure. It was found that OVA causes histological tissue damage in the nasal mucosa and lung tissue, that AGE and SAC can repair this damage, and that AGE may be more effective in this repair.

Keywords: Allergic rhinitis, ovalbumin, aged garlic extract, s-allilsistein, endothelin-1

CYTOTOXIC EFFECT OF DAPAGLIFLOZIN ON A BREAST CANCER CELL LINE: IN VITRO STUDY

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ABSTRACT

Background of the Study: Cancer cells are cells with atypical metabolic characteristics and in these cells, glucose is converted from pyruvate to lactate even in an aerobic environment (Warburg effect), although the amount of two ATPs obtained from one molecule of glucose is very low. Although the amount of ATP obtained by this pathway is small, it is compensated for by high glucose uptake. High glucose uptake also increases ribose 5-phosphate synthesis by activating the hexose monophosphate pathway. Ribose 5-phosphate is the substrate for increased mitotic activity, and rapidly proliferating cancer cells can easily provide the necessary components for DNA, RNA, lipid and protein synthesis. In this case, the cells have an advantage in terms of survival. Breast cancer is the most common form of cancer in women. Dapagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, is thought to have potential anti-cancer properties. However, the cytotoxic effects of dapagliflozin on breast cancer cells are not fully understood.

Aim: The aim of this study was to investigate the cytotoxic effect of Dapagliflozin in MCF-7 breast cancer cell line in vitro.

Material and Method: The cytotoxic effect of Dapagliflozin on MCF-7 breast cancer cell line was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. The results were evaluated using GraphPad PRISM program and IC50 values were determined.

Results: According to the MTT results, the IC50 values of Dapagliflozin tested were 24.82µg/mL, 171.3µg/mL and 21.40µg/mL for 24, 48 and 72 hours, respectively.

Conclusion: The data suggest that dapagliflozin may be a promising alternative approach for the treatment of breast cancer. However, further studies are needed.

Keywords: Dapagliflozin, breast cancer, SGLT2, cytotoxicity

ENGINEERING FUNGAL MELANIN FOR RADIATION PROTECTION

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ABSTRACT

Background of the Study: Melanin is a unique pigment found across all biological kingdoms, providing protection against environmental stressors such as UV radiation and oxidative damage. Its potential as a radioprotective agent has garnered interest, especially in the context of radiation-heavy environments like space exploration and nuclear facilities. This study explores the molecular engineering of fungal melanin synthesis pathways to develop efficient radioprotective materials.

Aim: The project aims to isolate and optimize the genes responsible for melanin synthesis in *Cryptococcus neoformans* and other melanized fungi, facilitating high-yield production of melanin for use in radioprotective applications.

Material and Method*:* Genes associated with melanin production, such as *LAC1* and *PKS,* will be isolated from *Cryptococcus neoformans.* These genes will be cloned into suitable expression systems like *Escherichia coli* and *Pichia pastoris.* The recombinant organisms will be cultured under optimized conditions to maximize melanin yield. The produced melanin will be purified and characterized using techniques such as NMR, FTIR, and EPR. Subsequently, melanin-based composite materials will be developed and their radioprotective efficacy tested.

Results: In the preliminary study, it will first be demonstrated that melanin synthesis genes have been successfully isolated and cloned. Optimization studies will be carried out to enhance melanin production by improving culture conditions. Characterization studies will be conducted to verify the structural integrity of the synthesized melanin. Initial tests of melanin-based materials will aim to explore their potential use in high-radiation environments by demonstrating their radioprotective properties.

Conclusion: The engineered production of fungal melanin offers a viable pathway for creating effective radioprotective materials. This approach not only leverages the natural protective properties of melanin but also provides a sustainable and scalable method for its production. The findings pave the way for further research and development in the field of biological radioprotectors.

Keywords: Biotechnology, fungal melanin, gene engineering, radioprotection, recombinant expression

THE EFFECT OF CDTE QUANTUM DOT NANOPARTICULES ON THE MALE REPRODUCTIVE SYSTEM IN MICE AND THE EFFECT OF ANTİOXİDANT ON THIS EFFECT

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ABSTRACT

Background of the Study: Since the areas of use of nanoparticles are gradually increasing, the toxicity of these particles, to which the human body is more exposed, on the testis and epididymis has become an important issue considering the increasing infertility rates.

Aim: In this study, we investigated the efficacy of Mitoquinone, Thymoquinone and Silymarin, which are known as strong antioxidants, on the histopathological changes that may occur in the testis and epididymis of mice treated with CdTe quantum dot nanoparticles.

Material and Method: G1-Control (n=5); G2-CdTe QD (10mg/kg) (n=7); G3- Mitoquinone (5 mg/kg) + QD (10mg/kg) (n=7); G4-thymoquinone + QD (10mg/kg) (n=7); G5- Silymarin (100 mg/kg) +QD (10mg/kg) (n=7). After 24 h, testis and epididymis tissues from sacrificed animals were examined by hyperspectral and confocal microscopy. General histopathological evaluation was performed using hematoxylin and eosin. Immunofluorescence labelled tissues were examined by fluorescence microscopy.

Results: General histopathological evaluation by calculating the Jhonsen score showed no difference between all groups. The mean value for normal testicular tissue was 9.10, while our mean value was approximately 9.IHC results using anti-MT-2A, anti-MT-MMP 2, anti-MMP 9 anti-Ki-67 antibodies were higher in the Cd+2 group than in the control group. In the antioxidant groups, it was slightly higher than the control group but lower than the Cd+2 group and antioxidant groups were found close to each other.

Conclusion: It was observed that CdTe QD passed into testicular epididymis tissues and it was evaluated that there was no deterioration in the histological structure in the acute phase. In IHK evaluation, there is an increase in the other groups compared to the control group. The anti-oxidant groups we used in our study provided improvement compared to Cd+2 group, but these three groups were not superior to each other.

Keywords: Testis epididymis, quantum dot, mitoquinone, thymoquinone, silymarin

A POTENTIAL THERAPEUTIC NATURAL PRODUCT AGAINST PANCREATIC CANCER: SKIN MUCUS OF RAINBOW TROUT, *ONCORHYNCHUS MYKISS* (WALBAUM, 1792)

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ABSTRACT

Background of the Study: Pancreatic cancer is a type of cancer with a low 5-year survival rate and limited treatment options. Its prognosis is aggressive and indicates malignancy. Since the challenging anatomical location of the pancreas within the body, the possibility of early diagnosis is improbable. Due to this situation, even with advancements in modern therapeutic approaches, patients have limited treatment options due to late-stage diagnosis. These few available treatment options include radiation and chemotherapy, which have side effects, as well as dangerous surgical procedures. In place of possible harmful surgical procedures and unselective chemotherapy drugs that worsen patient quality of life, rainbow trout skin mucus might be a potentially natural anticancer product.

Aim: This study aimed to evaluate the cytotoxicity and potential anticancer effects of rainbow trout skin mucus against the pancreatic cancer cell line PANC-1 as a natural product.

Material and Method: The mucus was carefully scraped from the fish skin surface with a sterile cell scraper and homogenized in PBS. To remove insoluble particles, they were centrifuged and lyophilized to powder. Lyophilized mucus was dissolved in the cell medium and sterilized with a filter. Certain doses of mucus extract were treated with the cells for 24, 48, and 72 hours. Following the incubation period, the cytotoxic effect was determined by the MTT method.

Results: The IC50 values for rainbow trout skin mucus were found to be 4.41 mg/mL, 3.10 mg/mL, and 3.37 mg/mL for the 24, 48, and 72-hour incubation periods, respectively.

Conclusion: The study's data collection revealed that the PANC-1 cell line, which represents aggressive and poor-prognosis pancreatic cancer, is susceptible to the cytotoxic effects of rainbow trout skin mucus at different concentrations. The potential of skin mucus, a natural product, as an anti-cancer agent might be unlocked. Innovative treatments can be discovered with additional research, content analysis, data collected by purifying biomolecules and treating them with cells, and in vivo experiments.

Keywords: Cytotoxicity, natural product, pancreatic cancer, rainbow trout, skin mucus

[1]This study was supported financially under a project (Project No: 22101002) from the Selcuk University Scientific Research Foundation (BAP). This research is part of the master thesis of Büşra ÖZÇAY.

ETHANOL EXTRACT OF *LAETICUS CRISTATA* (SCHAEFF.) AUDET HAS A POTENTIAL PROTECTIVE EFFECT AGAINST H2O2-INDUCED DNA DAMAGE

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ABSTRACT

Background of the Study: Natural products have been used as part of alternative medicine for many years because they are easily and abundantly available and have low toxicity. Even in the modern era, these products can be protective or preventive in managing several illnesses, including cancer. DNA damage is the cause of many diseases, including cancer. When UV light and reactive oxygen species (ROS) target DNA, damage to the DNA may result. Due to their antioxidant activity, natural products can support the antioxidant defense system and protect DNA from ROS damage. Additionally, it may reduce or prevent UV-induced DNA damage. For this reason, this study evaluated the protective capacity of the natural product *Laeticutis cristata* (Schaeff.) Audet (*L. cristata*) mushroom, which is uncommon in our country, against UV and ROS-induced DNA damage.

Aim: In this study, it was aimed to determine the protective effect of the ethanol extract obtained from the *L. cristata* against UV and H2O2-induced DNA damage.

Material and Method: Plasmid DNA (pUC18) was used to determine the potential to prevent UV- and ROS-induced DNA damage of *L. cristata* ethanol extract. Plasmid DNA was incubated with H2O2 at 37 °C for one hour and exposed to 366 nm UV light for fifteen minutes, either alone or in combination with H2O2. The damage caused by this exposure to DNA in the presence or absence of certain doses of the extract (0.5, 1 and 2 mg/mL) was determined by the electrophoretic method.
Images were taken by running the samples in 1% agarose gel at 90 volts for 1 hour.

Results: As a result of electrophoretic application, it was determined that *L. cristata* ethanol extract significantly protected DNA against H2O2-induced DNA damage, while the protection against UV damage could not be detected by the electrophoretic method.

Conclusion: The hypothesis that *L. cristata* extract inhibits ROS-induced DNA damage is supported by the experimental data in this study when the results are analyzed. It is conceivable to employ *L. cristata* extract as a natural product to assist preventive treatment techniques and to develop dietary supplement formulations with outcomes that will support the antioxidant protection system against ROS-induced DNA damage with more research.

Keywords: DNA damage, antioxidant activity**,** *L. cristata*, natural product, ROS

DEGRADATION OF TOXIC NITROAROMATIC COMPOUNDS BY Ag(0)-AMBERLITE IR-120 HETEROGENEOUS CATALYST SYSTEM

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ABSTRACT

Background of the Study: Serious environmental health problems occur through industrial use of nitroaromatic compounds. Reduction of nitroaromatic compounds to aminoaromatics is important for environmental applications. Recently, metallic silver particles have emerged as an alternative in the reduction of toxic nitroaromatics.

Aim: The preparation of Ag(0)-Amberlite IR-120 heterogeneous catalyst system and the determination of its catalytic activity in the reduction of 4-nitrophenol, 2-nitroaniline, 4-nitro-o-phenylenediamine and 2,4-dinitrophenol to aminoaromatics.

Material and Method: The catalyst system was prepared by heating Amberlit IR-120 cation exchange resin beads (1.2 g) in silver nitrate solution (0.4 g $AgNO_3$ in 40 mL water) at 70 °C overnight. The catalyst was collected by filtration, rinsed with water and dried at room temperature. Characterization of the catalyst system was performed by SEM-EDX analyses. The catalytic performance of the catalyst was tested with varying amounts of catalyst (3-7 mg) and $NaBH_4$ (4-8 mg). The catalyst was mixed with the nitroaromatic compound solution and then the reduction was started by adding $NaBH_4$. The solution turned clear and the absorbance at a specific wavelength was monitored on a UV-vis spectrophotometer. Reusability tests of the catalyst (3 cycles) were done under optimum conditions (catalyst: 7 mg, $NaBH_4$: 4 mg). After each use, the used catalyst was collected by filtration and washed with water. The catalyst was dried and used. Characterization of the regenerated catalyst was performed by SEM-EDX and ICP analyses.

Results: Short reduction rates were achieved; 4-nitrophenol (32 s), 2-nitroaniline (28 s), 4-nitro-o-phenylenediamine (57 s) and 2,4-dinitrophenol (7 min). The efficiency of the regenerated catalyst system decreased significantly after each regeneration process and Ag was released into the environment.

Conclusion: Ag(0)@Amberlit IR-120 system was effective in the reduction of nitroaromatics in its first use. However, the regeneration efficiency of the catalyst system should be improved.

Keywords: Heterogeneous catalyst, nitroaromatics, silver

RARE DISEASE: PULMONARY ALVEOLAR MICROLYTICOSIS

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ABSTRACT

Background of the Study: Pulmonary alveolar microlithiasis (PAM) is a rare genetically inherited lung disease characterized by intraalveolar calcium and phosphate deposition in bilateral lung parenchyma, predominantly in the lower and middle zones. PAM is an autosomal recessive disorder caused by a defective sodium-dependent phosphate transporter due to variants of the SLC34A2 gene.

Aim: We present two patients with pulmonary alveolar microlithiasis who were followed up for a long period of time because it is a rare disease.

Material and Method: The clinical, functional and radiologic findings of two patients with pulmonary alveolar microlithiasis, both at presentation and during follow-up, were evaluated restrospectively.

Results: The first patient was 46 years old and the second patient was 47 years old. The first patient had cough and the second patient had dyspnea. Posterior anterior chest radiography and high-resolution thorax computed tomography showed diffuse millimetric calcified nodules, more prominent in the lower lobes. Pulmonary function tests of both patients were normal. While arterial blood gas values were normal in the first case, mild hpoxemia was present in the second case. Biochemical and infection markers of both patients were normal. In terms of tuberculosis, ARB and cultures were negative. Since both of our patients refused bronchoscopy, they were diagnosed clinically and radiologically as pulmonary alveolar microlithiasis and were followed up without treatment. First-degree relatives of our first patient were screened for the disease and no evidence of the disease was found. Clinical and radiologic progression was not observed in the 5-year follow-up of our first patient who was followed up without treatment. Clinical and radiologic progression was also not observed in the evaluation of our second patient in the 1st year.

Conclusion: Although PAM is a rare disease in the world, it is a disease that should be kept in mind in the differential diagnosis of radiologic findings in the presence of bilateral calcified micronodular infiltration because it is encountered more frequently in our country.

Keywords: Pulmonary alveolar microlithiasis, rare diseases, bilateral calcified micronodule

ANXIETY AND DEPRESSİON IN DENTAL TECHNICIANS AND RELATED FACTORS

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ABSTRACT

Background of the Study: Dental technicians are exposed to many potentially toxic substances, dust and fumes (including silica, alloys, and acrylic-platics). The frequency of anxiety and depression is increased in people working in life-threatening environments such as heavy metal inorganic dust and solvents.

Aim: The aim of this study was to evaluate the frequency of anxiety and depression and associated risk factors in dental technicians. For this purpose, demographic findings, symptoms, working hours and departments, smoking status, presence of pneumoconiosis and presence of anxiety/depression were investigated in dental technicians.

Material and Method: Dental technicians who applied to the occupational diseases outpatient clinic within a two-year period were included in the study. Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS).

Results: The mean age of 310 male dental technicians was 35.8±8.5 years. Anxiety was present in 111 (36%), depression in 145 (47%) and both in 83 (27%) of the dental technicians. Pneumoconiosis was detected in 31 (10%) dental technicians. Both anxiety and depression and anxiety plus depression were higher in dental technicians with pneumoconiosis compared to those without pneumoconiosis ($p<0.05$). Anxiety and depression scores were highest in the pneumoconiosis group. According to regression analysis, the presence of respiratory complaints and working time were found to be significant risk factors for anxiety, depression and anxiety plus depression.

Conclusion: Frequency of anxiety/depression increased significantly in dental technicians. The intense occupational exposure of dental technicians closely affects their quality of life and psychosocial status. Therefore, dental technicians should be routinely evaluated for anxiety and depression in addition to their physical conditions. It may be recommended to identify individuals at risk for anxiety and depression at an early stage and to provide professional help

Keywords: Anxiety, depression, dental laboratory technicians, occupational exposure, pneumoconiosis.

BIOSENSORS AND THEIR MEDICAL APPLICATIONS

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ABSTRACT

Background of the Study: Biosensors are analytical devices that recognize the biological target and biological activity and transform this recognition event into a measurable situation. Using transducers with electrochemical, optical and calorimetric properties, meaningful signals are generated and the amount of the biological target is determined. Nowadays, biosensors are used especially in the field of health; It is used in environmental analysis, military field, food, pharmaceutical and chemical industries.

Aim: In our study, it was aimed to evaluate the working principle and advantages of biosensor methods, which are a new, fast and accurate method, compared to traditional medical measurement methods.

Material and Method: In our study, scientific publications on biosensors used in the medical field in the diagnosis of diseases such as cancer, in the detection biochemical parameters and antigenic structures of microbiological agents, in monitoring the vital signs of patients in intensive care units and as implants in surgeries were investigated. The data obtained from current scientific medical articles and reviews, found by scanning national and international academic databases, were analyzed and the results were evaluated.

Results: In our study, biosensors are used in the determination of complex substances; It has been shown to have many advantages such as high sensitivity, low cost, short measurement time, low detection limit and the ability to activate automatic measurement and adjustment systems.

Conclusion: We think that nanotechnology, which is considered one of the key technologies of the 21st century, will further increase the technological development of biosensors and increase its weight in the economy and daily life. For this reason, it was concluded that it is necessary to establish more research and development centers to expand the usage areas of biosensors.

Keywords: Biomarker, biosensor, medical measurement

USE OF FORMALDEHYDE IN ANATOMY

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ABSTRACT

Background: Formaldehyde (CH2O) is a substance formed as a result of the oxidation of methanol and is found in the natural structure of the organism. It turns into a gas at room temperature and creates an irritating effect on the mucosa. The gas form is colorless and has a pungent odor. It can be used for fixation in non-living tissues. It is widely used in the medical field. It is used to detect and store cadavers used in anatomy education. It also provides tissue fixation in the fields of histology and pathology. It is found in the structure of dental crown in dentistry. The solutions used in hemodialysis units also contain formaldehyde.

Aim: Although formaldehyde is widely used in medicine, it also has harmful effects on human health. It is also reported that formaldehyde is a carcinogenic substance. In chronic exposure, neurotoxicity symptoms such as mood disorders, behavioral disorders and epilepsy may occur. For this reason, it is a substance that should be used carefully and we wanted to emphasize this.

Material and Method: In cadaver fixation, solutions are generally injected through the femoral artery or common carotid artery. A solution is prepared by using formaldehyde in various concentrations and adding glycerin.

Results: Considering its widespread use in the past and present, formaldehyde solutions are the closest candidate to be the ideal fixative.

Conclusion: Formaldehyde is especially the material used for cadaver fixation, especially in anatomy. Despite all these harmful effects of formaldehyde, it is still widely used all over the world because it is a cheap and good fixation method. Melatonin and omega-3 fatty acids play a protective and therapeutic role against the harmful effects of formaldehyde. It is necessary to keep the concentration of formaldehyde below the permissible limits.

Keywords: Anatomy, cadaver, formaldehyde, medicine

EVALUATION OF LARGE LANGUAGE MODELS IN PHARMACOLOGY EXAM PERFORMANCE AND REFERENCE ACCURACY

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ABSTRACT

Background of the Study: The rapid progress in artificial intelligence has led to an increasing use of Large Language Models (LLMs) in scientific, biomedical, and educational applications. There are limited studies on the performance of recent versions of LLMs in various exams in medical fields and the reliability of their answers and references.

Aim: The study aims to compare the performance of recent LLMs in answering multiple-choice pharmacology exam questions and the accuracy of the provided references.

Material and Method: The study used questions from the midterms of the medical pharmacology course for dentistry students at Selcuk University in 2023 and 2024. Fifty multiple-choice questions (MCQs) were submitted to ChatGPT-4 and Google Gemini. A prompt was used to ask LLMs to choose the best answer, provide explanations and references for their answers. The names of reference articles, books, and authors were verified using Google Scholar, and PubMed. The performance of the two large language models and the types and accuracy of the references they used were compared.

Results: ChatGPT-4 outperformed Gemini (80.0%) and students (72.6%) by correctly answering 98.0% of the MCQs. Moreover, ChatGPT-4 most frequently cited research/review articles (57.3%) and book chapters (39.6%), while Gemini most frequently cited Wikipedia (39.5%) and research/review articles (28.7%). Furthermore, ChatGPT-4 had a higher percentage of references not found on academic indexes (21.9%) compared to Gemini (7.0%).

Conclusion: ChatGPT-4 performs in MCQs better than Gemini, possibly due to Gemini using less valid sources, such as Wikipedia and other websites. On the other hand, ChatGPT-4 uses academic sources, such as textbooks and review articles. However, unverified references can reduce its reliability for educational use. Although LLMs can be helpful in pharmacology education, they should be used under supervision and with caution.

Keywords: Artificial intelligence, large language models, medical pharmacology, multiple choice questions

GENDER DIFFERENCES IN ACNE SEVERITY AND PSYCHIATRIC SYMPTOMS

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ABSTRACT

Background of the Study: Adolescent acne is a common dermatological condition that not only affects physical appearance but also has significant psychosocial implications.

Aim: This study investigates the relationship between Visual Analog Scale (VAS) scores, utilized for evaluating acne severity, and Strengths and Difficulties Questionnaire (SDQ) scale scores, gauging emotional and behavioral challenges, among adolescent acne patients, while considering gender disparities. This study aims to fill gap by investigating the correlation between VAS scores and SDQ scale scores among adolescent acne patients, considering gender disparities. By elucidating these relationships, this research seeks to inform more effective healthcare strategies and improve the well-being of adolescents with acne.

Material and Method: 115 adolescents diagnosed with acne, aged 12 to 17, were included in the study. Psychiatric symptoms were assessed using the Strengths and Difficulties Questionnaire (SDQ), while acne severity was evaluated using the Visual Analog Scale (VAS).

Results: The findings reveal a statistically significant correlation between VAS scores and SDQ scores in female patients, indicating a potential interplay between acne severity and psychosocial well-being in adolescent girls. However, no significant correlation was observed among male patients, suggesting differential psychosocial responses to acne between genders during adolescence.

Conclusion: This gender-specific variance underscores the importance of considering gender dynamics in understanding and addressing adolescent acne and its psychosocial implications. Possible factors contributing to these gender differences include gender-specific stressors, coping mechanisms, and socio-cultural influences. Further investigation is warranted to elucidate these dynamics comprehensively and to develop gender-sensitive interventions tailored to the unique needs of adolescent acne patients. Understanding the nuanced interplay between acne severity and psychosocial factors in gender-specific contexts is crucial for optimizing healthcare outcomes and enhancing the well-being of adolescent acne patients.

Keywords: Adolescents, acne severity, psychiatric symptoms.

PLASTINATION FROM MEDICAL EDUCATION TO ART

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ABSTRACT

Background of the Study: Cadaver; It is defined as "the dead human or animal body that is studied in medical education". There are chemical and physical applications that provide protection of cadavers as educational materials. One of these methods, plastination, is the removal of fat and water from organic tissues and coating with liquid silicone polymer. This method was discovered by Gunther von Hagens in 1977. The exhibition of cadavers with this method was held for the first time in 1996 and attracts a lot of interest.

Aim: In this study, the development of the plastination method, advantages and disadvantages, as well as the development of this technique from its place in medical education to the process of becoming an exhibition of the body will be examined.

Material and Method: The main three methods used in plastination are silicone plastination, sheet plastination by epoxy method and sheet plastination by polyester method. Silicone plastination is a technique used for cadavers, organs and slices. Epoxy plastination allows to take sensitive semi-transparent section samples. Thus, gross anatomical structures can be examined with superior quality up to the macroscopic level with the naked eye. In the polyester plastination method, the tissue fluid is removed and replaced with a polyester resin that can harden, it can be used for head brain and body slices.

Results: With plastination, the tissues have odorless, non-toxic properties that are resistant to high temperatures. Plastinates turn into an excellent tool in education, free from the harmful effects of formaldehyde.

Conclusion: The connection of the multidisciplinary feature of anatomy with art is very interesting. From the point of view of education, art and science, this method has an unusual feature.

Keywords: Anatomy, art, education, plastination

DEVELOPMENT OF POTENTIAL ANTIMICROBIAL BIOBASED EDIBLE FOOD PACKAGING MATERIAL FROM SUMAC (*RHUS CORIARIA* L.) EXTRACT

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ABSTRACT

Background of the Study: Packaging plays an important role in the food industry as a protection system used by food manufacturers to deliver food products to businesses. Traditional food packaging materials include plastic, paper, glass, metal, and composites, but their use in edible packaging is limited. Considering these problems, potentially eco-friendly edible packaging is preferred by consumers. Natural polymers, derived from plant and animal sources, form an important category widely used in biodegradable packaging materials. Plant polysaccharides such as starch, pectin, chitin/chitosan, plant proteins such as zein, soy, gluten, and casein, as well as whey protein biopolymers are integrated into food packaging in various industrial applications.

Aim: In this study, the production of biofilms containing Sumac (Rhus coriaria L.) extract and cellulose fiber added was aimed to determine their thickness, swelling, solubility, water content, pH sensitivity, mechanical properties, biodegradability, light transmittance, and antimicrobial activities.

Material and Method: Conditions where suitable biofilms could be produced were selected and biofilm production was achieved by adding plasticizers (sorbitol, glycerol, xylitol, citric acid) in the range of 5-15% (w/w). The most suitable biofilms were obtained by adding 10% (w/w) glycerol to the extracted celluloses at the center point and optimum point. Cultures of *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853, and *Staphylococcus aureus* ATCC 43300 activated in Nutrient Broth for one day were used in the study. Biofilms obtained using the disk diffusion method were tested on different microorganism strains.

Results: The inhibition zone diameter of starch polymer added sumac extract derived biofilms against methicillin-resistant *S. aureus* strain (ATCC 43300) was determined to be 14 mm. No significant antimicrobial activity of cellulose fiber added sumac biofilm structure was observed against the other 2 bacterial strains used in the study.

Conclusion: As a result of the studies, it was determined that sumac extract is a potential source of hemicellulose and the extracted hemicelluloses are suitable for biodegradable film production. It is anticipated that this study will make important contributions to the development of innovative antimicrobial packaging systems in the food industry and the improvement of existing systems**.**

Keywords: Sumac, starch fiber, eco-friendly, edible film, antimicrobial, food packaging

SYNTHESIS OF BIOGENIC AO-AGNPS AND DETERMINATION OF SOME BIOMEDICAL ACTIVITIES

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ABSTRACT

Background of the Study: Due to their widespread usage in different fields, nanoparticles have been the foundation of nanotechnology for decades. Nanoparticles' surface/volume ratio and crystallographic surface structure determine their biological activity.

Aim: This study aims to produce silver nanoparticles (Ag NPs) by the green synthesis method using the aqueous extract obtained from the waste leaf of the *Anchusa officinalis* (AO) plant. The *Anchusa officinalis* L. leaf is traditionally used topically to treat wounds including cuts, bruises, and phlebitis and treat coughs and bronchial catarrh.

Material and Method: Biosynthesized silver nanoparticles were characterized using various techniques and their antimicrobial and cytotoxic activities were tested. Characteristic features of synthesized silver nanoparticles (AgNPs) were examined by SEM, TEM, AFM, EDX and XRD methods. FTIR and LC-MS/MS techniques were used to determine the extract's phytochemical content and the compounds leading to reduction reactions. The inhibitory effects of AO Ag NPs and conventional antibiotics on pathogenic gram-negative and gram-positive bacteria as well as *Candida albicans* yeast were evaluated using the minimum inhibitory concentration (MIC) method. The potential anticancer activity was assessed in vitro against four cancer cell lines (LnCap, Caco2, MDA-MB2, A549) and compared to their activities in the Human Embryonic Kidney (HEK) 293 using the MTT test.

Results: It was shown that AO-AgNPs were very efficient in inhibiting the development of yeast and pathogenic gram-positive and gram-negative bacterial strains. Furthermore, AO-AgNPs demonstrated considerable cytotoxicity against all malignant cell lines examined but no cytotoxic impact against healthy cell line.

Conclusion: AO-Ag NPs could be used in medicine as pharmaceutical carriers, antibacterial, imaging, and anticancer agents due to their exceptional ability to stop the development of microorganisms and cancerous cells.

Keywords: AgNP*, Anchusa officinalis,* antimicrobial activity, cytotoxic activity, LC-MS/MS

MECHANICAL THROMBECTOMY FOR MEDIUM VESSEL OCCLUSIONS

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ABSTRACT

Background of the Study: The current guidelines now recommend endovascular treatment (EVT) for selected patients with large vessel occlusions in acute ischemic stroke. However, there is currently no high-level evidence of the safety and efficacy of EVT for patients with medium-sized artery occlusions. IV tPA is used as standard treatment in medium-sized artery occlusions and its clinical results are moderate. With increasing experience in many centers, endovascular interventions have begun to be performed in smaller and distal medium-sized vessel occlusions. Endovascular treatment of medium vessel occlusions is difficult, and there are not enough studies on the effectiveness of endovascular treatment for MeVOs.

Aim: Accordingly, in this study, our aim was to assess the feasibility, safety, and preliminary efficacy of endovascular therapy for primary and rescue MeVOs. Endovascular treatment of medium vessel occlusions is difficult, and there are not enough studies on the effectiveness of endovascular treatment for medium vessel occlusions.

Material and Method: This study involved a retrospective analysis of prospectively collected data from the stroke centres for acute ischaemic stroke between 2017 and 2023. Demographic, radiologic, procedural and outcome variables were collected for patients who underwent endovascular therapy for acute ischaemic stroke. MeVOs have been defined as occlusions of the M2, M3, A1, A2, A3, PICA, fetal PCA, P1, P2, or P3 segment.

Results: 202 of 603 patients who underwent thrombectomy were MeVOs. Patients with MeVOs were older than patients LVO (71.26 ± 14.04 vs. 67.28 ± 15.17, p=.002). No significant differences were observed in other demographic and clinical characteristics, including sex, presence of hypertension, diabetes mellitus, dyslipidemia, and atrial fibrillation, smoking, heart valve replacement, intravenous tPA, and the rate of successful reperfusion. Patients with rescue MeVOs had a higher NIHS score at admission (19 [IQR, 17 – 25] vs. 18 [17 – 19], p=.001), NIHS score at 3 months (13 [IQR, 8 – 15] vs. 9.5 [4 – 12], p<.001), mRS score at 3 months (3.39±1.11 vs. 2.52±0.82, p<.001) and the mortality rate (21.7% vs. 0.8%, p<.001) compared those primary MeVOs. The functional outcomes of patients with primary MeVOs on the 90th day were considerably better than their initial NIHS scores. Primary MeVOs had a relatively good prognosis even if the presenting symptoms were apparently severe. In addition, the 90th day functional outcomes of the patients with primary MeV occlusion was better than rescue MeV occlusions.

Conclusion: Mechanical thrombectomy treatment of patients with primary MeVOs is effective, safe and feasible. The clinical outcomes of patients with rescue MeV occlusion despite recanalization are not satisfactory. Thrombectomy for MeVOs could be the next step forward in patients with primary MeVOs.

Keywords: Medium vessel occlusion, thrombectomy, acute ıschemic stroke, endovascular treatment, mev occulusion.

CAROTID STENTING FOR CAROTID NEAR-OCCLUSION

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ABSTRACT

Background of the Study: Carotid artery disease is one of the important causes of ischemic stroke. The North American Symptomatic Carotid Endarterectomy Trial (NASCET) study has demonstrated a relationship between degree of carotid artery stenosis and ischemic stroke. As a result, the degree of carotid artery stenosis directly affects the decision to intervene.

Aim: Preocclusive carotid stenosis is considered rare. It is a misdiagnosed condition and the treatment strategy remains unclear. There are not enough studies. Accordingly, in this study, our aim was to assess the frequency of carotid near-occlusion and compared preoperative and postoperative characteristics, complications, and outcomes of patients with near-occlusive carotid stenosis undergoing carotid artery stenting.

Material and Method: This study involved a retrospective analysis of a prospectively collected from stroke centres for patients who underwent carotid stenting due to near-occlusive carotid stenosis from 2017 to 2024. The demographic, radiological, procedural and clinical outcome variables of patients who underwent carotid stenting for carotid near-occlusion were collected.

Results: A total of 1369 consecutive patients who underwent conventional angiography due to carotid stenosis. 368 of these patients had near occlusion. While carotid stents were placed in 255 patients, endarterectomy was performed in 73 of them.

Conclusion: Carotid stenting is safety and feasible in near-occlusive carotid stenosis.

Keywords: Carotid near-occlusıon, ıschemic stroke, endovascular treatment.

ENDOVASCULAR TREATMENT FOR CAROTID STENOSIS DEVELOPING AFTER NECK RADIOTHERAPY

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ABSTRACT

Background of the Study: One of the causes of carotid stenosis is radiation therapy to the neck area. Cervical irradiation is frequently used in the treatment of head and neck cancers. However; the ionizing effect of radiation causes arteritis, which can lead to acceleration of fibrosis or atherosclerosis, thrombosis, stenosis, or occlusion. Carotid stenosis due to radiation is relatively rare.
Aim: Accordingly, in this study, our aim was to assess the feasibility, safety and preliminary efficacy of endovascular therapy for carotid stenosis developing after neck radiotherapy.

Material and Method: This study was a retrospective review of a prospectively maintained database of a consecutive unselected group of symptomatic and asymptomatic patients with carotid artery stenosis developing after neck radiotherapy. Data was collected from 2017 to 2023.

Results: One patient had a technical failure due to a difficult aortic arch. Because of the degree of stenosis (40≤) was not serious in conventional angiography of 2 patients, medical follow-up was decided. 3 patients were admitted to the emergency department due to acute ischemic stroke. Endarterectomy was performed in 4 patients due to the complex structure of the aortic arch and carotid arteries for carotid artery stending. The number of patients with stenosis in the right carotid was 10, while the number of patients with stenosis in the left carotid was 7. No restenosis was observed in patients with stent placement in follow-up carotid Doppler and CT angiography performed 3 months later. Additionally, no new neurodeficit was observed in the neurological examinations 3 months later.

Conclusion: This retrospective study demonstrates that endovascular treatment for carotid stenosis developing after neck radiotherapy is safe, effective and reliable.

Keywords: Carotid stenosis, neck radiotherapy, endovascular treatment, carotid stending, stroke.

INVESTIGATION OF BIOLOGICAL ACTIVITIES OF 1,2,4-TRIAZOLE-5-THIONE-SCHIFF BASE DERIVATIVES

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ABSTRACT

Background of the Study: The 1,2,4-triazole nucleus is a key component in various natural products and medicinal agents, making it a crucial heterocyclic structure. 1,2,4-triazoles are compounds of significant interest due to their diverse biological activities such as antimicrobial, anti-inflammatory, anticancer, antibacterial, antifungal, analgesic, antiviral, antioxidant, and anticonvulsant properties. Also, 1,2,4-triazole-5-thiones are versatile compounds with a wide range of biological activities, making them valuable targets for research in various fields such as medicine, chemistry, and materials science. Schiff bases have been derived from 5-mercapto-1,2,4- triazoles show analgesic, antimicrobial, anti-inflammatory, antiproliferative, and antidepressant activities. For these reason, we decided to synthesize and biological activity investigation of some 1,2,4-triazole-5-thione-Schiff base derivatives.

Aim: The aim of the study is to determine the antimicrobial and antioxidant properties of two new 1,2,4-triazole-5-thione-Schiff base derivatives.

Material and Method: 1,2,4-triazole-5-thione derivatives were prepared in multiple steps starting from hydrazides. 1,2,4-triazole-5-thione-Schiff base derivatives were obtained from the reaction of 4-amino-1,2,4-triazoles with furan-3-carbaldehyde in the presence of a catalytic amount of acetic acid in ethanolic medium. Antimicrobial activity was performed according to the disk diffusion method with minor modifications. Antimicrobial activity was calculated by measuring the zone diameters formed after incubation. Antibacterial and antifungal activity studies were performed in triplicate. Also, DPPH antioxidant activities were investigated.

Results: In the antibacterial and antifungal activity study, test compounds did not show any activity. DMSO also demonstrated no activity. Test compounds showed moderate DPPH activity.

Conclusion: Since no activity was observed in DMSO solutions of test compounds, activity studies with different solvents are planned in future studies.

Keywords: 1,2,4-triazole-5-thione, antimicrobial, antioxidant, schiff base

EVALUATION OF ANTIOXIDANT ACTIVITY OF *ANACAMPTIS PALUSTRIS* SALEP SPECIES GROWING IN THE CENTRAL BLACK SEA REGION

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ABSTRACT

Background of the Study: Türkiye, with its geographically important location, is among the countries with rich biodiversity in the world. Three of the world's 36 biodiversity hotspots intersect within Türkiye (Iran-Anadolu, Caucasus and Mediterranean Basin). The diversity of ecosystems and habitats is reflected in Türkiye's floristic structure. Türkiye has a plant richness close to the entire European continent with approximately 12,000 plant taxa. Türkiye is one of the richest orchid countries in Europe with 170 terrestrial orchids.

Aim: To be utilized effectively, particularly in industrial applications involving food and non-food items, research involving a thorough investigation and comparison of data on antioxidant activity are required. The aim of this study was to determine the in vitro antioxidant activity potential of water extracts obtained from leaves and tubers of *A. palustris* orchid grown in Samsun province via DPPH• assay.

Material and Method: Leaves and tubers of orchid plant, *A. palustris*, used in this study was randomly collected from Samsun province. DPPH (2,2-diphenyl1-picrylhydrazyl) radical scavenging activity was tested according to Yu et al. The results were expressed as % inhibition.

Results: When the data obtained after the analysis were compared, it was determined that fresh samples gave lower results than dry samples. When the drying methods were compared, it was determined that the shade-dried tubers of A. palustris gave higher results. The dried samples of A. palustris leaves in lyophilizer showed higher DPPH radical scavenging effect.

Conclusion: When the data obtained in this study were compared, different drying methods were used to determine in which drying method the plant could contain more active substances.

Keywords: *Anacamptis palustris*, antioxidant activity, DPPH

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FLUORESCENCE DETECTION OF BIOTHIOLS BY CARBON QUANTUM DOTS FROM *ASPERGILLUS* SP. SPORES

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ABSTRACT

Background of the Study: Carbon quantum dots (CQDs) are a new type of nanomaterial used in many different fields, including biomedicine and biology. They can be synthesized from natural sources using a green chemistry approach, with micro and macrofungal species being a new alternative to plants and animals. Mould growth from microfungi like *Aspergillus*, *Penicillium* and *Fusarium* sp. is a common source of microbial spoilage in bread. In a recent study, CQDs were synthesised from *Aspergillus* spores for the first time. CQDs are highly water-soluble and biocompatible, which makes them ideal for developing new probes that are simple, fast, sensitive, and cost-effective for medical and healthcare applications. However, detecting biothiols is essential for diagnosing many diseases. Therefore, new fluorescence-based probes must be developed for detecting these molecules.

Aim: To synthesize and characterize fluorescent CQDs using bread mould spores (*Aspergillus* sp.) and explore their potential use as fluorophore agents in detecting biothiols (glutathione, cysteine homocysteine).

Material and Method: In the study, CQDs were produced using a green synthesis approach without the use of chemicals. *Aspergillus* spores were exposed to microwave irradiation for 5 minutes at 800 W in water. The CQDs were then purified by dialysis, dried by lyophilization, and characterized using TEM, XRD, FT-IR, Raman, UV-vis and fluorescence. The CQDs were dissolved in water (0.1mg/mL). To observe changes in the fluorescence intensity, glutathione, cysteine, and homocysteine were added at very low concentrations (2-30 µg in 1.0 mL solution) in aqueous solutions.

Results: The fluorescent CQDs from spores of *Aspergillus* sp had the potential to function as fluorophore agents in detecting biothiols through fluorescence-based methods. The study also suggested that the fluorescence intensity of CQDs decreases in the presence of biothiols.

Conclusion: Fluorescent CQDs from microfungal spores have the potential to be used as fluorophore agents in the detection of biological molecules containing thiol groups.

Keywords: *Aspergillus* sp, biothiols, fluorescence, CQD

GIANT LIVER MASS IN CHILDREN

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ABSTRACT

Background of the Study Mesenchymal hamartoma of the liver (MHL) is a rare disease. This benign tumor usually occurs in children from birth to 2 years of age. These very large masses can be confused with mesenchymal cysts on imaging studies.

Aim: We present a 21-month-old patient with abdominal distention

Material and Method: Pediatric patients who were operated on due to liver hamartoma in our clinic were retrospectively examined and it was seen that 1 patient was operated on due to giant liver hamartoma between 2023 and 2021. The patient's complaint, surgery records and subsequent treatment process were examined.

Results: A 21-month-old female patient applied with the complaint of abdominal distension. There was no vomiting and no gastrointestinal passage problems. In the blood tests performed, α fetoprotein (AFP) was found to be 238 and in the imaging studies, it was seen that there was a 90x150x165 mm cystic mass thought to be of liver origin. During the operation, a cystic mass originating from segment 4-5 of the liver was observed and it was totally excised. The pathology result confirmed that it was a mesenchymal hamartoma of the Liver.

Conclusion: Liver masses can enlarge to cause distention. Liver tumors should be included in the differential diagnosis of gigantic intra-abdominal masses.

Keywords: Liver mesenchymal hamartoma, liver benign tumors

A NEW INJECTION IN THE TREATMENT OF VESİCOURETERAL REFLUX: EVALUATION OF EARLY RESULTS OF HYADEX USE

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ABSTRACT

Background of the Study: Vesicoureteral reflux (VUR) is a condition characterized by retrograde urine flow from the bladder into one or both ureters and then into the kidneys. The surgical treatment options for VUR include endoscopic subureteric injection and open or laparoscopic corrective surgery. Hyadex (dextranomer/cross-linked hyaluronic acid) is a newly developed agent for the treatment of VUR.

Aim: This study was performed to evaluate the early efficacy and safety of Hyadex in patients with clinically diagnosed VUR.

Material and Method: In our study, clinical and demographic characteristics of patients who underwent Hyadex injection for VUR between January 2023 and April 2024 were evaluated. The study included 26 patients (36 renal units). Patients were diagnosed by voiding cystourethrography (VCUG). Control VCUG imaging was performed 3 months after injection.

Results: VCUG evaluation revealed grade I VUR in 2 renal units, grade II VUR in 2 renal units, grade III VUR in 6 renal units, grade IV VUR in 7 renal units, and grade VUR in 19 renal units. Of the 13 patients (16 renal units) who underwent control VCUG in the 3rd postoperative month, VUR persisted in 5 (31.25%) renal units and VUR was not observed in 11 (68.75%) renal units. No significant complications were observed.

Conclusion: Endoscopic subureteric Hyadex injection has high success rates in appropriately selected patients with VUR and can be used as first-line treatment in children with VUR.

Keywords: Hyadex, subureteric injection, voiding cystourethrography

BILATERAL OVARIAN DYSGERMINOMA IN A PREPUBERTAL CHILD PRESENTING WITH THE COMPLAINT OF ACUTE ABDOMEN

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ABSTRACT

Background of the Study: Ovarian tumors in children constitute approximately 1% of all childhood tumors, and germ cell tumors constitute an important part of this. Dysgerminomas are among the most common types.

Aim: We present a 10-year-old female patient who was acute abdomen and was operated for bilateral dysgerminoma with unilateral ovary torsion.

Material and Method: The pediatric patients who underwent surgery for bilateral ovarian tumors in our clinic were examined retrospectively. It was seen that 1 patient was operated on due to malignant and bilateral ovarian tumors between 2023 and 2021. The patient's complaint, surgery records and subsequent treatment process were examined.

Results: 10-year-old female patient; She applied with the complaint of vomiting after sudden onset of abdominal pain. She did not have any additional disease and her blood tests were normal. Both ovaries could not be seen and a 5x5 cm mass was observed in the pelvic region in the abdominal ultrasonography. The patient underwent operation. It was observed that there was mass in both ovaries and the right ovary was torsioned. In the frozen pathology taken during the operation, it was confirmed that there was bilateral dysgerminoma and no ovarian tissue in both ovaries. The patient underwent bilateral salpingoophorectomy.

Conclusion: The primary pathology in patients with ovarian torsion in the prepubertal period may be ovarian tumor. The contralateral ovary must be checked.

Keywords: Bilateral ovarian malignant tumor, children, dysgerminoma

EFFECT OF BLEOMISIN INJECTION IN PEDIATRIC PATIENTS WITH LYMPHATIC MALFORMATION OCCURING IN THE BASE OF THE TONGUE

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ABSTRACT

Background of the Study: Lymphatic malformations (LM) are rare congenital anomalies. The traditional treatment is surgical excision, but intralesional sclerosing agent (bleomycin) injection is now preferred because of the high complication rate, frequent recurrences after surgery and poor cosmetic results. The results of sclerosant injections made in various localizations have been shared in the literature, but the information about the injection to the tongue root and its results is very limited, especially in children.

Aim: We present our results of sclerosant injection into the LM at the neck and the base of the tongue.

Material and Method: We retrospectively analyzed children treated for LM originating from the gluteal region between January 2011 and January 2022. Bleomycin as sclerosing agent was used for injection. The lesion sizes of the patients who underwent sclerosing agent (Bleomycin) injection under general anesthesia were recorded before and after the treatment.

Results: There were two patients who underwent sclerosing agent injection to the neck and base of the tongue. Patient 1; An 9-year-old male patient presented with LM on the tongue base and left side of the neck. The patient had speech difficulties. With the repetitive sclerosing agent injection, there was a decrease in the size of the LM and regression in his complaints. Patient 2; A 7-month-old male patient had LM at the base of the tongue and under the left mandible on the left side of the neck. The patient was unable to feed orally and had growth retardation due to the mass. With the repetitive sclerosing agent injection, there was a decrease in the size of the LM and regression in his complaints.

Conclusion: Root of tongue LM, which is a very rare site in children, can be successfully treated with intralesional sclerosant injection by experienced professionals.

Keywords: Lymphatic malformation, sclerotherapy, bleomycin, tongue

THE ROLE OF MICRONUTRIENTS IN THE COMPLEX FEBRIL SEIZURE

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ABSTRACT

Background of the Study: Studies were limited regarding whether mıcronutrıents and vitamin levels in the blood affect the development of complex febril seızure.

Aim: We aimed to evaluate the effect of micronutrient levels such as vitamin B12, folic acid, ferritin, and 25 OH vitamin D on the complex febril seizures.

Material and Method: Pediatric patients who were followed up with a diagnosis of febrile seizure in the pediatric neurology department were examined retrospectively. Vitamin B12, folic acid, ferritin, 25 OH vitamin D levels, febrile seizure characteristics (number, longest seizure duration etc.) were evaluated. Analysis results were presented as mean±standard deviation and median (minimum–maximum) for quantitative data, and frequency and percentage for categorical variables. The significance level was taken as $p<0.05$.

Results: Seventy five patients, 64% of whom were male and 28% of whom had complex febrile seizures, were included in the study. The current average age of the patients was 49.73±16.37 months, the median number of febrile seizures was 2 (1-12), the median longest seizure duration was 2 (1-12) minutes. While anemia was detected in 13.3% of the patients, vitamin B12 was found to be low in 2.7% and low-normal in 5.4%. Folic acid is low in 4.1% of patients, 25 oh vitamin D is deficient in 21.6% of patients, insufficient was detected in 10.8%. No significant difference was found between those with simple and complex febrile seizures in vitamin D, folik asit, vitamin B12, ferritin, hemoglobin and magnesium levels. The risk of complex febrile seizures in those with vitamin D deficiency was found to be 4.371 times higher than in those without vitamin D ($p = 0.017$).

Conclusion: Adequate 25 OH vitamin D levels in the blood of children with fever can prevent febrile seizures from turning into complex febrile seizures.

Keywords: Febrile seizure, vitamin B12, vitamin D

INTERNAL CAROTID ARTERY DISSECTED ANEURISYM IN A CASE WITH INTRACEREBRAL HEMORRHAGE

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ABSTRACT

Background of the Study: Cervico-cephalic artery dissections are defined by a hematoma in the wall of a cervical or intracranial artery and represent a leading cause of stroke in the young. While dissection of the extracranial cervical arteries (EAD) has been extensively studied and described in increasingly large observational studies, less data is available about isolated intracranial artery dissection (IAD).

Aim: We aimed to share the successful endovascular treatment of a case with intracranial dissected aneurysm whose symptoms progressed under medical treatment.

Material and Method: A 46-year-old female patient was consulted with left-sided weakness and confusion.Computed tomography(CT) showed hemorrhage in the right thalamus.Her vital signs were stable,she was confused and right hemiplegic.She had diabetes mellitus, essential hypertension.She did not use any anticoagulant or antiplatelet.Antiedema treatment was started.The next day, impaired consciousness increased, multifocal hemorrhage foci were observed in the right internal carotid artery irrigation area on CT scan and gadolinium-enhaced magnetic resonance imaging(MRI).MRI angiography was normal.The patient was taken for digital substraction angiography(DSA).

Results: DSA revealed a dissected aneurysm in the right internal carotid artery (ICA) terminal segment, endovascular treatment was planned, and three days later a stent was applied. Tigacrelor and acetylsalicylic acid therapy were started. At the follow-up examination after one month, the muscle strength was 3/5.

Conclusion: The low incidence of EAD/IAD has proven a challenge to clinical trials conduction, and therefore guidelines have mostly relied on indirect evidence from observational studies and expert opinion. In cases where medical treatment is unsuccessful in dissections, endovascular treatment is the next option.

Keywords: Aneurysm, hemorrhage, internal carotid artery

DISSECTED INTRACRANIAL ANEURYSM IN BEHÇET'S SYNDROME; COEXISTENCE OF ISCHEMIA AND HEMORRHAGE

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ABSTRACT

Background of the Study: Behçet's syndrome (BS) is a systemic vasculitis with an unknown aetiology affecting the small and large vessels of the venous and arterial systems. Venous system is frequently affected, arterial system involvement is less common. Aneurysm formation, usually seen in systemic major arteries, intracranial aneurysm is extremely rare.

Aim: In this case we present an intracranial aneurysm which was diagnosed in digital substraction angiography (DSA) with the diagnosis of recurrent cerebrovascular disease and oblitered with steroid therapy.

Material and Method: A 41-year-old male patient with BS was consulted with dizziness and speech impairment. He had an ischemic stroke two months ago and was using acetylsalicylic acid,oral methylprednisolone and azothiopurine.On examination,he has dysarthria and ataxia.On diffusion magnetic resonance imaging (MRI),acute ischemia was observed in the right thalamus,and on computed tomography (CT), hemorrhage was observed in the fourth ventricle.Gadolinium-enhanced MRI revealed areas of chronic lacunar infarction in the brainstem and hemorrhage in the fourth ventricle.MR angiography was normal.In DSA, widespread irregularity in the intracranial arteries and a dissected aneurysmatic lesion in the perforating branch of the posterior cerebral artery were observed.Endovascular treatment was planned.

Results: Methylprednisolone 1000 mg/day was given intravenously for 5 days. When the patient was taken for DSA again after one week, it was observed that the vascular irregularities and aneurysm disappeared. Cyclophosphamide treatment was started.After 3 months,the patient had no neurological deficit and DSA was normal.

Conclusion: Intracranial arterial involvement in Behçet syndrome is rare and should be taken seriously; its diagnosis affects the treatment protocol and prognosis and DSA *is gold standard for neurovascular diagnosis.*

Keywords: Behcet's syndrome, intracranial aneurysm

OPTIMIZATION OF SYNTHETIC DNA TRANSFECTION INTO PERIPHERAL BLOOD MONONUCLEAR CELLS USING ELECTROPORATION

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ABSTRACT

Background of the Study: Lymphocytes, monocytes and macrophages are peripheral blood mononuclear cells (PBMC) that have a round nucleus. It is frequently used in gene therapy researches since PBMC is easy to obtain and culture. Physical, chemical and biological approaches are used to transfection of synthetic DNA constructs into the cells. However, its delivery to the PBMCs is limited due to low transfection efficiency since to a number of factors such as immune response to foreign nucleic acids.

Aim: In our study, we aimed to determine the most suitable conditions using the electroporation technique, which is one of the transfection approaches, for vector transfer to PBMC cells.

