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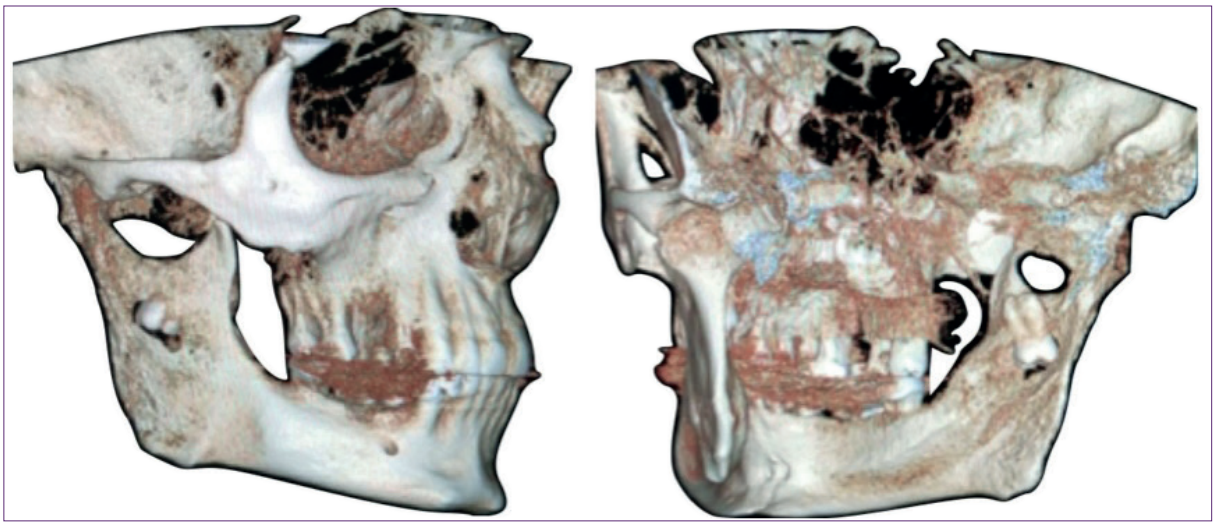

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RELATIONSHIP OF ADAMTS WITH BIOCHEMICAL PARAMETERS IN METABOLIC SYNDROME

METABOLİK SENDROMDA ADAMTS'LERİN BİYOKİMYASAL PARAMETRELER İLE İLİŞKİSİ

Semiramis DOĞAN ZEYBEK[1], Adnan Adil HİŞMİOĞULLARI[2]

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ABSTRACT

Objective: The aim of our study was to investigate the potential of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteins, which are thought to change in levels as a result of the deterioration of the extracellular matrix in the vessels due to the development of atherosclerosis, to be used as biochemical markers in individuals with metabolic syndrome and diabetes.

Material and Methods: Our study was carried out with the participation of 10 female individuals diagnosed with diabetes in the experimental group, 10 female individuals diagnosed with metabolic syndrome and 11 healthy female individuals the aged 25-65 years. Biochemical analyses and anthropometric measurements were performed and blood samples were taken. Serum was separated from the blood samples. ADAMTS-1 and ADAMTS-9 serum levels were analysed using ELISA method.

Results: When the amount of ADAMTS-1 protein was analysed, ADAMTS-1 level was found to be statistically ($p<0.01$) significantly lower when the experimental and control groups were compared. When the amount of ADAMTS-9 protein was analysed, similarly statistically significant ($p<0.01$) decreases were found between the groups.

Conclusion: This is a preliminary study showing that ADAMTS-1 and ADAMTS-9 proteins can be used in the early diagnosis and treatment of metabolic syndrome and in the pathogenesis of the disease. However, this potential needs to be investigated in detail.

Keywords: ADAMTS-1, ADAMTS-9, diabetes mellitus, metabolic syndrome, atherosclerosis

ÖZ

Amaç: Çalışmamızda ateroskleroz gelişimine bağlı olarak damarlardaki ekstraselüler matriksin bozulmasının neticesinde düzeylerinde değişiklik olabileceği düşünülen a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteinlerinin metabolik sendromlu ve diyabetli bireylerde biyokimyasal belirteç olarak kullanılma potansiyellerini ortaya koymaktır.

Gereç ve Yöntemler: Çalışmamız, 25-65 yaş aralığında deney grubunda diyabet tanısı konulmuş 10 kadın birey, metabolik sendrom tanısı konulmuş 10 kadın birey ve sağlıklı 11 kadın bireyin katılımıyla gerçekleştirilmiştir. Çalışmadaki bireylerin biyokimyasal analizleri ve antropometrik ölçümleri yapılıp kan örnekleri alındı. Alınan kan örneklerinden serumları ayrıldı. ADAMTS-1 ve ADAMTS-9 serum düzeyleri ELISA yöntemiyle analiz edildi.

Bulgular: ADAMTS-1 protein miktarı incelendiğinde deney ve kontrol grupları karşılaştırıldığında ADAMTS-1 düzeyi istatistiksel olarak ($p<0,01$) anlamlı derece düşük bulunmuştur. ADAMTS-9 protein miktarı incelendiğinde ise gruplar arasında yine benzer şekilde istatistiksel olarak anlamlı ($p<0,01$) azalmalar tespit edilmiştir.

Sonuç: ADAMTS-1 ve ADAMTS-9 proteinlerinin metabolik sendromda erken teşhis ve tedavi sürecinde ve hastalığın patogenezinde kullanılma potansiyellerinin mevcut olduğunu gösteren bir ön çalışma niteliğindedir. Fakat bu potansiyelin detaylı olarak araştırılması gerekmektedir.

Anahtar Kelimeler: ADAMTS-1, ADAMTS-9, diabetes mellitus, metabolik sendrom, ateroskleroz

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INTRODUCTION

Metabolic syndrome (MetS) is a condition that rapidly expands its area of influence because of today's modern lifestyle and leads to significant health problems (1). Metabolic syndrome is characterised by a combination of various disorders, including abdominal obesity, insulin resistance, glucose intolerance or type 2 diabetes, hypertension, dyslipidemia, and cardiovascular diseases (2). The prevalence of this syndrome is increasing rapidly all over the world. This situation decreases the living standards of the individual and leads to an increased burden on the healthcare system (3). In this context, the early diagnosis and prevention of MetS is of great importance (4).

The pathophysiology of Metabolic Syndrome is complex and is a blend of genetic factors, environmental factors, and lifestyle. Insulin resistance (IR), which is one of its important components, leads to elevated blood glucose levels as a result of the inefficient utilisation of glucose by cells (5). Obesity, especially abdominal obesity, is one of the main components of MetS and increases IR through the unbalanced release of adipokine (2). Another important component of MetS is dyslipidemia, the deterioration of the lipid profile in the blood. This disorder includes an increase in low-density lipoprotein (LDL) cholesterol and a decrease in high-density lipoprotein (HDL) good cholesterol. In metabolic syndrome, hypertension is often seen with an increased risk of cardiovascular diseases (6, 7). Finally, coronary artery diseases may occur as an important consequence of metabolic syndrome. This is related to the progression of the atherosclerosis process (8). There are many criteria in the diagnosis of metabolic syndrome. These criteria commonly include abdominal obesity, elevated fasting blood glucose, low HDL levels, and high triglyceride levels (9).

The main aim of this study was to investigate the potential of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) parameters as biochemical markers in metabolic syndrome. ADAMTS proteins consist of metalloproteinases that have roles in remodelling of extracellular matrix (ECM) (10). ADAMTS proteins, especially ADAMTS-1 and ADAMTS-9, are related to the disruption of the extracellular matrix of vessels and have important effects on the initiation and progression of atherosclerosis (11). In our study, we will examine the effects of these proteins in metabolic syndrome and their potential as markers by comparing ADAMTS-1 and ADAMTS-9 levels in individuals with metabolic syndrome and diabetes with those in the control groups.

MATERIAL and METHODS

Participants

The study was carried out with the participation of 10 female individuals between the ages of 25-65 who were previously diagnosed with diabetes, 10 female individuals diagnosed with MetS and 11 healthy female individuals. The experimental group was selected from female individuals diagnosed with MetS or DM because of clinical evaluations at Balıkesir University Faculty of Medicine Hospital. The control group consisted of individuals without any chronic disease and underwent regular health checks. Only female patients were included in the study because of the low number of such studies on women and in order for our study to contribute to filling this gap. In addition, it was aimed to better understand the effects of gender differences in the biochemical parameters. Patient and control group participants were informed and written consent was obtained. Approval for the research was obtained from Balıkesir University Faculty of Medicine Ethics Committee with the decision numbered 2016/111 on 16.11.2016.

Anthropometric measurements

The weight, height and waist circumference of the participants were measured and the body mass index (BMI) was calculated. These data obtained in our study were compared between the groups and the data were statistically analysed.

Anthropometric measurements of the patient and control groups were performed using standard methods. For body weight and height, a mechanical scale with a height gauge was used. The waist circumference was measured at the navel level and recorded.

Blood samples and analyses

Blood samples were taken from the participants after 8-12 hours of fasting. These samples were centrifuged at +4℃ for 10 min and the serum portion was separated. The serum portions obtained were stored at -80℃. ADAMTS-1 and ADAMTS-9 levels of the sera obtained from the blood samples were measured by ELISA test kits. In addition, the blood glucose, HbA1c, insulin, LDL, HDL, triglyceride, and total and cholesterol levels of the experimental and control groups were investigated. All biochemical analyses in the blood samples of the study group were performed using an autoanalyzer device in the Biochemistry Laboratory of Balıkesir University Health Practise and Research Hospital and the Biochemistry Laboratory of Balıkesir University Faculty of Medicine.

Statistical analysis

The analysis of the data obtained in the study was performed using the SPSS Statistical Package for Social Sciences (IBM SPSS Corp., Armonk, NY, USA) 20.0 statistical software. Numbers, mean, and percentage values were used to describe the data. The Kolmogorov-Smirnov test was employed to determine whether the variables followed a normal distribution. The non-parametric Mann-Whitney U test was used to compare the two groups. The significance of the relationship between the variables was assessed using the Spearman Correlation test. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

RESULTS

Age and anthropometric measurement values of ındividuals

The mean ages of the experimental and control groups were 45.10±13.60 years, mean body weights were 87.14±22.23 kg, mean BMI (kg/m^2) was 34.60±10.21, and mean waist circumference was 101.35±23.90 cm (Table 1).

Table 1: Age and anthropometric measurements of the participants (Mean±SD)

	n	Average	±SD	Minimum	Maximum
Age (years)	31	45.10	13.60	26	71
Body weight (kg)	31	87.14	22.23	47	129
BMI (kg/m)^2	31	34.60	10.21	19.56	55.11
Waist circumference (cm)	31	101.35	23.90	63	141

BMI: Body Mass Index

Table 2: Age, height, weight and BMI values of the individuals participating in the study (Mean±SD)

	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m²)
Control (n=11)	34.5±6.2	162.3±5.1	60.2±8.4	22.9±2.3
MetS + DM (n=20)	46.7±8.9	158.7±6.0	72.4±11.3	28.7±4.6

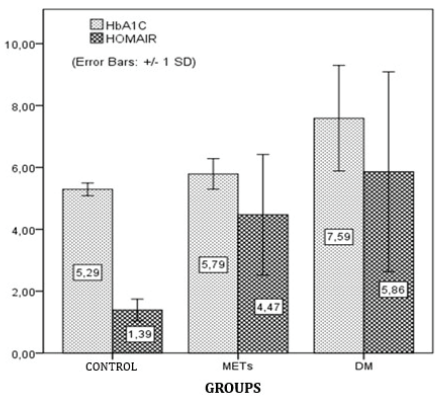

Figure 1: Mean HbA1c and HOMA-IR values of the study groups.

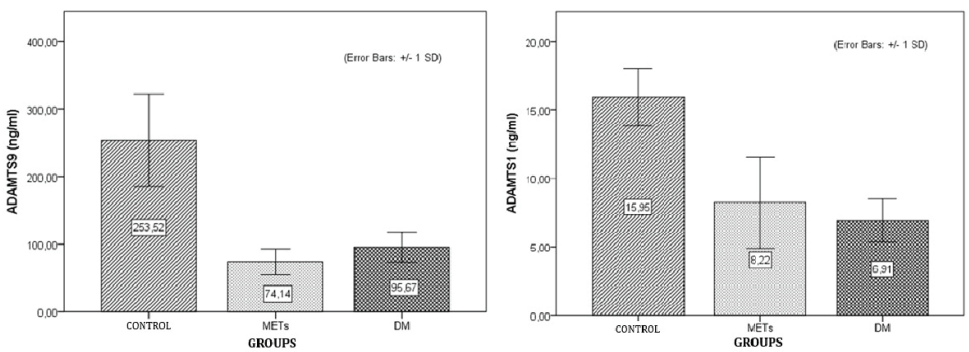

Figure 2: Mean ADAMTS-1 and ADAMTS-9 values of the study groups.

The mean age of the patient group was 45.6±10.2 years and 47.3±9.8 years, while the mean age of the control group was 42.1±8.7 years (Table 2). The waist circumference, BMI, and waist-to-hip ratio of the patient group were found to be significantly higher than those of the control group (p<0.01).

Mean HbA1c and HOMA-IR values of the study groups

The HbA1c value of the control group in the study was 5.29±0.21 ng/ml. The metabolic syndrome and diabetes groups were 5.79±0.5 ng/ml and 7.59±1.71 ng/ml, respectively (Figure 1). According to the mean HbA1c values, statistically significant differences were found between the control group and the diabetes group (p<0.01), between the control group and the metabolic syndrome group (p<0.05) and between the metabolic syndrome and diabetes groups (p<0.05).

According to HOMA-IR values, healthy individuals in the control group were 1.39±0.35 ng/ml, individuals with metabolic syndrome were 4.47±1.95 ng/ml, and individuals with diabetes were 5.86±3.23 ng/ml (Figure 1). According to this result, a sta-

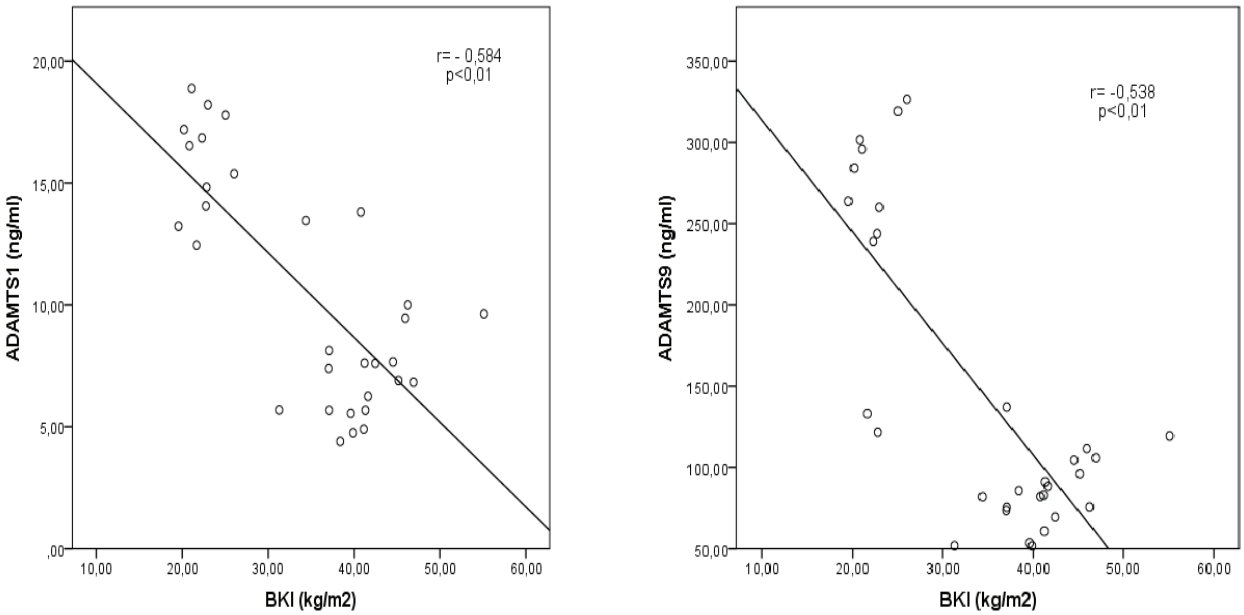

Figure 3: Relationship between BMI and ADAMTS-1 and ADAMTS-9 values

Table 3: Results of anthropometric and biochemical measurements of the study groups (Mean±SD)

	Control (n:11)		METs (n:10)		DM (n:10)	
	Average	±SD	Average	±SD	Average	±SD
Body weight (kg)	60	7.25	97.95	7.80	106.18	9.71
BMI (kg/m^2)	22.30	1.96	39.93	4.71	42.79	5.47
Waist circumference (cm)	72	6.56	114.70	8.23	120.30	11.83
Glucose (mg/dl)	89.82	6.00	104.6	15.15	153.30	48.66
Cholesterol (mg/dl)	157.64	13.58	201.20	28.28	195.10	22.73
LDL (mg/dl)	91.11	14.39	123.28	28.76	108.96	12.47
HDL (mg/dl)	52.55	9.95	50.80	11.60	56.20	17.30
TG (mg/dl)	70.27	22.07	135.60	50.76	149.70	50.50
HbA1c	5.29	0.21	5.79	0.50	7.59	1.71
HOMA-IR	1.39	0.35	4.47	1.95	5.86	3.23
ADAMTS-1 (ng/ml)	15.95	2.11	8.22	3.33	6.91	1.56
ADAMTS-9 (ng/ml)	253.52	68.59	74.14	18.79	95.67	22.25

BMI: Body Mass Index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TG: Triglyceride HbA1c: Haemoglobin A1c HOMA-IR: İnsülin direct test

tistically significant difference was found between the control group and the metabolic syndrome group ($p<0.05$). Again, a statistically significant difference was found between the control group and the diabetes group ($p<0.05$). However, no statistically significant difference was found between the metabolic syndrome and diabetes group ($p>0.05$).

ADAMTS levels

The mean ADAMTS-1 values of the control, metabolic syndrome and diabetes groups were 15.95±2.11 ng/ml, 8.22±3.33 ng/ml and 6.91±1.56 ng/ml, respectively (Figure 2). ADAMTS-1 levels in the patient group were significantly lower than those in the control group ($p<0.01$).

The mean ADAMTS-9 levels of the control, metabolic syndrome and diabetes groups were 253.52±68.59 ng/ml, 74.14±18.79 ng/ml and 95.67±22.25 ng/ml, respectively (Figure 2). ADAMTS-9 levels of the patient group were significantly lower than those of the control group ($p<0.01$). A significant decrease was found in the metabolic syndrome and diabetes groups ($p<0.05$) and no significant difference was observed between the metabolic syndrome and diabetes.

BMI and ADAMTS-1 and ADAMTS-9 values
According to Spearman's correlation analysis, a statistically significant and negative correlation was found between BMI (kg/m^2) and ADAMTS-1 (ng/ml) and ADAMTS-9 (ng/ml) measurements (r = 0.584; p<0.01) (Figure 3).

Anthropometric measurements and biochemical parameters
When the control group was compared with both the metabolic syndrome and diabetic patient group; mean HbA1c, glucose, HOMA-IR values and LDL (mg/dl), TG (mg/dl) and cholesterol (mg/dl) were found to be statistically significant (p<0.05). However, no significant difference was found in the mean HDL (mg/dl). HbA1c, ADAMTS-9 and glucose values were found to be statistically different in the metabolic syndrome and diabetic patient groups (p<0.05) (Table 3).

DISCUSSION

The results of our study indicate that ADAMTS-1 and ADAMTS-9 parameters are biochemical markers in the diagnosis of metabolic syndrome and diabetes. It is thought that the deterioration of the extracellular matrix decreases ADAMTS proteins. It is possible that this may have caused the development of atherosclerosis in the patient. This highlights that ADAMTS proteins may play a role in the formation and progression of vascular complications seen in patients with metabolic syndrome and diabetes.

These findings are consistent with other studies in the literature. For example, Santamaria et al. reported that ADAMTS-1 levels were associated with cardiovascular diseases and MetS (12). Similarly, in a study conducted by Wei et al., it was stated that ADAMTS-9 loci are associated with type 2 diabetes mellitus, insulin resistance, and coronary artery disease (CAD) risk factors. The Adamts-9 level showed a significant correlation with coronary artery disease and metabolic syndrome (13). However, as indicated in these studies, further research is required for ADAMTS proteins to be used as biochemical markers.

Low levels of ADAMTS-1 and ADAMTS-9 may be associated with increased inflammatory processes and oxidative stress. MetS and DM are known to cause increased chronic inflammation and oxidative stress. In the study of Boesgaard et al. on ADAMTS-9, the ADAMTS-9 parameter was found to be significantly lower in patients with diabetes (14). These results support the possible effect of ADAMTS-9 in diabetic complications. Wang et al. reported that ADAMTS-1 levels were low in obese individuals, and this may be associated with insulin resistance (15). This finding supports the decrease in ADAMTS-1 levels observed in our study. In a 2020 study, Li et al. examined the effect of ADAMTS-1 on cardiovascular diseases and found that low levels of ADAMTS-1 increased the cardiovascular risks of patients (16). This study shows that the ADAMTS-1' protein can be used in the early diagnosis and treatment of cardiovascular diseases.

According to the study conducted by Hoo et al., a decrease in the amount of ADAMTS-9 was observed in mice fed a high-fat diet and with increased insulin resistance. The results obtained in this study are consistent with our results (17).

However, more extensive and long-term studies are required to evaluate the potential for clinical use of ADAMTS-1 and ADAMTS-9 as biochemical markers. Future studies may show that ADAMTS proteins can be used in monitoring vascular complications and developing treatment strategies in patients with MetS and DM.

It is concluded that our preliminary study revealed that ADAMTS-1 and ADAMTS-9 proteins can be used in the early diagnosis and treatment of metabolic syndrome. However, it was concluded that this potential should be investigated in detail. Future studies need more data to better understand the clinical uses of these proteins. In addition, the potential of ADAMTS proteins to be used as therapeutic targets should also be investigated.

Ethics Committee Approval: This study was approved by Balıkesir University Faculty of Medicine Ethics Committee (Date: 16.11.2016, No: 2016/111).

Informed Consent: Written informed consent was obtained from patient who participated in this study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.A.H.; Data Acquisition- S.D.Z.; Data Analysis/Interpretation- S.D.Z.; Drafting Manuscript- S.D.Z.; Critical Revision of Manuscript- A.A.Ş.; Final Approval and Accountability- S.D.Z.; Supervision- A.A.Ş.

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CAN LYVE-1 MOLECULE BE A DIAGNOSTIC BIOMARKER IN PATIENTS WITH ADVANCED LUNG CANCER?

LYVE-1 MOLEKÜLÜ İLERİ EVRE AKCİĞER KANSERLİ HASTALARDA TANISAL BİR BİYOBELİRTEÇ OLABİLİR Mİ?

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ABSTRACT

Objective: Primary lung cancers that originate from epithelial cells are classified as carcinomas. Close monitoring of patients with predisposing conditions can enhance early diagnosis rates and facilitate the implementation of therapeutic approaches. LYVE-1 protein is localized to the lymphatic endothelial layer. This study aimed to evaluate the clinical utility of the serum protein and circulating mRNA of LYVE-1 in lung cancer.

Materials and Methods: We performed ELISA and Real-time PCR to measure and compare the serum protein and circulating mRNA levels of LYVE-1 in the peripheral blood of 60 patients with advanced lung cancer and 20 controls.

Results: Serum LYVE-1 protein and gene expression levels were significantly higher in patients with lung cancer than in controls (p=0.001). There was no association between LYVE-1 (both protein and gene) clinical parameters. The outcome of the ROC analysis, serum LYVE-1 protein (AUC: 0.873) or *LYVE-1* gene (AUC: 0.921) in lung cancer patients.

Conclusion: As far as we are aware, this study represents the first investigation to compare the protein and mRNA levels of *LYVE-1* in the blood samples of lung cancer patients. Additional research involving a larger cohort of subjects will be necessary to gain a deeper understanding of the mechanisms and consequences of LYVE-1 inhibitors in lung cancer.

Keywords: LYVE-1, lung cancer, lymphatic biomarker

ÖZ

Amaç: Primer akciğer kanserleri epitelyal hücrelerden türeyen karsinomlardır. Predispozan bozukluğu olan hastaların yakın takibi, erken tanı ve küratif tedavi yöntemlerinin oranlarında artış sağlayabilir. Hyaluronan için bir reseptör molekülü olan LYVE-1, lenfatik endotelde ifade edilir. Bu çalışma, akciğer kanserinde LYVE-1'in serum proteini ve dolaşımdaki mRNA'sının klinik faydasını değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: İleri evre 60 akciğer kanseri hastası ve 20 sağlıklı kontrolün periferik kanında LYVE-1'in serum protein ve dolaşımdaki mRNA seviyelerini ölçmek ve karşılaştırmak için ELISA ve Gerçek Zamanlı PCR uygulaması gerçekleştirildi.

Bulgular: Akciğer kanserli hastalarda serum LYVE-1 protein ve gen ekspresyon düzeyleri kontrol grubuna göre anlamlı derecede yüksek bulunmuştur (p=0,001). Akciğer kanseri hastalarında serum LYVE-1 protein ve gen ekspresyon düzeyleri ile klinik parametreler arasında bir ilişki bulunmamıştır. ROC analizine göre eğri altında kalan alanlar hesaplandığında; serum LYVE-1 proteini ve geni için AUC (Area Under Curve) değerleri sırasıyla 0,873, 0,921 şeklinde bulunmuştur.

Sonuç: Bu çalışma, akciğer kanseri hastalarının kan örneklerinde LYVE-1'in protein ve mRNA düzeylerini karşılaştıran ilk çalışmadır. Akciğer kanserinde LYVE-1 inhibitörlerinin mekanizmalarını ve sonuçlarını daha derinlemesine anlamak için daha geniş bir denek grubunu içeren ek araştırmalara ihtiyaç duyulmaktadır. Bu tür araştırmalar, bu alana ilişkin değerli bilgiler sağlamada faydalı olacaktır.

Anahtar kelimeler: LYVE-1, akciğer kanseri, lenfatik biyomarker

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INTRODUCTION

Lung cancer is the primary cause of cancer-related deaths globally, with a 5-year survival rate of 18%. As traditional therapies are largely insufficient for curative intent, the early diagnosis and identification of molecular targets are being investigated. The identification of diagnostic, prognostic, and predictive markers is crucial in clinical practice because lung cancer represents a heterogeneous group of diseases with diverse biological and clinical characteristics. A new hot topic to debate is finding non-invasive techniques, such as circulating biomarkers, to increase the rate of early diagnosis and targets for curative treatment modalities in lung cancer (1). The molecular mechanisms underlying lung cancer and its metastasis require further investigation because the disease is often metastatic at the time of diagnosis (2).

The lymphatic capillaries are composed of a single layer of lymphatic endothelial cells (LECs). In contrast to the anatomical structure of the blood vessels, the endothelium of the lymphatic system is surrounded by a muscle layer, pericytes, and a continuous basement membrane. Owing to this function, lymph node outflow is avoided (3). In addition, the endothelial cells of the lymphatic system are covered with elastic filaments that adhere to the extracellular matrix. Therefore, intercellular pressure is prevented during the collapse of the lymphatic capillary tissues due to the transformation of the lymphatic capillaries. As the pressure increases, the filaments elongate and overlap the lymphatic openings of the endothelial cells, allowing the lymphatic fluid, macromolecules, and cells to enter, which causes the capillaries to open. Lymph node metastasis, classification, prognosis, and treatment of malignant tumours are among the most important factors in determining the form. Although the clinical features of metastasis are known, their molecular mechanisms are limited, and LECs are pivotal in the development and maintenance of the lymphatic system. Moreover, the vascular system plays a fundamental role in regulating immune responses. Studying lymphatic endothelial cells (LECs) in both *in vivo* and *in vitro* models is often challenging, as these models can only partially replicate their behaviour and phenotypes. It has been demonstrated that LECs gradually lose their lymphatic markers over time, highlighting their adaptability and diversity in vivo. Given that LECs uniquely express LYVE-1, coating them with hyaluronic acid (HA) has been found to preserve their phenotypes and functions (4).

Most cancers, especially carcinomas, metastasize to the lymph centres from which they drain. Systemic spread can be stopped by attacking the higher endothelial vessels (HEV) in the paracortex (5, 6). Be that because it may, at show, the components by which tumours assault and move interior the lymphatics are not totally caught on to get to lymphatic capillaries and action to exhausting lymph centre points in the midst of secure observation, safe adjust, and assurance of disturbance (7, 8). These consolidate chemotaxis, haptotaxis, and heading by means of haptotaxis aerotaxis, which connects to the vascular surface through receptors such as integrins and MMPs (cross section metalloproteinases) and ADAMs (A Disintegrin and Metalloproteinases) (9-11). This brief review may be a new the coming approximately instrument for lymphatic segment counting of the broad polysaccharide HA and its key lymphatic structure and safe cell receptors LYVE-1 and CD44, respectively, that some tumours can also use this centre to help with nodal metastasis.

Most HA-binding proteins belong to the superfamily of Link proteins, all of which share a shared hyaluronan-binding domain called the “Link” module. LYVE-1 was characterised in the pooled Human Genome Sciences/TIGR EST database (12,13).

LYVE-1 exhibits a molecular mass of 60-70 kDa, A distinguishing feature of LYVE-1 is its uniqueness, as it is not found in its closest homologous receptors, such as CD44. Similar to CD44, the most closely related receptor to LYVE-1, it was bound -residue hydrophobic domain containing a cysteine residue (14).

Genetic changes in the *LYVE-1* gene play an important role in patients with lung cancer. Alterations in the expression of this gene contribute to a more aggressive cancer phenotype. However, how exactly do changes in the *LYVE-1* gene influence cancer development? Our goal is to provide valuable insights for clinicians in guiding treatment decisions and slowing or halting cancer progression. We believe that this study will make a crucial contribution to the field. In our research, we found elevated levels of LYVE-1 protein in the serum of lung cancer patients, which can be easily obtained. These findings indicate that LYVE-1 holds potential as a biomarker for lung cancer, as its protein levels can be evaluated in patients to support diagnosis or prognosis. We hope that our findings will guide diagnostic approaches. Additionally, no similar studies were found in the literature comparing both protein and mRNA levels of LYVE-1 in lung cancer serum, making our study a valuable addition to the existing body of research.

