



## RESEARCH ARTICLES

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*Klinik Olarak Evre 2 Malign Melanom Hastalarında Nötrofil ve Trombosit Lenfosit Oranlarının Sentinel Lenf Nodu Mikroskopik Metastazı Hakkındaki Öngörü Etkileri*

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# THE PREDICTIVE EFFECT OF NEUTROPHIL AND PLATELET LYMPHOCYTE RATIOS ON MICROSCOPIC SENTINEL LYMPH NODE METASTASES IN STAGE 2 MALIGNANT MELANOMA PATIENTS\*

## KLİNİK OLARAK EVRE 2 MALİGN MELANOM HASTALARINDA NÖTROFİL VE TROMBOSİT LENFOSİT ORANLARININ SENTİNEL LENF NODU MİKROSKOPİK METASTAZI HAKKINDAKİ ÖNGÖRÜ ETKİLERİ

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\*The study has been presented as an oral presentation during the 44th National Meeting of the Turkish Society of Plastic Reconstructive and Aesthetic Surgery in Antalya, on November 2022.

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### ABSTRACT

**Objective:** Malignant melanoma is a life-threatening disease, and the stage affects both treatment and survival. Staging is based on the microscopic Breslow thickness of the tumor, ulceration, and lymph node status. This study compares the neutrophil and platelet lymphocyte ratios of patients with and without micrometastatic sentinel lymph nodes and assesses the predictive value of these ratios.

**Materials and Methods:** The study includes patients who had a sentinel lymph node biopsy between January 2017 and December 2021 that resulted in a T value of T2b or T3a with neither any regional lymph node metastasis (N0) nor any systemic metastasis (M0). These patients were diagnosed with Stage 2A melanoma. The study evaluates the relationships patients' sentinel lymph node status and gender have with their neutrophil and platelet lymphocyte ratios.

**Results:** The study includes 65 patients, 24 of who are female and 41 of whom are male. The mean age is 55.58 years (Range = 18 to 87). The neutrophil, lymphocyte, and platelet counts and neutrophil and platelet lymphocyte ratio (NLR and PLR) did not significantly differ between the patients with and those without micrometastasis ( $p>0.05$ ). The difference showed no significance whether adjusting for age and gender. A significant difference was found between the patients with and those without micrometastasis with respect to gender ( $p=0.009$ ), with the percentage of males being significantly higher in the patients with micrometastasis.

**Conclusion:** Neutrophil and platelet lymphocyte ratios have not been found to be independent predictors of sentinel lymph node micrometastasis in Stage 2A malignant melanoma, with male patients in this stage having a higher risk of sentinel lymph node micrometastasis.

**Keywords:** malignant melanoma, micrometastasis, neutrophil lymphocyte ratio, platelet lymphocyte ratio, sentinel lymph node biopsy

### ÖZ

**Amaç:** Yaşamı tehdit eden bir deri kanseri olan malign melanomda hastalığın evresi tedavi yaklaşımını ve sağkalımı etkilemektedir. Malign melanomun evrelemesinde, tümörün mikroskopik olarak ölçülen Breslow kalınlığından, ülserasyondan ve olası bir lenf nodu tutulumundan faydalanılmaktadır. Bu çalışmada sentinel lenf nodu biyopsileri mikroskopik olarak metastazsız (negatif) ve metastazlı (pozitif) olan hastaların nötrofil lenfosit ve trombosit lenfosit oranları karşılaştırıldı ve bu oranların metastaz üzerinde bir öngörü etkisi olup olmadığı değerlendirildi.

**Gereç ve Yöntem:** Ocak 2017–Aralık 2021 tarihleri arasında sentinel lenf nodu biyopsisi ameliyatı yapılmış olan, Breslow kalınlığına göre T değeri T2b veya T3a olan, makroskopik lenf nodu metastazı olmayan (N0) ve sistemik (uzak) metastazı da olmayan (M0) evre 2A malign melanom hastaları çalışmaya dahil edildi. Sentinel lenf nodu biyopsisi durumları ile cinsiyet arasındaki ilişki ve sentinel lenf nodu biyopsisi ile nötrofil ve trombosit lenfosit oranları arasındaki ilişki değerlendirildi.

**Bulgular:** Çalışmaya 65 hasta dahil edildi. Hastaların 24'ü kadın, 41'i erkekti. Hastaların ortalama yaşı 55,58 idi (18–87). Yaş ve cinsiyete göre düzeltme yapmadan ve yapılarak gerçekleştirilen değerlendirmeler sonucunda mikrometastaz açısından nötrofil, lenfosit, platelet sayıları, nötrofil lenfosit oranı (NLO) ve platelet lenfosit oranları (PLO) değerleri bakımından istatistiksel olarak anlamlı fark saptanmadı ( $p>0,05$ ). Sentinel lenf nodu biyopsisi mikrometastazı olan ve olmayan hastalar arasında cinsiyet bakımından istatistiksel olarak anlamlı fark olduğu saptandı ( $p=0,009$ ). Mikrometastazlı grupta erkek yüzdesinin mikrometastazsız gruptan daha yüksek olduğu saptandı.

**Sonuç:** Evre 2A malign melanom hastalarında, nötrofil ve trombosit lenfosit oranları, sentinel lenf nodundaki mikroskopik metastazı bağımsız bir değişken olarak öngörememektedir. Bu evredeki erkek hastalarda sentinel lenf nodu mikrometastazı riski kadın hastalara göre daha yüksektir.

**Anahtar Kelimeler:** Malign melanoma, mikrometastaz, nötrofil lenfosit oranı, sentinel lenf nodu biyopsisi, trombosit lenfosit oranı

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## INTRODUCTION

Malignant melanoma of the skin may be a life-threatening cancer, and the stage of the disease affects both the treatment and survival (1). With the introduction of new treatment options, the 5-year survival rate may exceed 95% in Stage 1 patients, whereas it may be as low as 10% in Stage 4 patients (1). Thus, early diagnosis and precise therapy are mandatory before the regional and systemic spread of the disease (2).

The microscopic Breslow thickness of the primary tumor, the presence of ulceration, and lymph node metastasis status are utilized when staging malignant melanoma (3). The risk of lymph node metastasis increases with greater Breslow thickness and the presence of ulceration (1). During the routine work-up with physical examination and imaging studies, the patients may not have experienced any macroscopic lymph node spread; however, thick and ulcerated melanomas are prone to microscopic metastases that may be detected with sentinel lymph node biopsies (4, 5). This method enables the pathological staging of the patient, from which additional surgeries and oncological treatments may be planned (4, 5).

Neutrophil and platelet lymphocyte ratios (NLR and PLR) are used in order to evaluate patients' immune status and can be calculated from peripheral blood cell counts (6). Wade et al. suggested utilizing these ratios in order to predict the sentinel lymph node spread of malignant melanoma (6). In addition to tumor thickness and the presence of ulceration, NLR and PLR have been demonstrated to be able to predict lymph node metastasis in malignant melanoma (7). The aim of this study is to evaluate the sentinel lymph node biopsies of patients with T2b and T3a tumors based on Breslow thickness who have no clinical lymph node metastasis (N0) nor any systemic metastasis (M0). The study assesses the absence or presence of microscopic nodal disease by comparing the NLR and PLR and investigates the predictive effect these ratios have regarding micrometastasis.

## MATERIALS and METHODS

The study was approved by the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 22.03.2022, No: 06). Informed consent was obtained from each patient.

The study has a retrospective design and utilizes institutional digital health records. This study includes patients with T2b and T3a malignant melanoma who have neither any macroscopic lymphatic (N0) nor systemic (M0) metastases, who are classified as having stage 2A malignant melanoma, and who then have had sentinel lymph node biopsies. Patients over 18 years of age were included without any limitation in terms of gender. The study period occurred between January 2017-December 2021 to ensure that the same surgical team performed the operations and monitoring throughout the process, thereby minimizing any procedural or technical treatment variations.

This study includes patients who had had peripheral blood cell

counts two weeks prior to surgery and had no overt clinical or serological infectious disease during these counts in order to avoid infection-related changes to their neutrophil, platelet, and lymphocyte values. The study excluded patients whose operations and pathological evaluations had been performed at other institutions, as well as patients who'd had no sentinel lymph node biopsy. The study also excluded pediatric patients, patients with an overt clinical or serological infectious disease two weeks pre-surgery, and patients whose malignant melanoma stage was other than 2A.

The study recorded each patient's age; gender; absolute neutrophil, platelet, and lymphocyte counts; neutrophil and platelet lymphocyte ratios; and the absence or presence of microscopic sentinel lymph node metastasis. Sentinel lymph node biopsy status was assessed with regard to gender and neutrophil lymphocyte and platelet lymphocyte ratios.

## Statistical analysis

The study used the program R (version 2.15.3; R Core Team, 2013) for the statistical analyses, with minimums, maxima, means, standard deviations, medians, frequencies, and percentages being used to report the data. The normal distribution of the quantitative data was assessed with both the Shapiro-Wilk test and graphical evaluations. The two-group comparison of the normally distributed variables was performed with the independent groups t test. The Pearson chi-square test was used to compare the gender percentages. The groups were corrected for age and gender, and the analysis of covariance (ANCOVA) test was performed to evaluate the groups, with a  $p < 0.05$  being accepted as statistically significant.

## RESULTS

The study includes 65 patients, of who 24 are female and 41 are male. The patients' mean age is 55.58 years (Range=18–87). The blood count values, neutrophil and platelet lymphocyte ratios, and sentinel lymph node biopsy status are presented in Table 1. The ages of patients showed no statistically significant difference with regard to the presence or lack of sentinel lymph node micrometastasis ( $p > 0.05$ ).

Table 2 exhibits the comparison of the blood count values and neutrophil and platelet lymphocyte ratios with respect to the sentinel lymph node micrometastasis status. In addition, the data have been evaluated with respect to this status' dependence on gender. When not classifying the data according to age and gender, the sentinel lymph node micrometastasis status shows no statistically significant difference regarding absolute neutrophil, platelet, and lymphocyte values or NLR and PLR ( $p > 0.05$ ). When classifying the data according to age and gender, sentinel lymph node micrometastasis status still showed no statistically significant difference in terms of absolute neutrophil, platelet, and lymphocyte values or NLR and PLR ( $p > 0.05$ ).

Table 3 demonstrates the relationship between sentinel lymph node micrometastasis status and gender. A statistically signi-

**Table 1: Demographic data, peripheral blood count values ad sentinel lymph node status**

	Minimum – Maximum (Median)	Mean±Standard deviation
Age	18-87 (57)	55.58±16.45
Neutrophil	2.5-8.4 (4.8)	4.95±1.36
Lymphocyte	0.5-3.6 (2)	2.06±0.59
Platelet	159-507 (266)	265.26±64.73
Platelet lymphocyte ratio	59.16-476 (131.17)	140.87±62.43
Neutrophil lymphocyte ratio	1-15 (2.4)	2.77±1.96
	n	%
<b>Gender</b>		
Female	24	36.9
Male	41	63.1
<b>Sentinel lymph node biopsy</b>		
Negative	44	67.7
Positive	21	32.3

n: Number of Patients

**Table 2. The relationship between the blood count values and the sentinel lymph node status**

	Negative sentinel lymph node biopsy	Positive sentinel lymph node biopsy	p (independent of gender, tested with independent groups t test)	p (dependent on gender, tested with ANCOVA)
	Mean ± Standard Deviation	Mean ± Standard Deviation		
Neutrophil	4.83±1.34	5.21±1.42	0.285	0.518
Lymphocyte	2.08±0.62	2.03±0.51	0.750	0.953
Platelet	268.48±73.47	258.53±41.62	0.567	0.569
Platelet lymphocyte ratio	143.47±71.19	135.44±39.10	0.631	0.563
Neutrophil lymphocyte ratio	2.78±2.27	2.75±1.08	0.965	0.770

**Table 3. The relationship between sentinel lymph node micrometastasis status and gender demonstrated as number of patients and percentage**

	Negative sentinel lymph node biopsy	Positive sentinel lymph node biopsy	p value (Pearson chi – square test)
	Number of patients (Percentage)	Number of patients (Percentage)	
<b>Gender</b>			<b>0.009*</b>
Female	21 (47.7)	3 (14.3)	
Male	23 (52.3)	18 (85.7)	

ificant difference was found between patients with and those without sentinel lymph node micrometastasis with respect to gender (p=0.009), with a higher percentage of male patients in the group with micrometastasis, while no such difference was found in the group without micrometastasis.

## DISCUSSION

Although malignant melanoma is not the most common skin cancer, it has a higher mortality risk than other skin cancer

types (8). The survival rate has increased with the developments in diagnostic studies and oncological treatments (2). Similar to all types of cancer, stage is the main predictor of survival in malignant melanoma, with precise staging being mandatory during the initial diagnosis (3).

If regional and systemic metastases are not detected in the initial evaluation and imaging examinations, the primary lesion should be excised and sent for appropriate pathological

examination regarding such things as the measurement of the Breslow thickness and the assessment of the ulceration status (5, 8). A Breslow thickness greater than or equal to 1.0 millimeter and the presence of ulceration necessitate a sentinel lymph node biopsy in order to detect any possible microscopic metastasis (4, 8).

Many novel studies have been performed in order to find new and independent variables apart from the Breslow thickness and the presence of ulceration in order to detect any possible sentinel lymph node micrometastasis (1, 6, 7, 9-12). Such an independent variable may help predict the stage of the disease even prior to invasive procedures such as the excision of the primary lesion and a sentinel lymph node biopsy. Mancuso et al. found interleukin and interferon levels of the peripheral blood to help predict metastasis in early stage malignant melanoma (9). Neutrophil and platelet lymphocyte ratios of the peripheral blood have also been found to help predict the survival and response to oncological treatments for advanced stage malignant melanoma (11-14). This study has been hypothesized with respect to the current usage of NLR and PLR in malignant melanoma.

Robinson et al. evaluated 1,489 patients with malignant melanoma and found NLR to be able to help predict microscopic sentinel lymph node metastasis (7). In fact, they defined cut-off values for both microscopic and clinical metastases and evaluated patients in all stages (7). The present study included only Stage 2A malignant melanoma patients in order to avoid confounding factors such as the Breslow thickness and the presence of ulceration. The study has assessed the predictive value of NLR and PLR for local and regional metastases in the early stage and found these two factors to not be independent variables.

High NLR and PLR have been found to be inversely proportional to survival rate in many studies (11, 14). However, Wade et al. demonstrated NLR and PLR to have different patterns in early and advanced stage malignant melanoma (6), finding high NLR to enable the regression of the primary tumor in the early stage of the disease (6). Another study by Wade et al. screened patient databases and prepared a nomogram called MelRisk for detecting sentinel lymph node micrometastasis in malignant melanoma (10). Patient's age, Breslow thickness, the presence of ulceration, the anatomic localization of the primary tumor, and NLR were able to be added to this nomogram, allowing the probability of micrometastasis to be found as a percentage (10). They found the predictive effect regarding sentinel lymph node micrometastasis to be augmented upon NLR being included in the nomogram (10). The current study has been based on the findings from MelRisk and compared the NLR and PLR values for patients in the same early stage of malignant melanoma. However, the study did not find these variables to help predict sentinel lymph node micrometastasis in Stage 2A melanoma patients.

Several studies have found a relationship between the probability of sentinel lymph node micrometastasis and gender with regard to malignant melanoma (15-17). Scoggins et al. found the primary tumor to have worse pathological properties and higher metastasis potential in male patients (15). Joosse et al.

found local tumors to metastasize less and survival rates to be better in female patients (16). Mervic et al. showed similar results and found the survival rate to be better in the female patients with metastasis (17). A higher percentage of males occurred in this study in the group with micrometastasis, and this finding is in concordance with the literature.

The strengths of this study are that it includes patients who were at the same disease stage, who had the same surgeons perform their operations, who had the same pathologists evaluate the specimens, and who had the same nuclear medicine physicians mark the sentinel lymph nodes. Future studies may increase the number of the patients and/or separately evaluate the patients at other disease stages who have no clinical metastases in order to be able to assess the predictive effect of NLR and PLR on sentinel lymph node micrometastasis for different patient groups.

## CONCLUSION

NLR and PLR as independent variables failed to predict sentinel lymph node micrometastasis in Stage 2A malignant melanoma patients, and these variables should be reevaluated over a larger patient cohort. Also, male patients were found to have a higher risk of sentinel lymph node micrometastasis in this stage.

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**Ethics Committee Approval:** This study was approved by İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 22.03.2022, No: 06).

**Informed Consent:** Informed consent was obtained from each patient.

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# PROBIOTICS' EFFECT ON NUTRITIONAL STATUS, APPETITE HORMONES, AND INFLAMMATION IN PRE-OBESE WOMEN

## PROBİYOTİKLERİN PRE-OBEZ KADINLARDA BESLENME DURUMU, AÇLIK-TOKLUK HORMONLARI VE İNFLAMASYON DURUMU ÜZERİNE ETKİSİ

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### ABSTRACT

**Objective:** Obesity is one of the most common health problems in the world. The following methods are currently used to treat obesity: medical nutrition therapy, behavioral therapy, medical treatment, and surgical therapy. Discussions are still ongoing regarding whether changing the plasma levels of the hormones that regulate satiety and appetite can be used as a new add-on therapy to pre-existing obesity treatments to decrease food consumption. One possibility for changing the levels of the hormones that control hunger and satiety involves the use of probiotics. This study has been planned and conducted to examine the effects of probiotics on appetite, inflammation, and nutritional status in overweight individuals.

**Materials and Methods:** The study includes 35 overweight females aged 19-30 years old to who a questionnaire form had been applied prior to the study. Probiotics were given to these individuals for 8 weeks. Food consumption records and anthropometric measurements were taken and a body composition analysis conducted three times during the study. Inflammatory markers such as CRP, TNF- $\alpha$ , and IL-6 and the hormone analyses (i.e., leptin, adiponectin, cholecystokinin, ghrelin, GLP-1, and PYY) were evaluated at the beginning and end of the study using the ELISA method.

**Results:** The women were asked about their physical activity status, and their appetite hormones and inflammatory markers were analyzed both at the beginning and end of the study. When taken together, the study found the individuals' anthropometric measurements, inflammatory markers, and hunger hormone levels to have decreased significantly and their satiety hormone levels to have increased significantly after the use of probiotics ( $p<0.05$ ). However, no significant changes occurred regarding their energy, macronutrients, fiber intake, or physical activity levels ( $p>0.05$ ).

**Conclusion:** The study clearly shows an appropriate probiotic strain to be able to significantly affect anthropometric measurements, inflammatory markers, and appetite hormones in overweight individuals without any other intervention.

**Keywords:** Obesity, probiotics, nutritional status, hormones, inflammatory markers

### ÖZ

**Amaç:** Obezite dünyadaki en yaygın sağlık sorunlarından birisidir. Obezite tedavisinde güncel yöntemler; tıbbi beslenme tedavisi, davranış terapisi, tıbbi tedavi ve cerrahi tedavidir. Mevcut obezite tedavilerine yeni bir ek tedavi olarak açlık tokluk hormonlarının plazma düzeylerinin değiştirilmesinin besin tüketimini azaltıp azaltmayacağı halen tartışılmaktadır. Açlığı ve tokluğu kontrol eden hormonların düzeylerini değiştirme yöntemlerinden birisi probiyotik kullanımıdır. Bu çalışma hafif şişman bireylerde probiyotiklerin iştah, inflamasyon ve beslenme durumu üzerindeki etkilerini incelemek amacıyla planlanmış ve yürütülmüştür.

**Gereç ve Yöntem:** Bu çalışmaya yaşları 19-30 arasında değişen 35 hafif şişman kadın dahil edilmiştir ve çalışma öncesinde anket formu uygulanmıştır. Probiyotikler katılımcılara 8 hafta boyunca verilmiştir. Çalışma sırasında 3 kez besin tüketim kayıtları, antropometrik ölçümler ve vücut kompozisyonu analizi yapılmıştır. CRP, TNF- $\alpha$  ve IL-6 gibi inflamatuvar belirteçler ve hormon analizleri (Leptin, Adiponektin, Kolesistokinin, Ghrelin, GLP-1, PYY) ELISA yöntemiyle çalışmanın başlangıcında ve sonunda değerlendirilmiştir.

**Bulgular:** Çalışmanın başında ve sonunda fiziksel aktivite durumu sorgulanmış olup, açlık tokluk hormonları ve inflamatuvar belirteçler değerlendirilmiştir. Çalışmanın sonunda başlangıç durumuna göre antropometrik ölçümlerde, inflamatuvar belirteçlerde ve açlık hormon düzeylerinin önemli ölçüde azaldığını ancak probiyotik kullanımı sonrasında tokluk hormonu düzeylerinin istatistiksel olarak anlamlı arttığını tespit edilmiştir ( $p<0,05$ ). Ancak enerji, makrobesin, lif alımı ve fiziksel aktivite düzeyinde istatistiksel olarak fark olmadığı tespit edilmiştir ( $p>0,05$ ).

**Sonuç:** Çalışmamız, uygun probiyotik süşunun kilolu bireylerde başka herhangi bir müdahaleye gerek kalmadan antropometrik ölçümleri, inflamatuvar belirteçleri ve iştah hormonlarını önemli ölçüde etkileyebileceğini açıkça göstermektedir.

**Anahtar Kelimeler:** Obezite, probiyotikler, beslenme durumu, hormonlar, inflamatuvar belirteçler

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## INTRODUCTION

Probiotics are healthy supplements consisting of live bacteria. The morphemes *pro* and *biota* that form the term probiotic mean “for life” (1). Over the past few years, probiotics have experienced tremendous growth. Probiotics are live bacteria that provide health benefits for their hosts (2). Additionally, oral probiotics have the ability to modify the composition of the intestinal microbiome, and this altered microbiome may have an impact on the host’s inflammatory pathways as well as how they metabolize glucose and lipids (3). Furthermore, changes in the gut microbiome have been demonstrated in other contexts to influence these host responses (4). As a result, probiotics have been recommended as a treatment approach for obesity due to their ability to effectively lower waist circumference and body mass index (BMI) (5). Based on animal studies, the use of probiotics has been shown to positively impact the hormones that regulate appetite and satiety (6, 7). People need to change their lifestyle habits and adopt new ones to avoid becoming overweight. The most important lifestyle factor is eating habits. One of the main reasons why people gain weight is an imbalance between energy intake and energy expended. This balance requires modifying food consumption when the energy intake exceeds the energy expended (8). Individuals’ eating habits are influenced by various factors, with the changes in the hormones regulating appetite and satiety having a particular impact on physical change and body weight. The peripheral control of food intake is managed by cholecystokinin (CCK), peptide YY (PYY), glucagon-like peptides 1 and 2 (GLP-1 and GLP-2), ghrelin, and other hormones that are released by the gastrointestinal system. Among these hormones, ghrelin is the only orexigenic peptide that is known for increasing hunger. Other hormones known as anorexigenic peptides suppress appetite and send repletion signals (9). In fact, by enlarging the number of functional microorganisms in the intestinal microbiota and being involved in the intestinal lumen, probiotics oversee changes in the plasma levels of the hormones that are released from the gastrointestinal system for regulating appetite and satiety. Therefore, probiotics have been argued to play a significant role in controlling appetite and food consumption (10). The following methods are used for treating obesity: medical nutrition therapy, behavioral therapy, medical treatment, and surgical therapy. Behavioral and medical nutrition therapy are indispensable parts of treatment (11). Discussions are still ongoing regarding whether changing the plasma levels of the hormones that regulate satiety and appetite can be used as a new add-on therapy to pre-existing obesity treatments for decreasing food consumption. Of course, one possibility for changing the levels of the hormones that regulate appetite and satiety is the use of probiotics. However, current research in these direction instead involves animal experiments and only a limited number of clinical trials. Therefore, more studies are needed to clearly demonstrate the effects of probiotics on the hormones that regulate satiety and hunger (12). This study has been planned and conducted to observe the effects of probiotics on hunger and satiety hormones, inflammation, and nutritional status in overweight individuals.

## MATERIALS and METHODS

### Study design

The study is a prospective clinical study.

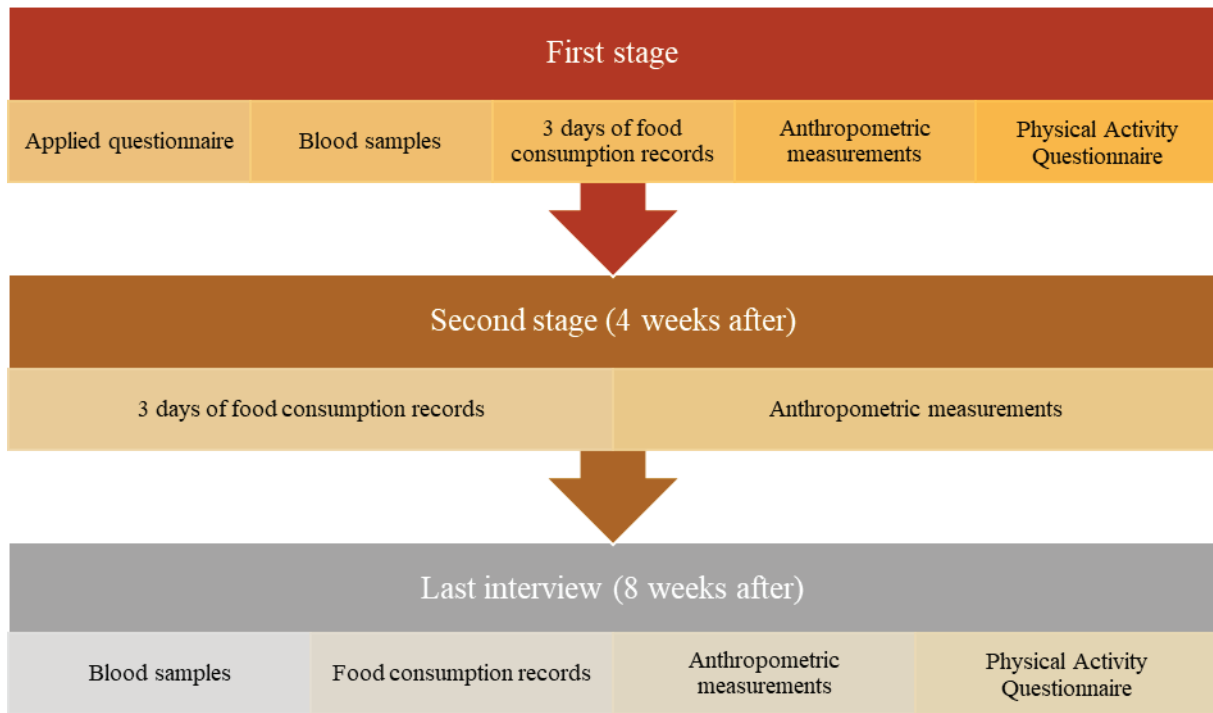
### Participants

The study includes 35 healthy, overweight, female adult participants between 19-30 years of age. The inclusion criteria are that the participants have no chronic illnesses and a BMI between 25.00-30.00 kg/m<sup>2</sup> and to not be dieting for weight loss or any other purpose during the study. The exclusion criteria are being male, being pregnant, lactating, using tobacco or alcohol, chronic illnesses (mild allergies excepted), permanent use of medication, ingestion of antibiotics within three months of the first examination, having used probiotics for at least three months before the first examination, and the regular use of vitamin and mineral supplements. This research was conducted between September 2019-April 2020.

### Procedures

The first stage of the study was carried out in the Nutrition and Diet Polyclinic of the Gazi University Faculty of Health Sciences Department of Nutrition and Dietetics. The study has included individuals living in Ankara. All individuals who were found to comply with the inclusion and exclusion criteria were explained the study in detail, and all who then agreed to participate in the study signed a voluntary consent form in accordance with the Declaration of Helsinki (World Medical Association, 1968). The study protocol was approved by the TOBB University of Economics and Technology Faculty of Medicine Clinical Research Ethics Committee (Approval no. 043 dated July 24, 2019).

The study has been planned in three stages. The first stage of the study is the acceptance stage and involves applying a questionnaire to the individuals. After receiving their anthropometric measurements, blood samples, food consumption records, and International Physical Activity Questionnaires taken, the participants started the probiotics usage (13). The researcher explained to them how to use probiotics, and providing the probiotics weekly ensured the follow-ups, which is the second stage involving taking the individuals’ food consumption records and anthropometric measurements. The third stage is the final stage of the study, with the last interview also applying a questionnaire to the individuals; their anthropometric measurements, International Physical Activity Questionnaire, and blood samples were taken again, and then the individuals were recorded as having finished the study (13). The subjects were not given any dietary treatment and nutrition training during the study period. The study design is summarized in Figure 1. During the first interview, probiotics with no product label were given to the individuals in seven daily packs. Probiotics were re-delivered to the participants at their individual weekly interviews, during which their usage status was monitored. The study emphasizes the effectiveness of the combined strains on the obesity treatment, the given probiotic strains being *Lactobacillus acidophilus* L1 (NBIMCC-8759; 2.9x10<sup>9</sup> colony forming units [CFUs]), *Lactobacillus rhamnosus* liobif (ATCC-7469; 2.9x10<sup>9</sup> CFUs), *Bifidobacterium longum* LBL-01 (NBIMCC-8329;



**Figure 1:** Study design

2.9x10<sup>9</sup> CFUs), and *Saccharomyces boulardii* (CNCM I-3799; 1.3x10<sup>9</sup> CFUs).

The researcher received the anthropometric measurements (i.e., body weight [kg], height [cm], waist circumference [cm], hip circumference [cm], and neck circumference [cm]) of the individuals participating in the study in accordance with techniques of anthropometric measurements.

Body weight measurement was made using the Tanita BC 418 brand bioelectrical impedance analyzer (BIA). The individuals' measurements were made on an empty stomach in the morning with their shoes taken off and while wearing a minimal amount of clothing. The measurement was recorded at a sensitivity of 0.1 kg (14).

All individuals' food consumption records were taken for three consecutive days (two days on weekdays, one day on the weekend) during Week 0 of the study, during Week 4, and during the final week (Week 8) of the study. For cases where not enough information about the number of nutrients in the meals was available, the book titled *Standard Recipes* was used to illustrate the amount of food recommended for one portion of a meal (15). The *Beslenme Bilgi Sistemi* [nutritional information system] (BEBiS) package program was used to evaluate the obtained data (16).

For the biochemical analyses conducted within the scope of the study, two blood vials (approximately 7 mL) were drawn from all individuals, one at the end of the first interview and the second at the end of the study. Blood samples were taken from all individuals after eight hours of fasting. These blood

samples were rapidly centrifuged for 10 min at 1000 x g using a YUDA 800D® brand centrifuge. After centrifugation, the supernatant portion was separated out, and the serum portion was transferred to four 1.5 mL Eppendorf tubes with the help of a Pasteur pipette. Serum samples were stored in a deep freezer at -32°C until the time of analysis.

Blood samples were drawn at baseline and after eight weeks of intervention (final stage of the study) following an overnight fast. Inflammatory markers such as CRP, TNF-α, and IL-6 and hormones assays (i.e., leptin, adiponectin, cholecystokinin, ghrelin, GLP-1, PYY) were carried out using the enzyme-linked immunosorbent array (ELISA). Measurements for all serum parameters were done in duplicate, and mean concentrations were calculated.

#### Data analysis

The obtained data were evaluated in the package software IBM SPSS Statistics version 21 (IBM SPSS Corp., Armonk, NY, USA). Frequency and percentage distributions were examined for the demographic variables. In addition, descriptive statistics (e.g., means, standard deviations, minima, and maxima) are provided for the analyzed variables. The normality assumptions of the variables were examined using the Kolmogorov-Smirnov test. The independent samples t-test was used for independent variables with two levels, the dependent samples t-test was used for the dependent variables, and the one-way analysis of variance (ANOVA) F test was used for variables with three or more levels. The results were accepted at a 95% confidence interval and a significance level of p<0.05 (17).

## RESULTS

After screening for the inclusion criteria, a total of 35 subjects were recruited. Their mean age is 22.8±0.48 years (Range=19-30). Table 1 shows the IPAQ-SF (MET-min/week) evaluation for each individual. For Week 0, the average IPAQ-SF (MET-min/week) result for the participants was 1,141.98±547.97, whereas at the end of the study (Week 8), the average was 1,201.07±503.46 (MET-min/week). No statistical difference was found between the first and last IPAQ-SF results (p=0.122).

Information on energy intake, macronutrient intake, and fiber intake are shown in Table 2. The average caloric intake was 1,609.37±310.51 kcal/day at the beginning of the study, 1,552.90±393.32 kcal/day in Week 4, and 1,535.52±364.79 kcal/day in Week 8. During the study, each participant had a similar average caloric intake (p>0.05). The study found no significant differences regarding daily energy intake, macronutrient

intake, or fiber intake (p>0.05). Table 3 shows the participants' BMI and anthropometric assessment results. The average weight of all participants was 73.16±5.90 kg before the use of probiotics, 72.48±6.04 kg at Week 4, and 71.36±5.87 kg at the end of the study (p<0.05). The average BMI of all individuals was 27.32±1.30 kg/m<sup>2</sup> at the beginning of the study, 27.01±1.42 kg/m<sup>2</sup> in Week 4, and 26.64±1.37 kg/m<sup>2</sup> in Week 8 (p<0.05). All anthropometric measurements were significantly reduced by using probiotics (p<0.05) except the waist-hip ratio (p>0.05).

Table 4 shows the evaluation for each participant's biochemical parameters. The GLP1 and leptin levels significantly increased after probiotics intake, while the cholecystokinin, ghrelin, peptide YY, and adiponectin levels decreased significantly (p<0.05).