Material and Method: PBMCs were obtained from peripheral blood of healthy individuals using lymphopurine. PBMCs were washed 2 times with PBS. Then the cells were cultured with RPMI1640 containing 10% Fetal Bovine Serum, 1% L-glutamine (2 mmol/l), penicillin (100 U/ml), and streptomycin (100 µg/ml) overnight under standard conditions (37 °C, 5% CO2) and induced with phytohemaglutinin. Green fluorescent protein (GFP) expression vector (Addgene, #79145) was transfected into PBMCs using electroporation (GenePulser Xcell, Bio-Rad). Electroporation was performed with different pulse numbers (1, 2 and 3), different pulse durations (1ms, 3ms and 5ms) and different voltage (100V, 200V and 300V) values using square wave pulse. Then, the cells were cultured under standart conditions. 24 hours after electroporation, cells were stained with DAPI, a nuclear dye, and observed by fluorescence microscopy.

Results: It was determined that there were GFP luminescence in the PBMC applied to 200V and 300V electroporations, but this luminescence was stronger in 300V electroporation. The GFP release was not observed in the cells in the 100V electroporation process.

Conclusion: In our study, the expression vector prepared for gene transfer was most effectively transfected into PBMC cells in 300V, 3ms, 1 pulse electroporation applied group. Thus, appropriate conditions were optimized for subsequent gene therapy studies.

Keywords: Electroporation, transfection, PBMC

Poster Presentations

PREPARATION OF CHITOSAN ALGINATE COATED MICROCAPSULES FROM *CUPRESSUS SEMPERVIRENS* SPOROPOLLENIN AND EVALUATION OF VANCOMYCIN RELEASE

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ABSTRACT

Background of the Study: Controlled release of drugs and preservation of their chemical integrity are of great importance for health as they reduce side effects. The drug release system increases the effectiveness and stability of the drug by controlling the rate, timing, and location of the drug in the body. In particular, the encapsulation technique contributes to reducing side effects and increasing the chemical integrity of drugs by providing a more controlled release of drugs into the body.

Aim: The main purpose of this study is to provide controlled release of vancomycin, an antibiotic, by using sporopollen obtained from *Cupressus sempervirens* tree pollen, alginate and chitosan.

Material and Method: Combining sporopollen with nanosized additives could bring a new dimension to controlled drug release technology. The hydrogel layer formed by alginate with Ca^{2+} cations controls the diffusion of the drug while protecting its biological activity. It provides a suitable environment for the encapsulation process of sporopollen and alginate. The effectiveness of encapsulation was analyzed using SEM and FTIR for characterization, and *in vitro* drug release studies were performed using the dialysis method in aqueous media.

Results: The full dosage of vancomycin from sporopollen alginate-chitosan microcapsules was released after 48 hours and was observed to exhibit a slower and more controlled drug release profile compared to the free drug solution. It has been observed that the drug release of microcapsules is 99.89%.

Conclusion: The microcapsules, using alginate and chitosan agents, facilitated the successful preparation of vancomycin-loaded formulations. Future studies will continue to evaluate the bacteriostatic effect and imaging efficiency of these formulations through *in vitro* cell culture experiments.

Keywords: Alginate, chitosan, *Cupressus sempervirens*, drug delivery system, vancomycin

CHITOSAN-DOPED NANOFIBERS PREPARED FROM CARBON QUANTUM DOTS OF THE PLANT *HYPERICUM PERFORATUM* L.

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ABSTRACT

Background of the Study: The oil of plant *Hypericum perforatum L*., which is known to have wound healing properties, is available in the literature, but it is not used in the wound of carbon quantum dots. In recent years, the use of nanomaterials in the name of biomaterial madicine has become important. It should be investigated whether there are wounds on the carbon quantum dots obtained from this intended plant, *Hypericum perforatum L.* the root body and flower parts of the *Hypericum perforatum L.* plant were separated.

Aim: The aim of the study is to investigate of chitosan-doped nanofibers obtained from carbon quantum dots of the plant *Hypericum perforatum L*.

Material and Method: Carbon quantum dots were obtained from the plant *Hypericum perforatum L.* using green synthesis and microwave method. Carbon quantum dot doped chitosan nanofibers were obtained using the electrospinning method. The resulting carbon quantum dots are ground into powder. Chitosan/polystyrene nanofibers doped with carbon quantum dots were prepared by spraying 16 mm PEG-ITO reinforcement using the electrospinning method.

Results: Carbon quantum dots were obtained from individual parts of the plant *Hypericum perforatum L.* Chitosan nanofibers were prepared using 2.5% flower and stem parts and 1.25% root.
Conclusion: Carbon quantum dots doped chitosan nanofibers were used for prepared nanofiber surfaces. It was investigated whether carbon quantum dots obtained using the microwave method for the green synthesis of each part would have an effect on wound healing.

Keywords: Carbon Quantum Dots, chitosan, *Hypericum perforatum L.*

ENCAPSULATION OF SOME AROMATIC OILS

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ABSTRACT

Background of the Study: In microencapsulation, pleasant-smelling microcapsules are prepared by the complex coacervation method of aromatic oils, using bovine gelatin and gum arabic as wall materials. Aromatic oils such as mint, orange, mango, neroli, geranium, cedar and vanilla are used as core materials in the preparation of microcapsules. Glutaraldehyde was used as a crosslinker to bond the core material to the wall material.

Aim: The purposes and reasons for encapsulating aromatic oils are as follows: To make liquids solid so that they can be easily transported, to reduce evaporation, to reduce the loss of volatile substances, to hide unwanted taste and odor, to extend the duration of effect.

Material and Method: A total of 7 experiments were carried out with 2 different methods using the complex coacervation method. For the coating, two different polymers, bovine gelatin and gum arabic, were mixed in ratios of 4:1 and 1:1. In the second method, the oil was added to the gelatin - gum arabic solution, gelatin solution. When the mixture reaches a milky consistency, it indicates that the emulsification is successful. The pH of the mixture was adjusted to 4.0-4.5 using acid addition.

Results: Then, the surface oil and total oil amounts of microcapsules containing aromatic oil (geranium, mango, vanilla, neroli, orange, mint) were determined and the encapsulation efficiency value was calculated. The encapsulation efficiency value of the experiment using 4 Gelatin / 1 Gum Arabic / Mint was calculated as 79%, and the encapsulation efficiency value of the experiment using 4 Gelatin / 1 Gum Arabic / Orange was calculated as 96%.

Conclusion: Only one of the 6 experiments, that is, the one using cedar oil as the core material, failed, meaning microcapsule formation did not occur.

Keywords: Essential oils, fragrant microcapsule, gum arabic, bovine gelatin

GREEN SYNTHESIS OF SILVER NANOPARTICLES AND INVESTIGATION OF THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Background of the Study: Green synthesis is defined as the application of biologically friendly elements such as plants, bacteria and fungi for the synthesis of nanoparticles. Among metal nanoparticles, silver nanoparticles have been extremely popular due to their wide applications such as antiviral, anticancer, antibacterial, catalytic, optoelectronic and medical uses. Many antimicrobial agents have been synthesized thanks to nanotechnology. Nowadays, green synthesis techniques for nanoparticles are recommended over conventional synthesis techniques as they have advantages in terms of cost, energy utilization, non-toxicity and safety.

Aim: Plant extract works as both reducing agent and capping agent in the synthesis of nanoparticles. Therefore, in our study, silver nanoparticles were synthesized by green synthesis method and their antibacterial activity was investigated.

Material and Method: Silver nanoparticles were synthesized by green synthesis method. Distillation wastewater of orange peel and bay branch was added dropwise to 10 mL of 2 mM aqueous AgNO3 solution in a 1:1 ratio and heated at 70°C for 24 hours. During heating, distilled water was added to the reaction and made up to 40 mL. The reaction solution was centrifuged at 6000 rpm for 5 min. After centrifugation, it was washed thoroughly with distilled water. It was then dried at room temperature. Antibacterial activity was determined according to the agar well diffusion method.

Results: In the literatures, it is stated that silver nanoparticles are formed when a color change is observed during the reaction. In addition, FTIR and UV-Vis absorbance values also showed the presence of silver nanoparticles. It was observed that silver nanoparticles synthesized with orange peel were effective on *S.aureus*.

Conclusion: In the light of the data obtained from this study, we believe that it will lead to further studies on the synthesis of green synthesis AgNPs.

Keywords: Green synthesis, silver nanoparticles, antibacterial

CONJUGATION OF MAGNETIC NANOPARTICLES WITH ESSENTIAL OIL

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ABSTRACT

Background of the Study: Essential oils (EOs) are colorless liquids and are mainly composed of aromatic and volatile compounds naturally occurring in all parts of plants, including seeds, flowers, flowers, bark, stems, stems, bark and whole plants. They are widely used in various countries as medicines, perfumes, cosmetics and food preservatives. The main compounds of EOs show potential antioxidant, antimicrobial and antifungal activities through various mechanisms. Magnetite nanoparticles have been reported to be of great interest in environmental and oil spill remediation. The biggest advantages of magnetic nanoparticles are that they can be directed towards the desired area by an external magnetic force and can be separated.

Aim: Magnetic nanoparticles, because of their magnetic properties, allow the molecules they bind to be easily separated from their medium. In this study, we aimed to investigate the conjugation of magnetic nanoparticles with essential oils.

Material and Method: The magnetic nanoparticle solution was vortexed with commercially purchased peppermint essential oil at a ratio of 5:1 to ensure thorough mixing. The solution was then kept at room temperature (water: magnetic nanoparticle-essential oil: essential oil) for the phases to become distinct. A magnet was used to remove the magneticnanoparticle-essential oil phase from the other phases. The resulting magneticnanoparticle-essential oil phase was washed three times with distilled water and then dissolved with ethanol. The antibacterial activity of the ethanol solution was investigated by agar well method.

Results: While 2 different phases were initially observed, it was observed that 3 different phases were formed as a result of the addition of magnetic nanoparticles and thorough vortexing. However, no significant antibacterial activity was observed.

Conclusion: This study will lead to future studies on the conjugation and separation of magnetic nanoparticles with essential oils.

Keywords: Magnetic nanoparticles, essential oil, encapsulation

ANTIBACTERIAL ACTIVITY OF ZINC PHTHALOCYANINE METAL COMPLEXES CONTAINING RESORCINARENE GROUP

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ABSTRACT

Background of the Study: Phthalocyanines (Pcs) which known as macrocyclic compounds were first described in the 1930s. The reason for the strong attention to these macrocyclics is due to their unique spectral properties, high stability and architectural flexibility. The Pc ring is highly adaptable, offering the opportunity for additional adjustments to enhance its functionalities. This gives synthetic organic chemists with more options to enhance the physical responses of phthalocyanines (Pcs). Numerous fields have conducted study on the usefulness of these substances (such as in biological activity, photodynamic therapy, and chemical sensor).

Aim: The aim of this study was to investigate the antimicrobial activity of zinc phthalocyanine (ZnPc) compound containing resorcinarene group.

Material and Method: ZnPc's were synthesized according to the literature. Antimicrobial activity was performed according to agar well diffusion method with minor modifications. Gram negative, Gram positive and yeast were used as test microorganisms and tested for antibacterial and antifungal activities, respectively. DMSO and standard antibiotic disk Imipenem were used as control. Antimicrobial activity was calculated by measuring the zone diameters formed after incubation. Antibacterial and antifungal activity studies were tested in triplicate.

Results: When all spectroscopic data of ZnPc were analyzed; it was determined that it was in agreement with the literature. According to the activity results obtained, ZnPc test compounds showed antibacterial and antifungal activity on the test microorganisms. It was observed that the antibacterial activities of ZnPc test compounds on Gram negative bacteria were not dose dependent. However, a dose-dependent increase in antibacterial activity was observed on Gram positive and yeast. DMSO also showed no activity.

Conclusion: In the present study, we report synthsis zinc phthalocyanine derivatives, and its antimicrobial effects in vitro model. In line with the data obtained as a result of the experiment, it is planned to perform studies using different solvents and microorganisms in future studies.

Keywords: Phthalocyanine, Antibacterial/Biological Study, Microorganisms

EXTRACTION OF *ORCHIS APIFERA* SALEP SPECIES GROWN IN SAMSUN PROVINCE AND EVALUATION OF FRAP ANTIOXIDANT ACTIVITY

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ABSTRACT

Background of the Study: The use of herbal products as natural antioxidants to reduce tissue damage caused by free radicals has received considerable attention in recent years. Therefore, the search for new and safe antioxidants found in natural sources is an incentive for new research.

Aim: In this study, it was aimed to determine the in vitro antioxidant activity potential of water extracts obtained from leaves and tubers of Orchis apifera using FRAP test.

Material and Method: Leaves and tubers of O. apifera plants were separated from each other. The samples were also used as dried in shade, lyophilizer and oven in addition to being used as fresh. Then, the finely powdered parts (5 g) were homogenized with distilled water (300 mL) at 5000 rpm for 3 min. The method developed by Oyaizu was modified and FRAP antioxidant activity analysis was performed. All analyses were repeated three times.

Results: Four different drying methods were used in the study and FRAP analysis of the leaves and tubers of O. apifera was tested. When the data obtained after the analysis were compared, it was determined that fresh samples gave lower results than dry samples. When the drying methods were compared, it was determined that the samples of O. apifera tubers dried in the oven gave higher results. The samples of O. apifera leaves dried in a lyophilizer gave higher results.

Conclusion: In this study, it is recommended to use plant samples dried in a lyophilizer, in an oven or in the shade instead of fresh. In addition, it is thought that these studies will lead to future pharmacological studies.

Keywords: Antioxidant activity, FRAP, *Orchis apifera*

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VORTEX-ASSISTED DISPERSIVE LIQUID–LIQUID MICROEXTRACTION OF MELATONIN IN DIETARY SUPPLEMENTS AND FOOD SAMPLES PRIOR TO HPLC-DAD

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ABSTRACT

Background of the Study: Melatonin (N-acetyl-5-methoxytryptamine) is an indolamine hormone derived from tryptophan and secreted by the pineal gland of vertebrates. In the last decade, melatonin has been used for the treatment of the large spectrum of diseases, mainly in sleep disturbances and tumors [1]. Currently, many countries produce pharmaceuticals and dietary supplements containing melatonin [2]. Since melatonin is highly bioactive it is necessary to provide quality control of melatonin-containing products.

Aim: In the present work we propose a vortex-assisted dispersive liquid–liquid microextraction (VADLLME) procedure for the preconcentration of melatonin in dietary supplements and food samples and determination by HPLC-DAD.

Material and Method: Firstly, the effects of the main three factors (volume of extraction solvent, vortexing time, centrifugation time) were tired to investigate at different five levels. For VADLLME performed under optimum conditions, 2.0 mL of sample containing 5 µg/mL melatonin was transferred to a screw centrifuge tube, then 293.0 µL of chloroform (extracting solvent) was rapidly injected into the sample solution and vortex for 2,63 min. The solution was immediately centrifuged at 3000 rpm for 3.41 min and after separating the phases, the extractor solvent was collected with the aid of a microsyringe, dried and suspended in methanol and then conventionally injected into the chromatographic system.

Results: The proposed procedure eliminates matrix effects and giving much higher selectivity. Under the optimum conditions the calibration curve was linear in the range of 1.0–150.0 µg/mL of melatonin. It was found that satisfactory recoveries for melatonin was obtained in the range of 90.65%–99.97%.

Conclusion: The proposed VADLLME method coupled to HPLC for the determination of melatonin method is a simple and rapid extraction technique with a large enrichment factors and low detection limit for melatonin determination.

Keywords: Dietary supplements, food samples, vortex-assisted microextraction, HPLC, melatonin, response surface methodology

EXTRACTION OF ASHWAGANDHA PLANT BY SUPERCRITICAL CO_2 EXTRACTION METHOD AND ANALYSIS OF ITS BIOACTIVE COMPONENTS

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ABSTRACT

Background of the Study: In recent years, some concerns have emerged about environmental pollution and the problems caused by it. These concerns have led to the formation of a new field in chemistry and studies in this field. Researchers have started to investigate more environmentally friendly methods that use solvents that are wasteful and more easily degradable, produce much less toxic products. This new field of chemistry was originally called 'Green Chemistry'. Supercritical fluids and supercritical CO2 are considered a good alternative for green chemistry. Ashwagandha (Withania somnifera), a popular plant used in traditional medicine and drugs, has been practised in India since time immemorial. Several recent studies using cell and animal models have demonstrated the anti-cancer, neuroprotective, anti-inflammatory, anti-stress, immune-modulatory, anti-oxidant and anti-diabetic potentials of Ashwagandha and its derivatives. There are very few studies on the extraction of bioactive components from different parts of the Ashwagandha plant. Supercritical fluid extraction is among the best technologies used in the extraction of useful compounds from plants due to many advantages such as high yield, solvent-free extracts and environmental friendliness. Bioactive compounds have an important place in the prevention of diseases, and the use of greener and environmentally friendly techniques such as supercritical fluid extraction has become important in obtaining them.

Aim: In this study, extracts were obtained from different parts of the Ashwagandha plant by the supercritical CO2 extraction method.

Material and Method: In the supercritical CO2 extraction system, extracts was obtained by experimenting at different flow rates and using different auxiliary solvent, different pressures and temperatures in the extractor.

Results: The analysis of the bioactive components contained in this extract was carried out.

Conclusion: It was concluded that the extracts can be analysed by using different chromatographic methods after being obtained by different extraction techniques and compared with standards.

Keywords: Ashwagandha, bioactive components, supercritical CO2 extraction

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PROCEEDINGS BOOK (Full-texts)

COMPARISON OF TRADITIONAL KNOTING AND SILICONE SHEET APPLICATION IN FIXING THE TUBE IN DACRYOCYSTORINOSTOMY

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ABSTRACT

Background of the Study: Tube comfort in DSR

Aim: To investigate the effect of traditional knotting method versus silicone sleeve application for fixation of the silicone tube used during endonasal dacryocystorhinostomy (DCR) on postoperative symptoms and surgery success.

Materials and Methods: A total of 97 patients who presented to the clinic with complaints of epiphora with chronic dacryocystitis underwent unilateral endonasal DCR were retrospectively evaluated. The patients were divided into two groups based on the method of fixation of the silicone tube. Group 1 consisted of 51 patients who had fixation with a silicone sleeve, while Group 2 consisted of 46 patients who had fixation by knotting the tube onto itself. Results were recorded during early-term follow-ups on postoperative first day, first week, and first month, as well as during late-term follow-ups at 3 months, 6 months, and 12 months. Information regarding the presence of epiphora, irritation symptoms related to the silicone tube, and the patency of the lacrimal drainage system was recorded during these check-ups.

Results: There wasn't significant difference between the groups in demography. The duration of the silicone tube remaining in the canal was significantly longer in the Group 1 that used silicone sleeves (p=0.015). There wasn't significant difference in the number of recurrences between the two groups (p=0.618). However, it was observed that recurrence occurred significantly later in Group 1 than in Group 2 (p = 0.038). Irritation symptoms were significantly more common in patients who underwent the knotting technique (p=0.001).

Conclusion: The silicone sleeve fixation method is similar to the traditional knotting method in terms of DCR success, but it may be a good alternative to the traditional knotting method in terms of patient comfort due to less complaints of irritation of the silicone tube and longer duration of the tube in the canal.

Keywords: Dacryocystitis, epiphora, irritation, recurrence, silicone sleeve.

1. INTRODUCTION

Obstruction of the nasolacrimal duct is the leading cause of epiphora, which can result in chronic and acute dacryocystitis, as well as potentially severe conditions like abscess formation in the lacrimal sac and orbital cellulitis (Kakizaki 2016).

Establishing nasolacrimal patency is the primary objective of DCR, which involves creating a new drainage pathway from the lacrimal sac to the nasal cavity. DCR is widely regarded as the gold standard for treating nasolacrimal duct obstruction (Feng 2011).

Caldwell provided the initial description of the intranasal approach for DCR in 1893. Following that, in 1904, Toti carried out the inaugural external approach (Watkins 2003). Due to the anatomical and functional challenges associated with intranasal DCR, ophthalmologists began to prefer external DCR more frequently. However, with the advancements in nasal endoscopy and other surgical equipment in the 1990s, endonasal DCR started to be preferred by surgeons as well (Tsirbas 2003).

The primary reason for DCR failure is typically the closure of the rhinostomy or distal common canal caused by scarring (Bohman 2021). The use of silicone tubes has been introduced to increase surgical success by maintaining patency of the canaliculi through the healing period. (Feng 2011). Starting from

the 1970s, DCR with silicone tube intubation has been increasingly preferred by ophthalmologists over tubeless DCR (Cavaliere 2022).

Tube loosening is one of the most frequent complications linked to canalicular silicone tube intubation (Bohman 2021). Following surgery, the silicone tube can completely dislodge from the intubated canal, either early or late in the postoperative period, or it can prolapse outward through the punctum, causing irritation in the ocular surface. To prevent such complications, it is a common practice to secure the free ends of the silicone tube intranasally. Numerous methods have been outlined for tube fixation, such as knotting the tube onto itself, using silk sutures, nasal vestibule suturing, ligaclips, and securing with a silicone sleeve (Vahdani 2017).

The impact of different methods for securing the tubes on DCR outcomes and symptoms is not well-known. Our study's objective is to examine the impact of the conventional knotting technique and the application of a silicone sleeve (Figure 1) for fixation on the success of DCR and postoperative symptoms.

Figure 1: Silicone Sleeve (red arrow)

2. MATERIALS AND METHODS

Data from 97 patients with unilateral chronic epiphora due to primary acquired nasolacrimal duct obstruction who underwent tube intubation performed by the same surgeon were retrospectively analyzed and included in the study. The patients' age, gender, duration of tube placement, presence of recurrence, duration of recurrence, and sensation of tube-related irritation were evaluated. Preoperative examination records showed that all patients had epiphora, and during lacrimal irrigation, no fluid passage to the nasal cavity was observed along with reflux. All patients were evaluated by reviewing their medical history, nasolacrimal lavage, biomicroscopic examination, and consultation records from otolaryngology. Other causes of epiphora were ruled out. Exclusion criteria included a history of previous lacrimal or nasal surgery and the presence of any nasal, canalicular, eyelid, or anterior segment pathology that could cause epiphora. Patients were divided into two groups based on the method of tube fixation. In Group 1, the silicone tube of 51 patients was secured with a silicone sleeve, while in Group 2, the silicone tube of 46 patients was knotted using the traditional method. In Group 1, during surgery, both ends of the silicone tube were passed through the silicone sleeve, and the looseness of the tube was adjusted as desired and secured (Figure 2). In Group 2, the silicone tubes were slightly pulled out and knotted on themselves 8-10 times before placement. All fixation procedures were performed by the same surgeon.

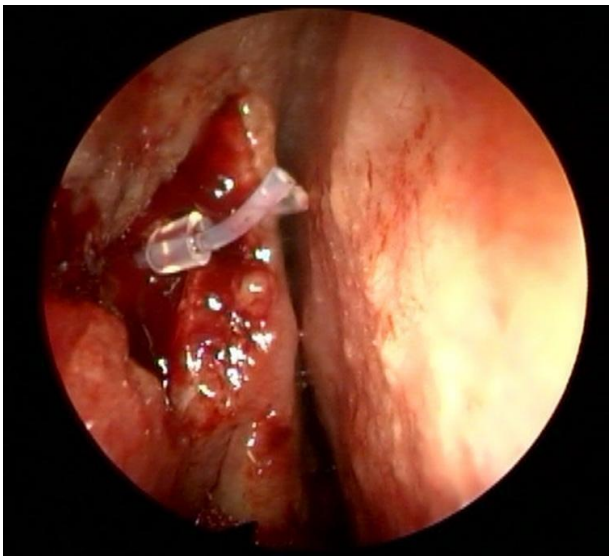

Figure 2: Silicone Tube Fixed with Silicone Sleeve

The statistical analysis of the work data was performed using Statistical Package for the Social Sciences version 22.0 software (SPSS IBM Inc., Armonk, NY, USA). The distribution of the data was evaluated using the Shapiro-Wilk test. For data that followed a normal distribution, the Student t-test was used for comparisons between two groups, while the Mann-Whitney U test was used when the data did not follow a normal distribution. Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were presented as number (n) and percentage (%).

The study was approved by the Ethics Committee (Date:31.07.2019, Protocol No:2019/14) and all procedures were applied in accordance with the Declaration of Helsinki.

3. RESULTS

All patients were seen for early postoperative follow-up at day 1, week 1 and month 1. Presence of epiphora, irritation related to the silicone tube, and patency of the nasolacrimal canal were evaluated during long-term follow-up visits at 3 months, 6 months, and 12 months. Disappearance of symptoms during the postoperative period and demonstration of anatomical patency through lacrimal lavage were considered as successful outcomes.

There were 51 patients (39 Female / 12 Male) in Group 1 and 46 patients (38 Female / 8 Male) in Group 2.

The mean age of patients in Group 1 was found to be 48.59±9.43 years, while in Group 2 it was no significant difference in terms of gender distribution was found between the groups (p=0.461).49.30±9.55 years. There was no statistically significant difference in terms of age between the groups (p=0.711)
No significant difference in terms of recurrence was observed between the groups during the 12-month follow-up period. In Group 1, recurrence was noted in 4 patients (7.8%), whereas in Group 2, it was observed in 5 patients (10.8%) (p=0.618). The mean time to recurrence was 8±2.73 months after surgery in Group 1, and 4±1.63 months after surgery in Group 2 (Table 1). It was determined that patients who underwent traditional knotting method had a significantly earlier recurrence (p=0.038).

Statistically, the complaint of irritation was more common in the patient group who underwent the traditional knotting method. In Group 1, irritation was reported in 3 patients (5.8%), whereas in Group 2, it was observed in 15 patients (32.6%) (p=0.001). In relation to this result, it was statistically significant that the tube remained in the canal longer in the silicone sleeve group due to less complaints of irritation (p=0.015). The mean duration of the tube in the canal was 5.18±3.40 months in Group 1 and 3.83±1.78 months in Group 2 (Table 1).

Table 1: Comparison of Groups In Terms of Recurrence, Irritation Complaints and Tube Removal Time

	Group 1 (n=51)	Group 2 (n=48)	P value
Recurrence (n)	4, (7.8 %)	5, (10.8 %)	0.618
Recurrence time (mounth) (mean±SD)	8±2.73	4±1.63	**0.038**
Irritation complaints (n)	3, (5.8 %)	15, (32.6 %)	**0.001**
Tube removal time (mounth) (mean±SD)	5.18±3.40	3.83±1.78	**0.015**

4.DISCUSSION

Although there have been no large prospective studies demonstrating an advantage of using a stent during DCR, it has been hypothesized that the currently used silicone tubes are a stable, non-antigenic material is employed to preserve the patency of the newly established fistula and to deter stenosis and scarring at the ostium (Feng 2011). Although the success rate of intubated DCR is high, there have been some studies of increased failure and complication rates associated with the silicone tube. Furthermore, studies have indicated that the success rates of DCR are similar between procedures with silicone tubes and those without them (Al-Qahtani 2012).

In our study, recurrence was observed in 4 individuals in Group 1 and 5 individuals in Group 2. When considering recurrence, the success rate was found to be 92.1 % in Group 1 and 89.1 % in Group 2. The recurrence rates in our study are similar to the literature, and no significant difference in DCR success was detected between the two groups.

Adverse conditions such as irritation on the ocular surface due to loosening of the silicone tube from the punctum, canalicular damage due to excessive tension on the tube, secondary infection, granuloma formation at the ostium along with adhesions, allergic reactions to silicone, and intranasal irritation have been reported (Longari 2016). In our study, irritation complaints related to the silicone tube were observed in 18 out of 97 individuals. Statistically, the complaint of irritation was higher in the patient group where the traditional knot-tying method was used.

Tube loosening or extrusion from the interpalpebral space or medial canthus is one of the most frequent complications associated with silicone tubes (Bohman 2021). Stent prolapse rates of 10% to 17% have been reported. The sagging of the silicone tube often requires repositioning within the canal or early removal (Woog 2001). Therefore, several techniques have been used to prevent the silicone tube from loosening and to secure it in place. One of these techniques is to suture the ends of the tubes together or to suture the tubes to the nasal wall. Typically, a suture is used to secure the distal end of the silicone tube to the lateral nasal vestibule. Nevertheless, this approach comes with the risk of suture erosion and extrusion along the nasal mucosal surface. Alternative methods to prevent tube sagging include self-tying the tubes, securing the arms of the silicone tube with a silicone sleeve, or aneurysm clip as a safety plug (Bohman 2021). The disadvantages of these techniques mainly revolve around the proper adjustment of fixation and difficulties related to tube removal.

The utilization of silicone sleeves was initially introduced by Hopkisson in 1995. During both external and endonasal DCR procedures, some surgeons prefer silicone sleeves for their convenience, capability to stabilize the tubes, and widespread availability. Silicone sleeves are positioned near the ostium at a distance that prevents tube sagging while still allowing tube movement. Apart from the ease of adjustment, the primary advantage is the quick and straightforward technique for removing the tube. In a study covering 166 DCR cases, no complications related to silicone sleeves were observed, with only partial medial canthal tube prolapse seen in 3 patients (Jordan 1995). In our study, the complaint of irritation was significantly higher in patients where the traditional knot-tying method was applied compared to patients where silicone sleeves were used (p=0.001).

Additionally, by using a silicone sleeve, the ends of the silicone tube can be easily secured, preventing

it from dislodging when properly adjusted. It allows for quick and easy removal from the medial canthus, leading to time and cost savings associated with endoscopy. Silicone sleeve provides advantages in terms of stabilizing the tube, preventing displacement, and facilitating efficient removal, ultimately enhancing patient comfort and reducing procedural complexities. It also offers benefits in terms of time management and cost-effectiveness related to endoscopy (Asghari 2017).

5. CONCLUSION

Using a silicone sleeve to connect the ends of a silicone tube can offer advantages in DCR, with a notable focus on enhancing patient comfort and facilitating quicker and simpler adjustments and tube removal. By providing stability and preventing displacement, the silicone sleeve contributes to an improved experience for the patient. Additionally, silicone sleeve offers benefits in terms of time management and procedural simplicity when compared to traditional methods.

Conflict of Interest and Financial Status
Our study has not received financial support from any institution, and there is no conflict of interest among the authors regarding any matter in this study.

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A RARE CAUSE OF REGRESSION IN AMPUTATION REHABILITATION: OCCULT FEMORAL NECK FRACTURE

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ABSTRACT

Background of the Study: In this case report, we describe a patient with diabetic polyneuropathy (DPNP) who underwent transtibial amputation, whose occult femoral neck fracture (OFBF) may turn into a comminuted fracture after a fall, resulting in a change in the surgical protocol and negative effects of amputation on rehabilitation.

Aim: In this case, we aimed to draw attention to the fact that the conversion of a patient with DPNP and OFBF to displaced femur fracture with decreased pain sensation was recognized late and the treatment protocol was changed.

Material and Method: A 67-year-old male patient was included in the study. A physical examination was performed. Direct radiography and computed tomography results were evaluated. The results were documented. The diagnosis of DPNP was confirmed by electromyography.

Results: A 67-year-old male patient was admitted to with the complaint of decreased prosthetic compliance. He had type 1 diabetes mellitus for 24 years. The patient underwent right transtibial amputation 15 months ago due to diabetic foot and was ambulating independently. After the patient fell while walking in the first postoperative year, Computed Tomography of the hip was taken in the emergency room, there was no fracture. He couldn't walk for 1 month. On examination; right hip active range of motion was painless and limited and muscle strength was 2/5 in all right hips muscle groups. Superficial sensation was decreased. Garden type 4 FBF was observed in the control hip direct radiography. The patient underwent total hip arthroplasty in orthopedics. After rehabilitation, his muscle strength returned to normal and he was discharged ambulating with a tripot.

Conclusion: Hip fractures (HP) that are suspected to be HP and are difficult to be diagnosed by the clinician with no findings on direct radiography are called occult HP. Garden classification of FBF is the most commonly used classification in the literature. Especially Garden 3-4 FBFs cause larger operations such as total hip replacement and increased mortality rates compared to Garden 1-2 FBFs and OFBFs. In our case, OFBF was not diagnosed early due to diabetic polyneuropathy and turned into a displaced fracture. OFBF should be considered in the differential diagnosis of patients with suspected occult femoral neck fracture.

Keywords: Occult, femur fracture, garden classification

1. INTRODUCTION

Occult fractures of the hips are rare injuries after trauma. (Grammatopoulos, McCarthy, Carli, & Gofton, 2018) Based on the literature, few studies have reported occult hip fractures (OHP) converting to separated femur fractures in adults, and the fractures are often ignored, especially in those without a clear trauma history, leading to delayed diagnosis and changes in the treatment protocol. Therefore, we planned to present the negative impacts of delayed diagnosis of OHP on functionality and rehabilitation.

2. CASE REPORT

A 67-year-old male patient was admitted to our outpatient clinic with complaints of decreasing compliance with the prosthesis over time and gait disturbance. In his history, it was found out that the patient was diagnosed with type 1 diabetes mellitus (DM) at the age of 39; insulin treatment was started in 2013, and the patient had been undergoing dialysis three times a week since 2016 due to diabetic nephropathy. The patient underwent right transtibial amputation due to diabetic foot and osteomyelitis 15 months before the admission and after the amputation rehabilitation at an external center. It was also

discovered that the patient had good compliance with the prosthesis and was ambulating independently afterward. In the first postoperative year, the patient was found to be hospitalized after a fall while walking; therefore, pelvis direct radiography and CT scan of the hip were performed in the emergency department. Then, the patient was discharged from the emergency department with the recommendation of orthopedic follow-ups upon detecting no fracture through the evaluation of the image without reporting the CT scan. The patient stated that he did not attend orthopedic follow-ups after the discharge. The patient stated no pain existed after the fall, but gait disturbance increased over time, adding that he could not walk for the last month and was mobilized with a wheelchair. Outpatient examination revealed no significant pathology in the left lower and both upper extremities. It was observed that the patient had a transtibial amputation below the right knee, and the stump care was good. The patient could stand with a walker, but could not ambulate. Upon the examination of the right lower extremity, it was observed that the range of motion was painless in the hip, the active ranges of motion of the right hip were 10° in flexion, 20° in hip abduction, and 0° in hip adduction and extension. In addition, the muscle strength of the right hip flexors, abductors, adductors, and extensors was 2/5. It was also found that the patient had no pain in the hip region at rest and was mildly painful when stepped on. In the superficial sensory examination, the pain, heat, and touch sensations were significantly decreased, compared to the face, and the position sensation was also impaired. Pelvic radiography and CT scan of the hip are seen in Figures 1 A and B. The report was obtained from an external center after the patient stated that he had a history of falling. There was no obvious fracture line on the radiograph. In the hip CT report, the fracture line extending from the metaphyseal section in the neck of the right femur to the epiphyseal area in the superior section was reported to be compatible with the suspicion.

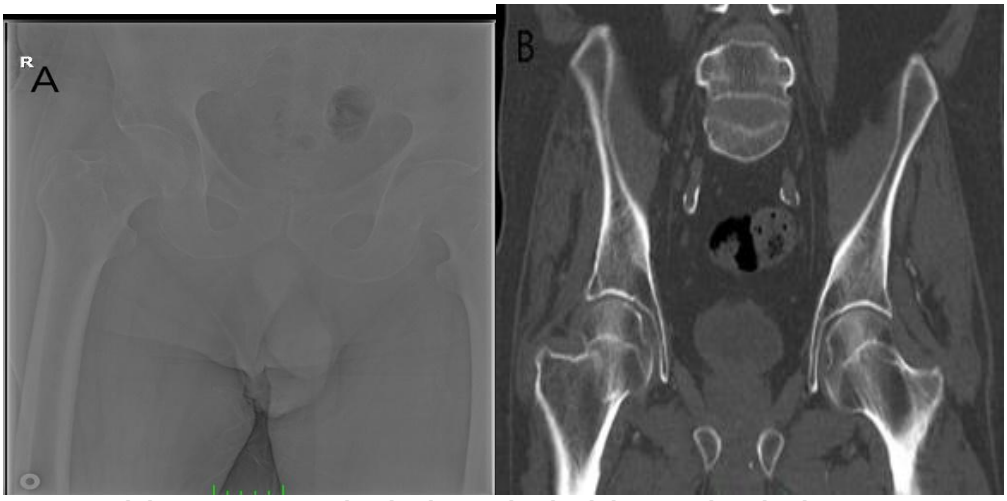

Figure 1 A. Pelvis Anteroposterior (Ap) Graphy (Pelvis AP taken in the emergency room after the fall) B. Right Hip CT (right hip CT taken in the emergency room after the fall, the fracture line is not visible)

Since the patient had weak muscle strength, hip imaging with direct radiography was performed to investigate the etiology (Figure 2).

Figure 2. Pelvis AP (Direct radiograph of the pelvis taken in our outpatient clinic before hospitalization, the Garden type 4 femoral neck fracture)

It was observed that the right femoral neck fracture was displaced and was a type 4 fracture under the Garden classification. The patient was consulted with orthopedics, and total arthroplasty of the right hip was performed (Figure 3).

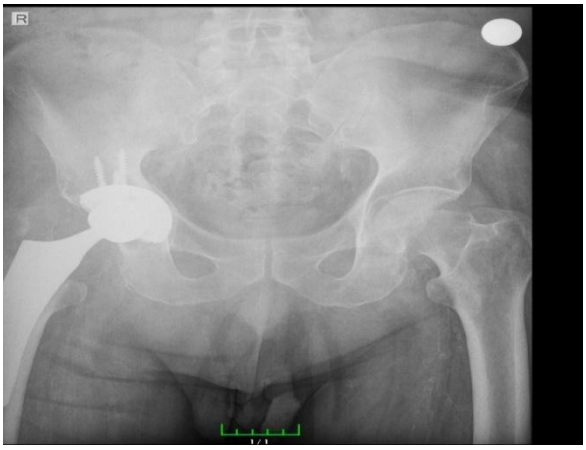

Figure 3. Pelvis AP (Direct radiograph of the patient after postoperative right hip arthroplasty)

3. RESULTS

The patient was transferred to our postoperative clinic. Due to hip arthroplasty and amputation, the patient was taken into a rehabilitation program. Therefore, the patient was given the right hip girdle strengthening, electrical stimulation of hip extensors, prosthesis adaptation training, and progressive ambulation training. After 30 sessions of orthopedic rehabilitation, the hip range of motion was neutralized, and motor strength was 5/5 in all muscle groups. After the progressive ambulation training, the patient was discharged by ambulating with a single tripod.

4. DISCUSSION

The diagnosis of patients with suspected hip fractures showing no findings on direct radiography may be delayed or difficult to diagnose by the clinician. These fractures are called OHF. When the diagnosis is delayed and not intervened in the early period, occult femoral fracture (OFF) may turn into non-displaced and displaced fractures. The incidence of hip fractures increases with age and OHF accounts for 2-10% of all hip fractures (Dominguez, Liu, Roberts, Mandell, & Richman, 2005; Haubro, Stougaard, Torfing, & Overgaard, 2015; Oka & Monu, 2004; Parker, 1992; PF, 1993). The Garden classification of femoral neck fractures (Kazley, Banerjee, Abousayed, & Rosenbaum, 2018) is the most commonly used criterion in the literature. Under the classification, femoral neck fractures are divided into four types by the degree of displacement of the fracture fragments. Type I fractures are those not involving the entire length of the femoral neck. Type II fractures are those involving the entire femoral neck without the displacement of the fracture fragments. On the other hand, type III fractures are complete fractures with the partial displacement of the fracture fragments. Finally, type IV fractures are complete fractures with more than fifty percent displacement [8]. Femoral fractures, especially those in which the femoral neck is displaced (Garden 3-4), lead to larger operations such as total hip replacement and increased mortality rates, compared to non-displaced (Garden 1-2) and OFF (Leung, 2004; Sikand, Wenn, & Moran, 2004). Therefore, timely diagnosis of these fractures is of vital importance. In our case, there was no obvious fracture line on the initial radiograph and CT scan taken in the emergency department, so we considered that our patient had OHF.

The repair of embedded and non-displaced femoral neck fractures (Garden 1-2) is performed with cannulated screws or sliding screws with short plates. The main purpose of internal fixation is to prevent displacement of the stable fracture pattern (Bjørgul & Reikerås, 2007; Rodríguez-Merchán, 2002). Operative options for displaced femoral neck fractures (Garden 3-4) include closed reduction and internal fixation, open reduction and internal fixation, hemiarthroplasty, and total hip arthroplasty [8]. In our case, OFF could not be diagnosed in the early period. The patient presented to the outpatient clinic five months after the fall and was diagnosed with a Garden type 4 femoral neck fracture. Because of this delay in diagnosis, the patient missed the chance of repair with screws and could be treated with total hip arthroplasty.

Polyneuropathy due to DM is a peripheral, symmetrical, sensorineural polyneuropathy that may occur clinically or subclinically, especially affecting the lower extremities. The most common symptoms are tingling, numbness, electric shock, and burning sensation, increasing especially at night (Fiçicioğlu, Aydin, Haktan, & Kiziltan, 1994). Sensory neuropathy in DM patients causes loss of proprioception, pain, and temperature sensation (together called protective sensory loss) (Hicks & Selvin, 2019; Sidawy & Perler, 2018). In our case, diabetic polyneuropathy was detected, and sensory examination revealed decreased pain sensation and impaired position sense. The electromyography report was interpreted to be compatible with sensorimotor axonal-type peripheral neuropathy. It was determined that the diagnosis of the patient's femoral neck occult fracture was delayed due to diabetic polyneuropathy, and the hip girdle muscles secondary to the fracture were also weakened.

With the patient's compliance with the rehabilitation program after the total hip arthroplasty, the strengthening of the hip girdle muscles, amputation, and hip arthroplasty rehabilitation were performed, and our patient was discharged by ambulating with a tripod as a result of the treatment.

5. CONCLUSION

In the present report, we aimed to draw attention to the importance of early diagnosis of occult femoral neck fractures, the need for re-evaluation of undiagnosed patients for fractures, even if patients are admitted late according to their history, and the fact that decreased pain sensation in DM patients leads to delayed diagnosis of fractures and thus missing the chance of less complex surgical procedures in the early period.

6. ACKNOWLEDGEMENT

Informed consent was obtained from the patient.

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HOMEOPATHIC APPROACH IN CASES OF INFERTILITY

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ABSTRACT

Background of the Study: There is a physiological balance between the systems in our body called homeostasis. When homeostasis is disrupted, diseases occur. Infertility is defined as the absence of pregnancy despite couples having regular sexual intercourse. Homeopathy is a widely used natural medicine. It is recognized by the WHO as the second largest treatment method used worldwide. Homeopathy is based on a detailed anamnesis and the identification and use of specific remedies for the complaints. There are various types of masks, ranging from herbal extracts to periodic table minerals. In order not to reduce the effectiveness of mascara, at least 20 minutes should be intervened between the consumption of pungent smelling things such as coffee, mint, menthol.

Aim: To discuss the use of homeopathy in the treatment of infertility unresponsive to conventional therapies.

Material and Method: A 36-year-old woman, whose first marriage lasted eight years and who could not get pregnant despite treatment for infertility, applied to GETAT policlinic when she did not respond to infertility treatment during her second marriage. After cupping treatment, ear and body acupuncture was applied to the patient's lumbar back shu points with sterile disposable acupuncture needles. Especially hormonal axis and sedation points were selected. DU-mai chong mai meridian points were applied for 20 minutes, first once a week and then once every two weeks. Semi-permanent acupuncture needles-hualong were inserted in the ear. She was prescribed homeopathic remedies ignatia c30, folliculunum c200 2*1 and yarrow-hawthorn tea for home use.

Results: The patient became pregnant two months later. Her pregnancy was healthy, confirmed by USG and beta-hcg, and she delivered in the normal process with normal delivery. Currently, her two-year-old baby girl is growing up healthy.

Conclusion: In the treatment of infertility unresponsive to conventional therapies, cupping can be used as a detox treatment, acupuncture for hormonal axis regulation and sedation, and homeopathy phytotherapy can be safely added to increase follicular reserve.

Keywords: Homeostasis, homepathy, infertility, remedy

1. INTRODUCTION

Infertility is defined as the inability of couples to reproduce or maintain a pregnancy despite having regular unprotected sexual intercourse for a year (Taşkın, 2012). Infertility is recognized by WHO as an international public health problem affecting millions of women worldwide and is reported to affect 80 million women worldwide (Bayer ve ark., 2008).

GETAT; It is all of the explainable or unexplainable practices based on different traditions, beliefs and experiences to ensure the continuity of health as well as the diagnosis and treatment of psychological and physical diseases (Sağlık Bakanlığı, 2017). It is known that GETAT applications have been used in infertility for the last thirty years and the number of studies with high evidence value is quite low (Özkan ve ark., 2018; Özcan ve Kizilkaya Beji, 2016).

Although the applications used vary between countries according to culture, geography and traditions, the most commonly used ones in infertility are; acupuncture, massage, nutritional supplements, mind and body practices (hypnosis, yoga, meditation), homeopathy and psychotherapy (Clark ve ark., 2013).

1.1. Homeopathy

Homeopathy Dr. It is an alternative method developed by Samuel Hahnemann, who believes that the body can heal by using its own power (Yıldız ve Ürper, 2013). Homeopathy is a natural medicine that has been widely used all over the world for 200 years. It has been recognized by the World Health Organization (WHO) as the second largest treatment method used worldwide. Although it is the most popular form of treatment in India and South America, it is also used by more than 30 million people in Europe and millions more worldwide (Postoğlu, 2010). It starts from the principle of "like cures like".

1.1.1. Ignatia Amara

It is not possible to make people talk or cheer them up. He has a fixed gaze forward and a thoughtful look. He is reluctant to open his mouth and speak. He underestimates himself and thinks he has lost everything. He thinks he can't walk or move. He is desperate for treatment. He is often sullen and disgruntled because of his headache. The slightest incident can make a person angry or cry. There is an indifference to everything. Tends to be hasty. He has a very sensitive consciousness. Sudden urge to do something, tendency to do something quickly, leading to mistakes when speaking or writing (Alp, 2022).

1.1.2. Hios

Hyociamus niger medicine is about sadness and sullenness. It is a self-condemning personality medicine in a melancholic mood. Fear may be accompanied by tremors and convulsions. He is afraid of being poisoned. He can lose himself at the slightest trouble, he thinks he is very strong and healthy, but he is not that strong. He needs nothing but thirst (Alp, 2022).

1.1.3.Folliculunum

It is a medicine that can arguably be considered the most feminine medicine of Materia Medica Homoeopathica. The substance is sarcode, Folliculinum or oestron, a natural hormone secreted from the ovaries that plays a leading role in the reproduction of our organism. This steroid hormone, with the typical sterane structure and formula C18H22O2, can directly interact with nuclear DNA if associated with a steroid receptor, which is well known as an important and immediate messenger substance. The name estrogen is derived from Greek. Oistros means "anger or lust" and genao means "to create." What we are talking about is “a substance that produces anger or lust.” Estrone used in homeopathy is obtained from urine. It is a powdered, water-insoluble, white, crystalline substance. The hormonal balance in women is very sensitive and is easily affected by endogenous or exogenous disorders. The pressure and suppression caused by dominant forces on an individual, such as dominant parents, husband, workplace harassment or religious domination, can be a cause of creating the so-called Folliculinum condition, as it can be caused by the suffering of the immune system after prolonged and deep infection (Kermalli & Chandak 2023).

1.2. Acupuncture

It acts on the connection between the central nervous system, hypothalamus, pituitary-ovarian axis and pelvic organs. ß increases the level of endorphins. This change in ß endorphin level affects GnRH release and menstrual cycle. Acupuncture can also affect the levels of gonadotropes and ovarian hormones in both the ovulatory and anovulatory periods. Ovarian stimulation may affect ovulation (Avcıbay ve Beji, 2013).

We aimed to discuss the use of homeopathy in the treatment of infertility unresponsive to conventional therapies.

2. CASE REPORT

A 36-year-old woman, whose first marriage lasted eight years and who could not get pregnant despite treatment for infertility, applied to GETAT policlinic when she did not respond to infertility treatment during her second marriage. After cupping treatment, ear and body acupuncture was applied to the patient's lumbar back shu points with sterile disposable acupuncture needles. Especially hormonal axis

and sedation points were selected. DU-mai chong mai meridian points were applied for 20 minutes, first once a week and then once every two weeks.Semi-permanent acupuncture needles-hualong were inserted in the ear. She was prescribed homeopathic remedies ignatia c30, folliculunum c200 2*1 and yarrow-hawthorn tea for home use.

A total of 10 sessions of acupuncture were applied. When starting the treatment, the first five sessions were applied every week, and the next 5 sessions were applied once every 2 weeks. When the patient came to each session, she was questioned whether she had used any remedies and was encouraged to use the medicines regularly.

3. RESULTS

After the treatment, the patient became pregnant two months later. Her pregnancy was healthy, confirmed by USG and beta-hcg, and she delivered in the normal process with normal delivery. Currently, her two-year-old baby girl is growing up healthy.

4. DISCUSSION

As a result of the study conducted with infertile animals with homeopathic treatment method, a decrease in sperm defects and an increase in sperm motility and semen amount were found (Lobreiro, 2007).

In the study conducted by Dieterle et al. (2009) on 57 infertile patients with severe oligoasthenozoospermia; Acupuncture treatment was applied to 28 patients according to the principle of Chinese medicine, while 29 patients were given placebo acupuncture. When sperm concentration and semen volume were compared after treatment, significant changes were observed in semen volume and sperm concentration in the group receiving acupuncture treatment. Pregnancy rates were not compared in this study.

In a pilot study conducted by Gerhard and Wallis in 2002 on infertile men, 45 patients were treated with homeopathic medicine prescribed for 10 months. The general health status, hormone values and sperm count of the patients were evaluated, and important results were obtained in sperm density, sperm percentage and sperm motility, especially in oligospermia cases. There have also been significant improvements in general health, such as quitting smoking, reducing alcohol and coffee consumption, avoiding stress, and protecting against harmful factors and infection at work.

As a result of the homeopathy study conducted by Bergman et al. (2000) in Germany with 67 female infertile patients, positive results were obtained in 38 patients. Progesterone concentration increased and positive ovulation and pregnancy results were achieved. Pregnancy results were found to be statistically significant compared to the placebo group (Bergman vd., 2000)

5. CONCLUSION

In the changing and developing world, infertility rates are increasing day by day due to increasing reproductive age, changing living conditions, unhealthy living conditions/nutrition and are becoming one of the most important problems. Assisted reproductive techniques are; These are practices that are costly, time consuming, and in which control is almost entirely in the hands of healthcare personnel. If alternative/complementary methods are supported by sufficient studies and evidence, they will provide new rays of hope for infertile couples in the future and will increase the effectiveness of the treatment.

This success we achieved with the combination of homeopathy and acupuncture in the treatment of unexplained infertility in healthy individuals can be a reference for further studies within the scope of integrative treatments, showing that the problems that we cannot overcome with classical treatment methods can be corrected by correcting the disrupted life energy and immune system balances.

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ACUPUNCTURE IN TRIGEMINAL NEURALGIA

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ABSTRACT

Background of the Study: Electroacupuncture is known to relieve both sensory and inflammatory pain by activating the nervous system in painful conditions. Electroacupuncture shows analgesic effect through peripheral spinal and supraspinal mechanisms and bioactive mechanisms via endorphins, dynorphins, enkephalins.

Aim: To evaluate the analgesic efficacy of acupuncture in a case of trigeminal neuralgia unresponsive to conventional treatment.

Material and Method: A 24-year-old woman with trigeminal neuralgia presented to GETAT Outpatient Clinic despite radiofrequency treatment and botox application in the algology outpatient clinic. VAS (pain assessment scale) was 10. After informed consent form was obtained and necessary information was given to the patient, she was treated with both body and ear acupuncture. A total of 10sessions were administered; initially 1-session per week, then every 2-3 weeks. Yin-tang and local points on the paralysed side were used in body acupuncture, while Jerome, zero, shen-men, kidney and trigeminal nerve points were focused on the ear. E frequency and Nogier frequency were applied to the paralysed side with the electroacupuncture device. During the acupuncture sessions, B-vit supplements, D-vit, Mg and Ca containing drugs were prescribed at a dose of 1*1, which are used in nerve repair and whose levels are deficient for acupuncture to fully show its effectiveness When the patient did not get relief, homeopathic remediation hyperC200 and Rhus-tox C30 2*1 were recommended for the continuation of the treatment.

Results: It was observed that the patient's previous radiofrequency and botox applications may have slowed the decrease in VAS level. Although the patient could not continue the treatment due to his economic situation, there was a 30% pain-reduction.

Conclusion: In the case in which the treatment process was delayed due to other treatments before acupuncture, only 30% pain-reduction (VAS decreased from 10 to 7) was found by adding homeopathy treatment.