MATERIAL AND METHODS

This study investigated 60 lung cancer patients and 20 control cohorts that were collected between 2015 and 2016. Of the 20 individuals in the healthy control group, 15 were male, 10 were over the age of 60, and 12 were smokers. Serum protein and circulating LYVE-1 levels were measured using ELISA in the two cohorts. Our study on human materials was approved by the İstanbul University Ethics Committee (Date: 27.08.2014, No: 1311). This study was conducted according to the Declaration of Helsinki (1989), and all the volunteers were informed about this study’s content.

Blood samples were collected from both patients and healthy controls via venipuncture, ensuring that the blood clotted at room temperature. Serum preparation involved centrifugation at 4000 rpm for 10 min at room temperature. For storage, the initial admission samples were frozen immediately at -80°C prior to treatment, while the follow-up samples were frozen at -20°C. Patient classification was performed on the basis of disease staging using the AJCC and IUCC systems, complemen-

Table 1: Primer sequences

Gene Name	Forward primer F (5′-3′)	Reverse primer R (3′-5′)
LYVE-1 NM_016164	TCCTATCCTCCTACCTCCAAAG	CGTATCCTCAGCCTTGTTCTATT
GAPDH NM_002046	GCTCTCTGCTCCTCCTGTTC	ACGACCAATCCGTTGACTC

ted by a comprehensive clinical evaluation, which included detailed medical history, physical examination, and blood tests. Eligibility for treatment was determined using specific criteria, including a performance status of ECOG ≤ 2, an absolute neutrophil count exceeding 1500/µL, and a platelet count greater than 100,000/µL. A multidisciplinary approach was employed for treatment planning to ensure comprehensive care.

Determination of protein levels of the LYVE-1 molecule

An ELISA kit ((Catalog no: CSB-EL013282HU, Shanghai Yehua Biological Technology Shanghai, China) was used to measure the serum LYVE-1 level. A volume of 40 µL of serum samples and 10 µL of LYVE-1 antibody were added to all antibody-coated wells using an automated pipette. Additionally, 50 µL of the prepared standards were introduced into the designated wells. Subsequently, 50 µL of the streptavidin-HRP solution was added to each well. The microplates were incubated at 37°C for 1 h to facilitate the formation of the antibody-antigen-antibody complex. Following the incubation, the wells were washed five times with 300 µL of washing solution and thoroughly dried. Next, 50 µL of Chromogen Reagent A was added, followed by 50 µL of Chromogen Reagent B, and the plate was incubated at 37°C for 10 min to allow for colour development. The enzymatic reaction was terminated by the addition of 50 µL of the stop solution. The absorbance values of the samples were measured at 450 nm using an ELISA reader (ChroMate 4300 Microplate Reader, USA).

The concentrations of the samples were determined using a standard curve generated from the absorbance values of known standards. These calculated concentrations were automatically compared with the experimentally measured concentrations.

Determination of the Gene Expression Levels RNA isolation
A volume of 200 µL of serum was transferred into 1.5 mL Eppendorf tubes, followed by the addition of 800 µL of TRIzol reagent. The mixture was homogenised and incubated at room temperature for 5 min. Subsequently, 200 µL of chloroform was added to the tubes, and the mixture was incubated at room temperature for another 5 min. The samples were centrifuged at 12,000×g for 15 min, resulting in phase separation. The upper aqueous phase was carefully transferred to new 1.5 mL Eppendorf tubes, and 550 µL of isopropanol was added. The mixture was incubated at room temperature for 5–10 min and centrifuged again at 12,000×g for 10 min. The supernatant was discarded, and the resulting pellet was washed with 1 mL of 75% cold ethanol, followed by cortexin. The sample was centrifuged at 7,500×g for 5 min, and the supernatant was discarded. The RNA pellet obtained was dissolved in RNase-free water.

Complementary DNA (cDNA) synthesis
Complementary DNA (cDNA) synthesis was performed from total RNA using a commercially available kit. For this process, 6 µL of total RNA, 0.5 µL of random hexamer primers, and 6 µL of dH2O were added to 0.5 mL PCR tubes. were sequentially mixed and placed in a conventional PCR device. The resulting cDNA samples were stored at -20°C for subsequent use.

Real-Time Polymerase Chain Reaction (RT-PCR)
The expression levels of *LYVE-1* molecules were analysed by Real-Time PCR on The LightCycler® 480 System (F. Hoffmann-La Roche Ltd) using the SYBR GreenMaster PCR Kit (Jena Bioscience GmbH Dortmund Germany). mRNA expression normalisation was performed using *GADPH*. RT-PCR conditions were selected according to the GreenMaster PCR protocol. The primers listed in Table 1 were used.

Following the RT-PCR position, "melting curve analysis" was performed. The aim of this is to confirm whether the RT-PCR products formed are the desired true products and to exclude non-specific products and secondary structures such as primer dimers. This is done by analysing the melting temperature (Tm) of the RT-PCR products. For this purpose, the samples were subjected to an increasing temperature rise of 0.2°C per second from 55°C to 95°C. In this process, melting curves in the range of approx. 75-80°C occur, depending on the composition of the RT-PCR product.

In the RT-PCR reaction, the cycle at which the fluorescence level exceeds the threshold value that can be measured by the instrument is called Ct (cycle treshold). According to the Ct value obtained, the gene expressions were calculated and evaluated using the $2^{-\Delta\Delta Ct}$ method.

$$\Delta\Delta Ct = (Ct_{target} - Ct_{referance})_{sample} – (Ct_{target} - Ct_{referance})_{control}$$

$$2^{-\Delta\Delta Ct} = 2^{-[(Cttarget - Ctreferance)sample – (Cttarget - Ctreferance)control}$$

Statistical analysis
The clinical parameters of the study groups and expression results were analyzed using SPSS version 30. Continuous variables were classified using median values as a cut-off point. The expression levels was evaluated by using Kolmogorov-Smirnov test. To compare the patient and healthy groups, the Wilcoxon rank test was applied to target LYVE-1 protein and gene expression levels. Spearman-rho correlation analysis was used to assess the relationships between the LYVE-1 protein and gene. ROC curve analysis was performed for evaluation. The Mann–Whitney U test was used to examine the effect of the clinical parameters LYVE-1 levels. Assuming an independent two-sample t-test based on the description. We calculated the standardised effect size and power range using the Power G program. This means the study is well-powered to detect a statistically signi-

Table 2: Arithmetic mean (x), standard deviation (sd), median (m), minimum (min) and maximum values (max) of protein levels in the lung cancer patients and healthy control groups

		LYVE1 protein (ng/ml)				
		Mean (x)	Standard Deviation (sd)	Median (m)	Minimum (min)	Maximum (max)
Group	Patient	22.44	18.92	10.80	6.20	62.90
	Control	7.26	1.92	7.70	2.70	10.50

Table 3: Arithmetic mean (x), standard deviation (sd), median (m), minimum (min) and maximum values (max) of gene expressions in the lung cancer patients and healthy control groups

		LYVE1 gene expression				
		Mean (x)	Standard Deviation (sd)	Median (m)	Minimum (min)	Maximum (max)
Group	Patient	4.60	3.55	3.70	0.65	22.02
	Control	1.03	0.67	0.88	0.20	2.50

ficant difference in LYVE-1 protein levels between lung cancer patients and healthy controls at a significance level of p=0.05.

RESULTS

Serum protein LYVE1 levels were found to be higher in patients with lung cancer compared with the healthy control group, and a statistically significant difference was determined (p=0.001). The gene of *LYVE-1* mean (x), standard deviation (sd) and median (m) values are 4.60±3.55; 3.70 ng/ml and in healthy controls it was 1.03±0.67; 0.88 ng/ml. *LYVE-1* gene expression levels showed statistical significance when compared with the healthy control group (p=0.001) (Table 2 and 3).

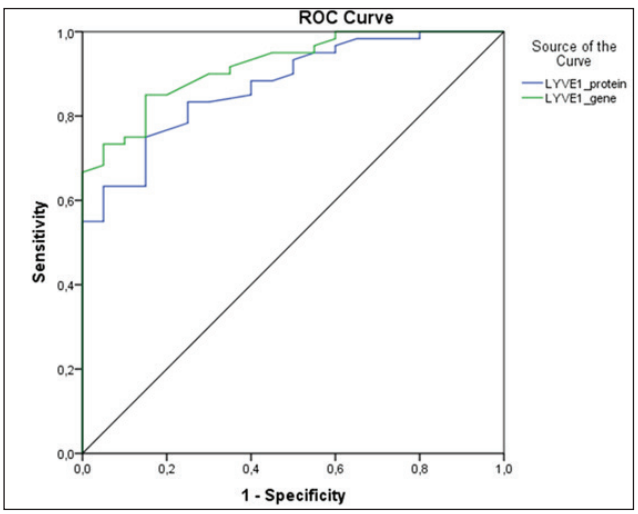
ROC analysis was performed for each test. Consequently, ROC analysis, AUC values for LYVE-1 protein and gene expression levels in NSCLC cancer patients were calculated, 0.892 and 0.920 respectively (Figure 1). Based on the AUC, the diagnostic value of *LYVE-1* gene expression was higher than the LYVE-1 protein level.

Boxplots depicting the variations in serum markers between patients with lung cancer and healthy controls are presented. Serum LYVE-1 protein is shown by boxplots with p<0.05 between NSCLC patients and healthy controls. The centre line in boxplots indicates the median for each data set (Figure 2 and 3).

According to Spearman's rank correlation test, a good correlation was found between the LYVE-1 protein and *the LYVE-1* gene. (Spermans Spearman's rho 0.690 p-value <0.001).

Table 4 displays the statistical significance between the serum LYVE-1 protein and gene expression values and various clinico-

Area Under the Curve



Test Result Variable(s)	Area
LYVE1_protein	.873
LYVE1_gene expression	.920

Figure 1: Receiver operating characteristic (ROC) curves for each test. The area under the curve for the LYVE-1 protein and LYVE-1 gene were 87.3% and 92.0%, respectively.

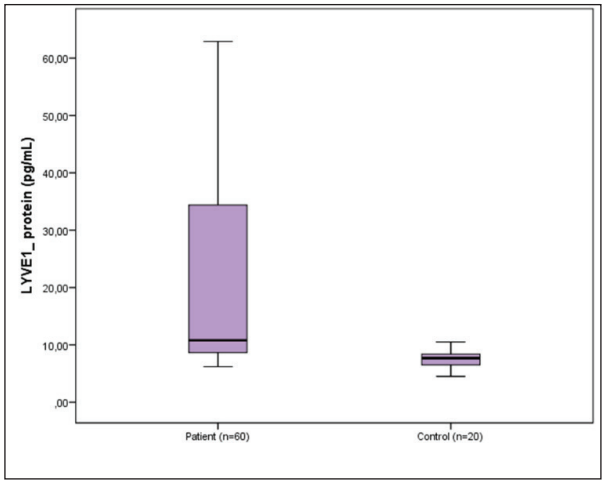


Figure 2: Serum LYVE-1 protein levels (ng/ml) in lung cancer patients and controls (p=0.001)

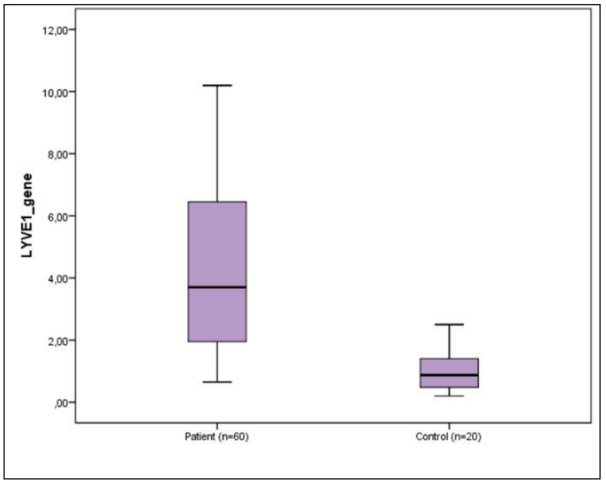


Figure 3: Serum LYVE-1 gene levels (ng/ml) in lung cancer patients and controls (p=0.001)

Table 4: Results of comparisons between serum LYVE-1 protein LYVE-1 gene expression and various demographic and disease

Characterictics		Number of patients	P value	P value
Age	<60	30	0.450	0.510
	>60	30		
Smoking	Yes	35	0.310	0.420
	No	25		
Gender	Male	48	0.550	0.510
	Female	12		
Stage	III	28	0.802	0.251
	IV	32		
Metastasis	Yes	32	0.782	0.232
	No	28		
Histological	NSCLC	50	0.732	0.689
	SCLC	10		
Type	Adeno	30	0.796	0.136
	Squamoz	30		

SCLC: Small cell lung cancer
P value: Statistical significance (Mann-Whitney U testi)
NSCLC: Non-small-cell lung cancer

pathological variables. As for both serum LYVE-1 protein and gene expression of these variables age, stage, gender, metastasis, histological type showed is not a statistically significant with the level of the biomarker. Our results indicate a concordance between LYVE-1 protein levels and mRNA expression in the serum of both control and lung cancer patients. Notably, both protein and mRNA expression levels were found to be significantly higher in the control group than in the lung cancer patient group.

DISCUSSION

Numerous biomarkers have been used clinically, with many protein-based assays readily available. Advances in the application of specific antibodies have significantly enhanced clinical diagnosis using biomarkers. While proteomic studies conducted worldwide have employed comprehensive protein-based approaches to identify improved biomarkers, the detection rates of traditional protein biomarkers have reached their limits.

We proposed circulating mRNA as a novel biomarker. mRNA is a valuable molecule. This method is capable of detecting genes with low protein expression levels as well as non-coding RNA precursors and gene products with limited cellular secretion. Although circulating mRNA is generally considered unstable in RNase-rich blood, our findings confirm that mRNA levels remain stable for at least 24 h after blood sample collection.

In contrast to DNA, RNA molecules possess the unique ability to indicate dynamic, daily physiological and pathological processes occurring within the human body, providing opportunities for early disease detection (15). In this study, we demonstrated the feasibility of detecting and quantifying mRNAs circulating in the human bloodstream.

Lung cancer has the highest mortality rate worldwide. In spite of the fact that the adequacy of diagnosis and treatment in lung cancer has improved recently. As of late, there is developing evidence that identifying biomarkers can progress lung cancer diagnosis, guess and treatment. Thus, the search for novel biomarkers can advance the conclusions and prediction of lung cancer. In our research, circulating mRNA has proven to be an exceptionally sensitive molecule for detecting diseases and systemic inflammatory conditions. Nevertheless, this approach has certain limitations. It may pose challenges in cases involving organs with high blood flow, such as the liver, which could be affected by disease progression. In addition, the target disease may remain latent, as observed in patients with progressive liver conditions. Considering these challenges, it is anticipated that advancements in circulating RNA research in the coming years will pave the way for novel diagnostic and monitoring approaches, enhancing our ability to detect and manage diseases effectively (16).

Through cell culture studies, *LYVE-1* genes will be identified. This study can determine when they begin to express themselves in vascular structures. Therefore, further research can be designed. Research can be planned according to different cultures. - Migration to show the link between CD44 and the immune system Establishment of LYVE-1 as a specific biomarker. for lymphatic vasculature presents a potential minimally invasive approach (17). Quantifying LYVE-1 levels can yield valuable information for the diagnosis of lung cancer. Various methods have been employed to detect LYVE-1, an integral membrane glycoprotein (18, 19). In this study, real-time PCR was used as the molecular method for LYVE-1 assessment. Over the past decade, there has been increasing attention towards the development of rapid diagnostic techniques. However, the growing sensitivity of detection methods introduces a challenge in differentiating between insignificant alterations and lesions that may progress to malignant cancer. In the past decade, there has been a growing focus on the development of rapid diagnostic techniques. However, the enhanced sensitivity of the detection methods presents a challenge in distinguishing between inconsequential alterations and lesions that can progress to malignant cancer.

LYVE-1 is specifically found in the lymphatic capillary endothelium and becomes active in the endothelium of the spleen, liver sinusoidal endothelial cells (LSEC), and macrophages (19, 20). During cancer progression, the presence of LYVE-1 increases within the endothelial cells of the lymphatic system. A dense concentration of LYVE-1-expressing lymphatic vessels is associated with an elevated occurrence of regional lymph node metastases (21).

LYVE-1 is emerging as a valuable diagnostic and prognostic biomarker in a range of cancers (22, 23). LYVE-1 plays a crucial role in processes such as hyaluronic acid homeostasis and the regulation of migration to the lymph nodes. The lymphatic system serves as a key pathway for early-stage cancer metastasis. LYVE-1 has been extensively used to identify tumour-associated lymphatic vessels in various cancer types. The intensity of LYVE-1 expression, as determined through immunohistochemistry, is an important tool for assessing lymphatic system infiltration and lymphangiogenesis in cancers (24). Based on the existing literature, our study revealed that both the protein and mRNA levels of LYVE-1 were significantly elevated in lung cancer patients compared to healthy controls.

Lymphangiogenesis is considered a key indicator for evaluating metastasis and predicting the prognosis of patients with gastric cancer. This study assessed the potential of LVD-tagged LYVE-1 to enhance lymphangiogenesis. Immunohistochemical analysis revealed that LVD in the plant-based medicinal food treatment with the dense-dose group showed a significantly lower incidence of recurrent tumours. These findings suggest hat PBMF exerts an inhibitory effect on lymph node formation (25).

In a cell culture study by Prevo et al., LYVE-1 was capable of binding and internalising hyaluronan *in vitro*, providing valuable insights into the potential role of LYVE-1. Although it is not yet confirmed whether LYVE-1 circulates in vivo, substantial ectodomain fragments of LYVE-1 have been identified (26). In our research, we used readily accessible serum samples from patients with lung cancer. Our findings revealed that serum LYVE-1 levels exhibited an inverse relationship with the size of the primary tumour and served as a significant predictor of both lymph node involvement and distant metastases. Moreover, lung cancer patients with lower serum LYVE-1 levels demonstrated poorer prognoses compared with those with higher levels (27).

Additionally, Two et al. proposed that LYVE-1 enhanced the adhesion of hyaluronan-high-expressing HS-578T cells to LYVE-1-transfected COS-7 cells (28). Similarly, Ito et al. observed elevated levels of hyaluronan on the surfaces of highly metastatic murine breast carcinoma cells and their surrounding stroma, with lymph node-infiltrating breast cancer cells expressing high levels of LYVE-1 via hyaluronic acid in vivo (29).

One of the advantages of our study is that the determination of LYVE-1, which can be a biomarker among those with advanced stage lung cancer at both the protein level and gene expression from an easily obtainable serum sample, will contribute to the literature.

LYVE1 expression was found to be elevated in tissue from patients with lung cancer, with significantly higher protein levels of the lymphoid-specific marker LYVE-1 observed in these samples compared with normal tissue. Immunohistochemical analysis further confirmed that *the LYVE-1* gene was expressed at higher levels in lung cancer tissues than in normal tissues (30). Similarly, our study was designed using serum instead of tissue samples, and LYVE-1 mRNA levels were found to be significantly higher in lung cancer patients compared with the control.

Our study has several limitations. First, we did not conduct LYVE-1 immunostaining on tumour. Secondly, cytokine levels were not assessed in patient blood samples, even though these levels are likely to exhibit changes relative to serum LYVE-1 levels in lung cancer patients. Third, although we evaluated serum LYVE-1 levels in lung cancer patients before and following treatment, further research is required to evaluate the temporal effects of therapy on these levels.

This study showed that both serum LYVE-1 protein and mRNA levels were significantly higher in patients with lung cancer. The current research demonstrated that increased levels of serum LYVE-1 protein, as well as mRNA, were markedly elevated in individuals diagnosed with lung cancer. LYVE-1 protein levels were not correlated with the stage of NSCLC, tumour size, or the presence of metastasis. These findings suggest that both the LYVE-1 protein and gene may have diagnostic value for advanced lung cancer. Despite the fact that the serum values of both LYVE-1 protein and gene are elevated in lung cancer patients, the *LYVE-1* gene is of more diagnostic importance than the results of the ROC analysis according to sensitivity and specificity.

To the best of our knowledge, *LYVE-1* serum mRNA expression in patients with advanced lung cancer has not been previously studied. We found that LYVE-1 was under-expressed in the blood of patients with NSCLC. In contrast to the literature that has reported its overexpression in various solid tumours. The present study contributes to the literature in that, to the best of our knowledge, this study is one of the studies examining the serum LYVE-1 protein and gene and their combined levels in lung cancer patients. Further trials with larger patient populations are necessary to determine the clinical importance of these biomarkers in patients with lung cancer. This study investigated the mRNA and protein levels of *LYVE-1* mRNA in patients with lung cancer.

CONCLUSION

The management of lung cancer has increasingly shifted towards a biomarker-driven approach. With the rapid development of effective targeted therapies, efforts have been made by organisations to establish best practices regarding the necessary tests and their appropriate target populations. Targeted testing enhances the coverage of relevant genomic regions, facilitates the identification of significant alterations, and ensures that crucial molecular data are available in a timely manner to guide therapeutic decision-making.

Serum-based assays provide several advantages compared with tissue-based tests.including being non-invasive, rapid, and easily reproducible over time. However, they may exhibit lower sensitivity compared with tissue-based tests and, therefore, cannot serve as a standalone diagnostic tool for patients with NSCLC. A range of tissue- and blood-based assays are available for biomarker analysis, each possessing distinct advantages and limitations that clinicians must consider when selecting the appropriate assay. Genomic abnormalities related to tumours, identified through biopsy or plasma analysis, provide comparable benefits. Studies based on non-invasive methods using patient blood are highly valuable for monitoring treatment response and detecting the emergence of acquired resistance before radiographic or clinical progression becomes evident.

The recognition of LYVE-1 as an accurate biomarker for lymphatic vasculature presents a potential non-invasive strategy for managing patients with lung cancer. The identification and measurement of LYVE-1 can yield crucial information for lung cancer diagnosis in clinical practice. Several methods exist to identify the LYVE-1 molecule, including immunohistochemical techniques. In the past decade, significant attention has been devoted to developing rapid techniques for its detection. However, enhancing the sensitivity of these detection methods remains a challenge, particularly in distinguishing between substantial changes and lesions that could progress to malignant cancer.

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NOT *PON1 L55M* BUT *ACE* I/D VARIANT MIGHT BE A RISK FACTOR FOR OSCC IN THE TURKISH POPULATION

TÜRK POPÜLASYONUNDA ACE I/D VARYANTI OSCC İÇİN BİR RİSK FAKTÖRÜ OLABİLİR FAKAT PON1 L55M RİSK FAKTÖRÜ DEĞİLDİR

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ABSTRACT

Objective: Oral squamous cell carcinoma (OSCC) covers more than 90% of the malignant neoplasms in the mouth. It has been shown that angiotensin-converting enzyme (*ACE*) and paraoxonase (*PON1)* gene variants were associated with several cancers. Therefore, we investigated the possible association between *ACE* insertion/deletion (I/D)-*PON1* L55M variants and OSCC development risk in a Turkish population.

Material and Methods: A total of 155 people (104 healthy controls and 51 OSCC patients) made up the study population. These variants were genotyped using polymerase chain reaction (PCR) and/or restriction fragment length polymorphism (RFLP) assays.

Results: *ACE* I/D allele frequencies were significantly different between patients and controls. The *ACE* D allele was higher in the patient group compared to the control group, while the I allele was more prevalent in controls than patients (*p<0.0000001*). When the patients and controls were examined based on the II+ID vs. DD genotype and II: ID vs. DD, a statistically significant correlation was found (*p = 0.0000001* and *p = 0.008691,* respectively). The genotype and allele distribution of *PON1* L55M did not significantly differ between the groups.

Conclusion: In conclusion, our study showed that the *ACE* I/D variant D allele is a risk factor for the development of OSCC in Turkey. This study contributes to more studies to confirm that *ACE* I/D plays a role as a genetic risk factor for OSCC.

Keywords: Oral squamous cell carcinoma, angiotensin-converting enzyme, paraoxonase, variant

ÖZ

Amaç: Oral skuamöz hücreli karsinom (OSCC), ağızdaki malign neoplazmların %90'ından fazlasını kapsar. Anjiyotensin dönüştürücü enzim (ACE) ve paraoksonaz (PON1) gen varyantlarının birçok kanserle ilişkili olduğu gösterilmiştir. Bu nedenle, Türk popülasyonunda ACE I/D-PON1 L55M varyantları ile OSCC gelişim riski arasındaki olası ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntemler: Çalışma popülasyonu toplam 155 bireyden oluşmaktaydı (51 OSCC hastası ve 104 sağlıklı kontrol). ACE I/D - PON1 L55M varyantları, PCR ve RFLP analizleri kullanılarak genotiplendi.

Bulgular: ACE I/D alel frekansları hastalar ve kontroller arasında anlamlı şekilde farklılık göstermiştir. ACE D aleli, hasta grubunda kontrol grubuna kıyasla daha yüksek iken, I aleli kontrollerde hastalara kıyasla daha fazlaydı (p<0,0000001). Hastalar ile kontroller, II+ID vs. DD genotipine (p=0,0000001) ve II: ID vs. DD'ye (p=0,008691) göre karşılaştırıldığında istatistiksel olarak anlamlı bir ilişki gözlendi. PON1 L55M genotipi veya alel dağılımı açısından hastalar ve kontroller arasında anlamlı bir fark bulunamadı (p>0,05).

Sonuç: Sonuç olarak çalışmamız ACE I/D varyant D alelinin Türkiye'de OSCC gelişimi için risk faktörü olduğunu gösterdi. Bu çalışma, ACE I/D'nin OSCC için genetik bir risk faktörü olarak rol oynadığını doğrulamak amacıyla yapılacak daha fazla araştırmaya katkı sağlamaktadır.

Anahtar Kelimeler: Anjiyotensin dönüştürücü enzim, oral skuamöz hücreli karsinom, paraoksonaz, varyant

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) covers more than 90% of the malignant neoplasms in the mouth (1). Risk factors for OSCC include tobacco and alcohol consumption, chronic inflammation, viral infection, and betel quid chewing (2). Despite being administered with surgery, radiation, and chemotherapy, OSCC is still considered to have a poor prognosis (3). Multiple genetic alterations cause OSCC because of chronic exposure to environmental carcinogens. There has been an association between common polymorphisms in inflammation, angiogenesis, and thrombosis-related genes and a higher risk of OSCC.

A zinc metallopeptidase in the cell surface, angiotensin-converting enzyme (ACE), is the renin-angiotensin system (RAS) system enzyme that plays the physiologic role of converting angiotensin I (Ang I) into angiotensin II (Ang II) and inactivating bradykinin. Ang II has been shown to have proliferative, angiogenic, and promitotic effects and therefore plays a role in the growth and proliferation of the tumour cells through the Ang II type 1 receptor (4). ACE may promote tumor cell proliferation, angiogenesis, migration, and metastatic behavior (5). There are 25 introns and 26 exons in the *ACE* gene (17q23.3 locus), which encode the ACE enzyme (6). Intron 16 contains a functional polymorphism as the deletion (D allele) and/or insertion (I allele) (7). *ACE* I/D variant (rs1799752) may affect Ang I-converting enzyme function and *ACE* gene expression. There is an association between the D allele presence, higher production of angiotensin II, and higher activity of the ACE enzyme than the I allele. Several studies have been recently conducted on the role of the *ACE* I/D variant in the risk of several cancers, but the results of these studies are contradictory.

Paraoxonase-1 (PON1) has strong lipophilic antioxidant properties and is an antioxidant enzyme maintain the antioxidant-oxidant balance. Human PON1 is related to a family of three serum paraoxonase, including PON3 and PON2. However, PON1 continues to be the most famous member of this family (8). At the same time, PON1 binds to involve high-density lipoprotein (HDL) an esterase in scavenging reactive oxygen species. Studies have found the participation of oxidative stress in the proliferation of cells and the malignant transformation process, damaging DNA and other biological molecules, leading to the occurrence of the tumour (9). The *PON1* gene is located on the seventh chromosome and the short arm at the q21-q22 locus. Replacing 55 leucines (L genotype) with methionine (M genotype) at the third exon caused 55 *PON1*-L55M (rs854560). The *PON1*-55M allele was shown to be associated with increased PON1 activity compared with the *PON1*-55L allele (10). This variant was associated with multiple cancer development.

Thus, the objective was to investigate the potential association between the risk of developing OSCC and ACE I/D-PON1 L55M variations in a Turkish population.

MATERIAL AND METHODS

Study population

ACE and *PON1* variants were studied in 51 OSCC patients (mean age: 61.51±13.07 years) pathologically confirmed and 105 age-matched healthy individuals (mean age: 59.50±9.39 years) with no disease history as a control group. This study obtained samples from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hitit University. All patients underwent oral examination, detailed medical history, and pathological diagnosis in the study. All patients underwent oral examination, detailed medical history, and pathological diagnosis in the study. Our study's control group consisted of people without a history of cancer at any site, leukoplakia, erythroplakia, or any oral precancerous diseases. In compliance with the 2008 Declaration of Helsinki's ethical guidelines, all patients and subjects gave their written informed consent before taking part in the study. The Hitit University ethics commission gave the study its clearance (Date: 19.12.2017, No: 2017/200).