Table 4 also illustrates the evaluations regarding the inflammation markers and glucose and insulin levels. After the use of probiotics, the CRP, IL-6, and TNF-α levels dropped to 0.21 mg/L, 4.09 ng/L, and 40.64 ng/L, respectively. Statistically, the

**Table 1:** The evaluation of IPAQ-SF (MET-min/week)

	0 WK		8 WK		t	p
	$\bar{X}\pm SD$	Min-Max	$\bar{X}\pm SD$	Min-Max		
IPAQ (MET-min/WK)	1,141.98±547.97	330-2,772	1,201.07±503.46	495-2,772	-1.585	0.122

Paired samples t-test, MET: Metabolic unit, IPAQ: International Physical Activity Questionnaire, WK: week, p<0.05

**Table 2:** Individuals' daily intake of energy and nutrients

Nutritional information	0 WK		4 WK		8 WK		p
	$\bar{X}\pm SD$	Min-Max	$\bar{X}\pm SD$	Min-Max	$\bar{X}\pm SD$	Min-Max	
Energy (kcal/day)	1,609.37±310.51	1,069.03-2,166.13	1,552.90±393.32	874.63-2,427.74	1535.52±364.79	881.94-2,326.92	0.374
Energy (kcal/Weight kg/day)	22.14±4.85	15.25-37.48	21.51±5.49	12.58-31.82	21.68±5.57	11.22-36.02	0.322
Protein (g/day)	63.12±11.50	37.66-84.63	59.43±19.03	25.18-95.57	61.15±13.25	28.61-91.37	0.395
Protein (%)	15.82±1.92	10.90-20.20	15.22±2.62	10.20-20.80	16.20±2.66	9.50-21.90	0.365
Animal proteins (g/day)	38.47±10.46	17.34-61.59	35.46±13.90	10.38-65.11	38.39±10.97	19.89-62.79	0.360
Vegetable protein (g/day)	24.64±6.76	14.18-42.37	23.97±8.23	8.55-40.52	22.75±7.86	8.72-39.80	0.362
Protein (g/Body weight kg/day)	0.83±0.16	0.57-1.25	0.87±0.27	0.38-1.31	0.81±0.18	0.41-1.41	0.586
Fat (g/day)	68.00±13.58	42.52-110.58	68.15±15.62	38.88-103.96	68.11±17.70	29.09-114.67	0.999
Fat (%/day)	38.26±4.61	30.60-47.60	40.33±7.12	30.60-59.70	40.05±6.00	29.10-58.70	0.391
Carbohydrate (g/day)	182.10±47.69	103.19-285.72	171.72±58.85	60.65-315.79	166.01±51.19	74.50-294.14	0.077
Carbohydrate (%)	44.91±5.34	33.10-54.80	43.49±7.36	26.70-56.30	42.81±6.07	29.20-58.70	0.324
Fiber (g/day)	17.44±6.00	7.24-32.22	16.59±6.06	5.26-30.82	16.05±6.20	5.55-30.99	0.315

ANOVA F test: p<0.05, kcal: Kilocalories, WK: Week



**Table 3:** Individuals' BMI and other anthropometric measurements

Characteristic	0 WK			4 WK			8 WK			F	p
	$\bar{X} \pm SD$	Min-Max	Median	$\bar{X} \pm SD$	Min-Max	Median	$\bar{X} \pm SD$	Min-Max	Median		
Body weight (kg)	73.16±5.90	57.80-90.90	73.10	72.48±6.04	57.60-91.20	72.90	71.36±5.87	56.50-87.00	71.30	56.33	<0.001*
BMI (kg/m <sup>2</sup> )	27.32±1.30	25.29-29.60	27.40	27.01±1.42	24.11-29.56	27.71	26.64±1.37	24.62-29.32	26.67	42.15	<0.001*
Waist circumference (cm)	83.17±6.05	70-98	83	82.95±6.17	70-97	83	82.20±6.13	69-96	82	30.10	<0.001*
Hip circumference (cm)	105.95±5.83	93-121	106	105.37±6.06	93-122	105	104.54±5.85	93-120	104	35.53	<0.001*
Waist/Hip ratio	0.78±0.06	0.63-0.87	0.80	0.78±0.05	0.64-0.87	0.80	0.78±0.05	0.64-0.87	0.80	1.79	0.182
Body fat mass (%)	36.66±3.49	28.60-44.50	36.90	36.48±3.20	29.10-44.30	36.80	36±3.26	28.70-42.60	36.10	4.64	0.020*
Body fat mass (kg)	27±4.38	19.10-40.40	26.30	26.70±4.29	19.30-40.40	25.40	26.06±4.06	19.50-37	25.90	10.38	<0.001*
Body fat Free mass (kg)	45.94±3.44	36.80-54	45.60	45.86±3.02	38.30-52.50	45.80	45.38±3.10	38.70-52.90	45.20	2.5	0.110
Total body water (kg)	33.82±2.23	28.40-39.50	33.50	33.64±2.21	28-38.40	33.50	33.45±2.18	28.30-38.70	33.50	4.63	0.013*

ANOVA F test: p<0.05\*, WK: Week

**Table 4:** Evaluation of individuals' biochemical parameters

	0 WK	8 WK	Difference (0 WK-8 WK)	p
	$\bar{X} \pm SD$	$\bar{X} \pm SD$		
Cholecystokinin (ng/L)	53.81±30.42	52.52±29.10	-1.31	<0.001*
GLP 1 (pmol/L)	79.32±43.88	80.16±43.15	0.86	<0.001*
Ghrelin (ng/ml)	4.92±2.84	4.80±2.65	-0.12	<0.001*
Leptin (ng/ml)	25.36±15.96	25.87±15.37	0.49	<0.001*
Peptit YY (pg/mg)	340.90±203.17	332.19±195.82	-8.71	<0.001*
Adiponectin (mg/L)	25.40±15.46	21.61±13.55	-3.78	<0.001*
Fasting Blood Glucose (mg/ dl)	92.57±10.87	92.37±8.57	-0.30	0.170
Insulin (mIU/L)	29.98±19.94	28.14±16.94	-1.83	<0.001*
CRP (mg/L)	2.36±1.47	2.15±1.50	-0.21	<0.001*
IL-6 (ng/L)	228.70±142.81	224.61±130.08	-4.09	<0.001*
TNF-α (ng/L)	369.29±241.98	328.64±195.56	-40.64	<0.001*

Dependent samples t test \*p<0.05, GLP-1: Glucagon-like peptide-1, TNF-α: Tumor necrosis factor alpha, IL 6: interleukin 6, pmol: Picomole, ng: Nanogram, dL: Decilitre, mIU: Milliunit, WK: Week

reduction in the inflammation markers is significant (p<0.05). Furthermore, observations regarding the changes in glucose and insulin levels reveal a significant positive development for insulin (p<0.05), whereas blood glucose levels after fasting exhibited no significant results (p=0.870).

## DISCUSSION

Gut microbiota are effective in the development and treatment of obesity. Eating habits are the most important reason for changes in microbiota. One effective way for increasing the

number of healthy microorganisms in microbiota is the use of probiotics (18). New methods have been developed for treating obesity in addition to pre-existing methods, and one recently discussed possible treatment method involves the use of probiotics. Studies have shown that manipulating the gut microbiota can be an alternative treatment model for obesity and that the use of probiotics can provide this effect (19). Food consumption and the levels of hunger-satiety hormones have been reported to only change through a change in gut microbiota using probiotics. Probiotic supplements have also been shown to improve individuals' anthropometric measurements (20).

Jung et al. researched the effects of probiotics on the adiposity of obese individuals. *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY103 were used as the probiotic strains. Compared to the values at the beginning of their study, significant changes were observed in terms of weight, waist-hip ratio, and BMI through the use of probiotics without even changing physical activities or the amount of food consumed (21). Kadooka et al.'s study intended to evaluate the effects of the *Lactobacillus gasseri* SBT2055 strain on the adipocytes of obese individuals, comparing the anthropometric measurements from the beginning and end of the study over both the test and control groups. The reduction in body weight, BMI, and waist-hip ratios was considered significant in both groups (22). Minami et al.'s study further aimed to evaluate the effects of the *Bifidobacterium breve* strain on the body fat values of 80 overweight people and also identified their body fat, BMI, and waist-hip ratio values to have been reduced (23). Minami et al. as well as the other studies identified a reduction in body weight, BMI, and waist-hip ratio without even changing physical activity habits or the amount of food consumed. Only the reduction in anthropometric measurements for the waist-hip ratio was not statistically significant ( $p > 0.05$ ). The present study chose to use *Lactobacillus acidophilus* L1 (NBIMCC-8759;  $2.9 \times 10^9$  CFUs), *Lactobacillus rhamnosus* liobif (ATCC-7469;  $2.9 \times 10^9$  CFUs), *Bifidobacterium longum* LBL-01 (NBIMCC-8329;  $2.9 \times 10^9$  CFUs), and *Saccharomyces boulardii* (CNCM I-3799;  $1.3 \times 10^9$  CFUs) based on previous studies (20, 21, 23). As shown in the literature, this study found body weight and BMI to reduce significantly ( $p < 0.05$ ). The study also found the waist-hip ratio to show no significant change, similar to the previous literature (23). These results show the use of probiotics even without changing physical activities or the amount of daily caloric intake to be able to clearly improve anthropometric measurements. Yet, this study considers that study duration and the strains used as probiotics may vary the effects on anthropometric measurements and body composition.

An individual's food consumption is affected by many factors, with change in hunger and satiety hormones in particular being among the most important physiological factors affecting food consumption and body weight (9). The use of probiotics can lead to a change in hormone levels. Of course, the leptin, ghrelin, adiponectin, GLP-1, Peptide-YY and cholecystokinin hormone levels are known to be able to change by consuming probiotics (18).

Zarrati et al.'s study on overweight and obese individuals aimed to observe the extent to which the *Lactobacillus acidophilus* LA5, *Lactobacillus casei* DN001, and *Bifidobacterium lactis* BB12 strains affect inflammation markers and hormone levels by consuming yogurt with probiotics. The study found leptin levels to have reduced by the end of the research (24). Behrouz et al. conducted a study on overweight individuals with nonalcoholic high-fat livers in order to evaluate the effects of probiotics and prebiotics on leptin and adiponectin glycemic parameters. They gave the *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Bifidobacterium breve* strains to the probiotic group and 8 gr maltodextrin to the prebiotic group during the 12-week-long weekly follow-ups. At the end of the research, they showed the use of probiotics to have led to significant increases in leptin and adiponectin (25). Another study found obese individuals who'd used the *Pediococcus pentosaceus* LP28 strain over a period of 12 weeks to have shown no significant effects regarding their leptin and adiponectin levels (26). The current study also evaluated the effects of probiotics on cholecystokinin, GLP-1, ghrelin, leptin, PYY, and adiponectin levels. The comparison of the beginning and end of the study has shown the leptin and GLP-1 levels to have increased and the levels of cholecystokinin, ghrelin, peptide PYY, and adiponectin to have decreased. The changes in all hormone levels were statistically significant in the current study ( $p < 0.05$ ). The study has shown that taking probiotics without even changing eating habits or physical activities can change the levels of hormones regulating satiety and appetite. Therefore, the study can clearly speculate the use of probiotics to be able to have a positive impact on managing hormones; however, the results do not sufficiently indicate at which specific duration of probiotics intake this impact actually occurs.

Obesity is associated with changes in immunity, chronic low-grade inflammation, and high circulating proinflammatory cytokines. However, how obesity precisely triggers inflammation remains unclear. Several hypotheses have been proposed, one being that an overload of nutrients in adipocytes triggers intracellular stress, leading to the activation of inflammatory cascades. A second hypothesis suggests that overloading adipocytes with fat overwhelmingly increases the infiltration of macrophages. These processes can result in the subsequent differentiation and activation of cytotoxic T cells that initiate and proliferate inflammatory cascades. A third hypothesis suggests that, as adipose tissue grows, the tissues become relatively hypoxic. Hypoxia within adipose tissue can activate inflammatory pathways. A final hypothesis is that overloaded adipocytes can directly activate the immune pathogen sensors that cause chronic inflammation (27). Probiotics have a significant effect on the gut barrier by stimulating  $\beta$  cells to produce IgA (28). Probiotics influence the production of cytokine (29), and the inflammation period depends on pro-inflammation and anti-inflammation cytokines. In fact, a lower-level chronic inflammation period, which is typical for obesity, can be improved (30). Furthermore, the use of probiotics can diminish the levels of such cytokines as CRP, TNF- $\alpha$ , and IL-627. To evaluate inflammation markers with regard to the *L. Reuteri* strain, Hansen

et al. divided participants into two random groups, providing one group with the *L. Reuteri* strain and the other group with a placebo. They observed the effects for 12 weeks, and after comparing against the control group at the end of the study, they found no significant results regarding IL-1 $\beta$ , IL-6, IL-8, and IL-10 levels (31). Kullisaar et al.'s study exhibited the use of the *L. Fermentum* strain to lead to changes in lipid profiles and inflammation markers. Their research analyzed the inflammation marker IL-6, and when comparing the test results against the control group, they found a significant difference in the IL-6 levels (32). Kobylak analyzed the extent to which probiotics affected the levels of diabetic individuals' resistance to insulin by providing them with the *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, and *Propionibacterium* strains for eight weeks. At the end of the research, Kobylak found no significant differences in IL-6 and TNF- $\alpha$  appetite levels between the control (placebo) and test (probiotics) groups (33). Bernini et al.'s study aimed to evaluate the effects of the *Bifidobacterium lactis* strain on inflammation markers and on the lipid profile (34). Meanwhile, the current study's comparison between Week 0 and Week 8 found the levels for the pro-inflammatory factors CRP, IL-6, and TNF- $\alpha$  to have reduced significantly. Therefore, this study can state that, due to probiotics ability to improve inflammation markers, they can be used effectively as an alternative therapy.

#### Limitations

The study has several limitations. The first was not having a control group due to the emergence of COVID-19 as the study was being conducted. The second is the use of various different bacteria strains in the content of the probiotics may have had varying impacts on the study, as discussed above. Finally, the number of participants and duration of the study are also considered another limiting factor.

#### CONCLUSION

Studies investigating the effects of probiotics on health are increasing these days. One such research area involves investigating the effectiveness of probiotic usage in the treatment of obesity. According to this study's results, the use of probiotics with appropriate strains can increase the success rate when treating obesity, in addition to other treatment approaches that are applied. However, more studies are needed in order to rule further on these findings.

**Ethics Committee Approval:** This study was approved by TOBB University of Economics and Technology Faculty of Medicine Clinical Research Ethics Committee (Date: 24.07.2019; Approval no. 043).

**Informed Consent:** Informed consent was obtained from each patients.

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- İ.Ü., H.Y., M.Y.A.; Data Acquisition- İ.Ü.; Data Analysis/Interpretation- İ.Ü.,

H.Y.; Drafting Manuscript- İ.Ü., H.Y.; Critical Revision of Manuscript- H.Y., M.Y.A.; Final Approval and Accountability- İ.Ü., H.Y., M.Y.A.; Material and Technical Support- İ.Ü., H.Y.; Supervision- H.Y., M.Y.A.

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# C-ERB B2 EXPRESSION IN COLORECTAL CARCINOMA; ITS RELATIONSHIP WITH TUMOR GRADE AND OTHER PROGNOSTIC FACTORS

## KOLOREKTAL KARSİNOMLARDA C-ERB B2 EKSPRESYONUNUN TÜRÖR DERECESİ VE DİĞER PROGNOSTİK FAKTÖRLERLE İLİŞKİSİ

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### ABSTRACT

**Objective:** The multidisciplinary management of colorectal carcinoma (CRC) is based on tumor stage, grade, and presence of vascular invasion. In this approach, information derived from the molecular characteristics of the tumor may be missed. There is a need to determine new prognostic parameters.

Human epidermal growth factor 2 (c-erb B2) protein overexpression is implicated in the development of various tumor types, while the data on CRC is ambiguous. This study aimed at determining the frequency of c-erb B2 overexpression in CRC, checking it against prognostic parameters, as it has a special role in breast and gastric cancers.

**Material and Methods:** A total of 71 colectomy specimens diagnosed as CRC at the Mustafa Kemal University Medical Faculty, Department of Pathology between January 2007, and December 2012, were included in this study. The c-erb B2 overexpression of tumor cells was detected by using immunohistochemistry (IHC).

**Results:** Among the 71 CRC cases, 34 (47.9%) of them were c-erb B2 score 0, 22 (31%) of them were score 1, 10 (14.1%) of them were score 2, 5 (7%) of them were score 3.

**Conclusion:** The overexpression of c-erb B2 protein was detected in 21.1% of CRC cases whereas no significant relationship with prognostic parameters was determined.

**Keywords:** ERBB-2 protein, colorectal neoplasms, prognosis

### ÖZ

**Amaç:** Kolorektal karsinomlarda multidisipliner yaklaşımda tümör evresi ve derecesi ile vasküler invazyon varlığı esas alınmaktadır. Bu yaklaşımda tümörün moleküler karakteristik bilgileri atlanabilmektedir. Bu tümörler için yeni prognostik parametrelerin belirlenmesine ihtiyaç duyulmaktadır. Birçok tümör tipinin gelişiminde c-erb B2 proteini aşırı ekspresyonu gösterilmiştir, ancak kolorektal karsinomlarda bu konudaki sonuçlar net değildir. Bu çalışmada meme ve mide kanserinde özel bir rolü olan insan kaynaklı epidermal büyüme faktörü 2 (c-erb B2) proteini aşırı ekspresyonunun kolorektal karsinomlardaki frekansını belirlemek ve prognostik parametrelerle ilişkisini araştırmak hedeflenmiştir.

**Gereç ve Yöntemler:** Ocak 2007 ile Aralık 2012 tarihleri arasında Mustafa Kemal Üniversitesi Tıp Fakültesi Patoloji Bölümü'nde kolorektal karsinom tanısı alan 71 kolektomi örneği bu çalışmaya dahil edilmiştir. Tümör hücrelerinin c-erb B2 protein ekspresyonu, immünohistokimyasal yöntemle araştırılmıştır.

**Bulgular:** Yetmiş bir kolorektal karsinom olgusundan 34 (%47,9) tanesi c-erb B2 skor 0, 22 (%31) tanesi skor 1, 10 (%14,1) tanesi skor 2, 5 (%7) tanesi skor 3 olarak değerlendirilmiştir.

**Sonuç:** Kolorektal karsinom olgularının %21,1'inde c-erb B2 proteini aşırı ekspresyonu tespit edilmiştir; ancak prognostik parametrelerle anlamlı bir ilişkisi izlenmemiştir.

**Anahtar Kelimeler:** ERBB-2 proteini, kolorektal neoplazmlar, prognoz

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## INTRODUCTION

Colorectal carcinoma (CRC) is one of the major worldwide health problems. It is the fourth most common cause of malignancies in the world and the second most common cause of cancer related deaths in the United States (US) and the western world (1).

The development of new cytotoxic agents and surgical techniques has improved the survival of CRC patients. But it is still not capable of preventing recurrence when patients become refractory to these modern chemotherapeutic regimens. Therefore, it is of great interest to identify molecular biomarkers for predicting outcome, therapeutic response, and potential therapeutic targets in CRC patients.

One of these biomarkers is the human epidermal growth factor 2 (c-erb B2) receptor. It has an oncogene located on the chromosome 17q21. Its increased expression plays a key role in cell proliferation, cell differentiation, inhibition of apoptosis and tumor progression. The overexpression of c-erb B2 has been reported in many epithelial tumors including breast, ovarian, gastric, colon and lung cancers. It is associated with more aggressive disease, poorer prognosis and high risk of recurrence (2, 3). Therefore, there are numerous ongoing researches for directed therapy alternatives for this target. Trastuzumab (Genentech, Inc. Roche Group, San Francisco, CA, USA), a humanized monoclonal antibody, targets the extracellular domain of the human epidermal growth factor 2 receptor. Its therapeutic benefit has been demonstrated in breast cancer and gastric cancer (4, 5).

Several studies evaluating c-erb B2 in CRC resulted in a large debate because overexpression rates varied between 0 and 84% (6). To optimize the wide range of these results, technical approaches should be standardized, and large-scale studies should be done.

This study aims to investigate the correlation of c-erb B2 expression with known prognostic parameters and to determine the availability in practical use.

## MATERIALS and METHODS

This study was carried out at the Department of Pathology of the Mustafa Kemal University University Medical Faculty. A total of 71 cases diagnosed as CRC by processing their colectomy specimens at the Mustafa Kemal University Medical Faculty Department of Pathology between January 2007 and December 2012 were included in the study.

The prognostic parameters such as age, gender, and tumor localization, are determined for each patient respectively. The hematoxylin-eosin stained sections in the archive obtained from the formalin-fixed, paraffin-embedded blocks of colectomy specimens were evaluated for determining other prognostic predictors such as the microscopic tumor type, the histopathologic grade, the presence of lymphovascular invasion and lymph node metastasis, depth of invasion, and pathological Tumor-

lymph nodes-metastasis (TNM) stage at diagnose duration. For microscopic type and histopathologic grade, the World Health Organization (WHO) 2010 classification was used.

For the immunohistochemical (IHC) process, new sections obtained from paraffin-embedded blocks were incubated with 1/800 diluted Polyclonal Rabbit Anti-Human c-erb B2 oncoprotein, for 30 minutes in an autostainer (DAKO Autostainer Link 48).

All sections were evaluated by two independent pathologists. Regarding c-erb B2 IHC staining, the scoring system for gastric cancer is used. Scores from 0 to 3 were used to determine the density of staining. No staining at all or incomplete membrane staining in <10% of the tumor cells was given the score 0, faint incomplete membrane staining in >10% of the tumor cells was given the score 1, weak to moderate staining of the entire membrane in >10% of the tumor cells was given the score 2, and strong staining of the entire membrane in >10% of the tumor cells was given the score 3. Scores of 0 and 1 were labeled as negative staining. Scores of 2 and 3 were labeled as positive staining (5, 7). The data was statistically analyzed by the Statistical Package for Social Sciences (SPSS) version 21 by using the Pearson chi-square test.

Approval was received for this study from the ethics committee of the Mustafa Kemal University Faculty of Medicine (Date: 04.10.2012, No: 24-2012/59). This study was exempted from informed consent because it is a retrospective study using previous data and materials from the archive.

## RESULTS

The ages of the cases were between 24 and 88 (mean 58). There were 32 (45.1%) males and 39 (54.9%) females with a male-to-female ratio of 1:1.2. According to IHC c-erb B2 staining results, no expression or membrane staining in <10% of the tumor cells was observed in 34 (47.9%) cases, were scored 0 and labeled as negative. Faint incomplete membrane staining in >10% of the tumor cells was observed in 22 (31%) cases, were scored 1 and labeled as negative (Figure 1). Weak to moderate staining of the entire membrane in >10% of the tumor cells was observed in 10 (14.1%) cases, were scored 2 and labeled as positive (Figure 2). Strong staining of the entire membrane in >10% of the tumor cells was observed in 5 (7%) cases, were scored 3 and labeled as positive (Figure 3). Overall, 15 (21.1%) cases were stained positive with c-erb B2.

Regarding the morphologic types of colorectal carcinoma, among 64 adenocarcinoma (not otherwise specified) cases, 14 (21.9%) of them were observed positive for c-erb B2 overexpression. Out of 6 mucinous adenocarcinoma cases, 1 (16.7%) of them was positive for c-erb B2 overexpression and the only signet ring carcinoma case was c-erb B2 negative (Table 1).

The tumor was localized at the proximal colon in 31 cases, 7 (22.6%) of them were positive with c-erb B2. Among 40 distal colon sited cases, 8 (20%) of them were positive with c-erb B2.

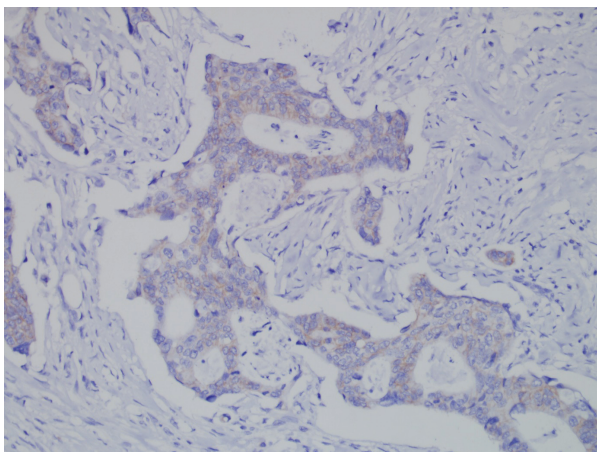


Figure 1: Score 1 c-erb B2 expression (x100)

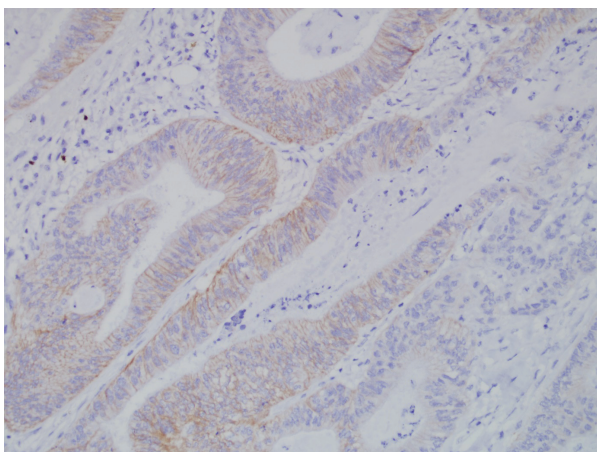


Figure 2: Score 2 c-erb B2 expression (x100)

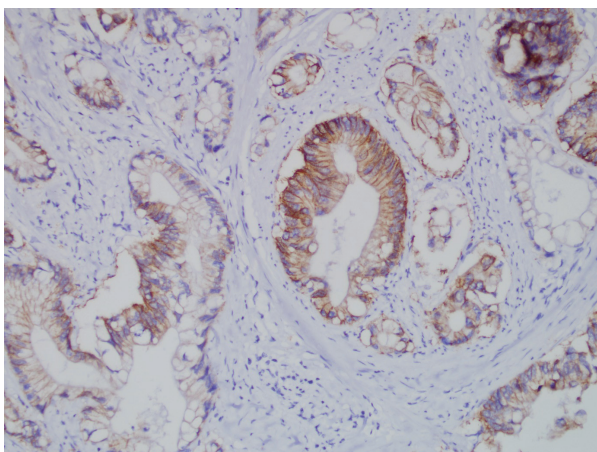


Figure 3: Score 3 c-erb B2 expression (x100)

The c-erb B2 overexpression were statistically similar between tumor localization sites (Pearson correlation (P))=0.792).

According to histopathologic grade evaluation, 64 cases were considered as low grade, 15 (23.4%) of them were positive with c-erb B2. Seven cases were considered high-grade tumors, but none of them were stained positive with c-erb B2. Statistically

c-erb B2 staining features were similar between tumor grades ( $p=0.332$ ).

According to pathological TNM stage procedure, 4 (5.6%) cases were considered as pT1, 8 (11.3%) cases were pT2, 9 (12.7%) cases were pT3 and 50 (70.4%) cases were pT4. In addition, 38 (53.5%) cases were considered as pN0, 20 (28.2%) of them were pN1, 13 (18.3%) of them were pN2.

The tumors have invaded to submucosa in four cases, 2 (50%) of them were positive with c-erb B2. In eight cases, the tumors have invaded to muscularis propria and 1 (12.5%) of them was positive with c-erb B2. None of 9 cases that have invaded to pericorectal tissue were positive with c-erb B2. In addition, among 50 cases with serosal invasion, 12 (24%) of them were positive with c-erb B2. According to invasion depth, c-erb B2 overexpression was statistically similar ( $p=0.953$ ).

Lymphovascular invasion was detected in 37 cases, 8 (21.6%) of them were positive with c-erb B2. Among the other 34 cases without lymphovascular invasion, 7 (20.6%) of them were positive with c-erb B2. The overexpression of c-erb B2 was statistically similar according to the presence of lymphovascular invasion ( $p=0.915$ ).

Among 33 cases with lymph node metastasize, 7 (21.2%) of them were c-erb B2 positive and 26 (78.8%) of them were negative. Out of 38 cases without lymph node metastasis, 8 (21.1%) of them were c-erb B2 positive and 30 (78.9%) of them were negative. The overexpression of c-erb B2 was statistically similar according to the presence of lymph node metastasis ( $p=0.987$ ).

## DISCUSSION

Colorectal carcinoma affects a substantial patient population with an estimated 132,700 incident cases in the US in 2015 (8). Even with improvements in surgical treatment and its association with adjuvant chemotherapy, CRC still causes high mortality today. Thus, it justifies the search for new therapeutic targets and markers that can be used to define prognosis beside the standard criteria. The current study was undertaken with a view of establishing the positivity status and the potential role of c-erb B2 overexpression by IHC.

To date, several studies have reported that the frequency of c-erb B2 protein overexpression varies widely from 0 to 84% in CRC, also its prognostic significance is controversial (6). This debate might be attributed to several causes such as differences in scoring systems for c-erb B2 protein overexpression, technical approach, and heterogeneity of the study population. In our study, c-erb B2 protein overexpression was found to be positive in 15 (21.1%) patients among 71 CRC cases.

In a study conducted in Vienna, the ratio of c-erb B2 protein overexpression in CRC cases was 30%, more similar to our result. They also found no significant association with tumor grade, gender, localization, or survival (9). In our study, no significant relationship was found between c-erb B2 overexpression and standard prognostic predictors such as tumor grade, presence of lymph node metastasis and lymphovascular invasion,

**Table 1:** Clinicopathologic features of the CRC individuals and number of cases presenting c-erb B2 overexpression

Variable	Total no:71	C-erb B2 overexpression	p
Gender (M:F)	32:39 (1:1.2)		
Mean age (range in years)	58 (24-88)		
Tumor site			0.792
Proximal colon	31 (43.7%)	7 (22.6%)	
Distal colon	40 (56.3%)	8 (20%)	
Tumor type			
Adenocarcinoma (not otherwise specified)	64 (90.1%)	14 (21.9%)	
Mucinous adenocarcinoma	6 (8.5%)	1 (16.7%)	
Signet ring cell adenocarcinoma	1 (1.4%)	0	
Histopathologic grade			0.332
Low grade	64 (90.1%)	15 (23.4%)	
High grade	7 (9.9%)	0	
Tumor invasion depth			0.953
Submucosa	4 (5.6%)	2 (50%)	
Muscularis propria	8 (11.3%)	1 (12.5%)	
Pericorectal tissue	9 (12.7%)	0	
Serosa	50 (70.4%)	12 (24%)	
Lymphovascular invasion			0.915
Positive	37 (52.1%)	8 (21.6%)	
Negative	34 (47.9%)	7 (20.6%)	
Lymph node metastasize			0.987
Positive	33 (46.5%)	7 (21.2%)	
Negative	38 (53.5%)	8 (21.1%)	

M: Male, F: Female

tumor localization site and invasion depth. In this aspect, the present study agreed with most other reports.

In a study conducted in Egypt, the ratio of c-erb B2 protein overexpression in CRC was 9.5% and no relationship between c-erb B2 overexpression and prognostic parameters of CRC was found (10). Kavanagh et al., found this ratio in rectal and colon cancer individuals at 7% and 11% respectively. They observed no correlation between c-erb B2 overexpression and age, gender, lymphovascular invasion, stage, perineural invasion or tumor size (11). In a study by Jesus et al., c-erb B2 protein overexpression was correlated with stage, prognosis and mortality of CRC; this ratio was 48.1% and no correlation was investigated (12).

Contrary to these results, in a study conducted in the US, Half et al. found a significant correlation between c-erb B2 overexpression (cytoplasmic staining) and tumor differentiation in colon cancer. They documented cytoplasmic staining in 63.5% of primary tumors, with strong membranous staining in 5% of primary CRC. Membranous but not cytoplasmic c-erb B2 staining has been strongly associated with gene amplification (13).

In a study performed in Pakistan in 2014, aimed to evaluate the frequency and staining pattern of c-erb B2 protein overexpression by using IHC in adenocarcinoma of the gastrointestinal tract, c-erb B2 positivity was found in 66% of CRC individuals. They found a highly significant association between tumor grade and c-erb B2 status but none with lymph node involvement (14). Seo et al. have observed that c-erb B2 protein overexp-

ression and gene amplification were found in about 6% of CRC individuals. Human epidermal growth factor 2 gene amplification was more frequently found in the rectum than in the right or left colon, and a high concordance rate between IHC and dual color Silver in situ Hybridization methods has been demonstrated (15).

Elezoğlu et al. conducted a study with CRC individuals to investigate the relationship between c-erb B2 IHC staining and prognostic factors. They found a significant relationship between c-erb B2 staining and both stage and grade, whereas none were found with other prognostic factors or survival (16). Also, in a study conducted in Sweden in 2009, the ratio of c-erb B2 positively stained CRC cases was 54% (17).

In a study conducted in Iran, the ratio of c-erb B2 protein overexpression in CRC was 40 % and no significant relationship between c-erb B2 overexpression and prognostic parameters of CRC was found (18). Sayadnejad et al. performed a study with 50 invasive CRC patients, positive immunostaining was detected in 24% of the cases. They found no significant relationship between the expression of c-erb B2 and clinicopathological features (19).

In a study conducted in Istanbul, a total of 123 colorectal resection cases were studied, c-erb B2 immunohistochemical staining was observed in 61 cases (50%). Of these 61 cases, 19 (31%) had poor, 26 (43%) had moderate and 16 (26%) had strong intense membranous staining. They found a significant



relationship between c-erb B2 and distant metastasis, but no relation between c-erb B2 score and other parameters such as lymphocytes infiltrating the tumor, Crohn-like response, tumor nodules, presence of a mucinous component and poorly differentiated area (20).

Siena et al. conducted an open-label, phase 2 study recruited 78 patients with unresectable, recurrent, or metastatic colorectal adenocarcinoma who had required to have RAS and BRAF-V600E wild-type tumors, received and progressed on at least two previous treatment regimens from 25 clinics and hospitals in Italy, Japan, Spain, the UK, and the USA. Based on c-erb B2 expression, patients were allocated into one of three cohorts: 53 (68%) of them were c-erb B2 IHC 3+ or IHC 2+ and in-situ hybridization (ISH) -positive, 7 (9%) of them were IHC 2+ and ISH-negative, and 18 (2%) of them were IHC 1+ (21).

## CONCLUSION

It would appear that we have not found a significant relationship between c-erb B2 overexpression and prognostic parameters such as tumor grade, lymph node metastasis, lymphovascular invasion, tumor localization site and invasion depth despite its crucial role in the occurrence of some tumors and its predicting value of worse prognosis for them. Furthermore, c-erb B2 protein overexpression may have a predictive role for targeted therapies like trastuzumab in CRC cases when standard therapeutic regimens are not efficient.

Although the prognostic significance of c-erb B2 has been demonstrated in gastric and breast cancer long since, it is still controversial in CRC. Nevertheless, because it is a target for anti c-erb B2 therapies and to evaluate these alternative managements in CRC, extensive studies using standardized techniques on large series are needed.

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**Ethics Committee Approval:** This study was approved by Mustafa Kemal University Faculty of Medicine (Date: 04.10.2012, No: 24-2012/59).