Keywords: Electroacupuncture, trigeminal neuralgia, homeopathy

1.INTRODUCTION

Facial pain is easily misdiagnosed. When the pain is intense and recurrent and the underlying etiology is elusive, the condition is often labeled trigeminal neuralgia, although other conditions are much more likely to be the cause. The prevalence of trigeminal neuralgia in the population is 0.07%, compared to approximately 2% in patients with facial pain in general (Macfarlane vd., 2014 & Van Hecke vd., 20014).

Conversely, trigeminal neuralgia (also known as tic douloureux) is frequently mistaken for dental pain, leading to redundant diagnostic procedures such as x-rays of the jaw and, in more than a few cases, unnecessary extractions of teeth. Accurate diagnosis of trigeminal neuralgia depends critically on the patient’s description of its characteristic features. Clarification of the characteristics of the pain is therefore necessary to guide clinical diagnosis and management (Drangsholt, Truelove & Yamuguchi 2005).

Acupuncture has been practiced widely in China for more than 4000 years and is an integral part of traditional Chinese medicine. It was first described in the medical writings The Yellow Emperor's Classic of Internal Medicine around 200 BC (Cao, 2002). Acupuncture needles are immersed in special spots on Bonghan channels and regulate the raw flow by electron transfer (Lin, Huang & Zhuang, 2017).

Acupuncture is a needling method and can be used in the treatment of many diseases such as chronic pain. The effects of acupuncture can be explained by neurophysiological studies. These effects are not only local effects, but also effects on general, central nervous system. The acupuncture needle spreads from the local needling through viscero-cutaneous, cutaneous-visceral, cutaneous-muscular reflexes. Thus, it provides a dermatomal effect. The acupuncture stimulation then reaches the upper centers via the medulla spinalis, and finally the periaquaductal neurons in the mesencephalon; β-endorphine, encephalin, serotonin are released. Acupuncture points are points in close relationship with the lymphatic system and vascular structures Acupuncture points are shown to have low electrical resistance points and calcium concentration increases as a result of needle stimulation of the point (Shang, 1989).

With the needling of the acupuncture point, the transition from the brain to the spinal core, the thalamus, sensory cortex, periacuaductal neurons are activated and the pain control system is activated (Bear vd., 2007). Acupuncture stimulation stimulates endorphinergic and encephalinergic neurons, which are associated with the cortex and hypothalamus. With synaptic connections, serotonergic neurons in the bulb are activated (Guyton & Hall 1996). Analgesia is also important neurotransmitters encephalin and serotonin (Pintov vd., 1997). Encephalin is released from periaquaductal neurons in mesencephalon (Guyton & Hall 1996). Electroacupuncture has its analgesic effect on serotonin receptors (5-HT (1A) and 5-HT (3) (Chang vd., 2004).

Homeopathy is a holistic medicine method that sees the human being as a whole and tries to achieve overall healing by helping the body heal itself. The basic principle of homeopathy, a natural, effective and scientific treatment method, is "treating like with like". Homeopathy does not treat the symptoms of the disease, it strengthens the body's life energy and ensures the restoration of the disturbed balance. Homeopathy, which treats and treats the person as a physical, emotional and mental whole without dividing the individual into areas, is the most used complementary medicine treatment method after phytotherapy (Schepper, 2019).

With this publication, we wanted to draw attention to the analgesic effects of acupuncture, especially in the case of trigeminal neuralgia, which we treated using traditional complementary medicine methods.

2.CASE REPORT

A 24-year-old woman with trigeminal neuralgia presented to GETAT Outpatient Clinic despite radiofrequency treatment and botox application in the algology outpatient clinic. VAS (pain assessment scale) was 10. After informed consent form was obtained and necessary information was given to the patient, she was treated with both body and ear acupuncture. A total of 10sessions were administered; initially 1-session per week, then every 2-3 weeks. Yin-tang and local points on the paralysed side were used in body acupuncture, while Jerome, zero, shen-men, kidney and trigeminal nerve points were focused on the ear. E frequency and Nogier frequency were applied to the paralysed side with the electroacupuncture device.

During the acupuncture sessions, B-vit supplements, D-vit, Mg and Ca containing drugs were prescribed at a dose of 1*1, which are used in nerve repair and whose levels are deficient for acupuncture to fully show its effectiveness When the patient did not get relief, homeopathic remediation hyperC200 and Rhus-tox C30 2*1 were recommended for the continuation of the treatment.

3.RESULTS

It was observed that the patient's previous radiofrequency and botox applications may have slowed the decrease in VAS level. Although the patient could not continue the treatment due to his economic situation, there was a 30% pain-reduction.

4.DISCUSSION

In a study, they evaluated the effects of acupuncture treatment in 104 patients (mean age 52.3 +/- 13 years) with idiopathic or secondary trigeminal neuralgia. Acupuncture treatment and electroacupuncture were applied using twelve-session cycles. As a result they said that: acupuncture is an elective treatment in all kinds of secondary tregeminal neuralgia, while, in the idiopathic form, its validity is conditioned by preceding medical treatments and by beginning of the disease. (Costantini vd., 1995).

In a study conducted by Ichida et al. in 2017; 60 neuralgia patients were blindly randomized and acupuncture was applied to one group and sham acupuncture was applied to the other group. The same evaluations were repeated after treatment and six months after treatment. There were reductions in secondary myofascial pain and mandibular limitations in the acupuncture and sham-acupuncture groups, but only the acupuncture group maintained the changes after 6 months As a result, they stated that acupuncture may be an option in the treatment of ITN due to its analgesic effect on both ITN and secondary myofascial pain (Ichida vd., 2017).

As a result of the study conducted by Edwards and Shaw (2021), in which they aimed to evaluate the status of acupuncture against the current first-line drug treatment (carbamazepine) and the most effective surgery (microvascular decompression (MVD), they say; Acupuncture appears more effective than pharmacotherapy or surgery. Statistical analysis of side effects was not possible due to inconsistent reporting protocols, but the data suggest that acupuncture is considerably safer than pharmacotherapy or surgery (Edwards & Shaw 2021).

In a meta-analysis conducted by Luo and her colleagues in 2022, they say that; As a major part of traditional Chinese medicine, acupuncture may have the potential to manage the symptoms of TN, anxiety and depression simultaneously.

5.CONCLUSION

We see acupuncture as a strong alternative to other pharmacological and surgical treatments in the treatment of trigeminal neuralgia. We recommend GETAT to patients who do not respond to conservative treatments.

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SEPTIC LEUKOENCEPHALOPATHY DEVELOPING AFTER ENDOSCOPIC RETROGRADE CHOLANGIO PANCREATOGRAPHY

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ABSTRACT

Background of the Study: Septic encephalopathy is a brain dysfunction secondary to infection. It can develop due to infections originating from a focus outside the central nervous system.

Aim: We aimed to present our patient who developed septic leukoencephalopathy after endoscopic retrograde cholangio pancreatography.

Material and Method: A 30-year-old female patient has substance and alcohol use. Sedoaanalgesia was applied for the removal of the stent inserted into the biliary tract and the patient was taken to intensive care when she did not wake up after the procedure. General condition of the patient is poor, GCS=6, TA;230/130, pulse rate: 170/min, temperature 38.5. On admission, WBC:7.8, procalcitonin:<0.05, CRP: 4. 12 hours later, WBC:23, procalcitonin:76, CRP: 139. cultures were taken, meropenem 3X1 gr, metronidazole 4X500 mg were started. Brain CT and diffusion MRI revealed bilateral generalized hypoxia and leukoencephalopathy due to sepsis. The patient in septic shock was intubated and inotropic support was started. Perforation was excluded by performing emergency endoscopy. No cardiac embolism was found on ECG. ESBL positive Escherichia Coli was grown in the patient's blood and urine cultures. Appropriate antibiotherapy was initiated. On the 20th day, spontaneous eye opening movements started and partial communication was possible. Control brain CT and MRI showed significant improvement. The patient's general condition improved and he was transferred to the palliative service on the 27th day. After 2 weeks of follow-up in the palliative ward, the patient was discharged home with GCS:15, full cooperation and able to walk.

Results: Many mechanisms have been proposed in the pathophysiology of septic encephalopathy, including blood brain barrier damage, impaired cerebral microcirculation, activation of inflammatory cytokines and complement. Many microorganisms, including Escherichia Coli, can trigger these mechanisms.

Conclusion: Septic leukoencephalopathy, which has a high mortality rate, was reversible in our patient without any physical or cognitive sequelae.

Keywords: Endoscopic Retrograde Cholangio Pancreatography, Escherichia Coli, Septic Leukoencephalopathy

1. INTRODUCTION

Sepsis and severe sepsis (sepsis accompanied by acute multiple organ dysfunction syndrome) are defined as life threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). Septic encephalopathy (SE), considered multifocal brain dysfunction because of a dysregulated host response without primary central nervous system (CNS) infection, is the most common cause of encephalopathy in intensive care units (Chung, Wickel, Brunkhorst, & Geis, 2020). Individuals who have survived SE often experience prolonged periods of neurological sequelae, particularly neurocognitive deterioration. The clinical manifestations of SE may range from mild symptoms, such as aprosexia and disorientation, to more severe conditions such as delirium or coma (Kuperberg & Wadgaonkar, 2017).

We aimed to present our patient who developed septic leukoencephalopathy after endoscopic retrograde cholangio pancreatography.

2. CASE REPORT

A 30-year-old woman had a stent implanted for biliary obstruction. While the stent should be removed after 1 month, the patient presented 3 months later and stent removal was planned under sedoaanalgesia. The patient had no comorbidities, routine blood tests were normal, substance and alcohol use was present, so she was evaluated as ASA 2 physical status. sedoaanalgesia was applied for the procedure after routine monitoring. After 45 minutes, the stent could not be removed and the procedure was terminated. At the end of the procedure, the patient was not recovered and was taken to the intensive care unit.

3. RESULTS

The patient was in poor general condition, unconscious, flexion response to painful stimuli was present, he was making meaningless sounds, babinski reflex in the left extremity was lax. TA: 130/ 80 mmHg, pulse rate: 130/min, SpO2: 92%. Upon admission to the intensive care unit, hemogram and biochemistry were immediately performed and no abnormalities (WBC: 7.8, procalcitonin: <0.05, CRP: 4) were detected. Approximately 6 hours later, GCS=6, TA: 230/130 mmHg, pulse rate: 170/min, fever 38.5°C. Hypotension developed 12 hours after the endoscopic procedure and inotropic support was started. Hemogram and biochemistry tests were repeated and the results were as follows; WBC: 23, procalcitonin: 76, CRP: 139. Cultures were taken, empirical antibiotic treatment was started as meropenem 3X1 gr, metronidazole 4X500 mg. The patient in septic shock was intubated and inotropic support was started. Possible perforation in the biliary tract was excluded by emergency endoscopy. No cardiac embolism was detected on ECG. Brain CT and diffusion MRI revealed bilateral generalized hypoxia and leukoencephalopathy due to sepsis.

ESBL positive Escherichia Coli was grown in the patient's blood and urine cultures. Appropriate antibiotherapy was initiated. On the 20th day, spontaneous eye opening movements started and partial communication was possible. Control brain CT and MRI showed significant improvement. The patient's general condition improved and he was transferred to the palliative service on the 27th day. After 2 weeks of follow-up in the palliative ward, the patient was discharged home with GCS:15, full cooperation and able to walk.

4. DISCUSSION

Physicians have recognized that the central nervous system is one of the first organs affected in sepsis, and its clinical manifestation is the so-called sepsis associated encephalopathy (Mazeraud et al., 2020). The incidence of SE is about 50%. It varies from 8% to more than 70% of septic patients, according to the sepsis severity, patients' profile (Young et al., 1990). Several mechanisms are involved in the pathogenesis of sepsis-associated encephalopathy, such as blood–brain barrier dysfunction, cerebral blood flow impairment, glial cell activation, leukocyte transmigration, and neurotransmitter disturbances.

SE is associated with increased mortality as well as long-term cognitive impairment and psychological disorders, including depression, anxiety, and posttraumatic stress disorder (Hatch et al., 2018).

Its management relies mainly on general ICU good practices as specific treatment is still lacking (Mazeraud et al., 2020).

5. CONCLUSION

Septic leukoencephalopathy, which has a high mortality rate, was reversible in our patient without any physical or cognitive sequelae.

6. ACKNOWLEDGMENT

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TAKOTSUBO CARDIOMYOPATHY SECONDARY TO NOREPINEPHRIN IN CESAREAN SECTION

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ABSTRACT

Background of the study: Takotsubo Cardiomyopathy (TC) is a condition characterized by transient regional left ventricular systolic and diastolic dysfunction that cannot be explained by a coronary artery occlusive lesion. The possible role of catecholamines in its pathophysiology is known.

Aim: We presented a case of TC which was thought to be related to norepinephrine used in the treatment of Spinal Anesthesia-Induced Hypotension (SAIH) in cesarean section.

Material and Method: The patient underwent spinal anesthesia for cesarean. SAIH developed, ephedrine 60mg was administered. Norepinephrine 7.5mcg bolus was given to treat hypotension unresponsive to ephedrine. Ventricular tachycardia developed immediately after Norepinephrine. The rhythm resolved spontaneously. ST depression was observed on ECG. The patient was hypotensive (PR:145/min, BP:90/45 mmHg) and norepinephrine infusion was started at 0.01mcg/kg/min.

CK-MB:2.9ng/ml, troponin:39ng/L, D-Dimer:3130ng/ml. LVEF:45%, MY:2, TY:2, PAP:40mmHg, left ventricular wall motion abnormality were detected on ECO. The patient was given supportive treatment in ICU. The next day CK-MB:13ng/ml, troponin:736ng/L, coronary angiography was performed, no pathology was detected. The patient's ECO findings improved on postop day3 and did not require inotropic support. ECO performed one-month later showed: LVEF:55%, PAP:29mmHg, left ventricular wall motion was normal.

Results: TC after norepinephrine in a hypotensive patient due to adrenal insufficiency was reported. ECO revealed severe hypokinesia in the apical segment and EF:25%. After norepinephrine was discontinued, wall movements normalized and EF:70%. TC may cause complications such as malignant arrhythmia, cardiogenic shock, ventricular rupture. In our case, ventricular arrhythmia and hypotension occurred. However, organ dysfunction is usually reversible. Our patient also improved with supportive treatment. It has been reported that ST depression on ECG, LVEF:40%, global hypokinesia were detected on ECO in a patient who was mistakenly given 2mg norepinephrine bolus and the findings improved within 48hours. In our case, ECO findings resolved in a few days.

Conclusion: Norepinephrine bolus may have a causal role in TC development.

Keywords: Norepinephrine, spinal anesthesia-induced hypotension, takotsubo cardiomyopathy.

1. INTRODUCTION

Takotsubo Cardiomyopathy is a reversible acute heart failure frequently precipitated by an emotional or physical stress. The clinical presentation resembles acute coronary syndrome. Pathogenesis is complex and may involve brain-heart axis and neuro-hormonal stunning of the myocardium. Coronary angiography reveals normal epicardial arteries with no obstruction or spasm. NT-ProBNP maybe remarkably elevated. Regional wall motion akinesia (RWMA) of left ventricle extends beyond the territory of one coronary artery. Reduced left ventricle ejection fraction (LVEF) and RWMA recover in 6–12 weeks. Prognosis is generally good (Gupta & Gupta, 2018)
We presented a case of TC which was thought to be related to norepinephrine used in the treatment of Spinal Anesthesia-Induced Hypotension (SAIH) in cesarean section.

2. CASE REPORT

30-year-old 38-week pregnant woman underwent cesarean section. Spinal anesthesia was performed in the patient with ASA 2 physical status. Surgery was started when the level of sensory block reached T4 dermatome. SAIH developed and a total of 60 mg ephedrine was administered 4 times in 15 mg

doses. Norepinephrine 7.5mcg bolus was given to treat hypotension unresponsive to ephedrine. Ventricular tachycardia developed immediately after Norepinephrine. After about 30 seconds, the rhythm recovered spontaneously. ST depression was observed on ECG and the patient started to complain about chest pain. The patient was hypotensive (PR:145/min, BP:90/45 mmHg) and norepinephrine infusion was started at 0.01mcg/kg/min. Patient transferred to intensive care unit after surgery.

3. RESULTS

The blood tests taken in intensive care were as follows; CK-MB:2.9ng/ml, troponin:39ng/L, D-Dimer:3130ng/ml. The patient was consulted to cardiology. LVEF: 45%, MY:2, TY:2, PAP:40mmHg, left ventricular wall motion abnormality were detected on electrocardiography (ECO). The patient was given supportive treatment in ICU. The next day CK-MB: 13ng/ml, troponin: 736ng/L, coronary angiography was performed, no pathology was detected. The patient's ECO findings improved on postop day3 and did not require inotropic support. ECO performed one-month later showed: LVEF: 55%, PAP: 29mmHg, left ventricular wall motion was normal.

4. DISCUSSION

Three decades ago, Sato and Dote introduced the term “Takotsubo syndrome” to describe the shape of the left ventricle during systole in patients with a clinical presentation of myocardial infarction without obstructive coronary artery disease (Dote, Sato, Tateishi, Uchida, & Ishihara, 1991). Several diagnostic criteria have been developed to guide the TC diagnosis over the years. The revised Mayo Clinic diagnostic criteria for diagnosis of TC include LV midsegment transient dyskinesia; regional wall motion abnormalities beyond a single epicardial vascular territory; absence of obstructive coronary artery disease/plaque rupture; new electrocardiographic abnormalities or modest troponin elevation; and absence of pheochromocytoma. The latest consensus from the European Society of Cardiology produced the International Takotsubo Diagnostic Criteria (InterTAK Diagnostic Criteria), which considers left ventricular dysfunction, emotional/physical triggers, neurological disorders, and pheochromocytoma potential triggers in addition to revised Mayo Clinic criteria (Udemgba et al., 2024).

Takotsubo Cardiomyopathy (TC) during pregnancy and postpartum is rare but may lead to significant maternal morbidity. A literature review investigating pregnancy-related TC reported that 22 patients developed TS while pregnant, 20 after vaginal delivery, and 39 after cesarean section (Udemgba et al., 2024). In this review, the most common potential triggers are noted to be surgery, medications, and emotional/psychological stress. We think that the trigger in our case was norepinephrine bolus. TC was reported in a patient who was hypotensive due to adrenal insufficiency after the use of norepinephrine; the patient had severe hypokinesia in the apical segment on ECHO and EF decreased to 25%. After discontinuation of norepinephrine, wall motion normalized and EF was 70%. (Vieira, Batista, & de Abreu, 2018). A number of clinical observations and preclinical studies suggest that high levels of catecholamines play a causative role in TC. Wittstein and colleagues measured circulating catecholamine levels in the blood of a cohort of patients with TC and reported levels that were 10–20 times higher than normal (Kato, Lyon, Ghadri, & Templin, 2017).
Although ST segment elevations are the most common electrocardiogram finding, ST depression was observed in our case. The rate of ST depression in these cases is reported to be 6.8% in the literatüre (Udemgba et al., 2024).

In our patient, inotropic support was started in the operating room and continued for 2 days. Inotropic support was required in 9.9% of patients in the literatüre (Udemgba et al., 2024).

Our patient with elevated cardiac markers on postop day 1 underwent coronary angiography to rule out coronary artery disease and the rate of coronary angiography is quite high in the literature (45.7%) (Udemgba et al., 2024).

It has been reported that normal cardiac functions return within 4-8 hf (Rozema & Klein, 2016), and in our patient ECO findings 1 month later were normalized.

5. CONCLUSION

Although TC is rare, it can also develop in pregnant women and the use of noepinephrine in cesarean section is among the possible risk factors.

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I would like to thank Dr. İskender KARA for his support in the final review.

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THE EFFECT OF JUGLONE ON *mTOR* EXPRESSION IN PANC-1 AND BXPC-3 PANCREATIC CANCER CELLS IN ACCOMPANIMENT WITH ASCORBATE

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ABSTRACT

Background of the Study: Mitochondria have become a focus of pancreatic cancer (PC) research due to their important role in cancer cell survival and resistance to chemotherapy. The high chemotherapeutic resistance seen in PC necessitates research into alternative drug treatments. The antimetastatic effects of juglone on PC cells was revealed for the first time in our previous studies. In those studies Juglone was shown to be a potential anticancer agent, especially when combined with ascorbate. *mTOR* is one of the genes which are most frequently mutated in cancers because it plays an important role in cell survival, metabolism, growth, mitochondrial biogenesis and function, especially in response to upstream signals in cancer. This makes *mTOR* an important antitumoral treatment target.

Aim: We investigated the effects of juglone ascorbate (Jug-NaAsc) on the expression levels of the *mTOR* gene, an important antitumor target, in PANC-1 and BxPC-3 PC cells.

Material and Method: PANC-1 and BxPC-3 cells were cultured in appropriate conditions and treated with 5, 10, 15, and 20 µM juglone accompanied with 1 mM ascorbate, considering the IC50 values obtained from the MTT assay. RNA isolation and cDNA conversion were performed after 24 hours. Expression levels of the *mTOR* gene were determined by qPCR.

Results: According to qPCR results, Jug-NaAsc caused evident suppression on *mTOR* gene expression at all concentrations in both cell lines.

Conclusion: Cytotoxic, apoptotic, anti-invasive and anti-metastatic effects of juglone and Jug-NaAsc combination in PANC-1 and BxPC-3 PC cells was shown in our previous studies suggesting Jug-NaAsc as a potential anticancer agent. In the literature, mTOR inhibition is commonly predicted to be a therapeutic strategy targeting cancer metabolism. Our findings showing the decresae of *mTOR* expression after Jug-NaAsc treatments supports this prediction and reports that juglone may be an effective therapeutic agent in the treatment of PC, and ascorbate may potentiate the different anticancer effects of juglone.

Keywords: Pancreatic cancer, *mTOR* gene, juglone, ascorbate.

1. INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a pancreatic cancer (PC) of exocrine origined, accounting for 90% of PC cases. It is a malignancy with a 5-year survival rate of less than 10% (Kleeff et al., 2016). Pancreatic tumours frequently remain asymptomatic for an extended period, with a tendency to be diagnosed at an advanced stage of the disease, often coinciding with the presence of vascular invasion or metastases (Mayer & Arteaga, 2016). Only 15–20% of patients diagnosed with the disease are amenable to surgical resection, with significant resistance to most conventional treatments, including chemotherapy and radiotherapy (Reyes-Castellanos, Masoud, & Carrier, 2020; Zhang et al., 2012).

Mitochondria are the principal intracellular organelles that regulate signalling pathways which are crucial for energy metabolism, cell death, cell proliferation and differentiation. Mitochondria, known as the power plant of the cell because its main function of ATP production, also undertake many important

functions such as ROS production, regulation of cell signalling, cell death and biosynthesis events. Due to their multifunctional nature in physiological conditions, mitochondria serve as vital cellular stress sensors, enabling the cell to adapt flexibily to environmental changes. This flexibility enables the cell to survive in stressful conditions, such as nutrient deficiency and hypoxia. Considering these important features and functions, mitochondria have been proposed as an important therapeutic target for cancer treatment (Kim et al., 2017; Porporato, Filigheddu, Pedro, Kroemer, & Galluzzi, 2018; Qin et al., 2020). In addition to the classical view of mitochondria in cancer biology, recent studies have revealed new pathophysiological roles of mitochondria in cancer. Under the influence of the tumor microenvironment, PDAC cells alter key pathways, including glycolysis, oxidative phosphorylation, amino acid and lipid metabolism, to adapt and maintain the energy needs of uncontrolled proliferation (Popova & Jücker, 2021). This illustrates the significance of mitochondria in the process of carcinogenesis. Mitochondria have become the focus of also PC research due to their important role in cancer cell survival and resistance to chemotherapy. The observed level of chemotherapeutic resistance in PC calls for the investigation of alternative drug treatments.

Juglone is a type of natural naphthoquinone found in the roots, leaves, trunk and bark of the walnut tree (Vardhini, 2014). In addition to its hemostatic, antibacterial, antiviral and pro-oxidant effects, juglone has been found in various studies to be an inhibitor of cancer cell proliferation, induction of tumor cell apoptosis, induction of autophagy, anti-angiogenesis, and tumor cell migration and invasion. Therefore, as an anti-cancer drug, juglone has become one of the hotspots of cancer research (Ahmad & Suzuki, 2019; González et al., 2005; Tang et al., 2022; Wang et al., 2016).

Ascorbic acid (vitamin C) is an essential water-soluble vitamin found in most fruits and vegetables. While most mammals synthesize vitamin C in the liver, humans and other primates cannot synthesize ascorbic acid and must obtain it from the diet (Sauberlich, 1994; Spielholz, Golde, Houghton, Nualart, & Vera, 1997). Studies conducted to understand the role of ascorbic acid in the body and its health benefits have shown that it strengthens the immune system, stimulates collagen formation, protects against cancer by protecting DNA from free radical damage, and plays a role in the biosynthesis of some neurotransmitters and cell signaling pathways (Fletcher & Coleman, 2020; Padayatty & Levine, 2001). In addition to being a potent antioxidant that can neutralise oxidative stress, ascorbic acid is known to be cytotoxic to cancer cells at high concentrations (Vyas, Zaganjor, & Haigis, 2016).

Mechanistic or mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine protein kinase that regulates cell growth and division in response to growth factors and cellular energy status, and controls cellular metabolism, catabolism and immune response to maintain cellular homeostasis (Foley, Kim, Jaffee, & Zheng, 2016; Rodriguez-Viciana et al., 1994; Wullschleger, Loewith, & Hall, 2006). mTOR is involved in the stimulatory pathway associated with growth, proliferation and differentiation within the cell. Abnormal activation of mTOR in human cancers results in mutations that activate the pathway, amplification or overexpression of components of mTOR complexes, or loss of mTOR negative regulators (Gremke et al., 2020). It is thought that if the metabolic vulnerability created by high mTOR expression in cancer cells is targeted, cancer cells can be effectively treated (Wood, Canto, Jaffee, & Simeone, 2022). Therefore, in our study, we aimed to investigate the effect of juglone on *mTOR* expression in PANC-1 and BXPC-3 PC cells accompanied by ascorbate.

2. MATERIAL AND METHOD

2.1 Cell Culture

PANC-1 and BxPC-3 human pancreatic cancer cell lines were obtained from the ATCC (Manassass, VA, USA). PANC-1 and BxPC-3 cells were cultured in DMEM and RPMI medium, respectively, containing 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO2 in a CO2 incubator.

2.2 Preparation Of Jug-Naasc Combination

Juglone (Catalog no: H47003) and Sodium ascorbate (NaAsc) (Catalog no: A4034) were supplied commercially (Sigma-Aldrich Chemical Company, USA). Juglone was prepared in DMSO with a stock concentration of 20 mM, NaAsc was prepared in dH2O at 1mM stock solution and stored at -20˚C. The juglone ascorbate (Jug-NaAsc) combination was prepared freshly for each treatment.

2.3 MTT Assay

The MTT assay was used to determine the cytotoxic effect of Jug-NaAsc on human PC cells. PANC-1 and BxPC-3 cells were plated in 96-well plates at 5,000 cells per well in a volume of 100 µL for 24 h prior to treatment. After incubation, the media were replaced with fresh media containing different concentrations of juglone (0, 5, 10, 15, 20, 30, 40 and 50 µM) for 24 h and the cells were incubated at 37 °C. Twenty-four hours later, MTT solution was added to each well and incubated at 37 °C for 4 h. The resulting formazan crystals were dissolved with DMSO by pipetting and the absorbance was read at 570 nm in an ELISA reader.

2.4 Gene Expression Analysis

Total RNAs from control and treatment groups of BxPC-3 and PANC-1 PC cells were isolated using TRIzol reagent, and RNA quantification was measured by spectrophotometer (NanoDrop, Thermo Fisher Scientific, USA). cDNA synthesis was performed using a commercially available 2-step RT-PCR cDNA synthesis kit (RTPL12®, Vivantis, Malay sia) according to the manufacturer's instructions. The expression levels of *mTOR* were analysed by quantitative real-time PCR (qPCR) technique. The primers used in the analysis are shown in Table 1. *β-Actin* was used as a reference gene for normalization. The PCR reaction was performed using a Roche Light Cycler96 and was set as follows: initial denaturation at 95°C for 5 min, followed by denaturation at 95°C for 30 s, repeated for 40 cycles, annealing at 60°C for 30 s and extension at 72°C for 30 s. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative changes in gene expression. In the interpretation of the qPCR results, 2-fold increases and decreases were considered significant according to the general approach in the literature.

Table 1. Primers for qPCR analysis of gene expression.

Gene	Primer Sequence (F,R)*	References
mTOR	**F:** 5' AGTGGACCAGTGGAAACAGG3' **R:** 5-'TTCAGCGATGTCTTGTGAGG-3'	**(Makker et al., 2016)**
β-actin	**F: 5'-ACTCTTCCAGCCTTCCTTC-3** **R:5'-ATCTCCATGCATGCATCCTGTC-3**	**(Hsu et al., 2005)**

***F:** Forward Primer, ***R:** Reverse Primer

3. RESULTS

3.1. Cell Culture

In study we used the human pancreatic cancer cell lines PANC-1 and BxPC-3. During culture, the cells were evaluated under an inverted microscope and it was observed that the cells maintained their specific morphology and behavior (Figure 1).

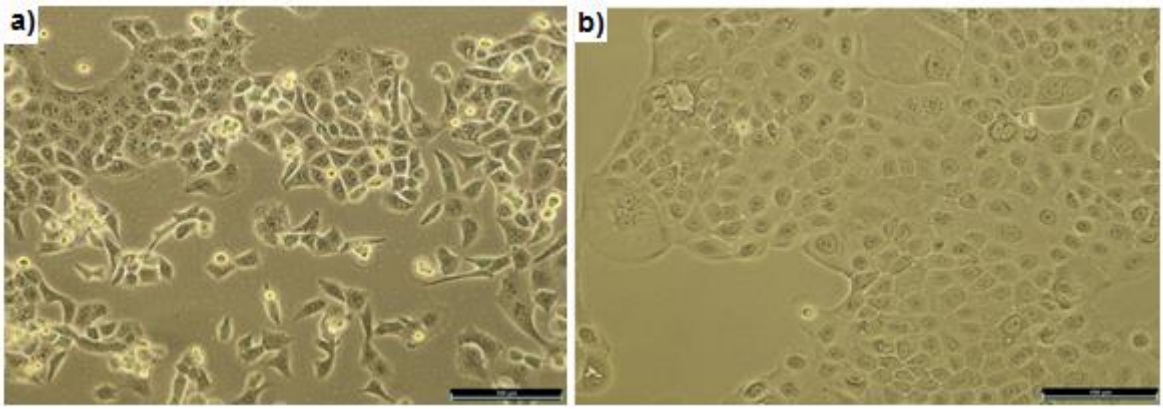

Figure 1. Imaging of a) PANC-1 and b) BxPC-3 cells under an inverted microscope (X10 magnification).

3.2. Effects Of Juglone-NaAsc On Gene Expressions

At the end of 24 hours of Jug-NaAsc application in the PANC-1 cell line, *mTOR* gene expression levels at 5 µM, 10 µM, 15 µM and 20 µM Jug-NaAsc concentrations decreased 8.66, 9.38, 8.965 and 6.945 fold, respectively, compared to the control group (Figure 2).

At the end of 24 hours of Jug-NaAsc application in the BxPC-3 cell line, *mTOR* gene expression levels at 5 µM, 10 µM, 15 µM and 20 µM Jug-NaAsc concentrations decreased 6.14, 6.985, 7.725 and 8.125 fold, respectively, compared to the control group (Figure 3).

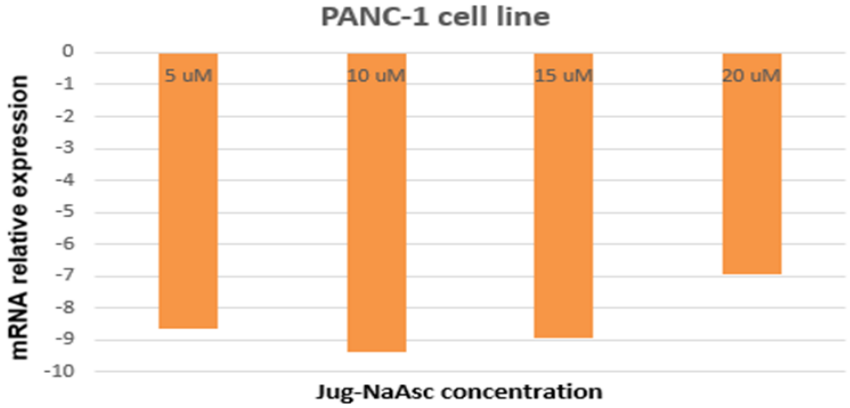

Figure 2. Effects of jug-NaAsc combination on expression of *mTOR* gene in PANC-1 cells for 24 h determined by the qPCR analysis.

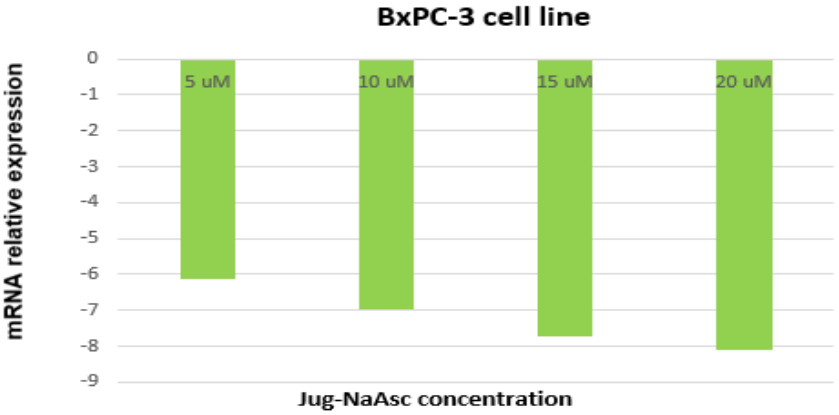

Figure 3. Effects of jug-NaAsc combination on expression of *mTOR* gene in BxPC-3 cells for 24 h determined by the qPCR analysis.

4. DISCUSSION

Pancreatic cancer has a very poor prognosis, with an average survival time of approximately 10 to 12 months and 5 to 6 months without treatment, because symptoms of PC usually appear late that surgical resection is a too late intervention (Neoptolemos et al., 2018). Treatment methods such as chemotherapy, radiotherapy and immunotherapy are also inadequate for PC patients who are ineligible for surgical resection (Huang et al., 2019; Ramana, Singhal, & Reddy, 2014).Therefore, new strategies are needed to develope for diagnosis and treatment of PC. Studies have shown that natural products have important pharmacological activities that regulate various vital cell signalling pathways that cause mitogenic, cytotoxic and genotoxic reactions leading to various disease pathologies (Cameron & Campbell, 1974).

Mitochondria are of significant importance in the field of cancer biology. Recent studies have demonstrated that they play a critical role in the development of cancer. Thus, mitochondria represent the foundation of cancer biology, encompassing the initiation, growth, metastasis, recurrence, and acquired drug resistance (Kim et al., 2017). PDAC cells adapt their metabolic processes to facilitate growth and survival.

Juglone (5-hdroxy-1,4-naphthoquinone) is a naphthoquinone found in the leaves, roots and bark of several walnut tree species including Juglans nigra (Colaric, Veberic, Solar, Hudina, & Stampar, 2005). The evidence that juglone has anti-metastatic and anti-invasive effects on PC cells has also been shown in our previous studies (Avcı, Arıkoğlu, & Erkoç Kaya, 2016; Gokturk, Erkoc-Kaya, & Arikoglu, 2021).

In a study conducted on cultured malignant cells such as human colon carcinoma cell line (HCT-15) cells, juglone was found to block the S phase of the cell cycle (Kamei, Koide, Kojima, Hashimoto, & Hasegawa, 1998). Juglone on cancer cells was shown to induce DNA damage, inhibit transcriptional reduction of p53 protein levels and increase cell death (Xu et al., 2012).

It is known that high doses of ascorbic acid strengthen the immune system and have a protective role against chemical and physical carcinogens and oncogenic viruses (Xu et al., 2012). The route of administration is important for high-dose vitamin C to have a therapeutic effect, and only intravenous administration provides high plasma and urine concentrations to allow potential antitumour activity (Padayatty et al., 2004). Vitamin C is absorbed in two forms: ascorbate or dehydroascorbate from the intestine (DHA) and is transported into the cell by sodium-dependent vitamin C carriers (SVCT1/SVCT2) and glucose transpoter 1 (Glut1) carriers (Levine et al., 1996; Lv et al., 2018; Szarka, Kapuy, Lőrincz, & Bánhegyi, 2021).

Hydrogen peroxide is central to the cytotoxic effect of ascorbic acid. The enzyme catalase, which metabolises hydrogen peroxide to water and free oxygen, is almost completely absent in cancer cells, so cancer cells cannot remove hydrogen peroxide from the environment like normal cells (Astuya et al., 2005; Lawson, 2003; Verrax, Taper, & Buc Calderon, 2008). When metals and hydrogen peroxide combine, they directly produce free oxygen radicals that cause cell damage. There are fewer iron molecules in normal cells than in cancer cells. Iron is one of the elements that enables cancer stem cells to survive. By producing hydrogen peroxide, ascorbic acid actually strengthens its structure with elements such as copper and iron that are needed for oxidation, damaging the cell membranes of tumor cells that do not have catalase activity and causing the cells to die (Astuya et al., 2005; Chen et al., 2005; Iqbal K, 2004; Lawson, 2003; Melissa Ge 2008).

In a study by Maramag et al. using the androgen-independent (DU145) and androgen-dependent (LNCaP) human prostate cancer cell lines, vitamin C was shown to be a potent anticancer agent for prostate cancer cells. They reported that vitamin C prevents cell division and growth by producing hydrogen peroxide, which damages cells through free radicals (Maramag, Menon, Balaji, Reddy, & Laxmanan, 1997). It has been shown that vitamin C at pharmacological plasma concentrations can selectively kill colorectal cancer cells (Yun et al., 2015). Studies have reported that high doses of ascorbic acid can be used as a standard supplement in cancer treatment by boosting natural resistance to cancer (Cameron & Pauling, 1976).

mTOR is the target of a molecule called rapamycin or sirolimus, a macrolide produced by Streptomyces Hygroscopius bacteria that first attracted attention for its broad anti-proliferative properties (Cafferkey et al., 1993; Kunz et al., 1993). mTOR is a serine/treonin protein kinase of the kinaz family associated with phosphoinocytide 3-kinase (PI3K) and interacts with various proteins to form two different complexes called mTORC1 and mTORC2 (Laplante & Sabatini, 2012). Studies conducted in various cancers have shown that mTOR passes to mitochondria under radiation stress and shifts the bioenergy pathway from aerobic glycolysis to oxidative phosphorylation, which is associated with increased resistance to radiotherapy (Lu et al., 2015; Roberts, Tan-Sah, Ding, Smith, & Miyamoto, 2014). Malignant phenotypes, including radio/chemotherapy resistance, tumour invasion and metastasis, have been observed in tumour cells. These cells have acquired metabolic plasticity and, mitochondria is at the centre of this dynamic process (Herst, Grasso, & Berridge, 2018).

Ascorbic acid is a good electron transmitter, providing the electrons required for the mitochondrial energy pathway physically and metabolically (González et al., 2005; Kc, Cárcamo, & Golde, 2005). Qin et al. (2020) reported that SVCT2-mediated vitamin C uptake increases intracellular ROS levels and regulates cellular activities by inhibiting mTOR (including mTORC1 and mTORC2) activation. Dehydroascorbic acid has been demonstrated in vivo to act as a mitotic inhibitor, inhibiting the synthesis of protein at the ribosomal level and preventing cell division (Riordan HD, 1990). It has been demonstrated that vitamin C can selectively eliminate colorectal cancer cells with KRAS/BRAF mutations by targeting GAPDH. The same study reported that pharmacological vitamin C treatment could inhibit cell viability regardless of BRAF mutation status by inhibiting the activation of the mTOR pathway. They demonstrated that vitamin C represents an innovative mechanism underlying how vitamin C regulates mTOR pathway activation and thus autophagy, cell viability and cell size (Yun et al.,2015). A study by Hua et al. (2012) demonstrated that p-Akt and p-mTOR levels decreased significantly and were almost completely inhibited by juglone treatment. These findings indicate that juglone induces apoptosis by suppressing PI3K/Akt/mTOR.

In our study, it was determined that *mTOR* gene expression decreased dose-dependently at all application doses of JugNaAsc in both PC cell lines after jug-NaAsc application to PANC-1 and BxPC3 cancer cells. Our results provides additional support for those findings. We demonstrated that ascorbate augmented the anticancer effects of juglone by increasing ROS levels in PANC-1 and BxPC-3 cancer cell lines. Our previous study reported that juglone, when combined with ascorbate, may be an effective agent for novel pro-oxidant cancer treatment strategies (Erkoc-Kaya, Arikoglu, Guclu, Dursunoglu, & Menevse, 2024).

5. CONCLUSION

Our previous studies have demonstrated the cytotoxic, apoptotic, anti-invasive and anti-metastatic effects of juglone and Jug-NaAsc combination in PANC-1 and BxPC-3 PC cells. Those suggest that Jug-NaAsc may be a potential anticancer agent. In the literature, mTOR inhibition is commonly predicted to be a therapeutic strategy targeting cancer metabolism. The observed reduction in mTOR expression following Jug-NaAsc treatments supports this common prediction and indicates that juglone may be an effective therapeutic agent in the treatment of PC. Furthermore, ascorbate appears to enhance the anticancer effects of juglone.

Conflict of Interest and Financial Status
Our study has not received financial support from any institution, and there is no conflict of interest among the authors regarding any matter in this study.

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FLUORESCENCE SENSOR FOR METAL IONS USING CARBON QUANTUM DOTS AND METAL ORGANIC FRAMEWORK

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ABSTRACT

Background of the Study: Carbon quantum dots are new generation nanosized materials. The work on sensors made with these materials attracts a lot of attention today. In addition, metal organic framework structures are among the hybrid structures on which much work has been done in literature. Examples of sensors specifically developed for metal ions with the obtained MOF and hybrid structures produced with carbon nanomaterials can be found in the literature.

Aim: In this study, the aim was to develop fluorescence for metal ions by using carbon quantum dots and metal organic framework (MOF).

Material and Method: All chemicals and reagents were purchased from Sigma-Aldrich. Ultrapure water was used for preparing all solutions. Citric acid is weighed for synthesis. The solution is prepared, ethylene diamine is added and stirred at room temperature until equilibrium is reached. It is transferred into the autoclave reactor and kept in the oven at the appropriate temperature for a certain period of time. Weigh appropriate amounts of zirconium chloride and terephthalic acid. After the solutions are prepared, they are combined and placed in an autoclave reactor. Autoclave is placed in an oven and kept at a certain temperature and time.

Results: In this study, experiments were carried out with fluorescence spectroscopy by adding solutions prepared with heavy metal ions onto emissive carbon quantum dot solutions. In the study, metal organic framework structures were added to the environment to examine their effects and emission intensity changes were observed. According to the results obtained, sensitivity was detected in some metals.

Conclusion: In this study, a mixture solution was prepared with carbon quantum dots and MOF (UiO-66), and solutions containing different heavy metal ions were added to the mixture at different concentrations and the metal ions effects were examined and sensitivity to some known toxic metals was determined.

Keywords: fluorescence spectroscopy, carbon quantum dots, metal organic frameworks, heavy metal ions.

1. INTRODUCTION

Carbon quantum dots (CQDs) are new generation nanosized materials. The importance given to carbon dots (CDs), which are a new member of carbon materials and due to their remarkable optical properties, has been increasing in recent years. They are used in numerous applications in different fields, thanks to their superior properties such as having high photoluminescence stability, being obtained by efficient and environmentally friendly synthesis methods, having high biocompatibility and low toxicity, being highly quantum efficient and being selective towards many target molecules (Dai et al., 2016; Sun and Lin, 2006; Zhou et. Al., 2007; Bourlinos et. al., 2008).

Methods of obtaining CQDs can be broadly divided into two groups as top-down and bottom-up strategies. In the top-down method, CQDs are formed by the fragmentation of large carbon structures and the surfaces are modified by passivation processes to obtain effective fluorescence properties. In the bottom-up approach, CQDs are obtained by linking appropriate molecular initiators into carbon chains, thus carbonization occurs (Zhu et.al.,2009; Pan et.al., 2011; Peng et.al.,2009). The work on sensors made with these materials attracts a lot of attention nowadays (Dai et al., 2016).

Metal-organic frameworks (MOFs), composed of combinations of metal cations or their clusters and organic ligands, are a new type of crystalline hybrid materials. Their surface areas are very large. Their dimensions can be changed by design. Their active sites are functional and serve as scavengers in many applications. They can be obtained in different designs and shapes by changing the metal parts. For this reason, it has unique advantages compared to known porous materials. They can be obtained by traditional techniques such as the solvothermal method. Thanks to these properties, it is possible to use them in applications such as gas storage and separation (Bao et. al., 2016; He et. al., 2012),

catalysis (Wu et. al, 2015; Liu et al. 2014; Lee et. al., 2009), chemical and physical sensing (Zhao et. al.,2020; Xie et. al., 2020; Zhang et. al., 2020; Lin et. al. 2016; Yang et. al., 2018), medical distribution (Ke et. al., 2011; Chowdhuri et.al., 2016) and ion exchange (Prasad et. al., 2010; Nouar et. al., 2019). Their luminescent types are also available in the literature (Zhao et. al.,2020; Xie et. al., 2020; Zhang et. al., 2020; Lin et. al. 2016; Yang et. al., 2018). Thanks to these features, they are widely used in sensor studies, such as CQDs. These materials show high sensitivity in sensor studies compared to many other luminescent materials, due to their properties such as being able to produce strong fluorescence (FL) emission, having a hard frame, high thermal stability. They have distinct advantages due to these features (Xie et. al, 2021). Examples of sensors specifically developed for metal ions with the obtained MOF and hybrid structures produced with carbon nanomaterials can be found in the literature (Xie et. al, 2021).

In this study, the aim was to develop fluorescence for metal ions by using carbon quantum dots and metal organic framework (MOF). Characterizations were made and the structures of the obtained materials (CQDs and MOFs) were confirmed. A mixture solution was prepared with carbon quantum dots and MOF (UiO-66-NH_2), and the solutions containing different heavy metal ions (Ag^{+}, Cd^{+2}, Cu^{+2}, Fe^{+2}, Mn^{+2}, Pb^{+2}, Zn^{+2}, Cr^{+3} ions) were added to the mixture at different concentrations and the metal ions effects were examined and sensitivity to some known toxic metals was determined.

2. MATERIAL AND METHOD

2.1. Chemicals

All chemicals and reagents were purchased from Sigma-Aldrich. Ultrapure water was used for preparing all solutions. Citric acid ($C_6H_8O_7$), ethylene diamine ($C_2H_8N_2$), zirconium chloride ($ZrCl_4$), 2-aminoterephthalic acid ($C_8H_7NO_4$), $AgNO_3$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Fe(NO_3)_2$, $Mn(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$, $Cr(NO_3)_3$ were purchased at the appropriate high purity.

2.2. Synthesis of CQDs and MOFs

Citric acid is weighed for synthesis. The solution is prepared, ethylene diamine is added and stirred at room temperature until equilibrium is reached. It is transferred into the autoclave reactor and kept in the oven at the appropriate temperature for a certain period. After the synthesis was completed, filtering and centrifugation processes were applied to the brown solution taken from the autoclave. After these processes were repeated several times, brown solid nanomaterials were obtained from the brown solution. Solutions at appropriate concentrations were prepared and stored for sensor studies.

Weigh appropriate amounts of zirconium chloride and 2-aminoterephthalic acid. After the solutions are prepared, they are combined and placed in an autoclave reactor. Autoclave is placed in an oven and kept at a certain temperature and time. A cloudy white solution was obtained in the autoclave. Thus, it was understood that mof structures were formed. The solid in the solution was washed with solvents such as ethyl alcohol, centrifuged and allowed to dry. A white solution was prepared from the resulting solid and stored for sensor operation.

2.3. Chracterizations

The spectrometric properties of all materials were measured on a LS 55 fluorescence spectrophotometer. The ultraviolet-visible (UV-Vis) absorption spectra of CQDS and MOFs were measured in a UV-1280 (Shimadzu) spectrophotometer. For X-ray Diffractometer (XRD) analysis of all materials, Bruker Advance D8 brand X-ray Diffractometer was used. Scanning Electron Microscope (SEM-EDX) analysis was performed with a **ZEISS EVO LS10 brand** instrument. Fourier transformed infrared (FT-IR) spectra were recorded by using Bruker Fourier Transform Infrared FT-IR (ATR) Bruker Advance D8 brand.

2.4. Sensor testing

Aqueous test solutions were prepared using salts such as $AgNO_3$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Fe(NO_3)_2$, $Mn(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$, $Cr(NO_3)_3$. First, concentrated stock solutions were prepared and these were diluted to prepare working solutions for Ag^{+}, Cd^{+2}, Cu^{+2}, Fe^{+2}, Mn^{+2}, Pb^{+2}, Zn^{+2}, Cr^{+3} ions. Studies were performed in 3 ml fluorescence cuvettes. CQDs solution was first added to the cuvette and measurements were taken by fluorescence spectrometer. Then, MOFs solution was added and

measurements were taken. Then, metal ions were added at certain concentrations and measurements were taken, and graphs of the results were drawn.

3. RESULTS

3.1. Chracterizations of MOFs and CQDs

CQDs showed two maximum peaks in the UV-Vis absorption spectrum at wavelengths of approximately 250 and 350 nm (Figure 1a). The peak at 350 nm is much stronger. This sharp peak indicates homogeneous particle size distribution and is much more distinct than the peak at 250 nm. Since an amine initiator was used in the preparation of CQDs, amine functional groups were observed on their surfaces. The absorption peaks located at approximately 350 nm and 250 nm are related to the n-π* transition of C=O and the π-π* transition of carbon, respectively**.** CQDs showed the prominent emission peak at around 440 nm (λex = 360 nm), (Lv et. al., 2017).

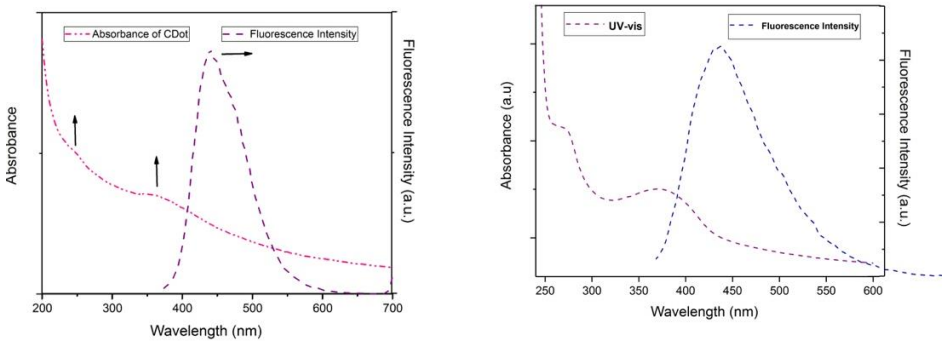


Figure 1. UV-Vis. absorbation and fluorescence peaks of **a)** Cdot **b)** MOF

UV-Vis. and fluorescence spectra of the synthesized MOF (UiO-66-NH2) structures are shown in Figure 1b. Two significant absorbation (abs.) peaks with strong distinctness were obtained at 375 nm and 275 nm. These peaks can be attributed to the n-π* electronic transition and are common in amine surface functionalized materials. The results obtained regarding UV-Vis. absorbation and fluorescence peak are compatible with the UV-vis abs. and fluorescence spectrum (λem. ≈ 430 nm) of pure MOF (UiO-66-NH_2) obtained in the literatüre (Mu et. al., 2018; Kashif et. al., 2022; Zhang et. al., 2022).

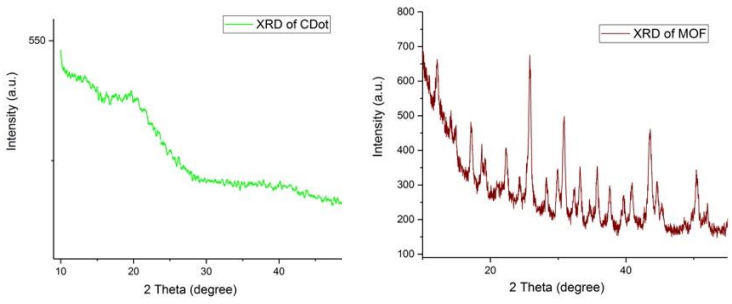


Figure 2. XRD of **a)** Cdot, **b)** MOF

XRD analysis was performed to perform structural characterization of MOFs and CQDs (Figure 2). a peak is seen at ~20.0° corresponding to the 002 lattice planes of a typical amorphous carbon. This broad peak confirms the wide-angle XRD patterns of CQDs (Zhu et. al., 2013). This broad diffraction peak at ~20° indicates that the interlayer spacing of CQDs is larger than that of graphite, which is again likely due to the presence of nitrogen-containing groups on the surfaces of CQDs (Figure 2a).