Genotyping

We used the DNA Isolation Kit (PureLink Genomic DNA Mini Kit; Invitrogen) to isolate the genomic DNA from the peripheral cells. As previously mentioned, these variations were genotyped using PCR and/or the restriction fragment length polymorphism (RFLP) approach (11, 12). *ACE* I/D genotypes were determined by PCR. Forward 5'-CTGGAGACCACTCCCATCCTTTTTTTT-3' and reverse 5'-GATGTGGCCATCACATTCGTCAAGT-3' were used for the reactions. Reactions were set up in a volume of 25 µL containing 1.5 µL of each primer, 12.5 µL master mix, 7.5 µL H2O, and 2 µL deoxyribonucleic acid (DNA). After initial denaturation at 95°C for 7 min (min) and 94°C for 45 seconds (sec), the reaction mixtures were subjected to 35 cycles of 60°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. After staining with ethidium bromide, we examined the PCR products on 2% agarose gels and observed them using a UV transilluminator. PCR revealed the variant as a roughly 490-bp fragment when the insertion (I) allele was present and as a roughly 190-bp fragment when the insertion (D) allele was absent. With the forward: 5'-GAA GAG TGA TGT ATA GCC CCA G-3' and reverse: 5'-TTT AAT CCA GAG CTA ATG AAA GCC-3' primers, the *PON1* L55M variant was amplified. In order to digest the amplified 170 bp product, NlaIII was used. The L allele remained intact, but the M allele was digested into 126- and 44-bp fragments.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 20.0 for Windows (IBM SPSS Corp., Armonk, NY, USA) was used for data analysis. The standard deviation and mean were used to present the continuous quantitative variables. The relationships between ACE I/D and PON1 L55M variants and the patients' demographic/clinical characteristics were analysed with the $\chi 2$ test, analysis of variance (ANOVA) statistics, or Fischer exact test. The associations between the genotypes of these variations and the allele distribution were determined using odds ratios (ORs) and 95% confidence intervals (CIs). A p-value of 0.05 was considered statistically significant.

RESULTS

The 51 OSCC patients and 105 controls were genotyped for *ACE* I/D and *PON1* L55M variants. Table 1 shows the participants' clinical and demographic characteristics. Table 2 shows the distributions of the genotype and allele of the *ACE* and

Table 1. Baseline clinical and demographic features of patients with OSCC

Characteristics	Controls (n=105)	Patients (n=51)
Gender, female/male, n (%)	35/70 (33.3/66.7)	18/33 (35.3/64.7)
Age, mean ± SD, years	59.50±9.39	61.51±13.07
Job		
Farmer, n (%)		11 (26.2)
Housewife, n (%)		12 (28.6)
Worker, n (%)		15 (36.7)
Officer, n (%)		3 (7.1)
Unemployed, n (%)		1 (2.4)
Smoking		
Yes, n (%)		5 (11.9)
No, n (%)		30 (71.4)
Ex-smoking, n (%)		7 (16.7)
Smoking Duration		
10-20 years, n (%)		2 (15.4)
20-30 years, n (%)		6 (46.2)
>30 years, n (%)		5 (38.5)
Daily cigarette consumption		
One package, n (%)		4 (33.3)
> One package, n (%)		8 (66.7)
Alcohol consumption, Yes/No, n (%)		8/34 (19/81)
Frequency of alcohol consumption		
Daily, n (%)		5 (62.5)
Social drinker, n (%)		3 (37.5)
Family history, Yes/No, n (%)		7/35 (16.7/83.3)
Response to treatment, Yes/No, n (%)		29/12 (70.7/29.3)
Patients status		
Alive, n (%)		35 (83.3)
Exitus, n (%)		7 (16.7)
Disease State		
Complete response, n (%)		27 (65.9)
Stable disease, n (%)		2 (4.9)
Metastatic disease, n (%)		12 (29.3)
Disease area		
Intra-oral, n (%)		3 (7.1)
Floor of the mouth, n (%)		3 (7.1)
Buccal, n (%)		1 (2.4)
Roof of the mouth, n (%)		3 (7.1)
Tongue, n (%)		12 (28.6)
Lip, n (%)		16 (38.1)
Oral mucosa, n (%)		1 (2.4)
Tonsil, n (%)		2 (4.8)
Cheek mucosa, n (%)		1 (2.4)

Table 2. Genotype and allele distribution of *ACE* I/D and *PON1* L55M variants in the groups

***ACE* I/D**			
	OSCC patients n = 51 (%)	**Control group n=105 (%)**	**p**
Genotypes			
I/I	4 (7.8)	27 (25.7)	
I/D	10 (19.6)	54 (51.4)	>0.05
D/D	37 (72.5)	24 (22.9)	
II + ID: DD	4+10:37	27+54:24	**0.0000001**
II: ID + DD	4:10+37	27:54+24	**0.008691**
Alleles			
I	18 (17.65)	108 (51.43)	**<0.0000001**
D	84 (82.35)	102 (48.57)	
***PON1* L55M**			
Genotypes	**OSCC patients n = 51 (%)**	**Control group n=105 (%)**	**p**
L/L	24 (47.1)	48 (45.7)	
L/M	19 (37.2)	47 (44.8)	>0.05
M/M	8 (15.7)	10 (9.5)	
MM + LM: LL	8+19:24	10+47:48	0.8744
MM: LM + LL	10:47+48	10:47+48	0.2593
Alleles			
L	67 (65.68)	143 (68.09)	0.6705
M	35 (34.32)	67 (31.91)	

The results that are statistically significant are shown in boldface

PON1 variants in the groups. The *ACE* I/D variant genotype distribution was not statistically different between the OSCC patients and controls (p>0.05). *The ACE* I/D D allele frequency significantly differed between the patients and controls. The patient group had a higher *ACE* I/D D allele than the control group, while the I allele was more prevalent in controls than patients (p<0.0000001). A statistically significant association was observed between II + ID vs. DD genotype and II: ID vs. DD (p=0.0000001, p= 0.008691, respectively). The OSCC patients and controls had no significant association regarding any allele or genotype frequency of the *PON1* L55M.

DISCUSSION

Several countries worldwide have seen the incidence of OSCC, which is a severe public health issue (13). Oral carcinogenesis is characterised by several epigenetic and genetic alterations as a complex pathological process, allowing the change of biologically healthy cells into functionally altered cells due to higher invasiveness, cell proliferation rates, and metastases. The RAS as a hormonal system causes increased cell proliferation through the active peptide Ang II signaling, stimulating neovascularization. Increasing evidence shows that vascular endothelial growth factor (VEGF)-mediated angiogenesis is promoted by ang signaling in malignancy by indirectly modulating the vascular cell growth during angiogenesis and directly impacting stromal cells and tumours (14). Matsushima-Otsuka et al. found an increase in the expression of Ang-II type 2 receptor (AGTR2) and Ang-I type 1 receptor (AGTR1) with the progression of OSCC (15). In contrast, AGTR2 exhibited a more pronounced increment than AGTR1, leading to a decrease in the AGTR1 to AGTR2 ratio in advanced-stage cases. Higher levels of ACE occurred in the mouth, larynx, and skin (16). Inhibition of ACE activity in in vitro and in vivo animal models results in suppression of tumor growth and angiogenesis. In addition, epidemiologic studies also found the reduced risk and mortality rate of cancers through ACE inhibitors (17).

Bioinformatic analyses showed that ACE inhibitors had therapeutic potential in OSCC (16). *The ACE* I/D variant, characterized by the absence or presence of a 287-bp Alu repetitive sequence, forms ~50% of the ACE levels. Half of the plasma ACE level may be displayed in homozygote II compared to the homozygote DD, while an intermediate level is displayed by heterozygote DI (17). Several studies have examined the contribution of this variant to the etiology of cancers among various organs, including the breast, lung, gastric, prostate, oral, and others (18). However, the results are contradictory. In a meta-analysis evaluating a total of 25 studies, it was found that in 3914 cancer patients, the *ACE* I/D variant was not related to all cancer risks (19). Also, several studies found *ACE* genotypes unrelated to various types of cancer, such as endometrial cancer and lung cancer (20, 21). However, a study found that the *ACE* D allele had a significant association with hepatocellular carcinoma risk in patients with HCV and a correlation with advanced stage and higher tumour growth (22). Furthermore, Yigit et al. showed an association between prostate tumour metastasis and the PSA level and genetic variation in the *ACE* I/D genotypes (23). Vairaktaris et al. found an association between *ACE* I/D and the progress of oral oncogenesis (17). In addition, Chung et al. found that *the ACE* D/D homozygous genotype was significantly higher in the subjects with oral precancerous lesions in Taiwanese subjects than in the controls (24).

It has been demonstrated that increased levels of reactive oxygen species (ROS) or free oxygen radicals during oxidative stress (OS) drive carcinogenesis by inducing metabolic dysfunction that damages biological macromolecules, including DNA. In this context, DNA bases are oxidated by ROS, forming chromosome aberrations and mutagenic lesions and activating the chemical carcinogens into highly reactive compounds (25). PON1, with its highly lipophilic antioxidant characteristics, is involved in eliminating ROS as an esterase enzyme. PON1 helps detoxify carcinogenic lipid-soluble ROS and organophosphate chemicals produced by lipid peroxidation in addition to its protective function against OS, which is believed to be involved in carcinogenesis (26). It has been reported that lower PON1 activity is associated with different disorders, including senile and diabetic cataracts (27), chronic renal failure (28), age-related macular degeneration (29), and hyperthyroidism (30). In a study measuring serum arylesterase and PON activity in OSCC patients and controls, it was shown that PON and arylesterase activities were decreased in OSCC patients (31). In addition, Metin et al. found that the serum PON1 activity levels were lower in OSCC patients than in controls (32). In a meta-analysis evaluating 19887 cases, 23842 controls, and 43 case-control publications, *PON1* L55M was significantly associated with the overall cancer risk (9). The stratified analyses of the cancer type showed the role of the *PON1*-L55M variant as a risk factor in the incidence of breast cancer, prostate cancer, and haematologic cancer (9). In another meta-analysis, it was found that there was a statistically significant difference between *PON1* L55M and cancer risk (33). The stratified analyses of ethnicity showed a statistically significant higher cancer risk in Caucasian populations. Santana et al. reported that *PON1* rs662 but not *PON1* L55M was associated with poor survival in patients with OSCC (34).

In this study, we evaluated whether *ACE* I/D and *PON1* L55M variants are risk factors for OSCC in the Turkish population. To the best of our knowledge, this is the first study to evaluate the relationship between these variants and the risk of OSCC in our population. We found an association between OSCC and the *ACE* I/D variant D allele. *ACE* D allele was higher in OSCC patients than in healthy controls (Table 2). Also, there was a significant association according to II + ID vs. DD genotype and II: ID vs. DD in comparison of the patients with the controls. The *PON1* L55M variant genotype distribution did not find any statistical difference between the OSCC patients and controls.

There are several limitations to this analysis. First, only two variants of these genes were evaluated. Second, the gene-gene and gene-environment interactions were not investigated for this variant due to a lack of original information. Finally, this study did not express the ACE and PON1 expression levels.

CONCLUSION

In conclusion, our research indicates that the *ACE* I/D variant D allele may be linked to an increased risk of developing OSCC in Turkish patients. This study adds to the body of research confirming the role of *ACE* I/D as a genetic risk factor for OSCC.

Ethics Committee Approval: This study was approved by Hitit University (Date: 19.12.2017, No: 2017/200).

Informed Consent: Written informed consent was obtained from all the participants of the study.

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Author Contributions: Conception/Design of Study- A.F.N., Ö.G., S.Y.; Data Acquisition- A.F.N., M.K.T., S.Y., Ö.G.; Data Analysis/Interpretation- A.F.N., S.Y., N.K.; Drafting Manuscript- A.F.N., S.Y.; Critical Revision of Manuscript- A.F.N., S.Y.; Final Approval and Accountability- A.F.N., S.Y., N.K.; Material and Technical Support- A.F.N., Ö.G., S.Y., N.K.; Supervision- A.F.N., Ö.G., S.Y., N.K., M.K.T.

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ARRHYTHMIAS DEVELOPING DURING ACUTE RHEUMATIC FEVER: A LONG-TERM SINGLE CENTRE EXPERIENCE

AKUT ROMATİZMAL ATEŞ ATAĞI SIRASINDA GELİŞEN ARİTMİLER: UZUN DÖNEM TEK MERKEZ DENEYİMİ

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ABSTRACT

Objective: This study aimed to evaluate the prevalence, types, and clinical significance of arrhythmias observed during acute rheumatic fever (ARF) episodes and their association with the severity of carditis in a single-centre paediatric cohort.

Material and Methods: A retrospective review of medical records from 118 patients diagnosed with ARF based on the revised Jones criteria was performed. Electrocardiograms (ECGs) recorded during the acute phase were analysed for arrhythmias, including atrioventricular (AV) blocks, supraventricular and ventricular arrhythmias, and conduction disturbances. Patients were stratified into mild-moderate and severe carditis groups. Statistical analyses were conducted using SPSS 26.0, with p-values<0.05 considered significant.

Results: Among the 118 patients (mean age 10.5±1.7 years; 55% female), 51.6% exhibited first-degree AV block. The other arrhythmias included second-degree AV block (4.2%), complete AV block (0.8%), supraventricular tachycardia (0.8%), non-sustained ventricular tachycardia (0.8%), and junctional rhythm (1.7%). Supraventricular and ventricular extrasystoles were identified in 4.2% and 5.9% of the patients, respectively. Most arrhythmias occurred in the mild-to-moderate carditis group and resolved spontaneously or with minimal intervention. No arrhythmias were associated with mortality or long-term complications.

Conclusion: Arrhythmias during ARF are relatively uncommon but may reflect myocardial inflammation. While typically benign and self-limiting, vigilant monitoring is essential for timely management. Further research is needed to elucidate the underlying mechanisms and optimise treatment strategies for arrhythmias in ARF.

Keywords: Rheumatic fever, arrhythmia, carditis

ÖZ

Amaç: Bu çalışma, akut romatizmal ateş (ARA) sırasında gelişen aritmilerin prevalansını, türlerini ve klinik önemini değerlendirmeyi ve bu aritmilerin kardit şiddetiyle ilişkisini incelemeyi amaçlamaktadır.

Gereç ve Yöntemler: Revize edilmiş Jones kriterlerine göre ARA tanısı konulan 118 hastanın tıbbi kayıtları retrospektif olarak incelendi. Akut dönemde kaydedilen elektrokardiyogramlar (EKG) atriyoventriküler (AV) bloklar, supraventriküler ve ventriküler aritmiler ile iletim bozuklukları açısından değerlendirildi. Hastalar hafif-orta ve şiddetli kardit gruplarına ayrıldı. İstatistiksel analizler SPSS 26.0 kullanılarak yapıldı ve $p < 0.05$ anlamlı kabul edildi.

Bulgular: Çalışmaya dahil edilen 118 hastanın (ortalama yaş 10,5±1,7 yıl; %55'i kız) %51,6'sında birinci derece AV blok tespit edildi. Diğer aritmiler arasında ikinci derece AV blok (%4,2), tam AV blok (%0,8), supraventriküler taşikardi (%0,8), "non-sustained" ventriküler taşikardi (%0,8) ve "junctional" ritim (%1,7) yer aldı. Supraventriküler ve ventriküler ekstrasistol ise sırasıyla %4,2 ve %5,9 hastada saptandı. Aritmilerin büyük çoğunluğu hafif-orta kardit grubunda görüldü ve genellikle spontan olarak veya minimal müdahale ile düzeldi. Aritmilere bağlı ölüm veya uzun dönem komplikasyon bildirilmedi.

Sonuç: ARA sırasında gelişen aritmiler nadir görülmekle birlikte, miyokardiyal inflamasyonu yansıtabilir. Genellikle iyi huylu ve kendiliğinden düzelen bu durumların zamanında tanınması ve izlenmesi önemlidir. ARA'da aritmilerin altında yatan mekanizmaların ve tedavi yaklaşımlarının optimize edilmesi için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Romatizmal ateş, aritmi, kardit

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INTRODUCTION

Acute rheumatic fever (ARF) is a systemic inflammatory disease that develops as a non-suppurative complication of group A beta-haemolytic streptococcal (GABHS) pharyngitis. It primarily affects the joints, skin, brain, and heart. Cardiac involvement, known as pancarditis, is a hallmark of ARF, affecting the endocardium, myocardium, and pericardium (1). Myocardial inflammation can disrupt the normal conduction system of the heart, leading to various arrhythmias (2). Arrhythmias during ARF can range from sinus tachycardia, which is commonly seen due to fever and systemic inflammation, to more severe forms such as atrioventricular block, atrial fibrillation, and ventricular arrhythmias. While first-degree atrioventricular block, manifesting as prolonged PR intervals on electrocardiography (ECG), is frequently observed and considered a minor diagnostic criterion for ARF, higher-grade blocks or other complex arrhythmias are rare but clinically significant (2, 3). The presence of arrhythmias during ARF can provide insights into the extent of myocardial involvement and may influence the disease course and management strategies. Understanding these disturbances is crucial for timely recognition, appropriate monitoring, and therapeutic intervention, thereby potentially improving patient outcomes (4).

This study aims to provides insights into the long-term experience of arrhythmias observed during ARF exacerbations in a single centre cohort. Also aims analysing the types, frequencies, and clinical significance of arrhythmias observed in ARF, emphasising the need for heightened clinical awareness and further research into this underexplored aspect of the disease. By doing so, it seeks to contribute to the growing body of literature on the cardiac manifestations of ARF and their implications for long-term management.

MATERIAL AND METHODS

This study was conducted to investigate the prevalence, types, and clinical significance of arrhythmias observed during acute rheumatic fever (ARF). A retrospective analysis was performed on the medical records of patients diagnosed with ARF based on the revised Jones criteria (Gold standard for diagnosing ARF). Data from the period when patients were diagnosed with ARF and followed up with anti-inflammatory treatment were evaluated.

Study population

Patients who were admitted to our Paediatric Cardiology Department between January 2010 and June 2024 with a confirmed diagnosis of ARF were included in this study. All patients diagnosed with acute rheumatic fever (ARF) and having carditis were included in the study. Patients with carditis were examined in two groups: mild-moderate carditis and severe carditis. Patients with single valve involvement or mild insufficiency in both valves were considered to have mild-moderate carditis, whereas cases in which significant insufficiency was detected in one or more valves were considered to have severe carditis according to the Jones criteria. Patients with missing data in their files and whose ECG could not be accessed were excluded from the study even though the diagnosis was made. The exclusion criteria also included pre-existing cardiac diseases, electrolyte imbalances, or other systemic conditions that could independently cause arrhythmias.

Data collection

The demographic data, clinical findings, laboratory results, and echocardiographic evaluations of all patients were reviewed. ECGs recorded during the acute phase of ARF were analysed for evidence of arrhythmias, including:

- Atrioventricular (AV) block (first, second, or third degree)

- Supraventricular arrhythmias (e.g., premature atrial contractions, supraventricular tachycardia)

-Ventricular arrhythmias (e.g., premature ventricular contractions, ventricular tachycardia)

- Junctional rhythm

The presence of QT interval abnormalities and other conduction disturbances was also documented.

Statistical analysis

Data was analysed using Statistical Package for Social Sciences (IBM SPSS Corp., Armonk, NY, USA) Windows 26.0 software. Continuous variables were presented as mean±standard deviation (SD), and categorical variables were presented as frequencies and percentages. Comparisons between groups (patients with and without arrhythmias) were made using the chi-square test for categorical variables and the t-test or Mann-Whitney U test for continuous variables, as appropriate. A p-value<0.05 was considered statistically significant.

Ethical considerations

This study was approved by the Istanbul Medical Faculty Clinical Research Ethics Committee (Date: 13.12.2024, No: 24). The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

The research encompassed a cohort of 118 paediatric patients, which comprised 65 females (55%) and 53 males (45%), with a mean age of 10.5±1.7 years. Within this population, 61 children (51.6%) were diagnosed with first-degree AV block, which included 33 females and 28 males. As previously noted, all patients identified with ARF included in this investigation were children presenting with carditis. The diagnosis of carditis, whether silent or clinical, was established in accordance with the Jones criteria, and patients were subsequently classified as exhibiting mild, moderate, or severe carditis. Given that the established guideline prescribes anti-inflammatory treatment by categorising patients into two distinct groups-mild-moderate and severe carditis-we assessed our subjects by similarly dividing them into these two classifications. The cohort exhibiting mild-moderate carditis comprised 97 individuals (82% of the

Table 1. Characteristics of patients with (+) and without (-) first-degree atrioventricular (AV) block

Variables	Total (n=118)	First-degree AV Block (+) (n=61, 51.6%)	First-degree AV Block (-) (n=57, 48.4%)	p value
Age (years), mean±SD	10.5±1.7	10.8±1.9	10.9±1.6	0.71
Female gender, n, (%)	65 (55%)	33 (54%)	32 (56%)	0.67
Mild-Moderate Carditis, n	97	52	45	0.56
Severe Carditis, n	21	9	12	0.7
QTc (ms), mean±SD	402.2±30.1	424.4±16.3	415.5±25.1	0.65
Syncope, n	0	0	0	
Mortality, n	0	0	0	

Table 2: Major Criteria distribution of the patients

Major Criteria	Number of patients (n)	Percentage (%)
Carditis	118	100
Mild-moderate Carditis	97	82.2
Severe Carditis	21	17.8
Arthritis	84	71
Chorea	9	7.6
Eritema marginatum	3	2.5
Subcutaneous nodules	2	1.6

Table 3. Distribution of arrhythmias detected in patients based on the severity of the carditis

Arrhythmia types	Total (n)	Mild-moderate carditis (n)	Severe Carditis (n)
Complete AV block	1	1	-
2nd degree AV block	5	5	-
Mobitz type 1	3	3	-
Mobitz type 2	2	2	-
Supraventricular extrasystole	5	3	2
Ventricular extrasystole	7	4	3
Supraventricular tachycardia	1	1	-
Non-sustained ventricular tachycardia	1	1	-
Junctional rhythm	2	2	-

total patient population; 53 females and 44 males), whereas the subgroup that manifested severe carditis consisted of 21 individuals (18% of the total patients; 12 females and 9 males).

Upon the assessment of the ECG data from the cohort of patients, the measurements of the corrected QT interval (QTc) were concurrently obtained. The mean value was established at 402.2±30.1 milliseconds (ms). Statistical analysis revealed no significant difference between the average QTc values of patients exhibiting first-degree AV block and those without such a condition (Table 1).

In the assessment of the cohort of patients, all of whom presented with carditis, based on the identification of additional major criteria, it was observed that 84 patients (71%) exhibited symptoms of arthritis. The classification of arthritis adhered to the 2015 revised Jones criteria. Given that our nation is categorised within a high-risk community demographic, both polyarthritis and instances of monoarthritis, as well as polyarthralgia, were deemed to satisfy the criteria for a positive arthritis diagnosis. Sydenham's chorea was identified in 9 patients (7.6%), erythema marginatum in 3 patients (2.5%), and subcutaneous nodules in 2 patients (1.6%) (Table 2). These findings were largely consistent with the existing literature and established textbook references.

The medical records of the patients, along with the post-diagnosis therapeutic interventions and ECG data, were subjected to a retrospective analysis. The prevalence of patients exhibiting the development of arrhythmias is delineated in Table 3 and as a graphic in Figure 1. A complete AV block was identified in one patient, a second-degree Mobitz type I AV

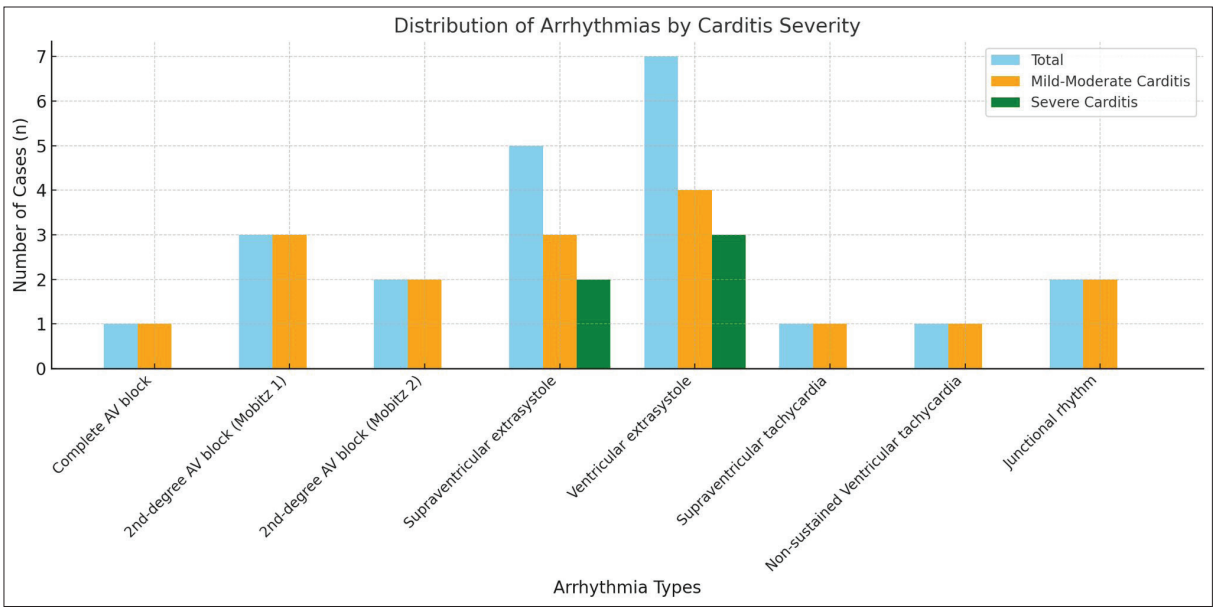

Figure 1: The types of arrhythmias by carditis severity

block was observed in three patients, and a Mobitz type II AV block was noted in two patients. All patients who manifested second- and third-degree AV blocks presented with mild to moderate carditis. Temporary pacemaker implantation was executed for the patient who experienced a complete block. The patient underwent monitoring for a duration of 1 day, after which pacing was discontinued following the resolution of the block, allowing the patient to return to a normal sinus rhythm.

Supraventricular tachycardia (SVT) was observed in one patient, non-sustained VT was noted in one patient, and junctional rhythm was recorded in two patients. The episode of SVT responded favourably to a single administration of adenosine and did not reappear. The patient diagnosed with non-sustained VT remained asymptomatic and was subsequently treated with a beta-blocker (propranolol). No recurrence of symptoms was documented during the follow-up period. The two patients presenting with junctional rhythm were also asymptomatic, and no therapeutic intervention was administered. The junctional rhythm resolved spontaneously during subsequent follow-up evaluations.

The four patients who exhibited SVT, non-sustained VT, and junctional rhythm were also those diagnosed with mild to moderate carditis. The corrected QTc value for the patient who had non-sustained VT was found to be within normal physiological limits.

Among the patients with arrhythmias identified during follow-up assessments, supraventricular extrasystole (SVE) was recorded in five individuals, whereas ventricular extrasystole (VES) was documented in seven individuals. Due to the limited number of patients with extrasystole, statistical analysis was not feasible; however, it was noted that extrasystoles were present in both patient groups, with a greater frequency observed in those with mild to moderate carditis.

DISCUSSION

Today, acute rheumatic fever continues to be one of the predominant aetiologies of acquired heart disease. Consequently, rheumatic heart disease is a significant contributor to morbidity (1). While the involvement of cardiac valves and pancarditis in rheumatic heart disease is well characterised through their pathogenesis in conjunction with clinical and imaging findings, the arrhythmias that arise during the progression of the disease are comparatively less delineated within the academic literature (4).

When assessing the association between acute rheumatic fever and the onset of arrhythmias, it is imperative to scrutinise the underlying inflammatory mechanisms that may precipitate cardiac complications. Contemporary studies have underscored the significance of elevated biomarkers such as C-reactive protein (CRP) in forecasting arrhythmic occurrences in affected individuals (2). Given that a 15-year retrospective analysis was conducted in our investigation, a numerical correlation was not performed due to the discrepancies in titres; nonetheless, acute phase reactants such as CRP and sedimentation rates were found to be positive according to the Jones criteria across all subjects.

PR prolongation on ECG, commonly referred to as first-degree atrioventricular (AV) block, constitutes a minor diagnostic criterion for acute rheumatic fever, yet it does not serve as a pathognomonic indicator of the condition. This phenomenon is observed with a prevalence of approximately 2% within the general population. The incidence among patients diagnosed with acute rheumatic fever ranges around 50% according to various literature sources (5, 6). Considering the commonality of PR prolongation in acute rheumatic fever, our retrospective study focused on the examination of other less prevalent arrhythmias.

Case series documented in the literature reveal that the arrhythmias experienced during an acute rheumatic fever episode may include second-degree and third-degree AV block, VT, and junctional rhythm (7). In our analysis, the overall rate of arrhythmias was determined to be 8.4% when factoring in SVT, VT, second-degree and third-degree AV block, as well as junctional rhythm; furthermore, when cases of supraventricular and ventricular extrasystole were included, the incidence escalated to 18.6%. This figure aligns closely with the existing literature values (2).

In the research conducted by Karacan et al., a comparative analysis of arrhythmia rates identified through standard ECG and 24-h rhythm Holter monitoring in patients with ARF revealed that arrhythmias were more frequently detected via the 24-h rhythm Holter compared to the standard ECG (accelerated junctional rhythm was observed in three patients on standard ECG, whereas it was present in nine patients-14%-during the 24-h rhythm Holter). Given that only standard ECG data were analysed in our study, the detection rate of junctional rhythm was noted to be lower (1.6%) and in another study, approximately 6% of individuals exhibited a junctional rhythm (2, 3). Furthermore, drawing upon literature insights, it would be appropriate to assert that, because junctional rhythm is typically asymptomatic, the 24-h rhythm Holter represents a superior modality for identifying such patients (3). Another characteristic of junctional rhythm is that it represents a rhythm disorder that corrects itself spontaneously and does not necessitate therapeutic intervention, as evidenced in our cases (2).

In the course of our investigation, we identified a total of six patients exhibiting advanced atrioventricular block; five patients presented with second-degree heart block: three classified as Mobitz Type 1, two as Mobitz Type 2, and one patient demonstrated complete heart block. Our rate of advanced atrioventricular block, which was ascertained to be 0.5%, was in proximity to the existing literature, where the incidence is reported to range from 1.5% to 5.5% (3-5, 7-9). There exist case reports within the existing literature that detail instances of temporary pacemaker implantation, including a case analogous to that of our patient who experienced complete atrioventricular block; however, in the majority of instances, it typically exhibits a propensity for spontaneous resolution without necessitating any form of intervention (10).

Supraventricular and ventricular extrasystoles and/or tachycardia manifesting during an acute rheumatic fever episode have been documented in few academic studies; given the occurrence of pancarditis in these individuals, it appears most plausible that these arrhythmias arise as a consequence of myocardial involvement. In our investigation, the instances that manifested SVT and non-sustained VT were characterised by mild to moderate carditis. Nevertheless, the occurrence of VT in the context of severe endocarditis affecting all four heart valves has been documented in the existing literature (11, 12).