**Informed Consent:** This study was exempted from informed consent because it is a retrospective study using previous data and materials from the archive

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- N.Ş.Ş., M.Y.; Data Acquisition- N.Ş.Ş., M.Y.; Data Analysis/Interpretation- N.Ş.Ş., M.Y.; Drafting Manuscript- N.Ş.Ş., M.Y.; Critical Revision of Manuscript- N.Ş.Ş., M.Y.; Final Approval and Accountability- N.Ş.Ş., M.Y.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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# EVALUATION OF PATIENTS WITH ORGANOPHOSPHATE POISONING IN THE EMERGENCY DEPARTMENT: A RETROSPECTIVE TWO-CENTER STUDY

## ACİL SERVİSTE ORGANOFOSFAT ZEHİRLENMESİ OLAN HASTALARIN DEĞERLENDİRİLMESİ: İKİ MERKEZLİ RETROSPEKTİF BİR ÇALIŞMA

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### ABSTRACT

**Objective:** The widespread use of organophosphates (OP) as pesticides in global agriculture, pose significant health risks in both developing and industrialized nations. Exposure through ingestion, occupational contact, and suicide attempts leads to high morbidity and mortality. This study will retrospectively analyze the demographic, clinical, and laboratory characteristics of adult patients aged 18 years and older who presented to emergency departments.

**Material and Methods:** The study is a two-center retrospective analysis of the Niğde Ömer Halisdemir University Training and Research Hospital, Clinic of Emergency Medicine and Kafkas University Health Practice and Research Hospital, Clinic of Emergency Medicine. Patient data, including demographics, clinical details, and laboratory parameters, were recorded. Analysis covered routine blood tests, pseudocholinesterase levels, and electrocardiography findings. Patients diagnosed with OP poisoning (international classification of diseases (ICD) code T60.0) between 15 June 2020 and 15 June 2023 were included in the study.

**Results:** The study included 24 patients meeting inclusion and exclusion criteria. Oral exposure (41.7%) and inhalation (25%) were common routes. The hospitalization rate was 91.7%, with only 4.2% requiring intubation. No in-hospital mortality occurred. Pseudocholinesterase levels correlated negatively with pH, bicarbonate, anion gap and positively with c-reactive protein (CRP). Duration of hospital stays was negatively correlated with Glasgow coma scale (GCS), pH, bicarbonate, and positively correlated with the anion gap.

**Conclusion:** This retrospective, two-center study provides comprehensive insights into the demographic profiles, clinical manifestations, and treatment outcomes of adult patients presenting with OP poisoning in emergency departments. In our study, most organophosphate poisonings were accidental. In this respect, it is important to provide safety equipment, safe farming conditions, limited access to hazardous substances, emergency education, public health campaigns and safe work practices in factories and farms to prevent organophosphate poisoning. Furthermore, this study underlines the importance of assessment of clinical parameters such as GCS, pH and HCO<sub>3</sub> in predicting the severity and duration of hospitalization.

**Keywords:** Organophosphates, poisoning, mortality, biochemicals markers

### ÖZ

**Amaç:** Organofosfatların (OF) küresel tarımda pestisit olarak yaygın kullanımını hem gelişmekte olan hem de sanayileşmiş ülkelerde önemli sağlık riskleri oluşturmaktadır. Yutma, mesleki temas ve intihar girişimleri yoluyla maruz kalma, yüksek morbidite ve mortaliteye yol açmaktadır. Bu çalışma, acil servislere başvuran 18 yaş ve üzeri yetişkin hastaların demografik, klinik ve laboratuvar özelliklerini retrospektif olarak analiz etmeyi amaçlamaktadır.

**Gereç ve Yöntem:** Çalışma, Niğde Ömer Halisdemir Üniversitesi Eğitim ve Araştırma Hastanesi, Acil Tıp Kliniği ve Kafkas Üniversitesi Sağlık Uygulama ve Araştırma Hastanesi, Acil Tıp Kliniği tarafından gerçekleştirilen iki merkezli retrospektif bir analizdir. Demografik bilgiler, klinik ayrıntılar ve laboratuvar parametreleri dahil olmak üzere hasta verileri kaydedilmiştir. Analizler rutin kan testlerini, psödokolinesteraz düzeylerini ve elektrokardiyografi bulgularını kapsamaktadır. Çalışmaya 15 Haziran 2020 ile 15 Haziran 2023 tarihleri arasında OF zehirlenmesi (uluslararası hastalık sınıflandırması (ICD) kodu T60.0) tanısı alan hastalar dahil edilmiştir.

**Bulgular:** Çalışmaya dahil edilme ve dışlanma kriterlerini karşılayan 24 hasta dahil edilmiştir. Hastaneye yatış oranı %91,7 olup sadece %4,2'si entübasyon gerektirmiştir. Hastane içi mortalite görülmemiştir. Psödokolinesteraz düzeyleri pH, bikarbonat, anyon açığı ile negatif; CRP ile pozitif korelasyon göstermiştir. Hastanede kalış süresi Glasgow koma skalası (GKS), pH, bikarbonat ile negatif; anyon açığı ile pozitif korelasyon göstermiştir.

**Sonuç:** Bu retrospektif, iki merkezli çalışma, acil servislere OF zehirlenmesi ile başvuran yetişkin hastaların demografik profilleri, klinik bulguları ve tedavi sonuçları hakkında kapsamlı bilgiler sunmaktadır. Çalışmamızda organofosfat zehirlenmelerinin çoğu kaza sonucu meydana gelmiştir. Bu açıdan, organofosfat zehirlenmelerini önlemek için fabrikalarda ve çiftliklerde güvenlik ekipmanları, güvenli tarım koşulları, tehlikeli maddelere sınırlı erişim, acil durum eğitimi, halk sağlığı kampanyaları ve güvenli iş uygulamaları sağlamak önemlidir. Ayrıca bu çalışma, GKS, pH ve HCO<sub>3</sub> gibi klinik parametrelerin değerlendirilmesinin hastaneye yatış şiddeti ve süresinin öngörülmesindeki öneminin altını çizmektedir.

**Anahtar Kelimeler:** Organofosfatlar, zehirlenme, ölüm, biyokimyasal belirteçler

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## INTRODUCTION

Organophosphates (OP) are chemical compounds commonly used in agriculture and known as pesticides (insecticides, herbicides, and rodenticides). Although poisoning from these substances is more common in developing nations, it is also a significant issue in industrialized nations (1-3). Accidental ingestion (oral), occupational exposure (through skin and inhalation) while working with pesticides, and unfortunately, suicide attempts are frequently observed. OPs are chemical compounds that cause high morbidity and mortality; therefore, early initiation of treatment in the emergency department will reduce morbidity and mortality rates (4). The lungs, eyes, skin, and gastrointestinal system are all capable of absorbing OPs well. In terms of how they affect the body, organophosphates stop the enzyme acetylcholinesterase (AChE) from working in synapses in a way that can't be undone. They also make nicotinic and muscarinic cholinergic receptors in the brain, the spinal cord, and the muscles work too hard with acetylcholine (ACh) (5). So, stimulating the muscarinic receptors makes cholinergic symptoms worse, like myosis, bradycardia, nausea, vomiting, abdominal pain, urination, increased bronchial and salivary secretions, and so on. The stimulation of nicotinic receptors makes fasciculations, tachycardia, muscle weakness, and high blood pressure worse. In addition, it causes seizures and loss of consciousness in the central nervous system (6). The diagnosis of OP is based on the identification of the exposure agent, the clinical findings of the patients, and laboratory tests (7). Decontamination, meticulous supportive care, aggressive antimuscarinic therapy (atropine), seizure control, and the administration of oximes are the cornerstones of treatment (5, 7). Niğde is located in the Central Anatolia region of Turkey, while Kars is situated in the Northeastern Anatolia region. The primary areas of focus for development in both cities are agriculture and animal husbandry. The Niğde Ömer Halisdemir University Training and Research Hospital and the Kafkas University Health Practice and Research Hospital (hereinafter referred to as hospitals) are the only tertiary care hospitals and the last referral institutions of the provinces. This study retrospectively analyzed the demographic, clinical and laboratory characteristics of adult patients aged 18 years and older presenting to emergency departments with OP poisoning and to contribute to the management of OP poisoning after the results were obtained.

## MATERIAL and METHODS

This study was approved by the Non-Interventional Research Ethics Committee of Kafkas University Application and Research Hospital (Date: 09.11.2023, No: 15). This study was designed as a two-center retrospective study. This study was conducted in accordance with the Declaration of Helsinki (2013 revision). The study was conducted at the Emergency Medicine Clinic of Niğde University Training and Research Hospital and the Emergency Medicine Clinic of Kafkas University Health Practice and Research Hospital. The data of all patients included in the study was recorded in a database set created using Excel. The demographic (age, gender), clinical (exposure agent, route of exposure,

treatment, hospitalization status, length of stay, and mortality status), and laboratory parameters of the patients were analyzed. Hemograms, biochemistry, cardiac markers, blood gases, and coagulation parameters were analyzed using routine blood tests. The levels of pseudocholinesterase requested and detectable in the patients were recorded. The electrocardiography (ECG) was also evaluated, and the findings were categorized as normal, sinus bradycardia, and sinus tachycardia. Findings considered to have an impact on clinical outcomes (length of stay, need for mechanical ventilation, or mortality) in prognosis are reported. Since the study was performed in retrospective design, written informed consent was not obtained from the patients.

### Inclusion criteria

The study included patients who applied to the study centers between June 15, 2020, and June 15, 2023, and had an international classification of diseases (ICD) ICD code T60.0 for OP poisoning with a history, clinic, and/or lab confirmation of the diagnosis.

### Exclusion criteria

Patients younger than 18 years old, pregnant women, patients whose medical history could not be found or who left the hospital without permission, patients whose file screening was missing information, patients whose ICD diagnosis code was given as OP poisoning but whose diagnosis could not be confirmed by history, clinical, and laboratory findings, and patients known to have a congenital pseudocholinesterase deficiency or non-OP diseases (advanced liver and kidney failure) were not included. In addition, pesticide poisonings not belonging to the OP group were excluded from the study and arrhythmic patients with known bradycardia and basal tachycardia were excluded.

### Statistical analysis

In a statistical analysis, the SPSS 22.0 (IBM SPSS Corp., Armonk, New York, USA) package program was used to evaluate the data. In descriptive statistics, categorical data were expressed as numbers and percentages and non-categorical data as mean±standard deviation (SD), or median and interquartile range (IQR). In the comparison of quantitative data, the normality and homogeneity of the variance distribution were checked first. The Shapiro-Wilk and Levene's tests were used for this. The student's t test was used to compare the averages of the data that exhibited a normal distribution; the Mann-Whitney U test or Kruskal-Wallis H test were used for those that did not. In the comparison of continuous variables with each other, Pearson's correlation analysis was used for normally distributed data, and Spearman's rho correlation analysis was used for non-normally distributed data. Since the study had a retrospective design and all patients over 18 years of age (minus exclusion criteria) were included in the study, apriori power analysis was not required. Since retrospective data was used in our study, a voluntary consent form was not required. All data was analyzed at 95% confidence interval (CI) and  $p < 0.05$  was considered statistically significant.

## RESULTS

A total of 24 patients who met the inclusion and exclusion criteria were included in the study. The median age of the patients was 34 years (IQR, 22.75–58.75). Thirteen (54.2%) of the patients were male, and 11 (45.8%) were female. The most common route of exposure in OP poisonings was oral in 10 patients (41.7%) and inhalation in six patients (25%). The most common form of poisoning was accidental exposure (n=15, 62.5%). All OP poisonings were from the pesticide group, and insecticide poisonings were the most common (n=19, 79.2%). Of the total number of patients in the study, 22 (91.7%) were hospitalized, and only one patient (4.2%) required intubation. The median

**Table 1:** Baseline characteristics of the patients

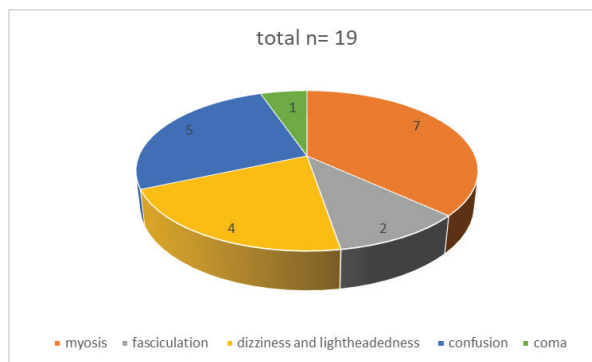
Variables	Total n=24
Age, years, median (IQR, 25-75)	34 (22.75- 58.75)
Gender, n (%)	
Male	13 (54.2)
Female	11 (45.8)
Most common exposure route, n (%)	
Oral	10 (41.7)
Nature of poisoning (mostly), n (%)	
Accidental	15 (62.5)
Source of poisoning (mostly), n (%)	
Insecticides	19 (79.2)
Hospitalization, n (%)	
Yes	22 (91.7)
Needing intubation, n (%)	1 (4.2)
GCS score at admission, median (IQR, 25-75)	15 (14-15)
In-hospital mortality, n (%)	
Yes	0 (0)

IQR: Interquartile range, GCS: Glasgow coma scale

**Table 2:** Organophosphate-related complaints in the patients

Variables	Total n=24
Complaints, n (%)	
Muscaranic	11 (45.8)
Muscaranic + nicotinic	3 (12.5)
Muscaranic + nicotinic + central	7 (29.2)
Muscaranic +central	3 (1.5)
Most common symptoms/findings, n (%)	
Muscaranic	
GIS (nausea, emesis)	21 (87.5)
Nicotinic	
Sinus tachycardia	9 (37.5)
Central	
Confusion	5 (20.8)
ECG findings, n (%)	
Normal	10 (41.7)
Sinus bradycardia	5 (20.8)
Sinus tachycardia	9 (37.5)

ECG: Electrocardiography, GIS: Gastrointestinal system



**Figure 1.** Specific physical examination findings in organophosphate poisoning

value of the Glasgow Coma Scale (GCS) score of patients admitted to hospital emergencies with OP exposure was 15 (14–15). No in-hospital mortality was found. These results are presented in Table 1. The patients' complaints were put into three groups: muscarinic, nicotinic, and central. Eleven patients (45.8%) had only muscarinic complaints, which included those with irritant eye symptoms. Three patients (12.5%) had muscarinic and nicotinic complaints, seven patients (29.2%) had muscarinic and nicotinic and central complaints, and three patients (12.5%) had muscarinic and central complaints. All patients (n=24) had muscarinic-type complaints or findings, with nausea and vomiting being the most common (n=21, 87.5%). Among specific physical examinations, myosis was found in seven patients (29.2%) and fasciculation in two patients (8.3%). Central complaints were observed in a total of 10 patients (41.7%) and included dizziness and lightheadedness in four patients (16.7%), confusion in five patients (20.8%), and coma in one patient (4.2%) (Figure 1). The OP-related complaints of the patients in the emergency department, intensive care unit, and ward follow-up are presented in Table 2. In addition, rhythm analysis revealed normal sinus rhythm in 10 patients (41.7%), sinus tachycardia in nine patients (37.5%), and sinus bradycardia in five patients (20.8%). Table 3 shows the specific treatments that were given to each patient as well as comparisons of their time in the intensive care unit and overall length of stay in the hospital based on their type of intoxication, gender, and ECG rhythm when they were admitted to the emergency room. The median length of stay of patients (n=22, 91.7%) who were hospitalized from the emergency department was 2.5 days (1-3.25), and the median length of stay in the intensive care unit was two days (1.25–3). The pseudocholinesterase levels were studied in a total of 12 patients. In cases of OP poisoning, patients were categorized into two groups: accidental and suicide attempts. In the suicidal group, the mean rank score for the duration of the intensive care stay was higher than in the accidental group (mean rank scores of 7.43 and 5.20, respectively). However, these differences were not statistically significant (p=0.266). In addition, it was found that female patients hospitalized in the intensive care unit were hospitalized for a longer period of time than male patients, but this difference was not statistically significant (mean rank scores of 8.00 and 5.43, respectively; p=0.2). The length of hospitalization was compared according

**Table 3:** Comparison of ICU length of stay and total length of hospitalization according to gender, type of poisoning and ECG

Variables	Total n=22		p	
Length of hospitalization (time), day, median (IQR, 25-75)				
ICU	2 (1.25-3)			
ICU+ward	2.5 (1-3.25)			
Length of hospitalization (time), day, mean rank				
	Suicide (n=5)	Accidental (n=7)		
ICU	7.43	5.20	0.266*	
ICU+ward	14.61	9.35	0.055*	
	Male (n=11)	Female (n=13)		
ICU	5.43	8.00	0.200*	
ICU+ward	12.50	10.30	0.416*	
ECG at admission in ED, mean rank				
	Normal	Bradycardia	Tachycardia	
ICU	2.00	6.40	7.33	0.352**
ICU+ward	5.55	16.90	13.78	0.003**

Abbreviations: IQR, interquartile range, ICU: Intensive care unit, ECG: electrocardiography, ED: Emergency department, \*Mann-Whitney U test; \*\*Kruskal- Wallis H test

**Table 4:** Correlations between biochemical parameters and clinical outcomes

Correlations	n	r	p*
Total length of hospital	GCS scale	-0.714	<0.001
	pH	-0.828	<0.001
	HCO <sub>3</sub> <sup>-</sup>	-0.773	0.003
	Anion gap	0.640	0.002
Pseudocholinesterase level	pH	0.591	0.043
	HCO <sub>3</sub> <sup>-</sup>	0.629	0.028
	Anion gap	-0.688	0.019
	CRP	-0.636	0.026

GCS: Glasgow coma score, HCO<sub>3</sub><sup>-</sup>: Bicarbonate, CRP: c-reactive protein, \*: Spearman's rho, n: Sample size, r: Correlation coefficient

to the ECG rhythm (normal, bradycardia, and sinus tachycardia) at the time of presentation to the emergency department; no significant difference was found between the groups in the length of intensive care stay (H(3)=2.086, p=0.352). However, a statistically significant difference was found between the groups in terms of total hospitalization duration rank mean scores (H(3)=11.884, p=0.003). According to the post hoc test (Mann-Whitney U), it was found that this difference between the groups was due to patients with normal ECG; patients with normal sinus rhythm had a shorter hospitalization time than patients with bradycardia and sinus tachycardia (p=0.002 for normal sinus-bradycardia, p=0.006 for normal sinus-sinus tachycardia, and p=0.328 for bradycardia-sinus tachycardia). The mean pseudo-cholinesterase level was 4934.41±3059.7. In total, 12 patients (50%) were treated with atropine and six patients (25%) with pralidoxime (PAM). A correlation analysis was performed between the continuous variables of the patients using the Spearman's rho test. The total length of hospital stays (n=22)

had a strong negative correlation with GCS, pH, and bicarbonate (HCO<sub>3</sub><sup>-</sup>) (r=-0.714, p<0.001; r=-0.828, p<0.001; r=-0.773, p=0.003, respectively) and a moderate positive correlation with anion gap (r=0.64, p=0.002). In addition, the pseudo-cholinesterase levels obtained from the patients (n=12) were positively correlated with pH and HCO<sub>3</sub><sup>-</sup> (r=0.591, p=0.043; r=0.629, p=0.028, respectively) and negatively correlated with anion gap and CRP (r=-0.688, p=0.019; r=-0.636, p=0.026, respectively). These relationships are presented in Table 4.

## DISCUSSION

OP poisonings affect both sexes almost equally and are more common in the young population (8). In our study, the male/female ratio was found to be close to each other. Although the rate of males (54.2%) was found to be higher in our study, this difference was not statistically significant (p>0.05). More than half of the patients in our study (n=13) were under the age of 35. In a study by Vucinic et al., they reported that the ratio of

men and women in OP poisonings was approximately equal in terms of gender, similar to our study, but in terms of exposure, the majority were suicide attempts (92%), and the rest were accidental poisonings (9). However, in this study, contrary to what Vucinik et al. reported, we found that the most common poisonings were accidental (62.5%). The main difference between these two situations is that serious poisonings from OP compounds typically result from suicidal oral ingestion in industrialized nations, whereas in developing nations, they typically result from occupational exposure, improper or non-use of protective equipment, or accidental ingestion. Most patients exposed to OP poisoning are usually exposed to these compounds through contact with insecticides and herbicides (10). In our study, insecticides were found to be the most common exposure agent (79.2%). The signs and symptoms of exposure after organophosphate poisoning can be quite wide-ranging. From general gastrointestinal complaints such as mild nausea, vomiting, or diarrhea to severe complaints and findings such as myosis, respiratory distress, bradycardia, fasciculations, seizures, confusion, or coma (11). The Peradeniya Organophosphorus Poisoning (POP) scale developed by Senanayake et al. to evaluate the severity of OP poisoning are used (12). This scale gives each of the five common clinical signs of OP poisoning a score between 0 and 2 points. A seizure is given a score between 0 and 1. The five signs are pupil diameter, respiratory rate, bradycardia, fasciculation, and altered consciousness. Patients are then classified as mild (score 0-3), moderate (score 4-7), or severe (score 8-11). However, due to the retrospective design of this study, a clinical severity scoring could not be performed on patients due to the missing or inadequate recording of the required parameters. Although 91.7% of the patients included in the study was hospitalized for follow-up in accordance with 114 national poison advisory recommendations, only one patient (4.2%) required intubation, and two patients (8.3%) had muscle fasciculation. No in-hospital mortality was found. Since the most common complaints in the study were gastrointestinal such as nausea and vomiting, only one patient needed intubation, two patients with fasciculations, no patients with seizures, and no mortality, the clinical poisoning in patients after OP exposure was not serious. The mechanism of OP intoxication involves phosphorylation of the active serine hydroxyl group. This leads to inactivation of the AChE region, which has an important role in neurotransmission (13). A low erythrocyte AChE or serum AChE level is expected in the confirmation of the diagnosis, but serum pseudocholinesterase is frequently studied for its usability in emergency services. Suppressed pseudocholinesterase activity is a well-known laboratory finding in patients with severe organophosphate poisoning (14). However, the interpretation of AChE and subgroup analysis is still controversial. The levels do not change in proportion with patient clinics; plasma pseudocholinesterase may be found normal or mildly low in emergency room admissions, even in severe poisoning (15-17). In this study, the pseudocholinesterase level was studied in half of the patients (n=12) diagnosed with organophosphate poisoning, and the mean pseudocholinesterase level (4934.41±3059.7) was found to be mildly low (normal range, 5.30–12.90 kU/L). To treat organophosphate or carbamate poisoning, the three main drugs are atropine, glycopyrrolate (GPR), which are anticholinergic drugs, and PAM, which turns

on cholinesterase (18). In this study, atropine was administered as an antidote in 12 symptomatic patients (50%) and PAM in six patients (25%). The atropine was also given to all patients who received PAM. Currently, there is some uncertainty about whether all cases of organophosphate toxicity should receive oxime. This is because some cholinesterase inhibitors bind permanently with cholinesterase after a certain period of time and progress to a process called “aging.” Therefore, PAM should be administered early (first 24-48 hours) and in adequate doses to selected patients (19, 20). We saw that in the clinical setting where PAM was used, the drug was given within the first 48 hours to people who had tachycardia, seizures, changes in consciousness like coma, and muscle fasciculations, in addition to relaxation effects. A study by Ke et al. looked at what factors affect the prognosis of people with OP. They found that the Acute physiology and chronic health evaluation (APACHE)-II score, body temperature, blood pressure, pH, and GCS score were all lower in deceased patients compared to the living; but the creatinine, white blood cell (WBC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinin kinase-myocardial band (CK-MB), blood glucose, blood lactic acid, and heart rate were higher in deceased patients (21). In this study, no in-hospital deaths were found. However, low pH, HCO<sub>3</sub><sup>-</sup>; GCS score, and high anion gap were found to be among the factors affecting prolonged intensive care unit hospitalization and total hospital stay. In poisoning, the clinic may be more serious in intentional exposures due to high intakes, the type of substance ingested, the need for specific antidotes, or patient-induced delays in treatment (22). In this study, the mean ranking for the duration of intensive care unit hospitalization, which could indicate clinical severity, was found to be higher in the suicide group. However, this was not found to be statistically significant. There are many studies in the literature examining the effect of gender on intentional exposures (22-24). In a study by Kim et al., there was no discernible difference between genders in terms of the level of intoxication (25). In this study, female patients in the intensive care unit were hospitalized for a longer period of time than male patients but these differences were not statistically significant. However, it was found that patients with sinus tachycardia and sinus bradycardia had longer hospitalizations, and this was statistically significant (p<0.005).

Limitations of the study include the retrospective design leading to data gaps, a small sample size, difficulty in generalizing findings due to data from two small geographic regions, and incomplete parameter records for assessing clinical severity.

## CONCLUSION

This retrospective, two-center study sheds light on the demographics, clinical manifestations, and treatment outcomes of adult patients presenting with organophosphate poisoning in emergency departments. The findings emphasize the predominance of accidental exposures, particularly through oral ingestion, and highlight the significance of early intervention in reducing morbidity and mortality rates. The study underscores the importance of assessing clinical parameters, such as GCS, pH, and HCO<sub>3</sub><sup>-</sup>, in predicting the severity and duration of hospitalization. The results contribute valuable insights for informed decision-making in the prevention and management

of organophosphate poisoning. Strategies such as ensuring regular use of personal protective equipment by those working in agriculture and industrial settings, promoting safe farming and industrial practices, implementing measures to restrict access to toxic substances, providing emergency training, conducting public awareness campaigns, and implementing public health measures can be effective in preventing organophosphate poisonings. By adopting these strategies, stakeholders can collaboratively work towards reducing the incidence of organophosphate poisonings and improving overall public health outcomes.

**Ethics Committee Approval:** This study was approved by Non-Interventional Research Ethics Committee of Kafkas University Application and Research Hospital (Date: 09.11.2023, No: 15).

**Informed Consent:** Informed consent was obtained from each patients.

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- A.V., T.D.; Data Acquisition- L.Ş., M.C.A., T.D., A.V.; Data Analysis/Interpretation- A.V., T.D., L.Ş., H.C.; Drafting Manuscript- A.V., T.D., M.C.A.; Critical Revision of Manuscript- H.C., L.Ş.; Final Approval and Accountability- A.V., T.D., L.Ş., M.C.A., H.C.; Supervision- A.V.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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## ANALYSIS OF POLYMORPHIC CHROMOSOME VARIANTS IN COUPLES WITH RECURRENT PREGNANCY LOSS

### TEKRARLAYAN GEBELİK KAYBI OLAN ÇİFTLERDE POLİMORFİK KROMOZOM VARYANTLARININ ANALİZİ

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#### ABSTRACT

**Objective:** This study evaluated chromosome polymorphisms (1qh+, 9qh+, inv9, 13ps+, 14ps+, 15ps+, 16qh+, 21 ps+, 22 ps+ and Yqh+) in a case group (n=1688) with two or more recurrent pregnancy losses (RPL) and a control group (n=80).

**Materials and Methods:** The control group was selected from 40 married couples who had no known hereditary disease, were not relatives, had healthy children, and had no history of miscarriage and/or stillbirth. Phytohemagglutinin-induced peripheral blood lymphocytes were cultured for 72 h. The Giemsa–Trypsin–Leischman (GTL) banding technique was applied to the obtained metaphase plates; thirty metaphase plates were examined at the 450-550 band level in each individual.

**Results:** A total of 488 individuals in the case group and 13 in the control group carried polymorphic chromosome variants. 9qh+ chromosome polymorphisms were more prevalent in the case group than in the control group (p=0.028). Other variants were also increasingly observed in the case group (p=0.014).

**Conclusion:** Our findings reveal a potential relationship between RPL and chromosome polymorphisms. Karyotype analysis and appropriate genetic counseling increase the chance of having healthy children in individuals with RPL.

**Keywords:** Recurrent pregnancy loss, cytogenetics, chromosome polymorphisms

#### ÖZ

**Amaç:** Bu çalışmada, iki veya daha fazla tekrarlayan gebelik kaybı (TGK) olan vaka grubu (n=1688) ve kontrol grubu (n=80) kromozom polimorfizmleri (1qh+, 9qh+, inv9, 13ps+, 14ps+, 15ps+, 16qh+, 21 ps+, 22 ps+ ve Yqh+) açısından değerlendirildi.

**Gereç ve Yöntem:** Kontrol grubu bilinen bir kalıtsal hastalığı olmayan, akraba olmayan, sağlıklı çocuğu olan, düşük ve/veya ölü doğum öyküsü olmayan 40 evli çiftten seçildi. Fitohemaglutinin ile indüklenmiş periferik kan lenfositlerinin 72 saatlik kültürü yapıldı. Elde edilen metafaz plaklarına Giemsa-Trypsin-Leischman (GTL) bantlama tekniği uygulandı. Her birey için yapılan kromozom analizinde 450-550 bant seviyesinde 30 metafaz plağı incelendi.

**Bulgular:** Polimorfik kromozom varyantı taşıyan bireylerin sayısı vaka grubunda 488 ve kontrol grubunda ise 13 olarak belirlendi. 9qh+ kromozom polimorfizminin kontrole göre vaka grubunda daha yüksek oranda olduğu belirlendi (p=0,028). Bununla birlikte, diğer bazı varyantların da vaka grubunda artış eğilimi gösterdiği gözlemlendi (p=0,014).

**Sonuç:** Bulgularımız TGK ile kromozom polimorfizmleri arasında bir ilişki olabileceğini kanıtladı. TGK olan bireylerde karyotip analizi ve uygun genetik danışma sağlıklı çocuk sahibi olma şansını artırmaktadır.

**Anahtar Kelimeler:** Tekrarlayan gebelik kaybı, sitogenetik, kromozom polimorfizmleri

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## INTRODUCTION

Recurrent pregnancy loss (RPL), or habitual abortion, is the spontaneous termination of two or more consecutive pregnancies before the 20th week of pregnancy. If RPL develops following a live birth, it is called secondary, and if there is no history of successful pregnancy, it is called primary RPL. RPL is observed in 1–3% of pregnancy losses (1). The risk is 31% on average in those who have experienced two or more pregnancy losses (2).

Many factors contribute to the etiology of RPL such as parental chromosomal anomalies, fetal anomalies, hypothyroidism, diabetes mellitus, anatomical anomalies of the uterus, antiphospholipid antibody syndrome, endocrine disorders, thrombophilia, immunological abnormalities, infections, and environmental factors. However, the cause of approximately half of RPLs remains unexplained (3).

Chromosomal polymorphisms are considered variations and are defined as differences in the size or staining of chromosome segments (4, 5). Heterochromatic regions are structures that contain tandem repeat sequences and do not encode active genes; they are evaluated as normal karyotype variations, and phenotypic reflections are not expected (6).

This study investigated the relationship between RPL and chromosomal polymorphisms. Cytogenetic analyses were performed on 1688 cases presenting with a history of RPL and a control group consisting of 40 married couples.

## MATERIALS and METHODS

### Determination of case and control groups

The current study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee Decision (Date: 08.09.2017, No: 233). Chromosomal polymorphisms were retrospectively evaluated in 1688 cases, 939 females and 749 males, who visited the Faculty of Medicine in the Depart-

ment of Medical Genetics at Afyon Kocatepe University, were diagnosed with RPL between 2007 and 2017, and underwent karyotype analysis. Of the 1688 cases evaluated, 665 participated as couples, as some spouses did not provide samples. This retrospective study was conducted on images and archive preparations by selecting archived files from patients who did not have any chromosomal abnormalities or consanguineous marriages. The control group consisted of 40 healthy married couples who had no known hereditary disease, were not relatives, could have healthy children, and had no history of miscarriage and/or stillbirth. Individuals in the control group signed a voluntary consent form, and cytogenetic analyses were performed. Structural and numerical anomalies were excluded from evaluation, and we focused only on chromosomal variants.

### Chromosome preparations

From the control group, 2 ml of peripheral blood were collected in heparinized tubes under sterile conditions, and closed lymphocyte culture was performed using standard techniques. The Giemsa-Trypsin-Leishman (GTL) banding technique was applied to metaphase plates. For each individual, 30 metaphase plates at the level of 450-550 bands were obtained. Other banding methods (C banding and NOR banding) were applied when deemed necessary. Three experts examined the metaphase plate using the Applied Imaging Cytovision Image Analysis System. The results were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013.

### Statistical analysis

Data were compared in the Statistical Package for the Social Sciences (IBM SPSS Corp., Armonk, NY, USA) version 25 software using the  $\chi^2$  test, and  $p < 0.05$  was considered significant.

## RESULTS

Of the 1688 cases evaluated retrospectively, 939 were female and 749 were male. There were 665 married couples in this case group. Their ages ranged from 17 to 54 years, and the

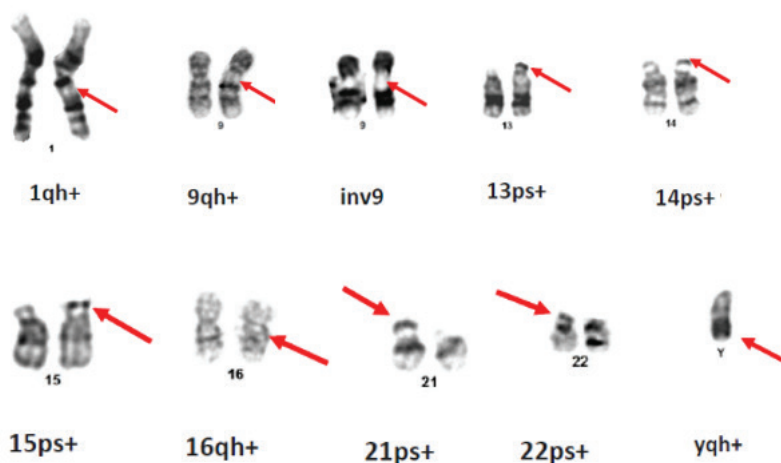


Figure 1. Chromosome images of 10 polymorphic variants detected in the case group

**Table 1.** The chromosomal polymorphisms and frequencies in the case group (%)

Polymorphism	Female (n=939)	%	Male (n=749)	%
1qh+	3	0.3	8	1.1
9qh+	170	18.1	130	17.2
inv9	11	1.2	7	0.9
13ps+	20	2.1	19	2.5
14ps+	29	3.1	19	2.5
15ps+	24	2.6	15	2.0
16qh+	3	0.3	4	0.5
21 ps+	42	4.5	32	4.3
22 ps+	18	1.9	23	3.1
Yqh+	-	-	1	0.1

**Table 2.** Distribution of 9qh+ chromosome polymorphisms in the case and control groups

	9qh+		Total	p
	Presence	Absence		
Case group	300	1388	1688	0.028
Control group	5	75	80	
Total	305	1463	1768	

**Table 3.** Presence of chromosome polymorphism in couples in the case and control groups

	Presence	Absence	Total	p
Case group	305	360	665	0.045
Control group	12	28	40	
Total	317	388	705	

**Table 4.** Presence of chromosome polymorphisms in individuals in the case and control groups

	Presence	Absence	Total	p
Case group	488	1200	1688	0.014
Control group	13	67	80	
Total	501	1267	1768	

average was 28 years. The ages of the controls, consisting of 40 married couples, ranged from 26 to 70 years, and the average was 41 years.