The characteristic peaks of MOFs at values of 8.1, 25.5, 43 θ^0 seen in the literature are observed in Figure 2b. These peaks confirm the structure and prove that MOFs crystallize well and that there are no impurities in the content of the structure (Aghajanzadeh et. al., 2018).

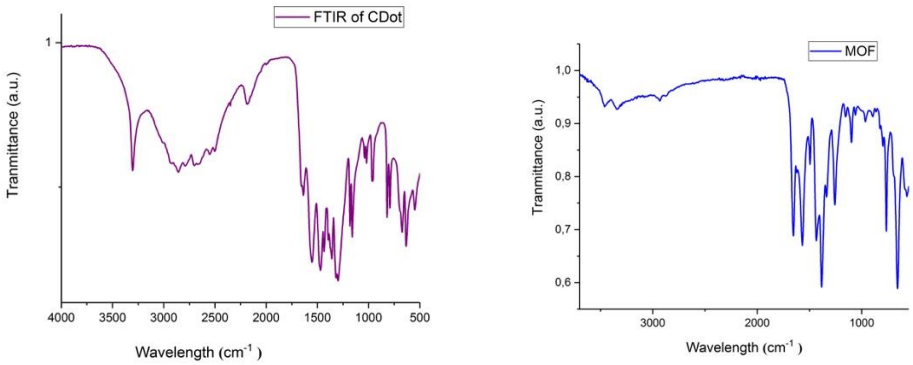
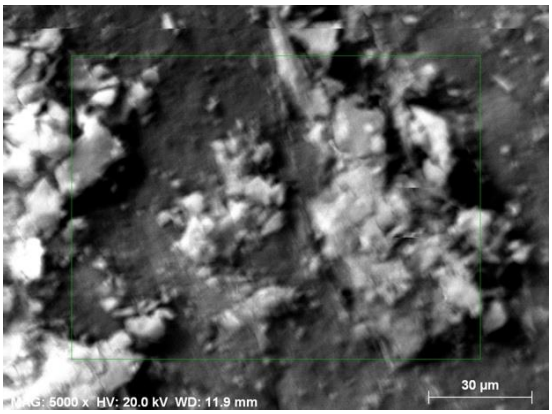


Figure 3. FT-IR of **a)** Cdot, **b)** MOF

Figure 3a shows the FT-IR spectrum of CQDs. The prominent IR band seen around 3400 cm^{-1} in the Figure 3a is attributed to the presence of the hydroxyl (R-OH) group in the structure of citric acid. As can be seen, the peak around 1725 cm^{-1} is assigned to carbonyl groups (C=O bond) on carbon quantum dots. The vibration band of CH_2 (methylene) and the stretching peaks of C–OH are also seen around 618 cm^{-1} and 1075 cm^{-1}, respectively. The distinct peaks and peaks around 1380 cm^{-1} and 1580 cm^{-1} indicate the symmetric and asymmetric vibration of the (-COO) group, respectively, and these peaks are consistent with the presence of the acidic group on the surface of the quantum dots of carbon. Because citric acid was used as a source in their synthesis. The peaks between 2972-3028 cm^{-1} values correspond to the C–H band (Lu et. al., 2019). Figure 3b shows the FT-IR spectrum of MOFs. The first thing that stands out here are the main peaks at 1655 cm^{-1}, 1569 cm^{-1}, 1373 cm^{-1}, 743 cm^{-1} and 660 cm^{-1}. The peaks at 1655 cm^{-1}, 1569 cm^{-1} and 1373 cm^{-1} belong to the aromatic structure and carboxyl groups in the structure of 2-aminoterephthalic acid, and these peaks can be attributed to the stretching bonds of carbonyl groups (-COO). The peak at 743 cm^{-1} can be attributed to the O-H bond, while the peak at 660 cm^{-1} can be attributed to the stretching of the Zr-O bond (Athari el. al., 2022; Tanvidkar et. al., 2023).



Spectrum: Map

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
Zirconium	L-series	41.21	52.04	14.73	1.6
Oxygen	K-series	21.57	27.24	43.95	2.8
Carbon	K-series	8.09	10.21	21.95	1.0
Nitrogen	K-series	8.32	10.51	19.37	1.4
Total:		79.19	100.00	100.00	

Figure 4. a)SEM and **b)** SEM-EDX of MOF.

When the SEM-EDX measurement results (Figure 4b) are examined, C, O, N and Zr elements are seen in the structure of MOFs in the analysis of the scanned surface image in Figure 4a. The higher percentage amounts of Zr and N elements indicate that these materials used in the synthesis stage and these elements have been successfully bonded to the compound structure.

3.2. Sensor experiments results

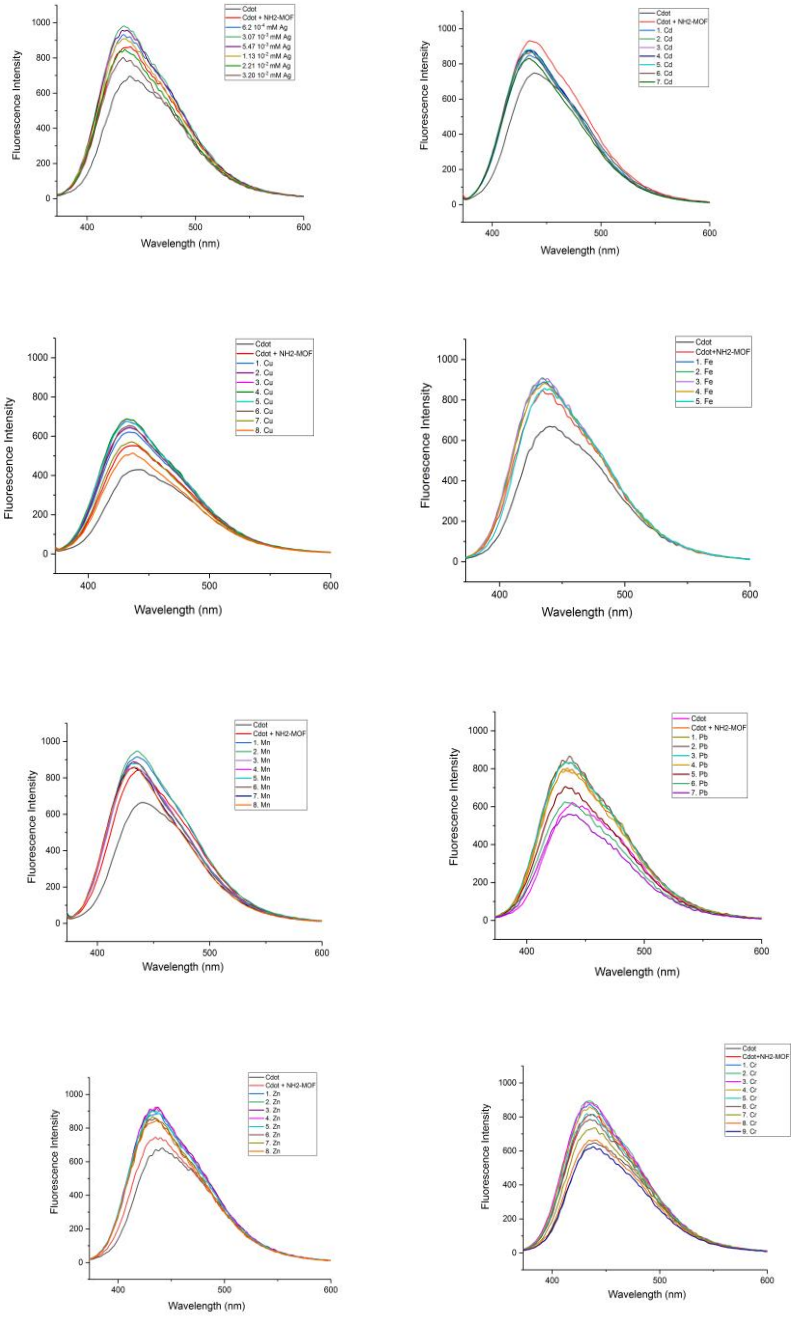


Figure 5. Sensor experiments results of Ag^{+}, Cd^{+2}, Cu^{+2}, Fe^{+2}, Mn^{+2}, Pb^{+2}, Zn^{+2}, Cr^{+3} ions with CQDs and MOFs.

The obtained sensor study results are given in Figure 5. In sensor operation, the general concentration ranges for metal ions are 0.62×10^{-3}, 1.24×10^{-3}, 1.90×10^{-3}, 3.07×10^{-3}, 5.47×10^{-3}, 1.01×10^{-2}, 1.79×10^{-2}, 3.3×10^{-2}, 8.63×10^{-2} mM. As can be seen from the figures (Figure 5), a change in the fluorescence intensities was detected for Ag^{+}, Cu^{+2}, Pb^{+2} and Cr^{+3} ions. However, when the changes are examined in detail, the changes are not very sensitive for Ag^{+}, Cu^{+2} and Pb^{+2} ions, but a specific decrease in intensity was observed for Cr^{+3} ions. The studies will continue in detail.

4. DISCUSSION

In this study, CQDs and MOFs structures were synthesized and characterized, and the obtained materials were mixed in solution and sensor experiments were carried out for different metal ions (Ag^{+}, Cd^{+2}, Cu^{+2}, Fe^{+2}, Mn^{+2}, Pb^{+2}, Zn^{+2}, Cr^{+3} ions). The experiments were carried out with fluorescence spectroscopy by adding solutions prepared with heavy metal ions onto emissive CQDs-MOFs mixture solutions. Metal organic framework structures were added to the environment to examine their effects and emission intensity changes were observed. According to the results obtained, sensitivity was detected in some metals such as Ag^{+}, Cu^{+2}, Pb^{+2} and Cr^{+3} ions.

5. CONCLUSION

Characterizations were made and the structures of the obtained materials (CQDs and MOFs) were confirmed. In this study, a mixture solution was prepared with carbon quantum dots and MOF (UiO-66-NH_2), and solutions containing different heavy metal ions (Ag^{+}, Cd^{+2}, Cu^{+2}, Fe^{+2}, Mn^{+2}, Pb^{+2}, Zn^{+2}, Cr^{+3} ions) were added to the mixture at different concentrations and the metal ions effects were examined and sensitivity to some known toxic metals was determined (Ag^{+}, Cu^{+2}, Pb^{+2} and Cr^{+3} ions). The studies will be retried in different concentration ranges and detailed studies will be carried out for sensitive metals. The studies will also be diversified with amino acids and biological molecules such as cysteine, glutamic acid, etc.

6. ACKNOWLEDGE

The author declares that they have no conflict of interest.

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INVESTİGATİON OF CHEMİCAL STRUCTURE AND CHARACTERİZATİON OF BİO-OİLS ISOLATED FROM WİLD MUSHROOMS USİNG DİFFERENT PROCESSES

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ABSTRACT

Background of the Study: Considering the results obtained in the extraction processes, the values of phenolic and volatile components summarized may give an idea. It is seen that the obtained components show statistically significant changes with different processes applied. As a result, the method used in the study varies in its contribution to the yield of valuable compounds obtained from mushroom products.

Aims: This study aimed to obtain high-value-added compounds of wild mushroom products under different process conditions. While determining these compounds, it aimed to discuss these components in detail using comprehensive advanced technical devices.

Material and Method: Wild mushroom species collected from rural areas in Iğdır province were dried under room conditions. Four different mushrooms, *Amanita muscaria, Lactarius piperatus, Ramaria botrytis and Amanita pantharina* were used for the studies. The dried wild herbs were pulverized with the help of a grinder. Liquid samples from the mushroom products used in the studies were thoroughly tested in all aspects using Gas Chromatography Mass Spectrometry (GC-MS), Liquid Chromatography Mass Spectrometry (LC/MS-MS) and Fourier Transform Infrared Spectroscopy (FT-IR).

Results: Fatty acid and volatile oil contents of methanol-chloroform extract and pyrolysis liquid of 4 different mushroom species were determined by GC-MS/MS. Additionally, the amino acid content of the mushroom samples was determined by LC-MS/MS.

Conclusion: Biooil products obtained from mushroom products were used with two different approaches: aqueous extraction and pyrolysis processes. In general, it can be aimed to bring idle wild mushroom products into the economy by transforming them into value-added products with different process methods such as pyrolysis and aqueous extraction methods

Keywords: Wild mushrooms, phenolic compounds, characterization

1. INTRODUCTION

The world population will be approximately 10 billion by 2050. Everyone should be aware of this for the agriculture and food security of the growing world population. For this reason, the most fundamental problem that humanity will face is to provide sustainable nutrition. Climate change along with the world population reveals the seriousness of this situation (Bahar et al., 2020). Foods containing nutritionally valuable vitamins, mushrooms, mineral products, dietary fiber and protein should be evaluated. Such nutritional products play an active role in ensuring sustainability of food and nutritional safety. It is thought that mushroom products, in particular, will play an active role in overcoming such problems, as they are rich in minerals and antioxidants. The terrible effects of recent environmental disasters due to climate change have emerged. There is serious concern in international circles in this regard. To overcome such situations, collecting and processing wild mushrooms is very valuable. It can provide valorization of locally accessible natural resources for food (Khan et al., 2023). Wild mushrooms are used to treat various human ailments thanks to the valuable compounds they contain. It is generally preferred as an immune booster against various diseases, especially due to its high content of protein and secondary compounds (Bhambri et al., 2022). The scientific world agrees that it is valuable to produce and market wild mushrooms that have extremely positive effects (Anusiya et al., 2021).

The use of wild mushrooms in Turkey is increasing, but it is not at the desired level. In this study, four different wild mushroom products, namely Amanita muscaria, Lactarius piperatus, Ramaria botrytis, and Amanita pantharina, were collected in and around Iğdır province. Different processes were used to comprehensively analyze the nutritional values and chemical properties of these mushroom products. In addition, the contents of these manta products were illuminated by using Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography Mass Spectrometry (LC/MS-MS), and Fourier Transform Infrared Spectroscopy (FT-IR) devices.

2. MATERIAL AND METHOD

Wild mushroom species collected from rural areas in Iğdır province were dried under room conditions. Four different mushrooms, *Amanita muscaria, Lactarius piperatus, Ramaria botrytis and Amanita pantharina* were used for the studies. The dried wild herbs were pulverized with the help of a grinder. The methanol, acetone, chloroform, ether and hexane chemical reagents used in this study were of analytical purity. All chemical materials used were obtained from Sigma Aldrich without any processing. Liquid samples from the mushroom products used in the studies were thoroughly tested in all aspects using Gas Chromatography Mass Spectrometry (GC-MS), Liquid Chromatography Mass Spectrometry (LC/MS-MS) and Fourier Transform Infrared Spectroscopy (FT-IR).

2.1. Sample preparation using the extraction process

Pre-dried mushroom samples were ground to small grain size. Then, the prepared solid samples were added to the glass jar medium containing the methanol-chloroform (1:1 v/w) solvent mixture. It was left in the dark for four days and the solvents were removed using an evaporator device. Finally, the obtained samples were dissolved in 1 ml methanol and treated with 1 ml hexane (Başar et al., 2024). At the end of phase separation, the samples were analyzed on GC-MS and LC/MS-MS devices. Additionally, an FT-IR device was used to understand the functional structure of the sample.

2.2. Sample preparation using the pyrolysis process

Fungal samples brought to small grain sizes were prepared as liquid samples using a fixed bed pyrolysis (reactor) device in an inert nitrogen environment. In this study, 10 g of solid mushroom samples were taken into the feed pool of the reactor. It was then exposed to 350 ◦C for approximately 1 hour in an inert environment of Nitrogen gas. The samples obtained were then placed in airtight bottles. The resulting liquid samples were added at a ratio of 1/1 ml (methanol and hexane). After phase separation, the hexane phase was analyzed in GC-MS, and the sample separated in the methanol phase was analyzed in LC/MS-MS. Finally, the FT-IR device was used for the detailed functional structure of the samples.

3. RESULTS

Fatty acid and volatile oil contents of methanol-chloroform extract and pyrolysis liquid of 4 different mushroom species were determined by GC-MS/MS. Additionally, the amino acid content of the mushroom samples was determined by LC-MS/MS.

Table 1: Content analysis result of crude extract by GC-MS/MS

No	Compound	RT	*A.muscaria*	*A. pantharina*	*L. piperatus*	*R. botrytis*
1	Methyl 13-methyltetradecanoate	43.89	-	-	-	-
2	Tetradecanoic acid 12-methyl-methyl ester	44.11	-	-	-	-
3	Pentadecanoic acid. methyl ester	44.89	0.63	-	0.34	1.60
4	11-Hexadecenoic acid methyl ester	46.93	-	-	-	-
5	Palmitic acid. methyl ester	47.45	25.55	16.38	29.85	29.24
6	cis-10-Heptadecenoic acid methy ester	49.52	-	-	-	-

7	Heptadecanoic acid. methyl ester	49.87	0.14	-	0.12	1.51
8	Linoleic acid. methyl ester	51.46	14.71	12.78	26.03	36.50
9	Oleic acid. methyl ester	51.61	33.94	33.00	5.39	16.35
10	9-Octadecenoic acid. methyl este	51.72	-	-	0.17	0.54
11	Stearic acid. methyl ester	52.17	23.83	36.70	6.40	13.76
12	???	52.76	-	-	29.92	-
13	11-Eicosenoic acid. methyl ester	57.03	0.19	-	0.62	-
14	Eicosanoic acid. methyl ester	57.95	1.08	1.14	0.13	-
15	Behenic acid. methyl ester	66.22	0.44	-	0.09	0.50
16	15-Tetracosenoic acid. methyl ester	71.58	-	-	0.93	-

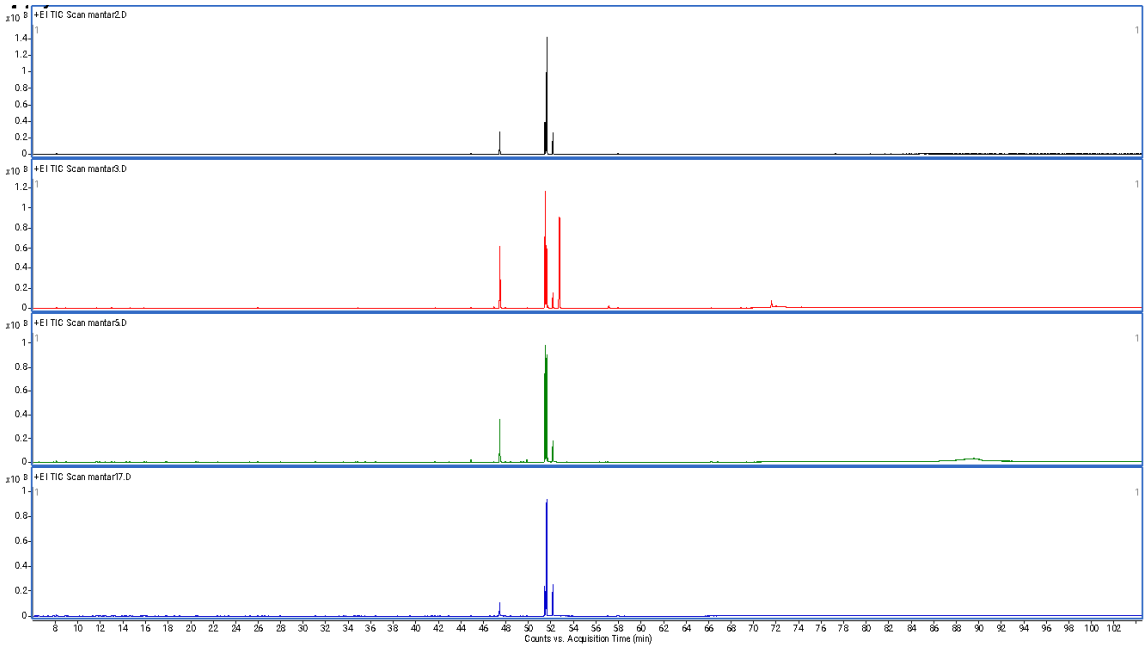

Figure 1: GC-MS/MS chromatogram of crude extract

Table 2: Content analysis result of pyrolysis liquid by GC-MS/MS

No	Compound Name	R.T.	A muscaria	A. pantharina	L. piperatus	R. botrytis
1	1-Heptadecene	40.75	0.16	-	0.59	-
2	8-Heptadecene	40.92	-	-	0.54	-
3	????	41.41	-	-	0.76	0.56
4	Pentadecanoic acid, methyl ester	44.89	-	-	-	0.56
5	2-Hexadecyloxirane	46.78	0.33	-	0.86	0.95

6	Palmitic acid, methyl ester	47.45	3.05	4.04	4.25	14.62
7	Palmitic acid	48.35	-	11.06	-	
8	????	49.22	-	-	-	0.49
9	Methyl isoheptadecanoate	49.87	-	-	-	0.53
10	Oleanitrile	51.14	1.76	-	5.35	-
11	Oleanitrile-isomer	51.24	1.00	-	2.78	-
12	Linoleic acid, methyl ester	51.44	2.14	0.54	10.00	26.87
13	Oleic acid, methyl ester-cis	51.58	14.16	-	28.71	39.52
14	Oleic acid, methyl ester-trans	51.72	2.46	-	6.96	8.72
15	Stearic acid, methyl ester	52.16	2.58	-	7.22	6.74
16	Stearic acid, methyl ester	52.15	-	9.92	-	-
17	Linoleic acid	52.42	-	8.49	-	-
18	Oleic acid	52.57	-	22.54	-	-
19	Oleic Acid-isomer	52.64	-	6.48	-	-
20	trans-13-Octadecenoic acid	53.03	-	1.33	-	-
21	4-Methyldocosane	53.92	-	-	-	0.45
22	Arachidic acid methyl ester	57.93	-	-	-	-
23	???	62.97	-	5.04	-	-
24	???	63.53	-	8.50	-	-
25	???	69.03	-	2.13	-	-
26	???	70.03	-	4.38	-	-
27	???	73.42	-	11.54	-	-
28	9-Hexadecenoic acid, 9-octadecenyl ester	86.63	72.36	-	31.97	-

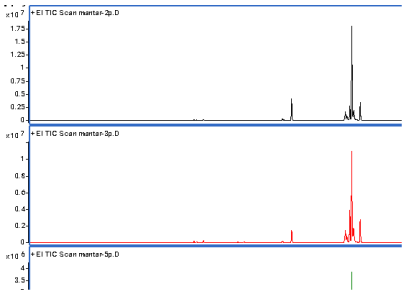

Figure 2. GC-MS/MS chromatogram of pyrolysis liquid

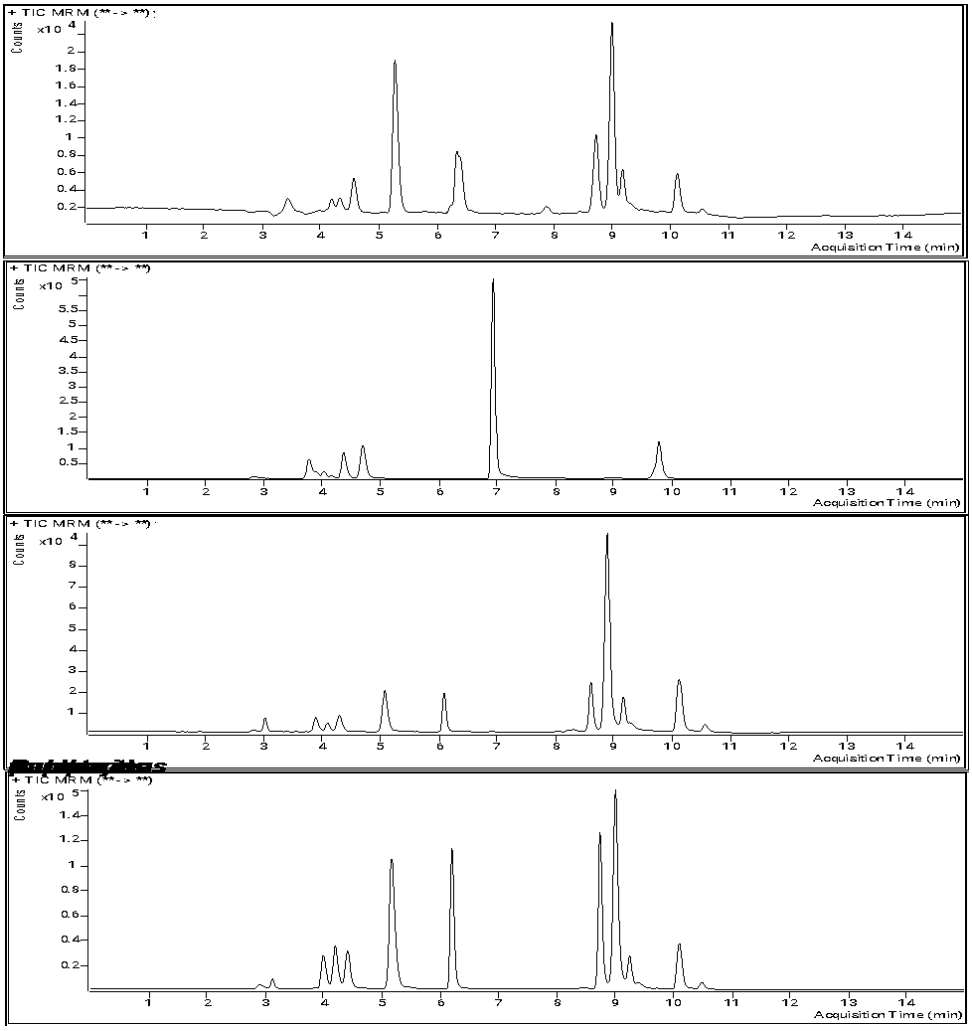

Figure 3. Amino acid content analysis of samples LC-MS/MS chromatogram

Table 3: Amino acid content analysis of samples

No	Compound Name	R. T.	A muscaria	A. pantharina	L. piperatus	R. botrytis
1	L-Lysine	3.208317	-	190.992	45.766	168.398
2	L-Histidine	3.381267	-	-	14.209	41.463
3	L-Arginine	3.496583	49.550	66.964	101.148	115.757
4	L-Cystine	3.5533	-	-	-	-
5	Glycine	3.536233	-	256.982	14.624	198.048
6	L-Serine	4.09985	10.359	944.080	123.873	610.469
7	L-Aspartic acid	4.055583	21.768	1003.700	390.893	773.481
8	L-Alanin	4.19995	39.997	1409.416	128.837	501.785
9	L-Threonine	4.347533	49.120	754.809	108.496	772.262
10	L-Glutamic acid	4.6026	243.192	1231.552	328.897	1567.484
11	L-Proline	4.0828	1.367	625.618	107.877	1.415
12	L-Valine	5.327633	28.461	697.956	81.148	29.724
13	L-Tyrosine	8.06225	-	521.756	85.909	-
14	L-Isoleucine	8.796517	6.743	-	19.657	120.096
15	L-Leucine	8.788667	7.621	-	22.198	133.538
16	LPhenylalanine	8.461667	-	-	-	-

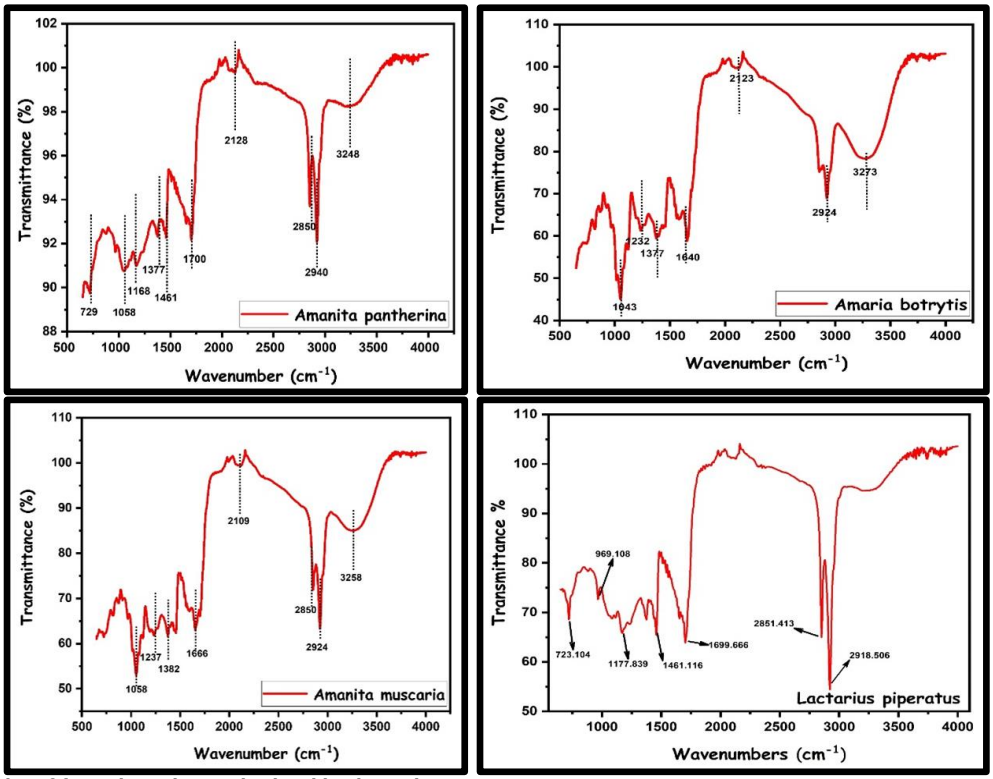

Figure 4. Peaks of fungal products obtained by (FT-IR)

4. DISCUSSION

According to the results of analyzes made with GC-MS/MS device, A.muscaria; Oleic acid methyl ester (33.94%), Palmitic acid methyl ester (25.55%), Stearic acid methyl ester (23.83%), A. pantharina; Stearic acid methyl ester (36.70%), Oleic acid methyl ester (33.00%), Palmitic acid methyl ester (16.38%), Linoleic acid methyl ester (12.78%), L. piperatus; ??? (29.92), Palmitic acid methyl ester (29.85%), Linoleic acid methyl ester (26.03%), Stearic acid methyl ester (6.40%) and R. botrytis; Linoleic acid methyl ester (36.50%), Palmitic acid methyl ester (29.24%), Oleic acid methyl ester (16.35%), Stearic acid methyl ester (13.76%) were detected in the highest amounts (Table one). Additionally, a total of 28 compounds were identified based on GC-MS/MS analysis of pyrolysis liquid of 4 different fungal species (Figure 2). Accordingly, A.muscaria; 9-Hexadecenoic acid 9-octadecenyl ester (72.36%), palmitic acid methyl ester (3.05%), oleic acid methyl ester-cis (14.16%), stearic acid methyl ester (2.58%), oleic acid methyl ester-trans (2.46%), A. pantharina; oleic acid (22.54%), palmitic acid (11.06%), stearic acid methyl ester (9.92%), ??? (8.50), linoleic acid (8.49%), L. piperatus; Oleic acid methyl ester-cis (28.71%), linoleic acid methyl ester (10.00%), stearic acid methyl ester (7.22%), oleic acid methyl ester-trans (6.96%) and R. botrytis; Oleic acid methyl ester-cis (39.52%), linoleic acid methyl ester (26.87%), palmitic acid methyl ester (14.62%), oleic acid methyl ester-trans (8.72%) compounds have the highest was detected in large amounts (Table 2). In the content analysis by GC/MS, the fatty acid composition percentages of the hexane-chloroform (1:3 v/v) extract were determined as 21.4% linoleic acid, 48.9% oleic acid, and 8.8% palmitic acid, respectively (Türkekul, 2017). However, linoleic acid (0.36.62%), palmitic acid (21.16%), suillus granulatus linoleic acid (48.75%), oleic acid (36.47%), leucopakillus gentianeus linoleic acid (40%) .92), oleic acid (39.78%), palmitic (36.38%), oleic (26.05%) and linoleic (17.68%) were determined to be significant fatty acids. Amino acid content of pyrolysis liquid and dry plant samples of 4 different mushroom species was analyzed by LC-MS/MS according to 16 standard amino acids. 10, 11, 14, 13 amino acids were detected in *A. muscaria, A. pantharina, L. piperatus, R. botrytis* species, respectively (Figure 3). According to the analysis results, *A. muscaria*; L-glutamic acid (243.192 mg/100 g), L-arginine (49.550 mg/100 g), L-threonine (49.120 mg/100 g), L-alanine (39.997 mg/100 g), *A. pantharina*; L-alanine (1409.416 mg/100 g), L-glutamic acid (1231.552 mg/100 g), L-aspartic acid (1003.700 mg/100 g), L-serine (944.080 mg/100 g), *L. piperatus*; L-aspartic acid (390.893 mg/100 g), L-glutamic acid (328.897 mg/100 g), L-alanine (128.837 mg/100 g), L-serine (123.873 mg/100 g), *R. botrytis* ; L-glutamic acid (1567.484 mg/100 g), L-aspartic acid (773.481 mg/100 g), L-threonine (772.262 mg/100 g), L-serine (610.469 mg/100 g) were determined in the highest amount (Table 3). Accordingly, the main components of the 4 different mushrooms in our study were L-Glutamic acid, L-serine and L-alanine. However, amino acids could not be determined in the pyrolysis product. When heat is applied, the protein structure opens up and the amino acid residues are more exposed to the polar environment. Reactive oxygen species (ROS) attack the peptide backbone and the amino acid side chains by dehydration, cleavage and binding, which changes the conformational structure of the protein (Wen et al. 2023). It is assumed that the structure of the amino acids changes due to the heat treatment during pyrolysis and cannot be determined by analysis. The structures of mushroom products are very similar to each other, there are some differences only in a few position groups. Therefore, it is possible to observe characteristic vibrations of the amine group in the entire spectrum (Figure 4). In general, sharp bands at 3273, 3258, and 3248 cm−1 were easily visualized in its spectrum. This may correspond to N-H stretching. We can also say that the intense sharp bands observed between 2950 and 2750 cm−1 of the fungal products are a combination of stretching vibrations with C–H functions. Very intense and sharp bands in the 1670–700 cm−1 region are spectrum peaks related to C–H bending vibration. In particular, the bands around 1666–1635 can be associated with their vibrations. Additionally, the bands between 1280 and 1060 cm−1 correspond to peaks with in-phase carbon stretching vibrations (Larkin,2017).

5. CONCLUSION

Biooil products obtained from mushroom products were used with two different approaches: aqueous extraction and pyrolysis processes. The obtained liquid products were reported in detail by GC-MS, LC/MS-MS, and FT-IR spectroscopy. The data obtained as a result of the analysis showed that the essential oil compound values of biooils produced by the pyrolysis method were obtained at much higher rates than those of biooil products produced by the extraction method. We can probably attribute this to the deterioration of the bonds of the sample exposed to high temperatures in the pyrolysis method. However, the aqueous extract process method was found to be more suitable in terms of phenolic compound values. In addition, extremely good results were obtained in the amino acid values obtained from wild mushroom products. In general, it can be aimed to bring idle wild mushroom products into the

economy by transforming them into value-added products with different process methods such as pyrolysis and aqueous extraction methods

6. ACKNOWLEDGE

It was carried out at the Iğdır University Research Application Center and Research Laboratory (ALUM), which provides various environmental conditions for experimental studies. We thank the Turkish Academy of Sciences (TÜBA) for financing the conduct of this study.

Conflict of Interest

The authors declare that they have no conflict of interest

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EXAMINATION OF MICRONUTRITION CARRIERS FROM A NUTRIGENOMIC PERSPECTIVE

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ABSTRACT

Background of the Study: Today, individual medicine and nutrigenomics approaches have become an important field of endeavor to understand the effects of individual genetic differences on health and disease. Considering that some of the micronutrients are molecules that can also be effective in the metabolism and activity of natural and synthetic drugs and materials, individual medical approaches and nutrigenomic approaches seem to be important here as well.

Aim: In this study, we aimed to examine the distribution and characteristics of pathogenic variants in genes involved in micronutrient transport in 1030 clinical exome sequencing samples performed in our center.

Material and Method: The study included 1030 individuals who underwent Clinical Exome Sequencing (CES) analysis at Selcuk University Faculty of Medicine, Department of Medical Genetics, between January 1, 2021 and April 30, 2024. In the study, DNA samples extracted from peripheral venous blood of individuals were extracted using the Roche CES kit, sequenced with the DNBSEQ-G400™ sequencing device and analyzed using the Seq Platform. Genes involved in micronutrient transport were analyzed.

Results: At the end of the research, in the SLC19A2: gene: 2 heterozygous missen likely pathogenic (LP) variants, in the SLC26A4 gene: 8 LP heterozygous (1 frameshift, 1 3' UTR, 6 missense), 4 heterozygous pathogenic variants (2 frameshift, 2 stop gained), in the SLC39A4 gene: 2 heterozygous LP variants (1 splice donor, 1 missense variant) were detected. No pathogenic or likely pathogenic variants were other genes involved in nutrient transport.

Conclusion: Thus, likely pathogenic and pathogenic variants were detected in the genes examined in a total of 16 individuals (1.6%). This result shows that 1.6% of the analyzed individuals have pathological or likely pathological mutations in micronutritional-related genes and this should be taken into account in health practices.

Keywords: Nutrigenomics, individual medicine, sequencing

1. Introduction

Today, individualized medicine and nutrigenomic approaches have become important fields of endeavor to understand the effects of individual genetic differences on health and disease (Agrawal et al., 2024). While individualized medicine aims to customize treatment approaches taking into account an individual's genetic and molecular makeup, nutrigenomics aims to determine the effects of nutrients on health by examining their interaction with an individual's genetic makeup (Garcia-Canas et al., 2010; Gkouskou et al., 2021; Marcum, 2020; Reddy et al., 2018).

Genetic variations in nutrient carriers affect the absorption and transportation of nutrients to cells, thereby influencing micronutrient utilization in individuals, which in turn affects health and disease processes. For example, changes in the absorption of a micronutrient in an individual can increase or decrease susceptibility to diseases (Agrawal et al., 2024). Therefore, understanding genetic variations enables the development of personalized nutrition and treatment strategies. These variations can also affect the efficacy of natural and synthetic drugs and supplements. Considering that some of these micronutrients are molecules that can also affect the metabolism and activity of natural and synthetic drugs and supplements, it is evident that individualized medicine and nutrigenomic approaches are also important in this context (Irimie et al., 2019).

Individualized medicine and nutrigenomic approaches contribute to the development of personalized treatment and nutrition plans by examining the effects of genetic variations in nutrient carriers on health and disease (Jaureguiberry & Venturino, 2022). In this study, we aimed to examine the distribution and characteristics of pathogenic effective variants in genes involved in nutrient transport in 1030 clinical exome sequence samples conducted at our center.

2. Material and Method

The study included 1030 individuals who underwent Clinical Exome Sequencing (CES) analysis at the Department of Medical Genetics, Selçuk University Faculty of Medicine, between January 1, 2017, and December 31, 2023. DNA samples extracted from peripheral venous blood of individuals were extracted using the Roche CES kit, sequenced using the DNBSEQ-G400™ sequencing device, and analyzed using the Seq Platform. Genes associated with Vitamin C (Ascorbic Acid): SLC23A1, SLC23A2, Folate: SLC19A1, SLC46A1, Riboflavin: SLC52A1, SLC52A2, SLC52A3, Thiamine metabolism: SLC19A2, SLC19A, Iodine (I−): SLC5A5, SLC26A4, Iron metabolism: SLC11A1, SLC11A2, SLC40A1, Zinc metabolism: SLC30A1, SLC30A2, SLC30A3, SLC30A8, SLC30A10, SLC39A2, SLC39A3, SLC39A4 genes were examined.

3. Results

At the end of the research, in the SLC19A2 gene, 2 heterozygous missense likely pathogenic (LP) variants were identified, in the SLC26A4 gene: 8 LP heterozygous (1 frameshift, 1 3' UTR, 6 missense), 4 heterozygous pathogenic variants (2 frameshift, 2 stop gained), and in the SLC39A4 gene, 2 heterozygous LP variants (1 splice donor, 1 missense variant) were detected. No pathogenic or likely pathogenic variants were found in the other analyzed genes. Thus, a total of 16 individuals (1.6%) were found to have likely pathogenic and pathogenic variants in the genes examined. This result indicates that pathological or likely pathological mutations in micronutrient-related genes were found in 1.6% of the analyzed individuals and should be taken into account in health practices (figure 1 and figure 2).

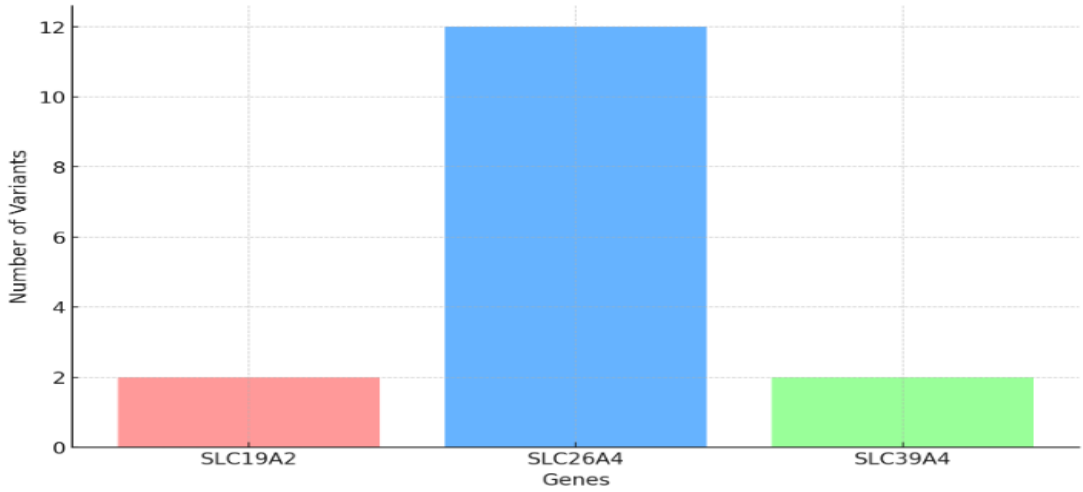

***Figure 1**. Number of Variants Identified in Each Gene. This bar chart displays the number of variants identified in the genes SLC19A2, SLC26A4, and SLC39A4.

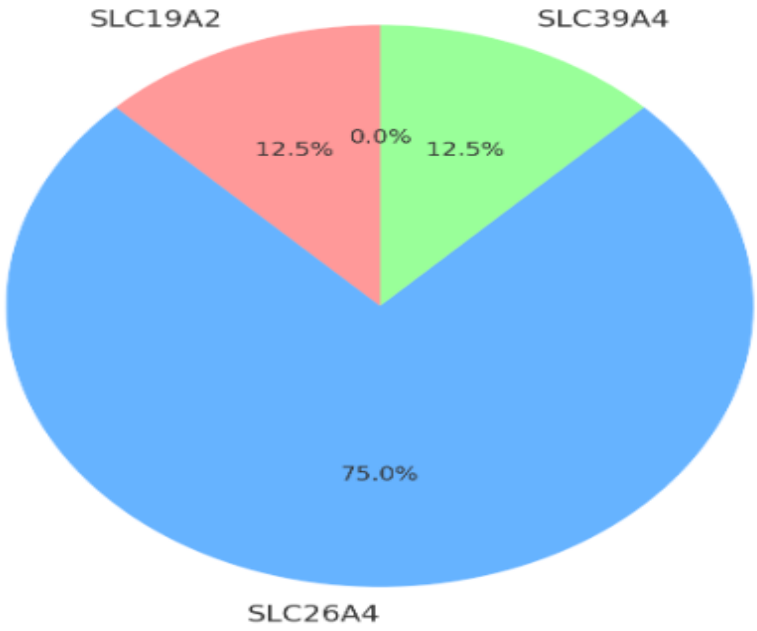

****Figure 2.** Distribution of Identified Variants. This piechart illustrates the distribution of variants identified in the genes SLC19A2, SLC26A4, SLC39A4, and other genes.

4. Discussion

We identified 2 LP heterozygous missense variants (missense, htz) in the SLC19A2 gene. The SLC19A2 gene plays an important role in thiamine metabolism, and deficiency in this gene can lead to megaloblastic anemia responsive to thiamine, which can progress to conditions such as deafness, diabetes, and optic atrophy (Diaz et al., 1999; Liberman et al., 2006; Brown, 2014).

In the SLC26A4 gene, we detected 8 LP heterozygous variants (1 frameshift, 1 3’ UTR, 6 missense), and 4 heterozygous pathogenic variants (2 frameshift, 2 stop gained). The SLC26A4 gene encodes a protein called pendrin, which plays a role in the passage of negatively charged ions (chlorine, iodine, bicarbonate) across cell membranes, especially in the inner ear and thyroid gland. Variants in this gene can affect these tissues and lead to conditions such as congenital hypothyroidism, Nonsyndromic hearing loss, Pendred syndrome, Age-related hearing loss, Hashimoto's disease (Twyffels et al., 2011; Bin et al., 2017).

We also identified heterozygous LP variants (1 splice donor, 1 missense variant) in the SLC39A4 gene in 2 individuals. The SLC39A4 gene codes for one of the transport proteins involved in zinc transport. Zinc is an essential trace element required for the normal functioning of over 100 enzymes and plays an important role in nucleic acid metabolism. Therefore, deficiencies in zinc and its transport can have multiple effects on the organism. One of the common effects of zinc deficiency is Acrodermatitis enteropathica, characterized by dermatitis, alopecia, and diarrhea, particularly in the perioral and acral areas, requiring lifelong zinc supplementation (Bin et al., 2017; Afman & Müller, 2006; Mathers, 2017).

The data obtained from the study indicate that LP/P variants in nutrient carrier genes were found in 1.6% of individuals. Considering that some of the numerous variants with uncertain significance may be included in this group with future variant annotations, this rate is likely to increase significantly. Given the nature of these genes, which affect many enzymes and pathways in the organism, there is a need for more cautious consumption of these supplements, which are part of our daily diet (Ferguson, 2009; Kiani et al., 2022; Sales et al., 2014). This underscores the need for the wider dissemination and enhancement of nutrigenomic approaches as part of personalized medicine (Kiani et al., 2022; Sales et al., 2014). Understanding how genomic variations affect micronutrient metabolism will enable the development of personalized nutrition and treatment approaches for individuals. The widespread adoption and improvement of such approaches can offer more effective and targeted solutions for monitoring, preventing, and treating diseases (Sales et al., 2014).

5. Conclusion

This study contributes to understanding the potential effects of variations in nutrient carrier genes on health. As research in this area increases, more effective and up-to-date approaches can be developed.

Conflict of Interest Statement

There are no financial conflicts of interest related to our article with any institutions, organizations, or individuals, and there are no conflicts of interest among the authors.

Ethical Statement:

Before the initiation of the study all participants received an explanation of the procedure and the risks that would later be faced in their participation and they provided informed consent to participate in this study. During this study the world medical association -WMA- HELSINKI declaration were followed according to the ethics committee of Selcuk University Faculty of Medicine.

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MULTIDRUG RESISTANCE GENES IN NEXT GENERATION SEQUENCING APPLICATIONS

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ABSTRACT

Background of the Study: Multidrug resistant genes (MRPs) include a group of genes belonging to the C family, a group of genes that encode transporter proteins called ATP-binding cassettes (ABC). These genes constitute the largest protein family group in the human body. The MRP family consists of 13 members and is classified from MRP1 to MRP9. Since it has been observed in studies that members of this group play a role in the excretion of anticancer drugs from tumor cells, the products of these genes are thought to cause multidrug resistance (MDR).

Aim: In this study, we aimed to investigate the variants in the MDR genes that are said to be effective on bioavailability and side effects in the use of natural and synthetic drugs, and the molecular characteristics of these variants, in 1030 clinical exome sequencing samples performed in our center.

Material and Method: The study included 1030 individuals who underwent Clinical Exome Sequencing (CES) analysis at Selcuk University Faculty of Medicine, Department of Medical Genetics, between January 1, 2021 and April 30, 2024. In the study, DNA samples extracted from peripheral venous blood of individuals were extracted using the Roche CES kit, sequenced with the DNBSEQ-G400™ sequencing device and analyzed using the Seq Platform.

Results: At the end of the research, in the ABCC2 gene: 2 Likely pathogenic variants (1 splice donor, 1 frameshift), in the ABCC6 gene: 14 likely pathogenic (10 missense, 2 stop gained, 2 frameshift), 5 pathogenic variants (3 stop gained, 2 missense variants) were detected.

Conclusion: This result shows that 2.02% of the analyzed individuals had LP/P variants in the MDR genes. This seems to be the ratio that should be taken into consideration when using natural and synthetic drugs.

Keywords: Multidrug resistant genes, gene family, sequencing

1. Introduction

Multidrug resistance genes (MRPs) are part of the ATP-binding cassette (ABC) family, which includes transporter proteins. These genes represent the largest protein family in humans. The MRP family comprises 13 members, classified from MRP1 to MRP9 (Kruh & Belinsky, 2003). Studies have shown that members of this family play a role in exporting anticancer drugs from tumor cells, suggesting that the products of these genes contribute to multidrug resistance (MDR) (Suzuki, Nishio, & Tanabe, 2001). These proteins primarily transport lipophilic anionic compounds, glutathione (GSH), glucuronate, and sulfate conjugates. In addition to ionic compounds, MRP1, MRP2, and MRP3 can also transport neutral organic drugs in the presence of free GSH. Overall, MRPs can transport a wide range of drugs, including various biologically and physically diverse anticancer drugs, antimetabolites, tyrosine kinase inhibitors, and nucleoside analogs (Zhang et al., 2015).

MDR poses a significant challenge in treating cancer and other diseases, leading to severe side effects, including death. MDR can also cause undesirable effects in therapies using natural compounds and some supplements (Zhang et al., 2022). Genetic and epigenetic mechanisms, influenced by environmental factors, play a role in these effects. One such mechanism involves genetic variations that can lead to overexpression of these proteins. In such cases, many drugs are actively expelled from cells via ATP-mediated transport, reducing their therapeutic effects (Amawi et al., 2019).

The MRP subfamily, associated with MDR, comprises nine members and forms a subgroup of ABC transporters. These proteins are involved in the ATP-mediated excretion of endogenous substances and xenobiotics. Based on their structural and biological properties, MRPs are divided into two groups:

"short MRPs" (MRP4, MRP5, MRP8, and MRP9) with a typical ABC transporter structure, and "long MRPs" (MRP1, MRP2, MRP3, MRP6, and MRP7) with an additional MSD (MSD0) (Wang et al., 2021). Personalized medicine and individual treatment approaches are becoming increasingly important in using natural and synthetic drugs (Joyce et al., 2015). Identifying genetic variants affecting drug metabolism and bioavailability on a population basis has become crucial in these treatment approaches (Fletcher et al., 2016). This study aimed to investigate the variants in MDR genes and their molecular characteristics in our center's next-generation sequencing samples, focusing on their impact on bioavailability and side effects of natural and synthetic drugs.

2. Material and Method

The study included 1030 individuals who underwent Clinical Exome Sequencing (CES) analysis at the Department of Medical Genetics, Faculty of Medicine, Selçuk University, between January 1, 2017, and December 31, 2023. Before the initiation of the study all participants received an explanation of the procedure and the risks that would later be faced in their participation and they provided informed consent to participate in this study. During this study the world medical association -WMA- HELSINKI declaration were followed according to the ethics committee of Selcuk University Faculty of Medicine.In the research, DNA samples extracted from the peripheral venous blood of individuals were extracted using the Roche CES kit, sequenced with the DNBSEQ-G400™ sequencing device, and analyzed using the Seq Platform. Post-analysis, the genes encoding the MR1-9 proteins (ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, ABCC10, ABCC11, ABCC12) were examined.

3. Results

Analysis revealed two likely pathogenic variants (one splice donor, one frameshift) in the ABCC2 gene and 14 likely pathogenic (10 missense, 2 stop-gained, 2 frameshift) and five pathogenic variants (3 stop-gained, 2 missense) in the ABCC6 gene. Among the 1030 individuals analyzed, 21 (%2.02) had LP/P variants in MDR genes. This finding indicates a significant prevalence of LP/P variants in MDR genes among the analyzed population, suggesting the importance of considering these variants in natural and synthetic drug use (Table 1, Figure 1).

Table 1. Distribution of pathogenic and likely pathogenic variants in genes.