The extant literature elucidates that advanced atrioventricular block is not uniformly correlated with valvulitis. Furthermore, an additional investigation revealed that junctional rhythms do not invariably correlate with clinical carditis. Our research corroborates their findings. Although arrhythmias constitute manifestations of cardiac involvement in rheumatic fever, they appear to arise earlier in the progression of the disease, preceding the onset of valvulitis, as evidenced by the markedly elevated levels of acute phase reactants (3, 6). Furthermore, the administration of steroid therapy to individuals diagnosed with severe carditis may exert an influence on the suppression of arrhythmias (13).

In conclusion, arrhythmias linked to ARF pose a considerable clinical challenge, often indicative of inflammatory heart involvement. The pathophysiology is mainly associated with rheumatic fever-induced cardiac damage, affecting electrical conduction. These arrhythmias are generally benign but can be life-threatening, warranting vigilant monitoring, especially in paediatric cases. Timely identification and treatment of acute rheumatic fever are vital to avert the long-term consequences of rheumatic heart disease and related arrhythmias. Continued investigation into the mechanisms and management of arrhythmias in acute rheumatic fever is critical for enhancing patient outcomes and mitigating morbidity.

Ethics Committee Approval: This study was approved by Istanbul Medical Faculty Clinical Research Ethics Committee (Date: 13.12.2024, No: 24).

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PREVALENCE OF PERI-IMPLANT DISEASE AROUND SUBCRESTAL PLACED IMPLANTS

SUBKRESTAL OLARAK YERLEŞTİRİLEN İMPLANTLARIN PERİ-İMPLANT HASTALIK PREVALANSI

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ABSTRACT

Objective: To investigate the long-term peri-implant health of subcrestal placed implants and identify the local factors influencing it.

Material and Methods: A total of 103 patients participated in this cross-sectional study. Subcrestal placed implants (n=322) were followed up. Patients were assessed for peri-implant health status at routine visits and the results were recorded. Binary logistic regression analysis was used to investigate factors influencing the peri-implant health status.

Results: The peri-implant health of 103 patients was analysed. The mean function time was 14.05±5.95 years. In total, 47.5% of the patients were found to be healthy. 31% had peri-implant mucositis and 21.5% had peri-implantitis. When all variables were analysed, it was found that peri-implant mucositis and peri-implantitis were significantly associated with the following outcomes: additional instruments adjunct regular brushing [Odds ratio (OR):11.23]; and type of prosthesis retention [Odds ratio (OR):4.032].

Conclusion: Peri-implant mucositis and peri-implantitis occur in approximately half of subcrestal placed implants, and the use of oral hygiene instruments in addition to regular brushing and screw-retained prostheses plays an important role in implant survival.

Keywords: Peri-implant mucositis, Peri-implantitis, Prevalence

ÖZ

Amaç: Subkrestal olarak yerleştirilen implantların uzun dönemli peri-implant sağlık durumlarını incelemek ve bu duruma etki eden lokal faktörleri belirlemektir.

Gereç ve Yöntemler: Çalışmaya 103 hasta katılmıştır. Subkrestal olarak yerleştirilen 322 implant takip edilmiştir. Hastalara rutin kontrollerinde peri-implant sağlık durumu taraması yapılmış ve sonuçlar kaydedilmiştir. İkili (binary) lojistik regresyon analizi ile peri-implant sağlık durumuna etki eden lokal faktörler araştırılmıştır.

Bulgular: Ortalama fonksiyon süresi 14,05±5,95 yıl olan hastaların %47,5'i sağlıklı tespit edilirken; %31'inde peri-mukozitis ve %21,5'sında peri-implantitis gözlenmiştir. Değişkenler incelendiğinde peri-implant mukozitis ve peri-implantitisin, diş fırçalamaya ek oral hijyen enstrümanlarının kullanımı [Odds oranı (OR): 11,23]; ve Protez retansiyon tipi [Odds oranı (OR): 4,032] ile anlamlı şekilde ilişkili olduğu görülmüştür.

Sonuç: Peri-implant mukozitis ve peri-implantitis, subkrestal olarak yerleştirilen implantların yaklaşık yarısında görülür ve diş fırçalamaya ek bakım enstrümanlarının kullanımı ve vidalı protezler implantın sağkalımında önemli bir yere sahiptir.

Anahtar Kelimeler: Peri-implant mukozitis, Peri-implantitis, Prevelans

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INTRODUCTION

The number of dental implants placed has increased dramatically with the increase in human life expectancy. Naturally, this increase has led to many mechanical and biological complications. Mechanical complications (screw loosening, porcelain chipping, abutment fracture) (1) are generally problems that can be managed without additional surgeries, whereas biological complications (peri-implant mucositis & peri-implantitis) (2) are very common after implant treatment (3). They often require revision of either the prosthesis or the implant. Peri-implantitis is preceded by peri-implant mucositis and is caused by bacterial plaque accumulation (4). Exposing irregularities and roughness in the implant topography to the oral environment rapidly leads to plaque accumulation, which, together with the colonisation of pathogenic oral bacteria (5), may lead to bone destruction and even implant loss. With the accumulation of a bacterial biofilm for 3 weeks, the response of the host is disturbed and an inflammatory response develops (6). However, this condition is usually reversible with the removal of the bacterial plaque (7). Peri-implant mucositis is a reversible condition limited to soft tissue inflammation (8). If the condition is left untreated and progresses, it will develop into peri-implantitis, which is pathological (4). Due to the differences between peri-implant and periodontal tissues, peri-implantitis is similar to periodontitis, but progresses more rapidly (9) and shows inflammatory bone destruction (8), but often does not follow a linear trend. The resulting gingival pocket and intraosseous defects, combined with the complex surface topography of the implant, cause anaerobic bacteria to accumulate in the reservoir areas, further exacerbating the disease (5). In contrast to peri-implant mucositis, peri-implantitis is characterized by increased bleeding on probing and increased pocket depth (≥ 6 mm), with additional signs of inflammation such as swelling, pain on palpation and/or spontaneous pain and pus (4) and radiographic sign as marginal bone loss ≥ 3 mm in the coronal part of the implant (8). Although it has been proven that peri-implantitis is due to a bacterial aetiology, it is still a matter of controversy as to which facilitating conditions cause it. The frequency of bacterial accumulation is determined by many patient- and implant-related factors, including; history of periodontitis (10), oral hygiene, implant malpositions, type of restoration, smoking, improper fit of the prosthesis margin (11), cement residues, implant surface characteristics, and systemic diseases (12). The prevalence of peri-implant disease varies between studies and populations. However, it is estimated to occur in approximately one in five implants and half of the patients (3). A variety of surgical and non-surgical treatment modalities have been developed for peri-implantitis, but there is no consensus on the most appropriate protocol. Therefore, the early diagnosis and timely treatment of peri-implant disease by identifying its causative factors is critical to prolonging implant survival and improving overall patient satisfaction and quality of life (4).

The aim of this study was to investigate the peri-implant health status of subcrestal placed implants and to determine the influencing local factors.

MATERIAL AND METHODS

Estimation of the required sample size

In order to determine the sample size, the study carried out by Romandini et al. in 2021 was taken as a reference (13). The prevalence of peri-implantitis and peri-implant mucositis was 56.6% and 31.7%, respectively. With an alpha level of 0.05 and a power (1-ß) of 0.80, the required sample size was 98 and the critical z was 1.64 (G Power version 3.1, Düsseldorf, Germany). To compensate for possible drop-outs, an additional 10 patients were added to the final sample size.

Allocation of the patients

This study was approved by the Ethics Committee of Istanbul University Faculty of Dentistry (Date: 21.11.2023, IRB No: 2023/42) and conducted in accordance with the Declaration of Helsinki as revised in 2011. The study included patients who came to the Department of Oral Implantology Faculty of Dentistry between November 2023 and September 2024 for routine check-ups or with any complaints, who had implant/implants placed in the department and who volunteered to participate in the study. Eligible patients for the study were enrolled after a detailed clinical and radiological evaluation. The study design was explained, and consent forms were signed.

Inclusion and exclusion criteria

The following inclusion criteria were defined for patient selection;

Patients who are willing to participate in the study and who have at least one existing implant and rehabilitated with implant-supported fixed prosthesis (at least 1 year of use) / over 18 years of age / implants with platform switching / who attend routine check-ups.

The exclusion criteria were as follows;

Uncontrolled systemic diseases that may affect the success of implant treatment (HbA1c>7, osteoporosis)/Patients taking medications that may delay bone turnover or wound healing (bisphosphonates, steroids)/ Smokers (>10 cigarettes per day)/ Patients requiring implant removal who have lost more than half of their bone support/Patients with implant-supported removable dentures.

Study variables

The following variables were analysed;

oral hygiene habits of patients (toothbrush, dental floss, interdental brush, water jet), type of prosthesis retention (screw retained-cemented), implant diameter (narrow-standard-wide), prosthesis cleanability (yes-no), history of periodontitis (yes-no), bridge or crown, number of implants, and prosthesis function time (years).

Study Design and Case Definition

The archive records of 103 patients and the measurements taken during the radiological examination were recorded. Gentle probing was performed to examine the peri-implant tissue he-

alth during clinical examinations (4). Probing depth, bleeding on probing, and pus and crestal bone changes were compared with previous examination findings obtained from patient records. Panoramic and periapical radiographs were taken to accurately detect changes in the marginal bone levels and peri-implant health. Peri-implant mucositis and peri-implantitis status were defined according to the study by Berglundh et al in 2018 (8).

Peri-implant mucositis: According to previous examinations; presence of increased bleeding on probing (with or without the presence of pus) without radiographic evidence of bone loss. The pocket depth may be increased.

Peri-implantitis: According to previous examinations; radiographic bone loss with bleeding on probing (with or without the presence of pus) and increased pocket depth.

Statistical Analysis

The normality of the distribution of the data was assessed using Kolmogorov–Smirnov. The chi-square test and Mann Whitney-U t-test were used to assess the similarity of the baseline variables between the groups. The effect of the variables was measured by binary logistic regression analysis. All variables were included in the regression model and a significant model was created by the backwards elimination method with Wald statistics. The fit and efficiency of the built model were assessed using the Hosmer-Lemeshow test and Nagelkerke R^2 test, respectively. Odds ratios (OR) and parameter estimates (β) were calculated for all the variables in the model that was built. $P<0.05$ was considered as statistically significant. All statistical analyses were performed using SPSS® (version 29.0.20.0) for Mac (IBM Corporation, Armonk, NY, USA, 2024) and were written according to the statistical guidelines of Altman et al. (14) The Strengthening Reporting of Observational Studies in Epidemiology Checklist (STROBE) was employed for the preparation of this manuscript.

RESULTS

In this study, 165 patients were screened between November 2023 and September 2024. 22 patients were heavy smokers (>10 cigarettes per day) and excluded. 12 patients with uncontrolled systemic disease, 12 patients requiring implant removal and 9 patients with implant-supported removable dentures were also excluded from the study. A total of 110 patients were evaluated clinically and radiographically. Clinical parameters were assessed around the implants with a periodontal probe (PCP-UNC 15, Hu-Friedy®, Chicago, USA). PD (Probing depth), BOP (Bleeding on probing), and pus and crestal bone changes were recorded, and patients were classified as healthy or peri-implant mucositis/peri-implantitis according to the peri-implant health criteria of Berglundh et al. (8), (Figure 1-5). 7 patients were excluded because their records were not accessible in the archive. Finally, 103 patients (48 females, 55 males) with 322 implants with platform switching were analysed. The majority of the implants (67%) had an internal conical connection. The remaining implants had an internal hexagonal connection.

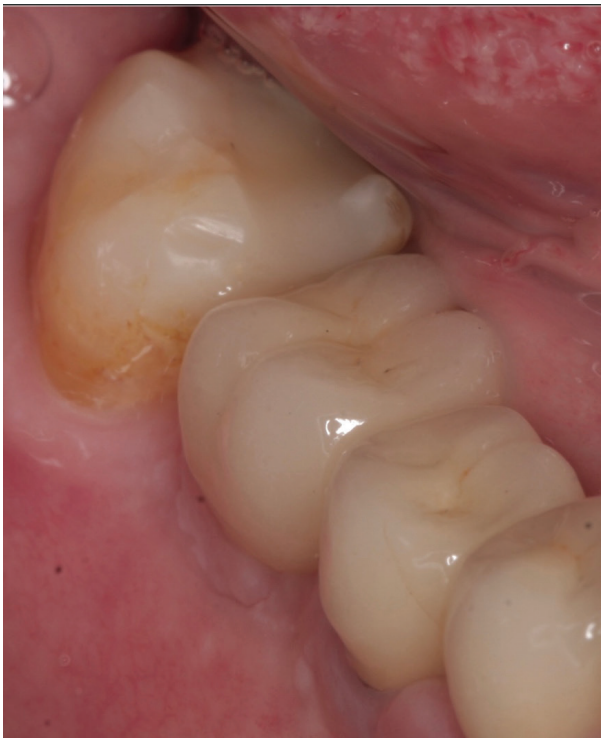

Figure 1: Clinical and radiological views of a healthy person after 5 years of function

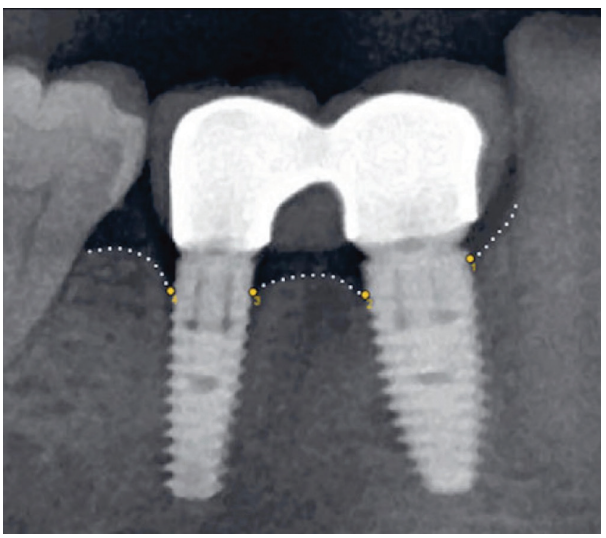

Figure 2: Panoramic radiographs were taken to monitor the progression of marginal bone loss

All implants exhibited a tapered apex, microgrooves in the neck area, and a rough collar with variable degrees of platform shifting. The distribution of implants according to the commercial manufacturers is listed in Table 1. The mean age of the study population was 53.64±11.16 years. Implants were considered healthy in 49 patients, whereas 54 patients had evidence of peri-implant mucositis or peri-implantitis in at least one implant. Peri-implant mucositis and peri-implantitis were found in 31% and 21.5% of patients, respectively (Table 2). The mean

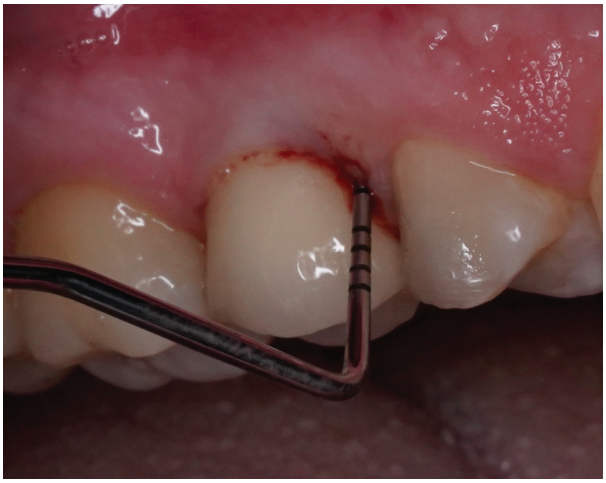

Figure 3: Peri-implant mucositis diagnosed by bleeding on probing after 2 years of function

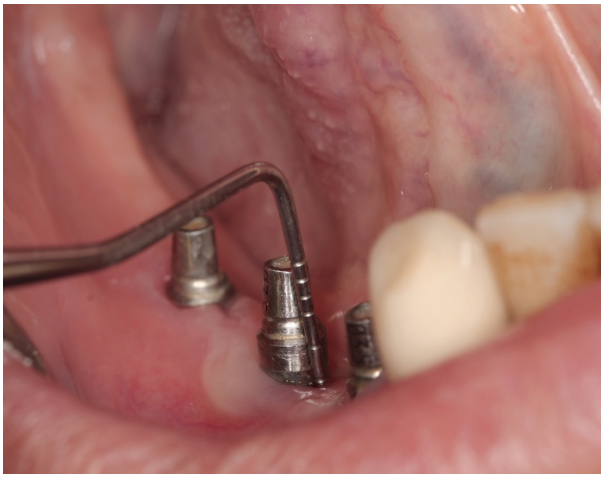

Figure 4: Clinical view and probing of an implant in region #45-46 after removal of the prosthesis

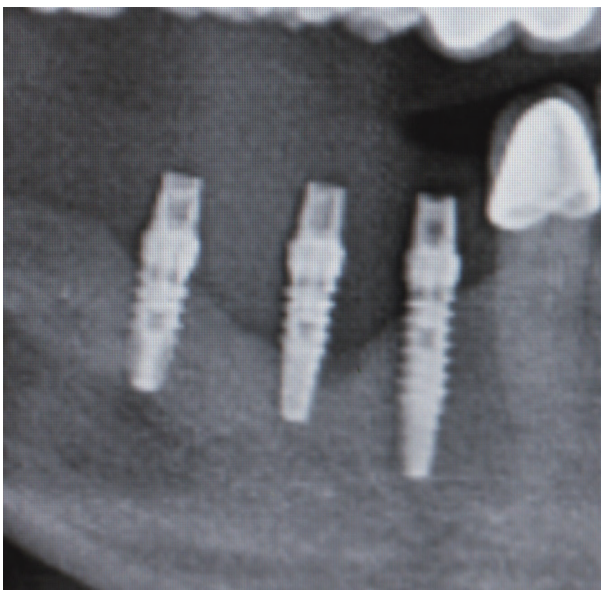

Figure 5: Radiographic examination indicated peri-implantitis

Table 1: Commercial manufacturers of implants

	N	Abutment connection	Degree of the conical connection
Biomet 3i	56	Internal hexagon	
Straumann BL	43	Internal conical	15°
Nobel (Parallel)	38	Internal conical	<12°
Mis (C1)	61	Internal conical	12°
Nucleoss (T6)	50	Internal hexagon	
Camlog (Conelog)	74	Internal conical	7.5°
Total	322		

Biomet 3i; Florida,USA; Straumann® (Straumann BL), Basel, Switzerland; Nobel®, Götheburg,Sweden; Mis (C1), Shlomi,Israel; Nucleoss®, İzmir, Turkey; Camlog (Conelog),Stuttgart,Germany

Table 2: Distribution of healthy and patients

	n (%)
Healthy	49 (47.5%)
Peri-implant mucositis	32 (31%)
Peri-implantitis	22 (21.5%)

n=number of patients

Table 3: Distribution of the patient cohort according to the variables

Physician-related factors	Healthy (n=49)	Diseased (n=54)	p
The type of Retention			0.39
Cement-retained	9 (19%)	6 (11%)	
Screw-retained	39 (81%)	48 (89%)	
İmplant Diameter			0.40
Narrow	20 (43%)	19 (36%)	
Standard	23 (49%)	32 (60%)	
Wide	4 (8%)	2 (4%)	
Prosthesis Cleanability			0.20
Yes	34 (71%)	45 (83%)	
No	14 (29%)	9 (17%)	
Patient-related factors			
History of Periodontitis			0.89
Yes	18 (38%)	22 (41%)	
No	30 (62%)	32 (59 %)	
Need for Implants for			0.23
Crown	7 (19%)	14 (33%)	
Bridge	30 (81%)	28 (67%)	
Additional Instruments Adjoining Regular Brushing			**<0.001****
Yes	41 (85%)	25 (46%)	
No	7 (15%)	29 (54%)	
Number of Implants[z] *(Mean)*	3.48±1.53	3.13±1.85	0.154
Function Time[z] *(Mean)*	13.46± 6.25	14.57± 5.67	0.340

***p<0.05, **p<0.01 χ2: Chi-square test (Categoric data), z: Mann-Whitney U t-test**
n = Number of patients

duration of function of the evaluated implants was 14.05±5.95 years (range 1- 24 years). The mean number of implants was 3.29±1.71. The distribution of the patient cohort according to the variables is listed in Table 3. Almost all of the participants brush their teeth at least once a day, while 35% used dental floss, 17% used an interdental brush, and 29% used a water jet as an adjunct. 64% of patients use additional instruments in addition to regular brushing (Table 4).

Table 4: Frequency of Using Oral Hygiene Instruments

Patient	Toothbrush	Dental floss	İnterdental brush	Water-jet	Additional instruments adjunct regular brushing
N (%)	102 (%99)	36 (%35)	17 (%17)	30 (%29)	**66 (%64)**

n = Number of patients

Table 5: Predictors in the Regression Model for Disease (Peri-implant mucositis&Peri-implantitis)

Variable	Explanation	β (Estimate)	Standard error	Wald	p	Odds Ratio
Function Time	Continuous	0.071	0.041	2.955	0.086	1.073
Additional Instruments Adjoining Regular Brushing	None	2.416	0.583	17.152	**<0.001****	11.23
The type of Prosthesis Retention	Cement-retained	1.395	0.699	3.979	**<0.046***	4.032

Peri-implant mucositis-peri-implantitis; 0=Healthy, 1=Disease
Hosmer and Lemeshow test p = 0.58
*p<0.05 **p<0.01; Nagelkerke's R^2= 0.316

A total of 8 variables were included in the regression analysis, but only 3 variables were used to form a meaningful model (p=0.58; Hosmer and Lemeshow test). In the model, the use of additional instruments adjunct regular brushing (OR:11.23 p<.001) and the type of prosthesis retention (OR:4.032 p=.046) were statistically significant for the odds of peri-implant mucositis and peri-implantitis. 31% of the variance in the dependent variables could be explained by the model (Table 5).

DISCUSSION

This cross-sectional study analysed 322 implants in 103 patients. A comprehensive set of variables including peri-implant mucositis and peri-implantitis was evaluated to define long-term peri-implant health. The prevalence of peri-implant disease at the patient level was found in %52 in this study, which was followed up for 14.05±5.95 years. Derks et al. found similar results (%45) in their study, which was followed up for 9 years (15). Ferreira et al. found a higher rate (73.5 %) of peri-implant disease in non-smoking patients (16). Obreja et al. found an increased rate of peri-implant disease (81.5%) in smokers and non-smokers who were followed up for 9 years (10). However, there is no clear evidence of a negative effect of heavy smoking on peri-implant disease in recent studies (17). Thus, smoking was not assessed in the study and heavy smokers (more than 10 cigarettes per day) were not excluded.

It can be hypothesised that the prevalence of peri-implantitis increases with longer function time. However, no statistically significant difference was found in the study regarding implant function time, which corresponds to the literature as well (18). Differing diagnostic criteria, rather than function time, cause

the prevalence of peri-implant mucositis and peri-implantitis to vary between studies (19). In many studies, the inflammation cut-off and the probing depth threshold are different (13). For example, Krebs et al. diagnosed peri-implantitis in 29.6% of patients with PD ≥ 4 mm with BOP, while this rate decreased to 15% when the bone loss threshold was used as ≥1.5 mm (20). Moreover; probing depth, which is the main parameter defining disease (2), cannot be measured objectively, mainly due to the threads of the implant. In addition, retrospective studies usually do not include data on initial bone loss (radiographic status several weeks after abutment placement), which is a limiting factor in defining peri-implantitis (19). There is a consensus on the need for long-term and regular clinical and radiographic evaluation of peri-implant tissues. Therefore, the threshold in this study was based on the initial peri-implant health status to track disease progression (8). This definition is supported by several recent studies (10).

Although there appears to be no debate in the literature about the negative impact of poor periodontal health on implant success, it is known that supportive care and maintenance therapy can improve the success rate of dental implants even in patients with a history of periodontal disease (21) unless there is a history of aggressive periodontitis (22). The main objective of supportive care and maintenance therapy in dental implantology is to maintain a healthy peri-implant mucosa and thus prevent the development of peri-implantitis. In cases where plaque-induced peri-implant mucositis has occurred, a well-designed therapy adjunct to oral hygiene motivation can help to restore the mucosa to a healthy state and prevent the development of peri-implantitis (23) In our department, patients are instructed on how to maintain good oral hygiene after each implant-supported rehabilitation, and follow-up appointments are scheduled at 6-month intervals and, if necessary, maintenance therapy will be given. Considering that 64% of the patients in this study used oral hygiene instruments in addition to regular brushing, the impact of oral hygiene motivation adjunct to maintenance therapy is undeniable. Many studies have linked poor oral hygiene to peri-implantitis (24). The results in the present study are also consistent with the literature, considering that the non-use of oral hygiene instruments adjunct regular brushing increased the odds of peri-implantitis (OR: 11.23). Similarly, Romandini et al. found the non-use of interdental flossing as an indicator of poor oral hygiene and peri-implantitis (13). Furthermore, Monje et al. found that the interval frequency of maintenance therapy was significant for both peri-implant mucositis and peri-implantitis (25).

All of the implants in the study were placed one to 3 mm subcrestal. The subcrestal placement of an implant is essential for long-term success. In a study conducted by Agrali and colleagues, it was observed that only 20% of implants (placed subcrestally) demonstrated marginal bone loss exceeding 2 mm (26). However, it causes more pronounced bone remodelling (27) and this may play a facilitating role in the development of peri-implant inflammation. For implants with crown margins ≤ 1.5 mm from the crestal bone, studies have shown higher odds ratios for peri-implantitis (15). Because subcrestal placement may reduce the accessibility of the prosthesis for cleaning and oral hygiene, some studies have defined subcrestal placement as a modifying factor of plaque control and peri-implantitis (28). Particularly with cement-retained prostheses, inadequate cleaning of the cement residue from the deep pocket epithelium, due to subcrestal placement, can lead to increased inflammation and peri-implant disease (29). The present results also show that the type of prosthesis retention (cement retained) is a strong predictor of peri-implantitis (OR:4.03). These findings are supported by many studies in the literature (30). On the contrary, Bayer et al. concluded that screw-retained prostheses may make little or no difference in the risk of peri-implantitis, but the evidence was considered low in this systematic review (31).

The main limitations of this study were the lack of randomization, as the study was conducted in a single university clinic. In addition, the presence of more than one brand of implant affects the generalizability. Another issue that needs to be considered is that the use of oral hygiene instruments affects plaque control, which was not investigated in the study. Although the aetiologies are the same, it is important to analyse peri-implant mucositis and peri-implantitis separately to identify the causative factors.

CONCLUSION

Within the limits of the study, peri-implant mucositis and peri-implantitis occurred in approximately half of subcrestal placed implants, and the use of oral hygiene instruments, in addition to regular brushing and screw-retained prostheses, played an important role in implant survival.

Ethics Committee Approval: This study was approved by İstanbul University Faculty of Dentistry (Date: 21.11.2023, IRB No: 2023/42).

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DEVELOPMENT AND EVALUATION OF BIOADHESIVE MUCOSAL DOSAGE FORMS OF PILOCARPINE HCL FOR XEROSTOMIA THERAPY

KSEROSTOMİ TEDAVİSİ İÇİN PİLOKARPİN HCL'NİN BİYOADEZİF MUKOZAL DOZAJ FORMLARININ GELİŞTİRİLMESİ VE DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Saliva maintains vital mouth functions and acts as a barrier to dental health. Xerostomia, which is characterised as a feeling of dry mouth, adversely affects the patient's quality of life. Palliative therapies, such as sialagogues, form the based on treatment of xerostomia. The FDA-approved sialagogue pilocarpine is currently recommended as the first-line medication for patients. Owing to its wide range of action, oral pilocarpine users may experience several negative side effects. The purpose of this study was to increase the duration of action and prevent systemic side effects of pilocarpine hydrochloride by designing and evaluating prolonged-release formulations of the drug using either xanthan gum, hydroxyethyl cellulose, or a combination of these two natural polymers buccal bioadhesive films.

Material and Methods: The films were analysed for their physicochemical, mechanical, bioadhesive, swelling, in vitro release, and in vitro cytotoxicity.

Results: Physicochemical and mechanical feature examinations revealed that the Xanthan-HEC combinations showed better results compared to the single polymer-used formulations. The *in vitro* dissolving profiles of all the optimal formulations showed a sustained release pattern with a steady-state plateau after an initial fast release. Using various release kinetic models to assess drug release kinetics revealed that the Higuchi and Korsmeyer-Peppas correlations are primarily followed by drug release from buccal films.

Conclusion: The findings show that the mucoadhesive buccal formulation is a viable method for pilocarpine localised distribution that is both safe and effective in treating xerostomia. Further in vivo studies are planned to assess the pharmacokinetic and histopathological effects of the formulation.

Keywords: Buccal delivery, buccal film, pilocarpine hydrochloride, sialogogues, xerostomia

ÖZ

Amaç: Tükürük salgısı hem ağız içi fonksiyonları hem de diş sağlığını korumaktadır. Bu nedenle ağız kuruluğu hissi ile karakterize olan kserostomi durumu hastanın yaşam kalitesini olumsuz yönde etkilemektedir. Sialagoglar gibi palyatif tedaviler, kserostominin tedavisinin temelini oluşturmaktadır. Mevcut hastalar için birinci basamak ilaç tedavisi olarak, Amerikan ilaç ve Gıda Dairesi tarafından onaylı pilokarpin HCl önerilmektedir. Oral yoldan pilokarpin kullanımı, geniş etki yelpazesi nedeniyle çeşitli olumsuz yan etkinin ortaya çıkmasına neden olabilmektedir. Çalışmanın amacı, pilokarpin hidroklorürün etki süresini arttırmak ve sistemik yan etkilerini önlemek için; ksantan sakızı, hidroksietil selüloz veya bu iki doğal polimerin kombinasyonunun kullanıldığı bukkal biyoadhezif filmlerinin hazırlanması ve ilacın uzun süreli salınımını sağlayan formülasyonlarının tasarlanıp değerlendirilmesidir.

Gereç ve Yöntemler: Bukkal filmler; fizikokimyasal, mekanik, biyoadheziflik, şişme, *in vitro* salım ve *in vitro* sitotoksisite açısından analiz edimiştir.