1qh+, 9qh+, inv9, 13ps+, 14ps+, 15ps+, 16qh+, 21 ps+, 22 ps+, and Yqh+ polymorphisms were observed in the case group (Figure 1). 9qh+ polymorphism was detected at the highest rate (17.2%), whereas Yqh+ polymorphism was detected at the lowest rate (0.1%) (Table 1).

9qh+ polymorphism was detected in 300 out of 1688 individuals in the case group and in five out of 80 individuals in the control group (Table 2). 9qh+ polymorphism was higher in the case group than in the control group ( $p < 0.05$ ). (Table 2).

Among couples in the case group, 9qh+ polymorphism was detected in a spouse in 163 couples and in both spouses in 37 co-

uples. In the control group, 9qh+ polymorphism was detected in a spouse of five couples. The distribution differed between the case and control groups ( $p < 0.05$ ). 9qh+ polymorphism was significantly higher in the couples in the case group.

Chromosome polymorphisms were detected in 305 of 665 couples in the case group and in 12 of 40 couples in the control group. There was a difference in the presence or absence of polymorphism between the pairs in the case and control groups ( $p < 0.05$ ). Polymorphisms were more frequently detected in the case group ( $p < 0.05$ ) (Table 3).

Chromosome polymorphism was detected in 488 of 1688 individuals in the case group and in 13 of 80 individuals in the control group. There was a difference between the case and control groups in the presence or absence of polymorphic va-

variants ( $p < 0.05$ ). Polymorphisms were observed significantly more frequently in the case group ( $p < 0.05$ ) (Table 4). In the retrospectively evaluated case group, the rate of coexistence of two chromosome polymorphisms in an individual was 3.5% (60/1688), whereas the rate of coexistence of three chromosome polymorphisms was 0.29% (5/1688).

## DISCUSSION

RPL is a serious health problem that affects couples who want children. Multiple genetic and/or environmental risk factors contribute to the etiology of RL (7, 8), which is observed in 1–5% of pregnancy losses (9-13). Chromosomal anomalies are responsible for 75% of spontaneous early pregnancy losses. More than 90% of fetal chromosome anomalies are numerical anomalies. The remainder consists of structural anomalies and mosaicism (14, 15). Chromosomal polymorphisms are normal variations that occur in 2–5% of the general population; they are usually found in the genetically inactive heterochromatic regions of chromosomes (7). A total of 576 (34.1%) polymorphic variants were detected in 320 female and 256 male individuals out of 1688 cases retrospectively evaluated in the case group. In our study, the 9qh+ polymorphism, which occurred most frequently, was detected in 170 female (18.1%) and 130 male (17.1%) individuals. In the case group, 9qh+ polymorphism was detected in one partner in 163 couples and in both partners in 37 couples. In the control group, the polymorphism was detected in only one individual among five couples. A difference was detected between the case and control groups: 9qh+ polymorphism was frequently observed in the case group couples. In a study conducted in Diyarbakır on 455 couples with RPL, chromosome polymorphism was detected in 8.4% of the case group and 4.9% of the control group. This study, like ours, found an association between RPL and chromosome polymorphisms. However, unlike our study, Akbaş et al. found that the distribution of chromosome polymorphisms in the RPL group was higher in males (11.3%) than in females (5.4%) (16). Other studies suggest that chromosomal polymorphisms contribute to recurrent miscarriages (17, 18). Although our results are compatible with the literature, they also show an accumulation of 9qh+ polymorphisms in Turkey's Afyonkarahisar region.

We did not observe a relationship between the distribution of chromosomal polymorphisms and gender in our study group, which agrees with other studies (18, 19). Sheth et al. reported a significantly higher number of polymorphic variants in women than in men (20), though this may be due to the sex imbalance in the study. However, a study in Mexico found a greater number of chromosomal polymorphisms in men in the RPL group than in women, and the distribution was similar in the control group (17).

Among chromosomal polymorphisms analyzed individually between the case and control groups, variants other than 9qh+ were similarly distributed. However, when evaluated as a percentage, polymorphic variants were increasingly observed in the case group. Though not statistically significant, this may indicate limitations in our control group. Literature data on the

deviation of polymorphic variants in RPL cases are variable (21, 22). Karaca et al. found that the frequencies of heteromorphism were similar in 384 case couples and 136 control couples (23). Moreover, several studies found no relationship between heteromorphisms and recurrent pregnancy loss (24-26). On the other hand, Nie and Lu focused on the Y chromosome and found that 16qh+ polymorphisms may cause RPL and infertility (27). In a study conducted with 842 couples with a history of RPL and/or infertility, polymorphic variants in heterochromatin regions were potentially related to RPL (27, 28).

Differences were detected in the distributions of chromosome variations evaluated collectively between the control and case groups. In the case group, the chromosomal polymorphisms were very high. Madon et al. karyotyped 842 individuals admitted for primary infertility or recurrent miscarriages and reported a very high rate of polymorphic variants: 28.82% in males and 17.19% in females. These data support the relationship between chromosomal polymorphisms and RPL. Many studies have shown that polymorphisms, especially in the heterochromatin region, are strongly associated with pregnancy loss (16, 22, 28-32). Polymorphic variants on chromosomes were considered "normal," as heterochromatin has no coding potential, and nucleolar regulatory regions (NOR) containing rRNA-encoding genes are therefore not reflected in the phenotype. However, studies conducted using improved molecular techniques suggest that fertility and viability genes are indeed located in heterochromatin. DNA sequencing of chromosome 9, on which we found qh+ in 17.7% (300/1688) of the case group cases in our study, showed that it is structurally highly polymorphic, is observed with many intra- and interchromosomal duplications, and contains the largest autosomal heterochromatin block (29). Many studies have been conducted using Sanger sequencing to investigate genetic factors in RPL cases. Genome-wide association studies aim to identify genomic regions and SNPs that may be associated with RPL; some polymorphisms in HLA genes, *FHIT*, *FAM154A*, *PDEA*, and *GRIK2* genes may be associated with increased risk, but no significant molecular marker has been identified (33, 34).

## CONCLUSION

Our study measured the incidence of RPLs in Afyonkarahisar and genetically analyzed them. Our results highlight the necessity and validity of cytogenetic analyses in couples with RPL. The data herein will guide the couples as they approach subsequent pregnancies. However, our study would benefit from larger case and control groups. More data obtained using molecular cytogenetic techniques, such as FISH and array-CGH, can also guide further research.

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**Ethics Committee Approval:** Ethics committee approval was received for the current study from the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee Decision (Date: 08.09.2017, No: 233).

**Informed Consent:** Informed consent was obtained from each patients.

**Peer Review:** Externally peer-reviewed.

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

**Conflict of Interest:** The authors have no conflict of interest to declare.

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# AN UPDATE OF CLINICAL TRIALS LANDSCAPE IN TÜRKİYE TÜRKİYE'DEKİ KLİNİK ARAŞTIRMA MANZARASININ GÜNCELLEMESİ

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## ABSTRACT

**Objective:** Clinical research is essential to enhance scientific knowledge and provide better patient outcomes. This work evaluated clinical trials in Türkiye between 2019-2023 and compared them with previous research.

**Material and Method:** Clinical trials in Türkiye registered in the ClinicalTrials.gov database between 01.01.2019 and 30.09.2023 were examined and evaluated. Trials were filtered by study type, study phase, recruitment status, funder type, location, condition, or disease. Comparative analyses were executed for different cities and countries.

**Results:** The total number of registered clinical trials in Türkiye was 12,563. The number of trials registered between 2019 and 2023 was 8,851. Of these, 68.21% were interventional and 9.3% were industry-sponsored. The majority of trials (61.13%) were completed, while 18.78% were still recruiting patients. Leading cities in Türkiye were İstanbul and Ankara with the highest trial numbers. Despite a remarkable increase in the total number of clinical trials in Türkiye, no considerable change was detected in the number of industry-sponsored trials. The majority of trials with unknown status (85%) were non-industry-sponsored in all countries in the comparative analysis. The highest numbers of trials were found in respiratory, pain/pain management and oncology in Türkiye among the evaluated fields including cardiovascular disease, genetic disorders, diabetes, and obesity.

**Conclusion:** The total number of clinical trials in Türkiye showed a considerable increase when compared with developed countries including Germany, France, and the United Kingdom, however, the reasons behind the lower number and stability in the yearly increase rate of industry-sponsored trials should be revisited by all stakeholders.

**Keywords:** Clinical trials, Türkiye, research

## Öz

**Amaç:** Klinik araştırmalar bilimsel bilgiyi geliştirmek ve hastalar için daha iyi sonuçlar elde etmek adına gerekli araçlardır. Bu çalışma Türkiye'de 2019-2023 yılları arasında yapılmış olan klinik çalışmaları değerlendirmek ve önceki analizle karşılaştırma yapmayı amaçlamaktadır.

**Gereç ve Yöntem:** Türkiye'de yapılmış olan klinik araştırmaları değerlendirmek için ClinicalTrials.gov veri tabanına 01.01.2019 ve 30.09.2023 tarihleri arasında kaydedilen klinik araştırmalar incelenmiştir. Araştırmalar çalışma türü, çalışma fazı, çalışmanın durumu, destekleyici türü, lokasyon, hastalık durumuna göre filtrelenmiştir. Türkiye'deki farklı şehirler ve farklı ülkeler için karşılaştırmalı analiz yapılmıştır.

**Bulgular:** ClinicalTrials.gov veritabanında Türkiye'de kayıtlı olan klinik araştırma toplam sayısı 12563'tür. 2019-2023 yılları arasında kaydedilmiş olan çalışma sayısı ise 8851'dir. Bu çalışmaların %68,21'si girişimsel, %9,3'ü endüstri tarafından desteklenen çalışmalardır. Çalışmaların çoğunluğu olan %61,13'ü tamamlanmıştır, %18,78'i ise hala hasta alımı yapmaktadır. En fazla klinik çalışma kayıtlı olan iller İstanbul ve Ankara'dır. Türkiye'de toplam klinik çalışma sayısında belirgin bir artış olmasına rağmen endüstri tarafından desteklenen çalışma sayısında anlamlı bir değişiklik saptanmamıştır. Karşılaştırmalı analize dahil edilen ülkelerin tamamında durumu bilinmeyen çalışmaların çoğunluğu (%85) endüstri tarafından desteklenmeyen çalışmalardır. Kardiyovasküler hastalık, genetik hastalıklar, diyabet ve obezitenin de dahil olduğu farklı tıbbi alanlar değerlendirildiğinde Türkiye'de klinik çalışma sayısının en fazla olduğu alanlar solunum hastalıkları, ağrı/ağrı yönetimi ve onkoloji olmuştur.

**Sonuç:** Türkiye'de klinik çalışma sayısı artmış ve Almanya, Fransa, Birleşik Krallık gibi gelişmiş ülkelerle karşılaştırıldığında benzer bir sayıya ulaşmıştır. Bununla birlikte, endüstri tarafından desteklenen çalışmaların sayısının daha düşük olması ve yıllık artışlarının sabit olmasının nedenleri tüm paydaşlar tarafından tekrar gözden geçirilmelidir.

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## INTRODUCTION

Clinical trials in Türkiye between 2009 and 2018 were investigated in an earlier report and published in 2020. It included the evaluation of the number of clinical trials, study type, study phase, and funder type by using ClinicalTrials.gov database as the global source (1, 2). Previous work has shown that the number of clinical trials in Türkiye was not as high as expected. According to the findings the total number of registered trials in Türkiye was 3,880, of which 42% were industry-sponsored trials, and 74.9% were interventional trials. The total number of clinical trials in Türkiye was reported to be less than developed countries with a comparable population (1).

Türkiye is a country with a growing population of over 80 million, so contributions to clinical research are essential to improve scientific knowledge in different disease areas and to achieve improvements in public health (1). In addition to the direct impact on scientific improvement and economic growth, clinical trials may offer patients the best access and treatment to new promising treatment options, especially in the management of serious conditions, such as rare diseases and oncology (3). The latest regulations and legislation for clinical trials in Türkiye are given on the website of the Turkish Medicine and Medical Devices Agency (4). Türkiye strives to improve the approval and permission process and keep clinical trials procedures updated in line with new developments in clinical research and technology.

In this work, we investigated and evaluated the current setting for clinical trials in Türkiye between 2019 and 2023. All registered clinical trials were evaluated according to study type, study phase, recruitment status, funder type, location, condition, or disease. Additionally, the main results were compared with selected countries.

## MATERIAL and METHODS

The clinical trials posted between 01.01.2019 and 30.09.2023 in the ClinicalTrials.gov database were analyzed to determine the current setting in Türkiye (2). The detailed evaluation was executed according to study type, recruitment status, study phase, funder type, gender, age, and location including different cities in Türkiye and other countries.

For the comparative analysis Germany (83.31 million), France (67.93 million) and the United Kingdom (67.73 million) were selected as the leading countries in the field of clinical research in Europe due to their comparable populations. Other countries including the United States of America (333.28 million), China (1.4 billion), the Russian Federation (143.55 million), South Africa (59.89 million), Egypt (109.3 million), Argentina (46.23 million), Thailand (71.69 million) were selected based on their location on different continents. The United States and China were selected as leading countries in the field of clinical trials globally. The database of the Worldbank was used for the population analysis of different countries selected for the comparative analysis (5).

For an analysis of different disease areas, specific terms were used for the condition or disease. The search in the database of

ClinicalTrials.gov was executed for respiratory diseases by using the keywords "respiratory disease", "respiratory failure", "asthma", "pneumonia", "chronic obstructive disease", "respiratory tract disease", "respiration disorder" or "respiratory insufficiency". The search for pain and pain management was executed by using the keywords "postoperative pain", "chronic pain", "acute pain", "back pain", "neck pain", "headache", "migraine", "analgesia", "pain management", "cervical pain" or "pain control". The search for oncology was executed by using the keywords "oncology", "cancer" or "tumor". The search for genetic diseases was executed by using the keywords "genetic disorder", "genetic disease", "genetic syndrome" or "chromosomal syndrome". The search for cardiovascular diseases was executed by using the keywords "cardiovascular disease", "coronary artery disease", "coronary syndrome", "heart failure", "arrhythmia", "hypertension", "cardiomyopathy", "valvular heart disease", "endocarditis" or "congenital heart disease". The search for diabetes was executed by using the keywords "diabetes mellitus", "diabetes" or "diabetic". The search for obesity was executed by using the keyword "obesity".

## Statistical analysis

The present work was designed as a descriptive study; therefore, a specific analytical statistical test was not conducted. The findings are given as numbers and percentages.

## RESULTS

The total number of registered studies in Türkiye accessible on ClinicalTrials.gov was 12,563 while the total number of registered studies between 2019 and 30 September 2023 was 8,851 which were evaluated in this analysis (2). The majority (68.21%) were interventional studies. Observational studies comprised the remaining 31.75% and there were four expanded access studies (0.04%). Among observational studies, there were 617 patient registries.

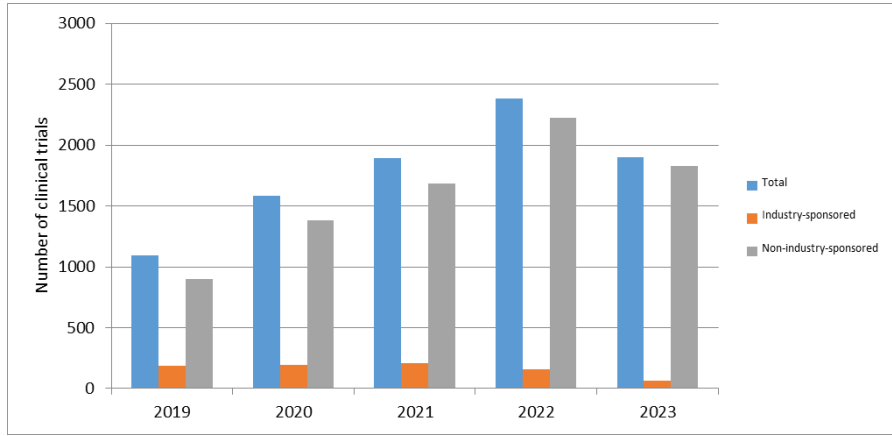
The number of registered studies increased each year between 2019 and 2022. The evaluation according to study type showed that 824 studies out of the total 8,851 were industry-sponsored, and that number increased between the years 2019 and 2021 but decreased between 2022 and 2023 (Figure 1).

Female participants were included in 8,560 studies, while male participants were included in 7,373. Five thousand six-hundred and eleven (5,611) studies included older adults. Older adults were described as participants over 65 years of age. The pediatric population was included in 1,928 studies which was defined by the ages between 0 to 17 years.

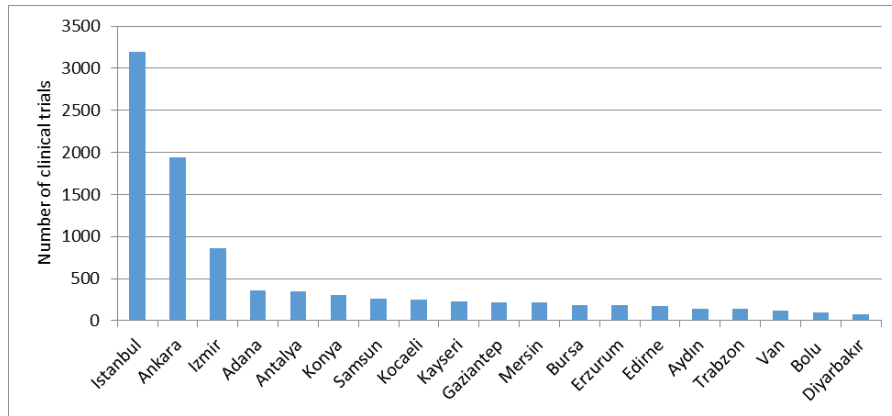
The cities with the highest number of studies were Istanbul and Ankara. Three thousand one-hundred and ninety-four (3,194) studies were conducted in Istanbul and 1,939 studies were conducted in Ankara. There was a decrease in the number of studies starting in Izmir and 865 studies across other parts of the country (Figure 2).

The studies were evaluated according to the study phase. Out of the total of 74 studies in Early phase 1/Phase 1, 53 studies



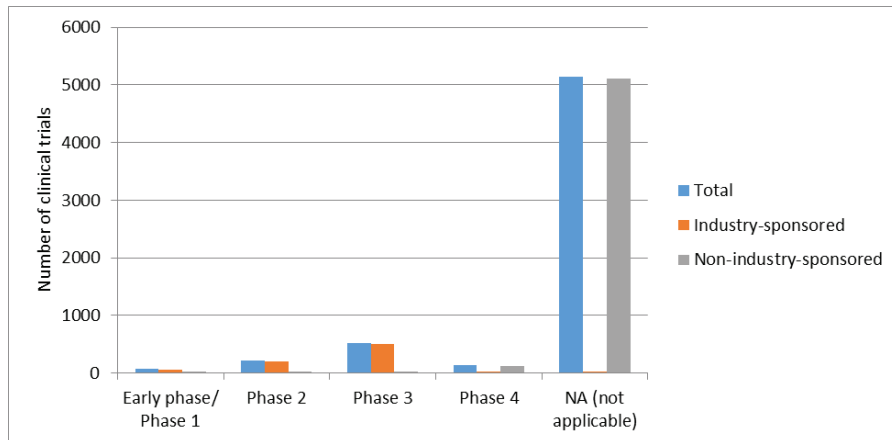


**Figure 1:** Number of registered clinical trials between 2019 and 2023 on ClinicalTrials.gov in Türkiye and their distribution according to funder type



**Figure 2:** Location of clinical trials in Türkiye\*

\*Multicenter clinical trials are in multiple locations which may lead to an excess in the total number of trials registered (8,851) in Türkiye.



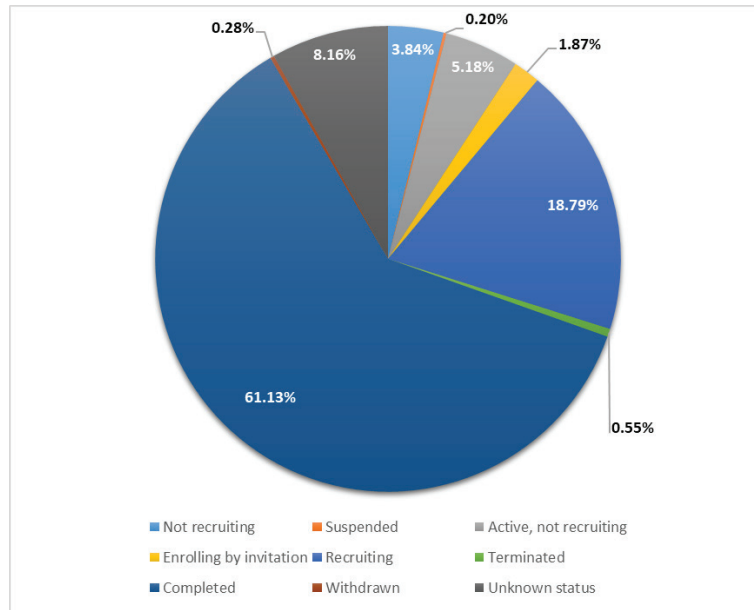
**Figure 3:** Distribution of clinical phase studies in Türkiye \*

\*“NA (not applicable)” studies did not have a phase definition registered in the database.

were industry-sponsored. The number of Phase 2 studies was 225, and Phase 3 studies were 525 with a high proportion of industry-sponsored trials (87% and 96%). The number of Phase 4 studies was 136 (14% industry-sponsored) and the number of studies without defined phases (“NA” studies; not applicable

studies) was 5,140 of which only 31 were industry-sponsored. The majority were non-industry-sponsored (99.4%) (Figure 3).

The percentage of industry-sponsored clinical trials conducted with drugs/pharmaceuticals and medical devices were 86.94%



**Figure 4:** Clinical trials in Türkiye according to recruitment status

and 4.19%, respectively. The rest (8.87%) consisted of trials with other interventions such as psychotherapy, surgery, diet, etc., and other types of clinical studies such as patient registries, development of biomarkers and diagnostic tests.

The evaluation according to recruitment status (including 8,847 studies and excluding 4 expanded access studies) showed that most of the trials (61.13%) were “completed” (n=5408). One thousand six-hundred and sixty-two (1,662) studies were still “recruiting patients” and 340 studies were “not recruiting” as of the last update on ClinicalTrials.gov (14.10.2023). The number of “withdrawn” studies was 25 (0.28%), 49 studies were “terminated” (0.55%) and 18 studies were “suspended” (0.20%) According to recruitment status 458 studies were “active but not recruiting”. There were 722 studies with unknown recruitment status (Figure 4).

The recruitment status is defined in the database as follows ; “Not yet recruiting”: The study has not started recruiting participants; “Recruiting”: The study is currently recruiting participants; “Enrolling by invitation”: The study is selecting its participants from a population, or group of people, decided on by the researchers in advance. These studies are not open to everyone who meets the eligibility criteria but only to people in that particular population, who are specifically invited to participate; “Active, not recruiting”: The study is ongoing, and participants are receiving an intervention or being examined, but potential participants are not currently being recruited or enrolled; “Suspended”: The study stopped early but may start again; “Terminated”: The study stopped early and will not start again. Participants are no longer being examined or treated; “Completed”: The study ended normally, and participants are no longer being examined or treated (that is, the last participant’s last visit occurred); “Withdrawn”: The study stopped early, before enrolling its first participant; “Unknown”:

A study on ClinicalTrials.gov whose last known status was recruiting; not yet recruiting; or active, not recruiting but has passed its completion date, and the status has not been verified within the past 2 years.

The United States (US), China, France, United Kingdom (UK), Germany, the Russian Federation, Egypt, Argentina, and Thailand were included in the comparative analysis of registered clinical trials. The total registered number of studies between 2019-2023 was highest in the United States (n=47,329) and China (n=18,406). The number of clinical studies in France (n=12,725) was higher than in Türkiye. The number of non-industry-sponsored trials were higher than industry-sponsored trials in all the countries except Germany, the Russian Federation and Argentina (Figure 5).

The comparative evaluation of France, Germany, the United Kingdom, and Türkiye demonstrated that there was a decrease in the number of clinical trials in Germany and the United Kingdom between 2020 and 2023. The number of clinical trials in France decreased starting from 2020 as well whereas the number in Türkiye increased between 2019 and 2022. Despite the decrease in registered trials in Türkiye in the first three quarters of 2023, the number of registered trials in Türkiye is higher than the numbers in France as of 2023 (Figure 6).

The number of clinical trials with “unknown status” was also evaluated in these countries which was highest in China and lowest in Argentina. The vast majority of trials with “unknown status” were non-industry-sponsored (Table 1).

Among the registered clinical trials in Türkiye 866 were conducted in “respiratory”, 852 in “pain/pain management”, 811 in “oncology”, 645 in “cardiovascular”, 230 in “genetic disorders”, 219 in “diabetes” and 158 in “obesity” disease areas. The number of industry-sponsored trials was higher in oncology (37.6%) and genetic disorders (46.5%) when compared with other disease areas (Figure 7).

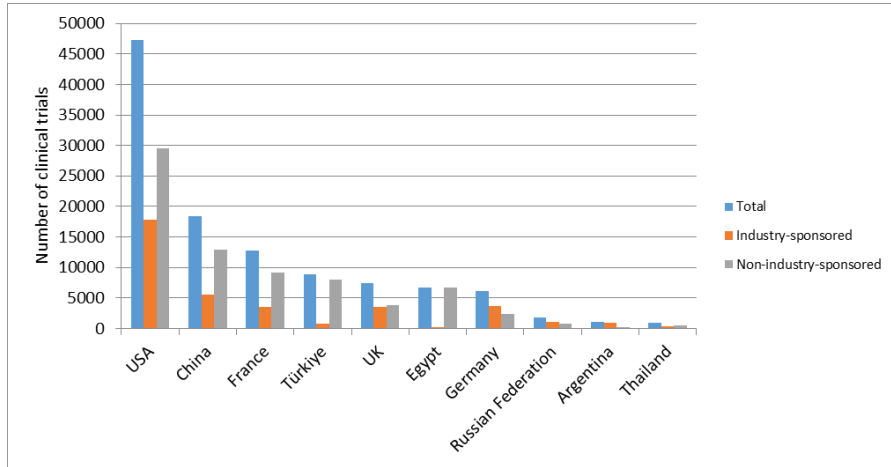


Figure 5: Comparison of clinical trial numbers between different countries (2019-2023)

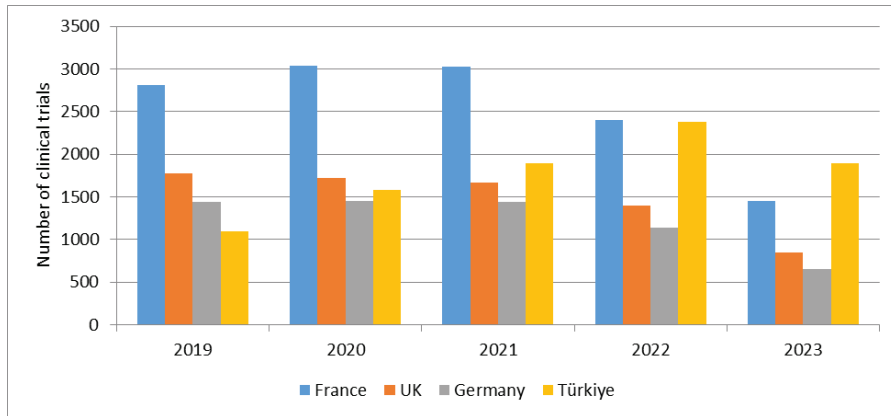


Figure 6: Comparison of numbers of clinical trials in different countries between 2019 and the first 3 quarters of 2023

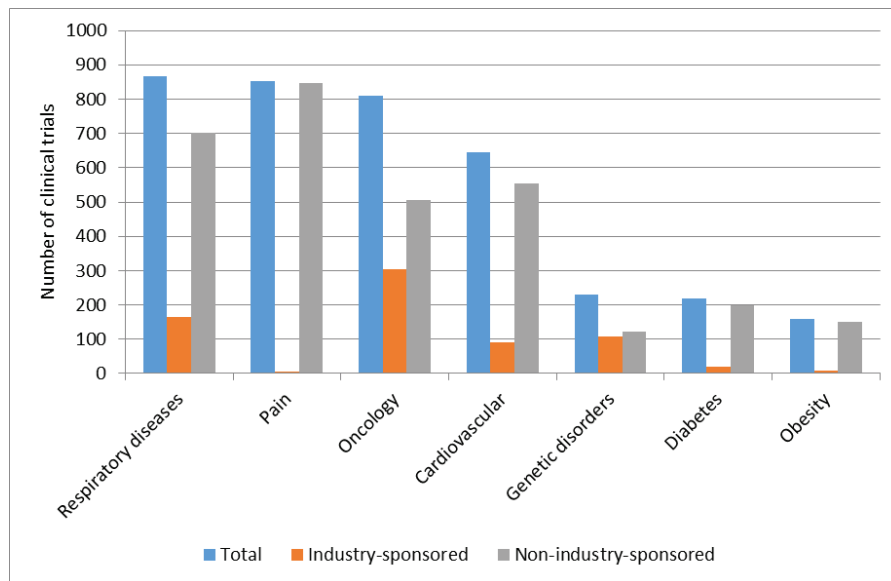


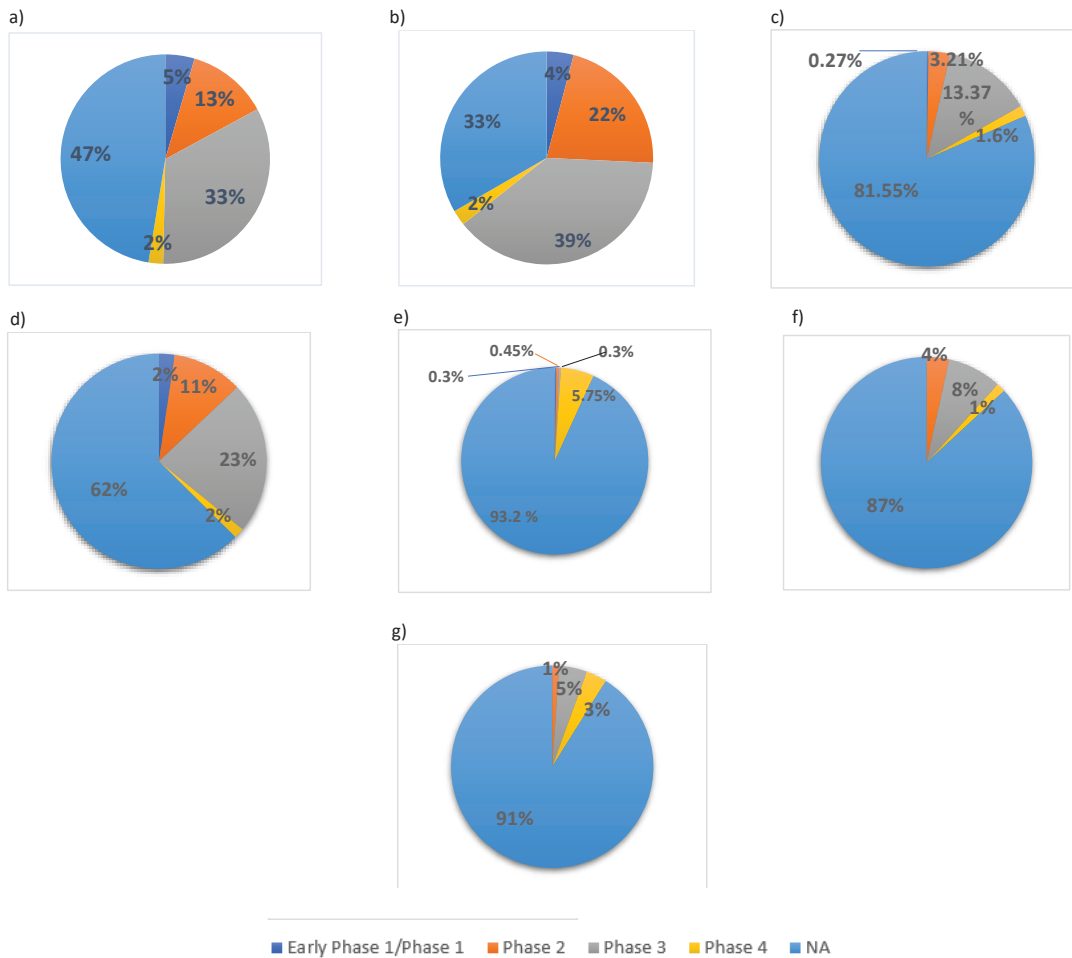
Figure 7: Clinical trials in different disease areas in Türkiye

**Table 1:** Number of clinical trials with “unknown status” and their distribution according to study funding type

	Total number	Industry-sponsored	Non-industry-sponsored
USA	1502	487	1015
China	3411	641	2770
France	1377	86	1291
Türkiye	722	19	703
United Kingdom	650	87	563
Egypt	1125	1	1124
Germany	347	53	294
Russian Federation	154	37	117
Argentina	49	9	40
Thailand	75	1	74

The number of clinical trials in oncology in Türkiye was 811 of which 74% were interventional and 26% were observational studies including 37 patient registries. Forty-seven percent (47%) of the interventional studies were “not applicable” which means that they did not have an applicable phase definition according

to the definition in the database. The vast majority of Early phase/Phase 1 (93%), Phase 2 (93.5%), Phase 3 (99.5%) and Phase 4 (35.7%) studies were industry-sponsored. Only one of the studies without a phase definition (NA; “not applicable” study) was industry-sponsored (Figure 8).