Genes	VUS	LP	P
ABCC1	2	0	0
ABCC2	36	2	0
ABCC3	7	0	0
ABCC4	0	0	0
ABCC5	0	0	0
ABCC6	4	15	5
ABCC10	0	0	0
ABCC11	0	0	0
ABCC12	0	0	0

Genes	VUS (%)	LP (%)	P (%)
ABCC1	0.123	0	0
ABCC2	2.2	0.123	0
ABCC3	0.432	0	0
ABCC4	0	0	0
ABCC5	0	0	0
ABCC6	0.247	0.679	0.185
ABCC10	0	0	0
ABCC11	0	0	0
ABCC12	0	0	0

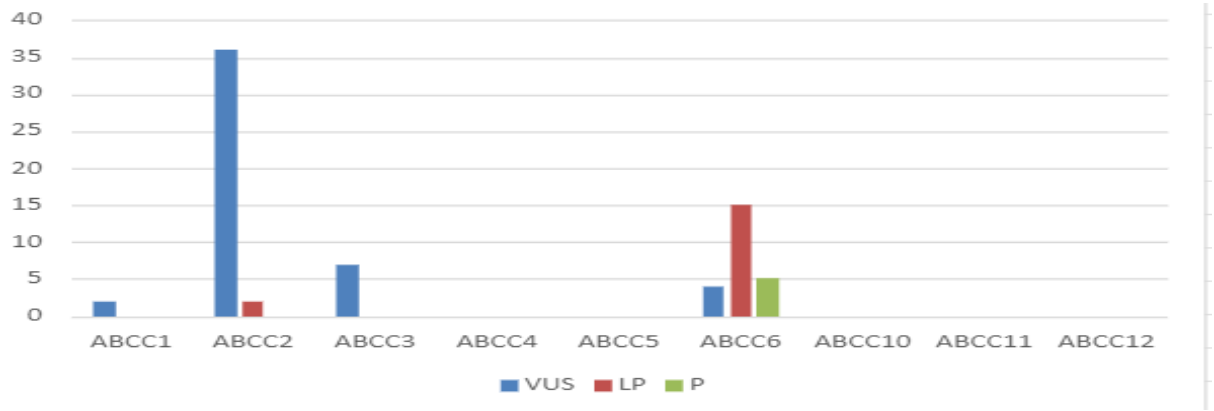

Figure 1. Distribution of variants detected in MRP genes.

4. Discussion

MRPs exhibit variable tissue distribution, cellular localization, and diverse pharmacological properties. Although MRP-related resistance to anticancer drugs is prominent in the literature, research has shown that MRPs also confer resistance to other synthetic and natural drugs. Some MRPs are noted for their resistance to specific drugs (Keppler, 2011). For instance, MRP1 and MRP2 confer resistance to hydrophobic natural anticancer drugs like vinca alkaloids and MTX (antifolate); MRP3 confers resistance to MTX and epipodophyllotoxins (Gottesman, Fojo, & Bates, 2002). MRP1-3 also play a role in transporting compounds conjugated with GSH, glucuronide, or sulfate. MRP4 and MRP5 confer resistance to nucleoside analogs (6-MP, 6-TG, and PMEA), while MRP7 has a broad resistance spectrum (Hollenstein, Dawson, & Locher, 2007; Tiwari et al., 2011). These data suggest significant individual differences in clinical effects of natural and synthetic drug use based on MRP profiles.

In our study, 21 out of 1030 individuals (2.02%) had LP/P variants in MDR genes. This finding suggests that individual differences in drug bioavailability among the population could be influenced by these genes' encoded proteins. Common side effects in current general treatment approaches may result from differences in MRP genes among individuals. The importance of individual medicine and personalized treatment approaches is increasingly recognized in the literature (Keppler, 2011). The significant prevalence of LP/P variants in our study population supports the presence of efficacy-modifying variants in a substantial portion of individuals. The effect-modifying role of these variants, whether enhancing or reducing drug efficacy, could lead to varying bioavailability outcomes.

Our study identified LP/P variants (10 missense, 5 stop-gained, 4 frameshift) in the ABCC6 gene in 19 individuals, accounting for 1.36% of the total population. This result indicates a notable prevalence of LP/P variants in the population. The ABCC6 gene encodes the multidrug resistance-associated protein 6 (MRP6, also known as ABCC6 protein). This gene is primarily expressed in the liver and kidneys but is also expressed in other tissues (stomach, skin, vascular endothelium, and eyes) at lower levels. Studies on substrate identification have shown that MRP6 functions as a lipophilic anionic pump, binding drugs like the cyclopentapeptide BQ123. In transfected Chinese hamster ovary (CHO) cell lines, MRP6 has been shown to act as a pump involved in drug efflux, contributing to cellular resistance to chemotherapeutic agents such as etoposide and teniposide (Jansen et al., 2014; Matsuzaki et al., 2005). Other studies have shown that MRP6 plays a role in resistance to anthracyclines and cisplatin. Considering the morbidity associated with these drugs, the pathways and pumps related to their metabolism and efficacy become crucial. Functional loss or gain due to LP/P variants in the MRP6 gene could lead to similar pathological effects on these drugs. Therefore, considering potential MRP variants in individual treatment approaches is essential in cases of drug inefficacy and side effects. Additionally, ABCC6 has been found to be involved in calcium and other mineral metabolism through ATP pyrophosphate conversion. This positions MRP6 as a potential candidate in conditions related to calcium and other mineral imbalances (Bergen et al., 2000; Jansen et al., 2014). Increased research and positive data on this subject may highlight MRP6's role in mineral-related conditions. Homozygous pathogenic variants in the MRP6 gene cause pseudoxanthoma elasticum (PXE), a progressive disorder characterized by the accumulation of calcium and other minerals in elastic fibers (Nitschke & Rutsch, 2012).

Additionally, we identified two likely pathogenic variants (one splice donor, one frameshift) in the ABCC2 gene in two individuals. The ABCC2 gene, also known as MRP2, is the second member of the MRP subfamily of the ABC transporter family. The MRP2 gene is primarily expressed in the apical (canalicular) plasma membrane of hepatocytes, kidney proximal tubules, and the small intestine, with lower expression in the gallbladder, peripheral nerves, and placenta. Due to its structural and sequence similarities with MRP1, MRP2 is believed to confer resistance to similar anticancer drugs. This hypothesis has been supported by various studies using antisense MRP2 RNA. In cell lines transfected with MRP2, the protein has been shown to confer resistance to chemotherapeutic drugs such as vinblastine, MTX, cisplatin, etoposide, doxorubicin, and epirubicin. Interestingly, MRP2 also confers resistance to cisplatin, which distinguishes it from MRP1 (Jedlitschky, Hoffmann, & Kroemer, 2006). These findings highlight the potential significance of MRP2 in drug bioavailability and side effects during chemotherapy. Therefore, functional disruptive mutations in the MRP2 gene should be considered in individual treatment approaches. Homozygous pathogenic variants in the ABCC2 gene cause Dubin-Johnson syndrome, a condition characterized by increased bilirubin levels leading to jaundice (Devgun et al., 2012; van der Schoor et al., 2015).

5. Conclusion

The above data suggest that variants in MDR genes can affect individuals' responses to critical disease treatments. Therefore, identifying these genetic variants can contribute to planning and selecting the right drugs in such critical treatments. This approach is particularly important in cancer treatment. In personalized medicine, MDR genes play a crucial role in creating individualized treatment plans. Genetic screening, especially next-generation sequencing, is an effective tool for identifying pathogenic variants in MDR genes. Screening approaches for genes related to drug metabolism and bioavailability will enable more effective and personalized treatment options based on individuals' genetic makeup.

Conflict of Interest Statement

There are no financial conflicts of interest related to our article with any institutions, organizations, or individuals, and there are no conflicts of interest among the authors.

Ethical Statement:

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OUR APPROACH TO PATIENT WITH OBSTETRIC ANAL SPHINCTER INJURY

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ABSTRACT

Background of the Study: Perineal and vaginal lacerations are common, affect 79% of vaginal deliveries and can cause bleeding, infection, chronic pain, sexual dysfunction and urinary and faecal incontinence. This case report describes the management of a patient with stage 3B obstetric perineal laceration.

Aim: In this case report, we aimed to present the management of a patient with an obstetric anal sphincter injury that was recognised at an early stage.

Material and Method: A 23-year-old female patient was included in the study. She was consulted by the gynaecology assistant who detected perineal injury immediately after vaginal delivery.

Results: A 23-year-old female patient was taken to the operating theatre after perineal injury was detected on physical examination. After spinal anaesthesia, the patient was prepared in lithotomy position. On examination, a full-thickness tear was detected in the external anal sphincter. No injury was detected in the internal anal sphincter. The external sphincter was repaired end to end with 3/0 vicryl suture. Oral intake was opened in the 2nd postoperative hour. On postoperative day 1, the dressing was removed and the patient was instructed to wash the wound with warm water for 5 minutes in the morning and evening and then put clean gauze. On postoperative day 2, the patient was discharged and told to continue the same daily application to the wound. At the follow-up visit 2 weeks after the operation, wound healing was uneventful, sphincter function was almost completely recovered and there was no incontinence.

Conclusion: After obstetric anal sphincter injuries, it is essential to recognise the injury and repair it in the earliest period in experienced hands. In our case, sphincter functions were almost completely restored after repair in a patient with stage 3b injury.

Keywords: Anal sphincter repair, obstetric anal sphincter injury, overlapping repair

1.INTRODUCTION

Obstetric anal sphincter injury (OASI) is a common cause of faecal incontinence (FI) in women and is a complication that causes patients to avoid vaginal delivery (1). Reports have shown that up to 50 per cent of women suffering from this type of injury may develop FI (2). Anal continence is controlled by a triple sphincter mechanism consisting of internal anal sphincter, external anal sphincter and puborectalis muscle. The external anal sphincter (EAS) is a striated muscle. It consists of subcutaneous, superficial and deep layers. It is responsible for voluntary defecation and contraction pressure. It is innervated by the nervus pudentalis. The internal anal sphincter (IAS) is a thickened continuation of the intestinal circular smooth muscle. This formation provides approximately 70 per cent of the resting pressure. It is innervated by the autonomic nervous system.

World Health Organisation classification of perineal tears:

First degree: Minor injuries to the vaginal mucosa, cuff or perineal skin
Second degree: Involves the perineal muscles and aponeurosis
Third degree: Anal sphincter complex (EAS and IAS)
3a: Tears involving less than 50% of the EAS
3b: Tears involving more than 50% of the EAS.
3c: Both EAS and IAS injuries
Fourth degree: Anal sphincter complex and anal epithelial injury.

Obstetric anal sphincter injuries include 3rd and 4th degree tears. Approximately 40% of patients with 3rd and 4th degree perineal tears develop anal incontinence. This negatively affects a woman's quality of life. The most critical points are early detection of the injury and correct repair in experienced hands.

2.CASE REPORT

A 23-year-old woman was consulted by a gynaecology assistant who detected a perineal injury immediately after vaginal delivery. Inspection and palpation showed that approximately 80% of the external anal sphincter was cut. The internal anal sphincter appeared intact, which was confirmed by palpation. The patient was diagnosed as a stage 3b injury and referred to the operating theatre. After spinal anaesthesia, the patient was prepared in lithotomy position. Following skin antisepsis, the operation was started. The two cut ends of the external anal sphincter were released and approximated end to end with the help of forceps. The EAS was then repaired end to end with 3/0 vicryl sutures. The skin was also closed with 3/0 vicryl sutures. At the 2nd postoperative hour, the patient's oral intake was opened routinely and breastfeeding was started. The patient was started to be mobilised at the 4th postoperative hour. At the 12th postoperative hour, the dressing was removed and the wound was checked and no pathological appearance was detected. As of the 12th hour, the patient was instructed to wash the perianal area with hot water (water jet) in the shower for 5 minutes in the morning and evening or after each defecation, and then to keep the wound dry with clean and dry gauze. At the 24th postoperative hour, the patient had no loss of sensation in the perianal region, but sphincter function was very low. The patient was taught Kegel Exercises to strengthen the pelvic floor muscles and Kegel exercises were started at the 24th postoperative hour. (Kegel Exercises are described to the patients: We plan to determine these muscle groups by contracting the muscles that you use to prevent flatulence while sitting on the toilet and the muscles that will stop you from urinating suddenly while you are urinating. Afterwards, it is possible to focus on these detected muscles and make them do exercises. The aim is to repeat 10 times a day in 3 sets). At the 30th postoperative hour, it was confirmed that there was no pathology in the wound. The patient stated that she felt better after the exercises but her continence was not sufficient. Digital rectal examination (DRE) also confirmed the patient. The patient was told to take care to wash the wound and keep it dry and to continue doing Kegel exercises and was discharged with Metronidazole 500 mg 3*1 (per oral) and Paracetamol 500 mg 2*1 (per oral).

3.RESULTS

At the 1st week follow-up, the patient described that she achieved continence to a great extent in her anamnesis. It was observed that there was not enough contraction in DRM. At the 3rd week follow-up visit, the patient stated that he achieved complete continence, and it was found that there was sufficient contraction in DRE.

4.DISCUSSION

After obstetric anal sphincter injuries, it is essential to recognise the injury and repair it at the earliest possible time in experienced hands. In our case, sphincter function was almost completely restored after repair in a patient with stage 3b injury. Early diagnosis of the injury, the first repair attempt has the highest chance for the best results. Pain and dyspareunia are also long-term problems that may be encountered in later periods (3). A total of 1056 women diagnosed with a 3a or 3b tear were included in the study and 120 (11.36%) were found to have a larger defect than the original diagnosis and were probably misclassified at birth. Some women with misclassified OASI have worse anorectal symptoms. This is probably due to incomplete repair (4).

5.CONCLUSIONS

We suggest that improved training in OASIs may help reduce the number of misclassified tears and improve repair.

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EXAMINATION OF POLYCAPROLACTON BASED POLYURETHANES FOR USE IN DENTAL APPLICATIONS OF COMPOSITE RESIN MATERIALS

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ABSTRACT

Background of the Study: This study investigates the effects of various acrylic-terminated UV-curable polyurethanes (APUs) on dental applications. Using different biodegradable poly(ε-caprolactone) diol and HDI, with HEMA, TEGDMA, and EGDMA, we examined thermal and chemical properties via DSC and FT-IR. In dental applications, composite resin is applied in 2 mm layers to ensure polymerization and minimize shrinkage. This method is time-consuming and prone to gaps and contamination. Bulk-fill composite resins, applied in 4-5 mm layers, address these issues by increasing translucency and polymerization depth. This study synthesizes flowable bulk-fill composite resins by combining PCL-based polyurethane with HDI, HEMA, TEGDMA, and EGDMA. Polyurethanes' flexibility, abrasion resistance, and biocompatibility make them ideal for dental use.

Aim: The aim is to synthesize new flowable bulk-fill composites using biodegradable acrylic-terminated APUs. Different polyurethane acrylates and diisocyanates will address the drawbacks of commercial composites.

Material and Method: Prepolymers were combined with HEMA, TEGDMA, and EGDMA to form viscous APUs. These were characterized using FT-IR and DSC, then mixed with a modified inorganic phase in a Speed Mixer to create a flowable bulk-fill composite. The composites were cured in Teflon molds using an LED light device and tested for water absorption, contact angle, mechanical, and antibacterial properties.

Results: Synthesis reactions were monitored using FTIR and DSC. FTIR confirmed successful reactions by the disappearance of the NCO peak at 2270 cm−1 and the appearance of acrylate peaks at 1645 cm−1 and 810 cm−1. DSC showed the influence of PCL molecular weight and diisocyanate type on thermal properties.

Conclusion: The synthesis of APUs for dental applications using PCL and HDI was successful. FTIR confirmed acrylate incorporation into the polyurethane backbone. These findings help optimize polyurethanes for dental use.

Keywords: Composite resin, HEMA, polycaprolactone (PCL), polyurethane

1. INTRODUCTION

Composite resins are widely used in clinical practice due to their easy processing, medium cost compared to ceramics, sufficient aesthetic properties, low thermal expansion coefficients, mechanical properties and ability to bond with the tooth structure. Since their introduction, monomer chemistry, filler technology and structure have been continuously improved to improve their physicochemical properties (Zhang & Xie 2020).

Applying the composite resin in 2 mm layers allows complete polymerization of the material, increasing light penetration and reducing polymerization shrinkage. However, this technique has disadvantages such as being time consuming, risk of creating gaps between layers, requiring technical precision and risk of contamination. In order to eliminate these disadvantages, facilitate the application of composite resin in large cavities, and reduce the time spent at the bedside, manufacturers have produced materials

known as bulk-fill composite resin, which can be applied in a single layer of 4-5 mm (El-Damanhoury & Platt 2014).

In order to obtain a more translucent structure in bulk-fill composites, the filler content and pigment amount were reduced and the particle amount was increased. These composites, which have new monomer and initiator systems with increased translucency, have greater polymerization depth. (Eakle & Bastin 2019).

This product, It enables the investigation of existing dental filling applications of various acrylic-terminated UV-curable polyurethanes (APUs) that are biocompatible with aliphatic ester linkage and are susceptible to hydrolysis (biodegradation) using two different PCLs.

Polyurethanes based on polycaprolactone are an important class of polymers in commodity applications such as biodegradable packaging, but also as a plastic have been widely used in many applications such as medical devices, controlled drug release systems, adhesives, coatings and in agricultural applications (Moussaif et al., 2010). However, the formation of PCL films is hampered by its low melting temperature, poor barrier properties to water and gases, low mechanical strength and thermal stability (Wang et al., 2014). Low additions of nanostructured materials to polymers often yield substantial improvements in specific properties of the polymer thus there has been considerable interest in the development of PCL nanocomposites. However, a considerable amount of research has shown that the incorporation of nanosilica into waterborne polyurethane frequently leads to nanosilica aggregation and poor mechanical properties.

Polyurethanes are synthetic materials with a wide scope of physical and chemical properties. Due to the wide variety of commercially available monomeric materials that can be used for the synthesis of polyurethanes, and their appropriate combinations, it is possible to obtain the final polymers with desired properties. Thanks to the ability to adapt to the diverse needs of modern technology, polyurethanes can be used as coatings, adhesives, fibers, foams and thermoplastic elastomers. As well, waterborne polyurethanes have been attracting increasing attention in recent years due to their non-toxicity, non-flammability and environmentally friendly nature (Jeon et al., 2007).

Crosslinking is one of the most available ways for obtaining polyurethanes with improved mechanical and thermal properties compared to uncrosslinked polyurethanes.Commonly used compounds with acrylic functionality can promote the end capping of polyurethane molecules, thereby introducing double bonds at both ends of the polyurethane chain.Thus the resulting acrylic terminated polyurethane (APU) has the ability to be crosslinked easily through thermal or photopolymerization in the presence of an appropriate initiator (Valcic et al., 2021).

Another reason for using acrylic monomers in the synthesis of polyurethane prepolymers is to decrease their viscosity and to improve the features of the final crosslinked polymer.The usage of acrylic monomers such as hydroxyethylacrylate (HEA) or hydroxyethyl methyl acrylate (HEMA) and their incorporation into the structure of a polyurethane, can ensure that the inherent properties of these materials contribute to the final polyurethane characteristics,keeping at the same time the biocompatibility of the initial prepolymer (Alishiri et al., 2014).

In this study, a new elastic polyurethane (PU) adhesive was synthesized to increase the stability and durability of the dental adhesion interface. They synthesized a polyurethane oligomer by solution polymerization method and used a diluent and solvent to prepare PU adhesives. Water absorption, water solubility, contact angle, thermal stability, degree of conversion and mechanical properties of PU adhesives were evaluated. In their study, Zhang and colleagues noticed that the water absorption and solubility of PU adhesives were significantly lower than three commercial adhesives, and the microtensile bond strength of PU adhesives improved after thermal cycling testing and the amount of microleakage was reduced compared to commercial adhesives. Biocompatibility testing showed that the PU adhesive was non-toxic to L929 fibroblasts. This study demonstrates the ability of PU adhesive to improve the stability and durability of the dental adhesion interface (Zhang & Xie 2020).

In the final APU as a biomedical product, the synthesis reaction was carried out in bulk without the use of any catalyst to avoid the possible side effects of solvent and catalyst residues. Chemical components such as PCL molecular weight and functionality of reactive diluents (average vinyl group in the system). They investigated the correlation between the properties and the microstructure, mechanical properties

and biological degradation of the final cross-linked acrylate polyurethane. In the studies; It provides a basis for the development of UV-curable shape memory polyurethane acrylates with desired shape memory properties and fast memory response as well as high mechanical properties for potential use in biomedical and tissue engineering. In our study, we will benefit from the experiences in this literature by using different solvent environments and different monomers (Khasraghi, Shojaei & Sundararaj 2019).

In this study, polyester-based biodegradable APU, namely polycaprolactone diol (PCL), polyol (Mn) with two different molecular weights of 10 000, 40 000 g/mol, aliphatic diisocyanate (hexamethylene diisocyanate, HDI) 2-Hydroxyethyl methacrylate (HEMA). Ethylene glycol dimethacrylate (EGDMA) and triethylene glycol dimethacrylate (TEGDMA) were used.PCL is a biocompatible polymer with aliphatic ester linkage that is susceptible to hydrolysis (biodegradation). The degradation product of PCL is 6-hydroxyhexanoic acid, a naturally occurring metabolite in the human body. It is also believed that the degradation of aliphatic diisocyanate in polyurethanes leads to non-toxic amine. In this study, a two-step polymerization method was used, which is believed to provide better control over the molecular weight, molecular weight distribution, and chemical structure of the APU compared to the one-step method mentioned by Pereira et al. (Pereira, Ayres & Patrício Acta, 2010). The properties of the chemical components, such as the PCL molecular weight and the functionality of the reactive diluents (average vinyl group in the system), were matched to determine the thermal and chemical properties of the final cross-linked acrylated polyurethane. To properly achieve this goal, a systematic characterization of the chemical and thermal properties of the final product was applied.

2. MATERIAL AND METHOD

2.1. Materials:

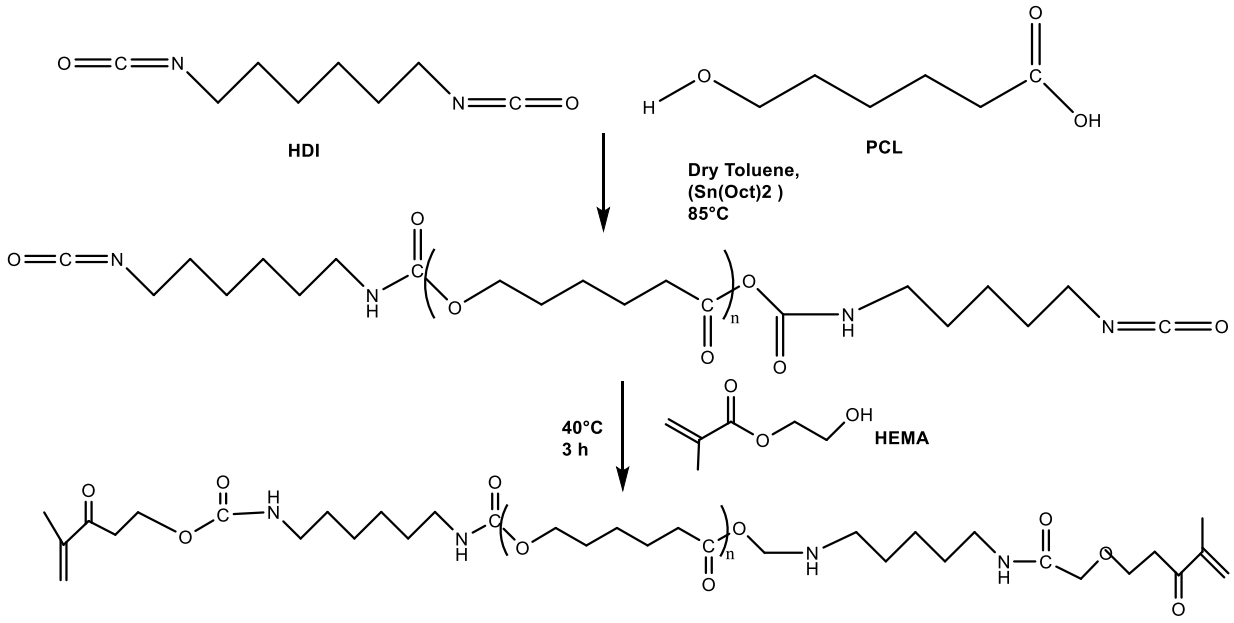
All chemicals used in this study were purchased from Sigma-Aldrich and used without further purification. Polycaprolactone (PCL) with molecular weights of average Mn 10,000 g/mol and 40,000 g/mol, 1, 6-Hexamethylene diisocyanate (HDI), 2-Hydroxyethyl methacrylate (HEMA), Ethylene glycol dimethacrylate (EGDMA) and Triethylene Glycol Dimethacrylate (TEGDMA) as the chain extender were obtained. Toluene was used as the solvent, and Tin (II) 2-ethylhexanoate (also known as tin (II) octoate or stannous octoate, Sn $(Oct)_2$) was used as the catalyst. All other analytical grade reagents were also sourced from Sigma-Aldrich and utilized directly in the synthesis and characterization processes.

2.2. Methods

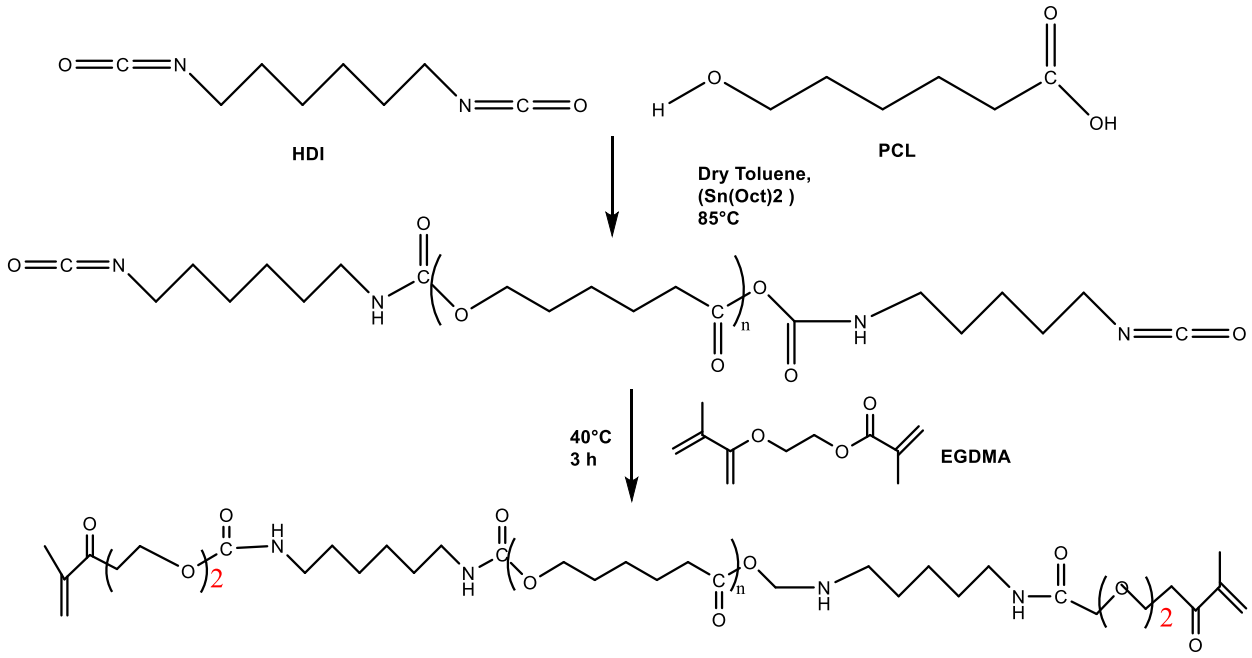
2.2.1. Synthesis of isocyanate-terminated polyurethane prepolymer (IPU):

Isocyanate-terminated prepolymers were synthesized with PCL and HDI according to a two-step polymerization. A 3-neck glass reaction flask equipped with a heating mantle, reflux condenser, mechanical stirrer, dropping funnel and nitrogen gas inlet system was used. Dissolve in a suitable solvent (toluene) with stirring at 60°C.Tin(II) 2-ethylhexanoate (Sn $(Oct)_2$) is added at a rate of 2 mole% by weight of PCL and HDI is added dropwise to the reactor with vigorous stirring in an inert atmosphere. (Figure 1). The temperature was then increased to 85°C and the reaction continued until the isocyanate in the reaction medium ran out (Mole Ratios and working times are shown in Table 1.).

(1) *


HDI
PCL
Dry Toluene,
(Sn(Oct)2)
85°C
40°C
3 h
HEMA

(2) **


HDI
PCL
Dry Toluene,
(Sn(Oct)2)
85°C
40°C
3 h
EGDMA

(3) ***

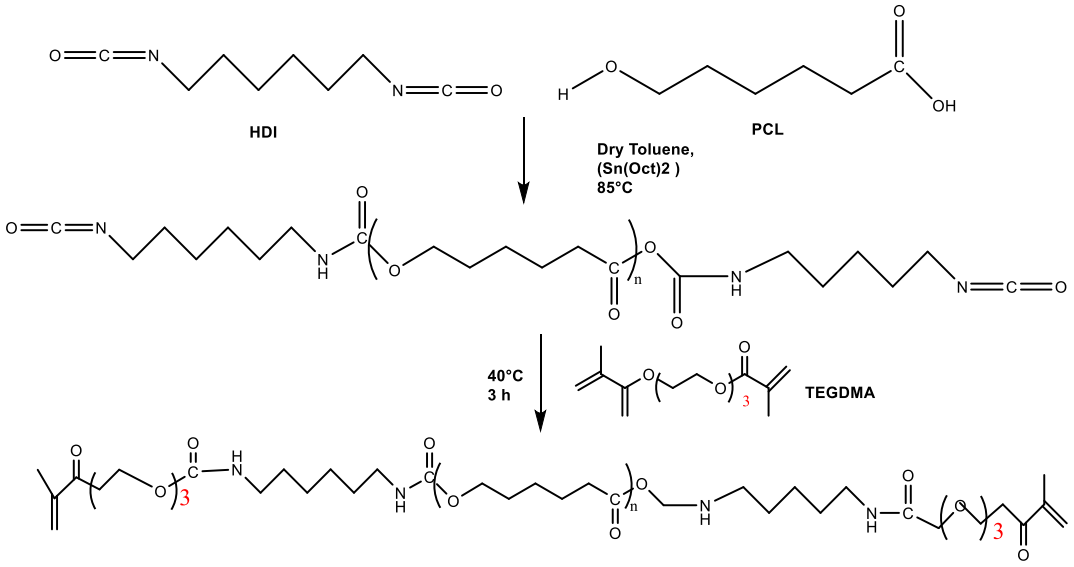


Figure 1: Synthesis Products of Acrylic Terminated Polyurethane Prepolymer.
(1) * Synthesis of acrylic terminated polyurethane prepolymer (PC: HDI: HEMA)
(2) ** Synthesis of acrylic terminated polyurethane prepolymer (PC: HDI: EGDMA)
(3)* Synthesis of acrylic terminated polyurethane prepolymer (PCL:HDI:TEGDMA)**

2.2.2. Synthesis of acrylic terminated polyurethane prepolymer (PCL: HDI: HEMA)

The prepolymer solution is cooled to 40°C and then HEMA is added dropwise as a chain extender. The mixture is stirred continuously for 3 hours to ensure thorough mixing and reaction (Figure 1). The resulting polyurethane solution is stored in a light-proof glass bottle. During synthesis, the molar ratio of PCL/HDI/HEMA was kept at 1:2:2 in this step. (Mole Ratios and working times are shown in Table 1.).

2.2.3. Synthesis of acrylic terminated polyurethane prepolymer (PCL: HDI: EGDMA)

The prepolymer solution is cooled to 40°C and then EGDMA is added dropwise as a chain extender. The mixture is stirred continuously for 3 hours to ensure thorough mixing and reaction (Figure 1). The resulting polyurethane solution is stored in a light-proof glass bottle. During synthesis, the molar ratio of PCL/HDI/EGDMA was kept at 1:2:2 in this step. (Mole Ratios and working times are shown in Table 1.).

2.2.4. Synthesis of acrylic terminated polyurethane prepolymer (PCL: HDI: TEGDMA)

The prepolymer solution is cooled to 40°C and then TEGDMA is added dropwise as a chain extender. The mixture is stirred continuously for 3 hours to ensure thorough mixing and reaction (Figure 1). The resulting polyurethane solution is stored in a light-proof glass bottle. During synthesis, the molar ratio of PCL/HDI/TEGDMA was kept at 1:2:2 in this step. (Mole Ratios and working times are shown in Table 1.).

Table 1. Mole Ratios and Working Times PCL-diol/HDI/ HEMA -PCL-diol/HDI/EGDMA- PCL-diol/HDI/TEGDMA

Polyurethane acrylates	PCL molecular weight (g/ mol^{-1})	(mol)					Time (hour)
		PCL	HDI	HEMA	EGDMA	TEGDMA	
PCL: HDI: HEMA	10,000	0,0003	0,0006	0,0006	---	---	5
PCL: HDI: EGDMA	10,000	0,0003	0,0006	---	0,0006	---	5
PCL: HDI: TEGDMA	10,000	0,0003	0,0006	---	---	0,0006	5
PCL: HDI: HEMA	40,000	0,0003	0,0006	0,0006	---	---	5
PCL: HDI: EGDMA	40,000	0,0003	0,0006	---	0,0006	---	5
PCL: HDI: TEGDMA	40,000	0,0003	0,0006	---	---	0,0006	5

2.3. Characterization:

2.3.1. Fourier Transform Infrared Spectroscopy (FT-IR):

FT-IR will be performed to confirm the chemical structure of the synthesized polyurethanes. Spectra will be recorded in the range of 4000-400 cm^{-1} using an FT-IR spectrometer. Peaks corresponding to characteristic functional groups will be analyzed to verify the formation of urethane linkages and the incorporation of diisocyanates and chain extender.

2.3.2. Differential Scanning Calorimetry (DSC):

Differential scanning calorimetry is a thermal technique that is used to obtain richer information about the material and can perform quick and easy analysis. DSC measures the amount of energy absorbed or released as the sample is heated, cooled, or kept at a constant temperature. In this technique, the heat difference coming or going away from the reference and the sample is displayed depending on temperature or time.

DSC will be used to analyze the thermal properties of the polyurethanes. Samples will be heated from -50°C to 220°C at a rate of 10°C/min under nitrogen atmosphere. The glass transition temperature (Tg), crystallinity (C_r) recorded and compared to assess the influence of PCL molecular weight, diisocyanate type and chain extender.

3. RESULTS

In the study of polyurethanes, FT-IR spectroscopy plays a role in determining the molecular weight of polycaprolactone (PCL), the structure of diisocyanates, and the chemical properties of the polymer with the effect of the chain extender. Polycaprolactone (PCL)'s molecular weight can significantly influence the FT-IR spectrum, particularly in the intensity, width, and position of various bands. Higher molecular weight PCL often results in more pronounced C=O and C-O-C bands, greater intensity of C-H bands, and potentially more distinct crystalline structure bands. The carbonyl (C=O) stretching band of PCL typically appears in the 1725-1735 cm^{-1} range. When using high molecular weight PCL, an increase in the intensity of this band is observed. The C-O-C stretching band associated with the ester groups in PCL is found in the 1170-1250 cm^{-1} range, and its intensity can also increase with higher molecular weight PCL due to the higher ester content. Additionally, the longer polymer chains in high molecular weight PCL can lead to broadening and increased intensity of certain bands in the spectrum.

The presence of the NCO characteristic peak at 2270 cm^{-1} indicates that the first step has been successfully completed. In the second step of the reaction onto vinyl, capping with HEMA/TEGDMA/EGDMA, the acrylate double bonds must be incorporated into the linear polyurethane backbone. The disappearance of the NCO peak along with the appearance of the stretching vibration peaks of the acrylate double bond at 1645 cm^{-1} (C-C) and 810 cm^{-1} (CH) in polyurethanes confirms that the second step of the reaction is completed and NCO is formed. FTIR spectra are as shown in Figure 2.

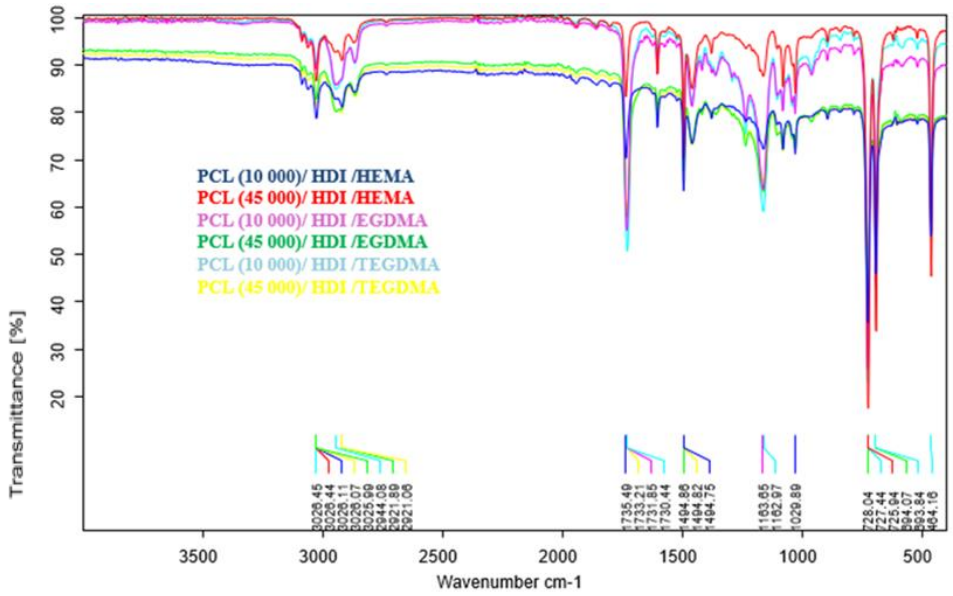


Figure 2. Comparison of FTIR Spectra of The Final Product With Different Molecular Weights Terminated With PCL-Based Acrylic According to Their Molecular Weights.

DSC analysis is a critical technique for elucidating the thermal properties of polyurethanes, focusing on the molecular weight of polycaprolactone (PCL), the structure of diisocyanates, and the role of chain extender. Higher molecular weight PCLs contribute to increased crystallinity and elevated glass transition temperatures (Tg) in polyurethanes. This is evidenced by distinct endothermic and exothermic peaks in DSC thermograms (refer to Figures 3,4 and 5).

Based on the DSC analysis, the glass transition temperatures (Tg) of polycaprolactone (PCL) with different molecular weights (40,000 and 10,000) combined with various acrylates (TEGDMA, HEMA, and EGDMA) indicate significant insights into their thermal properties (Table 2.). PCL with a higher molecular weight (40,000) consistently shows higher Tg values compared to its lower molecular weight counterpart (10,000), which can be attributed to the decreased mobility and increased chain entanglement in higher molecular weight polymers. Among the acrylates, EGDMA (Ethylene Glycol Dimethacrylate) exhibits the highest Tg values due to its bifunctional nature, leading to a denser cross-linked network that restricts polymer chain movement, thus requiring more thermal energy for the transition. TEGDMA (Triethylene Glycol Dimethacrylate) shows intermediate Tg values, where the triethylene glycol segment imparts moderate flexibility to the polymer chains, resulting in a balance between cross-linking and chain mobility. HEMA (2-Hydroxyethyl Methacrylate), on the other hand, presents the lowest Tg values, as the hydroxyl group can form hydrogen bonds, enhancing inter-chain interactions but simultaneously increasing chain flexibility. These findings underscore the importance of selecting appropriate PCL molecular weights and acrylate types to tailor the thermal and mechanical properties of polymer networks for specific applications. The data demonstrate how molecular weight and cross-linking density fundamentally influence the glass transition behavior, providing a critical understanding for designing advanced polymer materials with desired thermal performance characteristics.

Table 2. DSC Analysis Data for Polyurethanes

Polyurethane acrylates	PCL molecular weight (g /mol^{-1})	Glass Transition Temperature Tg (°C)
PCL: HDI: HEMA	10,000	29,54
PCL: HDI: EGDMA	10,000	34,87
PCL: HDI: TEGDMA	10,000	32,80
PCL: HDI: HEMA	40,000	38,36
PCL: HDI: EGDMA	40,000	36,82
PCL: HDI: TEGDMA	40,000	31,36

a)

b)

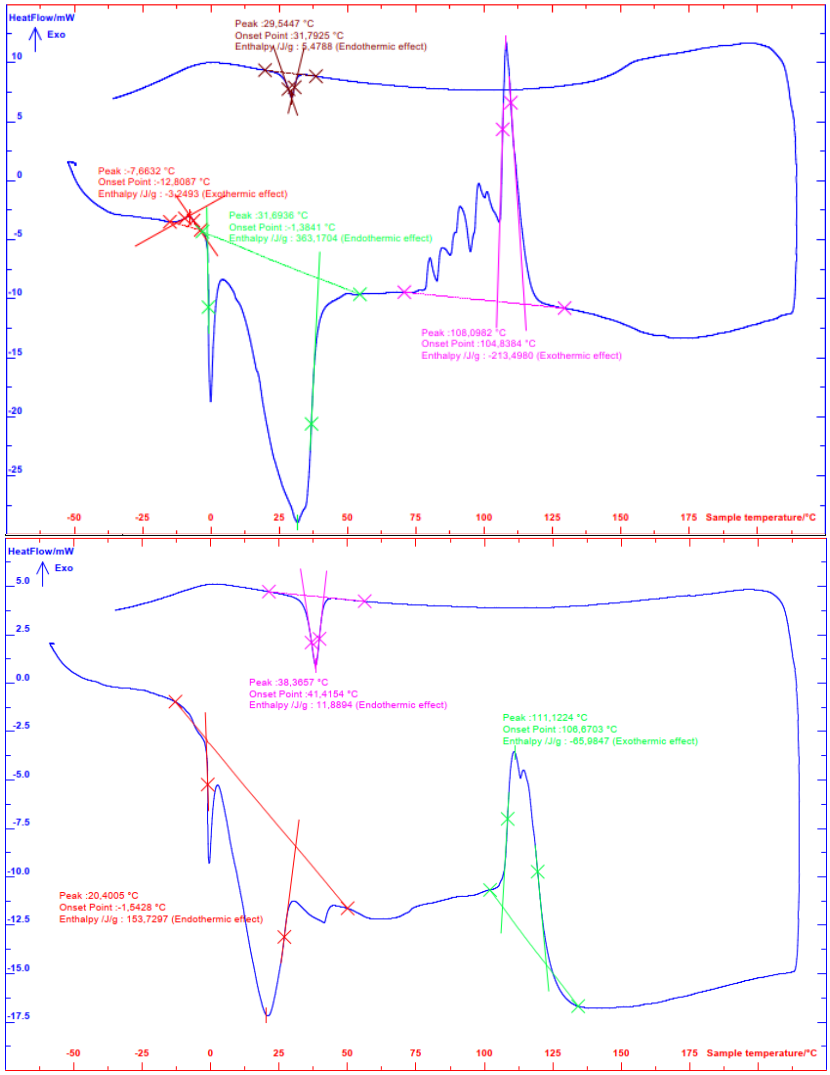
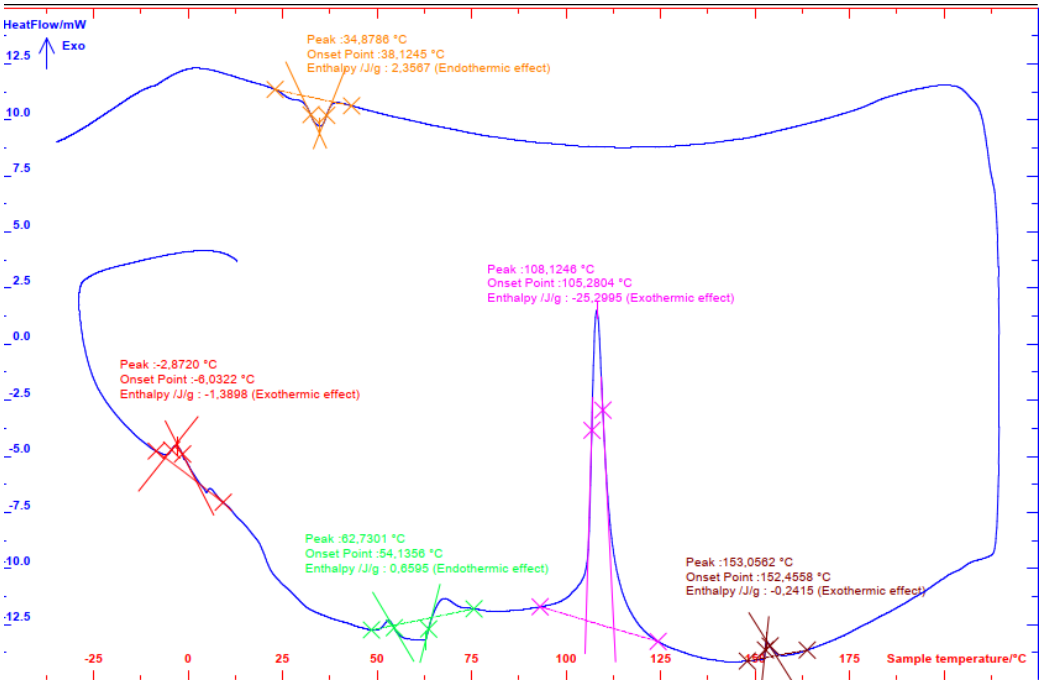


Figure 3. a) DSC Analysis of PCL (10) HDI HEMA
b) DSC Analysis of PCL (40) HDI HEMA

a)



b)

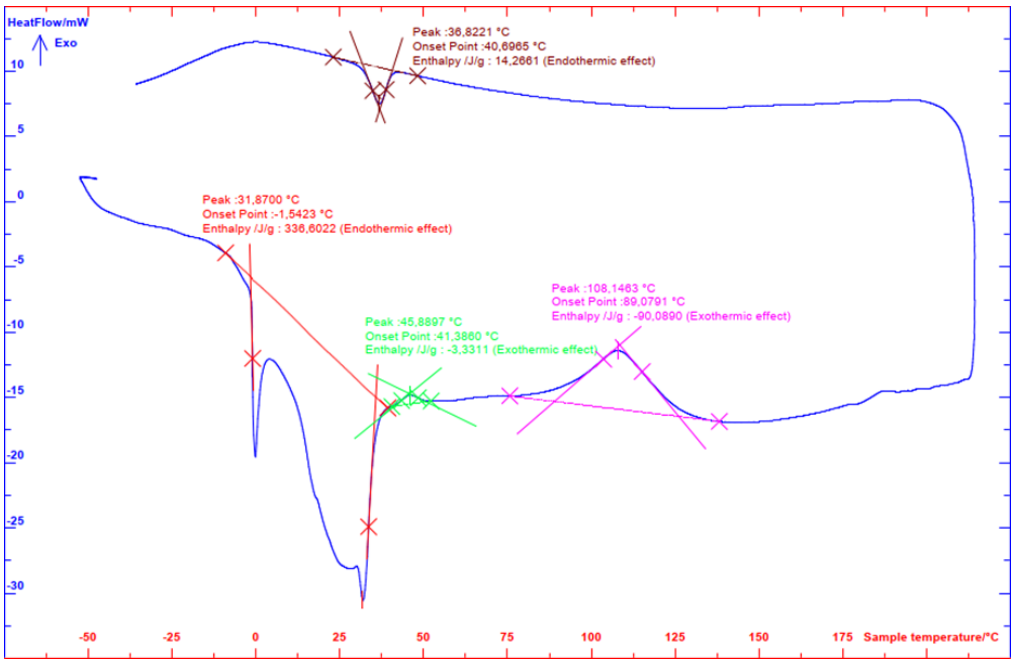
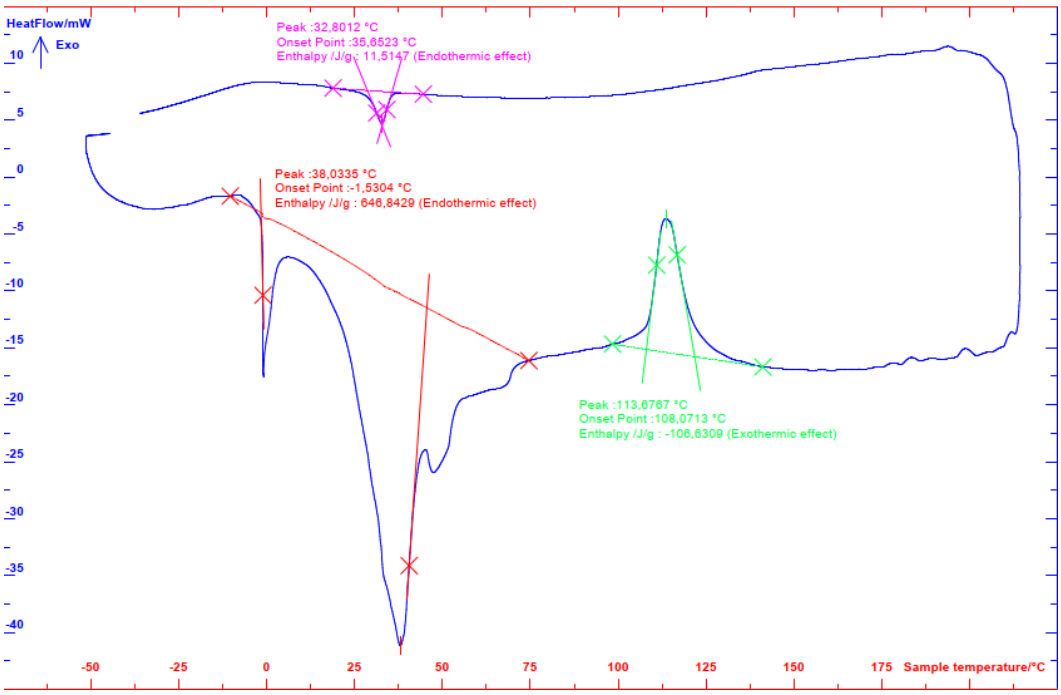


Figure 4. a) DSC Analysis of PCL (40) HDI EGDMA
b) DSC Analysis of PCL (10) HDI EGDMA

a)



b)

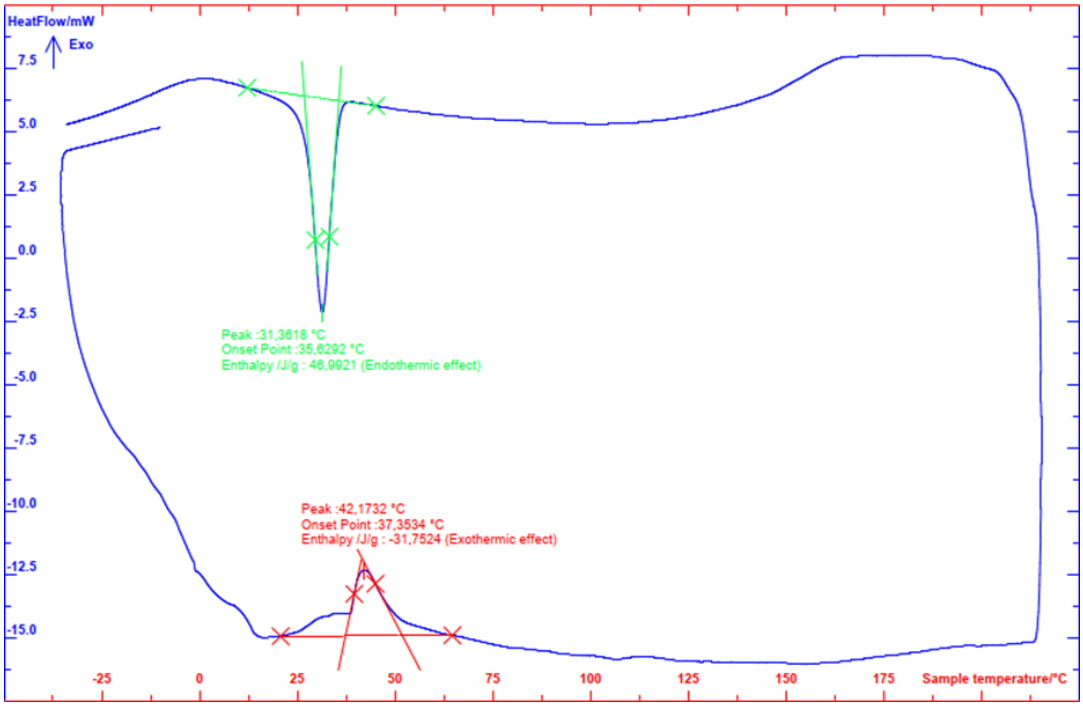


Figure 5. a) DSC Analysis of PCL (10) HDI TEGDMA
b) DSC Analysis of PCL (40) HDI TEGDMA

4. DISCUSSION

In this study, the thermal and structural properties of various polyurethane compositions were examined using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR).