Bulgular: Fizikokimyasal ve mekanik özellikler Ksantan-HEC kombinasyonlarının tek polimerin kullanıldığı formülasyonlara göre daha iyi sonuçlar verdiğini göstermiştir. Tüm optimal formülasyonların *in vitro* çözünürlük profilleri, başlangıçtaki hızlı salımdan sonra kararlı durum platosuyla birlikte sürekli salım modeli göstermiştir. İlaç salım kinetiğini değerlendirmek için kullanılan çeşitli salım kinetik modelleri öncelikli Higuchi ve Korsmeyer-Peppas korelasyonlarının gerçekleştiğini bukkal filmlerden ilaç salımının takip ettiğini göstermiştir.

Sonuç: Bulgular mukoadezif bukkal formülasyonun lokalize dağılımı için pilokarpinin kserostomi tedavisinde hem güvenli hem de etkili olarak uygulanabilir bir yöntem olduğunu göstermektedir. İleri çalışmalar olarak formülasyonun farmakokinetik ve histopatolojik etkilerini değerlendirmek için in vivo deneyler planlanmaktadır.

Anahtar kelimeler: Bukkal uygulama, bukkal film, pilokarpin hidroklorür, sialagog, kserostomi

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INTRODUCTION

Saliva is a protective factor for oral health and provides support for crucial oral functions as a digestion lubricant and buffering agent that modulates the pH levels in the mouth (1). Therefore, any kind of salivary dysfunction such as "xerostomia", generally used to describe the sense of dryness felt in the mouth (2). Currently, pilocarpine is indicated as the first-line drug for patients with radiation-induced xerostomia and Sjögren's syndrome (1). Due to its broad range of activity, various adverse effects , i.e. flushing, sweating, and nausea can be seen in oral pilocarpine users. Furthermore, the oral pilocarpine's stimulation effect is relatively short with a 5 to 10 mg dosage and a 3/4 times daily administration, which has a negative impact on the patient adherence to the therapy regime (3). Localised buccal drug delivery system increases the patient's compliance with ease of administration, reduced dose frequency, high drug accumulation, and prolonged residence time at the target site (3).

In the frame of this knowledge, the study aimed to design and *in vitro* characterise prolonged-release formulation of pilocarpine hydrochloride buccal bioadhesive films using either xanthan gum, hydroxyethyl cellulose, or a combination of these two natural polymers to increase the duration of action and extend the release duration, which may prevent systemic side effects of pilocarpine.

MATERIALS AND METHODS

Compounds and reagents

Pilocarpine Hydrochloride was kindly provided by the Bilim Pharmaceutical Company, Türkiye. Hydroxyethyl Cellulose (HEC; 250000 Mw, 145 mPas, 1% in H_2O/ 20°C) was purchased from Sigma-Aldrich (Darmstadt, Germany) and the Xanthan gum (1000-1400 mPas, 1% in H_2O/ 20°C) was purchased from the Doga Drug Company (Istanbul, Turkey). All other reagents were of analytical grade.

Preparation of the bioadhesive mucosal buccal films

Buccal films of the pilocarpine hydrochloride were prepared using the solvent casting technique. Various amounts of each matrix polymer were added to the required amount of water and left overnight under magnetic stirring to allow the polymers to obtain a bubble-free polymer dispersion. Pilocarpine hydrochloride (0.04% w/w) was dissolved in distilled water. As a penetration enhancer and a plasticising agent, propylene glycol (10% w/w) was added to the pilocarpine solution. Then, this mixture was added to the polymer dispersion and stirred on a magnetic stirrer until a homogeneous mixture was obtained. Afterwards, the homogenised gel was cast onto a glass petri dish (*r*=9 cm) and left to dry at 40 °C in a hot air oven (Nüve EN 40, Turkey) until a formed dry film was obtained. After drying, the films were stored in a desiccator at room temperature with 40% relative humidity for further experiments (4, 5).

Characterisation of Pilocarpine Hydrochloride Films

Physical appearance

The films' physical characteristics, including colour (visual inspection), transparency, softness, peelability (removal of the film from the Petri dish after drying), and homogeneity were visually examined (6).

Initial polymer solution viscosity measurement

The RV6 spindle Brookfield viscometer (Brookfield DV2, USA) was used to take measurement of the matrix polymers' viscosity were used to assess the formulations' pourability and spreadability (5).

Weigh uniformity and film thickness

The films were cut with a diameter of 1 cm from six different regions. The weight of the individual cut films was recorded, and the average and standard deviation of the film weight were calculated. A manual digital micrometre (QLR digit, IP4, PRC) was used to gauge the thickness of the produced films, and the samples for the thickness measurement were chosen similarly to the weight variation analysis (7).

Folding endurance

For each formulation, a little 4-4 cm strip of film was taken and folded in the same spot repeatedly creating an angle of 180° until it broke. The folding endurance of a film was measured by the number of times it could be folded in the same spot without tear (n=3) (8).

Surface pH

To measure the film's surface pH, it was allowed to swell in a glass Petri dish for 1 h while in contact with 10 mL of simulated saliva fluid (SSF) (KH_2PO_4 12 mM, NaCl 40 mM, $CaCl_2$ 1,5 mM adjusted with H_3PO_4 to pH 6.75). Using a pH meter, the surface pH was measured by placing a glass electrode close to the film's surface for one minute (n=3) (9).

Drug content and content uniformity

The buccal films were cut with a diameter of 0,6 cm and dissolved in 15 mL SSF in an orbital shaker (IKA, HS501, Germany) with the rotation speed adjusted to 200 rpm for 4 h. Then, the collected samples were filtered through membrane filters. The drug content of the films was quantified by the developed HPLC method (LC-20 AT model, Shimadzu, Japan). The chromatographic separation was accomplished on a C18 column (150x4,6mm, 5µm: Shim-pack VP-ODS, Shimadzu, Japan). HPLC was performed using an isocratic gradient; a mobile phase of 25:75 [MeOH: Buffer (10 µM sodium hexane sulphonate, 0.2% v/v Trimethylamine pH adjusted to 2.8 with o-phosphoric acid) at a flow rate of 1 mL/min. The pilocarpine hydrochloride peak was detected by a photodiode array detector at 214 nm (n=3, α=0.05) (10).

Measurement of Mechanical Properties

Mechanical properties

The mechanical properties of the pilocarpine films were measured using a texture profile analyser (TA.XT Plus, Stable Micro Systems, Surrey, UK) equipped with a 5-kg load cell (11). The tensile strength and elongation at break will be calculated as shown in the Equations;

$$\text{Eq1: Tensile Strength } (mm^2) = \frac{\text{Breaking Force (N)}}{\text{Initial Cross} - \text{Sectional Area of Sample } (mm^2)}$$

$$\text{Eq 2: Elongation at Break } (\%) = \left(\frac{\text{Increase in lenght at breaking point(mm)}}{\text{initial lenght}}\right) x100$$

***In vitro* bioadhesion studies**

The measurement was carried out using a texture analyser that was outfitted with a bioadhesion test rig and a 500 g load cell. The bioadhesive properties of the formulations were determined using bovine buccal mucosal tissue obtained from a local slaughterhouse. Mucosal membrane sections were attached to the holder of the texture analyser at 37±0.5°C. Formulations were placed at the lower end of the probe and the probe was lowered onto the bladder mucosa surface at a constant speed (1 mm/s). The contact force (0.05 N) was applied for 2 min. The area under the curve (mucoadhesion) was determined from the resultant force–distance graph (n=6) (12).

Swelling studies

A portion of each mucosal dosage film was divided into portions of 4 cm^2 (2x2 cm) and cut, placed in a stainless steel wire mesh, and the total weight (W1) was measured. Then, the mixture was submerged into a beaker containing 20 mL SSF at pH 6.75 (13). The samples were measured at predetermined periods (5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300 and 360 min). The samples were carefully withdrawn from the medium and the excess surface water was wiped off with filter paper and weighed (W2). After the experiment, the swollen films were dried at 60 °C for 24 h and kept in desiccators for over 48 h, and after they fully dried, the weighing was repeated (W3). These experiments were performed in triplicate (n=3, α =0,05) (5). The percentage of hydration and matrix erosion were calculated using the following equations (Eq.3-4):

$$\text{Eq 3: } \%\ of\ Hydration = \frac{W2-W1}{W2} X100$$

$$\text{Eq 4: } \%\ of\ Matrix\ Erosion = \frac{W1-W3}{W1} X100$$

***In Vitro* Drug-Release Studies**

***In vitro* drug-dissolution studies**

The PL release studies were assessed with a design closely similar to the USP 23 dissolution test apparatus 5 (paddle over disk) method using a dissolution tester (SOTAX, AT 7 Smart V230, Switzerland). The dissolution medium comprised 900 ml of SSF at 37±0.5 °C and paddle rotation speed was adjusted to 50 rpm. The buccal films were cut with a diameter of 2 cm and were fixed in a glass slide with a self-fabricated basket (50 mm diameter and 6 mm height) made from stainless steel with a sieve opening of approximately 850 µm (size No. 20, USP 23). The basket containing the sample was submerged in the dissolution medium. The manually collected samples at intervals of 0, 5, 1, 2, 3, 4, 5 and 6 h. were filtered through a 0.45 µM Millex syringe-driven filter (Millipore Cooperation, Bedford MA, USA) and quantified by HPLC (n=3). The samples were replaced with an equal volume of SSF maintained at the same temperature (14).

Drug Release Mechanisms

Based on the *in vitro* release data of the film formulations, four kinetic models with their corresponding relationships were constructed, as shown in Table 1 (15-17). The kinetic models' associated mathematical equations are shown in Eqs. (5)–(8) below;

Table 1: Various plots with corresponding kinetic mechanisms used to evaluate the in vitro dissolution data

Plot parameters	Kinetic Model	Release Dependency
Cumulative percentage drug release vs. time	Zero Order	The rate of drug release is independent of its concentration.
Log cumulative of percentage drug remaining vs Time	First Order	The rate of drug release is dependent on its concentration.
Cumulative percentage drug release vs. square root of time	Higuchi	Drug release through a matrix via diffusion based on Fick's law, which is square root time-dependent
Log cumulative percent drug release vs log of time	Korsmeyer-Peppas	The release regime depends on the "n" exponent value

Zero order; $Q_t = Q^0 + K_0 t$

Where Q_0 is the initial amount of the drug, Q_t is the cumulative amount of the drug released time *(t),* K_0 is the zero-order rate constant, and t is time in minutes.

$$Log\ Q_t = LogQ_0 + {K_1 t}/{2.303}$$

Where Q_0 is the initial amount of the drug, Q_t is the cumulative amount of the drug released time (*t*), K_1 is the first-order rate constant, and *t* is time in minutes.

Higuchi; $Q = K_H t^{1/2}$

where Q is the cumulative amount of drug released in time *(t),* K_H is the Higuchi release rate constant, and *t* is time in minutes.

Korsmeyer–Peppas;

$$F = (M_t/M) = K_p t^n$$

where F is the fraction of the drug released in time *(t), Mt* is the amount of the drug released at time *(t), M* is the total amount of the drug in the dosage form, K_p is the release rate constant, *n* is the diffusion or release exponent, *t* is the time in minutes, *'n'* is estimated from the linear regression of log *(Mt/M)* versus log *t.*

***In vitro* cell viability assay**

The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Caco-2 was used

to evaluate the cytotoxicity of pure Pilocarpine/Xanthan/HEC and selected buccal film formulations. Caco-2 cells (ATCC® HTB-37TM) are adherent cells derived from the human colorectal adenocarcinoma. Cells were incubated in Eagle's Minimum Essential Medium supplemented with 5% foetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin. The cells were grown in an incubator used for cell culture (Thermo Scientific, Hessen, Germany) at 37°C, 5% CO2. After attaining 80%–85% confluence, the cells were harvested. The cells (Caco-2, $4x10^4$ cells/mL) were seeded in a sterile, flat-bottomed 96-well tissue culture plate. After 24 h, the cells (except those in the control wells) were exposed to either pure Pilocarpine/Xanthan/HEC or buccal film formulations in the cell culture medium and incubated for 24 h. MTT stock solution (5 mg/mL) was prepared and 30 µL of the solution was added to each well and the plate was incubated for a further 4 h at 37 °C, 5% CO_2. At the end of the incubation time, formazan crystals formed by the mitochondrial dehydrogenase reduction activity were dissolved by adding 100 µL DMSO to each well. The optical density was measured on a Multiscan EX Micro-plate reader (Thermo Scientific, Essex, UK) at 570 nm. The obtained results were expressed as percentage inhibition relative to the control cells in which cell survival was taken as 100 %. The experiments were performed in triplicate (18).

Statistical analysis

In-vitro data obtained from each experiment will be subjected to statistical analysis using a computer programme, GraphPad-Prism 9.0 software, for a one-way analysis of variance (ANOVA) followed by the Newman–Keuls multiple comparisons test. $P<0.05$ will be considered to be indicative of significance.

RESULTS

Preparation of the bioadhesive mucosal buccal films

Table 2 displays the preliminary formulation details of the pilocarpine hydrochloride buccal films.

Characterisation of pilocarpine hydrochloride films

The weight of the drug-loaded buccal film formulations was determined using electronic balance, and the average weight ±SD of films was given in Table 3.

The polymer type and viscosity are responsible for the variance in the thickness (Table 3). Each film's pH was measured to be between 6.0 and 6.5, which is within the normal range for salivary pH (Table 3) (19). The folding endurance was increased with the increased amount of XG. All the films showed a good value of folding endurance, which is above 300 times/per film.

An HPLC method for the quantification of PL in the buccal film formulation was developed and validated. The determination correlation coefficient (r^2) was found to be 0.9997 with the linear regression equation. The limits of detection and quantification were determined as 0.162 and 0.256 µg/mL, respecti-

Table 2: Formulation components of the buccal film formulations

Formulation	PL(%)	HEC(%)	XG(%)	PG(%)	DW
F1	0.04	2.5	-	10	q.s.
F2	0.04	-	2.8	10	q.s.
F3	0.04	1.25	1.4	10	q.s.
F4	0.04	1.25	2.8	10	q.s.
F5	0.04	1.25	4.2	10	q.s.
F6	0.04	1.25	5.6	10	q.s.
F7	0.04	2.5	1.4	10	q.s.
F8	0.04	3.75	1.4	10	q.s.
F9	0.04	5.0	1.4	10	q.s.

(% w/w), PL: Pilocarpine, HEC: Hydroxyethyl Cellulose, XG: Xanthan Gum, PG: Propylene Glycol, DW: Distilled water

Table 3: Physicochemical characteristics of the prepared buccal films of PL

Formulations	Weigh (mg)	Thickness (mm)	Folding endurance (time/film)	Surface pH	Drug content (%)
F1	40.89±0.67	0.040±0.008	332±12	6.17±0.05	96.45±1.01
F2	56.64±0.54	0.056±0.002	315±11	6.23±0.08	97.75±1.02
F3	41.56±0.78	0.037±0.005	324±06	6.35±0.06	97.04±1.54
F6	58.21±1.12	0.045±0.006	321±04	6.45±0.05	98.77±1.03
F9	42.38±0.84	0.039±0.002	328±08	6.51±0.09	98.54±1.26

All values are expressed as mean ±S.D; n=6.
[a] percentage of the drug concerning the film weight.

Table 4: Mechanical properties of the prepared Bioadhesive buccal films of PL

Formulations	Tensile Strength (N/cm^2)	Elongation at the break (%)	Work of adhesion (mJ/ cm^2)
F1	1.659±0.154	4.260±0.089	4.016±0.026
F2	3.956±0.148	1.524±0.015	1.523±0.036
F3	2.827±0.295	3.260±0.024	3.625±0.049
F6	4.154±0.389	2.019±0.056	1.756±0.056
F9	1.896±0.256	5.126±0.045	5.260±0.025

All values are expressed as mean ±S.D; n=3.

vely. In pharmaceutical formulation content uniformity, one of the most important characteristics to guarantee the presence and consistency of the drug is the film's formulation (20). The percent drug content in the films was found to be between 96.45±1.01 and 98.77±1.03%. The data shows uniform distribution (Table 3). It can be concluded that the content homogeneity was not affected by the polymer or the polymer ratio.

Measurement of the mechanical properties

The Texture Profile Analyser was used to assess the mechanical characteristics of the buccal films based on Xanthan gum and HEC. Table 4 displays the findings of our measurements for the tensile strength, elongation at break, *and in vitro* bioadhesion work.

The mechanical strength of the films prepared with HEC varied between 1.659±0.154 and 1.896±0.256 N/cm^2, whereas xanthan gum contributed a greater mechanical strength to the films (3.956±0.148-4.154±0.389 N/cm^2). The results of the *in vitro* mucoadhesion study using PL buccal film formulations are given in Table 4.

Swelling studies

A correlation has been demonstrated between the swelling index and the mucoadhesive strength (Figures 1, 2) (21)-sufficient swelling guarantees that the polymer chains unfold and establish a connection with the buccal mucosa. The percentage hydration of the drug-loaded films (F1–F9) was measured for 6 h. The findings are shown in Figure 1. Notably, a steep slope during this phase indicates that the hydration rate was rapid in the first hour (Figure 1).

Comparisons between the films' initial and final weights upon immersion in simulated saliva were used to calculate the matrix erosion (%) profiles shown in Figure 2. Comparing the HEC-based formulation to the xanthan-based films revealed a substantial ($p \leq 0.05$) increase in matrix erosion (per cent), and the ratio of matrix erosion increased in direct proportion to the amount of HEC present (Figure 2).

***In vitro* drug-release studies**

In terms of the release profile (Figure 3), PL release ranged between 59.15±2.69% and 72.09±0.09% in the first 30 min in the HEC-based films (Figure 3). At 2 h, the release profile reached its maximum level (87.67±0.85 to 79.99±6.56%).

Within the first 30 minutes, 39.92±4.98% of PL was released

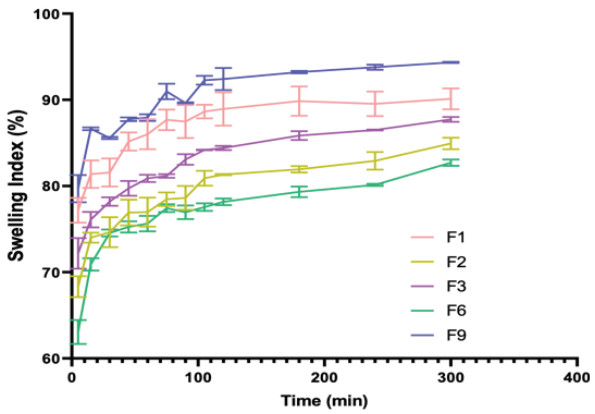

Figure 1: Swelling index of the mucoadhesive films
All values are mean ±SD, n=6

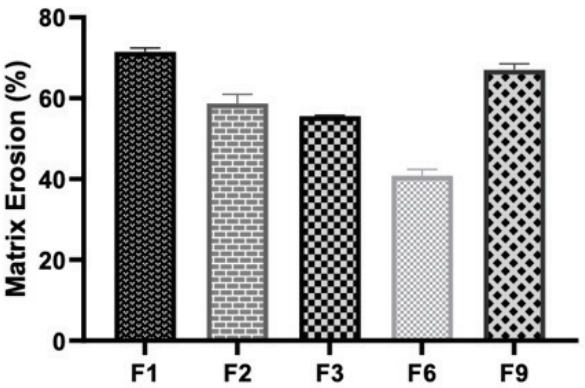

Figure 2: Matrix erosion of the mucoadhesive films
All values are mean ±SD, n=6

from the buccal film F3. The release profile reached its maximum level after 4 h (89.49±9.10%), and the release level was maintained until 6 h. Table 5 summarises the release parameters that were acquired through the process of fitting the experimental dissolution release data to the several kinetic equations that were assessed (22).

In our study, the xanthan-based films F2 and F6 fulfilled both the Higuchi and Korsmeyer–Peppas correlations (Table 5) (15). In the Higuchi model, the xanthan-based film exhibited the

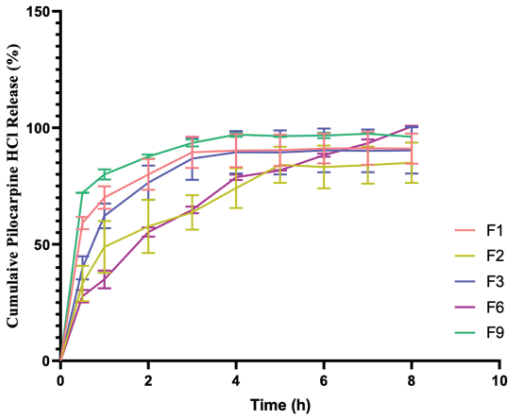

Figure 3: Pilocarpine hydrochloride release from the bioadhesive films over 6 h. Each film formulation contained the same amount of PL (10 mg) as the active agent.
All values are mean ±SD, n=6

Table 5: Estimated values of pilocarpine-release determination coefficient (r^2) and the Korsmeyer-Peppas kinetic model release exponent (n) for the buccal film formulations

Formulations	Zero-order Kinetics (r^2)	First-order Kinetics (r^2)	Higuchi Kinetics (r^2)	Korsmeyer-Peppas Kinetics (r^2.n)
F1	0.6871	0.6860	0.7819	0.8055 0.3281
F2	0.6372	0.8781	0.9909	0.9909 0.5768
F3	0.7259	0.7317	0.8475	0.9723 0.5129
F6	0.6871	0.8998	0.9905	0.9974 0.6178
F9	0.6998	0.7014	0.8114	0.8375 0.3425

best linear correlation, with an r^2 value of 0.9909 and 0.9905, respectively. In the Korsmeyer–Peppas model, the n-value of F2 was 0.5768 with a linear correlation of 2r ¼ 0.9909 while F6 was with an r^2 value of 0.6178 with a linear correlation of 2r ¼ 0.9974 whereas the combination of the polymers (F3) had an r^2 value of 0.5129 with a linear correlation of 2r ¼ 0.9723, indicating non-Fickian behaviour 40,52.

***In vitro* cell viability assay**

The effective and safe use of polymers as drug carriers in humans depends on several aspects, with toxicity being a key consideration (22). MTT was used in this investigation to assess the short-term cytotoxic effects of the chosen buccal film formulations. Based on the metabolically active cells reducing MTT to a coloured formazan product, the assay can be measured using spectrophotometry (23). The study formulations exhibited no cytotoxic impact and PL presented no physical risks to the endothelial cells. Additional research is required to shed light on the entire toxicological profile.

DISCUSSION

Because of its superior rheological characteristics, XG was employed as a thickening agent and mucoadhesive controlled-release excipient for the buccal formulations (24) along with HEC. Depending on the type, content, and temperature of the polymer, HEC is dissolved under different conditions to produce transparent solutions with different viscosities. With increasing temperature, the viscosity of the solution reduces (25). In addition to these mucoadhesive polymers/combinations, propylene glycol (PG) was also chosen as a hydrophilic permeation enhancer to improve drug partition into the mucosa to solubilise the drug and act as a plasticiser.

The thickness of the films was directly related to the dose accuracy, and the optimal thickness was necessary for adequate bioadhesion, whereby increased thickness reduces the mucoadhesion capability of the films (8). Each film's pH was measured to be between 6.0 and 6.5, which is within the normal range for salivary pH (Table 3) (19). The folding endurance was increased with the increased amount of XG. All the films showed a good value of folding endurance, which was above 300 times/per film. Mechanical studies were performed, where the tensile strength is the stretching force given to the sample at the time of breakage, while the elongation at break is the maximum change in the length of the polymeric film before breaking (6). These findings showed that films prepared with xanthan gum were more resilient to tension stress and underwent a plastic deformation, which is why the elongation at break happened

faster and earlier. Because the xanthan-based formulation has a smaller area under the force-distance curve than the HEC-based formulation, its work of adhesion is also lower. The HEC-based formulation, in contrast, exhibited an elastic deformation and more lasting elongation at break. It has a far wider region beneath the force-distance curve. As a result, the HEC-based formulation requires more force to adhere to it (26).

For the release studies, as the HEC concentration rose from 2.5% to 5%, the adhesiveness of the HEC films increased. The literature has demonstrated that when the concentration of polymer in the formulation increases, so does the work of adhesion value, which is compatible with our findings (27). For the film swelling, water entering the matrix allows drug molecules to diffuse out of the film and become available for mucosal penetration in the interim. As it may facilitate rapid film mucoadhesion with the buccal mucosa, rapid hydration is important during the first phase. It is assumed that HEC causes this quick hydration. However, after that, in the first phase, the hydration remained largely unchanged during the analysis. Moreover, there was no distortion or erosion, and the films maintained sufficient physical integrity until the completion of the experiment (9). The findings imply that greater matrix erosion may occur from the HEC-based films' with comparatively high swelling index. Lower matrix erosion was shown by the xanthan-based films, and this was correlated with a lower swelling index. PL release and HEC concentration were inversely correlated. The observed change in the viscosity of the polymer due to rapid hydration and the gelation process that affects the rate of drug dissolution could be the cause of this observation (5). In contrast, when xanthan and HEC were used together, the PL release was maintained more sustainably. The development of a thick gel layer that raised the viscosity of the polymeric film may have contributed to the decreased *in vitro* release during the first 4 h. This observation aligns with the findings of Akash et al., who suggested that thick gel formation and a dry interior core could cause a delay in drug release from xanthan films (28). Additionally, the drug's in-vitro release behaviour is impacted by the high viscosity of xanthan gum (29). This result also implies that PL partially diffuses with increasing diffusion path length across the gradually expanding hydrated matrix and the inflated polymer matrix. The phenomenon known as anomalous or non-Fickian diffusion is noted when the rates of liquid diffusion and polymer relaxation are equally large (30). However, according to various *in vitro* release models, the HEC-based films F1 and F9 were deemed inadequate and did not follow the Higuchi kinetics.

CONCLUSION

Hyposalivation can be caused by either long- or short-term cases that may be triggered by auto-immune diseases such as Sjögren syndrome, psychologic complications stimulated by stress, or radiotherapy of the neck and head region, which in turn may cause a syndrome named xerostomia. The objective of this study was to formulate a long-acting buccal dosage form to minimise the side effects and develop an alternative route of administration. Based on the results, it can be concluded that the mucoadhesive buccal formulation is a potential approach for effective as well as safe localised delivery of pilocarpine to treat xerostomia.

Data Availability: Data may be made available upon reasonable request to the corresponding author of the study. However, it must comply with the applicable legal restrictions.

Ethics Committee Approval: This manuscript does not contain any experiment that requires ethics committee approval.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.Y.P.; Data Acquisition- Ö.A.D., S.Y.K., N.S.B.; Data Analysis/Interpretation- A.Y.P., Ö.A.D.; Drafting Manuscript- Ö.A.D., N.S.B.; Critical Revision of Manuscript- A.Y.P.; Final Approval and Accountability- A.Y.P.; Material and Technical Support- Ö.A.D., S.Y.K., N.S.B.; Supervision- A.Y.P.

Conflict of Interest: The authors declare that there is no conflict of interest.

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EVALUATION OF SWALLOWING AND NUTRITION STATUS IN PARKINSON'S DISEASE

PARKİNSON HASTALIĞINDA YUTMA VE BESLENME DURUMUNUN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: One of the significant issues in Parkinson's disease (PD) is the risk of malnutrition due to taste and smell alterations at the early stages and the later onset of dysphagia. Dysphagia can cause complications like aspiration, which may accelerate the progression of the disease. Our study evaluates swallowing difficulties and nutritional status using objective questionnaires in PD patients who did not report any issues with swallowing or feeding.

Material and Methods: Forty patients had undergone swallowing and feeding-related tests during their outpatient visits.

Results: There was no significant relationship between the Gugging Swallow Screening (GUSS) score and body mass index (BMI), whereas there was a positive correlation between BMI and Mini Nutritional Assessment-Short Form (MNA-SF) scores (r=0.489, p=0.001). Additionally, there was a positive correlation between GUSS and MNA-SF scores (r=0.397, p=0.011).

Conclusion: In this study, various degrees of swallowing and feeding disorders were identified in most of our patients who did not complain of such issues. Therefore, it is considered crucial to conduct objective assessments of swallowing and feeding during each visit for PD patients, even if not mentioned by the patients or their caregivers. This early detection of dysphagia and malnutrition risk and taking necessary measures is believed to be important for ensuring proper management.

Keywords: Parkinson's disease, swallowing, nutrition, neurodegenerative disease

ÖZ

Amaç: Parkinson hastalığında, en önemli problemlerden biri tat ve koku değişikliğine bağlı malnütrisyon riski ve ilerleyen dönemde ortaya çıkan disfajidir. Disfaji aspirasyon gibi komplikasyonlara neden olabilmekte, hastalığın ilerleyişini hızlandırmaktadır. Bu çalışmanın amacı, kendisi ve bakım vereni, yutma ve beslenme bozukluğundan yakınmayan Parkinson hastalarında yutma güçlüğü ve beslenme durumunun değerlendirilmesidir.

Gereç ve Yöntemler: Poliklinik başvuruları esnasında yutma ve beslenme ile ilgili testleri yapılmış olan 40 hasta çalışmaya dâhil edilmiştir.

Bulgular: Hastaların BKİ ile GUSS skoru arasında anlamlı ilişki gözlenmezken, BKİ ile MNA-SF skorları arasında pozitif korelasyon gözlendi (r=0,489, p=0,001). Ayrıca GUSS ve MNA-SF skorlarının da pozitif korelasyon gösterdiği bulundu (r=0,397, p=0,011).

Sonuç: Bu çalışmada yakınması olmayan hastaların büyük kısmında çeşitli derecelerde yutma ve beslenme bozuklukları saptanmıştır. Bu nedenle hasta ve yakınları tarafından dile getirilmese de Parkinson hastalarında her başvuruda yutma ve beslenme ile ilgili objektif değerlendirmelerin yapılmasının disfajinin ve malnütrisyon riskinin erkenden saptanması ve gerekli önlemlerin alınmasını sağlayacağından önemli olduğu düşünülmektedir.