**Figure 8:** Evaluation of clinical trials in Türkiye in different disease areas according to study phase: a) Oncology, b) Genetic diseases, c) Cardiovascular disease, d) Respiratory diseases, e) Pain/Pain management, f) Diabetes, g) Obesity

The clinical trials in the genetic diseases area registered in Türkiye in the ClinicalTrials.gov database were mostly interventional trials (69%) most of which were Phase 3 trials (39%). There were 13 patient registries among observational trials. The majority of trials in Early phase/Phase 1 (85.7%), Phase 2 (94.5%), Phase 3 (95.45%), and Phase 4 (100%) were industry-sponsored. Only one of the "not applicable" studies (NA) was industry-sponsored (Figure 8).

The number of studies in the cardiovascular disease area in Türkiye between 2019-2023 was 645. Forty-two point five percent (42.5%) were observational trials including 67 patient registries. The majority of the Early phase/Phase 1 (100%), Phase 2 (92%) and Phase 3 studies (98%) were industry-sponsored. Eighty-one point fifty-five percent (81.55%) of the interventional trials were "not applicable" (NA) and most were non-industry-sponsored (97.4%) (Figure 8).

Our search in the respiratory diseases area comprised COVID-19 studies and lung cancer studies as well. There were 866 studies in respiratory diseases, 18.9% were industry-sponsored. Fifty-seven percent (57%) of the trials were interventional. Observational trials included 72 registries. The majority of the Early phase/Phase 1 studies (75%), Phase 2 studies (81%) and Phase 3 studies (95%) were industry-sponsored. Sixty-two percent (62%) of the interventional trials were "not applicable" when stratified according to phase definition (Figure 8).

The number of trials in pain and pain management was 852 of which five studies were industry-sponsored. There were 192 observational trials including 45 patient registries accessible in the database. Seventy-seven point four percent (77.4%) of the trials were interventional trials. The majority of the interventional trials (93.2%) were "not applicable" according to the phase definition from the database (Figure 8).

The number of studies in the diabetes area was 219. The percentage of industry-sponsored trials was 9.5%. Seventy-four (74) of the registered trials were observational including 12 patient registries. Of the 145 interventional trials there was no Early phase/Phase 1 study registered in the database. All the Phase 3 studies were industry-sponsored. There were only two Phase four trials of which one was industry-sponsored. The majority of the "not applicable" (NA) trials were non-industry-sponsored (Figure 8).

The trials in obesity in Türkiye comprised 90 interventional and 68 observational trials which included 15 patient registries. There was no Early phase/Phase 1 trial registered in the database. Ninety-one percent (91%) of the interventional studies were "not applicable" (NA) when stratified according to the phase description in the database. The percentage of Phase 4 trials was 3% (n=4), all of which were industry-sponsored (Figure 8).

## DISCUSSION

Like previous reports on clinical trials, the majority of clinical trials are being conducted in the United States and Europe. Clinical trials are vital tools of scientific advancement for all

stakeholders in the healthcare system. Patients, investigators, health authorities and funder bodies including both the pharmaceutical/medical device industry and public organizations/institutions benefit from clinical trials. In addition to scientific purposes, due to the economic impact of sponsored clinical trials on the healthcare system, all countries make improvements and increase awareness to get the maximum benefit.

In this work we analyzed the years 2019, 2020, 2021, 2022, and the first 3 quarters of 2023 and detected an increase in the number of clinical trials in Türkiye between 2019 - 2022 and a slight decrease in the first 3 quarters of 2023. When compared to the previous analysis, there was a considerable increase in the total number for the last five year period, however, the number of industry-sponsored trials (9.3%) was still lower than non-industry-sponsored trials throughout the period of our evaluation (1). Although it might not be the only factor, the registration of clinical research for publication requirement (6) asked by many scientific journals could have led to the high numbers of registered investigator-initiated studies in the ClinicalTrials.gov database (1). The influence of the increased awareness of researchers and the improvements in clinical trial regulations may also be other important factors. Although it was not possible to reach a definitive conclusion, the overall findings in our study show the enhancement and widespread research culture at the country level. As an expected result, in line with the population of cities, Istanbul, Ankara and Izmir are still the leading cities with the highest number of clinical trials.

The comparison with leading European countries in the field of clinical research demonstrated an increase in registered clinical trials in Türkiye. In the previous evaluation covering the years before 2019, the number of clinical trials in France, the United Kingdom and Germany was considerably higher than Türkiye. However, the present work covering the last five years demonstrated that the total number of trials in Türkiye was higher than Germany and the United Kingdom and even surpassed the numbers of France in the first 3 quarters of 2023. However, it should be taken into account that the number of industry-sponsored trials registered on ClinicalTrials.gov in France (27.5%), the United Kingdom (47.9%) and Germany (60.3%) were higher than in Türkiye (9.3%). There was also an unbalanced distribution of the number of industry-sponsored and non-industry-sponsored clinical trials among all countries. We also reviewed the number of trials with "unknown status" in different countries. Eighty-five percent (85%) of the trials with unknown status were non-industry-sponsored trials. The highest percentage was achieved in China where 18.5% of the clinical trials registered between 2019-2023 were unknown. The lowest percentage (4.4%) was obtained in Argentina where the vast majority of registered studies were industry-sponsored. This again may be evidence of tighter control and a higher quality of industry-sponsored studies.

Different disease areas were also evaluated such as oncology, respiratory diseases, genetic disorders, cardiovascular disease, diabetes, and obesity. The number of clinical trials in respiratory, pain/pain management, oncology and cardiovascular disease areas were prominently higher than in other disease areas with

a large number of industry-sponsored trials in oncology (37.6%). The search for respiratory disease trials in the database of ClinicalTrials.gov also included COVID-19 and lung cancer studies which may have contributed to the high number of trials in the respiratory disease area. Another fact and a possible limitation are that lung cancer trials are included in both respiratory disease and oncology trials which may have led to an overlap between respiratory and oncology trial evaluations. The high number of industry-sponsored trials in oncology could be an indication of an unmet need in this field.

In the evaluation according to different disease areas we detected an increase in the number of respiratory disease trials by 176% in 2020, but only 12% in 2021. Similarly, the number of trials increased by 79% in cardiovascular disease, 37% in oncology, 37% in diabetes and 21% in obesity in 2020 which may be related to the globally increased numbers of research activities during the COVID-19 pandemic. There was also an increase in the number of trials in the pain/pain management area by 9% in 2020, 38% in 2021 and 47% in 2022. There was no yearly increase in the number of trials in the field of genetic diseases.

The majority of the Early phase/Phase 1, Phase 2, and Phase 3 trials in oncology and genetic diseases area were industry-sponsored as well. We detected a remarkable number of trials in the pain/pain management area, most of which were investigator-initiated research activities. The number of Early phase/Phase 1 trials was highest in oncology (n=28) followed by respiratory diseases (n=12). Another interesting outcome was the low number of clinical trials in diabetes and obesity despite the prevalence of diabetes mellitus and obesity in our country (7, 8). There was no Early phase/Phase 1 trial in diabetes or the obesity disease areas either.

Regarding the intervention/treatment among industry-sponsored trials, it demonstrated that the vast majority (86.94%) of the trials were conducted with drugs/pharmaceuticals whereas only 4.19% of the trials were conducted with medical devices. The rest (8.87%) consisted of trials with other interventions such as psychotherapy, surgery, diet, etc., and other types of clinical studies including patient registries, development of biomarkers and diagnostic tests. By using the ClinicalTrials.gov database it was not possible to stratify if the clinical trials were supported by local or international pharmaceutical or device companies due to multinational collaborations in the field of clinical trials.

## CONCLUSION

Clinical trials that were registered on ClinicalTrials.gov between 2019 and the first three quarters of 2023 were included in this work. We provided an overview of the clinical trial setting in our country by filtering trials based on study types, recruitment status, study phases and evaluated different disease areas. To gain insights about our country's status on a global level we compared Türkiye with other countries. The total number of clinical trials in Türkiye increased and reached a high level when compared with developed countries including Germany, France, and the United Kingdom, however, the possible reasons for the lower number and the stability in the yearly increase rate of industry-sponsored trials should be revisited by all stakeholders of clinical trials.

**Ethics Committee Approval:** Information in the ClinicalTrials.gov database was used in the study.

**Peer Review:** Externally peer-reviewed.

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

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The author declared that this study has received no financial support

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# THE IMPACT OF INDIVIDUAL HEALTH PERCEPTION ON CYBERCHONDRIA BEHAVIOR

## BİREYSEL SAĞLIK ALGISININ SİBERKONDRI DAVRANIŞI ÜZERİNDEKİ ETKİSİ Mustafa FİLİZ<sup>1</sup>, Yalçın KARAGÖZ<sup>2</sup>

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### ABSTRACT

**Objectives:** The aim of this study is to determine the effect of individual health perception on cyberchondria tendency and to identify differences in individual health perception and cyberchondria tendency according to various demographic variables.

**Material and Methods:** The sample of the study consists of 400 individuals over the age of 18, residing in Istanbul, Türkiye. The individual health perception scale and cyberchondria severity scale were used as data collection tools. The t-test and ANOVA tests for difference analyses, correlation analysis to examine the direction of the relationship and multiple connection problem between sub-dimensions of the scale, and multiple regression analysis were used to determine the effect of individual health perception on cyberchondria tendency.

**Results:** The results indicate that participants exhibit sub-average tendencies towards cyberchondria but maintain favorable perceptions of their health. Upon examination, no significant variations in health perception were identified based on marital status, gender, age, income level, place of residence, healthcare facility type, or annual frequency of hospital visits ( $p>0.05$ ). Notably, individuals with a bachelor's degree manifested more pronounced cyberchondria behaviors compared to those with associate degrees or high school diplomas ( $p<0.05$ ). The data also underscores a significant correlation between individual health perceptions and the propensity towards cyberchondria ( $p<0.05$ ).

**Conclusion:** It was concluded that 49% of individuals' cyberchondria levels are explained by health perception. It was found that the education factor had a significant effect on individual health perception and cyberchondria tendency, but demographic factors such as gender, age, income, type of settlement, type of hospital, and annual hospital visit status did not have any effect. It is recommended to design customized informational campaigns or interventions aimed at specific educational groups to correct potential misunderstandings and improve health literacy.

**Keywords:** Health perception, disease perception, cyberchondria, digital health

### ÖZ

**Amaç:** Bu çalışmada bireysel sağlık algısının siberkondri eğilimi üzerindeki etkisinin belirlenmesi ve çeşitli demografik değişkenlere göre bireysel sağlık algısı ve siberkondri eğiliminin farklılıklarının belirlenmesi amaçlanmıştır.

**Gereç ve Yöntemler:** Araştırmanın örneklemi, Türkiye'nin İstanbul ilinde ikamet eden 18 yaş üstü 400 kişiden oluşmaktadır. Veri toplama aracı olarak bireysel sağlık algısı ve siberkondri ciddiyet ölçeği kullanılmıştır. Verilerin analiz edilmesinde, fark analizleri için t testi ve ANOVA testi, ölçek alt boyutları arasındaki ilişkinin yönünü ve çoklu bağlantı sorununu incelemek için korelasyon analizi ve son olarak bireysel sağlık algısının siberkondri eğilimi üzerindeki etkisini belirlemek amacıyla çoklu regresyon analizi kullanılmıştır.

**Bulgular:** Bireylerin siberkondri eğilimlerinin ortalamasının altında olduğu, sağlık algılarının ise iyi düzede olduğu bulgulanmıştır. Medeni duruma, cinsiyete, yaşa, gelir durumuna, yerleşim türü, hastane türüne ve yıllık hastaneye gitme sayısına göre bireylerin sağlık algısının değişmediği görülmektedir ( $p>0,05$ ). Lisans mezunu bireylerin siberkondri davranışları, önlisans ve lise mezunu bireylere göre daha yüksek bulunmuştur ( $p<0,05$ ). Bireysel sağlık algısının siberkondri eğilimi üzerinde anlamlı şekilde etkili olduğu tespit edilmiştir ( $p<0,05$ ).

**Sonuç:** Bireylerin, siberkondri düzeylerinin %49'unun sağlık algısı tarafından açıklandığı sonucuna ulaşılmıştır. Bireysel sağlık algısı ve siberkondri eğiliminde eğitim faktörünün önemli etkisi olduğu, ancak cinsiyet, yaş, gelir, yerleşim türü, hastane türü ve yıllık hastaneye gitme durumu gibi demografik faktörlerin herhangi bir etkisi olmadığı tespit edilmiştir. Belirli eğitim gruplarına yönelik potansiyel yanlışları düzeltmek ve sağlık okuryazarlığını artırmak amacıyla özelleştirilmiş bilgilendirme kampanyaları veya müdahaleler tasarlamak faydalı olacağı öngörülmektedir.

**Anahtar Kelimeler:** Sağlık algısı, hastalık algısı, siberkondri, dijital sağlık

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## INTRODUCTION

With the widespread use of the internet and digital devices, individuals have become more dependent on online information to alleviate their health-related concerns. The internet has facilitated people's access to health-related information. However, this easy access has also been associated with a range of negative consequences. In recent years, cyberchondria, a phenomenon where individuals tend to excessively search for online health-related information, often leading to heightened anxiety and stress levels, has emerged (1). Research found that individual health perceptions and cognitive and emotional evaluations regarding one's own health status, have a significant impact on cyberchondria behavior (2). Understanding the relationship between individual health perception and cyberchondria behavior is crucial in addressing potential negative outcomes of excessive online searches for health information (3). Cyberchondria is a term used to describe the excessive use of the internet to search for health information. Individuals with cyberchondria often worry excessively about their health and spend hours each day searching for information about possible illnesses. This anxiety can lead to stress and even depression (4).

Cyberchondria refers to the excessive search for medical care and health information in the online environment, triggered by anxiety and distress about a person's health condition (5). While online health information searching is a common behavior, cyberchondria becomes maladaptive when accompanied by excessive and escalating anxiety (6). The concept of cyberchondria has drawn attention in both clinical and research environments due to its association with increased distress, anxiety levels, and interference with daily activities (7). It is not just a tendency to search for health-related information on the internet, but also an abnormal behavior pattern and emotional state (8). However, it is important to note that cyberchondria is not a separate diagnostic category, but a behavior pattern observed among individuals with higher health anxiety (1, 2, 6, 7). Understanding the factors contributing to cyberchondria behavior is critical for developing effective interventions and strategies. Previous research examined various factors that could contribute to the development and maintenance of cyberchondria, including personality traits, health-related metacognition, cognitive bias, and emotional dysregulation (9). Studies have also explored the relationship between cyberchondria and other constructs such as self-esteem, health anxiety, and obsessive-compulsive symptoms (2).

Individual health perception refers to how people perceive their own health and well-being. People with a negative health perception are more likely to worry about their health and engage in unhealthy behaviors such as excessive internet use (10). There are several reasons why people with a negative health perception are more likely to be cyberchondriacs. Firstly, the internet provides people with a way to seek reassurance about their health. Secondly, the internet provides access to a wealth of information about health, which can be overwhelming for individuals with a negative health perception. Thirdly,

the internet provides a sense of control and mastery, which may appeal to people who feel they have little control over their health (4, 8, 11, 12). Cyberchondria refers to the excessive and obsessive search for online health information (13). Cyberchondria is particularly prevalent among individuals with high health-related anxieties and can lead to unnecessary utilization of health services and a decrease in overall quality of life (5).

Research suggests that health perceptions may play a pivotal role in cyberchondriac behavior (2). Individual health perception is defined as an individual's evaluation of their own health status and quality. Individuals with a negative health perception are prone to excessively and obsessively searching for online health information. This heightens health-related anxieties and leads to a worsening of cyberchondriac behavior (14). This article aims to contribute to the existing literature by examining the effect of health perceptions on cyberchondriac behavior. By investigating how individuals perceive their own health and the connection to cyberchondria, this study aims to illuminate the psychological factors underlying the issue. The findings derived from this research may be significant in understanding and addressing cyberchondria in clinical and preventive contexts. Understanding this issue can aid in the development of strategies to prevent and treat cyberchondriac behavior. It could also be crucial in optimizing the use of health services and improving the overall quality of life.

### Developing research hypotheses

The following model has been developed within the scope of the literature.

Under the model shown in Figure 1, the following hypotheses were developed.

H1: An individuals' health perception affects cyberchondria behavior.

While examining the main hypothesis, the influence of the individual health perception's sub-dimensions (Control Center/Precision/Importance of Health/Self-Awareness) on the sub-dimensions of cyberchondria (Compulsion/Extreme Anxiety/Extremism/Soothe/Distrust of the Doctor) has also been tested.

## MATERIALS and METHODS

### Population and sample

The population of the study consists of individuals aged 18 and over living in Istanbul. In the literature, there are criteria for determining the sample in the study. In the literature, it is accepted that when the population exceeds 100,000, 384 participants are considered to represent the population (15, 16). Based on this criterion, a minimum of 384 individuals is required for a 95% confidence interval.

In the research, a total of 411 participants were engaged, and based on the predefined criteria, this sample size was deemed adequate. However, 11 respondents were excluded from the analysis due to consistently selecting the same options or providing inconsistent answers. The study employed the conve-



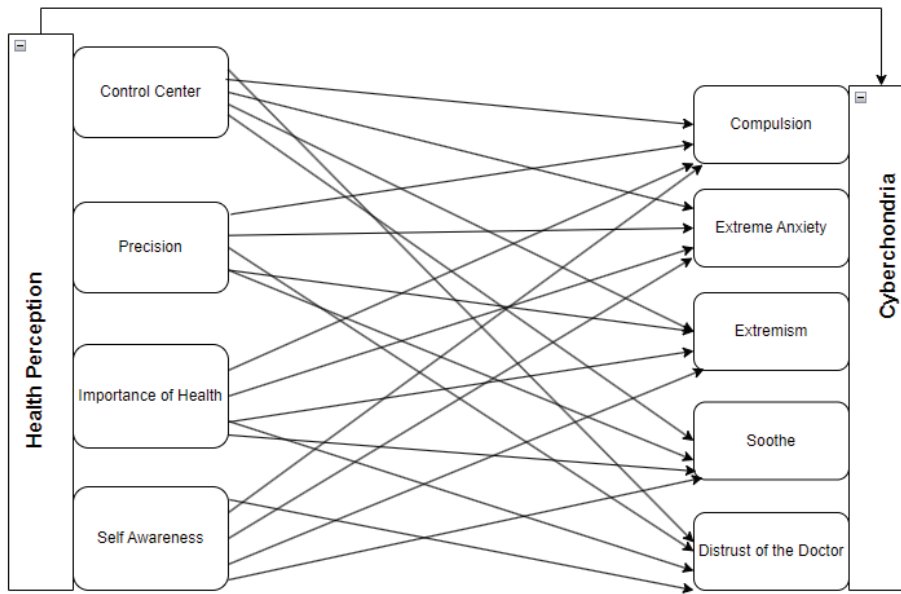


Figure 1: Research model

nience sampling method for participant selection, primarily due to its efficiency, simplicity, and cost-effectiveness (16).

#### Data collection tools

In the study, a questionnaire was used as the data collection tool, and the questionnaire consists of three sections.

Demographic information form: This form was created by the researchers. The form contains a total of eight questions, including the gender of the participants, educational level, income status, the type of residence where they spend their lives, marital status, age, annual average of hospital visits, and the type of hospital visited.

Cyberchondria severity scale: This scale was developed by McElroy and Shevlin (17). The scale consists of five dimensions and 33 statements. The dimensions are compulsion (eight statements), excessive worry (eight statements), excessiveness (eight statements), reassurance seeking (six statements), and distrust of medical profession (three statements). The Turkish validity and reliability study of the scale was conducted by Uzun and Zencir (18).

The Cronbach's alpha coefficient of the scale was determined as 0.962. Since this value is in the range of  $0.80 \leq \alpha < 1.00$ , the scale is highly reliable. The Cronbach's alpha coefficients of the sub-dimensions of the scale; the compulsion sub-dimension was determined as 0.905, the excessive worry sub-dimension as 0.868, the excessiveness sub-dimension as 0.793, the reassurance seeking sub-dimension as 0.771, and the distrust of the medical profession sub-dimension as 0.718.

Individual health perception scale: The scale was developed by Diamond et al.; the Turkish validity and reliability study was

conducted by Kadioğlu and Yıldız (19, 20). The scale consists of 15 items and four sub-dimensions. The scale is a five-point Likert type. A maximum of 75 points and a minimum of 15 points can be obtained from the scale. A high score indicates high health perception, and a low score indicates low health perception.

The Cronbach's alpha coefficient of the scale was determined as 0.982. Since this value is in the range of  $0.80 \leq \alpha < 1.00$ , the scale is highly reliable. The Cronbach's alpha coefficients of the sub-dimensions of the scale; the control center sub-dimension was determined as 0.706, the certainty sub-dimension as 0.700, the importance of health sub-dimension as 0.810, and the self-awareness sub-dimension as 0.801.

#### Collection and analysis of research data

After obtaining ethical approval for the study, the data was collected online from individuals living in Istanbul between the dates of June 16, 2023, and July 25, 2023. The survey was initially transferred to Google Drive in an appropriate manner. A Google survey form was created. The linked survey form was first tested by researchers on their personal phones to ensure functionality. Finally, the survey link was distributed to individuals residing in Istanbul via the Whatsapp application. The link was shared in various groups through university students living in Istanbul.

The study addresses individual health perception as the independent variable and the cyberchondria tendency as the dependent variable. The research type of this study is quantitative and correlational.

In an analysis of the data obtained, the normality distribution was first checked to decide which method to use. For this purpose, the study examined whether the average scores of

the scales and subdimensions show normal distribution. The skewness and kurtosis coefficient values, which are indicators of normality of the obtained data, were considered in making this decision. The skewness and kurtosis values of both scales and subdimensions show a distribution within the normal limits (+2.114/-2.297). Additionally, the homogeneity of variances was examined, and the p-values for the sub-dimensions of the scale were generally greater than .05. Based on this, the variances are homogeneously distributed (21).

In the study, parametric methods, specifically T-tests and ANOVA tests, were conducted to uncover differences among participants based on demographic variables. Before performing regression analysis, a correlation analysis was conducted to examine whether there was a multicollinearity problem between the subdimensions of the scale. To reveal the effect of the participants' levels of health perception on cyberchondria levels, a multivariate regression analysis was conducted. The IBM SPSS 25 package program was used in performing the analysis.

### Ethical declarations in the research

After determining the purpose and scope of the study, necessary forms were prepared to evaluate its ethical suitability and an application was made to the Artvin Çoruh University Scientific Research and Publication Ethics Committee (Date: 07.06.2023, No: 2023/6-18). For the individuals aged 18 and above who participated in the research, a short paragraph was included at the beginning of the survey to state the purpose of the study and their informed consent was obtained.

### RESULTS

When looking at the demographic characteristics of the individuals participating in the study, it shows that 53.8% (n:215) are male, 46.3% (n:185) are female, 49.8% (n:199) are between the ages of 18-25. 27.5% (n:110) are 26-35 years old, 16.8% (n:67) are 36-45 years old, 4.3% (n:17) are 46-55 years old and 1.8% (n:7) are 56 years old and above. Looking at the income situation, 1.5% (n:6) stated that their income is very bad, 8.8% (n:35) said their income is bad, 59.0% (n:236) said their income is at a medium level, 28.2% (n:113) said their income is good and 2.5% (n:10) said their income is very good. It shows that 7.8% (n:31) of the participant individuals live in a village,

20.3% (n:83) in a district/town, 45.0% (n:180) in a city center and 26.5% (n:106) in a metropolis. When looking at the educational status of the participants, it shows that 4.8% (n:19) are primary/middle school graduates, 12.0% (n:48) are high school graduates, 14.5% (n:58) are associate degree graduates, 64.3% (n:257) are undergraduate graduates and 4.5% (n:18) are postgraduate graduates. Participants generally receive services from public hospitals (46.0%, n:184), private hospitals (23.8%, n:95), university hospitals (11.8%, n:47), and city hospitals (18.5%, n:74). When evaluated in terms of the marital status of the individuals, it shows that 40.0% (n:160) are married and 60% (n:240) are single. In terms of participants' annual hospital visits, it shows that 63.5% (n:254) visit the hospital between 0-4 times, 26.5% (n:106) 5-8 times, and 10.0% (n:40) visit the hospital more than 9 times a year.

Table 1 provides basic values related to the sub-dimensions of the scale. In general, the variables of compulsion (1.51), excessive worry (2.32), and reassurance seeking (2.31) have an average of "never", excessiveness (2.87), locus of control (2.60), and certainty (2.99) have an average of "sometimes", and distrust of medical profession (3.95), importance of health (3.74), and self-awareness (3.71) have been answered as "usually" (Table 1).

In the study, a t-test was conducted to determine differences between the two groups. The results obtained are given in Table 2.

**Table 1:** Descriptive statistics related to factors

Factors	n	Mean	Standard deviation
Compulsion	400	1.51	0.70506
Extreme anxiety	400	2.32	0.84185
Extremism	400	2.87	0.76248
Soothe	400	2.31	0.81558
Distrust of the doctor	400	3.95	0.97310
Control center	400	2.60	0.77075
Precision	400	2.99	0.80119
Importance of health	400	3.74	0.62594
Self-awareness	400	3.71	0.68647

n: Total number

**Table 2:** Differences by gender and marital status

Sub dimensions	Variable	n	Mean	t	p
Health perception	Woman	185	3.12	1.419	0.157
	Male	215	3.18		
Cyberchondria	Woman	185	2.40	0.029	0.977
	Male	215	2.41		
Health perception	Married	160	2.41	0.346	0.730
	Single	240	2.39		
Cyberchondria	Married	160	3.16	0.330	0.739
	Single	240	3.14		

n: Total number, t: T Value, P: Level of significance

In Table 2, the individuals' health perception and cyberchondria behaviors do not change according to gender ( $p>0.05$ ).

In Table 2, the individuals' health perception and cyberchondria behaviors do not change according to marital status ( $p>0.05$ ).

In the study, an Anova test was conducted to reveal differences between three or more groups. The results obtained are given in Table 3.

In Table 3, the individuals' health perception and cyberchondria behaviors do not change according to variables such as age, income status, type of residence, type of hospital generally received service from, and average annual hospital visit situation ( $p>0.05$ ). However, the health perception and cyberchondria behaviors change according to the individuals' level of education ( $p<0.05$ ). To discern this variance, the choice of which Post Hoc multiple comparison tests to utilize is determined by examining the homogeneity test. If the p-value of the homogeneity test is greater than 0.05, it is concluded that the population variances are the same (homogeneous). If the

p-value of the homogeneity test is less than 0.05, it is decided that the population variances are different (21). Based on the conducted homogeneity test results, Levene's statistic for the perception of health was found to be 1.953 with a p-value of 0.071. The Levene's statistic for the perception of cyberchondria was determined to be 1.495 with a p-value of 0.178. Accordingly, it was decided that the variances for both the perception of health and cyberchondria are the same, indicating homogeneity. In this context, due to the greater sensitivity of the LSD test in producing results, the study decided to employ the LSD test.

It was found that the health perception levels of individuals with associate degrees (3.27) were higher than those with bachelor's degrees. Additionally, it was determined that the cyberchondria behaviors of individuals with bachelor's degrees (2.44) were at a higher level compared to those with associate (2.27) and high school (2.25) degrees (Table 3).

The results of the correlation analysis conducted for the sub-dimensions of the scale are given in Table 4.

**Table 3: Differences according to various demographic characteristics**

Demographic indicators	Variable	F	p	Reason of the difference
Age	Health perception	1.840	0.120	No Difference
	Cyberchondria	0.346	0.847	No Difference
Income status	Health perception	1.215	0.304	No Difference
	Cyberchondria	0.140	0.967	No Difference
Placement type	Health perception	1.630	0.182	No Difference
	Cyberchondria	0.358	0.783	No Difference
Education	Health perception	4.455	0.000	Associate degrees (3.27) Bachelor's degrees (3.12)
	Cyberchondria	3.947	0.001	Bachelor's degrees (2.44)/ Associate degrees (2.27) high school (2.25)
Hospital type	Health perception	0.304	0.823	No Difference
	Cyberchondria	0.295	0.829	No Difference
Annual average hospitalization status	Health perception	0.493	0.782	No Difference
	Cyberchondria	1.920	0.090	No Difference

F: F Value, P: Level of Significance

**Table 4: Relationships between study variables**

	1	2	3	4	5	6	7	8	9
1. Compulsion	1								
2. Extreme anxiety	0.542**	1							
3. Extremism	0.260**	0.636**	1						
4. Soothe	0.423**	0.648**	0.604**	1					
5. Distrust of the doctor	-0.191**	-0.005	0.101*	-0.037	1				
6. Control center	0.246**	0.172**	0.107*	0.137**	-0.151**	1			
7. Precision	0.152**	0.312**	0.205**	0.215**	-0.019	0.189**	1		
8. Importance of health	0.055	0.181**	0.174**	0.227**	0.101*	0.070	0.014	1	
9. Self awareness	0.004	0.014	0.104*	0.127**	0.108*	-0.132**	-0.059**	0.354**	1

\*: Correlation is significant at the 0.05 level (2-tailed), \*\*: Correlation is significant at the 0.01 level (2-tailed).

When Table 4 is examined, most of the relationships between the sub-dimensions are significant. Evaluating the correlation coefficients, since the tolerance values calculated for all variables are below 0.10 and VIF values are not above 10, it can be said that there is no multicollinearity problem (Table 4) (22).

Finally, a multiple regression analysis was conducted to determine the effect of the level of health perception on cyberchondriac behavior. The findings are presented in Table 5.

In Model 1, the regression model created to determine whether the sub-dimensions of the level of health perception affect the level of cyberchondria sub-dimension of compulsion is statistically significant (F:7.925; p:0.000<0.05). The adjusted R2 value is found to be 0.065. According to this result, it shows that 6% of the variance in the compulsion levels of individuals is explained by health perception. Accordingly, the sub-dimensions of control center ( $\beta=0.208$ , p:0.000<0.05) and certainty

( $\beta=0.097$ , p:0.026<0.05) have a positive effect on the level of cyberchondria. The dimensions of the importance of health and self-awareness have no effect on the compulsion sub-dimension (p>0.05) (Table 5).

In Model 2, the regression model created to determine whether the sub-dimensions of health perception level affect the level of excessive anxiety, which is the sub-dimension of cyberchondria, is statistically significant (F:15.983; p:0.000<0.05). The adjusted R2 value is found to be 0.131. This result shows that 13% of variance in the excessive anxiety levels of individuals is explained by health perception. Accordingly, the sub-dimensions of control center ( $\beta=0.112$ , p:0.034<0.05), certainty ( $\beta=0.303$ , p:0.000<0.05), and the importance of health ( $\beta=0.237$ , p:0.001<0.05) have an impact on the level of excessive anxiety. The self-awareness dimension does not have any effect on the excessive anxiety sub-dimension (p>0.05) (Table 5).

**Table 5: The impact of individual health perception on cyberchondria behavior**

Models	Dependent variable	Independent variable	$\beta$	t	p	F	Model (p)
Model 1	Compulsion	Control center	0.208	4.546	<b>0.000</b>	7.925	0.000
		Precision	0.097	0.110	<b>0.026</b>		
		Importance of health	0.059	0.027	0.610		
		Self-awareness	0.032	0.593	0.553		
		R <sup>2</sup> :.074Adjusted.R <sup>2</sup> :0.065. F:7.925					
Model 2	Extreme anxiety	Control center	0.112	2.133	<b>0.034</b>	15.983	0.000
		Precision	0.303	6.064	<b>0.000</b>		
		Importance of health	0.237	3.504	<b>0.001</b>		
		Self-awareness	-0.022	-0.355	0.722		
		R <sup>2</sup> :0.139;Adjusted.R <sup>2</sup> :0.131 F:15.983.					
Model 3	Extremism	Control center	0.070	1.423	0.156	8.542	0.000
		Precision	0.185	3.943	<b>0.000</b>		
		Importance of health	0.170	2.682	<b>0.008</b>		
		Self-awareness	0.084	1.448	0.148		
		R <sup>2</sup> :.080;Adjusted.R <sup>2</sup> :.070 F:8.542.					
Model 4	Soothe	Control center	0.103	1.980	<b>0.048</b>	12.123	0.000
		Precision	0.202	4.101	<b>0.000</b>		
		Importance of health	0.244	3.662	<b>0.000</b>		
		Self-awareness	0.101	1.657	0.098		
		R <sup>2</sup> :.109;Adjusted.R <sup>2</sup> :.100 F:12.123.					
Model 5	Distrust of the doctor	Control Center	-0.192	-2.983	<b>0.003</b>	3.907	0.004
		Precision	0.015	0.238	0.812		
		Importance of health	0.143	1.724	0.085		
		Self-awareness	0.079	1.045	0.297		
		R <sup>2</sup> :.038;Adjusted.R <sup>2</sup> :.028 F:3.907.					
Model 6	Cyberchondria	Health perception	0.486	7.479	0.000	55.938	0.000
R <sup>2</sup> :.124;Adjusted.R <sup>2</sup> :.121 F:55.938.							

$\beta$ : Effect Level, t: T Value, P: Level of Significance, F: F Value

In Model 3, the regression model created to determine whether the sub-dimensions of the health perception level affect the level of excessiveness, which is a sub-dimension of cyberchondria, is statistically significant ( $F:8.542$ ;  $p:0.000<0.05$ ). The adjusted R2 value is found to be 0.070. This result shows that 7% of the variance in the excessiveness levels of individuals is explained by health perception. Accordingly, the sub-dimensions of certainty ( $\beta=0.185$ ,  $p:0.000<0.05$ ) and the importance of health ( $\beta=0.170$ ,  $p:0.008<0.05$ ) have an impact on the level of excessiveness. The control center and self-awareness dimensions do not have any effect on the excessiveness sub-dimension ( $p>0.05$ ) (Table 5).

In Model 4, the regression model created to determine whether the sub-dimensions of health perception level affect the level of reassurance, which is a sub-dimension of cyberchondria, is statistically significant ( $F:12.123$ ;  $p:0.000<0.05$ ). The adjusted R2 value is found to be 0.100. This result shows that 10% of the variance in the reassurance levels of individuals is explained by health perception. Accordingly, the sub-dimensions of control center ( $\beta=0.103$ ,  $p:0.048<0.05$ ), certainty ( $\beta=0.202$ ,  $p:0.000<0.05$ ), and the importance of health ( $\beta=0.244$ ,  $p:0.000<0.05$ ) affect the level of reassurance. The self-awareness dimension does not have any effect on the reassurance sub-dimension ( $p>0.05$ ) (Table 5).