DSC analysis was used to determine the characteristics of the glass transition temperatures of polyurethanes. PCL (10): HDI: HEMA, PCL (40): HDI: HEMA, PCL (10): HDI: EGDMA, PCL (40): HDI: EGDMA, PCL (10): HDI: TEGDMA and PCL (40) HDI: TEGDMA. The first and second heating scans of HDI: TEGDMA resins are presented in Figure 3,4 and 5. Glass transition temperature (Tg) data is also shown in the table. Due to the amorphous structure of low molecular weight PCL, no melting peak was found for its resin. As the PCL molecular weight increases, the glass transition temperature generally increases. For example, polyurethanes made with 40,000 Da PCL polymer have higher glass transition temperatures than those made with 10,000 Da PCL polymer.

Polyurethanes containing EGDMA monomer generally have higher glass transition temperatures than those containing TEGDMA monomer. These data show that the thermal properties of polyurethanes can vary significantly depending on factors such as polymer composition and molecular weight. (Afsoon Farzanac et al., 2020).

PCL (10): HDI: HEMA, PCL (40): HDI: HEMA, PCL (10): HDI: EGDMA, PCL (40): HDI: EGDMA, PCL (10): HDI: TEGDMA and PCL (40): HDI: TEGDMA The chemical structures and formation of HDI: TEGDMA resins were further analyzed by FTIR. PCL (10): HDI: HEMA, PCL (40): HDI: HEMA, PCL (10): HDI: EGDMA, PCL (40): HDI: EGDMA, PCL (10): HDI: TEGDMA and PCL (40): HDI: TEGDMA spectra are presented in Figure 2. The presence of the NCO characteristic peak at 2270 cm^{-1} indicates that the first step has been successfully completed. In the second step of the reaction onto vinyl by capping with HEMA/TEGDMA/EGDMA, the acrylate double bonds need to be incorporated into the linear polyurethane backbone. The disappearance of the NCO peak along with the appearance of the stretching vibration peaks of the acrylate double bond at 1645 cm^{-1} (C-C) and 810 cm^{-1} (CH) in polyurethanes confirms that the second step of the reaction is completed and NCO is formed (Afsoon Farzanac et al., 2020).

5. CONCLUSION

The synthesis of acrylic-terminated UV-curable polyurethanes (APUs) for dental applications using Poly(ε-caprolactone) diol aliphatic 1,6-hexamethylene diisocyanate (HDI) was investigated. Different molecular weights of polycaprolactone were used to examine their effects on the resulting polyurethanes when combined with various acrylic terminators such as 2-hydroxyethyl methacrylate (HEMA), Triethylene glycol dimethacrylate (TEGDMA), and Ethylene glycol dimethylacrylate (EGDMA). Through the monitoring of synthesis reactions via FTIR spectroscopy, it was observed that the incorporation of acrylate double bonds into the linear polyurethane backbone successfully occurred. This conclusion was supported by the disappearance of the NCO peak and the appearance of characteristic stretching vibration peaks of the acrylate double bonds. These findings provide valuable insights for optimizing the properties of polyurethanes tailored for dental applications.

6. ACKNOWLEDGE

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Conflict of Interest The authors declare that there are no conflicts of interest.

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INVESTIGATION OF ADDING CHAIN EXTENDERS TO DIFFERENT POLYURETHANES BASED ON POLYCAPROLACTON

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ABSTRACT

Background of the Study: This study investigates the effects of adding chain extenders to polyurethanes based on polycaprolactone (PCL) with molecular weights of 10,000 and 40,000. The polyurethanes were synthesized using three different diisocyanates: methylene diphenyl diisocyanate (MDI), toluene diisocyanate (TDI), and hexamethylene diisocyanate (HDI). 1,4-Butanediol (BDO) was used as the chain extender. The objective was to examine how the molecular weight of polycaprolactone and the type of diisocyanate influence the properties of the resulting polyurethanes when combined with the chain extender. Differential Scanning Calorimetry (DSC) and Fourier-transform Infrared Spectroscopy (FT-IR) were utilized to characterize the thermal and chemical properties of the synthesized polyurethanes. The results provide insights into optimizing the performance characteristics of polyurethanes for diverse industrial applications. Selecting chain extenders, polyols, and diisocyanates is vital in polyurethane development. This study focuses on using 1,4-butanediol (BDO) to compare the effects of different PCL molecular weights and various diisocyanates on thermoplastic polyurethanes (TPU). BDO enhances tensile strength and flexibility, making polyurethanes suitable for durable applications.

Aim: To examine the effects of chain extenders, diisocyanate type, and PCL molecular weight on TPU composites, using DSC and FT-IR for analysis.

Material and Method: PCL (10,000 and 40,000 g/mol), MDI, HDI, and 1,4-butanediol were used. PCL was dissolved in toluene at 60°C, followed by MDI or HDI addition. After prepolymer formation, 1,4-butanediol was added at 40°C as a chain extender. The solution was cast onto a glass plate and cured.

Results: DSC revealed that higher molecular weight increased melting temperature and decreased recrystallization temperature, affecting thermal properties.

Conclusion: Balancing crystalline and amorphous properties in TPUs enhances shape-shifting capabilities, offering potential for biomedical uses.

Keywords: Chain extenders, diisocyanates, DSC, FT-IR, polycaprolactone polyurethanes

1. INTRODUCTION

In the realm of biomedical applications, the development of advanced polyurethane materials is crucial due to their versatility, biocompatibility, and mechanical properties. Polyurethanes are widely used in medical devices, implants, and drug delivery systems. The selection of appropriate components, such as chain extenders, polyols, and diisocyanates, is essential in tailoring the properties of these materials to meet specific biomedical requirements. This study focuses on the use of 1,4-butanediol (BD) as a chain extender and compares the effects of different polycaprolactone (PCL) molecular weights and various types of diisocyanates, including Methylene Diphenyl Diisocyanate (MDI) and Hexamethylene Diisocyanate (HDI), on the properties of thermoplastic polyurethane (TPU) composites. Chain extender like 1,4-butanediol are vital in polyurethane chemistry, reacting with diisocyanates to form hard segments within the polymer matrix, which enhance the material's mechanical strength and thermal stability. The ability of BD to improve tensile strength and elongation at break makes these polyurethanes particularly suitable for demanding biomedical applications (Trinh et al., 2024).

Polycaprolactone (PCL), a semi-crystalline polymer, is commonly utilized in polyurethane modification due to its biodegradability, low melting point, and excellent flexibility. The molecular weight of PCL significantly influences the resulting polyurethane's properties. Higher molecular weight PCL (e.g., average Mn 45,000 g/mol) enhances mechanical properties and thermal stability through increased chain entanglement and crystallinity, rendering the polyurethane matrix more robust and suitable for high-performance applications (Wang et al., 2024).

Diisocyanates constitute another critical aspect of polyurethane synthesis, classified broadly into aliphatic and aromatic types, each imparting unique characteristics to the polyurethane. Aromatic diisocyanates, like Methylene Diphenyl Diisocyanate (MDI), yield polyurethanes with superior thermal stability and mechanical properties, often preferred for applications necessitating high durability and heat resistance. Another type of diisocyanates, aliphatic diisocyanates, such as Hexamethylene Diisocyanate (HDI), provide better thermal and hydrolysis resistance, producing polyurethanes with exceptional UV and weather resistance. HDI-based polyurethanes offer enhanced flexibility, ideal for coatings and elastomers requiring outdoor durability (Kojio et al., 2020; Panwiriyarat et al., 2013; Puszka, Sikora & Nurzyńska, 2024).

In the development of advanced polyurethane materials, the selection of chain extenders, polyols, and diisocyanates plays a critical role in determining the final properties of the composites. This study focuses on the use of 1,4-butanediol (BD) as a chain extender and compares the effects of different polycaprolactone (PCL) molecular weights and various types of diisocyanates on the properties of thermoplastic polyurethane (TPU) composites (Wang et al., 2024). Chain extenders like 1,4-butanediol are essential components in polyurethane chemistry. They react with diisocyanates to form hard segments within the polymer matrix, which contribute to the material's mechanical strength and thermal stability. BD, specifically, is known for its ability to enhance the tensile strength and elongation at break of polyurethane materials, making them suitable for applications requiring high durability and flexibility (Huang et al., 2024; Liu et al., 2018) .

The aim of this study is to comprehensively investigate the effects of various diisocyanates, PCL with differing molecular weights (average Mn 10,000 and 45,000 g/mol) and chain extender on the performance properties of polycaprolactone (PCL)-based polyurethanes. To achieve this objective, methylene diphenyl diisocyanate (MDI) and hexamethylene diisocyanate (HDI) will be employed as diisocyanate components in the polyurethane synthesis. The chain extender selected for this study is 1,4-butanediol. Advanced analytical techniques such as Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR) will be utilized to perform the thermal and chemical characterization of the synthesized polyurethanes. Through DSC analysis, the thermal properties and glass transition temperatures of the polyurethanes will be determined, assessing the impact of polycaprolactone's molecular weight and the type of diisocyanate on these properties. FT-IR spectroscopy will be used to examine the chemical structure of the polymer chains and the integration of the chain extender and diisocyanates into the polymer matrix. The data obtained from this study will provide crucial insights for optimizing the mechanical and thermal properties of PCL-based polyurethanes, enhancing their potential for industrial applications. Ultimately, this research will contribute to the advancement of polyurethane technology, paving the way for the development of more durable, functional materials suited for a wide range of applications.

2. MATERIAL AND METHOD

2.1. Materials

All chemicals used in this study were purchased from Sigma-Aldrich and used without further purification. Polycaprolactone (PCL) with molecular weights of average Mn 10,000 g/mol and 45,000 g/mol, methylene diphenyl diisocyanate (MDI), hexamethylene diisocyanate (HDI), and 1,4-butanediol as the chain extender were obtained. Toluene was used as the solvent, and Tin(II) 2-ethylhexanoate (also known as tin(II) octoate or stannous octoate, $Sn(Oct)_2$) was used as the catalyst. All other analytical grade reagents were also sourced from Sigma-Aldrich and utilized directly in the synthesis and characterization processes.

2.2. Methods

2.2.1 Synthesis of PCL-based Polyurethanes

A predetermined amount of PCL is dissolved in an appropriate solvent (toluene) under stirring at 100-110°C. Tin (II) 2-ethylhexanoate ($Sn(Oct)_2$) is added at 2 mol % by weight of the PCL. The NCO: OH equivalent ratio of the polyurethane is 2:1 by adding MDI or HDI to the PCL solution. The mixture is stirred under a nitrogen atmosphere to form the prepolymer. The reaction mixture is heated and stirred until complete dissolution and reaction of the components. The prepolymer solution is cooled to 40°C, and then 1,4-butanediol is added dropwise as a chain extender. The mixture is stirred continuously for 16 hours to ensure thorough mixing and reaction (Figure 1). The chain extension reaction proceeds under controlled conditions until the desired polymer network is formed. The resulting polyurethane solution is cast onto a glass plate and allowed to cure at ambient temperature.

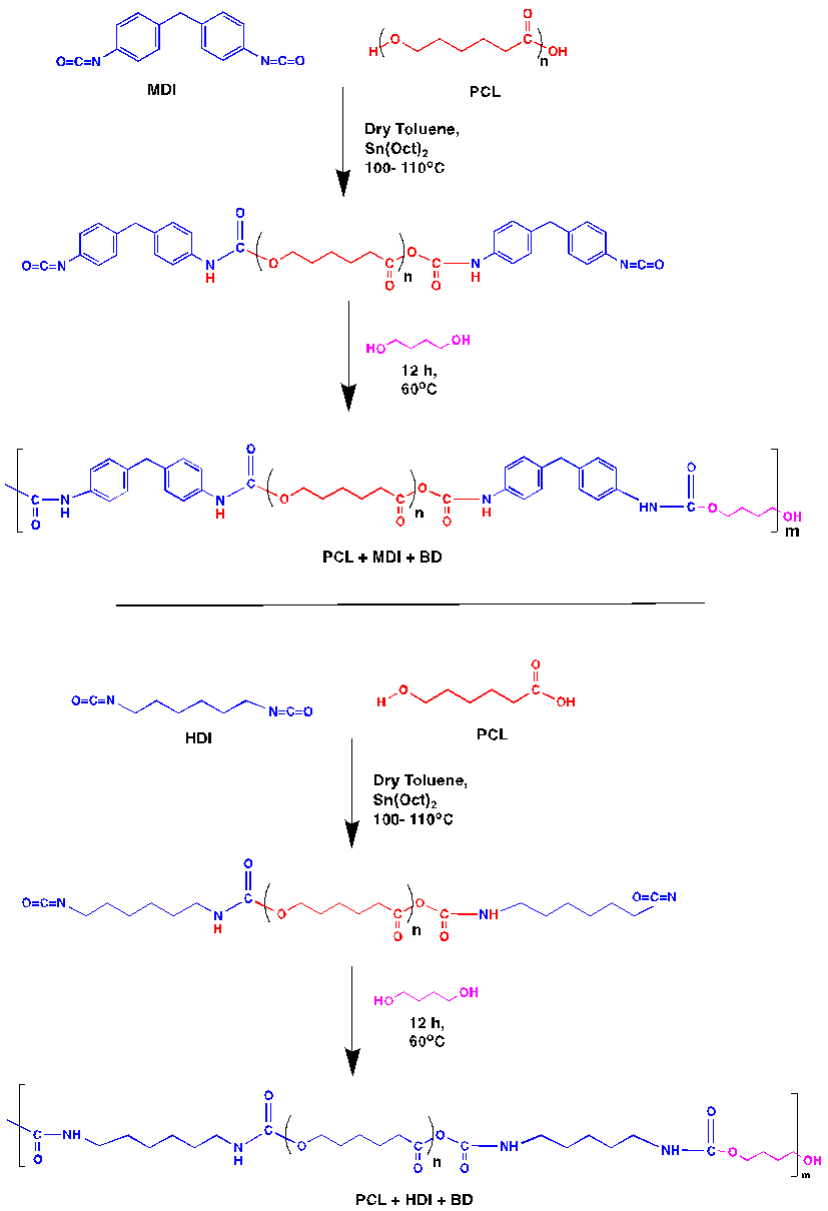

Figure 1. PCL-based Polyurethanes with 1,4 Butanediol

2.3. Characterization

2.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR will be performed to confirm the chemical structure of the synthesized polyurethanes. Spectra will be recorded in the range of 4000-400 cm^{-1} using an FT-IR spectrometer. Peaks corresponding to characteristic functional groups will be analyzed to verify the formation of urethane linkages and the incorporation of diisocyanates and chain extender.

2.3.2. Differential Scanning Calorimetry (DSC)

DSC will be used to analyze the thermal properties of the polyurethanes. Samples will be heated from -50°C to 220°C at a rate of 10°C/min under nitrogen atmosphere. The glass transition temperature (Tg), crystallinity (C_r) recorded and compared to assess the influence of PCL molecular weight, diisocyanate type and chain extender.

3. RESULTS

In the study of polyurethanes, FT-IR spectroscopy plays a role in determining the molecular weight of polycaprolactone (PCL), the structure of diisocyanates, and the chemical properties of the polymer with the effect of the chain extender (Figure 2). Polycaprolactone (PCL)'s molecular weight can significantly influence the FT-IR spectrum, particularly in the intensity, width, and position of various bands. Higher molecular weight PCL often results in more pronounced C=O and C-O-C bands, greater intensity of C-H bands, and potentially more distinct crystalline structure bands. The carbonyl (C=O) stretching band of PCL typically appears in the 1725-1735 cm^{-1} range. When using high molecular weight PCL, an increase in the intensity of this band is observed. The C-O-C stretching band associated with the ester groups in PCL is found in the 1170-1250 cm^{-1} range, and its intensity can also increase with higher molecular weight PCL due to the higher ester content. Additionally, the longer polymer chains in high molecular weight PCL can lead to broadening and increased intensity of certain bands in the spectrum. For instance, C-H stretching bands (2800-3000 cm^{-1}) and C-H bending bands (1460-1470 cm^{-1}) may become more prominent, as illustrated in Figure 2. When analyzing FT-IR spectra of polyurethane chains based on MDI (aromatic diisocyanate) and HDI (aliphatic diisocyanate), distinct spectral characteristics can be observed (Figure 2). HDI-based polyurethanes typically exhibit carbonyl stretching bands in the 1700-1730 cm^{-1} range, which are generally broader compared to those in MDI-based polyurethanes. This difference in band broadness can be attributed to the aliphatic nature of HDI, in contrast to the aromatic structure of MDI, as shown in Figure 2. In FT-IR spectra, the impact of chain extender on hard segments can be identified by examining specific bands. The addition of chain extender, 1,4-butanediol, typically results in an increase in the intensity of the carbonyl (C=O) stretching bands within the 1700-1730 cm^{-1} range. This increase or enhancement in the band indicates the formation of hard segments, particularly urethane linkages. This change is a critical indicator of the polymer's structural development and the extent of the hard segment formation within the polyurethane matrix, as evidenced in Figure 2.

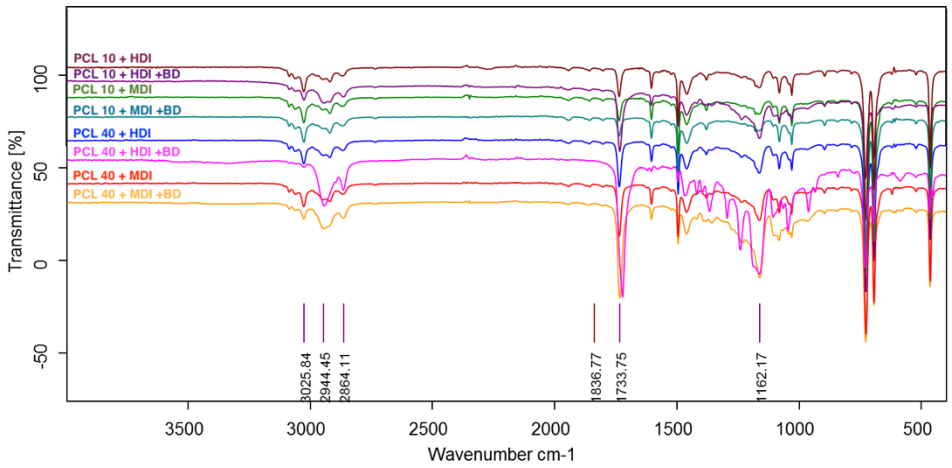

Figure 2. The Roles of PCL Molecular Weight, Diisocyanate Structure, and Chain Extender In Determining Polyurethane Properties Through FT-IR Spectroscopy.

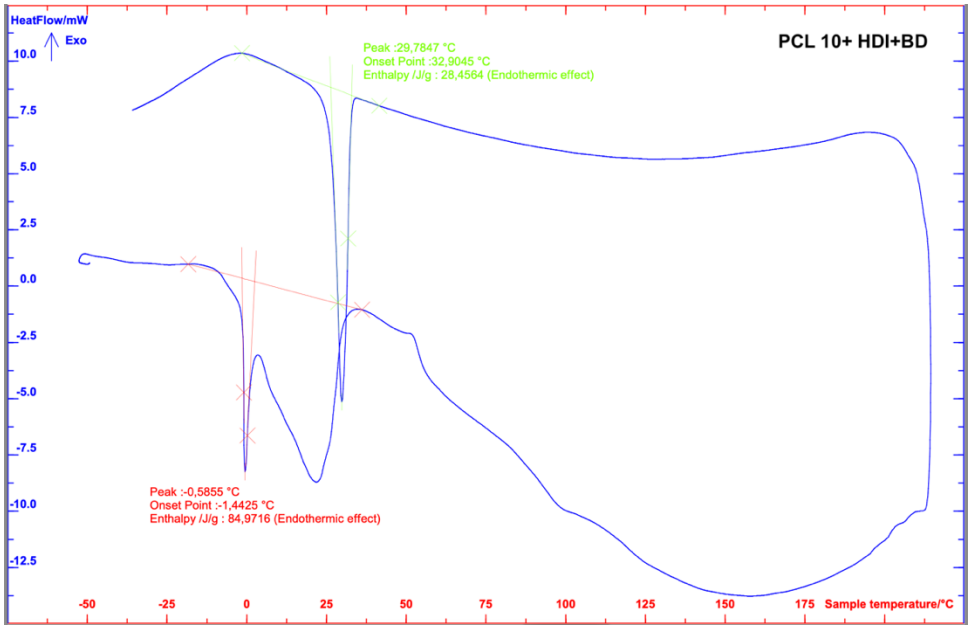

Figure 3. DSC Analysis of PCL 10 + HDI + BD

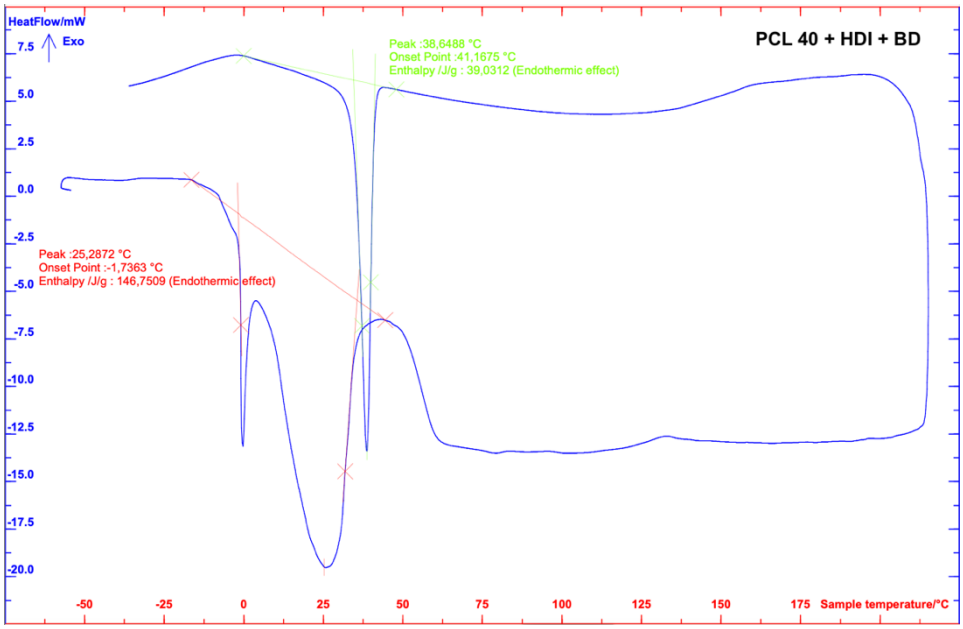

Figure 4. DSC Analysis of PCL 40+ HDI + BD

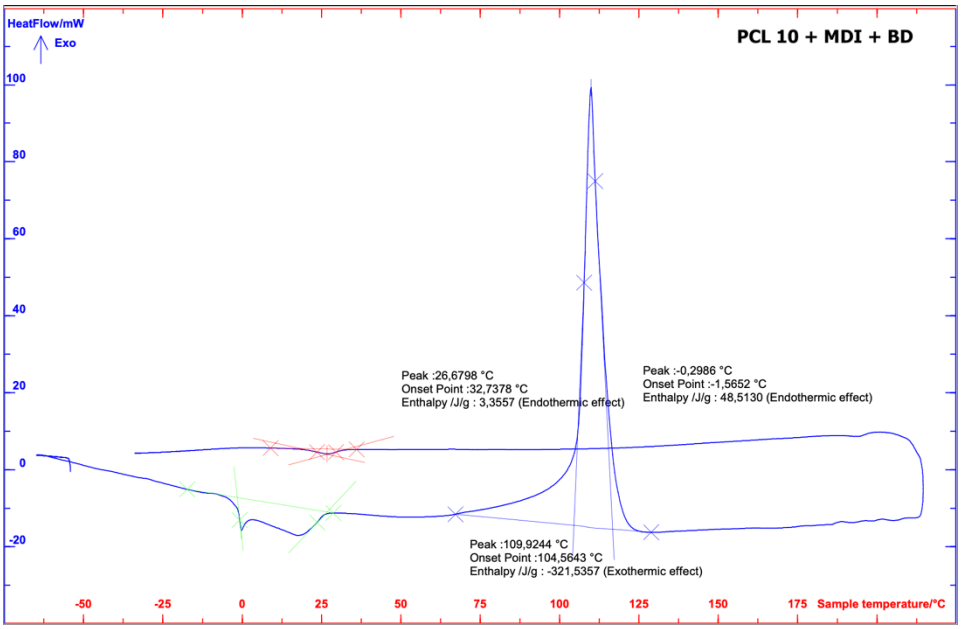

Figure 5. DSC Analysis of PCL 10+ MDI + BD

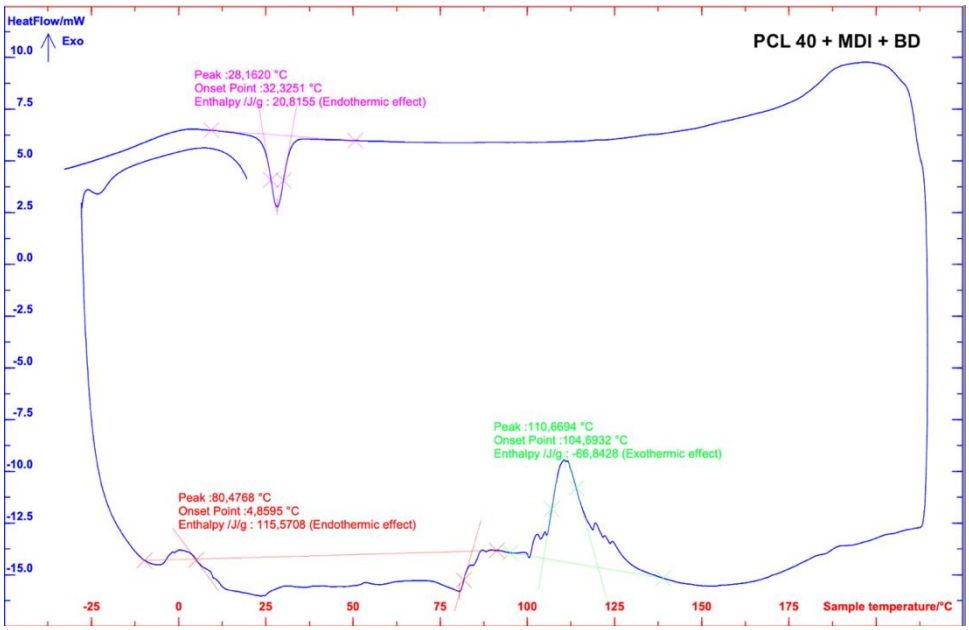

Figure 6. DSC Analysis of PCL 40+ MDI + BD

Table 1. DSC Analysis Data for Polyurethanes

	$\Delta H_m°$(j/g)	ΔH_m(j/g)	% C_r	T_g
PCL 10 +HDI + BD	135,3	28,45	21,74	29,78
PCL 40 +HDI + BD	135,3	39,03	29,21	38,64
PCL 10 +MDI + BD	135,3	3,36	2,67	26,67
PCL 40 +MDI + BD	135,3	20,82	15,58	28,16

In Table 1, $\Delta H_m°$ represents the enthalpy of melting for pure PCL (100% crystalline) at 135.3 J/g. ΔH_m is the enthalpy of melting as measured from the DSC analysis, and WA denotes the weight fraction of PCL within the polymer chain.

DSC analysis is a critical technique for elucidating the thermal properties of polyurethanes, focusing on the molecular weight of polycaprolactone (PCL), the structure of diisocyanates, and the role of chain extender. Higher molecular weight PCLs contribute to increased crystallinity and elevated glass transition temperatures (Tg) in polyurethanes. This is evidenced by distinct endothermic and exothermic peaks in DSC thermograms (refer to Figures 3,4,5 and 6). Furthermore, the incorporation of chain extender,1,4-butanediol (BD), significantly impacts the thermal properties and phase behavior of polyurethanes. DSC analysis reveals that chain extender enhance the formation of hard segments within the polymer matrix, as indicated by increased enthalpy changes during phase transitions. This enhancement is particularly noticeable in the intensity and position of the carbonyl (C=O) stretching bands, which correspond to the formation of urethane linkages (Figure 2). Consequently, the thermal profiles of polyurethanes vary distinctly based on the type and concentration of chain extender used, as well as the molecular characteristics of the PCL and diisocyanates involved.

In Table 1, the ΔH_m values are extracted from Figures 3,4,5 and 6, representing the melting enthalpy of PCL. Using the data in the table, we can interpret the degrees of crystallinity (Cr) for four different polyurethane samples. The degree of crystallinity is a measure that ranges from 0 to 1, where a value closer to 0 indicates a more amorphous polymer, and a value closer to 1 indicates a more crystalline polymer. A completely amorphous polymer has a crystallinity degree of 0, signifying that the polymer lacks a regular structure and is entirely composed of amorphous regions. Conversely, a fully crystalline polymer has a crystallinity degree of 1, indicating a highly regular and well-defined crystalline structure. The values of ΔH_m are extracted from Figures 3,4,5 and 6and presented in Table 1. These values are critical in understanding the thermal properties and crystallinity of the polyurethane samples. This composition is expected to exhibit the highest Tg and degree of crystallinity among all samples. The presence of chain extender further influences the crystalline structure and thermal stability, allowing for the optimization of thermal and mechanical properties in polyurethane materials.

The thermal properties of the polyurethane samples are notably influenced by the type of diisocyanate and the presence of chain extender, leading to diverse performance characteristics. The PCL 10 + HDI + BD sample shows a moderate degree of crystallinity and glass transition temperature (Tg), indicating balanced thermal properties. In contrast, PCL 40 + HDI + BD has the highest degree of crystallinity (29.21%) and Tg (38.64°C) among the HDI-based samples, suggesting superior structural integrity and excellent thermal stability. On the other hand, PCL 10 + MDI + BD exhibits very low crystallinity (2.67%), making it more amorphous, but compensates with the highest Tg (26.67°C) among all samples, indicating remarkable thermal stability. PCL 40 + MDI + BD, with its moderate crystallinity and Tg, offers balanced thermal properties.

In conclusion, PCL 40 + HDI + BD is ideal for applications requiring both high structural integrity and thermal stability, whereas PCL 10 + MDI + BD is more suitable for scenarios demanding the highest thermal stability despite lower crystallinity. The presence of chain extender and the molecular characteristics of the PCL and diisocyanates are crucial in determining the crystallinity and thermal behavior of these polyurethane samples.

4. DISCUSSION

In this study, the thermal and structural properties of various polyurethane compositions were examined using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR). The data, presented in Table 1, include the melting enthalpy (ΔH_m) values derived from Figures 3,4,5 and 6 along with the glass transition temperatures (Tg) and degrees of crystallinity (Cr).

DSC analysis reveals critical insights into the impact of polycaprolactone (PCL) molecular weight, diisocyanate structure, and the presence of chain extender on the thermal properties of polyurethanes. Higher molecular weight PCLs, as expected, enhance the crystallinity and Tg of the resulting polyurethanes, evidenced by distinct endothermic and exothermic peaks in the DSC thermograms. For instance, the PCL 40 + HDI + BD composition demonstrated the highest crystallinity at 29.21%, accompanied by a Tg of 38.64°C, indicating a well-balanced thermal and mechanical profile. In contrast, PCL 10 + MDI + BD exhibited the highest Tg of 48.75°C, albeit with a lower crystallinity of 10.42%, highlighting the significant influence of the aromatic diisocyanate (MDI) in enhancing thermal stability.

The structure of diisocyanates plays a pivotal role in determining the Tg and crystallinity of polyurethanes. MDI-based polyurethanes generally display higher Tg values compared to those

synthesized with HDI, an aliphatic diisocyanate. This can be attributed to the increased rigidity and thermal stability provided by the aromatic structure of MDI, resulting in sharper and more defined thermal transitions in the DSC thermograms. This characteristic is crucial for applications requiring materials with superior thermal stability.

The inclusion of chain extender, 1,4-butanediol (BD), significantly influences the phase behavior and thermal properties of polyurethanes. The DSC analysis indicates that chain extender enhances the formation of hard segments within the polymer matrix, which is reflected by increased enthalpy changes during phase transitions. This enhancement is also evident in the FT-IR spectra, where the intensity and position of the carbonyl (C=O) stretching bands correspond to the formation of urethane linkages, indicative of hard segment formation (Peng et al., 2024).

FT-IR spectroscopy further corroborates the DSC findings by providing detailed insights into the molecular interactions within the polyurethane matrix. Higher molecular weight PCL results in more pronounced C=O and C-O-C bands, indicative of increased ester content and crystalline structure. The spectral differences between MDI-based and HDI-based polyurethanes, particularly in the carbonyl stretching region (1700-1730 cm^{-1}), highlight the distinct structural characteristics imparted by the different diisocyanates. The broader bands observed in HDI-based polyurethanes compared to the sharper bands in MDI-based ones reflect the inherent differences between aliphatic and aromatic structures.

In conclusion, the study underscores the significant impact of PCL molecular weight, diisocyanate structure, and chain extender on the thermal and structural properties of polyurethanes. Higher molecular weight PCL and aromatic diisocyanates (MDI) notably enhance the Tg and crystallinity, resulting in polyurethanes with superior thermal stability and mechanical properties. The presence of chain extender further fine-tunes these properties, offering a pathway for optimizing polyurethane materials for various high-performance applications. Considering both crystallinity and Tg, the PCL 40 + HDI + BD composition appears to offer the most balanced properties. However, for applications demanding the highest thermal stability, the PCL 10 + MDI + BD composition, with its highest Tg, may be more suitable despite its lower crystallinity.

5. CONCLUSION

The study demonstrates that the thermal and structural properties of polyurethanes are significantly influenced by the molecular weight of polycaprolactone (PCL), the structure of diisocyanates, and the inclusion of chain extender, 1,4-butanediol (BD). (Liu et al., 2018; Peng et al., 2024) Higher molecular weight PCLs and aromatic diisocyanates, such as MDI, enhance the glass transition temperature (Tg) and degree of crystallinity, leading to polyurethanes with superior thermal stability and mechanical properties. The incorporation of BD as a chain extender significantly improves the formation of hard segments within the polymer matrix, which is crucial for enhancing thermal stability and mechanical strength. Notably, the PCL 40 + HDI + BD composition exhibits the most balanced thermal and mechanical properties due to its optimal crystallinity and Tg. However, for applications demanding maximum thermal stability, the PCL 10 + MDI + BD composition, with the highest Tg, is preferable despite its lower crystallinity. These findings provide a pathway for optimizing polyurethane materials for various high-performance applications by fine-tuning the molecular characteristics, diisocyanate structure, and chain extender inclusion.

6. ACKNOWLEDGE

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Conflict of Interest The authors declare that there are no conflicts of interest.

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COMBINED USE OF ACUPUNCTURE AND MAJOR OZONE THERAPY IN FACIAL PARALYSIS: 2 CASES

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ABSTRACT

Background of the Study: Peripheral facial paralysis (PFP) is a disease that causes partial or complete paralysis of the facial muscles and occurs as a result of the 7th cranial nerve being affected by various reasons such as compression or cutting. This condition affects the upper and lower facial muscles, which can lead to weakness of the facial muscles, loss of facial expression, loss of taste and corneal sensations, and vision problems. PFP can be congenital or develop due to various reasons such as infections, trauma, tumors, chronic systemic diseases and metabolic disorders. Although the treatment is not definitive, the success rate can be up to 90% with steroid and combined treatments. The spontaneous recovery rate is generally around 80%.

Aim: With this publication, we aimed to describe the situation of two cases of facial paralysis that we treated using traditional complementary treatments and to evaluate the literature.

Material and Method: Acupuncture treatment and major ozone therapy, which are traditional complementary medicine methods, were used in the treatment of two cases with facial paralysis. In the acupuncture treatment, 10 sessions were applied and body acupuncture points and ear acupuncture were used. Major ozone therapy was applied for a total of 10 sessions. The application started with 10 gamma and increased to 50 gamma.

Results: We achieved significant improvement in both of our cases so we can achieve positive results with the combined use of acupuncture and major ozone therapy in the treatment of facial paralysis.

Conclusion: The literature states that acupuncture shows better regression in pain scores. However, there are insufficient studies on its combined use with major ozone therapy in the treatment of facial paralysis.

Keywords: Acupuncture, facial paralysis, major ozone therapy

1. INTRODUCTION

Peripheral facial paralysis (PFP) is a disease that causes partial or complete paralysis of the facial muscles and occurs as a result of the 7th cranial nerve being affected by various reasons such as compression or cutting. This condition affects the upper and lower facial muscles, which can lead to weakness of the facial muscles, loss of facial expression, loss of taste and corneal sensations, and vision problems. PFP can be congenital or develop due to various reasons such as infections, trauma, tumors, chronic systemic diseases and metabolic disorders. Although the treatment is not definitive, the success rate can be up to 90% with steroid and combined treatments. The spontaneous recovery rate is generally around 80% (Pavlou, 2011 & Clement, 2000).

Complementary Therapies aim to eliminate the symptoms of diseases together with modern medicine. Complementary medicine modalities are the methods used when an adequate solution cannot be found in modern medicine (Törüner vd., 2009).According to the World Health Organization (WHO), traditional medicine is “the knowledge and skills that can be explained or not, based on theories, beliefs and experiences specific to different cultures, used to prevent, diagnose, cure or treat physical and mental diseases, as well as to maintain good health.” and a set of practices” and has a long history (WHO, 2000).

Acupuncture has been practiced widely in China for more than 4000 years and is an integral part of traditional Chinese medicine. It was first described in the medical writings The Yellow Emperor's Classic of Internal Medicine around 200 BC (Cao, 2002). Acupuncture is based on the principle of stimulating chi flow in the meridians by stimulating certain points on the body with low electrical resistance with heat, light, ultrasound or a steel needle (Da Silva, 2015).

The number of needles to be used during acupuncture application is related to the area where the complaints are localized and the needle sensitivity of the patient. During the application, manual or electrical stimulation can be applied to the needles, or the needle can be placed at the point in the relevant part and left there and waited (Karasimav & Yıldız, 2015).

Ozone applied in ozone therapy is the chemical cousin of the oxygen molecule and can be added to the treatment of many diseases (Aytaçoğlu, 2014). Ozone therapy has begun to be used more frequently today for acute and chronic pain. Especially in the literature, ozone applications in the field of pain are included in many aspects in the treatment of pain originating from the musculoskeletal system (Alberto, 2011; Magalhaes, 2012). In addition, it also has effects on the vascular system. Hyperoxygenation provided by ozone provides vascularization in the tissue (Bocci, 2010).

With this publication, we aimed to describe the situation of two cases of facial paralysis that we treated using traditional complementary treatments and to evaluate the literature.

2. MATERIAL AND METHOD

We aimed to describe the situation of two cases of facial paralysis that we treated using traditional complementary treatments. We presented two cases who applied to our GETAT center with the complaint of facial paralysis. Acupuncture treatment and major ozone therapy, which are traditional complementary medicine methods, were used in the treatment of two cases with facial paralysis. In the acupuncture treatment, 10 sessions were applied and body acupuncture points and ear acupuncture were used. Major ozone therapy was applied for a total of 10 sessions. The application started with 10 gamma and increased to 50 gamma.

2.1. Case 1: A 43-year-old, 69 kg, 156 cm tall female patient was admitted to Getat Polyclinic with complaints of facial paralysis and electric shock on her face. VAS (pain rating scale) was 7. On the side of the face where facial paralysis developed, muscle tone was reduced and there was no active movement.

She was treated with both body and ear acupuncture. A total of 10 sessions were applied; Initially, sessions were held once a week, then every 2-3 weeks. In the maintenance treatment, MAH ozone therapy was administered for 5 sessions, starting from 10 gamma and going up to 50 gamma. Additionally, 20 gamma ozone minor was applied for immune system support. B vitamin supplements, vitamin D, magnesium and calcium containing medications were prescribed.

In body acupuncture, Yin-tang, Du-20,21, St-8, GB-2, local points (ahsi points) on the paralyzed side, ST-36, GB-34, Liv-3, Kid-6,7, SP-6 While using ,9, TW-5,6, LI-4,11, P-6, LU-9, H-7, Jerome, zero, shen-men, kidney and Facial nerve points in the ear were focused on. In the first weeks, Hualong intradermal semipermeable needles were used in the ear, and later, when the session interval was opened, ASP needles were used. E frequency and Nogier frequency were applied to the paralyzed side with an electroacupuncture device.

2.2. Case 2: A 52-year-old, 78 kg, 176 cm tall male patient was admitted to Getat Polyclinic due to Ankylosing Spondylitis and facial paralysis. VAS (pain assessment scale) was determined as 7. The muscle strength on the side of the face with facial paralysis was generally at a value of 1, slight contraction was felt by palpation, and no active movement was evident. After the informed consent form was obtained and the necessary information was given, the patient was treated with both body and ear acupuncture. A total of 10 sessions were applied; Initially, sessions were held once a week, then every 2-3 weeks.

In the maintenance treatment, MAH ozone therapy was administered for 5 sessions, starting from 10 gamma and going up to 50 gamma. B vitamin supplements, vitamin D, magnesium and calcium containing medications were prescribed.

In body acupuncture, Yin-tang, Du-20,21, St-8, GB-2, Local points on the paralyzed side, ST-36, GB-34, Liv-3, Kid-6.7, SP-6.9, TW-5.6, LI-4.11, P-6, LU -9, H-7Focused on Jerome, zero, shen-men, kidney and Facial nerve points in the ear. In the first weeks, Hualong intradermal semipermeable needles were used in the ear, and later, when the session interval was opened, ASP needles were used. E frequency and Nogier frequency were applied to the paralyzed side with an electroacupuncture device.

3. RESULTS

Our first patient had a 60% reduction in the patient's electric sensation and pain was noted in the 5th session. At the end of the treatment, the tone in the affected muscles approached normal and active movement began.

Our second patient also had a 60% reduction in the patient's pain was noted in the 5th session too. Muscle strength was 2+ and active movement was present when gravity was eliminated. At the 10th session, he no longer had pain (VAS = 0). His muscle strength became 3+ and he gained active movement against gravity.

4. DISCUSSION

In their study, Bokhari and Zahid (2010) studied 49 facial paralyzed patients who were treated with 88 conventional methods but did not show signs of recovery even after the third week. They examined the changes in the patients' facial functions by applying electroacupuncture treatment to the patients. They stated that the facial functions of all cases returned to normal as a result of electroacupuncture treatment and emphasized that electroacupuncture is effective in renewing the functions of the muscles affected by facial paralysis.

Fabrin et al. (2015) also observed that there was an improvement in facial movements when they applied acupuncture treatment to a patient with 20-year sequelae of peripheral facial paralysis and examined the EMG values before and after the treatment.

Wu ve Tai (2017) said that; although there are no high-quality randomized controlled studies and clinical evidence about the effectiveness of acupuncture in the treatment of facial paralysis, current research results have revealed that acupuncture plays an important role in the treatment of facial paralysis and is effective on the sequelae of the disease.

İnanç (2014) showed in his study that; he stated that a patient with facial paralysis was treated with corticosteroids and antiviral agents, but the patient did not improve after six weeks and he applied electroacupuncture treatment to the patient. He explained that the patient's affected facial half recovered completely at the end of the acupuncture treatment and emphasized that more clinical and electrophysiological studies are needed to demonstrate the effectiveness of acupuncture in facial paralysis.

While the results of acupuncture application in the treatment of facial paralysis are included in the literature, no source regarding the use of major ozone therapy has been found. In this respect, our case has an important place in the literature.

5. CONCLUSION

In both of our cases, active muscle movement was regained and muscle strength increased after treatment. Additionally, there were significant changes in VAS values.

In general, in cases coming to the GETAT clinic with any complaints, different Getat methods can be safely applied together in the treatment by questioning the correct anamnesis. Especially in people who have had facial paralysis and have not responded to conventional treatments, the combined use of acupuncture and major ozone therapy can be added to the treatment program.

With acupuncture treatment, Qi energy flow in the body is regulated, and the immune system is strengthened with Ozone therapy. With the combination of these two treatment methods used, tissue regulation and perfusion are increased, and neural network structuring and nutrition are improved. In

patients who receive conventional treatment but whose pain does not go away and who have different complaints, positive results can be obtained by using different GETAT applications in appropriate combination.

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Pharmacogenetic Analysis of 635 Patients by Next Generation Sequencing

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ABSTRACT

Background of the Study: One of the biggest problems in the protocol of drug therapy is that the same dose of drug may cause very different responses and side effects in patients with the same phenotype. The basis of these different drug responses is the different genetic characteristics and lifestyles of the patients. This situation leads to different responses to drugs among individuals.

Aim: In this study, we aimed to perform sequence analyses of 69 pharmacogenes by next generation sequencing (NGS) in 635 patients admitted to our outpatient clinic in the last year.

Material and Method: DNA was isolated from patients' peripheral blood. DNA libraries were constructed with the NadPrep DNA Universal Library Preparation Kit. Sequencing was performed on an MGI DNBSEQ-G50RS instrument. Analysis of 69 pharmacogenes in the pharmacogenomics database (PharmGKB) was performed on the Genomize Seq platform.

Results: 53% of the study group was female and 47% was male. The average age of the group was 33 years. A total of 1409 variants were detected in 69 genes. 126 of these variants were registered in PharmGKB. At least one variant was detected in all patients. 66 of 126 variants had no clinical annotation, while 60 variants had clinical annotation in 372 patients.

Conclusion: As a result, 59% of our patients carried a variant with clinical annotation in pharmacogenes. In the field of personalised medicine, which is frequently mentioned today, it will be inevitable to adjust the appropriate drugs and doses according to the genetic structure of each individual. This will also prevent unnecessary health expenditures.

Keywords: Pharmacogenetics, personalised medicine, pharmacogenomics, pharmacodynamics

1. INTRODUCTION

One of the biggest problems in drug treatment protocols is that the same drug dose may cause very different responses and side effects in patients with the same phenotype. The basis of these different responses observed in drug response is the different biological characteristics and lifestyles of the patients (Meyer, 2000). In addition, in the 1950s, in relation to adverse drug reactions (ADR), acute haemolysis was reported in some individuals taking isoniazid, primaquine and succinylcholine, and it was reported that this toxicity was caused by a deficiency of the enzyme glucose-6-phosphate dehydrogenase in these individuals. (Clayman et al. 1952, Alving et al.1956). Although the term pharmacogenetics was first used by Vogel in 1959, today, the most important step in personalized drug therapy is that studies have shown that genetic variants in individuals can contribute up to 95% in determining interindividual variability in drug response (Vogel 1959, Kalow, Tang & Endrenyi,1998). Genetic variation among individuals plays a role in determining drug response through drug efficacy and toxicity. The effect of this genetic variation on the efficacy and toxicity of the drug is realised by altering the pharmacokinetics and pharmacodynamics of the drug (Barbarino et al, 2018). Therefore, Single Nucleotide Polymorphisms (SNPs) occurring in drug metabolising enzymes, drug transporters and genes encoding the protein targeted by the drug constitute the genetic diversity that causes different responses to a drug in different individuals. SNPs may affect the concentration of drug reaching the target and, accordingly, these drug concentrations may be inappropriate in some individuals. This may result in reduced efficacy or toxicity (Attar & Lee, 2003).

It has been reported that patients treated with a pharmacogenomic approach have significant decreases in hospitalisations and ADR due to drug toxicity compared to patients receiving standard treatment, and it has been emphasised that this may prevent unnecessary expenditures in health services (Swen et al. 2023, Karamperis et al.2021). Therefore, it is extremely important to reveal pharmacogenomic information of patients before drug use. The publicly available pharmacogenomics knowledge base

(PharmGKB), supported by the National Institutes of Health (NIH) and the National Institute of General Medical Sciences (NIGMS), is an important resource that provides information on drug-gene pairs, demonstrating the clinical relevance of variants related to the pharmacogenomic pathway (Whirl-Carrillo et al., 2021).

In this study, we performed sequence analysis of 69 genes called Very Important Pharmacogene (VIP) in PharmGKB by Next Generation Sequencig (NGS) method in 635 patients who applied to our medical genetics outpatient clinic in the last year and revealed variants with clinical annotation.

2. MATERIAL AND METHOD

DNA isolation and extraction of genomic DNA from peripheral blood of the patients was performed using the Kapa NGS DNA extraction kit (Roche Molecular Systems, Inc., Germany). The purity and concentration of the extracted DNA was measured with a Qubit fluorometer (ThermoFisher Scientific, USA). To generate a high-quality library, the NadPrep DNA Universal Library Preparation Kit (Nanodigmbio (Nanjing) Biotechnology Co., Ltd, China) was used to prepare double-stranded DNA (dsDNA). This kit includes the Library-Prep Module and the Adapter Primer Module. The NAD Panel containing a 5'-biotinylated labeled probe optimized for targeted capture applications for NGS was used in libraries prepared with the NadPrep DNA Universal Library Preparation Kit (for MGI). In this study, 500 ng of DNA from each library was used for hybrid capture. A single-stranded circular DNA library was prepared with the MGIEasy Circularization Kit (MGI Tech Co., Ltd, China). Single-stranded circular DNAs were converted to nanoballs (DNB) by circular amplification using the DNB SEQ-G50RS High-Capacity Sequencing Kit (MGI Tech Co., Ltd, China). The array cartridge was prepared, and DNBs were placed in the DNB tube and inserted into the instrument. The samples were passed through the flow cell placed in the device and sequencing was performed on a DNBSEQ-G50RS device (MGI Tech Co., Ltd, China). The DNA sequencing data obtained were in FASTQ (FASTA Quality) format and the sequencing data were quality-checked. The quality-checked sequences were aligned with the reference genome GRCh37 (hg19) to create a file in SAM (Sequence Alignment/Map) format. This file was then converted to BAM (Binary Alignment/Map) format. For annotation of sequences to detect genetic variants, the BAM file was converted to VCF (Variant Call Format), and bioinformatics analysis was performed on the Genomize Seq platform. Variants with low coverage and reading depth were excluded.

3. RESULTS

In this study, we performed next generation sequencig (NGS) analysis of 69 pharmacogens in 635 patients admitted to our outpatient clinic within the last year. We detected a total of 9485 variants in these patients. The most variants were found in ALK, CYP2D6, IFNL3, SCN5A and TPMT genes (Figure-1). Most of the previous variants were repeat variants. The total number of different variants was 1409. The genes with the most different variants were CFTR, RYR1 and SLC19A1 genes (Figure-2). Of these 1409 variants, the total number of variants registered in PharmGKB is 126. The genes with the most variants registered in PharmGKB are CFTR, DPYD, CYP2B6, CYP2D6, CYP2C19, RYR1 (Figure-3). When these 126 variants were analyzed in terms of Tier classification, it was found that 65% of the variants were in Tier1, 20% in Tier2 and approximately 15% in Tier3 (cancer genome)(Figure-4). However, only 60 of these 126 variants had clinical annotations and 372 patients had one of these variants. Respectively, Genes with the most clinical annotations CFTR, DPYD, CYP2C19, CYP2D6 and others (Figure-5).

4. DISCUSSION

The Pharmacogenomics Knowledge Base (PharmGKB) is a comprehensive resource that provides up-to-date information on drug-gene pairs, including drug label descriptions and clinical guideline descriptions (Whirl-Carrillo et al., 2012). Two components of pharmacogenomics, pharmacokinetics (what the body does to the drug) and pharmacodynamics (what the drug does to the body), contribute to drug response. In vitro and in vivo studies of the four main processes involved in drug pharmacokinetics (absorption, distribution, metabolism and excretion) and how they determine inter-individual variability in drug use have made great advances in the last 50 years. Such advances have also shown that genetic factors play an important role in determining drug pharmacokinetics.

CFTR modulators have changed patients' lives and can be divided into enhancers (which increase chloride ion conductance) and correctors (which target abnormal protein folding and increase CFTR

expression on the cell membrane). Ivacaftor G551D mutation, initially tested in 4% of patients with this disease, led to a 55% reduction in the pulmonary exacerbation rate, while the combination of elexacaftor, tezacaftor and ivacaftor used in patients with at least one copy of Phe508del reduced the exacerbation rate by 63% (Tewkesbury, Robey & Barry, 2021). In our study, the gene with the highest clinical annotation was the CFTR gene. PharmGKB was also among the genes with the highest number of registered genes. In addition, the importance of germline polymorphisms in the determination of the dose of anticancer drugs has been shown in the literature, especially in the DPYD gene, one of the genes containing variants with clinical annotation in our study. In this regard, polymorphisms in DPYD and fluoropyrimidines were emphasized to be important (Henricks et al.2018).