Anahtar Kelimeler: Parkinson hastalığı, yutma, nütrisyon, nörodejeneratif hastalık

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INTRODUCTION

The second most common neurodegenerative disease is Parkinson's disease (PD) following Alzheimer's disease, and the prevalence of the disease has been increasing in recent years (1). Early neurodegeneration of the substantia nigra pars compacta causes this disease, and it accounts for 80% of all parkinsonism cases. (2). Dopamine deficiency is the main neurobiochemical abnormality associated with this disease (3, 4). Accordingly, motor symptoms, including rigidity, tremor, and postural instability, especially bradykinesia, are the most common manifestations of PD (5). However, non-motor symptoms, including loss of smell, depression, anxiety, REM sleep behaviour disorder, constipation, bladder dysfunction, cognitive impairment, pain, and sensory complaints, may also occur as a result of the involvement of other brain regions during the earlier stages of the disease and during its progressive prognosis (6, 7).

Weight loss in patients with PD may occur due to different symptoms, which can start years before the diagnosis. (8-10). Affected individuals may experience a decrease in motor function and initial weight gain due to treatments, including levodopa and dopamine agonists, during the years following the onset of symptoms (11). Nevertheless, weight loss associated with an increase in the amount of energy consumption due to muscle hypertonia and dyskinesia occurs during the course of the disease with an increased risk of malnutrition. Reduced daily activities, including shopping and cooking, associated with disrupted motor symptoms are considered to play a role in decreased food and energy intake. Furthermore, alterations in stomach and bowel movements may cause bloating, discomfort, nausea, and early satiety. Decreased cognitive performance and sense of smell also affect eating behaviours, and individuals with cognitive impairment may forget whether they have eaten or not. It is well established that dysphagia, which may occur during the disease, also contributes to malnutrition. Dysphagia and malnutrition may worsen the existing clinical picture, cause complications such as aspiration, and accelerate disease progression in the affected patients. Early symptoms such as changes in appetite and nutrition, weight loss, and dysphagia, which can appear during the initial stages of the disease, may often be overlooked and not reported by the patient or their relatives. This contrasts with motor symptoms, which receive more clinical attention. Therefore, it is important to monitor these symptoms regularly for early recognition and to take the necessary precautions (11-13).

In this study, we evaluated dysphagia and nutritional status in patients with PD who did not present with complaints of dysphagia or malnutrition using objective questionnaires in an outpatient clinic.

MATERIAL AND METHODS

Selection of the participants

In this descriptive and cross-sectional study, 40 patients who were followed up at the Movement Disorders Outpatient Clinic of the Department of Neurology, Division of Behavioral Neurology and Movement Disorders, Department of Neurology, Istanbul Faculty of Medicine, Istanbul University were included. Patients who presented to the outpatient clinic between September 2022 and January 2023 and were diagnosed with possible PD based on the Parkinson's Disease Society Brain Bank diagnostic criteria were selected. (14). The patients underwent swallowing and feeding tests during their outpatient visits, and their demographic and clinical characteristics were captured. All patients were examined by neurologists specialised in Movement Disorders during outpatient clinic assessments, and their medical history, examination findings, neuroimaging results, and other investigations were noted in detail. The Gugging Swallow Screening (GUSS) test and Mini Nutritional Assessment-Short Form (MNA-SF) were used to analyse dysphagia and nutritional status during outpatient clinic visits. Patients were evaluated at outpatient clinic visits during which neither the patients themselves nor their relatives mentioned any swallowing complaints. Informed written consent was obtained from all patients. Ethical approval was obtained from İstanbul University Ethics Committee (Date: 22.02.2021, No: 94690).

Statistical analysis

Statistical analyses of the results were performed using the statistical software SPSS, Version 21.0 (IBM SPSS Corp., Armonk, NY, USA).

Hugging Swallow Screening Test

The GUSS test consists of two stages: the first stage is the indirect swallowing test, which is a preliminary assessment, followed by the second stage, the direct swallowing test, which includes three sub-tests. The first stage comprises the ability to maintain wakefulness for 15 min, to cough and/or clear the throat voluntarily at least twice, and to swallow saliva successfully without a change in voice or salivation. Patients who pass this first part of the GUSS with a full score of 5 continue on the second stage, which is a direct swallowing test with foods of variable fluidity. During the direct swallowing test, signs of aspiration were observed, including delayed swallowing (<2 s for liquids and semi-solids, >10 s for solids), involuntary cough (Before, during, 3 min after swallowing), drooling, and changes in voice. Assessment was performed after 5 teaspoons of semi-solid food intake. Those who were successful were then assessed with liquid (Starting with 3 mL, followed by increasing amounts of 5, 10, 20 and 50 mL). Patients who successfully scored full points at this stage were then tested with solid food (15).

Direct swallowing assessment was performed using yogurt for semi-solid foods, water for liquid foods, and biscuits for solid foods in our outpatient clinic. A total of 20 points can be scored with 5 points from the first section and a maximum of 5 points from each consistency thereafter.

The test scores were evaluated as follows for dysphagia and aspiration (15).

0-9 points: Severe

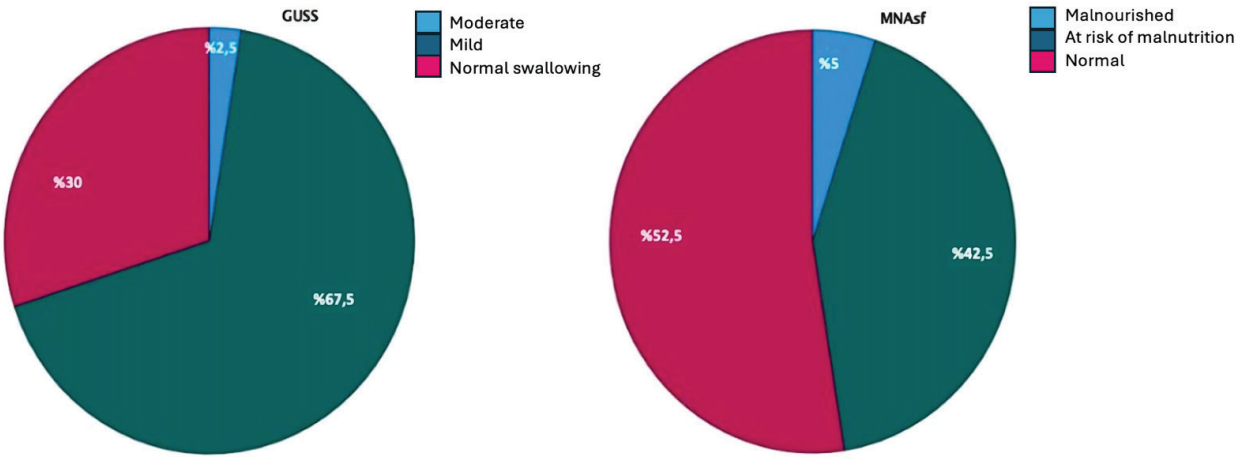

Figure 1: The Gugging Swallow Screening (GUSS) test and Mini Nutritional Assessment-Short Form (MNA-SF) results of the patients

10-14 points: Moderate

15-19 points: Mild

20 points: Normal swallowing

Mini nutritional assessment-short form

The Mini Nutritional Assessment (MNA) was conducted using six survey items (questions A-F1/F2), which are the MNA-SF with a maximum score of 14 points. These items cover the patient's weight loss, food intake, mobility, body mass index (BMI), psychological stress or acute illness, and depression or memory problems. Calf circumference may be appropriate in cases in which BMI measurement is not possible (16).

The test score is evaluated as follows for malnutrition:

12–14 points: Normal

8–11 points: At risk

0–7 points: Malnourished

RESULTS

Clinical characteristics and demographics of the patients

The present study included 40 patients [19 females (47.5%), 21 males (52.5%)] who presented to our outpatient clinic between September 2022 and January 2023 and were followed up with a diagnosis of PD. The mean age of the patients was 64.7±11.9 years, mean disease duration was 10.7±6.2 years, and mean follow-up duration was 10.2±5.9 years. Hoehn and Yahr staging scale of 7 patients were Stage 1, 17 were Stage 1.5, 10 were Stage 2, 2 were Stage 2.5, and 4 were Stage 3. The mean BMI of the patients was 27±7.66 kg/m^2. The mean levodopa equivalent dose administered to the patients was 1200.8±1152.70.

GUSS and MNA-SF test results

Upon review of the patients by swallowing using the GUSS test, 27 of 40 patients had mild dysphagia (67.5%) and 1 patient had moderate dysphagia (2.5%). Twelve patients had normal swallowing (30%). Upon review of the patients according to malnutrition using the MNA-SF test, 17 of 40 patients (42.5%) were at risk and 2 were malnourished (5%). The remaining 21 patients (52.5%) were normal (Figure 1).

There was no significant correlation between BMI and GUSS scores, whereas a positive correlation was observed between BMI and MNA-SF scores (r=0.489, p=0.001). There was a positive correlation between GUSS and MNA-SF scores (r=0.397, p=0.011). There was no significant correlation between the clinical parameters, levodopa equivalent dose, GUSS score, and MNA-SF test score.

All patients who were at risk in the swallowing test were referred to the Ear, Nose, and Throat (ENT) outpatient clinic for instrumental evaluation. Recommendations were made for swallowing safety and therapy. Patients at risk were interviewed by a dietitian, and oral nutritional supplements (ONS) were started based on their daily caloric needs and comorbidities.

DISCUSSION

Dysphagia and malnutrition may occur at various stages of PD. Recent studies have highlighted varying rates of malnutrition and dysphagia across different stages of PD and how these conditions evolve with disease progression. Coelho et al. revealed that patients in the late stages of PD exhibited significantly higher rates of dysphagia, often reaching critical levels that necessitate advanced interventions (17). Similarly, a meta-analysis by Kalf et al. showed that the prevalence of oropharyngeal dysphagia increased from the early to the advanced stages of PD, reaching nearly 100% in some advanced cases (18). These studies highlight a crucial link between disease progression and increased risk of malnutrition due to dysphagia. In this study, dysphagia was detected at various levels in a large proportion of patients, and malnutrition risk or malnutrition was observed in nearly half of the patients. These results are significant because patients who did not have swallowing or feeding difficulties according to either self-report or their caregivers' observations were evaluated, and swallowing dysfunction was observed in most patients.

Increased basal metabolism and energy expenditure due to motor symptoms play a pivotal role in the aetiology of weight loss in patients with PD. A reduction in body weight was reported in the advanced and early stages. Drug-related side effects, including nausea/vomiting, gastrointestinal problems (delayed gastric emptying, constipation), anorexia, insomnia, fatigue, irritability, and anxiety, may lead to malnutrition due to increased energy need associated with tremor, dyskinesia and rigidity, decreased energy intake and/or dry mouth due to conditions, including olfactory dysfunction, cognitive impairment, dysphagia and impaired manual dexterity (inability to prepare meals) (11). Many studies that compared patients with PD and healthy controls reported that PD patients had a lower BMI. Beyer et al. compared 51 PD patients with 49 healthy controls and reported that their patients lost an average of 3.3 kg more weight (10). Malnutrition may result in a higher risk of infection, delayed wound healing, reduced muscle strength and mobility, and an increased tendency towards depression. The number of hospital admissions and hospitalisations is higher, systemic infections have a much more severe course, durations of hospital stay are longer, and complication rates are higher in malnourished patients (19). Although malnutrition was mentioned neither by the patient nor the caregivers, 17 (42.5%) patients were at risk and 2 (5%) patients were malnourished in our cohort of 40 patients in this study. These results indicate the importance of evaluation via objective questionnaires in patients who did not raise relevant complaints.

Dysphagia is one of the most prevalent and significant causes of malnutrition in patients with PD. The main cause of dysphagia is considered to be the reduced rate of swallowing and slowed mastication, consistent with the nature of the disease, a hypokinetic movement disorder. Dysphagia may be subjectively reported by patients or caregivers or detected objectively using instrumental tools (20, 21). The rate of subjectively reported dysphagia was 68% among patients with late-stage PD in a cross-sectional study involving the Barcelona and Lisbon cohorts (22). Dysphagia was detected in more than 50% of participants with PD who did not report dysphagia among studies using instrumental tools, such as fiberoptic endoscopic swallowing or videofluoroscopic swallowing assessment (23, 24). The prevalence of oropharyngeal dysphagia was 35% in subjective reports and 82% in objective evaluations, and the rate of dysphagia in advanced stages was reported as 95%–100% in a meta-analysis, which reviewed the prevalence of dysphagia in all PD stages (17, 25). More than 20%–40% of individuals with PD were not aware of swallowing disorders, and only >10% personally reported symptoms of dysphagia (25, 26). Although subjective dysphagia was not reported, the rates of mild and moderate dysphagia were 67.5% and 2.5%, respectively, in the patients included in our study. In our study, there was no significant correlation between the Hoehn and Yahr stages and the levodopa equivalent dose level and test scores, which are considered to correlate with the motor symptoms of the disease. This finding indicates the fact that dysphagia may occur at any stage of the disease, not only when motor symptoms progress.

It is well established that dysphagia and weight loss are closely related in patients with PD. In a previous study that investigated the aforementioned relationship, 31% of the participants had dysphagia, and the BMI of the dysphagic group was considerably lower than that of the non-dysphagic group (13). Another study reported the rate of dysphagia as 43% in elderly participants, where 59% were malnourished and 35% were at risk (14). In this study, there was a positive correlation between dysphagia and BMI and malnutrition scores.

The major limitation of this study is that due to the retrospective design of the study and the absence of a relationship between dysphagia, malnutrition, and the Unified Parkinson's Disease Rating Scale, not all objective motor examination scores of the patients were available. The authors attempted to mitigate this limitation indirectly by using the Hoehn and Yahr staging and performing related analyses by considering that the levodopa equivalent dose increases as rigidity and bradykinesia increase. The retrospective design of this study may introduce selection bias, as patients who attend clinic visits could regularly differ in key characteristics from those who do not. Furthermore, the cross-sectional nature of the study limits our capacity to establish causal inferences regarding the relationship between clinical parameters, as the associations observed reflect correlations rather than causation. To better understand how dysphagia progression directly affects the nutritional status and clinical outcomes in PD patients over time, future longitudinal studies would be beneficial.

Patients with PD and their relatives are not aware that swallowing disorders may lead to certain complications like aspiration pneumonia, accelerating disease progression and causing increased mortality. Swallowing and feeding disorders of various degrees were found in most patients without complaints in this study. Therefore, although it is not mentioned by the patients or their relatives, it is important to perform objective evaluations in PD patients regarding swallowing and feeding at each admission, as this can enable early detection of dysphagia and malnutrition risk and take necessary precautions.

Ethics Committee Approval: This study was approved by İstanbul University (Date: 22.02.2021, No: 94690).

Informed Consent: Informed written consent was obtained from all patients.

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Author Contributions: Conception/Design of Study- H.H., B.B., B.S., E.Ş., B.Ü.; Data Acquisition- H.H., B.S., E.Ş., B.Ü.; Data Analysis/Interpretation- H.H., B.B., B.S., E.Ş.; Drafting Manuscript- B.S., E.Ş., B.Ü.; Critical Revision of Manuscript- H.H., B.B., B.S., E.Ş.; Final Approval and Accountability- H.H., B.B., B.S., E.Ş., B.Ü.; Material and Technical Support- H.H., B.S., E.Ş., B.Ü.; Supervision- H.H., B.B., B.S.

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A SINGLE CENTRE EXPERIENCE OF LOW C3/C4 LEVELS AND OTHER LABORATORY ASPECTS IN TURKISH CHILDREN WITH IMMUNOGLOBULIN A VASCULITIS

İMMÜNOGLOBULİN A VASKÜLİTLİ TÜRK ÇOCUKLARINDA DÜŞÜK C3/C4 DÜZEYLERİ VE DİĞER LABORATUVAR ÖZELLİKLERİYLE İLGİLİ TEK MERKEZ DENEYİMİ

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ABSTRACT

Objective: Immunoglobulin A vasculitis (IgAV) is the most prevalent systemic vasculitis in childhood. This intricate immune-mediated vasculitis affects the small blood vessels in several organ systems. Complements 3 and 4 (C3 and C4) are constituents of the complement system; serum C3/C4 measurements play a critical role in the diagnosis and follow-up of some immune diseases. Thus, the purpose of this study was to assess low C3/C4 levels and other laboratory results in children with IgAV.

Material and Methods: A total of 124 children—60 IgAV patients and 64 healthy controls—were assessed in this study. The C3 and C4 levels were quantified using a turbidimetric immunoassays method with manufacturer details. The extractable nuclear antigen antibody (ENA) panels were evaluated using the ELISA method. The results were evaluated statistically.

Results: The white blood cell count, neutrophil count, and neutrophil/lymphocyte ratio were higher in the patient group compared to the control group (p=0.015, p=0.013 and p=0.039, respectively). CRP, ESR, and random urine protein/creatinine ratio (RUPCR) increased in patients than controls (p<0.001, p=0.002 and p<0.001 respectively). There was no difference in the lupus anticoagulant activity and low C3/C4 levels between the groups (p>0.05).

Conclusion: IgAV, an IgA-mediated systemic small vessel vasculitis, affects many organs. As a result, it is crucial to evaluate the laboratory results in the follow-up of the patient and the possible complications.

Keywords: Immunoglobulin A vasculitis, C3, C4, immunoassay

ÖZ

Amaç: Eskiden Henoch-Schönlein purpurası olarak adlandırılan immünoglobulin A vasküliti (IgAV), çocukluk çağında en sık görülen sistemik vaskülit türüdür. Kompleman 3 ve 4 (C3 ve C4) kompleman sisteminin bileşenleridir ve bazı bağışıklık hastalıklarıyla ilişkili oldukları bulunmuştur. Bu nedenle bu çalışmada IgAV'li çocuklarda düşük C3/C4 düzeylerinin yanı sıra diğer laboratuvar bulgularını değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Bu çalışmada 60 IgAV hastası ve 64 sağlıklı kontrol olmak üzere toplam 124 çocuk değerlendirildi. C3 ve C4 düzeyleri, üretici ayrıntılarıyla birlikte türbidimetrik immünoanaliz yöntemi kullanılarak ölçüldü. Ekstrakte edilebilir nükleer antijen antikor (ENA) panelleri, enzime bağlı immünosorbent analizleri (ELISA) kullanılarak değerlendirildi. Sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Hasta grubunda lökosit sayısı, nötrofil sayısı ve nötrofil/lenfosit oranı kontrol grubuna göre daha yüksek bulundu (sırasıyla p=0,015, p=0,013 ve p=0,039). Ayrıca hastalarda kontrollere göre CRP, ESR ve rastgele idrar protein/kreatinin oranı (RUPCR) artmış bulundu (sırasıyla p<0,001, p=0,002 ve p<0,001). Düşük C3/C4 seviyeleri ve lupus antikoagülan aktivitesi açısından gruplar arasında anlamlı fark bulunamadı (p>0,05).

Sonuç: IgA aracılı sistemik küçük damar vasküliti olan IgAV, öncelikle böbrekleri, eklemleri, gastrointestinal sistemi ve cildi etkilemektedir. Sonuç olarak, potansiyel hasta durumlarını izlerken laboratuvar sonuçlarının değerlendirilmesi çok önemlidir.

Anahtar Kelimeler: İmmünoglobulin A vasküliti, C3, C4, immünolojik test

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INTRODUCTION

Immunoglobulin A vasculitis (IgAV), known as Henoch-Schönlein purpura, is the most prevalent vasculitis characterised by the involvement of small blood vessels in various organ systems. Abdominal pain, arthritis, and nonthrombocytopenic palpable purpura are its hallmarks. While some demographic studies estimate an incidence rate of 0.1-0.8 per 100,000 adults, the annual incidence rate of IgAV in children and adolescents under the age of 17 varies from 6.79 to 55.9 per 100,000 children across various nations (1). IgAV is uncommon in Africa, but the disease is more common in Southeast Asia and, to a lesser extent, in Europe and North America (2). Although the exact cause of HSP is unknown, IgAV aetiology appears to include environmental, genetic, and antigenic components. The 2012 Chapel Hill Conference described the condition as vasculitis with IgA1-dominant immune deposits, highlighting the role of IgA. The immune deposits primarily damage small arteries, such as capillaries, venules, or arterioles (3). Ten to forty percent of patients have gastrointestinal involvement, and ten to fifty-five percent have renal abnormalities (4). The major causes of morbidity and mortality include gastrointestinal involvement in the acute phase and renal involvement in the chronic phase (5). While the pathophysiology of IgAV remains poorly understood, the epidemiology, clinical symptoms, and prognosis of IgAV are well known. IgA1-dominant IgA deposits in the vessel walls are the most prominent pathogenic characteristic of IgAV, as the disease's name suggests. Aberrant IgA and IgA complexes are essential for the immunopathogenesis of IgAV.

The complement system performs various effector functions related to humoral immunity and inflammation. These proteins interact with other immune system components and with each other in a carefully regulated way (6). Increased cytokine, chemokine, and other innate defence molecule synthesis results from the complement system activation. Complement activation components, such as anaphylatoxin C3a and C5a, also cause the humoral adaptive immune response, the generation of reactive T cells and antibodies, and a marked increase in the detection of antigens by follicular dendritic cells and B cells. In addition, the complement system facilitates the removal of soluble immune complexes and cell debris, both of which run the risk of triggering autoimmunity and an immunological response against autoantigen (7). Complements 3 and 4 (C3 and C4) are constituents of the complement system and function in various pathways associated with complement activation. Certain autoimmune diseases can be diagnosed based on the levels of serum C3/C4. In patients with systemic lupus erythematosus (SLE), depletion may lead to a decrease in serum C3 and C4 levels (8). Furthermore, there is mounting evidence that antineutrophil cytoplasmic antibody-related vasculitis is pathophysiologically and progressionally connected with complement system activation (9).

Therefore, this study evaluated C3/C4 levels as well as other immunological markers in children with IgAV.

MATERIAL AND METHODS

Study population

This study evaluated 60 IgAV patients who were monitored between March 2024 and July 2024 in the Paediatric Rheumatology Department of Başakşehir Çam and Sakura City Hospital. The 2010 criteria of the European League Against Rheumatism, the Paediatric Rheumatology International Trials Organisation, and the Paediatric Rheumatology European Society (EULAR/PRINTO/PRES) served as the basis for the diagnosis of IgAV. The control group consisted of 64 age- and sex-matched healthy children who were admitted during the same study period. The exclusion criteria included concurrent chronic diseases, including other autoimmune disorders, and prior use of glucocorticoids or other immunosuppressive agents. Participants underwent complete blood count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), urine protein/creatinine ratio (RUPCR), antinuclear antibodies (ANA), lupus anticoagulant activity, antibodies to double-stranded DNA, β2 glycoprotein 1, anticardiolipin, and anti-neutrophil cytoplasmic (ANCA), and extractable nuclear antigen antibodies. Prior to their participation in the study, all patients and their families provided written informed consent in accordance with the 2008 Declaration of Helsinki's ethical principles. The Başakşehir Çam and Sakura City Hospital Ethical Committee approved the Project (Date: 26.06.2024, Approval Number: E-96317027-514.10-246491516).

Laboratory

C3/C4 levels were quantified using a turbidimetric immunoassay [method/instrument used, manufacturer details], with reference ranges of 0.9-1.8 g/dL for C3 and 0.1-0.4 g/dL for C4. The urinary protein-to-creatinine ratio was calculated using spot urine samples. The extractable nuclear antigen antibodies (ENA) panel was measured using enzyme-linked immunosorbent assays (ELISA).

Statistical analysis

The OpenEpi information software package, version 3.01 (www.openepi.com), and the Statistical Package for the Social Sciences program (IBM SPSS, version 20) were used to conduct the statistical analyses. The mean±standard deviation (SD) was used to display the data. The relationship between these variances and the patients' clinical and demographic characteristics was investigated using the chi-square ($\chi 2$) test, Fischer exact test, or analysis of variance (ANOVA) statistics. To evaluate the risk factors, 95% CIs and the odds ratio (OR) were employed. Each two-tailed p-value was considered significant if it was less than 0.05.

RESULTS

A total of 124 subjects, 60 IgAV patients and 64 healthy controls-were assessed in this study. Table 1 displays the patients' demographic and clinical characteristics.

We then evaluated the laboratory findings in the groups (Table 2). The white blood cell count, neutrophil count, and neutrophil/lymphocyte ratio were higher in the patient group compared to the control group (p=0.015, p=0.013, and p=0.039, respectively). Also, CRP, ESR, and urinary protein/creatinine ratio increased in patients compared with controls (p<0.001,

Table 1. Baseline demographic and clinical features of the subjects

	Patient group (n=60)	Control group (n=64)	p
Age (months) (Mean±SD)	107.7±45.7	100.8±46.4	0.407
Gender, n (%)			
Female	31 (51.7)	35 (54.7)	0.875
Male	29 (48.3)	29 (45.3)	
Age at diagnosis (month) (Mean±SD)	108.8±43.9		
Clinical findings, n (%)			
Cutaneous	59 (98.3)		
Gastrointestinal system	24 (40)		
Arthritis/arthralgia	19 (31.7)		
Renal	5 (8.4)		
Other	1 (1.7)		
Treatment, n (%)			
Conservative	9 (15.0)		
NSAID	23 (38.3)		
Steroids	27 (45.0)		
Azothioprine	3 (5.0)		
IV Ig	1 (1.7)		
Colchicine	3 (5)		

Table 2. Laboratory features of the groups

	Patient group (n=60)	Control group (n=64)	p
Haemoglobin (gr)	12.4±1.3	12.5±1.1	0.662
White blood cell count (/mm3) Mean (min-max)	8870.0 [3670.0-20200.0]	7870.0 [4220.0-24220.0]	**0.015**
Neutrophil count (/mm3) Mean (min-max)	4860.0 [1750.0-18490.0]	3865.0 [1400.0-19140.0]	**0.013**
Lymphocyte count (/mm3) Mean (min-max)	2895.0 [940.0-7940.0]	2715.0 [1230.0-6680.0]	0.779
Neutrophil/lymphocyte ratio (/mm3) Mean (min-max)	1.8 [0.1-12.7]	1.3 [0.2-6.8]	**0.039**
Platelet count (/µL) Mean (min-max)	352500.0 [184000.0-983000.0]	338500.0 [161000.0-515000.0]	0.266
CRP (mg/dL)	3.3 [0.1-85.8]	1.0 [0.1-40.1]	**<0.001**
ESR (mm/h) Mean (min-max)	12.0 [2.0-64.0]	8.0 [2.0-65.0]	**0.002**
Proteinuria	4 (6.7)	0 (0)	0.063
RUPCR	0.2 [0.0-11.8]	0.1 [0.0-0.9]	**<0.001**
ANA (+)	4 (6.7)	7 (10.9)	0.603
ENA panel	11 (18.3)	4 (6.2)	

Ku	3	1	
Scl-70	2	--	
Sjögren's syndrome type B	1	--	
Mitochondrial M2	3	--	
DFS70	2	1	0.074
Pm-Scl	3	0	
Centromere protein-B	1	--	
Ro-52	--	1	
Jo-1	--	1	
Complements			
Low C3	1 (1.7)	0 (0.0)	0.484
Low C4	1 (1.7)	0 (0.0)	0.484
Lupus anticoagulant activity (+)	1 (1.7)	0 (0.0)	0.484

CRP: C-reactive protein; ENA: extractable nuclear antigen; ESR: erythrocyte sedimentation rate; RUPCR: urine protein/creatinine ratio

p=0.002, and p<0.001, respectively). The low C3/C4 levels, ENA panel, and lupus anticoagulant activity were not differed between groups (p>0.05).

DISCUSSION

One of the most common forms of vasculitis in children, IgAV typically appears before the age of 10 years. IgA1-predominant immunological deposits and polymorphonuclear leukocyte inflammatory infiltration of small blood arteries are its defining characteristics (10). One of the five main immunoglobulins, IgA, is the predominant antibody of immunity and is essential for maintaining mucosal homeostasis in the gastrointestinal, respiratory, and genitourinary systems. It is made up of two heavy and two light chains, as well as an Fc-tail that can engage with Fc receptors and Fab regions that bind antigens (11). Immune-mediated vasculitis (IgAV), is linked to complement deposition, neutrophil recruitment, and IgA deposition. Approximately 90% of individuals with IgAV worldwide are young people. In contrast to other forms of systemic vasculitis, IgAV typically has a self-limiting course in children. Although some chemical and viral triggers exist, the fundamental aetiology of IgAV remains unknown. It has been that some cytokines, particularly IL-8, play a role in the pathophysiology of IgAV. The chemokine required to attract neutrophils, the main effector cells in IgAV, is IL-8 (12). IgA immune complexes, which are made up of galactose-deficient IgA1 and anti-IgA1 antibodies, have the ability to trigger IL-8 (13). Remarkably, these immune complexes increase the expression of IL-8 and other pro-inflammatory cytokines by activating the complement system, including C3a and C5a (14).

Complement is a crucial component of the innate immune system, which defends against all invasive pathogens (15). Moreover the complement system is known to play a number of other immunological and immunoregulatory functions, such as: (1) opsonising and solubilising native immune complexes (IC) made up of autoantigen and self-reactive antibodies; (2) generating and releasing the anaphylatoxin C3a and C5a, which draw inflammatory cells to the complement activation site; and (3) facilitating the binding of complement receptors CR1 on erythrocytes or CR3/CR4 on phagocytic myeloid cells to opsonised IC, which helps remove IC from the bloodstream (16). There is a clear mechanistic connection between complement activation and vascular damage because it causes neutrophil adherence and the development of neutrophil-platelet aggregates in vascular endothelial cells (17). It has been demonstrated that complement proteins can be activated by IgA. The blood contains complement in its dormant form, and there are three different ways that complement might be activated (18). Because IgA does not have a C1q binding site, it is unable to activate the complement's classical pathway. Nevertheless, it has been shown that IgA can activate the complement pathway that binds mannan and other pathways (11). Clinical practice frequently uses immunoassays to assess serum complement C3 and C4 levels to identify and track complement activation. Crucially, in individuals with ANCA-associated renal vasculitis, low serum C3 levels-without hypocomplementemia per se—are a reliable indicator of a poor renal prognosis (19). To improve the sensitivity of the systemic lupus erythematosus (SLE) categorisation criteria, hypocomplementemia involving C3 and C4 was proposed as an immunological criterion in 2009 (20). Although the complement system plays a role in the pathogenesis of IgAV, serum C3 and C4 levels have been reported to be within the normal range in most patients. However, decreases in C3/C4 levels may occur because of the depletion of complement components (21). Low C3 and C4 levels can be seen in active SLE (22). Low C3 levels can be seen in post-streptococcal glomerulonephritis and C3 nephritic factor-related disease (23). Low C4 levels can be associated with C1 inhibitor deficiency (24). Lower C3 and higher C4 levels were associated with a poorer prognosis in patients with IgA nephropathy (25). Lower C3 and C4, indicating complement activation, were associated with higher coronavirus disease 19 (COVID-19) severity (26).