In Model 5, the regression model created to determine whether the sub-dimensions of health perception level affect the level of distrust of the doctor, which is a sub-dimension of cyberchondria, is statistically significant ( $F:3.907$ ;  $p:0.004<0.05$ ). The adjusted R2 value is found to be 0.028. This result shows that 3% of the variance in the distrust of the doctor levels of individuals is explained by health perception. Accordingly, the sub-dimension of control center ( $\beta=-0.192$ ,  $p:0.003<0.05$ ) negatively affects the level of distrust of the doctor. Certainty, the importance of health, and the self-awareness dimensions have no effect on the distrust of the doctor sub-dimension ( $p>0.05$ ) (Table 5).

In Model 6, the regression model created to determine whether the level of health perception affects the level of cyberchondria is statistically significant ( $F:55,938$ ;  $p:0.000<0.05$ ). The adjusted R2 value is 0.486. This result shows that 49% of the variance in the cyberchondria levels of individuals is explained by health perception. As a result, hypothesis H1 has been accepted (Table 5).

## DISCUSSION

This study yielded important findings to reveal the effect of individual health perception on cyberchondria tendency and to determine health perception and cyberchondria behavior according to various demographic variables. In this section, the findings obtained in the study will be discussed by comparing them with the literature.

It was found that individuals' cyberchondria perceptions were below average and their health perceptions were at a good level.

In the literature, it is generally found that individuals prone to cyberchondria have more anxiety about their health status (23). The findings of this study show that this tendency is not present in a general sample, which could be a positive finding. The fact that individuals' cyberchondria tendencies are low and their personal perceptions are at a good level is considered as a positive situation. This shows that most individuals do not tend to overinterpret online health information that could lead to excessive concern. The fact that individuals generally perceive their health as good is a sign of a general health awareness and a positive health status.

It was found that individuals' health perceptions did not change according to marital status, gender, age, income status, type of settlement, type of hospital, and the number of annual hospital visits. It was found that the health perception levels of individuals with an associate degree were higher than those of individuals with a bachelor's degree. In the literature, it is particularly found that age and gender have a significant effect on health perception (24). However, this study shows that health perception varies depending on the cultural and regional factors of the sample data. It also shows that health perception is independent of these factors. However, found that the health perception of those with an associate degree is higher than those with a bachelor's degree, indicating that the level of education has an effect on health perception.

It was found that cyberchondriac behaviors of individuals did not change according to marital status, gender, age, income status, type of settlement, type of hospital, and the number of annual hospital visits. It was found that the cyberchondriac behaviors of individuals with a bachelor's degree were higher than those of individuals with an associate degree and high school graduates. This finding is consistent with the findings mentioned in the literature (25). This shows that cyberchondriac behaviors are also independent of these factors. However, the fact that individuals with a bachelor's degree have higher cyberchondriac behaviors than individuals with an associate degree and high school graduates is interpreted as indicating that a higher level of education may lead to a tendency to search for more online health information and misinterpret this information.

It was found that 49% of the variance in individuals' cyberchondria levels was explained by health perception. In the literature, a strong relationship has been noted between health anxiety and cyberchondria (26). This finding is consistent with the literature and confirms that individuals' general perceptions of their health play a significant role in determining cyberchondria levels. This demonstrates a significant impact in an individuals' general perceptions of their health status, how they interpret online health information and, as a result, the type of cyberchondriac behavior they exhibit. An individual who thinks they are healthy may be less likely to overinterpret specific health information. Delving deeper, it becomes evident that this relationship is rooted in the manner in which individuals interact with online health information. An individual's inherent

health perceptions significantly shape their interpretation and assimilation of online health data (27). In essence, an individual with positive health perceptions might approach online health content with a discerning perspective, thus reducing the propensity for unwarranted health anxieties. Conversely, those with negative or uncertain health perceptions might be more prone to misinterpreting or overemphasizing specific health information, leading to heightened cyberchondria tendencies (28). As highlighted by Glinert, the vast realm of online health information, if not navigated judiciously, can exacerbate health anxieties in susceptible individuals (29). Our findings resonate with this sentiment, suggesting that bolstering an individuals' accurate health perceptions and health literacy can act as potential mitigators against the spiral of cyberchondria (29).

Considering the research findings and results, the following suggestions could be useful.

More research is needed to better understand the relationship between cyberchondriac behaviors and health perception. This will allow us to better understand the impact of access to online health information on an individuals' health perceptions and behaviors.

Since there is a relationship between the level of education, cyberchondriac behaviors, and health perception, it is recommended to develop educational programs to increase health awareness to reduce these behaviors. These programs should provide training on how individuals can search for and interpret health information and should also help them develop a healthy health perception.

Since cyberchondriac behaviors are higher in individuals with a bachelor's degree, it is suggested to develop special preventive and intervention strategies focusing especially on this group.

Health professionals should be sensitive to a patients' behaviors and provide appropriate guidance and support when necessary.

More regulatory efforts are suggested regarding the presentation and accessibility of online health information. This could reduce the risk of excessive anxiety or cyberchondria based on misleading or confusing information.

The relationship between cyberchondria and psychopathologies experienced in the digital environment can be explored in-depth or through qualitative research.

It is anticipated that investigating the functional use of the internet as a factor in reducing cyberchondria could be valuable for further research.

#### **Study limitations**

**Sample Selection:** The sample in the study was selected using a convenience sampling technique, which is not a random selection method. While the convenience sampling technique might have been preferred due to its speed, ease, and cost-effectiveness, it limits the ability of the sample to represent the entire population. This is particularly the case where the

sample consists of individuals aged 18 and over living in Istanbul, which might not fully represent a wider population (e.g., individuals prone to cyberchondria across Türkiye or globally).

**Data Collection Time and Method:** The data was collected online between June and July 2023. The quality of responses from online data collection is lower compared to offline data collection because online responses are generally less thoughtful and provided more quickly. Additionally, due to the time and duration of data collection, seasonal factors or transient events could have influenced the results.

The absence of any questions regarding the frequency of internet usage from the participants is also considered a limitation of the study.

Considering the research findings and results, the following suggestions could be useful.

More research is needed to better understand the relationship between cyberchondriac behaviors and health perception. This will allow us to better understand the impact of access to online health information on an individuals' health perceptions and behaviors.

Since there is a relationship between the level of education, cyberchondriac behaviors, and health perception, it is recommended to develop educational programs to increase health awareness to reduce these behaviors. These programs should provide training on how individuals can search for and interpret health information and should also help them develop a healthy health perception.

Since cyberchondriac behaviors are higher in individuals with a bachelor's degree, it is suggested to develop special preventive and intervention strategies focusing especially on this group.

Health professionals should be sensitive to a patients' behaviors and provide appropriate guidance and support when necessary.

More regulatory efforts are suggested regarding the presentation and accessibility of online health information. This could reduce the risk of excessive anxiety or cyberchondria based on misleading or confusing information.

The relationship between cyberchondria and psychopathologies experienced in the digital environment can be explored in-depth or through qualitative research.

It is anticipated that investigating the functional use of the internet as a factor in reducing cyberchondria could be valuable for further research.

#### **CONCLUSION**

This study revealed that individuals generally perceive cyberchondria below average and perceive their health at a good level. This indicates that individuals do not tend to overinterpret online health information and generally perceive their health in a good way.

Also, considering various demographic and socioeconomic factors (marital status, gender, age, income status, type of settlement, type of hospital, and number of annual hospital visits), it found that an individuals' health perception and cyberchondriac behaviors do not change according to these factors. However, the level of education affected both health perception and cyberchondriac behaviors. Specifically, while the health perception levels of individuals with an associate degree were higher than those with a bachelor's degree; the cyberchondriac behaviors of individuals with a bachelor's degree were higher than those of individuals with an associate degree and high school graduates.

The most significant finding is that 49% of cyberchondria levels is explained by health perception. This finding indicates that health perception plays a significant role in determining how individuals interpret health-related online information and exhibit cyberchondriac behaviors.

In conclusion, this study has shown that individuals' health perceptions and levels of education have a significant impact on cyberchondriac behaviors. This indicates the need for further research to better understand the impact of online health information on health perceptions and behaviors.

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**Ethics Committee Approval:** This study was approved by Artvin Çoruh University Scientific Research and Publication Ethics Committee (Date: 07.06.2023, No: 2023/6-18).

**Peer Review:** Externally peer-reviewed.

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

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## EFFECT OF CHRONIC STRESS ON SERUM AND TISSUE LEVELS OF KLOTHO AND TNF- $\alpha$

### KRONİK STRESİN KLOTHO VE TNF- $\alpha$ 'NİN SERUM VE DOKU SEVİYELERİ ÜZERİNE ETKİSİ

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#### ABSTRACT

**Objective:** Chronic stress is recognized as a factor that affects almost all organs and tissues and disrupts homeostasis. Stress also directly impacts the inflammatory process. In addition, klotho is a protein associated with lifespan. This study investigates the relationship that chronic stress has with klotho and tumor necrosis factor alpha (TNF- $\alpha$ ), which is a key protein in the proinflammatory process.

**Materials and Methods:** The study involves 16 Wistar albino rats divided into control and stress groups. The study applies the protocol for the chronic unpredictable mild stress (CUMS) model and uses the enzyme-linked immunosorbent assay (ELISA) method to measure the TNF- $\alpha$  and Klotho levels in the serum, heart, aorta, and liver tissues.

**Results:** The serum samples obtained from the stress group were found to have a significant decrease in klotho levels ( $p=0.013$ ) and high TNF- $\alpha$  levels ( $p=0.042$ ). However, no significant difference was observed in the TNF- $\alpha$  or klotho levels in the heart, aorta, and liver tissues between groups.

**Conclusion:** Under chronic stress, a significant increase is observed in TNF- $\alpha$ , one of the main cytokines of inflammation, as well as a decrease in klotho hormone. This research endeavors to contribute to the existing body of knowledge concerning the interplay among stress, inflammation, and longevity.

**Keywords:** Chronic stress, inflammation, klotho, TNF- $\alpha$

#### ÖZ

**Amaç:** Kronik stres hemen hemen tüm organları/dokuları etkileyen ve homeostazı bozan bir faktör olarak kabul edilmektedir. Stres, enflamatuar süreç üzerinde doğrudan bir etkiye sahiptir. Bununla birlikte, Klotho yaşam süresi ile ilişkili bir proteindir. Çalışmamızda kronik stres ile proinflamatuar süreçte anahtar bir protein olan Tümör nekroz faktör alfa (TNF- $\alpha$ ) ve Klotho arasındaki ilişkiyi araştırdık.

**Gereç ve Yöntemler:** Çalışmamız kontrol ve stres gruplarına ayrılan 16 Wistar albino'dan oluşmuştur. Kronik öngörülemez hafif stres model protokolü uygulandı. Serum, kalp, aort ve karaciğer dokusundaki TNF- $\alpha$  ve Klotho düzeyleri ELISA yöntemi kullanılarak ölçüldü.

**Bulgular:** Stres grubundan alınan serum örneklerinin Klotho düzeylerinde anlamlı azalma ( $p=0,013$ ), TNF- $\alpha$  düzeylerinde ise anlamlı yükseliş ( $p=0,042$ ) olduğu belirlendi. Ancak; gruplar arasında kalp, aort ve karaciğer dokularındaki TNF- $\alpha$  ve Klotho düzeylerinde anlamlı bir fark gözlenmedi.

**Sonuç:** Kronik stres altında inflamasyonun ana sitokinlerinden biri olan TNF- $\alpha$ ' da belirgin bir artış, Klotho hormonunda ise azalma gözlemlendi. Araştırmamız; stres, iltihaplanma ve uzun ömür arasındaki etkileşime ilişkin mevcut bilgi birikimine katkıda bulunmaya çalışmaktadır.

**Anahtar Kelimeler:** Kronik stres, inflamasyon, Klotho, TNF- $\alpha$

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## INTRODUCTION

Homeostasis denotes the intricate process of governing and upholding cellular equilibrium in response to external stimuli. Stress is recognized as a disruptive factor that affects homeostasis (1). Stressors can cause changes in intercellular communication pathways within the body (2). In cases of chronic stress, alterations in hormones, proinflammatory cytokines, and neurotransmitters can lead to morphological changes in organs, tissues, and arteries (3). These biological changes can result in the development of cardiovascular pathologies, metabolic disorders, neurodegenerative changes, and associated functional impairments (4, 5). The chronic stress process triggers adaptations for maintaining organismal homeostasis (2). These adaptive mechanisms govern the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, which orchestrates the body's stress response. The release of mediator molecules through this pathway results in the restructuring of numerous biological structures at the cellular level, facilitating physiological adaptations to stress (6, 7).

Chronic stress has a direct impact on the inflammatory process (8). When looking at the pathogenesis of psychological stress and inflammation, they are noted to be parts of a mutually reinforcing cycle (3). During chronic psychological stress, the continuous active sympathetic discharge of the autonomic nervous system, metabolic response, gain in oxidative stress molecules, and activation of NF- $\kappa$ B constitute the basic pillars of the biological cascade (9). The correlation between psychological stress and leukocytes is of interest due to the effects stress has on the immune system. Psychological stress can cause the release of stress hormones such as cortisol and catecholamines (6). These hormones can affect the functions of leukocytes and the biomodulators that get released. Depression and stress can both directly and indirectly influence the secretion of proinflammatory cytokines (9).

Klotho is a protein associated with lifespan (10) and represents a transmembrane protein complex that assumes crucial functions in regulating the blood electrolyte balance (11). Klotho is a member of a biological axis along with FGF23 and is described as a cellular receptor coactivator (12). A substantial reduction is observed in the human population in serum concentrations of klotho after the age of 40, thus establishing it as one of the pivotal proteins associated with the aging process. Klotho is primarily synthesized in the body in the distal tubules, choroid plexus, and parathyroid gland (10-13). Kuro-o et al. observed overexpression of klotho gene expression levels using transgenic methods to result in a 30% longer lifespan in mice compared to the control group. Additionally, experiments that silenced klotho gene expression levels in mice led to rapid aging processes and systemic disorders such as cardiovascular disorders, neurodegeneration, and kidney failure (14, 15). The factors commonly associated with longevity, such as exercise, regular sleep, and balanced nutrition, are known to increase klotho levels (11). Meanwhile, chronic stress is widely accepted as a factor that negatively affects health and lifespan (16, 17).

Moreover, the relationship between chronic stress and klotho, which is known to be a factor that causes cellular and systemic tissue damage, has recently become a research area that draws attention (13-17).

Cytokines are biomolecules that regulate inflammation. They play an active role in many processes such as the immune system, infections, and tissue damage (8). The correlation between klotho and cytokines is intricately linked to cellular senescence, inflammation, and various other biological processes. In this context, klotho provides protection against oxidative stress by increasing the expression levels of antioxidant enzymes (18). Proinflammatory cytokines elicit an elevation in cell adhesion proteins, whereas klotho is hypothesized to exert its anti-inflammatory effects through the downregulation of relevant adhesion proteins (19). When looking at the other side of the equation, experimental increases in oxidative stress lead to a significant decrease in klotho expression (20). Within this scope, an inverse relationship has also been stated to be observed between proinflammatory cytokines and klotho's association with longevity, thus indicating a negative correlation (21). Further investigation of this relationship may help develop new treatment options for various health issues such as aging and inflammation.

Chronic unpredictable mild stress (CUMS) is a type of stress caused by the stressors to which a person is regularly exposed but which are unpredictable and mild in nature (22). CUMS differs from the acute stress that occurs in response to a specific event or situation. Chronic unpredictable mild stress can occur due to the small but constant stresses a person experiences in their daily life, of which they are generally unaware, but from which constantly feel a sense of tension and anxiety (23). The objective of this study is to examine the impact chronic mild stress has on the expression of klotho and TNF- $\alpha$ . To this end, the study aims to determine the levels of target proteins in the heart, aorta, liver, and serum of rats subjected to chronic unpredictable stress and examine their relationship with each other. The study is of the opinion that the data acquired from this research can make a valuable contribution to unraveling the intricate biological mechanisms that underlie the impacts of chronic stress on the human body.

## MATERIALS and METHODS

### Animals and standard procedures

The study obtained Presidency approval (Approval No: 3/4-2023 Date: 29.03.2023) from Kocaeli University Animal Experiments Local Ethics Committee. The study uses 4-month-old female *Wistar albino* rats (n=16) weighing 190-200 gr. The rats were divided into two groups in standard cages 2 weeks before the start of the study process and provided standard room conditions. The rats' menstrual cycles were considered and analyzed. During anesthesia, a combination of ketamine hydrochloride (90 mg/kg; Ketalar, Parke-Davis) and xylazine hydrochloride (12 mg/kg, 2%; Rompun, Bayer) was administered intraperitoneally to the rats. Euthanasia was performed by cervical dislocation after intracardiac blood collection. The

**Table 1.** Stressors applied in the chronic unpredictable mild stress (CUMS) model

1. Cage inclination, 45°/24 hours	5. Changing day-night cycle
2. Hanging by the tail, 1 minute	6. Cage shaking, 10 minutes
3. Buoyancy in cold water 4°C/5 minutes	7. Cage wetting 200 mL/24 hours
4. Buoyancy in hot water 45°C/5 minutes	8. Exchanging sawdust between cages

study applied the principles from the Guidelines for the Care and Use of Laboratory Animals.

### Stress model and groups

The study uses the CUMS protocol, which has been previously defined in the literature, as the stress model (22, 23). A total of eight different stressors were identified, and the order of the stressors was determined beforehand (Table 1). At the end of the experiment, anhedonic behaviors, which are considered an indicator of the depression status of animals, were closely monitored and measured.

During the 4th week of the experiment, a sucrose preference test was conducted to measure anhedonic behaviors in stressed animals. The test placed 2 water bottles in each cage and was applied for 6 days. One bottle contained 200 mL of a 20%-sucrose solution, and the other contained 200 mL of tap water. The rats were allowed to drink from both bottles during the first 5 days for habituation. The water bottles were changed every 12 hours. Sucrose consumption was calculated as the ratio of sucrose consumption to total consumption:

Sucrose Consumption=(Sucrose consumption x 100) / Total consumption

This study formed the rats into two groups (i.e., experimental and control groups) before applying the stressors to the experimental group for 28 days. The order in the application protocol was determined at random prior to the experiment.

### Tissue lysis procedures

Analysis was performed on the heart, aorta, and liver tissues and serum. To eliminate any blood contamination, the tissues underwent washing with a saline solution containing 0.09% NaCl. Subsequently, the tissues were weighed and homogenized in a 1:10 ratio of phosphate-buffered saline (PBS) at a pH of 7.4. The homogenization was carried out at a speed of 24,000 revolutions per minute using the T25 Basic Ultra Turrax homogenizer (IKA Werke, Breisgau, Germany). Following homogenization, the samples were centrifuged at 10,000 times the force of gravity for 15 minutes at a temperature of 4°C. The resulting homogenate was then divided into smaller tubes and preserved for further analysis based on tissue-specific measurements.

### ELISA method and biochemical procedures

Blood samples (3 ml) were taken from the left ventricle. Blood specimens were allowed to clot for two hours at room conditions and centrifuged for 15 minutes at a 1000g force at 4-8 °C. The supernatants were collected and diluted to a 1:10 ratio before the assay. Serum, heart, aorta, and liver tissues samples were stored at -40°C. Serum klotho and TNF- $\alpha$  levels were determined with the enzyme-linked immunosorbent assay (ELISA; Elabsience, Houston, USA) kits and measured with the Alisei

Quality System Seac Radim Company Analyzer (Rome, Italy)-ELISA reader based on the manufacturer's instructions (catalog number E-EL-R2580 for klotho; Catalog number E-EL-R2856 for TNF- $\alpha$ ). The results were multiplied by the dilution coefficient, and their concentrations were calculated according to the kit's standards. The ELISA kit has a sensitivity of 0.10 ng/mL and detection range of 0.16-10 ng/mL for klotho, while it has a sensitivity of 9.38 pg/mL and detection range of 15.63-1000 pg/mL for TNF- $\alpha$ . The modified Lowry method was employed to determine the protein content (24). The total protein concentration of the liver tissue was equalized prior to the ELISA process.

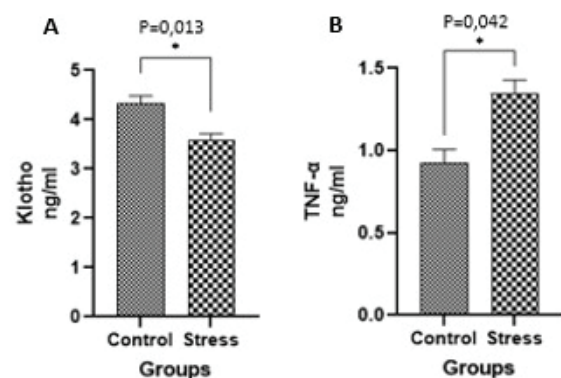
### Data analysis and statistics

The Kolmogorov-Smirnov analysis was performed regarding the normal distribution suitability test in the statistical evaluation of the results. The independent T-test was applied for the normally distributed values. The Mann-Whitney U-test was used for the non-normally distributed values, with p-values of 0.05 or less being considered statistically significant. The package program SPSS 22.0 (IBM SPSS Corp., Armonk, NY, USA) was used for the statistical analyses, while the package program GraphPad Prism8 was used for designing the figures.

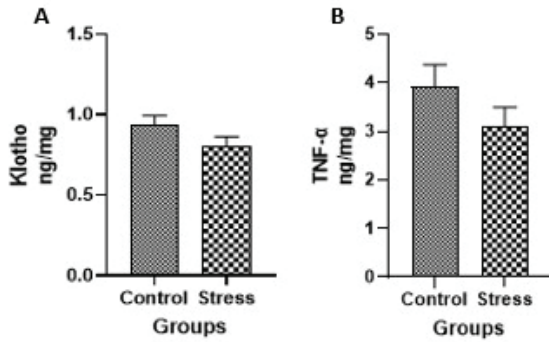
## RESULTS

The serum klotho levels were 4.08 $\pm$ 0.06 ng/ml for the control group and 3.72 $\pm$ 0.11 ng/ml for the stress group. The serum TNF- $\alpha$  levels were 1.05 $\pm$ 0.1 ng/ml for the control group and 1.34 $\pm$ 0.07 ng/ml for the stress group.

When comparing the klotho and TNF- $\alpha$  serum levels, a statistically significant difference was determined to be present between the control and stress groups (p<0.05). A significant



**Figure 1:** Relationship of klotho and TNF- $\alpha$  levels in serum between groups. A) Serum klotho (ng/ml) levels B) Serum TNF- $\alpha$  (ng/ml) levels.

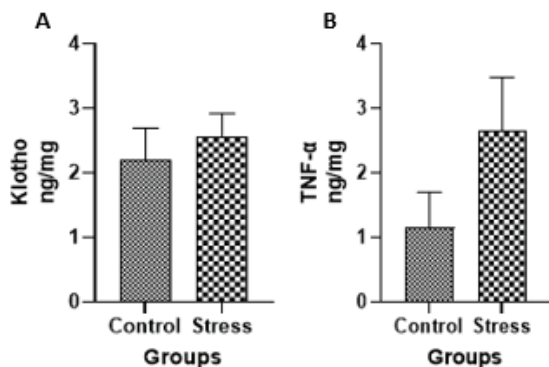


**Figure 2:** Mean klotho and TNF- $\alpha$  levels in liver tissue. A) Liver tissue klotho (ng/mg) levels B) Liver tissue TNF- $\alpha$  (ng/mg) levels.

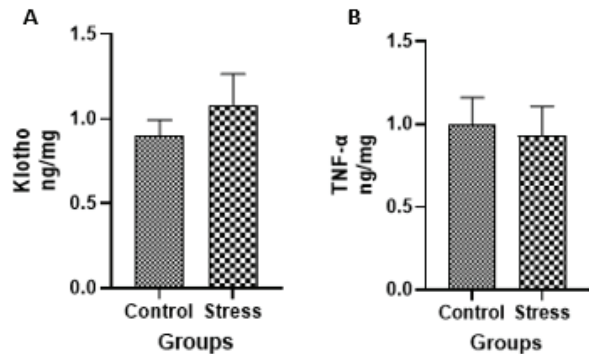
decrease was observed in the stress group rats regarding klotho in the serum ( $p=0.013$ ). When examining the TNF- $\alpha$  levels in the rats from the stress group, a significant increase was observed to have occurred ( $p=0.042$ ). The stress factor was determined to have decreased the serum klotho level and to have increased the TNF- $\alpha$  level. Figure 1 shows the comparison of the klotho-TNF $\alpha$  levels between the two groups.

When considering the klotho and TNF- $\alpha$  levels in the liver tissue, no statistically significant difference was found between the control and stress groups ( $p>0.05$ ). The mean klotho level in the liver tissue was  $0.93\pm0.05$  ng/mg for the control group and  $0.80\pm0.05$  ng/mg for the stress group. The mean TNF- $\alpha$  level in the liver tissue was  $3.91\pm0.46$  ng/mg for the control group and  $3.10\pm0.38$  ng/mg for the stress group. Figure 2 shows the mean klotho and TNF- $\alpha$  levels in the liver tissue for the two groups.

When comparing the klotho and TNF- $\alpha$  levels in the aortic tissue, no statistically significant difference was found between the control and stress groups ( $p>0.05$ ). Furthermore, the mean TNF- $\alpha$  levels were  $1.15\pm0.55$  ng/mg for the control group and  $2.65\pm0.83$  ng/mg for the stress group. An evident increase in TNF- $\alpha$  levels had occurred in the aortic tissue, but this was not considered statistically significant ( $p=0.052$ ). The mean klotho



**Figure 3:** Mean klotho and TNF- $\alpha$  levels in aortic tissue. A) Aortic tissue klotho (ng/mg) levels B) Aortic tissue TNF- $\alpha$  (ng/mg) levels



**Figure 4:** Mean klotho and TNF- $\alpha$  levels in heart tissue. A) Heart tissue klotho (ng/mg) levels B) Heart tissue TNF- $\alpha$  (ng/mg) levels.

level in the aortic tissue was  $2.20\pm0.48$  ng/mg for the control group and  $2.56\pm0.35$  ng/mg for the stress group. Figure 3 shows the mean klotho and TNF- $\alpha$  levels in the liver tissue for the two groups.

When comparing the klotho and TNF- $\alpha$  levels in the heart tissue, no statistically significant difference was found between the control and stress groups ( $p>0.05$ ). The klotho levels in the heart tissue were  $0.89\pm0.09$  ng/mg for the control group and  $1.08\pm0.18$  ng/mg for the stress group. The mean TNF- $\alpha$  levels in the heart tissue were  $0.99\pm0.16$  ng/mg for the control group and  $0.93\pm0.17$  ng/mg for the stress group (Figure 4).

## DISCUSSION

Stress is a term with broad significance and is employed to depict the physiological and psychological responses that arise within an organism due to the influence of a stimulus (1). Furthermore, stress has been identified as a disturbance of adaptation. In response to stress, organisms unleash a defense mechanism and are roused into action to restore homeostasis (2). As such, Selye contended stress to be a physiological response that is set into motion when an organism encounters a stimulus that challenges its ability to adapt (7).

In the initial stages of the stress response, the intermedia lateral region of the thoracolumbar spinal cord experiences an upsurge in activity in the preganglionic sympathetic neurons. The activation of these cells is transmitted to the paravertebral ganglia and subsequently to the chromaffin cells of the adrenal medulla (6). This stimulation brings about the fight-or-flight response, which was initially identified by Walter Cannon (1). Along with the biological damage and disorders that stress causes in tissues and organs, ischemia may also occur (25). The cardiovascular system and metabolic activity are the first biological systems to suffer in the face of stress (5).

Chronic stress-induced changes in an organism typically contribute to an increase in pro-inflammatory cytokines, including TNF- $\alpha$  (3). Several studies have suggested a correlation between stress and changes in the number of circulating T, B, and NK

cells. Evidence derived from both animal models and clinical studies supports the association between inflammation and depression. Exposure to inflammatory cytokines like TNF $\alpha$  or the use of cytokine inducers such as LPS has demonstrated significant behavioral changes in both human subjects and rats (8). Cross-sectional investigations have revealed associations between psychosocial factors, including low socioeconomic status, chronic job stress, caregiver tension, early-life adversity, hostility, and social isolation, as well as the circulating levels of TNF- $\alpha$ , C-reactive protein, and interleukin-6. Although the pathophysiology of the relationship between stress and immunology has not been fully elucidated, a clear association between the two has been established. TNF- $\alpha$  can contribute to increased neuroinflammation in the brain, potentially leading to impairment in nerve cells and connections. This can disrupt neuronal functions, consequently causing a decline in cognitive abilities such as memory loss. This effect may negatively impact neuronal health by enhancing the immune response in nerve cells, alongside other inflammatory factors such as NF- $\kappa$ B, COX-2, and IL-1 (4-8-26). A study conducted on medical students collected blood samples several weeks before, one day before, and immediately after their exams to investigate changes in proinflammatory cytokines. The study demonstrated psychological stress to be able to affect the production of proinflammatory and immune-regulatory cytokines. Students exhibiting high anxiety responses displayed markedly elevated production levels of TNF- $\alpha$ , IL-6, IL-1 receptor antagonist (IL-1Ra), interferon-gamma (IFN-gamma), and IL-10 in comparison to their counterparts without anxiety (3). Patients diagnosed with major depression have exhibited increases in the concentrations of inflammatory mediators such as chemokines, adhesion molecules, and prostaglandins in peripheral blood compared to controls, indicating the manifestation of all main features of inflammation (26). The similarity between the behavioral symptoms induced by cytokines and depression is striking (4). In both conditions, a physical and social withdrawal occurs accompanied by pain, fatigue, and decreased reactivity to reward (anhedonia). The severity of symptoms in both conditions is associated with increases in peripheral blood cytokine concentrations (9-27).

Stress factors are primarily perceived as neurobiological elements (28). When exposed to stressors, the brain primarily stimulates the synthesis of neuroendocrine hormones in the HPA axis (2). Differences arise in the biological pathway alongside the chronicity of the stressor (6). Chronic stress induces heightened synthesis of the high mobility group box-1 (HMGB-1) protein in microglial cells, mirroring conditions akin to ischemia and injury. Consequently, HMGB-1 amplifies the expression of TLR2-4 in immune cells, initiating a cascade of biological responses that prompt chemotactic cell mobilization and the secretion of TNF- $\alpha$  (29). The relationship between stress and inflammation is noteworthy, even in the absence of a microbial agent, as the immune response observed is quite remarkable. The current study shows similar results. When comparing the stress group with the control group, a significant increase in serum TNF- $\alpha$  levels was detected in the stressed group. How-

ever, no similar increase was observed regarding TNF- $\alpha$  levels in the tissue. No significant difference was detected in the heart, aorta, or liver tissues between the groups.

Klotho is a polypeptide with important roles in regulating the blood electrolyte balance (10). Klotho is defined as a co-activator of the cellular receptor FGF23 and is closely related to cellular aging (15). Studies have shown transgenic rats with an overexpression of klotho to have significantly longer lifespans, while those subjected to gene silencing experiments have shorter lifespans accompanied by many metabolic defects (10). Studies involving this interesting hormone-like protein have also shown the factors that support healthy living to increase klotho levels. For example, regular exercise, sleep, and a balanced diet have been reported to increase klotho levels (13-30). Conversely, klotho levels decrease in situations such as smoking, alcohol consumption, major depression, and obesity compared to control groups (17). One study investigating caregivers of children with autism and typically developing children reported psychosocial stress to reduce klotho levels, with the serum klotho levels of caregivers of autistic children with high-stress symptoms being significantly lower than those of the other caregiver group (16). A new *in vivo* study showing the relationship between psychological stress and klotho revealed klotho expression to be genetically increased and decreased in the nucleus accumbens (NAc), a brain region that regulates behavioral mood. When silencing klotho expression in mice, they exhibited major depressive behavior patterns, while an antidepressant effect was stated to occur in mice whose klotho expression had been increased (31). Drawing upon the current research findings, this study purports to lend credence to the existing literature. Remarkably, the study witnessed a striking reduction in klotho levels within the serums of rats that had been exposed to CUMs. In contrast to the control group, the klotho levels in the stress group exhibited a significant drop in their serum, despite no marked difference being discerned in their tissue klotho levels.

Klotho emerges as a newly identified hormone with high therapeutic potential (11). It has been linked to longevity, and its independent effect against stress provides a wide field of study (16, 17). Klotho protects against oxidative stress factors in an organism (20). The klotho protein has recently been described as a hormone that inhibits insulin/insulin-like growth factor-1 (IGF-1) signaling (11). The klotho protein activates the forkhead Box O (FOXO) transcription factors that are negatively regulated by the insulin/IGF-1 signal and induces the expression of manganese superoxide dismutase. This facilitates the removal of reactive oxygen species and provides resistance to oxidative stress. Therefore, inhibition of the insulin/IGF-1 signaling potentially contributes to klotho's anti-aging properties and is associated with increased resistance to oxidative stress (18). Another study observed a significant decrease in klotho expression in the RT-PCR analysis of cell lines that had been exposed to oxidative stress. Apoptosis has also been reported to be significantly reduced in cells experimentally overexpressing klotho (20). Proinflammatory cytokines increase the expressi-

on of adhesion proteins in cells. The klotho protein was exogenously applied to cell lines through the induction of TNF- $\alpha$  and reduced the expression of adhesion molecules (ICAM-1, VCAM-1). Klotho has been shown to activate a biological step toward reducing inflammation activity (19). Upon evaluating TNF- $\alpha$  and klotho levels together, this study observed that, while klotho levels had decreased in the stressed group, TNF- $\alpha$  levels had increased. The negative correlation between klotho and TNF- $\alpha$  is consistent with the literature. CUMS reduced the serum klotho level, which is associated with longevity. In contrast, the stress group showed a significant increase of the proinflammatory factor TNF- $\alpha$  levels in the serum.

#### Limitations of the study

The study used the ELISA method to measure TNF and klotho levels in the serum and heart, aorta, and liver tissues. In this way, the study determined the level differences in serum and tissues. However, the study's limitation lies in the absence of any histological assessment using the immunohistochemical method.

#### CONCLUSION

TNF- $\alpha$  is one of the main cytokines of inflammation, and this study observed a significant increase in TNF- $\alpha$  and a decrease in the klotho hormone to occur under chronic stress. The serum samples obtained from the stress group were found to have a significant decrease in klotho levels when TNF- $\alpha$  levels were high. However, no significant difference was found to have occurred in the TNF- $\alpha$  and klotho levels in the heart, aorta, and liver tissues among the groups.