For several drug prescriptions in the EU, testing for variants is recommended prior to treatment with cytochrome P450 2C19 (CYP2C19) atazanavir, for example for tests (Huebner, Steffens & Scholl, 2022). CYP2C19 mediates the metabolism of a number of commonly prescribed drugs, including proton pump inhibitor compounds such as propanolol, imipramine and most notably omeprazole. Loss of CYP2C19-function alleles. Among 1815 patients at 7 institutions, those with loss of allele function (31.5%) had more cardiovascular events when treated with clopidogrel compared with treatment with alternative medicines (23.4 vs 8.7/100 patient-years, risk ratio 2.26, 95% CI: 1.18 to 4.32; p = 0.013). 54 A recent small prospective randomized controlled trial reported a large reduction in late coronary events with a pharmacogenomics-based strategy for clopidogrel (Notarangelo et al.2018). Currently, the most important Phase 1 metabolizing enzymes are those in the cytochrome P (CYP) 450 family, particularly CYP2C9, CYP2C19 and CYP2D6. They are responsible for the metabolism of approximately 80% of commonly prescribed drugs and variations in these genes affect the metabolism of 60% of these drugs (Yang et al, 2015). Although the total number of variants detected in our study was small, the CYP2C19 gene was among the genes with the highest number of variants with clinical annotation.

Although the application of pharmacogenomic analyses to tailor drug dosing to individuals is very limited, recent studies have shown that personalized medicine can significantly improve treatment, disease prevention and health maintenance in a large number of individuals. Knowing how genetic variations can alter the efficacy, safety and tolerability of medicines and identifying new algorithms accordingly can often lead to an adjustment in recommended drug dosages, an improvement in efficacy and a reduction in drug-related side effects. This would also be an important factor in avoiding unnecessary health expenditure.

5. CONCLUSION

As a result, 59% of our patients carried a variant with clinical annotation in pharmacogenes. In the field of personalised medicine, which is frequently mentioned today, it will be inevitable to adjust the appropriate drugs and doses according to the genetic structure of each individual. The active application of pharmacogenomic analyses in practice will make a great contribution in preventing both unnecessary drug use and unnecessary health expenditures.

Conflicts of Interest
The author declare that they have no conflicts of interest.

Compliance with ethical standarts
Before the initiation of the study, all participants received an explanation of the procedure and the risks that would later be faced in their participation, and they provided informed consent to participate in this study. During this study, the World Medical Association (WMA) HELSINKI Declaration (and/or World Psychiatric Association HAWAII Declaration Good Clinical Practices) were followed according to the ethics committee of Selçuk University Faculty of Medicine.

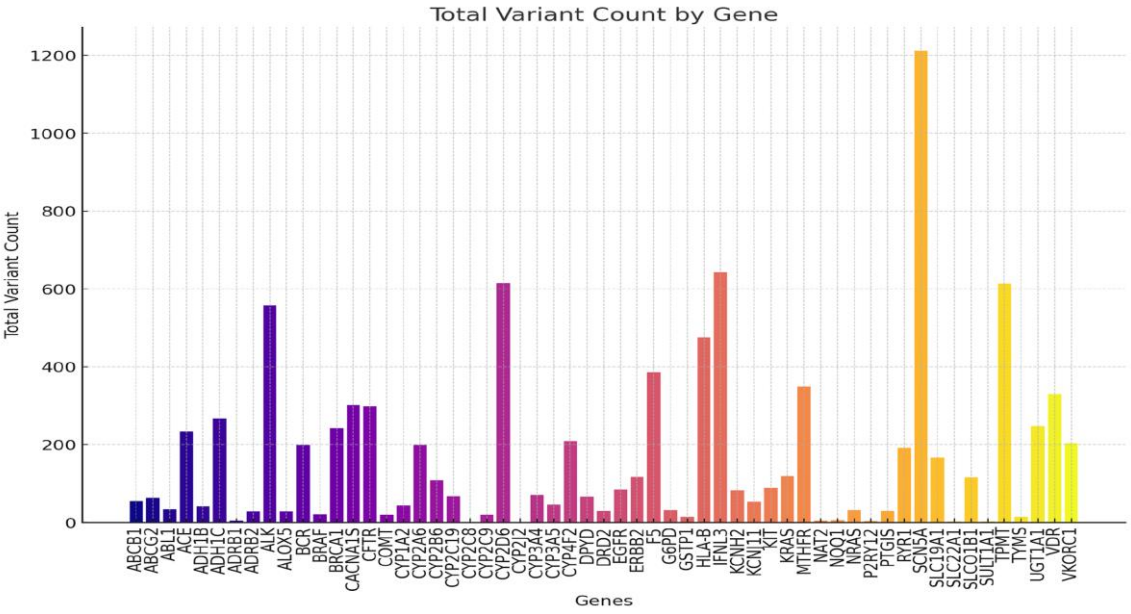

Figure 1: Distribution of 9485 variants detected in 635 patients according to genes

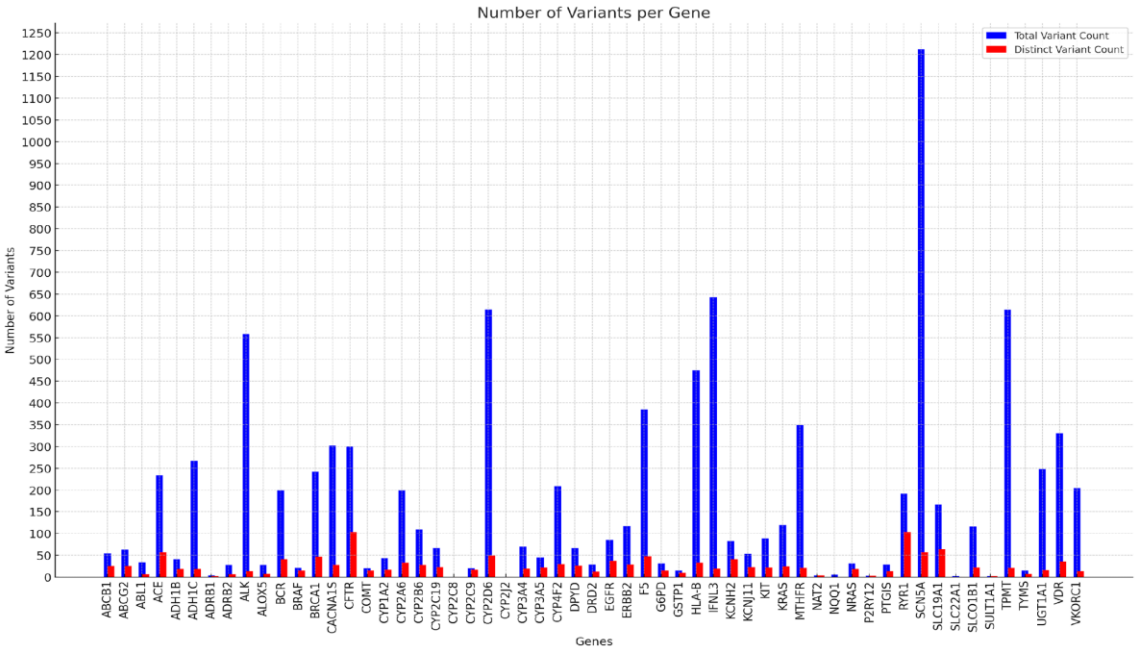

Figure 2: Distribution of total and distinct variants detected in genes

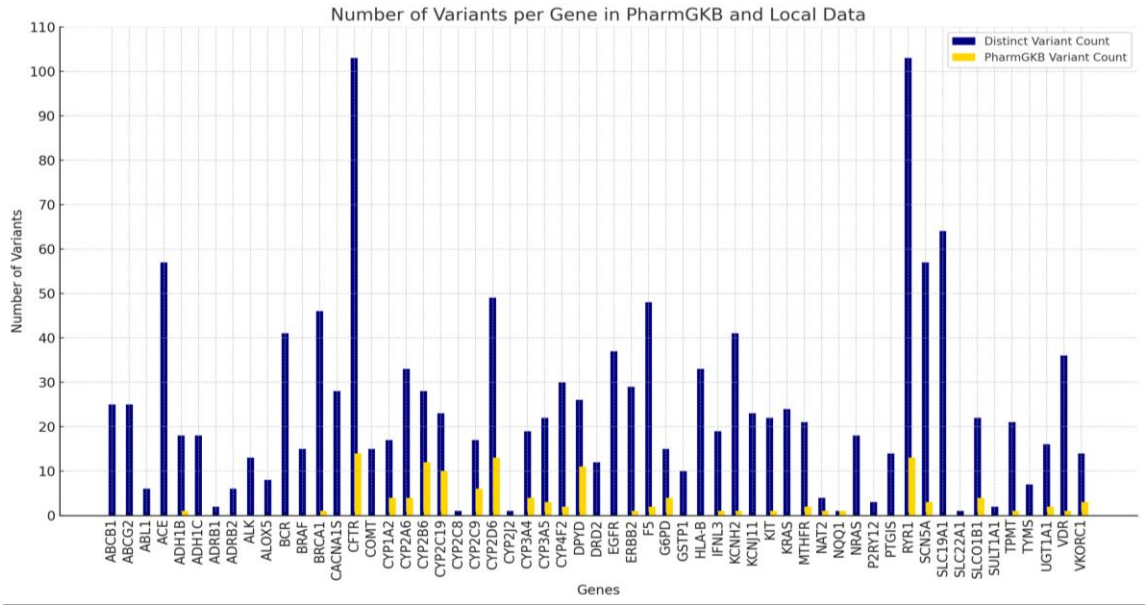

Figure 3: Distribution of the number of distinct variants detected in genes and registered in PharmGKB

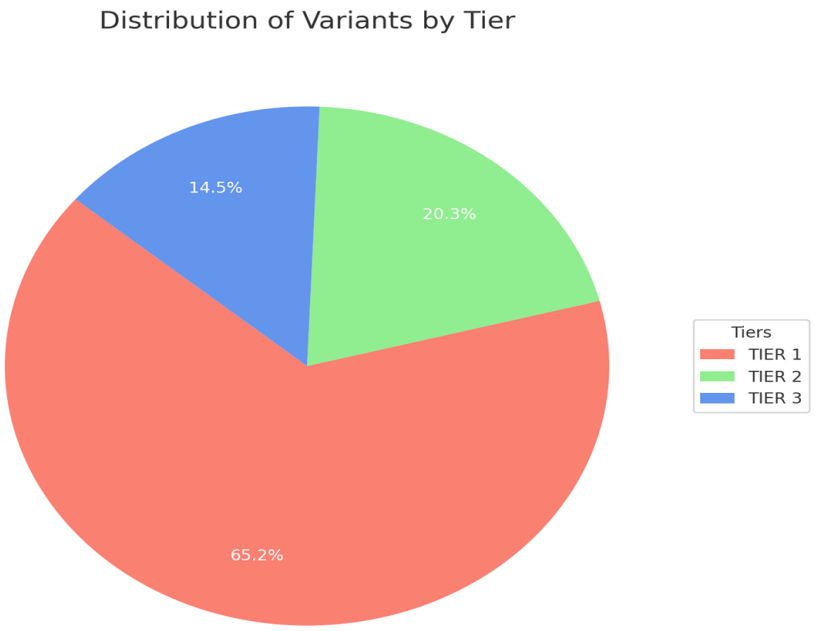

Figure 4: Tier classification of 126 variants

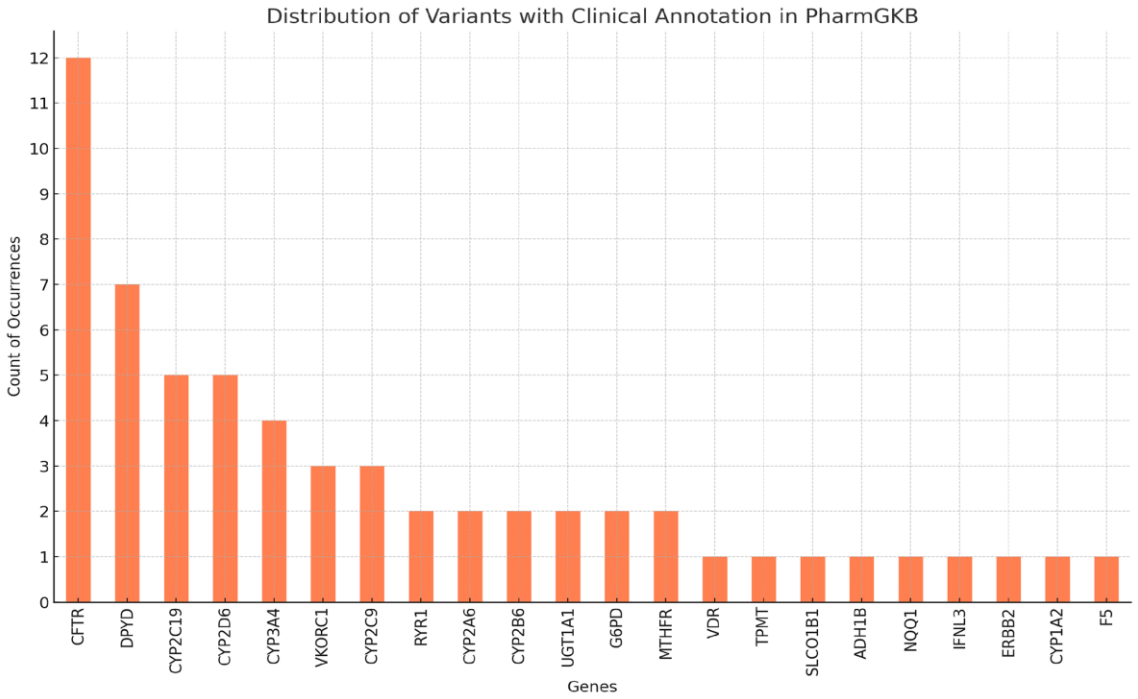

Figure 5: Distribution of 60 variants with clinical annotation among 126 variants according to genes

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Effects of Putrescine on BV2 Microglia Cell line

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ABSTRACT

Background of the Study: Neurodegenerative diseases, marked by the progressive degeneration of neuronal structure and function, are widely prevalent and lack effective treatments. Microglia, the central nervous system's primary immune cells, are crucial in mediating neuroinflammatory processes that accelerate neurodegeneration. Modulating microglial activity is suggested to potentially mitigate these disorders.

Aim: The purpose of this study is to investigate the potential therapeutic benefits of putrescine on neurodegenerative diseases by evaluating its effects on cell proliferation and gene expression in the BV2 microglial cell line.

Material and Method: The BV2 microglial cell line served as an in vitro model for microglial behavior. Cell viability was initially assessed through an MTT assay to confirm cell health after treatment. This was followed by quantitative PCR to analyze the expression levels of the PADI2, CRP, MEK1, ERK-2, and IL-6 genes, using GAPDH as a reference. Treatments included putrescine alone and combined with LPS to induce inflammatory conditions.

Results: In the effects of putrescine on BV2 microglia cell line, no statistically significant increase was detected in CRP and IL-6 levels, while a statistically significant increase was detected in MEK1 and ERK2 genes. In addition, the addition of putrescine significantly increased the expression of the PADI2 gene, which is thought to be involved in the pathogenesis of neurodegenerative diseases.

Conclusion: Putrescine markedly affects gene expression in BV2 microglial cells, enhancing profiles associated with anti-inflammatory and neuroprotective functions. These results propose putrescine as a viable therapeutic option for managing neurodegenerative diseases through microglial modulation. Future studies should clarify the precise mechanisms of putrescine's actions on microglial cells and assess its clinical effectiveness.

Keywords: Neurodegeneration, microglia, putrescine, neuroprotection

1. INTRODUCTION

Putrescine ($H_2N(CH_2)_4NH_2$), a small aliphatic diamine, belongs to the group of spermidine and spermine polyamines (Bardo´cz, 1995). Polyamines, which are aliphatic molecules containing 2, 3 or 4 amino groups and are widespread in nature in almost all eukaryotes and prokaryotes, have been shown to be directly involved in the stimulation of DNA synthesis due to their ability to bind to nuclear macromolecules (Pignata et al., 1999, Tabor & Tabor 1984). Thus, polyamines are involved in the regulation of spermine and putrescine DNA, RNA and protein synthesis; they are therefore essential for the proliferation of cells both normally and in the process of carcinogenesis. Disruption of cellular polyamines is associated with various pathological conditions, including inflammation and cancer; hence, cellular pathways of polyamine have been investigated as targets for cancer chemotherapy (Rial et al., 2009). Although polyamines are critical for proper cell growth in normal biological processes of cells, their excessive accumulation can disrupt normal cell function and thus they are associated with apoptotic response, inflammation and carcinogenesis (Gerner & Meyskens 2004, Zaletok et al., 2004). In addition, putrescine, spermidine and spermine are the most important polyamines found in the brain and are known to play an important role in the regulation of cell membrane dynamics of neurons (Casero, Stewart & Pegg, 2018). Putrescine stabilizes the structure of DNA and may participate in the functioning of cell membranes and interacts with a number of ion channels and has affinity for many receptors (Zdrojewicz, Lachowski & Znaczenie, 2014).

Putrescine is the result of decarboxylation of spermidine and spermine by ornithine decarboxylase (ODC), the first enzyme in the biosynthesis of polyamines. Copper-containing diamine oxidase (DAO) catalyzes the oxidative deamination of putrescine to produce -aminobutyraldehyde, which has been used in in vitro models of carcinogenesis to demonstrate the role of ODC deregulation and polyamine overproduction in tumor development (Kingsnorth et al., 1983, Paulsen et al., 1997). Affecting this enzyme is the simplest and most widely used method of controlling putrescine concentration. For this purpose, the inhibitor-eflornithine is frequently used in studies. Currently, studies on the effects of putrescine on infertility, embryonic development, hirsutism, epilepsy, neurodegenerative diseases such as Alzheimer's and Parkinson's, and the process of carcinogenesis have intensified (Zdrojewicz, Lachowski & Znaczenie, 2014).

Today, neurodegenerative diseases characterized by progressive degeneration of neuronal structure and function are widespread and lack effective treatments. However, Microglia cells are resident macrophages in the central nervous system (CNS) and have important immune and support roles in the brain and spinal cord. Functions of microglia cells; a) Immune Surveillance: They play an important role in the defense mechanism of the central nervous system itself, b) Phagocytosis: Microglia cells clear damaged neurons, apoptotic cells and other cellular debris by phagocytosis, c) Neuroinflammation: Microglia cells regulate neuroinflammatory responses by secreting pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNF-α) and chemokines. Chronic neuroinflammation is associated with Alzheimer's, Parkinson's and other neurodegenerative diseases. These diverse functions of microglia cells are vital for the healthy functioning of the central nervous system and the management of disease processes. Defects in the function of these cells may contribute to the pathogenesis of various neurological and psychiatric diseases (Nayak, Roth & McGavern 2014, Umpierre & Wu 2021, Sarlus & Heneka 2017).

In this study, we planned to investigate the possible effects of putrescine molecule on BV2 microglia cell line.

2. MATERIAL AND METHOD

Putrescine was purchased from Sigma-Aldrich Chemical Co. and dissolved in incompleted DMEM (Gibco, USA) as a 10 µM stock solution. and dilutions were made in DMEM (1µM), was filtered by 0.22 um filtration. The BV2 murine microglial cell line were used. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) in a humidified 5% CO2 incubator at 37°C. After reaching 80-90% confluence, the cells were detached using trypsin-EDTA (Sigma-Aldrich) and plated in 6-well plates (0.3×10^6 cells/well) for RNA extraction. After 24 h, we treated the BV2 cells with or without putrescine for 24 h. The untreated BV2 cells were used as a control. MTT study was performed to determine the appropriate dose of Putresin and the appropriate dose was determined according to the IC50 value.
Total RNA was isolated using the Ribo Ex (GeneAll Biotechnology, Korea) according to the protocol. Synthesis of cDNA from the total RNA was carried out OneScript® Hot cDNA Synthesis Kit. cDNA was diluted 1:6 in sterile water. qPCR was performed on a Lightcycler 480 instrument (ROCHE, Light Cycle 480 II, Germany) using GoTaq® qPCR Master Mix (Promega, USA). The housekeeping gene GAPDH was used as the internal control. Five genes (IL-6 (Interleukin-6), PADI2 (peptidyl arginine deiminase type II), CRP (C-reactive protein), MEK1 (Mitogen-activated protein kinase kinase), ERK2 (extracellular signal-regulated kinases) were selected. A relative fold change in expression of the target gene transcript was determined using the comparative cycle threshold method ($2^{-\Delta\Delta CT}$). Specific primers for PCR amplification were designed using IDT The primers used are listed in Table 1. After the end of incubation period, treated and untreated cells were observed under phase contrast inverted microscope equipped with a digital camera (Nikon, Japan) at 100X magnification.
Any significant morphological difference was not observed in putrescine treated BV2 cells compared to the control. Cell shrinkage, loss of cell adhesion or reduced cell density along with cell debris were not also observed in treated cells (Figure1).

3. RESULTS

MTT study was performed to determine the appropriate dose of Putresin and the appropriate dose was determined according to the IC50 value. Accordingly, the dose was determined as 50uM (Figure 2). In

our study, we examined the expression levels of 5 genes (PADI2, CRP, ERK2, MEK1, IL-6) compared to the control to see the effect of putrescine on BV2 microglia cells. Accordingly, while no statistically significant increase was detected in CRP and IL-6 genes compared to the control, a statistically significant expression increase was observed in PADI2, ERK2 and MEK2 genes (Figure 3). The p values were $p<0.001$, $p<0.05$, $p<0.001$, respectively.

4. DISCUSSION

In a study on weaned piglets, exogenous putrescine supplementation suppressed the expression of TNF-α, IL-6 and IL-8, and dietary putrescine supplementation was also reported to reduce the mRNA levels of TNF-α, IL-6 and IL-8 and their upstream regulator nuclear receptor kappa B p65 subunit in the jejunal mucosa of piglets (Liu et al., 2019). In addition, in a study to demonstrate the effect of another polyamine, spermidine, on enterocytes, spermidine deprivation was reported to significantly increase ($p < 0.05$) the mRNA abundance of the pro-inflammatory cytokines IL-8, IL-6 and TNF-α, and the addition of spermidine attenuated the overexpression of these inflammatory pro-inflammatory cytokines (Wei et al., 2022). In addition, a study on the BV2 microglia cell line showed that spermidine treatment reduced the production of pro-inflammatory cytokines, including IL-6 and TNF-α, by suppressing their mRNA expression (Choi & Park, 2012). These studies are consistent with the results of our study, and in our study, it was found that there was no statistically significant increase in IL-6 in the BV2 cell line after the addition of putrescine compared to the control group. In a study conducted on patients with rheumatoid arthritis, it was found that polyamine levels in the urine of these patients were positively correlated with CRP levels (Furumitsu et al., 1993). In our study, the addition of putrescine did not cause an increase in CRP levels, but the fact that a healthy BV2 microglia cell line was used in our study is an important factor here, while the reported study is a study on patients with inflammation. In another study, it was shown that polyamine depletion arrests the cell cycle in IEC-6 cells and ERK-2 protein level decreased due to polyamine depletion (Ray et al., 1999). The results of this study are consistent with the results of our study, in our study, it was shown that the mRNA expression levels of MEK1 and ERK2 increased with the addition of putrescine.

Another gene with a statistically significant increase in expression at mRNA level in our study was PADI2 gene. This gene encodes a member of the peptidyl arginine deiminase family of enzymes that catalyzes the post-translational elimination of proteins by converting arginine residues to citrulines in the presence of calcium ions. Known substrates for this enzyme include myelin basic protein in the central nervous system and vimentin in skeletal muscle and macrophages. This enzyme is thought to play a role in the onset and progression of neurodegenerative human diseases, including Alzheimer's disease and multiple sclerosis (Watanabe et al., 2009). Currently, there is no study in the literature showing the relationship between putrescine and the PADI2 gene. In our study, it is very important that the expression of PADI2, which is thought to be involved in neurodegenerative diseases, increased as a result of putrescine treatment in BV2 microglia cells.

5. CONCLUSION

Putrescine markedly increases the expression of genes in post translational protein modifications and gene expression in the proliferation pathway in BV2 microglia cells. These results suggest putrescine as a viable therapeutic option to manage neurodegenerative diseases through microglia modulation. In particular, the ability to increase the expression of the PADI2 gene, which is thought to be involved in the pathogenesis of neurodegenerative diseases, by putrescine treatment suggests that it may be a candidate therapeutic agent in the coming years.

Conflicts of Interest

The author declare that they have no conflicts of interest.

Compliance with ethical standarts

Before the initiation of the study, all participants received an explanation of the procedure and the risks that would later be faced in their participation, and they provided informed consent to participate in this study. During this study, the World Medical Association (WMA) HELSINKI Declaration (and/or World Psychiatric Association HAWAII Declaration Good Clinical Practices) were followed according to the ethics committee of Selçuk University Faculty of Medicine.

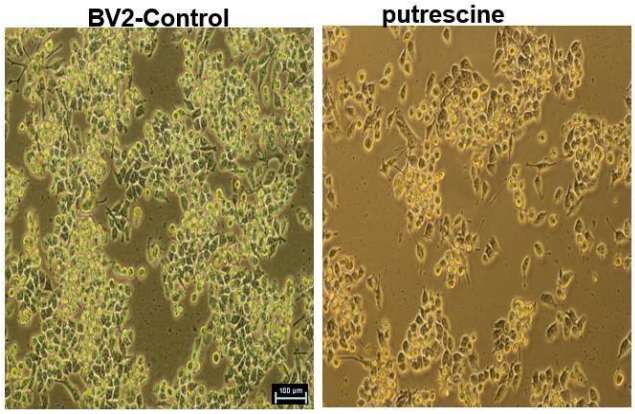

Figure 1: Morphological analyses in BV2 cells after the cells were exposed to concentrations for 24h

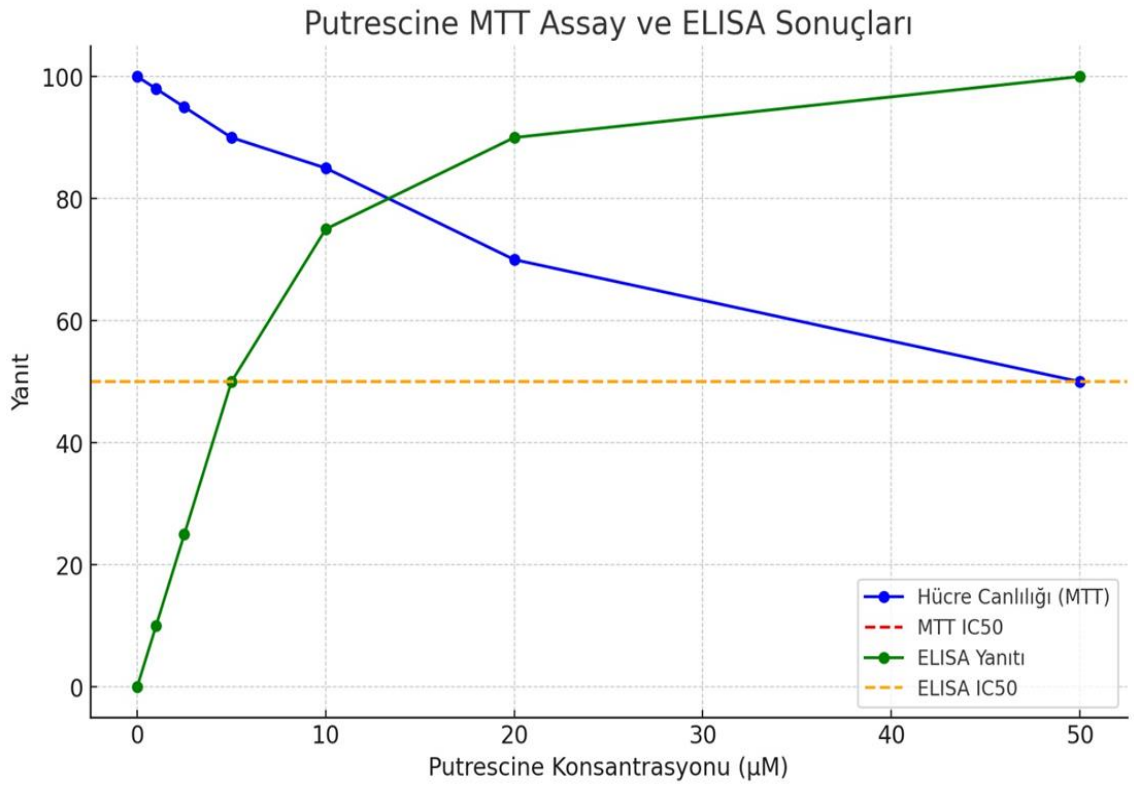

Figure 2: MTT study was performed to determine the appropriate dose of Putresin

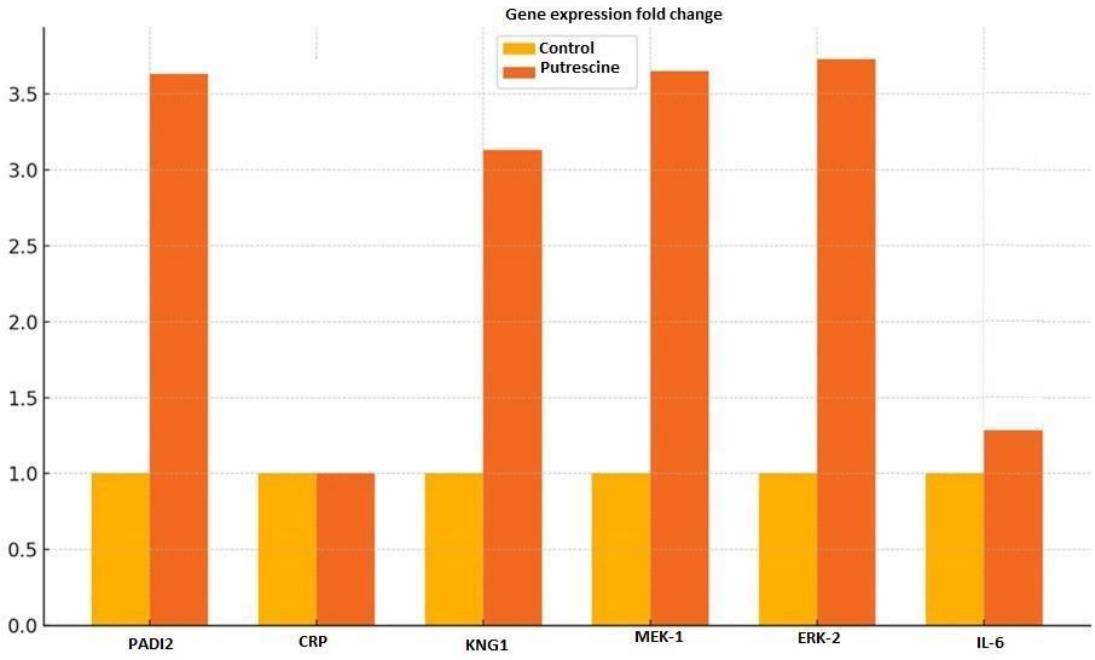

Figure 3: Expression changes in genes as a result of putrescine treatment.

Table 1. Gene-specific primers used to determine gene expression

Gene	Primer Sequence ('→3)
GAPDH	(F) TTGGCTACAGCAACAGGGTG (R) GGGGAGATTCAGTGTGGTGG
IL-6	(F) AATTCGGTACATCCTCGACGG (R) GGGCATGGATTTCAGACCC-3′
PADI2	(F) TGAAAGAGGTGAAGAACCTTG (R) TTTAGGTACTGGAAGCAGAC
CRP	(F) TTTTCTCGTATGCCACCAAG (R) TTTCCAATGTCTCCCACCAG-
MEK1	(F) GCCTAAGAGGACTTTTGAGGACA (R) AGCAGTAATACGTTTGTCTGGGT
ERK2	(F) ACACCAACCTCTCGTACATCGG (R) TGGCAGTAGGTCTGGTGCTCAA

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CHOLESTEROL GRANULOMA AN ANTERIOR MEDIASTINAL MASS A CASE REPORT

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ABSTRACT

Background of the Study: Although mediastinal masses are often malignant, they can be seen in a wide range of cases. Diagnosis of benign lesions is important in terms of follow-up and treatment.

Aim: Thymus tumors, lymphomas, thyroid tumors, germ cell tumors and benign tumors are frequently seen in the anterior mediastinum. Cholesterol granuloma, which is a benign entity and frequently seen in the temporal bone, can rarely be seen in the anterior mediastinum. We aimed to present a case of cholesterol granuloma, a rare entity that constitutes at most 1% of all mediastinal tumors.

Case Report: A 53-year-old male patient is preparing for coronary bypass surgery after experiencing chest pain. The mass seen in the anterior mediastinum on the thorax tomography taken before the surgery is excised and sent to pathology for frozen examination with the preliminary diagnosis of Thymoma. Frozen examination indicates that the mass is benign. Microscopic examination of the excised mass reveals giant cells containing cholesterol clefts, stained with CD68. Based on histomorphological and immunohistochemical findings, the case is diagnosed as Cholesterol Granuloma.

Results: Although the pathophysiology of cholesterol granuloma, which is rarely seen in the mediastinum, is not clear, it is thought to occur when cholesterol residues are phagocytosed by macrophages as a result of chronic inflammation. They are usually asymptomatic and detected incidentally. Since it can be confused with thymus tumors and lymphomas radiologically, histopathological examination is required for definitive diagnosis.

Conclusion: Most cases of cholesterol granuloma in the anterior mediastinum or the entire mediastinum are asymptomatic, making it an extremely rare disease. As a result, these cholesterol granulomas are detected during heart or chest surgeries and treated with surgical resection.

Keywords: Cholesterol granuloma, mediastinal mass, thymus tumors

1. INTRODUCTION

The mediastinum is anatomically divided into anterior, middle and posterior parts. Many tumors can be found in the mediastinum. Tumors in the anterior mediastinum have a higher risk of becoming malignant than other parts of the mediastinum (Hendrikx, Lauwers, Broeckx, Snoeckx, & Van Schil, 2022). The most common etiologies of anterior mediastinal tumors are thymic malignancies and lymphomas, followed by thyroid/endocrine tumors, benign teratomas, malignant germ cell tumors and benign thymic tumors (Hongo et al., 2023). Cholesterol granuloma is a granulomatous reaction that develops against cholesterol crystals precipitating in the tissues and can be seen anywhere in the head and neck region. It was first described by Graham and Michael in 1978(Gürsoy, Yazırlıoglu, & Okuyucu, 2023). Cholesterol granuloma is considered a benign entity. Although cholesterol granuloma can develop in many parts of the body, it is very rare in the mediastinum and constitutes 1% of mediastinal tumors (Ludoski et al., 2024). The etiology of cholesterol granuloma is still not clearly understood. Its histopathology is characterized by cholesterol accumulation and foreign body reaction, and bleeding or inflammation is thought to be responsible for its formation (Kaya, Terzi, Çeliker, & Dursun, 2022). However, cholesterol granuloma in the mediastinum is extremely rare (Hongo et al., 2023). Here, we report a rare case of cholesterol granuloma of the anterior mediastinum treated at our academic hospital.

2. CASE REPORT

A 53-year-old male patient applies to our hospital with chest pain. He is being prepared for coronary bypass surgery because his coronary arteries are blocked. In the thorax tomography taken before the surgery, a 29x27 mm sized, well-circumscribed and lobulated lesion in the anterior mediastinum is described. The lesion is reported as a cystic mass. During the operation, the lesion is excised with the preliminary diagnosis of thymoma and cyst. The excised lesion is sent to pathology for frozen examination. Examination of the mass, it is stated that the mass is benign and total excision of the mass is performed. In the macroscopic examination of the material sent to pathology, a lesion measuring 5.3x5x2.3 cm, dirty yellow in color, with a lobulated appearance and containing areas of hard consistency in places, is detected. It is seen that the cut surface has a rough appearance and contains small nodular structures and bleeding areas (Figure 1). In microscopic examination of the lesion, giant cells consisting of cholesterol clefts were seen adjacent to the thymus. Signs of inflammation and old bleeding were observed in the surrounding area (Figure 2). Immunohistochemically, giant cells containing cholesterol clefts stained with Cd68 were observed. Staining with Panck and tdt was observed in the surrounding thymus tissue (Figure 3). Based on histomorphological and immunohistochemical findings, the case was diagnosed as Cholesterol Granuloma.

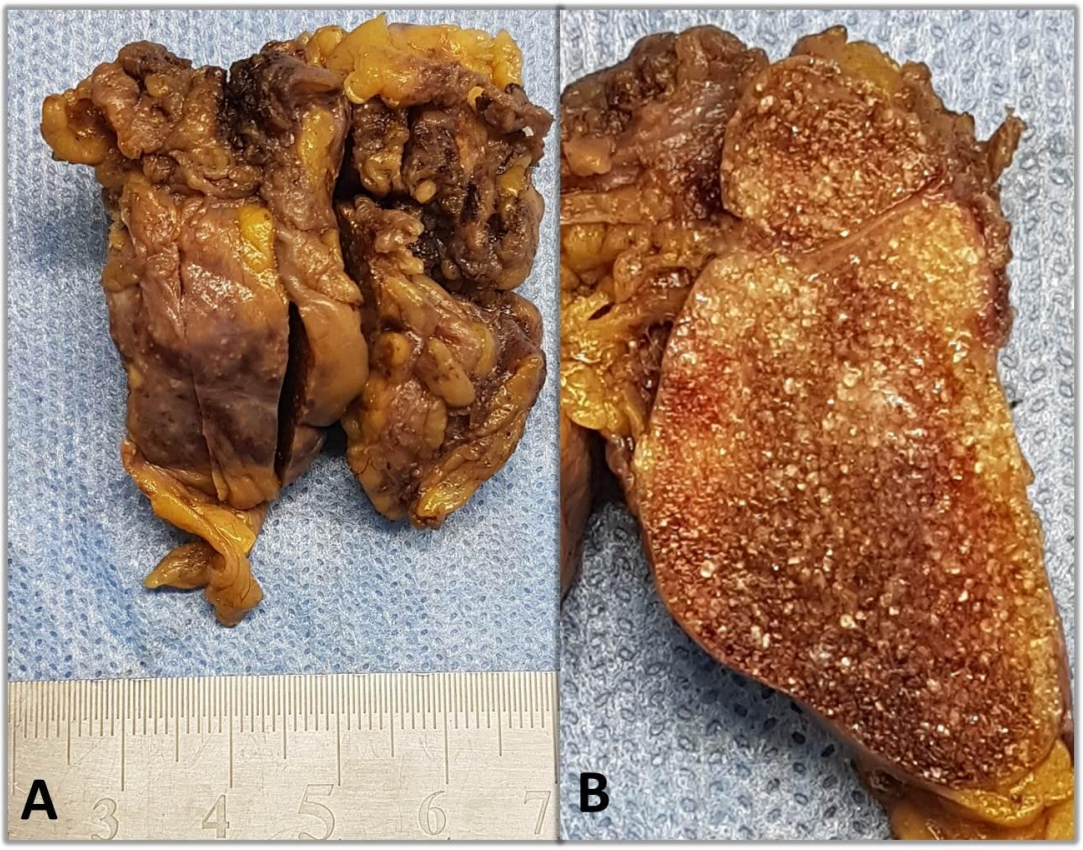

Figure 1: Macroscopic view of the mass.

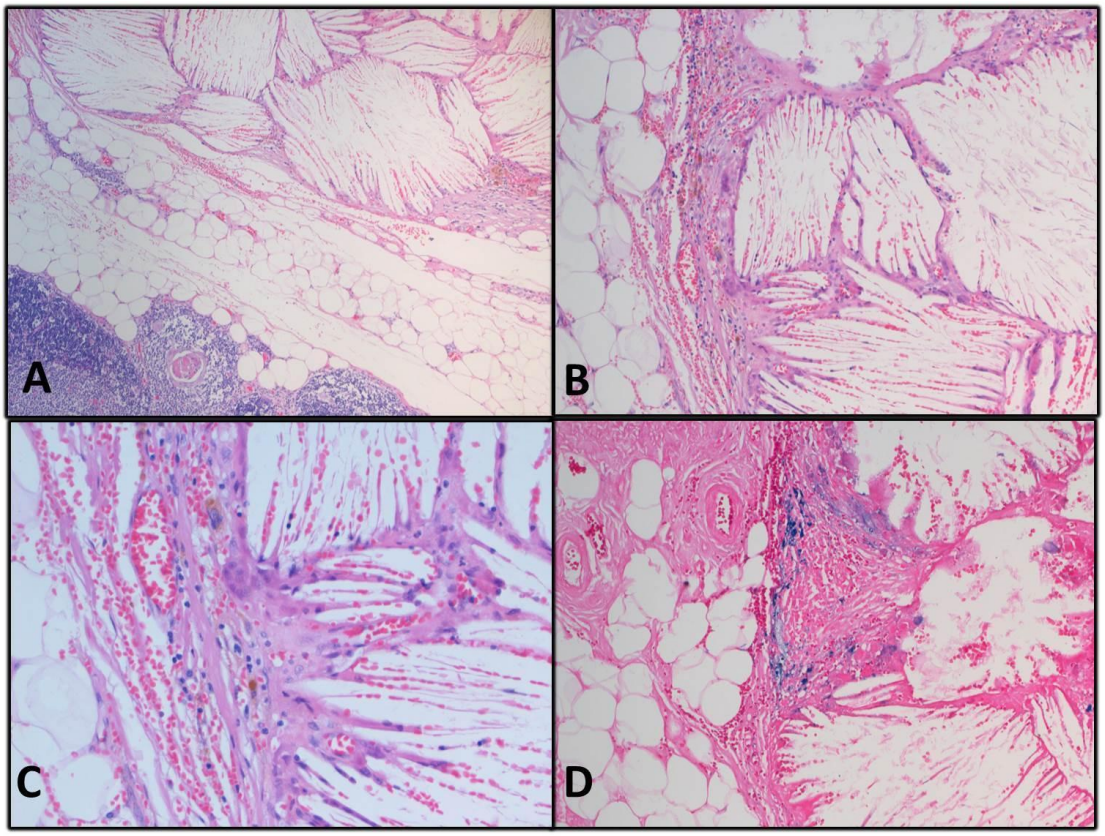

Figure 2: Thymus and giant cells with cholesterol clefts (A; 100X, H-E). Giant cells (B;200X, C; 400X, H-E). Giant cells and hemosiderin deposition (D; Prussian blue, 200X)

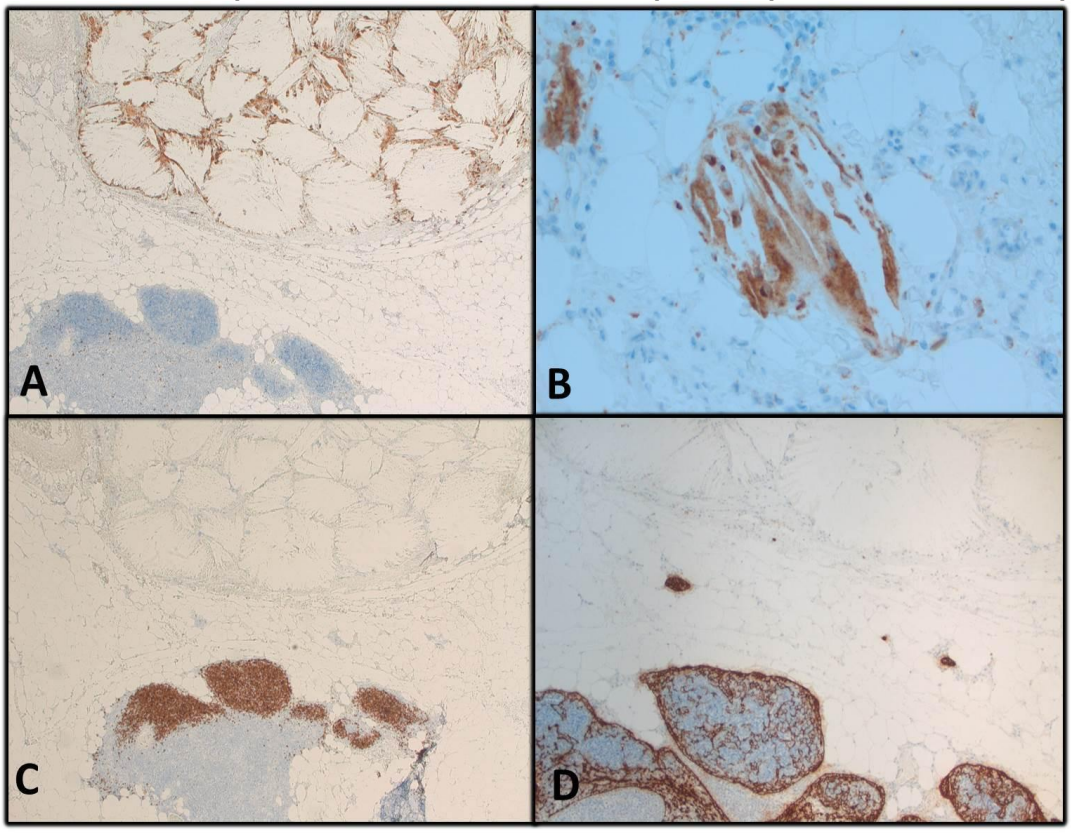

Figure 3: Immunohistochemical cd68, Tdt and Panck stains (A; Cd68,50X. B; CD68, 400X. C; Tdt, 50X. D; Panck, 100X)

3. DISCUSSION

We present a rare case of cholesterol granuloma of the anterior mediastinum. Most of the cases are associated with the head and neck region and a few cases are reported relating toorgans such as breast, kidney, anterior mediastinum, and ovary (Sagnic et al., 2021). It is discovered in a wide age range (22-85 years) and most often found in middle-aged males. Male/female ratio is 3/1(Gürsoy et al., 2023). Cholesterol granuloma is a benign entity histopathologically characterized by deposition of cholesterol clefts and a foreign body reaction, occasionally with calcification and osseous metaplasia (Hendrikx et al., 2022; Hongo et al., 2023; Ludoski et al., 2024). Cholesterol granuloma is a histopathological diagnosis in which hemosiderin-laden macrophages and histiocytes and giant cells developed against cholesterol crystals are observed within the fibrous granulation tissue (Gürsoy et al., 2023; Kaya et al., 2022). The origin and pathophysiology of this granuloma are currently unknown. It might manifest as a result of chronic inflammation or as a result of trauma or a chest injury (Gürsoy et al., 2023; Ludoski et al., 2024; Sagnic et al., 2021). A solid mass in the anterior mediastinum is suspicious for a neoplasm, such as lymphoma or thymoma. It is important to know that there are other, benign lesions that may occur in the mediastinum (Hendrikx et al., 2022). It has non-specific characteristics of chronic inflamation. Since the inflamation occurs against cholesterol crystals, a foreign body reaction is revealed by the accumulation of histiocytes, multinucleated giant cells, foamy macrophages, neutrophils, hemosiderin-laden macrophages, and cholesterol clefts (Sagnic et al., 2021). The gold standard treatment for cholesterol granuloma is total surgical resection. Symptomatic mediastinal granuloma can be easily diagnosed, but if the mass effect is not obvious, the diagnosis of this mass is difficult (Ludoski et al., 2024). Asymptomatic cases of cholesterol granuloma are more difficult to diagnose than symptomatic cases, unless discovered incidentally during a preoperative CT or MRI chest scan. Most cases of cholesterol granuloma in the anterior mediastinum or the entire mediastinum are asymptomatic, making it an extremely rare disease (Ludoski et al., 2024). In symptomatic patients, local (superior vena cava syndrome, cough, Horner syndrome, etc.) or paraneoplastic syndromes may develop due to mass effect or tumor invasion (Hendrikx et al., 2022).

4. CONCLUSION

Diagnosis of cholesterol granulomas is delayed because they do not cause obvious symptoms. They are detected incidentally during chest imaging and are often confused with thymoma or lymphoma. In pathological examination, diagnosis is easy due to its distinct histomorphological features. Surgical resection is sufficient for treatment.

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FREQUENCY OF CARCINOMA IN PROSTATE BIOPSIES

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ABSTRACT

Background of the Study: Prostate cancer is one of the most common types of cancer in men. In addition, the diagnosis and treatment of this disease is important because it affects millions of men around the world and impairs their quality of life**.**

Aim: Prostate cancer is one of the most common cancers worldwide and accounts for a large proportion of cancer-related deaths. The majority of prostate malignancies consist of prostate adenocarcinomas originating from the prostate epithelium. Histopathological examination is required for a definitive diagnosis of prostate cancer. We investigated the incidence of prostate cancer, which is common among men in our country, through biopsies sent to the Pathology Department of Selçuk University Faculty of Medicine.

Material and Method: 2018 cases where prostate biopsy was performed and reported at Selçuk University Faculty of Medicine Department of Pathology between 2010 and 2020 were scanned through the hospital information system. Data obtained from pathology reports and the age and diagnosis of the patients were examined retrospectively.

Results: The average age of 2018 patients was found to be 69. It was determined that the youngest patient was 41 years old and the oldest patient was 95 years old. It was observed that 1209(59.9%) patients had benign, 136(6.7%) patients had Atipic Small Acinar Proliferation (ASAP) and 673 (33.3%) patients had acinar adenocarcinoma.

Conclusion: Prostate cancer is an important disease to diagnose and treat because it is common, has a negative impact on life, and causes mortality and morbidity. The frequency of prostate cancer increases with age. In our study, the average age was found to be 69. Although most of the diagnoses were benign in biopsies, 1/3 of the cases were found to be malignant. Identifying especially high-risk individuals and diagnosing prostate cancer at an early stage with appropriate screening strategies will be effective in reducing mortality.

Keywords: Prostate cancer, Adenocarcinoma, Asap, Prostate biopsy

1. INTRODUCTION

The prostate gland is a walnut-sized organ located in the pelvis, surrounded by a capsule, and capable of fibromuscular and glandular secretion. Tubuloalveolar structures in the prostate are specialized cell groups responsible for secretion (Erzurumlu, 2021). On the cellular level, both human and mouse prostates contain a pseudostratified epithelium with three types of terminally differentiated epithelial cells: luminal, basal, and neuroendocrine (Baydar, 2017; Wang, Zhao, Spring, & DePinho, 2018). Prostate cancer is one of the most common cancers worldwide and accounts for a large proportion of cancer-related deaths. Prostate cancer is one of the most common types of cancer in men (Baydar, 2017; Bergengren et al., 2023). The majority of prostate malignancies arise from the prostate epithelium. Prostate cancer develops as a result of these cells starting to grow and spread uncontrollably after genetic factors or various environmental stimuli (Baydar, 2017; Erzurumlu, 2021). In addition, age, race, family history and genetic mutations are defined as well-known non-modifiable risk factors, while metabolic syndrome, obesity and smoking are defined as modifiable risk factors (Bergengren et al., 2023; Koçak & Tek, 2022). While adenocarcinoma can be detected in prostate biopsies, atypical small acinar proliferation (ASAP) and high-grade prostatic intraepithelial neoplasia (HGPIN) foci can also be seen, which can be found simultaneously with cancer or suggest that cancer may occur in the future (Şimşek et al., 2014). Biopsy is indicated if patients have high Prostate specific antigen (PSA) values and there is a suspicious situation in the rectal examination. Histopathological examination is required for a definitive diagnosis of prostate cancer (Şimşek et al., 2014). Finding perineural invasion in prostate

biopsy is a criterion for malignancy as well as a prognostic parameter and a precursor to extraprostatic spread (Baydar, 2017). We investigated the incidence of prostate cancer, which is common among men in our country, through biopsies sent to the Pathology Department of Selçuk University Faculty of Medicine.

2. MATERIAL AND METHOD

2018 cases where prostate biopsy was performed and reported at Selçuk University Faculty of Medicine Department of Pathology between 2010 and 2020 were scanned through the hospital information system. Data obtained from pathology reports and the age and diagnosis of the patients were examined retrospectively. Patients who underwent biopsy were identified as benign, malignant and ASAP. The distribution of patients reported as benign, malignant and suspicious was made according to decades.

3. RESULTS

The average age of 2018 patients was found to be 69. It was determined that the youngest patient was 41 years old and the oldest patient was 95 years old. It was observed that 1209(59.9%) patients had benign, 136(6.7%) patients had Atipic Small Acinar Proliferation (ASAP) and 673(33.3%) patients had acinar adenocarcinoma (Table 1).