In this study, we evaluated low C3/C4 levels and other laboratory findings in Turkish IgAV patients. As far as we know, this

study is the first study on this subject in our country. Calvo-Río et al. reported that the most common laboratory finding in their study was leukocytosis, with a rate of 36% in Spanish children (27). We found that the white blood cell count, neutrophil count, and neutrophil/lymphocyte ratio were higher in the patients than in the control group. In our patient group, there was leukocytosis compared with the control group. We also showed that CRP, ESR, and RUPCR increased in patients compared with the control group (Table 2). Simple, quick, and sensitive, RUPCR is closely linked to kidney injury and can determine the extent of it. Traponi et al. studied 150 Italian children with IgAV epidemiologically and clinically over 5-years (28). They found low C3/C4 levels in 10% of the patients. In the study by Luciana et al. in which they evaluated serum C3/C4 levels in children, a decrease was observed in only 2.15% of the cases (29). This was lower than the previous study. Calvo-Río et al. found low C3/C4 levels to be 12.8% in IgAV patients with nephritis (27). In our study, low C3/C4 levels did not show any difference between the patient and control groups (Table 2). A low C3/C4 level was detected in 1.7% of patients. This result was lower than the two studies conducted in Italy. This difference may be due to the fact that it was evaluated at different periods of the disease. Additionally, the ENA panel and lupus anticoagulant activity were not different between the groups.

Limitations

This study had some limitations. We had a comparatively modest patient population. The follow-up findings of the patients were not evaluated in the study. However, the advantage of the study is that it reflects data from a centre with high patient potential in Istanbul.

CONCLUSION

IgAV is an IgA-mediated systemic small vessel vasculitis affecting different systems. Although IgA vasculitis is typically a self-limiting disease, patients can develop life-threatening complications. Therefore, it is crucial to evaluate laboratory findings in the follow-up of complications that may develop in patients.

Ethics Committee Approval: This study was approved by Başakşehir Çam and Sakura City Hospital (Date: 26.06.2024, Approval Number: E-96317027-514.10-246491516).

Informed Consent: Prior to their participation in the study, all patients and their families provided written informed consent in accordance with the 2008 Declaration of Helsinki's ethical principles.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.D.; Data Acquisition- S.D., S.N.T.; Data Analysis/Interpretation- S.D.; Drafting Manuscript- S.D.; Critical Revision of Manuscript- S.D., S.N.T.; Final Approval and Accountability- S.D., S.N.T.; Material and Technical Support- S.D.; Supervision- S.D., S.N.T.

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INFUSION FROM *C. COGGYGRIA SCOP.* LEAVES ON THE HEPATIC OXIDATIVE STRESS IN MICE WITH DEXTRAN SODIUM SULPHATE (DSS)-INDUCED ULCERATIVE COLITIS

C. COGGYGRİA SCOP. YAPRAKLARINDAN ELDE EDİLEN SULU İNFÜZYONUN DEKSTRAN SODYUM SÜLFAT (DSS) İLE İNDÜKLENMİŞ ÜLSERATİF KOLİTLİ FARELERDE HEPATİK OKSİDATİF STRES ÜZERİNE ETKİSİ

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ABSTRACT

Objective: Since free radicals play a crucial role in ulcerative colitis (UC)-assotiated liver manifestations, antioxidants of plant origin have been proposed as potential therapeutic agents to counteract liver damage. This study aimed to elucidate the ameliorative effect of the aqueous infusion of the *C. coggygria* leaves (CCLAI) in comparison with a mesalamine in alleviating the oxidative stress in the liver of mice with DSS-induced UC.

Material and Methods: Two different doses of CCLAI (4% and 6%) or mesalamine (250 mg/kg body weight) were administered by oral gavage to C57BL/6 male mice once a day for 7 consecutive days. UC was induced with 3% DSS in drinking water for 5 days, except the normal and plant control groups that had access to water only.

Results: No statistical difference between all the groups was observed in the activities of AST and ALT, hepatic damage markers, suggesting that the oxidative alterations were not sufficient to cause liver damage, although oxidative stress occurred. The elevated activities of antioxidant markers (SOD, CAT, GR, GPx, GST) and increased GSH and NO levels in the colitis groups compared with the normal group may represent an initial defence mechanism against oxidative stress. These results indicate that CCLAI may attenuate oxidative stress, as demonstrated by decreased MDA levels and MPO activity and reversed levels of oxidative stress parameters towards the value of normal controls.

Conclusion: Our study provided evidence that CCLAI can reduce oxidative stress probably by scavenging ROS and modulating the oxidant/antioxidant balance in hepatic tissues.

Keywords: *Cotinus coggygria*, antioxidant activity, dextran sulphate sodium, liver, oxidative stress, ulcerative colitis

ÖZ

Amaç: Serbest radikaller ülseratif kolit (ÜK) ile ilişkili karaciğer bulgularında önemli bir rol oynadığından, bitki kökenli antioksidanlar karaciğer hasarına karşı potansiyel terapötik ajanlar olarak önerilmiştir. Bu çalışmanın amacı, C. coggygria yapraklarının sulu infüzyonunun (CCLAI), DSS ile indüklenmiş ÜK'li farelerin karaciğerindeki oksidatif stresi hafifletmede mesalamine kıyasla iyileştirici etkisini aydınlatmaktır.

Gereç ve Yöntemler: İki farklı dozda CCLAI (%4 ve %6) veya mesalamin (250 mg/kg vücut ağırlığı) C57BL/6 erkek farelere oral gavaj yoluyla ardışık 7 gün boyunca günde bir kez verildi. ÜK, sadece su verilen normal ve bitki kontrol grupları haricinde, 5 gün boyunca içme suyunda %3 DSS ile indüklenmiştir.

Bulgular: Karaciğer hasarı belirteçleri olan AST ve ALT aktivitelerinde tüm gruplar arasında istatistiksel bir fark gözlenmemiştir, bu da oksidatif stres oluşmasına rağmen oksidatif değişikliklerin karaciğer hasarına neden olmak için yeterli olmadığını düşündürmektedir. Normal gruba kıyasla kolit gruplarında antioksidan belirteçlerin (SOD, CAT, GR, GPx, GST) aktivitelerinin ve GSH ile NO seviyelerinin artması, oksidatif strese karşı bir ilk savunma mekanizmasını temsil ediyor olabilir. Bu sonuçlar, MDA seviyeleri ve MPO aktivitesinin azalması ile birlikte oksidatif stres parametrelerinin normal kontrol değerlerine doğru gerilemesinin de gösterdiği gibi, CCLAI'nin oksidatif stresi azaltabileceğini düşündürmektedir.

Sonuç: Çalışmamız, CCLAI'nin muhtemelen ROS'u temizleyerek ve hepatik dokulardaki oksidan/antioksidan dengeyi modüle ederek oksidatif stresi azaltabildiğine dair kanıtlar sağlamıştır.

Anahtar Kelimeler: *Cotinus coggygria*, antioksidan aktivite, dekstran sülfat sodyum, karaciğer, oksidatif stres, ülseratif kolit

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INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis, is an immune-mediated, "chronic and recurrent intestinal inflammatory disorder" that occurs as a result of the interaction of genetic predisposition, dysregulated immune responses, and environmental factors, leading to the impairment of mucosal barrier function (1). "UC is a chronic inflammatory disease characterised by relapsing and remitting mucosal inflammation of the intestinal lamina propria, extending from the rectum to proximal segments of the colon", and developing of widespread superficial mucosal ulceration. Clinical symptoms include recurrent bloody diarrhoea, weight loss, and abdominal pain (2). UC often occurs accompanied by the intestinal barrier dysfunction resulting in colonic leakage of harmful substances, such as gut microbiota-derived high lipopolysaccharide levels and bacteria, resulting in secondary liver injuries, which in turn aggravates UC. Long-term intestinal inflammation as well as excessive generation of ROS and imbalances in redox status can cause oxidative damage and colitis-induced liver injury. The association between the development of UC and liver complications was examined in several experimental studies and related to "liver gut cross talk" (3-8).

UC constitutes a global concern, but therapeutic treatments for this disease are often associated with limited efficacy in relieving the symptoms or side effects. For these reasons, alternative and/or complementary therapies such as herbal preparations may be used to develop an effective therapeutic approach for managing UC (2, 9).

Cotinus coggygria is a well-known Balkan traditional medicine used as an anti-inflammatory, wound healing, antimicrobial, and anti-haemorrhagic agent. Accordingly, the therapeutic potential of *C. coggygria* has been focused on by many researchers and outlined in the review by Matic et al. (10). Phytochemical screening showed the presence of polyphenolic compounds (quercetin, fustin, and taxifolin), anthocyanins, gallic acid methyl ester, galanin, myrcene, alphapinene, camphene, linalool, and alpha-terpineol in the leaf infusion (11). We have previously found that *C. coggygria* leaves showed *in vitro* antioxidative activity, scavenging oxidative radicals to terminate the radical chain reaction (12). Matic et al. (13, 14) reported that the pretreatment with the *C. coggygria* extract was effective in protecting against the pyrogallol-mediated hepatotoxicity by reducing oxidative stress. Taking into consideration the results of these previous studies, we hypothesise that CCLAI may exert an antioxidant effect by improving the antioxidant status in mice with DSS-induced UC.

MATERIALS AND METHODS

Plant material: Young shoots, which include leaves and branches of *C. coggygria*, were collected from the Bartın Province of Turkey in August 2017. After separation of the leaves from the other parts, they were dried at room temperature and powdered. The botanical authentication of voucher specimens was done by Prof. Dr. Şükran Kültür and deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE 93133).

Preparation of the infusion: Two concentrations (4/100 and 6/100) of CCLAI were prepared 1 h before each treatment by adding 4 g or 6 g of dried material to 100 mL of boiling distilled water and leaving for 30 min. The infusions were filtered through cotton lint.

Animals: The experimental protocol was approved by Istanbul University Aziz Sancar Experimental Medicine Research Institute Animal Experiments Local Ethics Committee on 26 October 2017. A total of 56 C57BL/6J male mice were housed at an adequate temperature of 22°C, under a 12-h day/night cycle, and fed a commercial diet with free access to drinking water. Animals underwent a one-week acclimatisation period.

Animal groups and UC induction: The animals were divided into seven groups with 8 animals each: Group I (C_{normal}), used as the normal control, was given sterile tap water. Group II, served as the UC control group (C_{DSS}). Groups III (C_{CCLAI4}) and IV (C_{CCLAI6}), V (DSS + CCLAI4) and VI (DSS + CCLAI6) or VII (DSS + M) were orally administered two different doses of CCLAI (4% and 6%) (2 ml/kg body weight) or mesalamine (250 mg/kg body weight) for 7 consecutive days. Groups V, VI, and VII were subjected to UC induced by drinking water containing 3% (w/v) DSS (MW 36.000-50.00; MP Biomedicals, USA) for 5 days (starting at day 3 until day 7). Blood samples were taken from each mouse into EDTA-containing tubes through direct intracardiac intervention 24 h following the last treatment. The livers were collected and processed for biochemical analysis.

Biochemical analyzes

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the blood samples were evaluated using Reflotron® analyser and commercially available strips (Roche Diagnostics, Switzerland). The protein concentrations were determined using the bicinchoninic acid (BCA) protein assay. The antioxidant status was evaluated by measuring the extent of lipid peroxidation (LPO) (15) and activities of antioxidant enzymes such as superoxide dismutase (SOD) (16), catalase (CAT) (17), glutathione reductase (GR) (18), and glutathione peroxidase (GPx) (19), glutathione-S-transferase (GST) detoxifying enzyme activity (20), myeloperoxidase (MPO) activity (21), as an indicator of inflammation, as well as reduced (GSH) glutathione (22) and nitric oxide (NO) (23) levels.

Statistical analysis

The results were analysed using Graphpad Prism 9.0 (GraphPad Software, San Diego, CA, USA). A one-way analysis of variance (ANOVA) followed by the Tukey–Kramer Test was performed for the evaluation of statistical differences. All data values were expressed as the mean±SD.

RESULTS

In this study, the antioxidative effect of CCLAI on hepatic oxidative stress was demonstrated in a mouse model of DSS-induced UC.

There was no change in either the AST or ALT levels of the DSS-treated animals (Table 1).

Table 1: AST and ALT levels in hepatic tissue samples of mice with DSS-induced UC.

Group	ALT (U/L)	AST (U/L)
C_{normal}	23.10±1.92	25.93±1.25
C_{DSS}	26.40±2.02	29.23±2.05
C_{CCLAI4}	24.88±6.17	27.19±1.90
C_{CCLAI6}	21.46±3.69	26.65±1.85
DSS+CCLAI4	24.07±4.19	28.89±2.36
DSS+CCLAI6	25.47±4.30	29.74±2.91
DSS+M	23.82±3.84	26.55±1.86

Values are means±SD, from 8 animals. ALT: Alanine Aminotransferase; AST: Aspartate transferase; C_{normal}: Normal control; C_{DSS}: Ulcerative Colitis Control Group; C_{CCLAI4}: 4% aqueous infusion of *C. coggygria* leaves; C_{CCLAI6}: 6% aqueous infusion of C. coggygria leaves; DSS: Dextran sodium sulphate induced ulcerative colitis group; M: Mesalamine-treated ulcerative colitis group

Table 2: Effects of CCLAI and mesalamine on oxidative stress markers in hepatic tissue samples of mice with DSS-induced UC

Group	C_{normal}	C_{DSS}	C_{CCLAI4}	C_{CCLAI6}	DSS+CCLAI4	DSS+CCLAI6	DSS+M
NO (nmol/g tissue)	97.13± 10.72	167.82± 37.10*	99.18± 15.87^{f}	115.47± 21.26^{f}	129.54± 24.83^{f}	139.03± 35.00	125.18± 26.56^{f}
GSH (nmol/g tissue)	484.05± 22.34	726.86± 46.27*	509.61± 20.21^{f}	508.67± 38.93^{f}	582.52± 65.68^{f}	625.71± 64.91$^{*;f}$	575.6± 62.71^{f}
MPO (nmol/g tissue)	0.94±0.14	2.54± 0.65*	1.18± 0.21^{f}	1.24± 0.25^{f}	1.79± 0.53$^{*;f}$	1.99± 0.53*	1.56± 0.28$^{*;f}$
GST (U/mg protein)	257.82± 14.19	402.14± 49.17*	250.42± 25.14^{f}	264.17± 15.34^{f}	291.36± 39.75^{f}	309.01± 33.79^{f}	272.70± 26.81^{f}
GPx (U/mg protein)	128.31± 9.11	290.32± 33.48*	138.16± 9.76^{f}	130.99± 13.41^{f}	169.13± 19.45$^{*;f}$	176.50± 26.20$^{*;f}$	157.51± 16.07^{f}
GR (U/mg protein)	100.12± 12.79	212.75± 22.23*	110.57± 11.03^{f}	113.44± 7.30^{f}	158.42± 14.83$^{*;f}$	161.15± 17.85$^{*;f}$	145.19± 14.18$^{*;f}$
CAT (U/mg protein)	139.37± 14.85	244.57± 9.15*	130.7± 19.81^{f}	139.76± 7.13^{f}	179.84± 13.06$^{*;f}$	184.05± 26.12$^{*;f}$	162.22± 21.48^{f}
SOD (U/mg protein)	2.52±0.28	4.66± 0.24*	2.71± 0.27^{f}	2.86± 0.14^{f}	3.16± 0.38$^{*;f}$	3.29± 0.21$^{*;f}$	3.53± 0.37$^{*;f}$
MDA (nmol/g tissue)	16.2±2.3	31.73± 6.21*	15.6± 2.5^{f}	18.0±3.4^{f}	23.6± 3.6$^{*;f}$	26.7± 2.8$^{*;f}$	22.0± 3.8$^{*;f}$
Protein (mg/ml)	6.26±0.62	5.05± 0.67*	5.13± 0.34*	5.09± 0.64*	5.03 ± 0.47*	4.91± 0.67*	5.38± 0.69

Values are means of 8 animals±SD, from. Activities were expressed as units SOD (one unit of SOD inhibits the rate of increase in absorbance at 560 nm by 50 %), mmol of H_2O_2 /min per mg of protein (for CAT), nmol of NADPH oxidised/min per mg of protein (for both GPx and GR), nmol conjugate formed/min per mg of protein (for GST), units of MPO per g wet tissue (1 U of activity was defined as the amount that consumes 1 mmol H_2O_2/min).
* Values significantly different from C_{normal}; fValues significantly different from C_{DSS} (one-way ANOVA followed by Tukey–Kramer Test), p<0.05; NO: Nitric oxide; GSH: Reduced glutathione; MPO: Myeloperoxidase; GST: Glutathione-S-transferase; GPx: Glutathione peroxidase; GR: Glutathione reductase; CAT: Catalase; SOD: Superoxide dismutase; MDA: Malondialdehit; C_{normal}: Normal control; C_{DSS}: Ulcerative Colitis Control Group; C_{CCLAI4}: 4% aqueous infusion of *C. coggygria* leaves; C_{CCLAI6}: 6% aqueous infusion of C. coggygria leaves; DSS: Dextran sodium sulphate induced ulcerative colitis group; M: Mesalamine-treated ulcerative colitis group

Regarding the oxidative stress markers, we observed a significant ($p<0.05$) increase in the activities of CAT, SOD, GR, GPx, and GST as well as hepatic non-enzymatic GSH and NO levels in the DSS mice group when compared with the untreated mice. As expected, DSS caused an increase in MDA levels, as a marker of lipid peroxidation, and MPO activity, as a marker of neutrophil infiltration in the hepatic tissue of mice with UC compared to normal controls ($p<0.05$). However, these levels were reversed in CCLAI- and mesalamine-treated animals towards the value of normal controls, although the difference was significant (Table 2).

DISCUSSION

Many drugs are converted to metabolites in the extramitochondrial, microsomal system in the hepatocytes that uses some oxidative enzymes and regulates redox homeostasis. Oxidative stress may impair redox homeostasis and cause intestinal barrier dysfunction, decreased immunity, and dysbiosis, which in turn can trigger intestinal dysfunction and secondary liver injury. Liver injury is a common complication or manifestation of UC and should not be neglected in the management of UC (24).

Liver complications after the induction of UC by the administration of DSS, an animal model that mimics UC symptoms, have been demonstrated in several studies (4-8, 25). In our earlier study (unpublished data), it was shown that a 7-day administration of CCLAI was able to significantly reduce the oxidative stress markers in the colon tissues of mice with DSS-induced UC. Considering these results, in this study, we evaluated the possible oxidative stress attenuating effect of CCLAI in the liver tissue caused by DSS-induced UC.

Two doses of CCLAI (4% and 6%) were evaluated in colitis mice, in comparison with a mesalamine, a metabolite of sulfasalazine used as a drug control, which was administered at a commonly used dose in animal studies (250 mg/kg body weight). Mesalamine is used for the treatment of IBD and was reported to be an efficient ROS scavenger (26). Dose selection for preparing the infusion from leaves was based on a preliminary experiment reported by Eftimov et al. (11), who showed that the administration of aqueous infusion from *C. coggygria* leaves at a concentration of 1; 2 and 4% for 30 days did not cause liver toxicity. The AST and ALT levels, used as a marker of liver damage, were in the normal range, suggesting that the oxidative changes found in our acute colitis model (3% DSS, v/v, for 5 days) may not be sufficient to cause liver injury, although oxidative stress may be induced by this exposure.

Cellular membranes, due to their high content of oxidisable polyunsaturated fatty acids (PUFAs), are extremely susceptible to free radical assaults. High levels of oxidants like lipid hydroperoxides (LOOH), the major primary products produced during the propagation stage of lipid peroxidation and their secondary products such as malonaldehyde and 4-hydroxynonenal, which may serve as "oxidative stress second messengers" can induce tissue oxidative stress and redox imbalance leading to impaired mucosal integrity (26). As lipid peroxidation can initiate the development and progression of degenerative processes in the digestive system, such as inflammation and cancer, scientists tried to find strategies to prevent and treat it. *In vivo* studies in animal models showed increased levels of MDA (lipid peroxidation products) and oxidative reactive sensitivity of the intestinal epithelium and liver (6-8).

ROS are produced during normal physiological cellular metabolism; however, an increased ROS production or decreased ROS-scavenging capacity disrupts redox homeostasis, causing oxidative stress damage. In fact, the activity of ROS-scavenging enzymes such as CAT, SOD, GR, and GPx has been shown to be significantly altered in the liver of UC (6-8).

Superoxide is the first generated ROS that is produced by transferring an electron to O_2 through the mitochondrial electron transport chain (mETC), NADPH oxidases (NOXs), and xanthine oxidase (XO) in biological systems. SOD, which converts superoxide into hydrogen peroxide and oxygen, plays a protective role against oxidative damage, because it neutralised radical electrons at an initial stage and terminates chain reactions (27). Superoxide is not harmful to cell function directly because its oxidising power is mild compared to the hydroxyl radical, but if not properly removed, it potentially may lead to cell death. Previous studies have demonstrated that under the condition of inflammation and oxidative stress in UC pathogenesis, SOD activity increases as a defence reaction against oxidative damage (28). Contrary to the previous studies that reported the decreased SOD activity in only the DSS-treated group (6, 8, 25), our study indicated that the liver tissue SOD activity was elevated in the colitis group, compared to the normal group. These results were found to be consistent with those reported by Rtibi et al. (7).

CAT and GPx catalyse the reduction of hydrogen peroxide (H_2O_2) or organic hydroperoxides to water and an oxygen molecule or the corresponding stable alcohols, respectively, using GSH as the reductant, which in turn is oxidised to form glutathione disulphide (GSSG). The reduced form of cellular GSH is a more prevalent antioxidant, functioning in the detoxification of reactive oxygen metabolites. GPx, together with GR, acts as an enzyme couple in the reduction of peroxides with concomitant oxidation of GSH to GSSG, which is converted back to GSH by GR in a NADPH-dependent manner, forming a GSH redox cycle, which is an important intracellular detoxication mechanism for peroxide elimination and maintenance of GSH levels. It was reported that these two enzymes together with GSH form an antioxidant barrier and protect the cells against peroxide damage and mucosal inflammation in mouse models of UC (28, 29).

In this study, the observed increase in GR and GPx activities in the DSS-induced colitis group may be due to high GSH levels. Considering that GR and GPx cooperate with GSH in the breakdown of organic hydroperoxides, the simultaneous induction of GPx activity and elevation of GSH levels may be an indicator of the antioxidant response to hepatic oxidative stress.

Increased generation of superoxide radicals induces SOD activity in the hepatic tissue of DSS-treated mice. However, an increase in SOD activity without a concomitant increase in CAT

and/or GPx activities may be damaging because increased SOD activity leads to an elevation of the levels of H_2O_2, which is a relatively stable ROS that must be eliminated by CAT or GPx. Therefore, a concomitant increase in CAT and/or GPx activity, as observed in the CCLAI- and mesalamine-treated UC groups, may have beneficial effects against oxidative stress

NO produced by iNOS contributes to tissue injury and inflammatory processes as a mediator of macrophage and neutrophil function. The production of high NO by activated macrophages in inflamed tissues can be toxic and lead to tissue damage. The generation of increased NO and O_2^- by activated macrophages can lead to the formation of highly cytotoxic peroxynitrite (-$ONOO^-$), which induces tissue injury through mechanisms leading to lipid peroxidation. It has been elucidated in both experimental animals and humans that an enhanced production of NO is associated with inflamed mucosa, which is characterised by macrophage infiltration and release of inflammatory mediators, the potent inducers of iNOS. Therefore, NO may participate in the pathogenesis of UC, augmenting the extent of tissue damage. It was reported that the NO levels and expression of the iNOS increased in experimental animal models of UC (30).

Myeloperoxidase is a membrane-bound, haem-containing peroxidase released exclusively by neutrophils, tissue macrophages and to a lesser extent by monocytes. MPO provides an estimate for activated neutrophil infiltration in tissues (31). H_2O_2, formed as a result of the dismutation of the superoxide radical, may react with the chloride ion via the myeloperoxidase enzyme which catalyses the formation of hypochlorous acid, which is a potent cytotoxic oxidant. Increased MPO activity has been observed in the inflamed colonic and hepatic mucosa in animal models with UC (6-8, 25). As the reduction of ROS production may be due to the downregulation of hepatic antioxidant defence activities due to the decrease of their substrates, our findings of a reversed levels of oxidative stress parameters in mice treated with CCLAI towards the value of normal controls were consistent with these observations.

We suggested that oral administration of CCLAI may counteract the hepatic oxidative stress through reduced production of ROS, as assessed by decreased MDA levels and MPO activity, reversed GST and antioxidant enzymes (SOD, CAT, GPx, and GR) activities as well as GSH and NO levels towards the normal values. As in previous reports (13, 14), *C. coggygria* has been proven to be a hepatoprotective agent under *in vivo* conditions, mostly through its antioxidant action in reducing oxidative damage.

Our results corroborate with previous studies indicating that antioxidants can comprehensively reduce oxidative stress derived from DSS-induced UC in the liver tissue (4-8).

CONCLUSION

The present study results revealed that CCLAI has a comparable therapeutic potential with mesalamine against DSS-induced oxidative stress, which indicates that CCLAI may be a therapeutic option to relieve UC.

Ethics Committee Approval: This study was approved by Istanbul University Animal Experiments Local Ethics Committee Presidency (Date: 26.10.2017, No: 35980450-050.01.04).

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PHYSICAL THERAPY INTERVENTIONS IN CHILDREN WITH CANCER

KANSERLİ ÇOCUK HASTALARDA FİZİKSEL AKTİVİTE GİRİŞİMLERİ

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ABSTRACT
Children with cancer experience severe adverse effects from cancer or as a sequela of cancer treatment. These side effects can affect major body systems such as musculoskeletal, cardiovascular and neurological systems and impact participation in daily living activities and thus quality of life. These sequelae are to be lessened with physical therapy, rehabilitation, or neuropsychological treatment according to the requirements of the patients. Thus, awareness of the rehabilitation needs of children with cancer is crucial to enhance a healthy life with quality. Recommendations concerning the quality of life among adults are included in the guidelines, but data including recommendations for children are limited.
Keywords: Paediatric, cancer, physical therapy, rehabilitation

ÖZ
Kanserli çocuklar, kanserin veya hastalığın tedavisinden kaynaklanan ciddi yan etkiler ve sekeller yaşamaktadır. Bu yan etkiler, en majör vücut sistemlerinden olan iskelet-kas, kalp ve solunum ile nörolojik sistemleri etkileyerek hastanın günlük yaşam aktivitelerini engellemekte ve hayat kalitesini bozmaktadır. Bu engellenmeler hastanın gerektirdiği her türlü fiziksel aktivite, rehabilitasyon ve nöropsikolojik tedaviler ile en aza indirilmeye çalışılmaktadır. Bu nedenle, kanserli çocuklarda rehabilitasyon ihtiyacının farkedilmesi kaliteli bir hayat için şarttır. Erişkinlerin hayat kalitesi için yapılan öneriler kılavuzlara dönüştürülmüş olmasına rağmen çocuk hastalar için öneriler halen kısıtlıdır.
Anahtar Kelimeler: Çocuk, kanser, fiziksel aktivite, rehabilitasyon

INTRODUCTION

Although progress in cancer treatments has resulted in increased survival rates in children, they have acute or chronic side effects. Two-thirds of children with cancer develop at least one chronic or long-term side effect after cancer treatment (1-3). The risk of long-term side effects is dependent on tumour-related factors (e.g.type of tumour, location within the body and extent of the cancer); treatment modality factors (e.g. extent and location of surgery, chemotherapy type and dosage, radiation therapy type, location and dosage); as well as patient-related factors (e.g. the child's age, gender, overall health at initial diagnosis of cancer; e.g any underlying chronic disease other than cancer, and neurological developmental age at the time of diagnosis) (3, 4).

Specific long-term adverse effects of cancer treatment on the musculoskeletal system include effects on muscle and soft tissues as well as on bone (1-5). The impact of surgery such as amputation or limb-salvage intervention may result in chronic pain, gait and balance dysfunction, and impact overall activity (1, 5). Mostly, children diagnosed with acute lymphoblastic leukaemia, bone tumours like osteosarcoma or Ewing sarcoma, central nervous system tumours and who have undergone stem cell transplantation have to cope with side effects involving the musculoskeletal system (1,3-5). Other than the musculoskeletal system, such cancer therapies, including chemotherapy and radiotherapy, lead to neurological system deficits, such as motor and sensorial nerve deficits, affecting the central and peripheral nervous systems (1, 3-5).

In patients with acute lymphoblastic leukaemia, (5-8), osteoporosis and avascular necrosis (5, 9-11), cardiotoxicity (12), and neurocognitive side effects such as peripheral neuropathy are the most common sequela (1, 3-6).

In patients with CNS tumours, complications depend on both the grade, size, and location of the tumour and also the type

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and extent of treatment modality (e.g. extent and location of surgery, chemotherapy type and dosage, radiation therapy type, location and dosage) and patient-related factors (5, 6, 13-15). Patients having craniospinal irradiation may experience neurocognitive deficits as well as spinal bony deformities. (5, 6, 16).

Children with bone tumours experience physical complications concerning muscles and bones according to chemotherapy or surgery; weakness, neuropathy, impaired balance, contractures among joints, and activity limitations (1, 3-6).

Physical therapy interventions may help affected children to repair and restore function and improve the quality of life not only in healthy children but also in children with cancer (1, 4-6).

CLINICAL AND RESEARCH CONSEQUENCES

Physical activity and rehabilitation programmes for children with cancer have shown benefits on both musculoskeletal and neurological and as well as cardiorespiratory systems and thus for improving quality of life (1, 3-6). Thus, early rehabilitation programmes that restore function are critical. Therefore, prevention and rehabilitation should be involved during the treatment plan for these patients even in the initial diagnostic phase (6). Rehabilitation should be integrated as a part of the palliative treatment of children with cancer since diagnosis, during chemotherapy, and after surgery with or without radiotherapy and should be maintained after completion of the therapies to lessen chronic side effects and improve the quality of life (1, 5, 6, 17).