As a result, TNF- $\alpha$  and klotho levels were found to be related to the changes caused by CUMS. By comparing the groups, the study established a relationship between the changes in serum and tissue. The study holds the conviction that the acquired data possess the potential to enrich the existing literature concerning the interconnections among stress, inflammation, and longevity.

**Ethics Committee Approval:** This study was approved by Animal Experiments Local Ethics Committee, Kocaeli University (Date: 29.03.2023, Approval No: 3/4-2023).

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# EVALUATION OF PHARMACY EMPLOYEES' USAGE OF HERBAL PRODUCTS AND KNOWLEDGE ABOUT FOOD-DRUG INTERACTIONS

## ECZANE ÇALIŞANLARININ BİTKİSEL ÜRÜN KULLANIMININ VE BESİN-İLAÇ ETKİLEŞİMLERİ HAKKINDAKİ BİLGİLERİNİN DEĞERLENDİRİLMESİ

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### ABSTRACT

**Objective:** This study was conducted to determine and compare herbal product use, attitudes toward herbal products, and the knowledge about food-drug interactions of pharmacy employees.

**Materials and Methods:** This descriptive cross-sectional study included 132 pharmacy employees, comprising 67 pharmacists, 40 pharmacy technicians, and 25 pharmacy foremen. Demographic characteristics of the participants, herbal product use and attitudes toward herbal products, questions concerning food-drug interactions, and the Food-Drug Interaction Knowledge Level Scale were evaluated. Data were collected through a web-based questionnaire and analyzed using appropriate statistical methods.

**Results:** Pharmacists used herbal products ( $p=0.008$ ) and counseled more ( $p<0.001$ ) than pharmacy technicians/foremen. Most pharmacy employees received questions from patients regarding herbal products. A total of 73.8% of pharmacy technicians/foremen believed that herbal products exert fewer side effects than conventional drugs, and 91.0% of pharmacists believed that herbal products are not regulated and that there is an interaction between conventional drugs and herbal products. The mean Food-Drug Interaction Knowledge Level Scale score of pharmacists was  $13.4\pm 3.96$ , whereas that of pharmacy technicians/foremen was  $7.9\pm 5.46$  ( $p<0.001$ ). Good or very good knowledge about food-drug interactions was found among 79.1% of pharmacists and 33.8% of pharmacy technicians/foremen. Profession (pharmacist vs pharmacy technician/foreman) and having additional education on herbal products significantly affected the scale scores ( $p<0.001$  and  $p=0.019$ , respectively).

**Conclusion:** Pharmacy employees are an important health group who provide counseling on herbal product use and food-drug interactions. Pharmacists' knowledge about food-drug interactions was higher than that of pharmacy technicians/foremen. Therefore, lifelong learning should be a part of the lives of these professional groups as in every profession.

**Keywords:** Alternative Medicine, complementary medicine, food-drug interaction, herbal medicine use

### ÖZ

**Amaç:** Bu çalışma ile eczane çalışanlarının bitkisel ürün kullanımı, bitkisel ürünlerle ilgili tutumları ve besin-ilaç etkileşimleri hakkındaki bilgilerinin belirlenerek karşılaştırılması amaçlanmıştır.

**Gereç ve Yöntem:** Çalışma tanımlayıcı tipte kesitsel bir çalışmadır. Çalışmaya 67 eczacı, 40 eczane teknikeri ve 25 eczane kalfası olmak üzere toplam 132 eczane çalışanı katılmıştır. Çalışmanın verileri bireylerin demografik özellikleri, bitkisel ürün kullanımı ve bitkisel ürünler hakkındaki tutumları, besin-ilaç etkileşimine dair sorular ve Besin-ilaç Etkileşimi Bilgi Düzeyini Saptamaya Yönelik Ölçek'i içeren sorulardan oluşmuştur. Veriler web tabanlı bir anket aracılığıyla toplanmış ve uygun istatistiksel yöntemler kullanılarak değerlendirilmiştir.

**Bulgular:** Eczacıların bitkisel ürünleri eczane teknikerleri/kalfalarına göre daha fazla kullandığı ( $p=0,008$ ) ve daha fazla danışmanlık yaptığı ( $p<0,001$ ) belirlenmiştir. Çoğu eczane çalışanı bitkisel ürünler hakkında hastalardan soru almaktadır. Eczane teknikerleri/kalfalarının %73,8'i bitkisel ürünlerin geleneksel ilaçlara göre daha az yan etkisi olduğunu, eczacıların %91,0'i ise bitkisel ürünlerin denetlenmediğini ve geleneksel ilaçlarla bitkisel ürünler arasında etkileşim olduğunu düşünmektedir. Ölçek sonuçlarına göre eczacıların ortalama ölçek puanları  $13,4\pm 3,96$  iken eczane teknikerleri ve kalfalarının ortalama ölçek puanları  $7,9\pm 5,46$ 'dır ( $p<0,001$ ). Eczacıların %79,1'i eczacı teknikeri/kalfalarının ise %33,8'i iyi ve çok iyi düzeyde besin-ilaç etkileşimi bilgisine sahiptir. Meslek grubunun (eczacı ve eczane teknikeri/kalfası) ve bitkisel ürünler hakkında ek eğitim alınanın ölçek puanlarını anlamlı olarak etkilediği tespit edilmiştir ( $p<0,001$  ve  $p=0,019$ ).

**Sonuç:** Eczane çalışanları bitkisel ürün kullanımı ve besin-ilaç etkileşimi konusunda danışmanlık yapan önemli bir sağlık grubudur. Eczacıların besin-ilaç etkileşimi bilgisi eczacı teknikeri/kalfasından yüksektir. Bu nedenle yaşam boyu öğrenme her meslekte olduğu gibi bu meslek gruplarında da hayatın bir parçası olmalıdır.

**Anahtar Kelimeler:** Alternatif Tıp, besin ilaç etkileşimi, bitkisel ilaç kullanımı, tamamlayıcı tıp

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## INTRODUCTION

Complementary and alternative medicine (CAM) approaches have become popular in recent years. The usage rate of these methods has been found to be 26% on average in European countries (1). A study reported that CAM usage in patients with cancer varied from 15% to 73%, with the average being 36% (2). Another study conducted in Türkiye showed that the usage rate of CAM was 22%–84% with an average of 46.2% (3). Patients with hypertension showed a CAM usage rate of 13%–86% (4). A small-scale study conducted in our country reported a CAM usage rate of 60%, indicating that CAM methods are used at varying rates in different disease groups and in the general population (5).

CAM methods are categorized as alternative medical therapies, biology-based therapies, manipulative and body-based therapies, and mind-body therapies (6). The usage of herbal medicines and herbal products is prominent among these methods (4, 7). In Türkiye, there is a lack of legal CAM applications; however, traditional and complementary medicine (T&CM) practices have been regulated by the Ministry of Health. Phytotherapy is a T&CM practice included in the regulation (8). Considering the products sold in pharmacies, there has been an increase in the use of herbal products over the years (9, 10).

Food–drug interactions are generally defined as those originating from the physical, chemical, physiological, or pathophysiological relationship between a drug and a food/nutrient or nutritional state (11). Although food or nutrients may affect the absorption, transport, metabolism, and excretion of drugs, drugs may also affect nutrient metabolism and nutritional status. This interaction between nutrients and drugs may result in failure to obtain the expected effect of nutrients and drugs, resulting in alteration of the pharmacokinetic and/or pharmacodynamic effect of the drug and thus failure in treatment (12). For instance, grapefruit juice is a CYP3A4 inhibitor and may increase the effect of the drug by more than five times when taken together with drugs. The use of some drugs may affect the function of the gastrointestinal system and result in loss of body electrolytes and fluid (13). Undesirable effects such as increased/decreased drug efficacy, toxicity, and changes in the patient's health status that may occur as a consequence of food–drug interactions should be considered by healthcare professionals in all areas of the healthcare system (12).

Pharmacists are the first healthcare professionals consulted by patients for drugs and herbal products (14). They have the responsibility of counseling individuals regarding the appropriate use of herbal products and drugs (15). Considering the widespread use of herbal products and drugs throughout the world and the important effect of nutrient–drug interactions on treatments, pharmacy employees should have the knowledge about the use of herbal products, their interactions with drugs, and health effects (16). In Türkiye, pharmacists take compulsory and elective courses on herbal products throughout their education and core education program to emphasize lifelong learning (17). Conversely, pharmacy technicians and foremen may also consult patients. However, there are limited data regarding the current attitudes toward herbal products and

food–drug interactions among this profession. Therefore, this study was conducted to determine and compare the knowledge of pharmacy employees regarding food–drug interactions and attitudes toward herbal products.

## MATERIAL and METHODS

A total of 132 pharmacy employees (67 pharmacists, 40 pharmacy technicians, and 25 pharmacy foremen) from 51 pharmacies participated in this descriptive study. Participants were included in the study after obtaining their written informed consent. The Ondokuz Mayıs University Clinical Research Ethics Committee approved this study (Date: 30.04.2021, No: 2021/249).

Data were collected online using Google Forms through a questionnaire, including “9 questions” on sociodemographic characteristics (age, sex, and educational status) and employment status (work experience and pharmacy ownership status), “4 questions” on herbal product practices (herbal product sale, use, and consultancy), “5 questions” on attitudes toward herbal products, and “3 questions” on food–drug interactions and the Food–Drug Interaction Knowledge Level Scale. When generating the survey questions, the knowledge and attitudes of pharmacy employees regarding herbal products and nutrient–drug interactions were determined.

The knowledge of pharmacy employees about food–drug interactions was determined using the Food–Drug Interaction Knowledge Level Scale developed by Karagöz et al. (18). The scale consists of 21 items in total. Each question has three options, viz., “True,” “False,” and “Don't know.” In the evaluation, each appropriate answer is equal to 1 point, and the others (incorrect and unknown) are equal to 0. Those who chose the “False” option in items 1 and 11 and those who chose the “True” option in the other items of the scale received 1 point for each item. When the total scores of the scale are evaluated, 0–5, 6–11, 11–15, and 16–21 points indicate “low,” “moderate,” “good,” and “very good” knowledge level, respectively. Cronbach's alpha coefficient of the scale is 0.731 (18).

Data were analyzed using the SPSS 20 (IBM SPSS Corp., Armonk, NY, USA) package program. We tested whether the data were suitable for normal distribution. Quantitative data were expressed as median (minimum–maximum), and qualitative data were expressed as percentage (%) (number). Comparisons were performed with two groups (as pharmacists and pharmacy technicians/foremen). The significance of the differences between two groups was determined using chi-square or Fisher's exact test. The significance of the differences between the mean values of two groups was determined using the independent samples t-test. Linear regression analysis was used to obtain the determinants of individuals' food–drug interaction scale scores. The significance level was accepted as 0.05.

## RESULTS

The majority of participants was women (69.7%), pharmacists (50.8%), and had an undergraduate degree (43.2%). A total of 63.6% of the participants had no additional education on herbal products other than undergraduate education (Table 1).

**Table 1:** Descriptive data of participants

Data*	Descriptive statistics
Age (year)	27 (19–62)
Work experience (year)	4 (1–33)
Sex (women)	69.7 (92)
Chronic disease (yes)	16.7 (22)
<b>Profession</b>	
Pharmacist	50.8 (67)
Pharmacy technician	30.3 (40)
Pharmacy foremen	18.9 (25)
<b>Education level</b>	
Primary and secondary education	3.8 (5)
High school	28.8 (38)
Associate degree	15.9 (21)
Bachelor's degree	43.2 (57)
Master's degree	8.3 (11)
<b>Status of the graduated school</b>	
State	88.6 (117)
Private	11.4 (15)
<b>Ownership</b>	
Owner	25.0 (33)
Employee	75.0 (99)
<b>Additional education on herbal products</b>	
Yes	36.4 (48)
No	63.6 (84)

\*: Quantitative data are expressed as median (minimum–maximum), and qualitative data are expressed as percentage (%) (number)

**Table 2:** Herbal product practices and attitudes and opinion on food–drug interactions according to pharmacy employee groups

Questions	Positive (yes/I agree) response (% , n)				p
	Total (n=132)	Pharmacists (n=67)	Pharmacy technicians/foremen (n=65)	Chi-square/Fisher's exact test value	
I sell herbal products in pharmacy.	84.1 (111)	88.1 (59)	80.0 (52)	1.602	0.206
I use herbal products for self-treatment.	78.8 (104)	88.1 (59)	69.2 (45)	6.999	<b>0.008</b>
I counsel patients on the use of herbal products.	81.1 (107)	95.5 (64)	66.2 (43)	18.535	<b>&lt;0.001</b>
I receive questions from patients about the use of herbal products.	87.1 (115)	94.0 (63)	80.0 (52)	5.788	<b>0.016</b>
Herbal products have beneficial effects.	97.0 (128)	98.5 (66)	95.4 (62)	1.087	0.297
Herbal products have fewer side effects than conventional medicines.	53.8 (71)	34.3 (23)	73.8 (48)	20.728	<b>&lt;0.001</b>
Herbal products have a placebo effect.	56.8 (75)	61.2 (41)	52.3 (34)	1.062	0.303
Herbal products are adequately regulated.	21.2 (28)	9.0 (6)	33.8 (22)	12.231	<b>&lt;0.001</b>
Herbal products have significant interactions with conventional medicines.	81.1 (107)	91.0 (61)	70.8 (46)	8.835	<b>0.003</b>
I inform patients about the interactions of the medicines they buy with food/drinks.	88.6 (117)	94.0 (63)	83.1 (54)	3.930	<b>0.047</b>
Patients ask me if the medicines they buy interact with any food/drink.	66.7 (88)	59.7 (40)	73.8 (48)	2.970	0.085
I think I have sufficient knowledge about the interactions of foods/drinks with medicines.	56.8 (75)	62.7 (42)	50.8 (33)	1.910	0.167

#: Percentage, n: Number

**Table 3:** Knowledge about food–drug interactions according to pharmacy employee groups

Scale items	True response (n, %)			Chi-square value	p
	Total (n=132)	Pharmacist (n=67)	Pharmacy technicians/foremen (n=65)		
1.* "The fasting status of an individual does not change effectiveness of a drug"	90.2 (119)	97.0 (65)	83.1 (54)	7.219	<b>0.007</b>
2. "Some antiulcer drugs, such as sucralfate, should be taken on an empty stomach as they bind to the protein in the nutrients"	75.0 (99)	79.1 (53)	70.8 (46)	1.223	0.269
3. "Energy restricted diets may increase sensitivity of certain stimulant drugs such as amphetamine"	55.3 (73)	61.2 (41)	49.2 (32)	1.910	0.167
4. "Taking a lipophilic drug with high fatty food/meal increases toxicity of the drug"	47.0 (62)	56.7 (38)	36.9 (24)	5.189	<b>0.023</b>
5. "Propranolol having antihypertensive effect increases the bioavailability of drugs, if taken with a high-carbohydrate meal"	22.0 (29)	20.9 (14)	23.1 (15)	0.092	0.762
6. "High fiber diets bind bile and diminish acids, and thus increases excretion of some drugs"	49.2 (65)	58.2 (39)	40.0 (26)	4.377	<b>0.036</b>
7. "High fiber and pectin foods delay absorption of some drugs such as digoxin"	39.4 (52)	47.8 (32)	30.8 (20)	3.990	<b>0.046</b>
8. "In general, a protein-poor diet causes a decrease in half-life of drugs and plasma clearance by decreasing albumin levels"	42.4 (56)	58.2 (39)	26.2 (17)	13.879	<b>&lt;0.001</b>
9. "Due to the components found in its chemical structure, grapefruit juice increases the blood concentration of calcium channel blocker drugs such as Felodipine"	63.6 (84)	80.6 (54)	46.2 (30)	16.914	<b>&lt;0.001</b>
10. "Chemotherapeutic drugs' bioavailability increases with grapefruit juice"	35.6 (47)	32.8 (22)	38.5 (25)	0.455	0.500
11.* "Vegetables containing vitamin K such as broccoli, cabbage, spinach, etc. increase the efficacy of warfarin-containing anticoagulant drugs"	23.5 (31)	34.3 (23)	12.3 (8)	8.903	<b>0.003</b>
12. "Taking Parkinson's disease drugs such as Levodopa, Methyl dopa with protein-rich foods decreases the absorption of the drug"	36.4 (48)	40.3 (27)	32.3 (21)	0.910	0.340
13. "Anticonvulsant drugs such as phenobarbital and phenytoin may disrupt folic acid, vitamin D and vitamin K metabolism"	53.0 (70)	68.7 (46)	36.9 (24)	13.339	<b>&lt;0.001</b>
14. "Antacid group drugs such as sodium bicarbonate reduce calcium absorption"	53.8 (71)	73.1 (49)	33.8 (22)	20.488	<b>&lt;0.001</b>
15. "As antihypertensive drugs containing ACE inhibitors cause hyperkalemia, they should not be consumed with foods rich in potassium such as bananas and green leafy vegetables"	51.5 (68)	74.6 (50)	27.7 (18)	29.098	<b>&lt;0.001</b>
16. "Long-term use of steroids adversely affects calcium metabolism"	71.2 (94)	88.1 (59)	53.8 (35)	18.839	<b>&lt;0.001</b>
17. "Cyclosporin-containing immunosuppressive drugs may increase blood pressure by increasing the sodium and water retention in the body"	49.2 (65)	71.6 (48)	26.2 (17)	27.313	<b>&lt;0.001</b>
18. "Drugs such as thiazides and loop diuretics trigger hypokalemia by increasing potassium excretion"	47.7 (63)	65.7 (44)	29.2 (19)	17.561	<b>&lt;0.001</b>
19. "Antacids and proton pump inhibitors adversely affect iron absorption by changing gastric pH"	56.8 (75)	80.6 (54)	32.3 (21)	31.357	<b>&lt;0.001</b>
20. "Monoamine oxidase inhibitors (MAOI) cause a hypertension crisis, if taken with high tyramine-containing foods such as processed and aged cheese, fermented salami, fermented sausage, sausage, chicken, and calf liver"	45.5 (60)	71.6 (48)	18.5 (12)	37.634	<b>&lt;0.001</b>
21. "Antidiabetic drugs with metformin active substance negatively affect the absorption of vitamin B12"	62.9 (83)	82.1 (55)	43.1 (28)	21.513	<b>&lt;0.001</b>

‰: Percentage, n: Number, \* The correct answer to the marked questions is "False," the correct answer to the other questions is "True."

**Table 4:** Food–drug interaction scale score and classifications according to pharmacy employee groups

	Pharmacist (n=67)	Pharmacy technicians/foremen (n=65)	Test value	p
Scale score (mean±SD)	13.4±3.96	7.9±5.46	-6.676	<0.001
<b>Classification (% , n)</b>				
Low	3.0 (2)	43.1 (28)		
Intermediate	17.9 (12)	23.1 (15)		
Good	52.2 (35)	21.5 (14)	35.691	<0.001
Very good	26.9 (18)	12.3 (8)		

SD: Standard deviation, %: Percentage, n: Number

**Table 5:** Determinants of individuals' food–drug interaction scale score

Dependent variable	Factors	Beta	t	p
Food–drug interaction scale scores	Age (year)	-0.032	-0.227	0.821
	Sex	-0.105	-1.321	0.189
	Profession	-0.443	-5.308	<0.001
	Work experience (year)	0.028	0.187	0.852
	Additional education on herbal products	-0.184	-2.377	0.019

Beta: Regression coefficient

Herbal product usages, attitudes toward herbal products, and opinions on food–drug interactions according to occupational groups are shown in Table 2. Pharmacists and pharmacy technicians/foremen mentioned that herbal products are sold in pharmacies at a high rate, with 88.1% by pharmacists and 69.2% by pharmacy technicians/foremen ( $p=0.008$ ). Pharmacy technicians/foremen believed that herbal products exert fewer side effects than those believed by pharmacists ( $p<0.001$ ). Pharmacists agreed more with the statement that herbal products interact with drugs ( $p=0.003$ ).

Almost all participants (90.2%) believed that an individual's hunger state changed the effectiveness of the medication taken. Most participants could not provide correct answers to questions on food–drug interactions in items 5 and 11 of the scale (Table 3). In most items of the scale, pharmacists had a higher rate of correct answers than pharmacy technicians/foremen.

The mean scale score of pharmacists was  $13.4\pm 3.96$ , whereas that of pharmacy technicians/foremen was  $7.9\pm 5.46$  ( $p<0.001$ ) (Table 4). Regarding the knowledge level, 3.0%, 17.9%, 52.2%, and 26.9% of pharmacists had low, intermediate, good, and very good knowledge level, respectively, whereas 43.1%, 23.1%, 21.5%, and 12.3% of pharmacy technicians/foremen had low, intermediate, good, and very good knowledge level, respectively. Most pharmacists had good and very good knowledge level, and most pharmacy technicians/foremen had low and intermediate knowledge level ( $p<0.001$ ) (Table 4).

According to the linear regression analysis (Table 5), being a pharmacist or pharmacy technician/foreman and having additional education on herbal products significantly affected the scale scores ( $\beta=-0.443$ ,  $p<0.001$ ,  $\beta=-0.184$ ,  $p=0.019$ , respectively). Age, gender, and work experience exerted no significant impact on scale scores ( $p>0.05$ ).

## DISCUSSION

This study investigated the current attitudes toward herbal products and knowledge on food–drug interactions of pharmacy employees. To the best of our knowledge, this is the first study to compare knowledge regarding herbal products and food–drug interactions between pharmacists and pharmacy technicians/foremen in Türkiye. Pharmacists have crucial responsibilities in ensuring the safe and appropriate use of medicines (19). Hence, considering the globally increasing demand on T&CM practices, it is essential to understand their attitudes toward herbal products (9).

In our study, the majority of pharmacists sold and received questions concerning herbal products from patients and informed patients about food–drug interactions as anticipated. These findings can be supported by several previous studies (20-22). Nevertheless, we also observed high response rates from pharmacy technicians/foremen for the same questions. In most of the community pharmacies in Türkiye, pharmacy technicians/foremen, although not primarily authorized, assist pharmacists and can counsel customers (23). Unfortunately, we found that only 50.8% of them believed that they had sufficient knowledge regarding the interactions of foods/drinks with medicines. It is well known that interactions between herbal products and drugs can increase or decrease the pharmacological or toxicological effects of product/drug components in the human body. A review study showed that the use of herbal or dietary supplements with warfarin may cause several side effects ranging from bleeding to fatal cerebrocranial hemorrhages (24). Herbal products are commonly used together with medications. A study conducted in the USA reported that 38% of people who used herbal drugs also used prescription drugs and 42% of them used over-the-counter drugs (25). Another study showed

that most pharmacists believed that they need more intensive training in the field of herbal products (26). These data indicate that the importance of education on food–drug interactions.

Most users of herbal products reported that they found these products safe (27). In the present study, the majority of pharmacy technicians/foremen believed that herbal products exert fewer side effects than conventional medicines. Most pharmacists agreed that herbal products have significant interactions with conventional medicines. One study reported that 37.8% of pharmacists were unsure about the safety of using herbal products, and at the same time, the majority of pharmacists believed that the use of herbal products with prescription drugs was not safe (20). Another study found that several physicians believed that phytopharmaceuticals were not safe and effective (26). Based on these studies, it can be concluded that healthcare professionals are skeptical about herbal products. In a parallel study, 54.3% of pharmacists reported that there was a food–drug interaction when herbal products were used with conventional drugs (21). The interaction between herbal products and drugs is a very critical issue in terms of health. Studies have demonstrated that pharmacists and physicians are more aware of this important issue (21, 26). In our study, pharmacy technicians/foremen believed that herbal products did not have significant interactions with traditional medicines, which can be explained by their food–drug interaction scale scores. The lower level of knowledge of pharmacy technicians/foremen on the subject may have caused them to provide less counseling to patients regarding food–drug interactions and to leave this responsibility primarily to pharmacists or physicians.

Furthermore, most pharmacists in our study agreed that herbal products were inadequately regulated. A previous study investigated the ingredients of herbal supplements and found that approximately 50% of the tested products were contaminated, and some products had unreported presence of stimulants (such as caffeine, synephrine, and ephedrine). The same study also found the presence of allergens, toxins, or animal ingredients in some products (28). Therefore, to prevent the abuse of people's attitudes toward herbal products, healthcare professionals should be involved in product inspection to ensure that herbal products are provided with clean ingredients.

In our study, the majority of pharmacists had good food–drug interaction scale scores, whereas pharmacy technicians/foremen had low scale scores. In the original scale development study, the food–drug interaction scale scores were different among health professional groups (18). The overall food–drug interaction scale scores of pharmacists were reported as inadequate in studies from Palestine and Jordan (29, 30). However, in another study, having a master's degree increased the awareness of herb–drug interactions (31). In the present study also, pharmacists with a master's degree had the highest food–drug interaction scale score (data not shown). Being a pharmacist or assistant and having additional education on herbal products were the determinants of individuals' food–drug interaction scale scores. Therefore, these results demonstrated that pro-

fessional specialization is valuable in terms of updating basic knowledge and acquiring novel knowledge.

Fasting and satiety states of individuals affect the gastrointestinal transit time of drugs and cause changes in the absorption and bioavailability of drugs. In satiety, gastric emptying slows down and the absorption time of drugs increases (32). Furthermore, the physiological response of the body to food intake, especially gastric acid secretion, may increase or decrease the bioavailability of some drugs (33). Protein intake causes a more significant difference in the clearance of drugs than carbohydrate and fat intake (18). In general, the correct response rate to the scale questions was higher among pharmacists than among technicians/foremen. Nonetheless, the correct answer rate to some questions was low among both pharmacists and technicians. In our study, for the question regarding the intake of drugs used for Parkinson's disease such as levodopa and methyl dopa with protein-rich foods, 36.4% of pharmacy employees could answer correctly. Moreover, 22% of them could answer correctly to the question whether the antihypertensive drug propranolol increases bioavailability when taken with a high-carbohydrate meal. For another question concerning drug interaction with grapefruit juice, the correct answer rate was 35.6%. Grapefruit juice acts by inhibiting presystemic drug metabolism mediated by CYP3A isoforms in the small intestine. This interaction may cause at least doubling of the plasma drug concentration (34). High intake of foods rich in vitamin K (e.g., broccoli, cabbage, and spinach) stimulates the excretion of warfarin, which consequently decreases the anticoagulant effect reduces the efficacy of the drug (35,36). In our study, the rate of finding this information correct was low (23.5%). Pharmacists provided a higher rate of correct answers than pharmacy technicians (34.3% vs 12.3%, respectively,  $p=0.003$ ).

## CONCLUSION

Pharmacists are important healthcare professionals who counsel and guide patients regarding herbal products and food–drug interactions. The attitudes and knowledge of pharmacy technicians/foremen in this field may be inadequate compared with those of pharmacists. Although pharmacists have better knowledge about food–drug interactions, they may need to improve themselves on knowledge regarding some food–drug interactions as well. Therefore, this study clearly indicates that both pharmacists and pharmacy technicians/foremen should update their knowledge regarding food–drug interactions to provide adequate counseling to their patients.

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**Ethics Committee Approval:** This study was planned following the Helsinki Principles, and ethical approval was obtained from the Ondokuz Mayıs University Clinical Research Ethics Committee (Date 30.04.2021, Decision number: 2021/249).

**Informed Consent:** Informed consent was obtained from each participants.

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# COMPARISON OF THE EFFECT OF TWO SURGICAL TECHNIQUES ON THE BIOMECHANICAL STRENGTH OF BICORTICAL SCREWS AND MINIPLATES IN THE ADVANCEMENT OF POLYURETHANE MANDIBLES

## POLİÜRETAN ALT ÇENELERİN İLERLETİLMESİNDE İKİ CERRAHİ TEKNİĞİN BİKORTİKAL VİDA VE MİNİPLAKLARIN BİYOMEKANİK DAYANIMINDAKİ ETKİSİNİN KARŞILAŞTIRILMASI

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### ABSTRACT

**Objective:** This study aims to compare the biomechanical strength of miniplate and bicortical screw fixation in polyurethane mandibles with bilateral sagittal split osteotomy using either the Obwegeser-DalPont (ODP) or Hunsuck-Epker (HE) surgical cutting techniques.

**Materials and Method:** Thirty-two (N=32) polyurethane mandible models were created from data obtained from a tomographic image of a patient. In these models, 4mm advancements were made using ODP and HE cutting techniques. For fixation, 1mm thick, 4-hole, 9mm spaced straight titanium mini plates and 8 titanium screws, 2mm in diameter and 5mm in length, or 6 bicortical screws, 2mm in diameter, were used. Based on the cutting and fixation techniques, four groups were formed, each with 8 (n=8) mandible models. These groups were named M-EPKER, M-DALPONT, B-EPKER, and B-DALPONT, respectively. Fixed models were placed in a universal testing machine, and linear, continuous, and progressively increasing forces were applied from the cutting region. Force magnitudes required to reach displacement values of 1mm, 3mm, and 5mm for the test interface were measured in Newtons (N) and statistically compared.

**Results:** To reach the 1mm displacement level of the test models, the B-EPKER group required a higher force magnitude than the M-EPKER group (p<0.05). At the 3mm level, the B-DALPONT group demonstrated higher force values compared to the M-DALPONT group (p<0.05). Similarly, at the same level, the B-EPKER group reached higher force values than the M-EPKER group (p<0.05). At the 5mm level, both groups using bicortical screws achieved higher values compared to the group using mini plates (p<0.05).

**Conclusion:** Within the limitations of this experimental study, it can be concluded that fixation procedures with bicortical screws in polyurethane models with mandibular advancement, regardless of the surgical technique, are more durable than those with mini plates.

**Keywords:** Epker, DalPont, mini plate, bicortical screw, orthognathic surgery

### ÖZ

**Amaç:** Bu çalışmanın amacı Obwegeser-DalPont (ODP) ya da Hunsuck-Epker (HE) cerrahi kesi yöntemleri kullanılarak bilateral sagittal split osteotomi uygulanan poliüretan alt çenelerde miniplak ve bikortikal vida fiksasyonlarının biyomekanik dayanımını karşılaştırmaktır.

**Gereç ve Yöntem:** Bu çalışmada bir hastanın tomografik görüntüsünden elde edilen verilerle üretilen 32 adet (N=32) poliüretan alt çene modeli kullanılmıştır. Bu modellerde ODP ve HE kesi teknikleri kullanılarak 4mm ilerletme yapılmıştır. Fiksasyon için 1 mm kalınlığında, 4 delikli, 9mm aralıklı 2 adet düz titanyum mini plak ve 8 adet 2mm çapında, 5mm uzunluğunda titanyum vidalar ya da 2mm çapında 6 adet bikortikal vida kullanılmıştır. Çalışmada kesi ve fiksasyon tekniklerine göre her birinde 8 (n=8) alt çene olan 4 grup oluşturulmuştur. Bunlar sırasıyla M-EPKER, M-DALPONT, B-EPKER ve B-DALPONT olarak isimlendirilmiştir. Fikse edilmiş modeller bir evrensel test cihazına yerleştirilerek kesiciler bölgesinden doğrusal, devamlı ve giderek artan kuvvet uygulanmıştır. Deney arayüzünün 1 mm, 3mm, 5mm yer değiştirme değerlerine ulaşması için gereken Newton (N) cinsinden kuvvet büyüklükleri ölçülmüş ve istatistiksel olarak karşılaştırılmıştır.

**Bulgular:** Deney modellerinin 1 mm yer değiştirme seviyesine ulaşması için B-EPKER grubunda M-EPKER grubuna göre daha yüksek kuvvet büyüklüğü gerekli olmuştur (p<0,05). 3mm seviyesinde ise B-DALPONT grubu M-DALPONT grubuna göre daha yüksek kuvvet değerlerine dayanım göstermiştir (p<0,05). Aynı seviyede B-EPKER grubu M-EPKER grubuna göre daha yüksek kuvvet değerlerine ulaşmıştır (p<0,05). 5mm seviyesinde ise her iki kesi tekniğinde de bikortikal kullanılan grup, miniplak kullanılan gruba göre daha yüksek değerlere ulaşmıştır (p<0,05).

**Sonuç:** Bu deneysel çalışmanın sınırları dâhilinde, alt çene ilerletmesi yapılan poliüretan modellerde cerrahi teknik fark etmeksizin bikortikal vida ile yapılan fiksasyon işlemlerinin miniplak ile yapılanlara göre daha dayanıklı olduğu söylenebilir.

**Anahtar Kelimeler:** Epker, DalPont, miniplak, bikortikal vida, ortognatik cerrahi

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## INTRODUCTION

Orthognathic surgical techniques are commonly employed in the treatment of dentofacial deformities (1). Among these, the bilateral sagittal split osteotomy (BSSO) technique is a preferred method for correcting asymmetries and deformations in the lower jaw. Its advantages include ease of application, rapid healing of the region between bone segments, no scar tissue formation as it is applied intraorally, and a low risk of facial nerve damage (2). BSSO is a complex osteotomy performed in three different planes. Over the years, various modifications have been proposed for the anatomical positions of the cutting lines (3). The most significant difference among them is the position of the cutting lines on the medial and lateral surfaces (4).

Among these modifications, the Obwegeser/DalPont (ODP) or Hunsuck/Epker (HE) osteotomies are techniques often used, particularly in procedures involving the advancement or setback of the lower jaw. In the ODP technique, two horizontal cuts are made on the lingual and buccal cortices above the mandibular foramen, and these cuts are connected by a vertical cut. Subsequently, the HE method was developed, considering that extending the medial cortical cut to the mandibular foramen was sufficient. The first osteotomy is performed horizontally on the inner surface of the ramus, approximately 0.5-1cm above the mandibular foramen, in a diagonal direction similar to the course of the external oblique line, moving anterior, buccal, and downward. The endpoint of the diagonal osteotomy in the anterior direction ends at the level of the lower molars. The osteotomy process is completed by making a vertical cut between this point and the basal process of the mandible. After reaching the cancellous bone with osteotomies, the ramus is separated from the mandibular body using osteotomes. The neurovascular bundle consisting of the inferior alveolar artery and nerve remains in the mandibular body. After the cuts are completed, the body and ramus are brought to the desired position and fixed with fixation methods (5).