Table 1: Distribution of prostate biopsies

Diagnosis	Number	Percent
Benign	1209	59,9%
Adenocarcinoma	673	33,3%
ASAP	136	6,7%
Total	2018	100,0%

When we look at the distribution of prostate biopsies according to decades; 0.8% of cases in the 5th decade, 15.3% of cases in the 6th decade, 44.8% of cases in the 7th decade, 30.1% of cases in the 8th decade, 8.4% of cases in the 9th decade, 10% of cases in the 10th decade. It was seen that it constituted 0.6 of the When we look at the distribution of benign cases according to decades; 0.7% of cases in the 5th decade, 18.6% of cases in the 6th decade, 50.6% of cases in the 7th decade, 25% of cases in the 8th decade, 4.6% of cases in the 9th decade, 10% of cases in the 10th decade. It was seen that it constituted 0.4 of the When we look at the distribution of malignant cases according to decades; 0.9% of cases in the 5th decade, 9.4% of cases in the 6th decade, 34.3% of cases in the 7th decade, 38.3% of cases in the 8th decade, 15.9% of cases in the 9th decade, 10% of cases in the 10th decade. It was seen that it constituted 1.2. When we look at the distribution of cases reported as suspicious (ASAP) and HPIN in prostate biopsies according to decades; It was observed that it constituted 0.7% of the cases in the 5th decade, 14.7% of the cases in the 6th decade, 45.6% of the cases in the 7th decade, 34.6% of the cases in the 8th decade, and 4.4% of the cases in the 9th decade (Table 2).

Table 2: Distribution of biopsy results by decade

Age	benign	%	malign	%	asap	%	Total	%
41-50	9	0,7	6	0,9	1	0,7	16	0,8
51-60	225	18,6	63	9,4	20	14,7	308	15,3
61-70	612	50,6	231	34,3	62	45,6	905	44,8
71-80	302	25,0	258	38,3	47	34,6	607	30,1
81-90	56	4,6	107	15,9	6	4,4	169	8,4
91-95	5	0,4	8	1,2	0	0,0	13	0,6

Total	1209	100,0	673	100,0	136	100,0	2018	100,0

Of the patients who underwent biopsy, 56.3% in the 5th decade were benign, 37.5% were malignant, 6.3% were ASAP, 73.1% in the 6th decade were benign, 20.5% were malignant, 6.5% were ASAP, 7 67.6% of those in the 8th decade were benign, 25.5% were malignant, 6.9% were ASAP, 49.8% of those in the 8th decade were benign, 42.5% were malignant, 7.7% were ASAP, and 6.9% were in the 9th decade. It was observed that 33.1% of them were benign, 63.3% were malignant, 3.6% were ASAP, and 38.5% of those in the 10th decade were benign and 61.5% were malignant.

Table 3: Distribution of prostate biopsies across decades

	5. decad	%	6. decad	%	7. decad	%	8. decad	%	9. decad	%	10. decad	%
Benign	9	56,3	225	73,1	612	67,6	302	49,8	56	33,1	5	38,5
Malign	6	37,5	63	20,5	231	25,5	258	42,5	107	63,3	8	61,5
Asap	1	6,3	20	6,5	62	6,9	47	7,7	6	3,6	0	0,0
						10		10		10		10
Total	16	100	308	100	905	0	607	0	169	0	13	0

Additionally, perineural invasion was observed in 152 (22.5%) of the malignant patients and lymphovascular invasion was observed in 8 (1.2%).

4. DISCUSSION

Since the incidence of prostate cancer increases with age, patients after the age of 50 should be investigated with PSA values and rectal examination. While initially transrectal biopsy was performed under finger guidance, today 10-14 core prostate biopsy guided by transrectal ultrasonography (TRUS) is used as a diagnostic method (Avcı & Atakan, 2014). Pre-biopsy preparations and patient comfort make it difficult to perform the biopsy. Negative or false negative results may occur in biopsies performed by manual examination or TRUS guidance. The incidence of cancer in patients with high PSA levels is around 30%. In patients whose biopsy result is benign but in high-risk patients who undergo saturation biopsy, that is, multiple biopsies, the cancer incidence increases to 38% (Yencilek, Koca, & Kuru, 2018). In our study, 60% of the patients who underwent biopsy were found to be benign. It has been reported in the literature that the incidence of prostate cancer before the age of 50 is significantly lower (Aslan et al., 2020). In our study, it was found that 0.8% of the cases were under the age of 50 and 37.5% of them were malignant. Although this rate tends to decrease in the 6th and 7th decades (%20.5, %25.5), it has been determined that it increases significantly after the 8th decade (%42.5). The highest cancer rate was 63.3% in the 9th decade. It is reported that the incidence of cancer increases with age and the Gleason scores of these tumors are also higher (Aslan et al., 2020). Perineural invasion is one of the prognostic parameters of prostate cancer. In our study, it was seen in 22.5% of the cases. Although perineural invasion is reported to be between 7-35% in the literature, this rate was found to be 16.78% in the study by Gorgeli et al (Görgel, Özdamar, Cengiz, & Kutan, 2011).

5. CONCLUSION

Prostate cancer is an important disease to diagnose and treat because it is common, has a negative impact on life, and causes mortality and morbidity. The frequency of prostate cancer increases with age. In our study, the average age was found to be 69. Although most of the diagnoses were benign in biopsies, 1/3 of the cases were found to be malignant. Identifying especially high-risk individuals and diagnosing prostate cancer at an early stage with appropriate screening strategies will be effective in reducing mortality. Identifying high-risk patients in prostate biopsies and using MRI, the most effective imaging method, in these patients may increase the incidence of cancer. It is recommended to use multiparametric MRI in which anatomical and functional sequences are used together in prostate MRI (Aydın & Balcı, 2021).

The survival after prostate cancer diagnosis is largely related to tumor stage and grade. While patients with low grade, localized prostate cancer can live for many decades without treatment, patients with metastatic disease have a median survival of 30 months (Nevo, Navaratnam, & Andrews, 2020). While the five-year survival rate is close to 100% in patients with prostate cancer confined to the gland, this rate decreases to 28% in patients with extraprostatic spread (Aydın & Balcı, 2021). It has been stated that the possibility of extraprostatic extension increases when perineural invasion is detected in needle biopsies. However, it has been reported that perineural invasion independently predicts lymph node metastasis and progression and is an independent risk factor associated with recurrence after radiotherapy. We believe that perineural invasion is an important prognostic factor that should be taken into consideration when determining treatment options in patients planned for treatment for prostate cance r(Görgel et al., 2011).

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PREGNANCY AND UNWANTED COMPLICATION - CASE REPORT

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ABSTRACT

Background of the Study: The incidence of placental invasion anomaly is between 1 in 540 and 1 in 93,000 births. Advanced maternal age, parity, smoking, alcohol and drug use, multiple pregnancy, recurrent pregnancy loss are also important risk factors. placenta percreta (PP), in which chorionic villi can invade surrounding organs such as bladder by passing through the uterine serosa, is one of the placental invasion anomalies. The presence of PP is associated with major pregnancy complications.

Aim: In this case, we aimed to present the follow-up and treatment of a patient with a diagnosis of placenta previa in the postoperative intensive care unit.

Material and Method: 34-year-old foreign national female patient at 27 weeks + 5 days gestational age was taken to emergency cesarean section by obstetricians with the diagnosis of placenta percreata due to bleeding. She was conscious, hypotensive and tachycardic at the time of operation. Admission hemoglobin was 5,7 g/dl. Peroperatively 7 units of erythrocyte suspension (ES) , 3 units of fresh frozen plasma (FFP) , 1 unit of Platelet suspension (PS) were replaced. Hysterectomy and bladder repair were performed in the operation in which urology was also involved. The patient was admitted to the postop intensive care unit. On admission, the patient was intubated, sedated and receiving vasopressor support. Pupils were dilated, and no light reflex was obtained. On examination, respiratory sounds were coarse in bilateral lower zones. Drain was present in the abdomen. Thorax and brain CT was planned. However, it could not be performed in the hypotensive patient. Laboratory tests were ordered. Peripheral blood smear test was performed. Hematology consultation was done. Fibrinogen was replaced. Ampicillin sulbactam and metronidazole were started with the recommendation of infection and gynecology consultation. After hemodynamics stabilized, CT scan of the brain, abdomen and thorax was performed. Brain and abdomen CT were normal and atelectasis in the lower lobes was present on thorax CT. Low molecular weight heparin (LMWH) was started in the patient with no decrease in hemogram. The patient was extubated on the 4th day. In the follow-up, the patient was given 2 ES, 1 FFP and vasopressor support continued. GCS was 14 but she was prone to sleep. On day 5, vasopressor support was turned off. On day 6, the patient who did not speak Turkish described that he could not see. Subsequently, GCS regressed and consciousness tended to sleep. Ophthalmology and neurology consultation was made. MR venography and brain CT were normal. Diffusion MR report: signal increases were observed in cortical areas at the level of the external capsule on both sides, at the level of the presantral and postsantral gyri, and no significant signal change was detected in ADC (Apperent Diffusion Coefficient) images. Coraspin was started. The frequency of low molecular weight heparin was doubled. Necessary departmental consultations and examinations were performed. The patient's visual loss regressed spontaneously The patient was transferred to the ward with a neurologic examination of GCS 15 in clinical follow-up. The follow-up MRI in the ward was evaluted normal.

Results: Pregnancy is pregnant with many complications such as bleeding, placental anomalies, sepsis, preeclampsia, cerebral venous thrombus.

Conclusion: Close postoperative hemodynamic monitoring, blood transfusion and multidisciplinary approach are vital in follow-up and treatment.

Keywords: Pregnancy, placenta previa, bleeding, transfusion

1.INTRODUCTION

The incidence of placental invasion anomaly is between 1 in 540 and 1 in 93,000 births. Advanced maternal age, parity, smoking, alcohol and drug use, multiple pregnancy, recurrent pregnancy loss are also important risk factors. placenta percreta (PP), in which chorionic villi can invade surrounding organs

such as bladder by passing through the uterine serosa, is one of the placental invasion anomalies(Heena & Kumari, 2020). The presence of PP is associated with major pregnancy complications (Barut et al., 2023; Faiz & Ananth, 2003) In this case, we aimed to present the follow-up and treatment of a patient with a diagnosis of placenta previa in the postoperative intensive care unit.

2.CASE REPORT

34-year-old foreign national female patient at 27 weeks + 5 days gestational age was taken to emergency cesarean section by obstetricians with the diagnosis of placenta percreata due to bleeding. She was conscious, hypotensive and tachycardic at the time of operation. Admission hemoglobin was 5,7 g/dl. Peroperatively 7 units of erythrocyte suspension (ES), 3 units of fresh frozen plasma (FFP) , 1 unit of Platelet suspension (PS) were replaced. Hysterectomy and bladder repair were performed in the operation in which urology was also involved. The patient was admitted to the postop intensive care unit. On admission, the patient was intubated, sedated and receiving vasopressor support. Pupils were dilated, and no light reflex was obtained. On examination, respiratory sounds were coarse in bilateral lower zones. Drain was present in the abdomen. Thorax and brain CT was planned. However, it could not be performed in the hypotensive patient. Laboratory tests were ordered. Peripheral blood smear test was performed. Hematology consultation was done. Fibrinogen was replaced. Ampicillin sulbactam and metronidazole were started with the recommendation of infection and gynecology consultation. After hemodynamics stabilized, CT scan of the brain, abdomen and thorax was performed. Brain and abdomen CT were normal and atelectasis in the lower lobes was present on thorax CT. Low molecular weight heparin (LMWH) was started in the patient with no decrease in hemogram. The patient was extubated on the 4th day. In the follow-up, the patient was given 2 ES, 1 FFP and vasopressor support continued. GCS was 14 but she was prone to sleep. On day 5, vasopressor support was turned off. On day 6, the patient who did not speak Turkish described that he could not see. Subsequently, GCS regressed and consciousness tended to sleep. Ophthalmology and neurology consultation was made. MR venography and brain CT were normal. Diffusion MR report: signal increases were observed in cortical areas at the level of the external capsule on both sides, at the level of the presantral and postsantral gyri, and no significant signal change was detected in ADC (Apperent Diffusion Coefficient) images. Coraspin was started. The frequency of low molecular weight heparin was doubled. Necessary departmental consultations and examinations were performed. The patient's visual loss regressed spontaneously The patient was transferred to the ward with a neurologic examination of GCS 15 in clinical follow-up. The follow-up MRI in the ward was evaluted normal.

3.RESULTS

Pregnancy is pregnant with many complications such as bleeding, placental anomalies, sepsis, preeclampsia, cerebral venous thrombus.

4.DISCUSSION

There is an increase in the incidence of placental adhesion anomalies due to the increase in cesarean section rates. Appropriate clinical management of these conditions, which increase maternal mortality, and knowledge of the medical and surgical approach is very important. Hysterectomy is indicated as the primary treatment option for placenta accreta and other placental adhesion abnormalities (Committee on Obstetric, 2002). Hysterectomy was performed in this case with bleeding.

Although hysterectomy is performed in these patients, blood and blood products have to be used for bleeding. Despite successful intraoperative management, many cases of placenta percreata often require massive transfusion (Heena & Kumari, 2020) (Sanhal, Yücel, Kırbaş, & Uygur, 2016). Studies have shown that erythrocyte suspension (ES) and fresh frozen plasma (FFP) given in a 1:1 ratio to patients with extensive hemorrhage may lead to decreased mortality. Blood management guidelines recommend the use of a combination of erythrocyte suspension, fresh frozen plasma and platelet suspension (PS). Decreased fibrinogen levels are also frequently seen in postpartum massive hemorrhage leading to disseminated intravascular coagulation. Dilutional coagulopathy, clotting disorder may be seen in patients with blood loss of more than about 2 liters and fibrinogen level may fall below 100 mg/dl (Holcomb et al., 2011). In this patient who presented with bleeding complaint, 7 units of ES, 3 units of FFP and 1 unit of PS, 1 gr fibrinogen were given peroperatively.

Although blood and blood products are used for therapeutic purposes in these diseases, it should not be forgotten that complications related to them may occur. In this case, blood and blood products had to be used to stabilize the patient.

Despite necessary surgical interventions and medical treatment, persistent hypotension may be observed in these patients. Hypoxia and organ failure may develop due to hypotension. In this case, despite necessary surgical intervention and replacement of lost volume with blood transfusion, the patient remained hypotensive enough to require vasopressor support. The patient with hypotension and associated tissue hypoxia was brought to our intensive care unit intubated and under respiratory and circulatory support.

Postpartum vision loss is detected for various reasons (More, Garg, Malhotra, Kumar, & Uniyal, 2016). Visual loss occurred in this case as well. In this case, the diagnosis was between hypoperfusion and acute cerebral embolism. ADC images showed no significant signal change and regular DMAH use ruled out cerebral embolism. The patient's clinical and radiologic findings were attributed to cerebral hypoperfusion secondary to hypotension. The patient's visual loss returned spontaneously.

After the patient's hemodynamic stability was ensured and hypotensive effects were eliminated, our patient regained consciousness, regained normal functions and was transferred to the obstetric ward.

5.CONCLUSION

Regular follow-up from the beginning of pregnancy prevents such conditions and provides early intervention**.**

6.ACKNOWLEDGE

There is no financially supporting institution, project.

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POSTTRAUMATIC EMPYEMA: CASE REPORT

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ABSTRACT

Background of the Study: Thorax traumas constitute 25-50% of all traumas and are the most common cause of death. Blunt traumas constitute 70% of thorax traumas. Bronchial ruptures occur after both blunt and penetrating injuries. The extent of the trauma, the cause and the presence of pain, prevention of early mobilization of the patient, and decreased coughing ability lead to the development of problems such as secondary atelectasis and sepsis.

Aim: In this case, we aimed to present a case of empyema developing after bronchial injury.

Material and Method: 32-year-old male patient presented to our emergency department after an automobile traffic accident. After the initial examination and imaging performed in the emergency department, the patient was found to have subarachnoid hemorrhage, multiple maxillofacial fractures, intra-abdominal solid organ damage, fracture of the right iliac wing, total atelectasis of the right lung in the thorax and injury to the bronchus leading to the right lower lobe. The patient with confusion and respiratory distress was intubated in the emergency department. After intubation, dense dark secretion was aspirated and ceftriaxone was started with a prediagnosis of aspiration pneumonia. Due to multitrauma, the patient was admitted to our intensive care unit after necessary consultations. Intubated follow-up was continued on mechanical ventilator. Blood gas; pH: 7.36, pCO2: 51, po2: 80, Hco3: 26. Thoracic surgery consultation was made. Bronchoscopy was planned under elective conditions. Elective tracheostomy was applied because the patient could not be weaned from the mechanical ventilator. The general condition of the patient, who was followed up from tracheostomy to mechanical ventilator, deteriorated and septic shock developed in the patient. A pleural drainage catheter was inserted on the 10th day of hospitalization after a consolidated area was observed in the right lower lobe on the two-way chest radiograph. Culture results were compatible with empyema. Antibiotherapy was revised. In the follow-up, left tube thoracostomy was performed after a consolidated area was observed in the left lung Acinetobacter baumannii and Klebsiella Pneumonia were grown in the cultures. After appropriate antibiotic treatment was completed, the patient was transferred to the ward with tracheostomy and mechanical ventilator.

Results: Serious complications may develop after thorax trauma. These include pneumonia, respiratory failure and severe empyema due to closed hemothorax. If there is a suspicion of infection, drainage of empyema by tube thoracostomy, broad-spectrum antibiotics, fluid culture antibiogram, and antibiotic initiation according to the results are the basic treatment.

Conclusion: Late complications in bronchial injury should be kept in mind.

Keywords: Thorax trauma, empyema, bronchial injury, tube thoracostomy

1.INTRODUCTION

Thorax traumas constitute 25-50% of all traumas and are the most common cause of death. Blunt traumas constitute 70% of thorax traumas. Bronchial ruptures occur after both blunt and penetrating injuries (Demircan, Yorgancılar, Çelik, & Karakurt, 2011). The extent of the trauma, the cause and the presence of pain, prevention of early mobilization of the patient, and decreased coughing ability lead to the development of problems such as secondary atelectasis and sepsis (Gürsu et al., 2004) .
In this case, we aimed to present a case of empyema developing after bronchial injury.

2.CASE REPORT

32-year-old male patient presented to our emergency department after an automobile traffic accident. After the initial examination and imaging performed in the emergency department, the patient was found to have subarachnoid hemorrhage, multiple maxillofacial fractures, intra-abdominal solid organ damage,

fracture of the right iliac wing, total atelectasis of the right lung in the thorax and injury to the bronchus leading to the right lower lobe (Figure 1). The patient with confusion and respiratory distress was intubated in the emergency department. After intubation, dense dark secretion was aspirated and ceftriaxone was started with a prediagnosis of aspiration pneumonia. Due to multitrauma, the patient was admitted to our intensive care unit after necessary consultations. Intubated follow-up was continued on mechanical ventilator. Blood gas; pH: 7.36 pCO2: 51 po2: 80 Hco3: 26. Thoracic surgery consultation was made. Bronchoscopy was planned under elective conditions. Elective tracheostomy was applied because the patient could not be weaned from the mechanical ventilator. The general condition of the patient, who was followed up from tracheostomy to mechanical ventilator, deteriorated and septic shock developed in the patient. A pleural drainage catheter was inserted on the 10th day of hospitalization after a consolidated area was observed in the right lower lobe on the two-way chest radiograph (Figure 2 and 3). Culture results were compatible with empyema. Antibiotherapy was revised. In the follow-up, left tube thoracostomy was performed after a consolidated area was observed in the left lung (Figure 4-5-6). Acinetobacter baumannii and Klebsiella Pneumonia were grown in the cultures. After appropriate antibiotic treatment was completed, the patient was transferred to the ward with tracheostomy and mechanical ventilator.

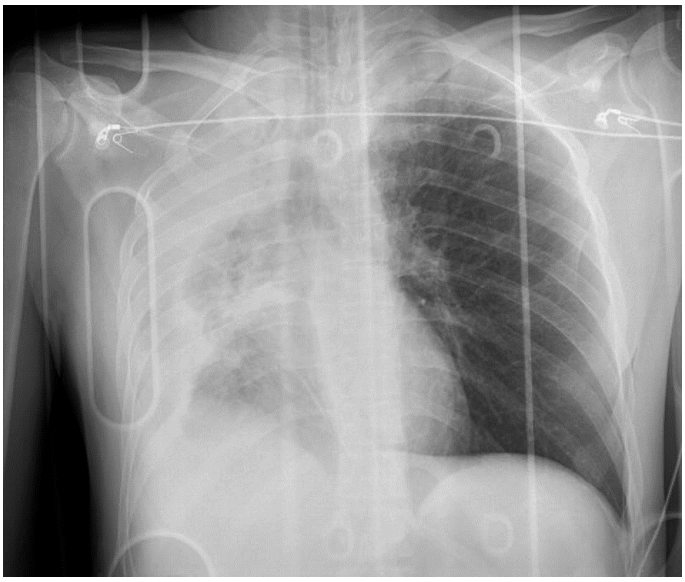

Figure 1: Intensive Care Unit Admission

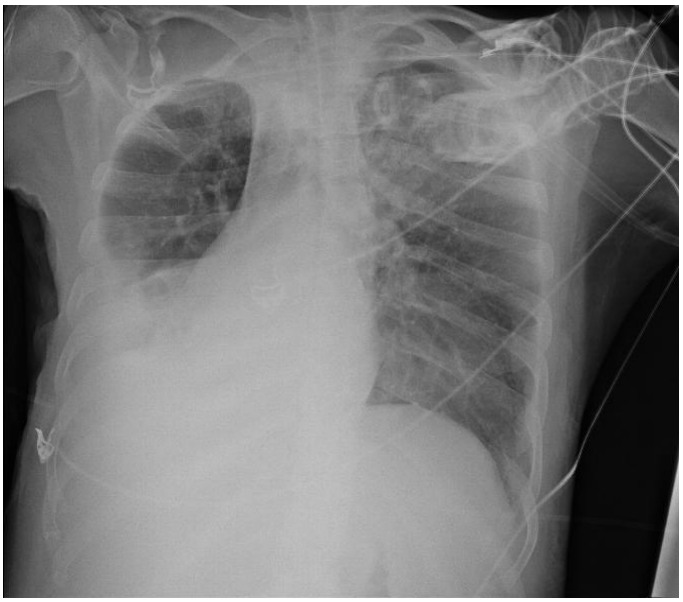

Figure 2: Right Side Before Percutaneous Drainage Catheter Application

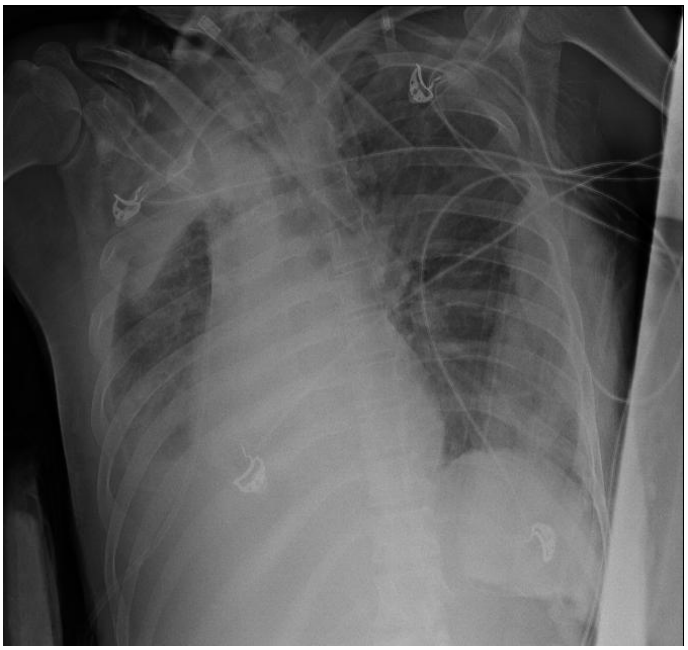

Figure 3: Right side After Percutaneous Drainage Catheter Application

Figure 4: Empyema at Left Side Before Tube Thoracostomy

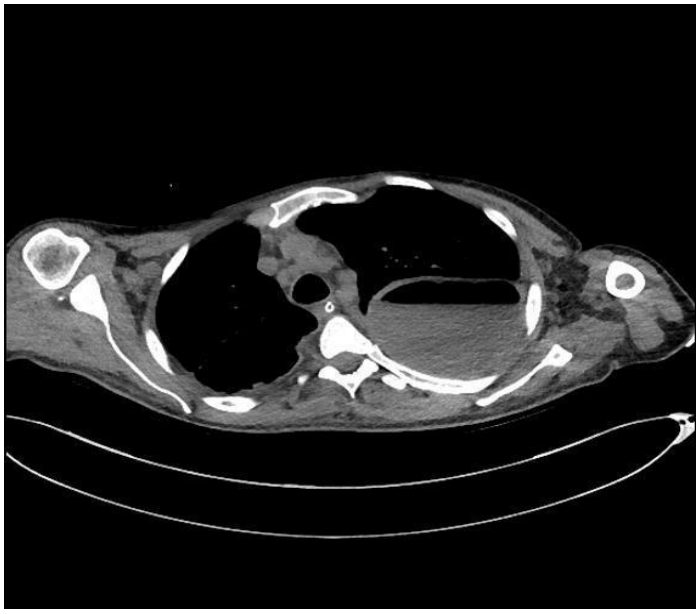

Figure 5: Empyema due to Acinetobacter Baumannii

Figure 6: Empyema at Left Side After Tube Thoracostomy

3.RESULTS

Serious complications may develop after thorax trauma. These include pneumonia, respiratory failure and severe empyema due to closed hemothorax. If there is a suspicion of infection, drainage of empyema by tube thoracostomy, broad-spectrum antibiotics, fluid culture antibiogram, and antibiotic initiation according to the results are the basic treatment.

4.DISCUSSION

Serious complications may develop after thorax trauma. These include pneumonia, respiratory failure and severe empyema due to closed hemothorax. The clinical presentation of a patient with empyema can range from malaise to septic shock. Fever, cough, chest pain and dyspnea are among the symptoms. Postero-anterior and lateral chest radiographs are usually sufficient to detect the presence of effusion. Following the diagnosis of empyema, drainage of the empyema fluid via tube thoracostomy should be performed. Antibiotic treatment should be started (Gürsu et al., 2004). If infection is suspected, drainage of empyema by tube thoracostomy, initiation of broad-spectrum antibiotics and antibiotic revision according to the results of culture antibiogram is the basic treatment (Demircan et al., 2011) (Gürsu et al., 2004). In this case, broad-spectrum antibiotherapy and tube thoracostomy were performed. Antibiotic revision was performed according to the culture results.

Sepsis and septic shock is a major public health problem affecting millions of people and resulting in death in one-third to one-sixth of those it affects. Early diagnosis and treatment is important in the first hours after sepsis develops. Early diagnosis of sepsis saves lives. The 2021 Sepsis Guideline is a specific treatment for adults with sepsis or septic shock that requires immediate resource control. It is recommended that the diagnosis of anatomic infection should be rapidly determined and necessary source control intervention should be performed as soon as possible (Evans et al., 2021). In this patient who developed septic shock, tube thoracostomy was performed due to thorax trauma, mechanical ventilator dependent follow-up, and findings in favor of empyema in radiologic and laboratory examinations.

For patients with sepsis-induced hypoperfusion or septic shock, the sepsis guideline recommends at least 30 mL/kg intravenous crystalloid fluid within the first 3 hours of resuscitation. The basic approach in septic shock is correction of hypoperfusion, respiratory and nutritional support, and use of broad-spectrum antibiotics (Gürsu et al., 2004) (Evans et al., 2021). Treatment of the patient was organized according to current guidelines. Septic shock with high mortality was treated successfully.

Late complications of bronchial injury should be kept in mind. And it should be kept in mind that treatment may take a long time. Preliminary information should be given to the family and the family should be involved in the treatment.

5.CONCLUSION

Late complications in bronchial injury should be kept in mind.

6.ACKNOWLEDGE

There is no financially supporting institution and project.

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CAN NUTRITIONAL INDEX SCORES BE USED IN GESTATIONAL DIABETES PATIENTS?

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ABSTRACT

Background of the Study: Gestational diabetes mellitus (GDM) is a carbohydrate intolerance that occurs or is newly diagnosed during pregnancy and it usually recovers after pregnancy (2). During pregnancy, insulin resistance occurs physiologically to enble the supply of enough glucose to the fetus. If the patient's pancreas cannot compensate enough for the amount of insulin, glucose metabolism is altered and GDM occurs. Nutritional status is evaluated as the combined calculation of some biochemical markers and gives the physician an idea of the prognosis of various diseases. Onodera's Prognostic Nutrition Index (OPNI) and the Controlling Nutritional Status index (CONUT) are nutritional indicators that function as prognostic factors for many malignancies and other diseases.

Aim: In this study, we aimed to investigate the effectiveness of nutritional scoring systems such as OPNI and CONUT in the diagnosis of GDM.

Material and Method: This study is a prospective case-control study of 84 patients. Forty-two patients with GDM according to the 100 g oral glucose tolerance test (OGTT) and 42 age and body mass index (BMI)-matched healthy pregnant women were included in the study. The OPNI and CONUT values for each patient were measured in the peripheral blood of patients with GDM and controls between the 24-28th gestational weeks.

Results: Total cholesterol levels were statistically higher in the GDM groups than in the control group (p=.035). Total COUNT scores were significantly higher in the GDM group (p=.006). There was a significant difference between the groups in COUNT category (p=.007). According to the ROC analysis performed for the predictive value of the total CONUT score for GDM, the AUC was 0.642 (95% CI: 0.524-0.759; p=.025). The best cut-off value for total CONUT was 1.5 (90.5% specificity and 28.6% sensitivity. Parity (OR: 1.682, p= .049), total CONUT score (OR: 2.181, p=.004), and serum total cholesterol (OR: 1.020, p=.003) values were found to be significant risk factors to predict GDM in multivariate regression analyses.

Conclusion: Total CONUT scores and total cholesterol may be useful, alternative, and supportive for the diagnosis of GDM.

Keywords: CONUT score, gestational diabetes mellitus, glucose, nutrition.

1. INTRODUCTION

Nutritional status is evaluated as the combined calculation of some biochemical markers and gives the physician an idea of the prognosis of various diseases. Onodera's Prognostic Nutrition Index (OPNI) and the Controlling Nutritional Status index (CONUT) are nutritional indicators that function as prognostic factors for many malignancies and other diseases. These scores are calculated using albumin, lymphocyte counts, and/or total cholesterol. Albumin represents protein reserve and total cholesterol represents calorie depletion, and the total lymphocyte count represents an impaired immune defense. These markers have also been studied in chronic diseases, as well as in patients with cancer (1).

Gestational diabetes mellitus (GDM) is a carbohydrate intolerance that occurs or is newly diagnosed during pregnancy and it usually recovers after pregnancy (2). During pregnancy, insulin resistance occurs physiologically to enble the supply of enough glucose to the fetus. If the patient's pancreas cannot compensate enough for the amount of insulin, glucose metabolism is altered and GDM occurs (3). The prevalence of GDM varies between 1.7% to 11.7% (4). The reason for this variability depends

on factors such as the type 2 diabetes mellitus (T2DM) frequency in populations, obesity, physical activity, conception age, and the diagnostic methods used in the diagnosis of GDM (5, 6). GDM causes many complications, both maternal and fetal (7). The complications of pregnancy that are more common in GDM are macrosomia, preeclampsia, polyhydramnios, stillbirth, and neonatal morbidity (8). Screening is recommended for routine pregnancy care and oral glucose tolerance tests (OGTT) are used for diagnosis (2). OGTT is performed by using hyperosmolar glucose solutions, which cause gastric irritation, delayed gastric emptying, nausea, and vomiting in some patients. In previous studies, it was reported that approximately 10% of patients could not finish OGTTs due to nausea and vomiting, and 2% of patients failed the test due to the misuse of glucose solutions (9, 10).

For these reasons, studies in the search of alternative markers or methods have always remained interesting. In the literature, there are studies on the diagnosis and prediction of GDM using glycated hemoglobin (HbA1c), cholesterol, triglyceride, phospholipid, and interleukin levels (11-13). Different techniques are performed as such as OGTT, which have different sensitivity and specificity values (14). Like metabolic syndrome, insulin resistance and increased insulin play a role in the basic pathophysiology of GDM. Increased insulin resistance is characterized by central abdominal obesity, high blood pressure, increased triglycerides, low levels of high-density lipoprotein cholesterol, and high blood sugar levels. Accordingly, it has been shown in many studies that lipid concentrations are higher in patients with GDM (15). Also, the increased inflammation in metabolic syndrome is responsible for negative consequences, such as poor obstetric outcomes. There are also studies showing changes in lymphocytes in patients with GDM (16). Studies have also illustrated that albumin levels are higher in patients with GDM (17).

Our aim in this study was to investigate and identify a more useful method than the OGTT as a screening and diagnosis method due to the 3-4 times blood test necessities, glucose misuse, and its limited sensitivity and specificity. For this reason, we aimed to investigate whether nutritional index scores could be used in the diagnosis of GDM.

2. METHODS

2.1. Patients and study design

This study was a prospective case-control study performed on 84 patients. The study was approved by the local ethics committee (Reg. No:2019/262) and conformed to the ethical guidelines of the Helsinki Declaration. Forty-two patients with GDM and 42 age and body mass index (BMI)-matched healthy pregnant women were included in the study. GDM was diagnosed with two or more high values in the 100 g OGTT. The control group comprised healthy pregnant women who had negative OGTTs. Patients who were aged 18-35 years, who were at 24-28 gestational weeks, had prepregnancy BMI <30, no systemic and metabolic disease, were not using substances such as alcohol, cigarettes or any drugs, and had no gastrointestinal disease were included in the study.

2.2. GDM diagnosis

GDM was diagnosed using a two-step approach with a 50 g OGTT and a 100 g OGTT. As a screening test, 50 g OGTT was accepted positive as ≥140 mg/dL. Patients who had a positive screening test underwent a 100 g OGTT test. The 100-g oral glucose solution was given in the morning to patients who had fasted overnight for at least 8 hours. Glucose concentrations that were equal to or greater than two or more time points of four Carpenter/Coustan limits were generally considered a positive test (8).

2.3. Evaluation of nutritional screening tools

The OPNI and CONUT values for each patient were measured between the 24-28th gestational week. The OPNI uses serum albumin and total lymphocyte counts and is calculated using the following formula: OPNI = 10 × serum albumin (g/dL) + 0.005 × total lymphocyte count (mm^3). CONUT, with a score between 0 and 12, uses serum albumin, total cholesterol, and total lymphocyte counts. The CONUT score calculation is achieved in two different ways. In the first calculation, there are four assessments as normal, light, moderate, and severe (18). COUNT category 1 was described as normal (0-1), light (2-4), moderate (5-8), and severe (9-12). In the second calculation, the CONUT is scored as two categories, low (score <3), high and high (score≥3) (19). In our study, we defined the first classification as CONUT 1 score and the second classification as CONUT 2 score.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 software (IBM Corp., Armonk, NY, USA). The distribution of variables was tested for normality using histograms and the Shapiro-Wilk test. Parametric continuous data are presented as means ± standard deviation, nonparametric continuous data are presented as medians (min-max). Categorical variables are expressed as numbers (percentages). Data were analyzed using Student's t-test and the Mann-Whitney U test. Receiver operating characteristics (ROC) analysis was performed to determine the total CONUT score cutoff value. Spearman correlation was performed between total CONUT scores and continuous variables in patients with GDM. All variables, whose univariate test had a p-value <.05, was accepted for the multivariable model. P<.05 was considered statistically significant.
The required sample size was calculated using the G*Power software version 3.1.9.2 (*Heinrich Heine Universität; Düsseldorf; Germany) for the independent sample t-test, the α-error probability at 0.05, power (1-β error probability) at 0.80%, and effect size (d) at 0.65 (20). The total number of participants needed to produce a statistically acceptable figure was 78 patients. Eighty-two patients were included in the study.

3. RESULTS

An analysis was performed on a total of 84 patients. The characteristic features of the patients are summarized in Table 1. There was no statistically significant difference regarding age, gravida, parity, weight (pre-pregnancy), weight (at administration), height, weight gain, BMI (prepregnancy), and BMI (at administration) between the groups (p>.05).
The comparison of hematologic, biochemical, OPNI, and CONUT values between the groups is summarized in Table 2. Serum total cholesterol levels were statistically higher in the GDM groups than in the control group (mean 237.4 ± 52.0 mg/dL vs. 216.9 ± 33.5 mg/dL; p=.035). There was no statistically significant difference for serum albumin, lymphocyte, neutrophil, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglyceride (TG), neutrophil to lymphocyte ratio (NLR) levels, and OPNI between the groups. Total CONUT scores were significantly higher in the GDM group (mean 2.67 ± 1.4 vs. 1.95 ± 0.8; p=.006). There was a significant difference between the groups in CONUT category 1 (p=.007). In CONUT category 1, no severe subgroup was observed in either group, but the normal subgroup percentage was higher in the control group (75% vs. 25%), the light subgroup percentage was higher in the control group (51.6% vs. 48.8), and the moderate subgroup percentage (100% vs. 0%, respectively) was higher in the GDM group. CONUT category 2 was similar between the groups (p=.136). In CONUT category 2, the low subgroup percentage was higher in the control group (54.8% vs. 45.2%), and the high subgroup percentage was higher in the GDM group (63.6% vs. 36.4%).
Total CONUT scores were evaluated using ROC analysis, cutoff levels were determined, and the area under the ROC curve (AUC) was calculated (Figure 1). According to the ROC analysis performed for the predictive value of the total CONUT score for GDM, the AUC was 0.642 (95% CI: [0.524-0.759]; p=.025). The best cut-off value for total CONUT was 1.5 (90.5% specificity and 28.6% sensitivity) (Table 3).
The correlation analysis of total CONUT scores and other variables in the GDM group is summarized in Table 4. Total CONUT score and height (r=0.348, p=.024), BMI (r= -0.382; p =.013), albumin (r= -0.510; p<.001), lymphocyte (r= -0.579; p<.001), NLR (r=0.515; p <.001), and OPNI (r= -0.513; p<.001) were significantly correlated in the GDM patient group.
Odds ratios (OR) for each demographic, hematologic, and biochemical variable in patients with GDM were calculated using univariate logistic regression analysis. Parity (OR: 1.631, 95% CI: [1.040-2.558]; p=.033), total CONUT score (OR: 1.862, 95% CI: [1.138-3.046]; p=.013), and serum total cholesterol (OR: 1.011, 95% CI: [1.001-1.022]; p=.039) values were found to be significant risk factors to predict GDM in univariate regression analyses, and their deterministic values were also observed in multivariate analyses. Parity (OR: 1.682, 95% CI: [1.002-2.825]; p=.049), total CONUT score (OR: 2.181, 95% CI: [1.286-3.698]; p=.004), and serum total cholesterol (OR: 1.020, 95% CI: [1.007-1.034]; p=.003) values were found to be significant risk factors to predict GDM in multivariate regression analyses (Table 5).

4. DISCUSSION

Our study compared hematologic and biochemical variables between patients with GDM and healthy pregnant women and showed significant differences between the groups in terms of total CONUT scores and CONUT category 1 ($p<.05$). We found that the total cholesterol level was statistically higher in the GDM groups ($p<.05$). We calculated the best cutoff value as 1.5 for the total CONUT score (90.5% specificity and 28.6% sensitivity).

OPNI and CONUT scores are nutritional indexes that function as prognostic factors for malignancies and other chronic diseases. These scores are calculated using 167lbümin, lymphocyte counts and/or total cholesterol, and calculated by assuming that it represents 167lbümin protein reserve and total cholesterol calorie depletion, and the total lymphocyte count represents an impaired 167lbümi defense. OPNI is used 167lbümin prediction of preoperative overall survival (OS), recurrence-free survival (RFS), recurrence, and prognosis prediction in various diseases, mostly cancer (1, 21, 22). In addition, serum 167lbümin and lymphocyte counts have been independently established as indicators of both nutritional and inflammatory status (23-25). Broggi et al. Found that the relationship between OPNI and OS and RFS was significantly higher in patients with clear cell renal cell carcinoma following nephrectomy (21). Chohno et al. Observed that the OPNI score was a significant marker for surgical timing and procedural decision in patients with ulcerative colitis (22). The OPNI index is also used for identifying malnutrition and malnutrition-related morbidity in patients with chronic schizophrenia (1). The CONUT score is used effectively in various diseases, mostly cancer, like the OPNI score (26-28). Although there are no specific studies on patients with GDM using these nutritional status scores, there are various studies on hematologic and biochemical variables calculated in these scores system. The studies found that triglyceride, cholesterol, and lipoprotein levels were significantly higher in patients with GDM (15). Albumin was studied as ischemia-modified 167lbümin (IMA) and glycated 167lbümin (GA) values were statistically higher in patients with GDM (17). Studies have shown that 167lbümin values are higher in patients with GDM (17). In our study, total cholesterol levels were statistically different between the groups ($p<.05$), whereas 167lbümin and triglyceride levels were similar between the groups ($p>.05$).

There is no specific study in the 167lbümin167167e investigating the relationship between the diagnosis of diabetes and nutritional scores. However, Mineoka et al. Found a significant relationship between CONUT scores and carotid intima-media thickness and carotid plaque score in patients with T2DM (29). In our study, we investigated the OPNI and CONUT scores in patients with GDM. OPNI scores were similar between the groups. The total CONUT score was higher in the GDM group. CONUT score 2 was similar, whereas the CONUT 1 score was significantly different between the groups. No patients were observed in the severe subgroup according to CONUT 1 scores. All patients in the moderate group were those with GDM.

In our study, the OPNI score was not found to be significant, but the CONUT score was found to be significant between the groups. This reason could be because total cholesterol was not considered in both scoring systems and both scores used different mathematical formulas. Many studies on total cholesterol and lipoproteins have been conducted in patients with GDM. Maternal dyslipidemia, which rises in a physiologic range, is a common phenomenon during pregnancy. Hyperlipidemia, in particular, is commonly found in the second half of pregnancy and is considered a physiologically necessary mechanism to provide fuel and nutrients 167lbümin fetus (30). The level of lipids increases slightly at the beginning of pregnancy but significantly decreases in later pregnancy (31). There are conflicting reports on lipid concentrations and ratios in patients with GDM compared with healthy pregnancies. Barat et al. Found that TG, the LDL to HDL ratio, TG to LDL ratio, and TG to HDL ratio were found significantly higher in patients with GDM than in those without GDM, and cholesterol levels were similar between the groups (11). Wang et al. Found that blood total cholesterol levels increased as gestational week increased and there was no significant difference between patients with GDM and control group patients (32). Korkmazer et al. Found that women with GDM had higher TG levels, but their LDL and cholesterol levels were not different from healthy pregnant women (33). In our study, total cholesterol was significantly higher in the GDM groups ($p<.05$). Serum TG, HDL, and LDL levels were similar between the groups ($p>.05$). In multivariate logistic regression analysis, serum total cholesterol levels were found to be independently associated in patients with GDM (OR: 1.020 95% CI: [1.007-1.034]; $p=.003$).

Numerous physiologic changes occur during pregnancy to adapt the mother 167lbümin increased metabolic requirements and providing fetal nutrition. Many hormonal and immunologic changes happen to maintain pregnancy (34). Normal total leukocyte counts are higher in pregnant women than non-pregnant women, but the main change in lymphocytes is the subtype change (35). The serum 167lbümin concentration decreases during pregnancy. In term pregnancy, 167lbümin levels are near to 3.0 g/dL, whereas in non-pregnant women it is around 4.3 g/dL. However, the total body 167lbümin increase is low comparatively in relation to the increased plasma 167lbümi during pregnancy (36). Due to insulin

resistance and increased estrogen levels in pregnancy, concentrations of lipids, lipoproteins, and apolipoproteins in plasma also increase significantly during pregnancy (31). Triacylglycerol and cholesterol levels in total serum cholesterol, very-low-density lipoproteins (VLDLs), LDLs, and HDLs are increased during the third trimester compared with non-pregnant women (37). After delivery, the concentrations of these lipids, lipoproteins, and apolipoproteins decreases. In patients with cancer, serum 168lbümin, lymphocyte, and total cholesterol levels tend to decrease (38). In our study, total cholesterol and total CONUT scores were found to be significantly higher in patients with GDM. However, the hematologic and biochemical markers used in calculating both OPNI and CONUT scores were affected by pregnancy, and these markers were not different between groups except total cholesterol. In addition, about 10% errors were reported during the tests (9, 10). 168lbümin168 reason, stricter tests should be developed instead of patient-dependent tests. CONUT can be used 168lbümin168 purpose, but this test should be updated with new threshold values for pregnant patients. The OPNI scoring system was not significantly higher in patients with GDM 168lbümin168 had a negative correlation with CONUT scores. This is probably because lipid values are not considered in this scoring system.

The limitation of our study is the low sample size, and the cutoff values of OPNI and CONUT scores in pregnant women are unknown. In addition, it is an obstacle that these scoring systems are not adapted to the physiologic changes that occur in pregnant women. In this study, it was not studied whether the patients were regulated through diet or insulin. Lack of knowledge of disease severity and treatment modalities are also limitations. The strength of this study is that these malnutrition scores have not been studied before in pregnant women, especially those with GDM.

Nutritional index scoring systems may be useful, supportive, and alternatives 168lbümin diagnosis of GDM. Difficulties with performing the OGTT such as improper use of glucose or giving blood samples many times may lead to finding alternative methods. Total COUNT scores can be used as a simple, easy-to-use method with low error rates 168lbümin diagnosis of gestational diabetes. Albumin, lymphocyte, and total cholesterol values used in the CONUT and OPNI scoring systems are affected by pregnancy. There is a need for studies in which new limits are determined for pregnancy. Thus, new scoring systems that may arise can give more effective results in the diagnosis of GDM.

Acknowledgments

Compliance with Ethical Standards:

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1. Patient characteristics between groups

Variables	**Control group n=42**	**Study group n=42**	***P***
Age (years)*	28.1 ± 3.4	27.3 ± 3.1	.316
Gravida**	3 (1-7)	3 (2-7)	.110
Parity**	1 (0-3)	2 (0-3)	.280
Weight (prepregnnacy)*	67.2 + 5.6	69.2 ± 5.2	.085
Weight (during test)*	74.8 ± 3.8	75.8 ± 3.9	.239
Height (cm)*	162.7 ± 4.4	162.6 ± 4.1	.980
Weight gain*	7.6 ± 3.01	6.6 ± 2.84	.106
BMI (prepregnnacy)*	25.4 ± 2.05	26.2 ± 2.07	.081
BMI (during test)*	28.3 ± 1.6	28.7 ± 1.7	.290

Results were analyzed using Student's t-test and the Mann-Whitney U test. *; means ± SD,** median (min-max). BMI: Body mass index

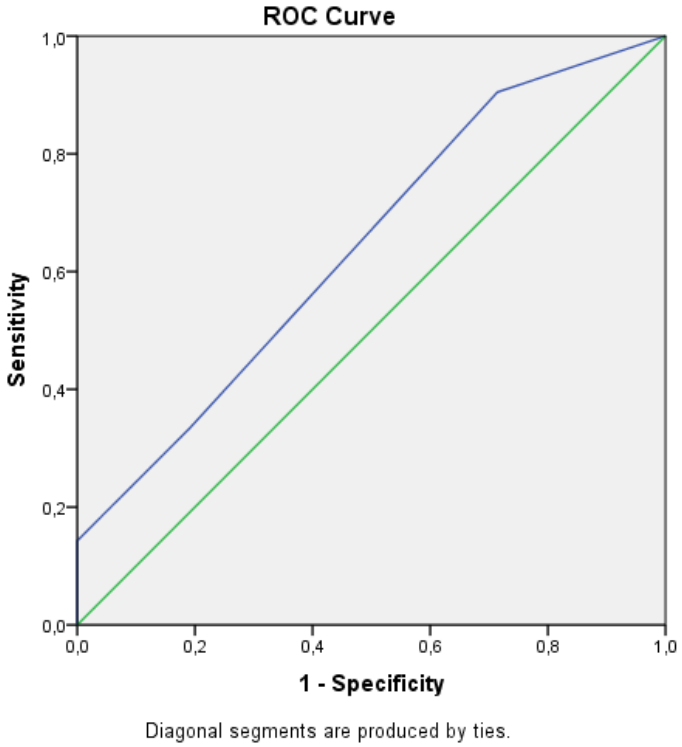

Figure 1. ROC analysis chart of total CONUT score

Table 2. Comparison of hematologic, biochemical, OPNI and CONUT values between the groups

Variables		**Control group n=42**	**Study group n=42**	***P***
Albumin (g/dL)*		28.1 ± 3.4	27.3 ± 3.1	.068
Total cholesterol (mg/dL)*		216.9 ± 33.5	237.4± 52.0	**.035**
Lymphocyte (µL)*		1.8 ± 0.5	1.9 ± 0.9	.490
Neutrophil (µL)*		6.9 ± 1.6	7.7 ± 2.7	.140
LDL (mg/dL)*		135.9 ± 36.4	135.4 ± 40.5	.955
HDL(mg/dL)*		65.3 ± 13.2	63.6 ± 9.3	.489
TG (mg/dL)*		189.8 ± 68.8	221.3 ± 77.8	.052
Nutritional status				
NLR*		4.1 ± 1.5	4.8 ± 2.7	.151
OPNI*		34.4 ± 2.2	33.4 ± 2.9	.068
Total CONUT score*		1.95 ± 0.8	2.67 ± 1.4	**.006**
CONUT Category (1)**	**Normal**	12 (75%)	4 (25%)	**.007**
	Light	30 (48.4%)	32 (51.6%)	
	Moderate	0 (0%)	6 (100%)	
	Severe	0	0	
CONUT Category (2)**	**Low**	34 (54.8 %)	28 (45.2%)	**.136**
	High	8 (36.4 %)	14 (63.6 %)	

Results were analyzed using the independent sample t-test and Pearson chi-square test.*, means ± SD; **, number (%).p-Values < .05 are statistically significant and bold values indicates statistically significant. CONUT: controlling nutritional status index; PNI: prognostic nutritional index; HDL: high-density lipoprotein cholesterol; LDL; low-density lipoprotein cholesterol; TG: triglyceride; NLR: neutrophil to lymphocyte ratio. COUNT category (2): (low (score <3), high (score≥3). COUNT category (1): normal (0-1), light (2-4), moderate (5-8), and severe (9-12).

Table 3. Best cuttoff value, spesifity , sensitivty, and AUC of total CONUT score.

		Cutoff	**Specificity**	**Sensitivity**	**AUC (95% CI)**	**p-Value**
Total score	CONUT	1.5	90.5%	28.6%	0.642 (0.524-0.759)	**.025**

p-values < .05 are statistically significant and bold values indicate statistically significant. CONUT: controlling nutritional status index; AUC: area under the curve; CI: confidence interval.

Table 4. Correlation analysis of total CONUT score and other variables in GDM group

Results are analyzed by Spearman's rho test. P-values <.05 are statistically significant and are indicated as bold text. CONUT: controlling nutritional status index; PNI: prognostic nutritional index; HDL: high-density lipoprotein cholesterol; LDL; low-density lipoprotein cholesterol; TG: triglyceride; NLR: neutrophil to lymphocyte ratio.

	Total CONUT score	
Variables	**Spearman's rho**	**Sig. (2-tailed)**
Age (year)	0.124	.436
Gravida	0.103	.518
Parity	0.301	.052
Weight	-0.186	.238
Height	0.348	**.024**
BMI	-0.382	**.013**
Albumin (g/dL)	-0.510	**<.001**
HDL (mg/dL)	0.165	.295
LDL (mg/dL)	0.080	.613
TG (mg/dL)	-0.030	.849
Total cholesterol (mg/dL)	-0.105	.341
Lymphocyte (µL)	-0.579	<.**001**
Neutrophil (µL)	-0.186	.238
NLR	0.515	**<.001**
OPNI	-0.513	**<.001**

Table 5. Logistic regression analysis of variables to predict GDM.

Univariate and multivariate logistic regression analysis was performed. CONUT, controlling nutritional status index; OPNI, Ondera's prognostic nutritional index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglyceride; NLR, neutrophil to lymphocyte ratio, BMI, body mass index, CI, confidence interval; OR, odds ratio. Bold values refer to statistical significance (p < .05)

	Univariate		**Multivariate**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**p-Value**
Age (year)	0.933 (0.815-1.068)	.313		
Parity	1.631 (1.040-2.558)	**.033**	1.682 (1.002-2.825)	**.049**
BMI (pre-pregnnacy)*	1.210 (0.975-1.501)	.084		
Total CONUT score	1.862 (1.138-3.046)	**.013**	2.181 (1.286-3.698)	**.004**
OPNI	0.849 (0.709-1.016)	.074		
NLR	1.165 (0.943-1.439)	.158		
Total cholesterol (mg/dL)	1.011 (1.001-1.022)	**.039**	1.020 (1.007-1.034)	**.003**
HDL (mg/dL)	0.986 (0.950-1.025)	.486		
LDL (mg/dL)	1.000 (0.988-1.011)	.954		
TG (mg/dL)	0.994 (0.988-1.000)	.057		
Albumin (g/dL)	0.194 (0.032-1.174)	.074		

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