In children with acute leukaemia, rehabilitation is feasible in all stages of the cancer even in the induction phase, and possibly the intensity of the physical activity should be modified according to the clinical condition of the patient both in the hospital and at home (6, 18). During the initial phases of treatment, patients may perform low- to moderate-intensity physical activity, including aerobic training and stretching and strengthening exercises (6, 19-22). During the maintenance phase of leukaemia treatment, more intensive physical activity and rehabilitation programme may be integrated (6, 19-22). However, no guideline so far has been established. In children with avascular necrosis, rehabilitation should focus on pain reduction and protection and improvement of functional impairments of the bones and joints (6, 23).

In children with CNS tumours, a careful evaluation of the patient is crucial to establish the appropriate time, the intensity and type of physical activity programme in the acute postoperative period. In the maintenance follow-up of these children, according to the clinical situation and improvement in the neurological deficits, the intensity and the modality of the rehabilitation programme can be modified (6, 14, 15, 24). The intensity and frequency parameters are the most challenging to define, especially for infants and those with neurological impairments.

In children with bone tumours who are candidates for surgery, low-to-moderate physical activity rehabilitation should begin before surgery to improve physical functioning and to reduce postsurgical complications. Post-surgical rehabilitation should be maintained until the individual has achieved maximum functioning (6, 25-27). Even after years, rehabilitation may restore minimal deficits in some patients. No guidelines but recommendations have been established (1, 3-6).

The physical activity and rehabilitation programme of the children with cancer should be managed by a multidisciplinary experienced team and modified according to the requirements of each individual patient. It is useful to develop a modified version of the FITT (Frequency, Intensity, Time, and Type) principles in children with cancer (6, 19, 22, 28-30). Patients and their caregivers should be integrated into the rehabilitation programme in every step and evaluation. The physical activities should be enjoyable and involve parents and siblings or even peers to improve compliance (6, 31). In recent years, numerous modalities, including mobile/tablet apps, video games, virtual reality, social media, and other web-based interventions, have been found to exist for the purpose of promoting physical activity and improving adherence among children, particularly in adolescents, in comparison to conventional exercises (1, 14, 15, 32, 33).

Healthy lifestyle behaviours and the integration of physical activity programmes into medical treatment regimens and posttreatment follow-up may also produce numerous health benefits such as improvement in cancer-related fatigue and sleep (33-36).

CONCLUSION

Evidence-based rehabilitation guidance for children with cancer is limited according to the current available literature. However, crucial points such as the awareness of early integration of rehabilitation programme even in the initial diagnosis of the cancer have already been established. This activity programme should not only be integrated to promote overall physical well-being but also to achieve psychosocial health in this population. Intensity, duration, and type of rehabilitation guidelines are still lacking and optimal activity programme according to each specific cancer type is not known; therefore, studies with large sample sizes should be planned for the establishment of guidelines for children with cancer.

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CASE REPORT / OLGU SUNUMU
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MANDIBULAR THIRD MOLARS IN THE SIGMOID NOTCH: A RARE STUDY AND CLINICAL MANAGEMENT

SİGMOİD ÇENTİKTEKİ MANDİBULAR ÜÇÜNCÜ MOLARLAR: NADİR BİR OLGU SUNUMU VE KLİNİK YÖNETİMİ

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ABSTRACT

Ectopic mandibular third molars (EMTMs) are uncommon, especially in atypical locations like the sigmoid notch. Their aetiology includes developmental anomalies, pathological conditions,trauma, and iatrogenic factors. EMTMs are uncommon and frequently asymptomatic, typically identified during routine radiographic examinations. Often associated with pathologies such as cysts and inflammation, EMTMs predominantly affect females after the fourth decade of life. This case report presents a 49-year-old male patient who presented with symptoms of swelling, limited mouth opening, and infection, attributed to an EMTM in the sigmoid notch associated with a dentigerous cyst. Radiological imaging revealed the precise location and extent of the EMTM and cyst. The choice of surgical approach, based on 3D imaging, should minimise risks and optimise outcomes. Surgical management involved an intraoral approach for extraction of the impacted tooth and enucleation of the cyst, leading to the resolution of symptoms and restoration of normal mouth opening. Literature review emphasises the significance of accurate diagnosis through advanced imaging techniques and tailored surgical strategies for the effective management of EMTMs.

Keywords: Ectopic third molar, sigmoid notch, dentigerous cyst

ÖZ

Ektopik mandibular üçüncü molar dişler (EMTM'ler), özellikle sigmoid çentik gibi olağan konumundan oldukça uzakta nadiren görülmektedir. EMTM etiyolojisinde gelişimsel anomaliler, patolojik oluşumlar, travma ve iatrojenik faktörler rol oynamaktadır. EMTM'ler genellikle asemptomatiktir ve rutin radyografik incelemeler sırasında tespit edilirler. EMTM'ler sıklıkla kist ve apse gibi patolojilerle ilişkili olarak yaşamın dördüncü dekadından sonra daha sıklıkla kadınları etkilemektedir. Bu olgu sunumu, sigmoid çentikte yerleşen bir EMTM ile ilişkili dentigeröz kist nedeniyle şişlik, ağız açıklığında kısıtlılık ve enfeksiyon şikayetleriyle başvuran 49 yaşında bir erkek hastayı rapor etmektedir. Klinik ve radyolojik muayene sonucunda EMTM'nin ve kistin lokalizasyonu ve etkilediği anatomik yapılar belirlenmiştir. Cerrahi yaklaşıma karar verilirken, üç boyutlu(3B) görüntüleme sonucunda elde edilen veriler göz önünde bulundurularak cerrahiden kaynaklanabilecek riskler en aza indirilmeye çalışılmıştır. İntraoral cerrahi yaklaşımla kist enükleasyonu ve gömülü diş çekimi ardından semptomlarda gerileme ve ağız açıklığında artış görülmüştür. Yapılan literatür taraması sonucunda EMTM'lerin etkin klinik yönetimi için doğru teşhis ve vakaya özgü cerrahi yaklaşımların önemi vurgulanmaktadır.

Anahtar Kelimeler: Ektopik üçüncü molar, sigmoid çentik, dentigeröz kist

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INTRODUCTION

Ectopic mandibular third molars (EMTMs) are relatively uncommon. They can occur in various regions of the mandible, such as the condyle, subcondylar, ascending ramus, sigmoid notch, and coronoid (1, 2). Some studies have indicated that ectopic mandibular third molars are more frequently observed in females and typically appear after the fourth decade of life. When considering their distribution, the condylar region is the most common site, followed by the coronoid process (3). Factors that may contribute to EMTMs include tooth development anomalies, pathological conditions, trauma, and iatrogenic causes (2). When a mandibular third molar is in an atypical position, it is commonly observed with pathologies such as cysts, hyperplastic follicles, and chronic inflammation (4). The diagnosis is often based on clinical signs, predominantly infection, pain, limited mouth opening, swelling, fistula, facial asymmetry, and temporomandibular joint (TMJ) dysfunction (3, 5, 6). Patients presenting with clinically limited mouth opening should be thoroughly evaluated because this condition may be associated with a history of trauma, infection, temporomandibular joint disorders(ankylosis, condylar hyperplasia, disc displacement without reduction), myositis ossificans, dental interventions, tumours such as osteochondroma, or radiotherapy (7). A comprehensive clinical assessment, detailed medical history, and appropriate radiological investigations are crucial for accurate diagnosis and management. Three-dimensional (3D) imaging is helpful in determining the most appropriate surgical approach, which may include intraoral, extraoral, or endoscopic procedures (8). This case report presents an EMTM in the sigmoid notch associated with a dentigerous cyst.

CASE PRESENTATION

A 49-year-old male presented to the Istanbul University Faculty of Dentistry complaining of swelling and limited mouth opening. During the examination, the masseter, medial pterygoid, and sternocleidomastoid muscles were affected. Panoramic imaging revealed an ectopic mandibular third molar (EMTM) in an inverted position within the sigmoid notch, leading to trismus and infection (Figure 1). In cone-beam computed tomography (CBCT), the affected tooth was located medially in the right sigmoid notch, with the crown positioned towards the outer surface of the mandible (Figure 2). CBCT revealed a tunnel-like lesion, which was a pathological cystic structure surrounding the tooth, extending from the surrounding tissue, causing destruction in the cortical layer. A radiolucent migration pathway extending to the anterior ramus was observed (Figure 3). An informed consent form and permission letter were obtained from the patient. After placing an extraoral drain, the patient was prescribed 600 mg clindamycin intramuscularly twice a day for 7 days and mouth-opening exercises (Figure 4). The tooth was extracted on day 7 when the mouth opening was 27 mm using an intraoral approach under local anaesthesia.

An incision was made along the anterior border of the mandibular ramus. A mucoperiosteal flap was elevated to reveal the retromolar trigone and external oblique ridges, reaching up to the coronoid process. A round steel bur was used to create a window in the anterior aspect of the ramus in the region identified in the preoperative radiographs (Figure 5). The tunnel-like bone destruction in the surgical field helped to determine the tooth migration pathway. The tooth was extracted by separating the crown and root into two pieces, and the cyst was removed (Figure 6).

After the extraction, the patient was prescribed 1 g amoxicillin orally for 1 week. Subsequently, the area was closed primarily, and no complications were observed. The pathological examination confirmed the preliminary diagnosis, revealing a dentigerous cyst and inflammation (Figure 7). The patient's mouth opening was 35 mm on the seventh postoperative day

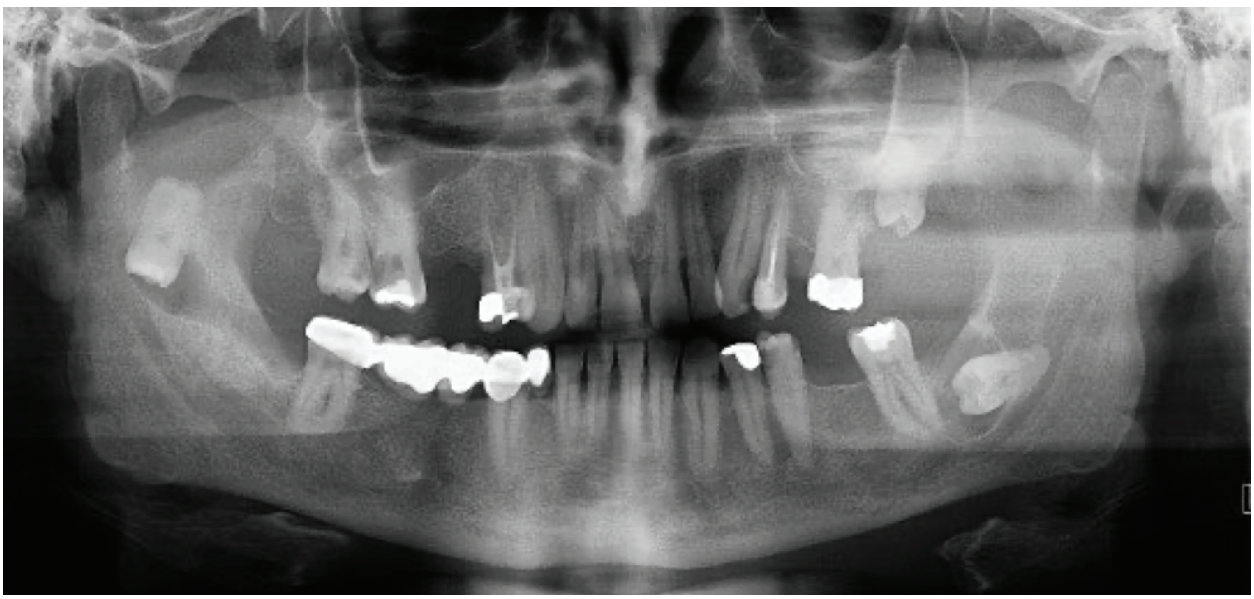

Figure 1: Preoperative panoramic radiograph showing that the third molar is located in the Sigmoid notch

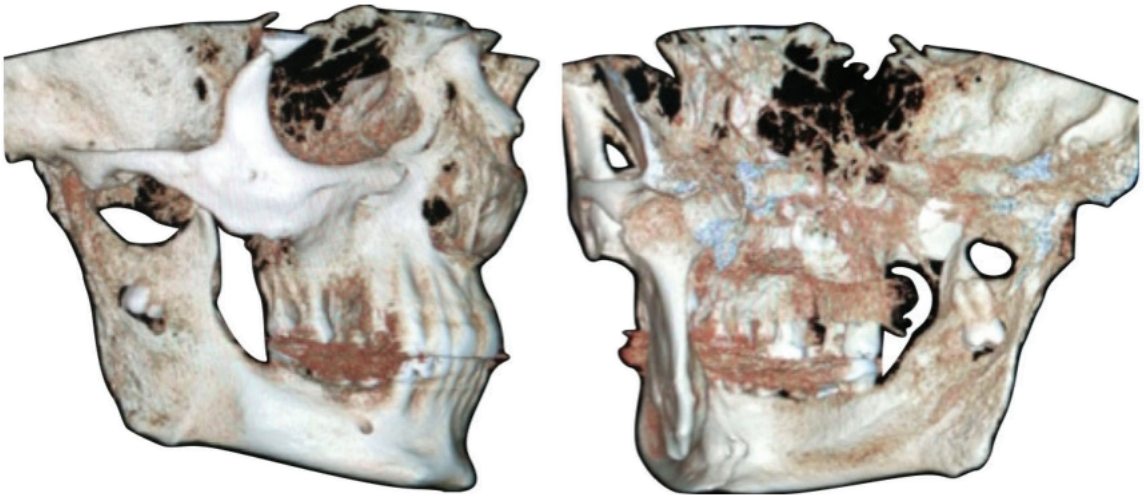

Figure 2: In the three-dimensional reconstruction of CBCT, the impacted tooth was found medially within the right sigmoid notch, with its crown oriented towards the external surface of the mandible

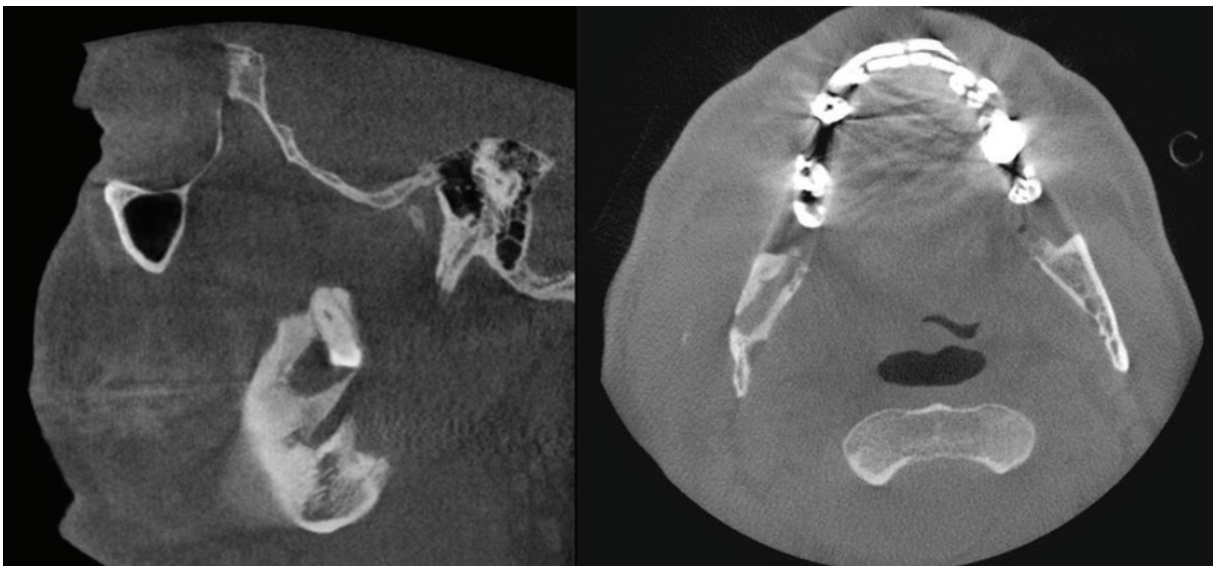

Figure 3: CBCT revealed a tunnel-like lesion, which is a radiolucent migration pathway extending to the right anterior ramus

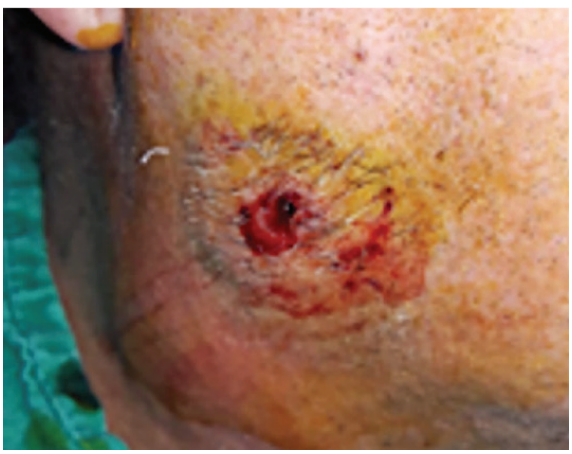

Figure 4: The site where extraoral drainage is performed

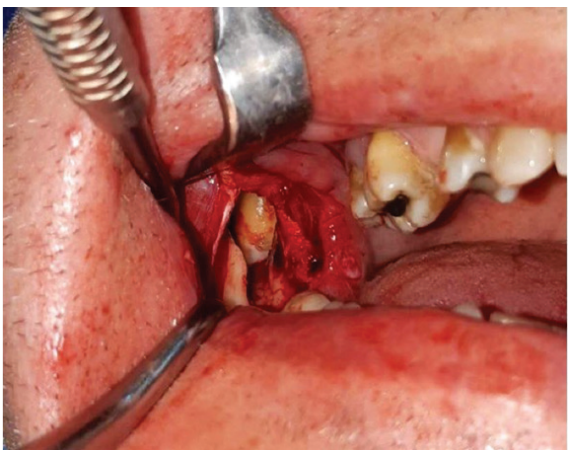

Figure 5: Exposure of the tooth in the sigmoid notch

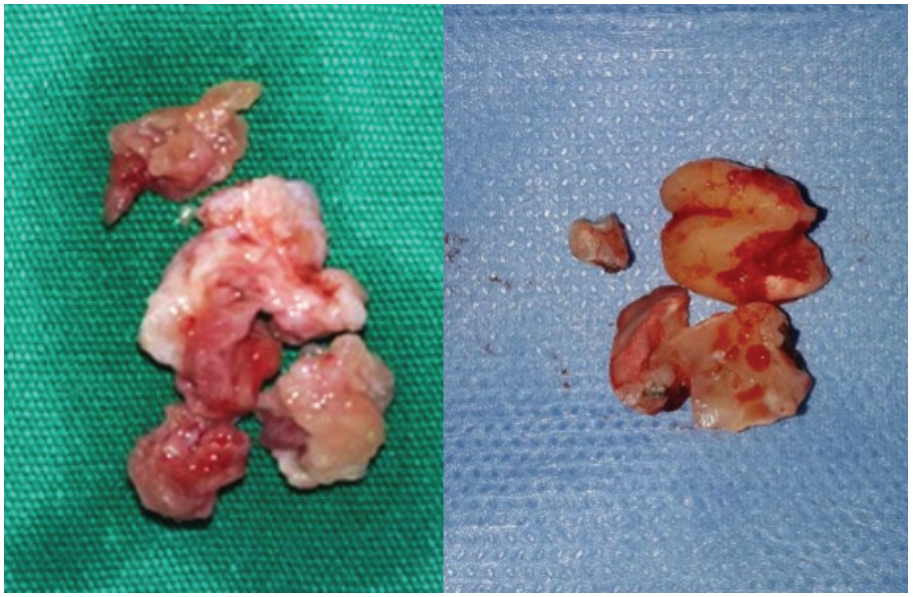

Figure 6: The image of the tooth and the cystic lesion

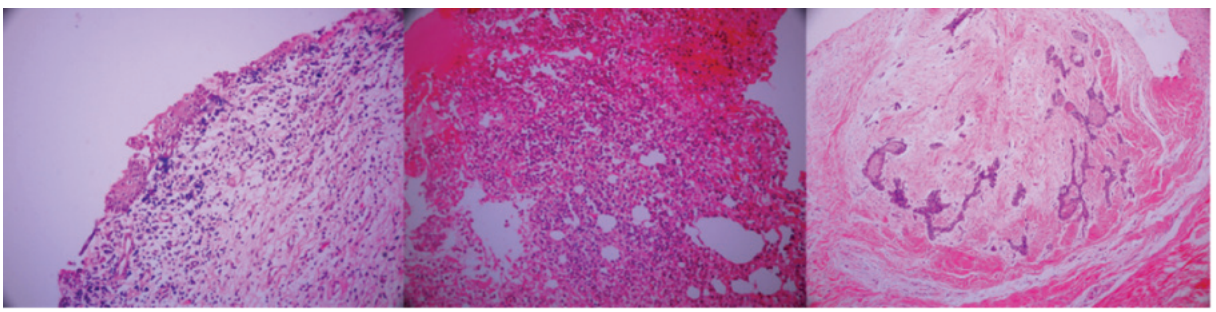

Figure 7: Histological image of the dentigerous cyst:the stratified epithelium demonstrating active inflammation (H&E 100x)

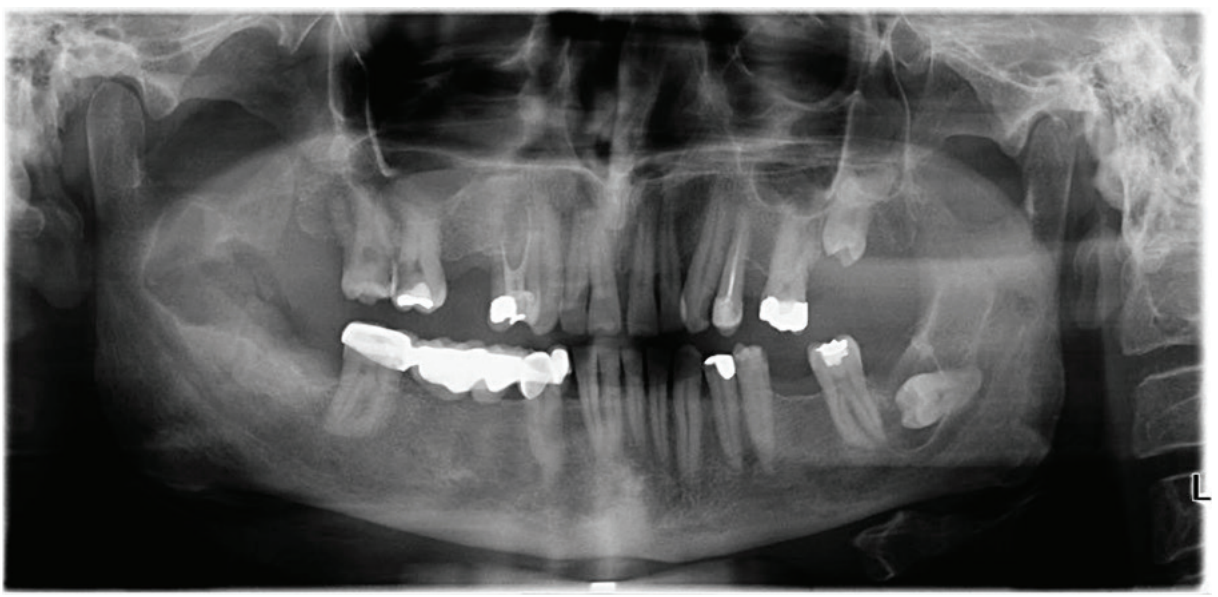

Figure 8: The panoramic radiograph on the seventh day of follow-up

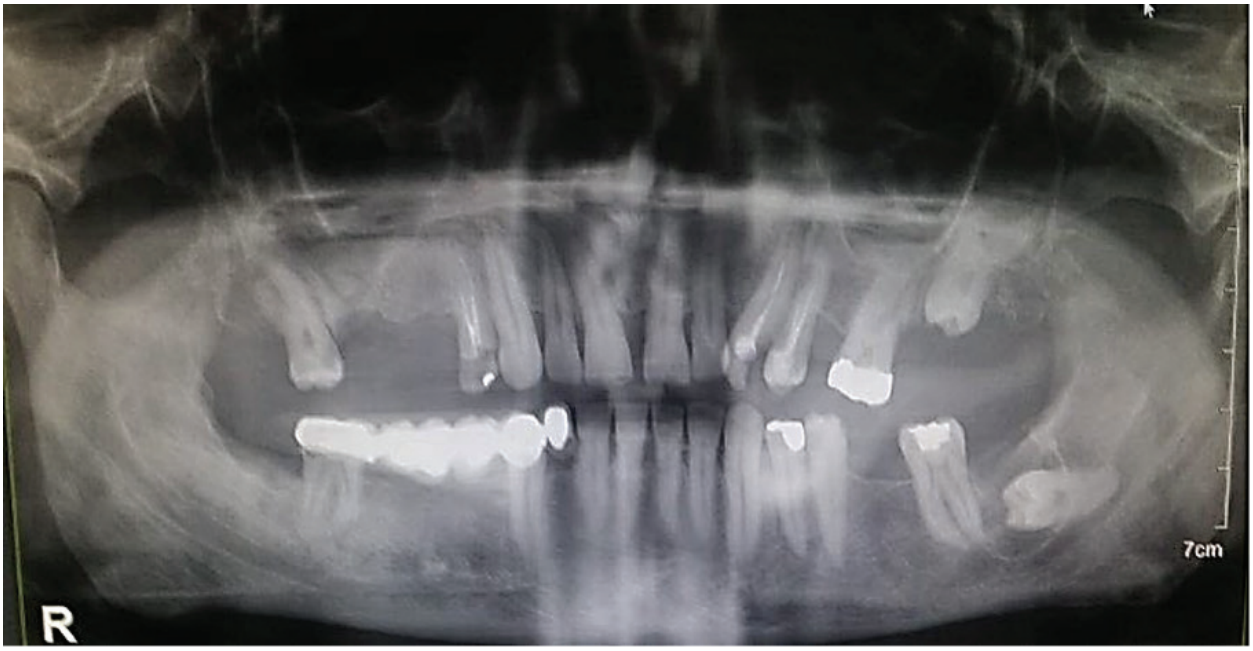

Figure 9: After 1 year of follow-up, the panoramic radiograph showed no signs of pathology and indicated bone healing

(Figure 8). Throughout the 1-year follow-up, the patient's mouth opening remained at 34 mm, with no reported symptoms, and panoramic X-rays did not reveal any pathology (Figure 9).

DISCUSSION

Ectopic teeth can occur in both the maxilla and mandible. In the mandible, they are located most commonly in the condyle and coronoid process regions, but they may also occur in the ascending ramus, lower border of the mandible, and sigmoid notch (6, 8, 9). Several theories have attempted to explain EMTMs, including trauma, abnormal eruption pathways, disruptions in the tooth root form, idiopathic causes, infection, craniofacial syndromes, cysts, and tumour pathologies. Capelli et al. reported a correlation between a decreased distance between the second molar and ramus and the likelihood of an EMTM (10). Adachi et al. reported that chronic inflammation in the coronal region could lead to the abnormal positioning of a tooth (11). Moreover, cystic fluids, particularly those associated with dentigerous cysts, have been suggested to be a potential cause of tooth displacement (12). The presence of a dentigerous cyst in our case, along with the displacement of the tooth, supports this theory. Another theory suggests that during the development of the lower third molar, it can be displaced due to the bone growth of the coronoid process (2). Following the classification of Wu et al., the EMTM in our case was positioned at level I in an inverted position and associated with a dentigerous cyst (13).

EMTMs are more common in women around the age of 40, although our case involved a male in his late 40s. Asymptomatic cases may be monitored annually based on the patient's health and tooth position. However, when an ectopic tooth coexists with the pathology, as in our case, surgery is inevitable. Without extraction and cyst enucleation, the risks include bone destruction, pathological alteration, bone weakening, and potential fractures.

While panoramic radiographs may be sufficient for diagnosing ectopic teeth, as in our case, 3D imaging is crucial to accurately determine the true position of the tooth and to assess the pathology. It also plays a significant role in selecting the surgical technique. In our case, a well-defined, radiolucent tunnel was observed, starting from the mandibular third molar and extending to the anterior edge of the ramus. In similar reported cases, the most common symptoms were pain, swelling, and trismus (13, 14). In our case, unlike the others, the trismus was severe due to the sternocleidomastoid muscle involvement. An intraoral approach provides a more aesthetic result, as it does not create an extraoral scar. It also avoids the possibility of damaging the facial nerve branches that can occur with extraoral approaches (14). The disadvantages of the intraoral approach include a limited field of view and the need to remove excessive bone to reach the tooth. In this approach, the retraction of soft tissues may cause damage to the lingual nerve (n. lingualis) and the inferior alveolar nerve (n. alveolaris inferior), which are in close anatomical proximity to the tooth. Excessive dissection and removal of bone to enhance the surgical field of view can increase the risk of soft tissue trauma and fractures (1, 5).

An endoscopic approach is very effective for minimally invasive access to this region. Endoscopic methods benefit from the magnification of the surgical field, improving visibility (15). However, using the equipment requires specialised training and the equipment is expensive (15). In our case, the following the drainage of the abscess, sufficient mouth opening was attained.

Intraoral access was deemed feasible in the 3D examination; therefore, an intraoral approach was preferred to mitigate the possible side effects associated with the extraoral approach.

CONCLUSION

EMTMs are rare. Asymptomatic cases can be followed up annually, while symptomatic cases require surgical intervention. The choice of surgical approach should be made according to the location of the EMTM. Three-dimensional radiological evaluation imaging is recommended for more precise planning.

Informed Consent: Written informed consent was obtained from patient who participated in this study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- J.A., S.Ç., C.İ., T.K.; Data Acquisition- J.A., B.G.; Data Analysis/Interpretation- S.Ç., T.K.; Drafting Manuscript- T.K., J.A., B.G.; Critical Revision of Manuscript- S.Ç., C.İ., T.K.; Final Approval and Accountability- J.A., T.K., B.G., S.Ç., C.İ.; Supervision- S.Ç., C.İ.

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