Rigid internal fixation is performed after BSSO to stabilize the distal and proximal segments, accelerating healing, enabling the patient to begin mandibular function as early as possible after the operation without the need for maxillomandibular fixation, and minimizing relapse. To achieve these goals, bicortical screws or monocortical miniplate-screw systems can be used. When choosing between these, certain factors are considered. Bicortical screws may be preferred when there is a mismatch between the distal and proximal bone segments. Furthermore, in cases where there is a lack of alignment between the buccal cortex and condylar position control, it has been reported that in addition to bicortical screws, one or two mini plates may be required. Bicortical screw fixation is less hardware-intensive and, therefore, more cost-effective. However, in fixation with bicortical screws, holes are made in the lingual cortex of the distal segment, which can lead to lingual nerve injuries. This is less common in miniplate-screw fixations (6). Another advantage of the miniplate fixation method is that it does not require the use of a cheek retractor. In cases of malposition during the

operation, correction is easier with the miniplate-screw method compared to the bicortical screw method (7).

When reviewing the literature, it becomes evident that there are numerous clinical or experimentally designed studies on plate-screw systems and bicortical screws used in BSSO fixation. However, there is no consensus on which fixation method is suitable and effective for which surgical technique. Surgeons often rely on their clinical experience when choosing a fixation method. However, the anatomical positions of the osteotomy lines can affect the strength. Therefore, this experimental study aims to compare the biomechanical strength of miniplate and bicortical screw fixations in polyurethane BSSO models prepared using ODP and HE surgical cutting lines. The tested null hypothesis in this study is that there is no difference in the force magnitudes required for the entire system to reach displacement levels of 1mm, 3mm, or 5mm when force is applied over the anterior cutting edge to BSSO models prepared with ODP and HE surgical cutting lines and fixed with mini plates or bicortical screws (8-10).

## MATERIAL and METHODS

### Ethical approval

This study protocol was reviewed and approved by the İstanbul University Faculty of Dentistry Clinical Research Ethics Committee (Date: 02.04.2021, No: 102).

### Sample size determination

As no similar publication with this research was found, a pilot study was conducted using 5 models in each group (n=5) to obtain data for effect size calculation. Using G\*Power software, with an effect size of 0.63, a 0.05 error probability, and an 80% power level ( $1-\beta$ ), and data entered as 4 groups, the non-centrality parameter was calculated as 12.99, the critical F value as 2.94, and the total number of subjects as 32 (N=32) (11). Therefore, it was decided to have 8 models in each experimental group (n=8).

### Polyurethane model construction

A cone-beam computed tomography of a patient planning for mandibular advancement was selected from the archive. The volumetric dataset was prepared to include the entire mandible. Then, sections with a thickness of 1 mm were prepared and saved in DICOM format. These were opened in 3D SLICER v4.11 software (<https://www.slicer.org/>). The mandible was segmented using both automatic thresholding and manual techniques to exclude the mandibular teeth. The acquired data were saved in stereolithography format. The surface of this virtual model was cleaned with Autodesk Meshmixer v3.5 (RRID: SCR\_015736) software and printed into a physical model using a FormLabs Form 2 SLA 3D printer (FormLabs Inc., Boston, MA, USA). This primary model was sent to a commercial anatomical model manufacturer (Selbones Inc., Kayseri, Türkiye) for mold preparation using molding silicone. After curing, the primary model was removed from the mold. Then, a 1:1 mixture of polyol and isocyanate was injected into the mold cavity. After curing, the model was removed from the mold and cleaned.

### Preparation of cutting lines and fixation

In this study, surgical cuts were made according to the ODP and HE techniques. First, cutting lines were drawn with a permanent marker on two primary polyurethane models, and silicone molds were prepared accordingly. These molds were used for standardization in each model. Fissure burs with a diameter of 2mm, a piezoelectric device, and an electric motor were used for making cuts on the medial and lateral surfaces. Controlled force was applied with surgical chisels and mallets for complete separation of the bone segments. In the HE-cut groups, the horizontal cut on the inside of the ramus was extended to the mandibular foramen. In the ODP-cut groups, the cut on the inside of the ramus was extended to the posterior border.

After the cuts were completed, guides for advancement were prepared for the two primary models with different cutting lines, each having four different advancement procedures. A 4mm advancement was performed in each model using these guides. Subsequently, models distributed to random groups were fixed with a total of 2 straight titanium mini plates (Medplates, Ramed Med., Izmir, Türkiye) with a 2.0mm hole diameter and a 9mm gap, and a total of 8 monochromatic screws (Medplates, Ramed Med., Izmir, Türkiye) with a diameter of 2.00mm and a length of 5mm, with one plate on each side, or a total of 6 bicortical screws (Medplates, Ramed Med., Izmir, Türkiye) with a diameter of 2mm and a length of 13mm, with 3 on each side, placed in a reverse L configuration.

### Experimental Groups and Testing Setup

According to the surgical cutting method and fixation system applied, in the groups where HE technique cuts were made and miniplate-screw systems were used for fixation, the group was named M-EPKER, and in the groups where bicortical screws were used, it was named B-EPKER. In the groups where ODP technique cutting lines were created and fixed with miniplate-screw systems, the group was named M-DALPONT, and in the groups where bicortical screws were used, it was named B-DALPONT. Accordingly, a total of 4 groups, each with 8 models (n=8), were created (Figures 1-4).

Models were randomly selected and placed on a platform specially made of stainless steel. The mandibles were horizontally fixed with a stainless-steel pin passing through both condyles. Additionally, the base of both angulus regions was placed on another pin parallel to the first one (Figure 5). Contact points were marked to ensure that all models were fixed in the same position. This platform was placed on a universal testing machine with a 50kg load cell (AG-IS, Shimadzu Corp., Kyoto, Japan). A non-pointed tip was used to apply linear, continuous, and gradually increasing force to the mandibles from the anterior cutting edge. As a result of this process, a load-displacement graph was drawn digitally. Using this graph, the force magnitudes required for each mandibular model to reach displacement levels of 1mm, 3mm, and 5mm were recorded (1 N=9.03kg).

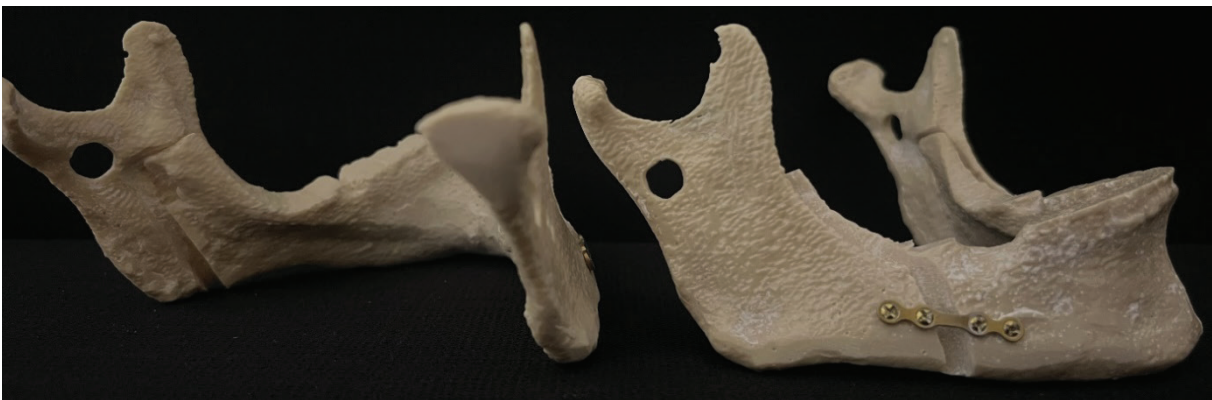


Figure 1: Model belonging to the M-EPKER group

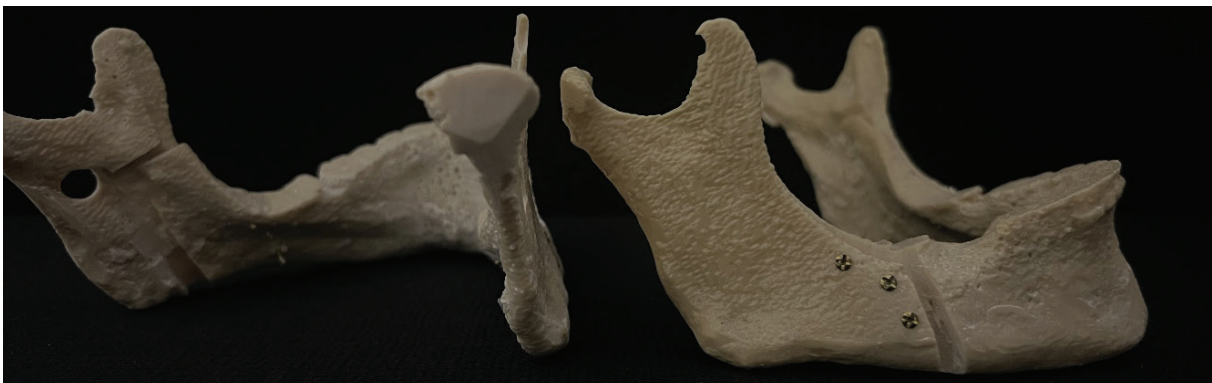


Figure 2: Model belonging to the B-EPKER group

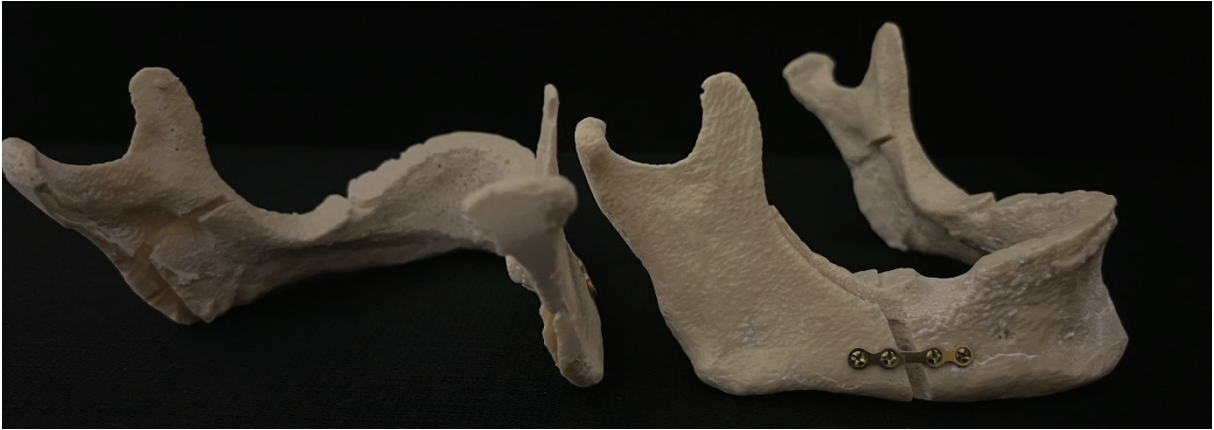


Figure 3: Model belonging to the M-DALPONT group

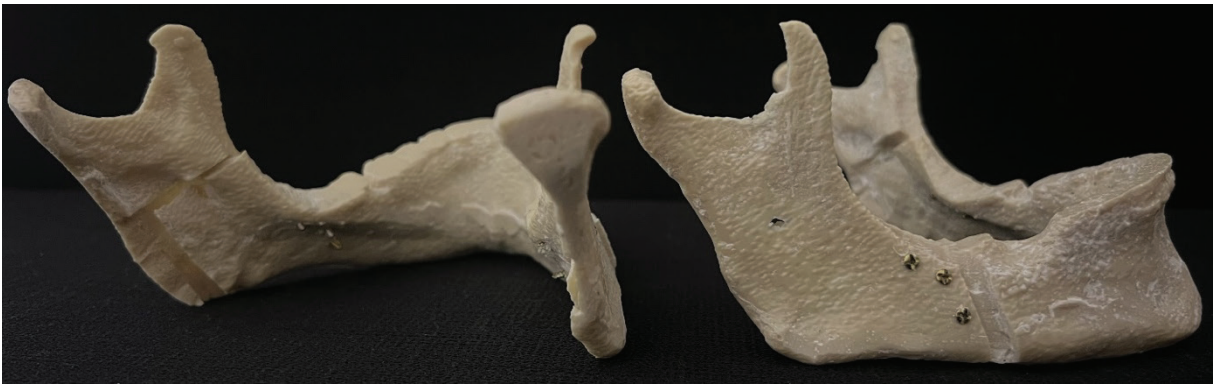


Figure 4: Model belonging to the B-DALPONT group



Figure 5: Placement of the model in the setup

#### Statistical analysis

In this study, statistical analyses were performed using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Descriptive statistical methods (mean, standard deviation) were used to evaluate the data. The distribution of variables was checked with the Shapiro-Wilk normality test, and the homogeneity of variances was checked with the Levene

test. Since the distribution of groups was normal, and variances were homogeneous, a one-way analysis of variance was used for multiple comparisons, and the Tukey HSD test was used for pairwise comparisons. A confidence interval of 95% was set, and  $p < 0.05$  values were considered statistically significant.

#### RESULTS

At the 1 mm displacement level, the strength of the B-DALPONT group was found to be statistically significantly higher than the M-DALPONT group ( $p=0.046$ ). At the 3mm displacement level, the strength of the B-EPKER group was statistically significantly higher than the M-EPKER group ( $p=0.026$ ). The strength of the B-EPKER group was also found to be statistically significantly higher than the M-DALPONT group ( $p=0.002$ ). Similarly, at the same level, the strength of the B-DALPONT group was found to be statistically significantly higher than the M-DALPONT group ( $p=0.008$ ). At the 5mm level, the attachment strength of the B-EPKER group was statistically significantly higher than the M-EPKER group ( $p=0.008$ ). The attachment strength of the B-DALPONT group was found to be statistically significantly higher than the M-EPKER group ( $p=0.027$ ). The attachment strength of the B-EPKER group was also statistically significantly higher than the M-DALPONT group ( $p=0.001$ ). Furthermore, the attachment strength of the B-DALPONT group was found to be statistically significantly higher than the M-DALPONT group ( $p=0.004$ ). There were no statistically significant differences in the strengths of the other groups (Table 1, 2).

**Table 1:** Mean ± standard deviation values of the forces required for the test groups to reach displacement levels of 1mm, 3mm, and 5mm, and the results of the one-way analysis of variance for each group (ANOVA)

Group	1mm (Mean±S.D. N)	ANOVA	3mm (Mean±SD, N)	ANOVA	5mm (Mean±SD, N)	ANOVA
M-EPKER	10.21±2.22	F=3.84 <b>p=0.02</b>	21.93±5.67	F=7.57 <b>p=0.001</b>	33.41±9.12	F=9.27 <b>p&lt;0.0001</b>
B-EPKER	12.29±2.32		29.52±5.68		46.76±8.14	
M-DALPONT	9.45±1.67		19.36±3.68		30.29±4.66	
B-DALPONT	12.44±2.33		28.21±4.80		44.84±7.74	

N: Newton, M: Miniplate, B: Bkortical screw, SD: Standard deviation, mm: Millimeter

**Table 2:** p-values obtained from pairwise comparisons using the Türkiye HSD test for the groups. Statistically significant values are indicated in bold

Groups	1mm	3mm	5mm
M-EPKER/B-EPKER	0.242	<b>0.026</b>	<b>0.008</b>
M-EPKER/M-DALPONT	0.895	0.737	0.845
M-EPKER/B-DALPONT	0.188	0.082	<b>0.027</b>
B-EPKER/M-DALPONT	0.063	<b>0.002</b>	<b>0.001</b>
B-EPKER/B-DALPONT	0.999	0.953	0.958
M-DALPONT/B-DALPONT	<b>0.046</b>	<b>0.008</b>	<b>0.004</b>

M: Mini plate, B: Bikortical screw, mm: Millimeter

## DISCUSSION

In this study, the biomechanical strength provided by plate-screw and bicortical screw systems used for the stabilization of mandibular advancements in BSSO models prepared with two different surgical cutting techniques was compared. Testing the hypothesis under clinical conditions requires a significant number of cases, which is challenging to achieve. Therefore, the experiment was preferred to be conducted in a laboratory setting. To investigate biomechanical strength in this environment, virtual or physical models are used. Virtual models are generated from tomographic data and are used in methods such as finite element analysis. These techniques are more focused on design applications in engineering sciences. Since this study involved materials with known characteristics that are commercially available, physical models were preferred. These models should mimic the lower jaw in terms of both shape and content. In addition, the morphology of facial bones affects both the frequency of fracture occurrence and the strength of plate-screw systems. Therefore, selecting an appropriate master model for the experiment is necessary. While human cadaver lower jaws are the most suitable material for modeling, their use is limited due to the lack of an adequate number and ethical concerns. Therefore, in vitro experiments have used sheep cadaver lower jaws, bovine and porcine rib bones, red oak, resin, and various other materials. In this study, the polyurethane models used were prepared from tomographic data obtained from an orthognathic surgery patient, poured into a silicone mold, and thus closely resembled clinical conditions in terms of morphological features and shape. Furthermore, it has been reported that rigid polyurethane foams with polyol and isocyanate mixture show similar resistance to insertion

and removal as natural bone (12). In this experimental setup, forces were applied from the incisor region. Although in some studies, forces are applied over the first molar teeth, it can be assumed that, in clinical conditions, patients may avoid this due to their proximity to the cutting lines. Furthermore, the incisor region is preferred because it is farther from the molar region, resulting in a longer lever arm. This was considered a possible situation that might negatively affect the strength of the structure.

In vitro experiments in which the biomechanical strength of plate-screw systems is examined are usually designed based on values obtained from clinical studies. It is reported that there is a significant decrease in chewing forces in patients after orthognathic surgery (13). Gerlach and Schwarz reported that the average chewing force was 69.91N one week after the miniplate fixation of mandibular angle fractures and increased to 130.43N at six weeks (14). Similarly, Harada et al. reported that the average chewing forces of mandibular prognathism patients were 66.5N at two weeks and 128N at four weeks after surgery, and they mentioned that the chewing forces were even higher than the preoperative values after six months (15). Joos and Vassalli reported that the extent of advancement and fixation method affected relapse and emphasized the need for high compression strength in the early postoperative period (16). In our study, the range of force magnitudes was selected based on this information to make the experiment more clinically relevant. On the other hand, two different approaches are used to evaluate the results. The first approach is to compare the displacement values obtained at each 10N force increment. The second approach divides the displacement into levels, such as 1mm, 3mm, and 5mm, and evaluates the force magnitu-

des required to reach these levels. In the second approach, the focus is on relapse values accepted in the clinical setting. Proffit et al. defined movements greater than 2mm in the horizontal and vertical planes and angular changes exceeding 2 degrees as relapse (17). Pepersack and Chausse reported that a 1.5mm movement of the lower jaw should be considered a relapse (18). Based on this information, force magnitudes required for the displacement values of 1, 3, and 5mm were compared to improve the similarity of the experimental setup to clinical conditions.

Clinical and laboratory studies examining fixation methods used after orthognathic surgery yield different results. Dolanmaz et al. compared the strength of titanium and absorbable plates in 5mm mandibular advancements performed with the Dalpont surgical technique on sheep jaws. They applied forces ranging from 10N to 140N to the jaws and reported a significant difference in plate strength between 10N and 50N, stating that titanium plates provided higher stability (19). Oğuz et al. compared standard titanium mini plates with locking plates in the same experimental setup and reported no statistically significant difference between the two methods. However, they noted that standard mini plates were preferable for loads up to 60N, and locking plates were superior for loads exceeding 60N (20). In our study, mini plates and bicortical screw systems were used to conform to routine clinical practices. These systems can be used together or individually for mandibular advancements. Both systems have their advantages and disadvantages, depending on their characteristics. When miniplate systems are used, there is less risk of trocar scarring on the face, and the risk of lingual nerve damage is lower. Additionally, making corrections is easier when malposition is detected. However, the plates need to be adapted to the bone surface appropriately; otherwise, the position may change when the screws are tightened. Bicortical screw fixation ensures that segments are compressed, and this leads to the resistance of irregular surfaces to cutting and compression forces. Bicortical screws can be preferred when there is insufficient conformity between bone segments. Bicortical screw fixation requires less hardware, which reduces the cost. Particularly, the use of three bicortical screws extending linearly to the upper border in the reverse L configuration, as preferred in this study, is considered an appropriate fixation approach (21). When the diameters of bicortical screws are evaluated in terms of stability, there is no significant difference in diameters, and small-diameter screws are sufficient for patient comfort (22, 23). In this study, standard material dimensions were preferred, and systems were evaluated when used individually, which is more suitable for this study since the study involved standard materials and shapes.

Takahashi et al. examined stress areas when different numbers and locations of plates were placed on models that underwent advancement using the Dal Pont (OD), Obwegeser-Trauner (OT), and Obwegeser (OB) methods in the mandibles using finite element analysis. They found less stress on the plates and around the screws in models using the OD method compared to the OT and OB techniques, and they emphasized the high

retention of plates placed near the upper limit of the mandible at the osteotomy line (24). They attributed this to the fact that the OD technique shortens the segment acting as a lever arm. Puricelli et al. used computer-assisted simulations with vertical cuts made closer to the mental foramen and reported that increased medullary bone contact resulted in reduced stress distribution and an advantageous effect in stress reduction (25). Şirin et al. compared double and single miniplate fixation techniques in sheep mandibles for Trauner-Obwegeser (TO) and Obwegeser-Dal Pont (OD) techniques. As a result, they reported that the OD technique provided higher strength with a single plate and emphasized that early postoperative stability achieved through surgical techniques could be different even if the fixation techniques were similar. They suggested that the OD technique could be advantageous (26). In this study, there were no bicortical screws, and sheep mandibles were used unilaterally. In our study, it was observed that using a single plate in both techniques, there was no difference in strength. The discrepancy between these two studies may be due to the use of fully mandibular polyurethane models with superior shape similarity in our study and the application of loads only in the incisor region. In the absence of muscles and other soft tissues in the experimental environment, the strength of the fixation systems is dependent on the interaction between the screw, plate, and bone or the material that mimics it. When forces are applied to the incisor region due to mastication, the corpus mandibula is forced to rotate medially bilaterally, causing a reduction in the distance between the first molar teeth. This situation could limit the single-plate-bearing models when the fixation lines are intact. This study has some limitations. Firstly, the study used only one lower jaw model, two fixation systems, two cutting techniques, and a 5mm advancement range. Therefore, the validity of the findings is limited to these scenarios. The model used in the study does not include factors such as muscle involvement, callus, and other tissues that reduce the movement between the segments. In addition, the linear and gradually increasing force application technique used in the study does not fully mimic the complex biting and chewing movements of the lower jaw. Although polyurethane models can mimic the lower jaw in terms of shape, they differ in material properties. For these reasons, the findings of this study may differ from those conducted under clinical conditions.

## CONCLUSION

Within the limits of this study, it can be concluded that, in polyurethane mandibles undergoing BSSO procedures using either Dalpont or Epker techniques and fixed with mini plates or bicortical screws, the surgical technique does not have a significant effect on strength. Furthermore, regardless of the preferred cutting techniques, bicortical screw systems are more likely to provide higher strength compared to miniplate usage.

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**Ethics Committee Approval:** This study was approved by İstanbul University Faculty of Dentistry Clinical Research Ethics Committee (Date: 02.04.2021, No: 102)

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# EVALUATING THE EFFECTS OF VARIOUS IRRIGATION SOLUTIONS ON THE FRACTURE STRENGTH OF ENDODONTICALLY TREATED TEETH

## KÖK KANAL TEDAVİSİ YAPILMIŞ DIŞLERDE FARKLI İRRİGASYON SOLÜSYONLARININ KIRILMA DAYANIMLARI ÜZERİNE ETKİSİNİN İNCELENMESİ

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### ABSTRACT

**Objective:** Various irrigation solutions can affect the root canal sealer to bind dentine and not remove microorganisms from the root canal system completely. This study's objective is to investigate the efficiency of fracture strength of various irrigation solution protocols.

**Material and Methods:** The study involves 60 single-rooted teeth. The working lengths were determined, and ProTaper Next (PTN; Dentsply-Sirona) files (X5 50.06) were used to prepare the root canal shaping. The teeth were then randomly divided into three (n=20) groups according to the different irrigation solutions: ethylene diamine tetra acetic acid (EDTA), Irritrol, and chlorhexidine (CHX). A fracture strength test was performed with the fracture value being recorded in Newtons (N). Data were statistically analyzed with the Kruskal-Wallis test (p<0.05).

**Results:** The EDTA group showed higher bond strength values than the CHX or Irritrol groups. No significant difference was found between EDTA and CHX (p>0.05). When using Irritrol as the final irrigation solution, fracture values were significantly lower compared to when CHX and EDTA were used (p<0.05).

**Conclusion:** EDTA and CHX have similar effects on fracture strength.

**Keywords:** Fracture strength, irrigation solutions, Irritrol, resin-based sealers

### ÖZ

**Amaç:** Farklı irrigasyon solüsyonlarının kök kanal patının dentine bağlanma gücünü olumsuz etkileyebileceğini ve mikroorganizmaların kök kanal sisteminden tamamen uzaklaştırılmadığı görülmüştür. Bu çalışmanın amacı, farklı irrigasyon solüsyonlarının kullanılmasının dişlerin kırılma dayanımları üzerindeki etkisinin değerlendirilmesidir.

**Gereç ve Yöntem:** Çalışmaya 60 adet çürüksüz, tek köklü mandibular premolar diş dahil edildi. Çalışma boyları belirlendi ve kök kanal şekillendirilmesi üretici firmanın talimatlarına göre ProTaper Next (PTN; Dentsply-Sirona) X5 50.06 apikal genişlik elde edilinceye kadar prepare edildi. Ardından, dişler kullanılan irrigasyon solüsyonlarına (Etilendiamin tetraasetik asit (EDTA), Irritrol, Klorheksidin (CHX)) göre rastgele 3 (n=20) gruba ayrıldı. Kırılma dayanım testi uygulandı ve bu kırılma değeri Newton (N) cinsinden kaydedildi. Gruplarda normal dağılım göstermemiştir. Çalışmamızda, Non-Parametrik test olan Kruskal-Wallis ve Mann Whitney U testleri uygulanmıştır.

**Bulgular:** Etilendiamin tetraasetik asit grubu CHX ve Irritrol gruplarına göre daha fazla kırılma dayanımı göstermiştir. Etilendiamin tetraasetik asit ve CHX grupları arasında anlamlı bir farklılık bulunmamıştır (p>0,05). Irritrol ise diğer gruplara göre anlamlı derecede daha düşük kırılma dayanımı göstermiştir (p<0,05).

**Sonuç:** EDTA ve CHX'nin irrigasyon solüsyonu olarak kullanıldıktan sonra kök kanal patına ve gutaperkanın adhezyonuna benzer etkide buldukları görülmüştür.

**Anahtar Kelimeler:** Irritrol, irrigasyon solüsyonu, kırılma dayanımı, resin içerikli kök kanal patları

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## INTRODUCTION

The removal of bacteria from the root canal system using appropriate instrumentation, medications, and irrigation solutions is crucial for good root canal therapy (1). Chemomechanical preparation of root canals is very important for providing disinfection (2). However, mechanical preparation of the root canal results in the production of a smear layer of 1-2  $\mu\text{m}$  thickness that includes necrotic debris, bacteria, and bacterial byproducts. This smear layer clogs the dentinal tubules and is responsible for the establishment of bacterial growth (3). It also prevents irrigation solutions, medicaments, and canal sealers from penetrating the dentinal tubules (4, 5). Ethylenediaminetetraacetic acid solutions (EDTA), which are chelating agents, and sodium hypochlorite (NaOCl) are often used to remove the smear layer. Different antimicrobial agents can be used as irrigation solutions. Chlorhexidine (CHX) can be preferred as the final irrigation solution in root canal treatment due to its low toxicity and extended-release antimicrobial agent. As a final irrigation solution, Irritrol Two-In-One with EDTA and CHX was just introduced. The manufacturer claims that it causes a small amount of demineralization in dentin and provides effectiveness by removing the smear layer less aggressively than conventional irrigation solutions (6).

Recent studies have shown that using various irrigation solutions may adversely affect how the root canal sealer binds to the dentine and may incompletely remove microorganisms from the root canal system (7).

The present study aims to evaluate the fracture strength of the bond between the root canal filling and root canal wall after using EDTA, Irritrol, and CHX. The study's null hypothesis is that no significant difference will occur between any of the three groups.

## MATERIALS and METHODS

Upon obtaining permission from the Ankara Yıldırım Beyazıt University Ethics Committee, this investigation began involving 60 caries-free single-root mandibular premolar teeth. Periodontal curettes were used to remove debris and soft tissue remnants from the root surfaces. Teeth were examined under a stereomicroscope at x20 magnification for the existence of fractures and cracks. Teeth with cracks, fractures, defects, or an open apex were not included in the study. All teeth were examined with radiographs taken at buccolingual and mesiodistal angles. Teeth with calcification, resorption, curved canals, or multiple canals were replaced with new teeth that met the eligible criteria. All teeth were selected to have buccolingual diameters of 4-6 mm and mesiodistal diameters of 2-4 mm. Until testing, the teeth were maintained in a 0.9% saline solution. After removing the crowns of teeth, the root lengths were standardized at  $13\pm 1$  mm (8). Working lengths were determined using an electronic apex locator (Propex Mini; Dentsply, Sirona, Ballaigues, Switzerland). Root canal shaping was prepared with ProTaper Next (PTN) using an endomotor (X-Smart Plus,

Dentsply-Sirona) until an apical width of 50.06 was obtained.

### Experimental groups

The teeth were randomly divided into three ( $n=20$ ) groups based on the different irrigation solutions to be used (i.e., EDTA, Irritrol, CHX). The apex of the teeth was coated with wax to prevent the irrigation solutions from extruding the apex.

**EDTA-Saline Group:** The root canals were irrigated with 2 mL of 5.25% NaOCl following each file change. All samples were irrigated with 5 mL of a 17% EDTA solution for 1 minute to eliminate the smear layer. Samples were then irrigated with 5 mL of saline.

**EDTA-CHX Group:** The root canals were irrigated with 2 mL of 5.25% NaOCl following each file change, and then all samples were irrigated for 1 minute with 5 mL of 17% EDTA solution to remove the smear layer. Next, the samples were irrigated with 5 mL of saline and lastly with 5 mL of 2% CHX.

**Irritrol Group:** The root canals were irrigated with 2 mL of 5.25% NaOCl following each file change. Samples were then irrigated with 5 mL of saline, followed by 5 mL of Irritrol. Afterward, all root canals were dried with ProTaper Next X5 paper points.

Root canals were filled with the single cone technique using ProTaper Next X5 (PTN) gutta-percha and AH Plus (Dentsply-Sirona), a resin-containing root canal sealer. Afterward, the samples were kept in a 100% humidity environment at 37°C for 7 days in order to cure the root canal sealer.

The specimens were embedded in acrylic blocks with their 2 mm coronal part exposed (9). The samples were stored in a humid environment with the help of a wet towel stop to prevent dehydration until the fracture test was applied. The fracture strength values were determined using an electromechanical servo universal testing machine (Besmak Ltd., Ankara, Türkiye). The study was carried out with reference to the experimental conditions of the previous study (10). The sudden decrease in the graph value displayed on the computer screen connected to the test device against the applied load was determined as the force value at the moment of fracture (Figure 1). This fracture value was recorded in Newtons (N).

### Statistical analysis

The obtained data were analyzed using the program IBM SPSS 21 (IBM SPSS Corp., Armonk, NY, USA). The Kolmogorov-Smirnow test was applied to determine whether the obtained data were normally distributed. The Kruskal-Wallis test, a non-parametric test, was then applied. The Mann-Whitney U test was used to determine whether differences between groups exists.

This study was approved by the Ankara Yıldırım Beyazıt University (AYBU) Health Science Ethics Committee (Approval No. 2022-1231 dated December 8, 2022).



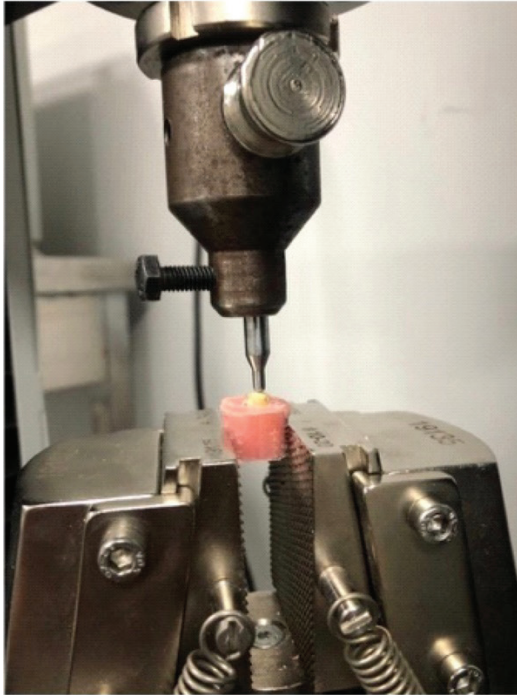


Figure 1: Applying the fracture strength test

## RESULTS

Table 1 shows the median and standard deviation values for the fracture strengths. While the EDTA group showed higher fracture strength values than the CHX and Irritrol groups, no significant difference was observed between the EDTA and CHX groups ( $p>0.121$ ). When using Irritrol as the final irrigation solution, fracture strength values were significantly lower than the CHX ( $p<0.012$ ) and EDTA values ( $p<0.000$ ).

Table 1: Fracture Strength Values (N) of the Experimental Groups

Irrigation solutions	Median	Min	Max	SD	P value
EDTA	353.36 <sup>x</sup>	240.57	450.88	59.14	
CHX	327.66 <sup>x</sup>	116.14	431.44	88.44	0.001
Irritrol	197.87 <sup>y</sup>	128.67	452.77	101.38	

Different superscript letters indicate statistically significant differences between groups.  $P<0.05$ , <sup>x,y,z</sup>: Columns, EDTA: Ethylenediaminetetraacetic acid; CHX: Chlorhexidine

## DISCUSSION

The aim of the treatment has been to provide a three-dimensional obturation that completely covers the dentin canals, accessory canals, and lateral canals, as well as the coronal and apical sections in the root canal system (11). The use of a canal sealer is important in terms of providing a connection between the gutta-percha and canal walls and for filling the canal spaces where gutta-percha cannot be reached. During root canal preparation, a smear layer forms that adversely affects the adhe-

sion of the sealer to the radicular dentin and dentinal tubules (12). This study has used AH-Plus root canal sealer due to its epoxy resin content and stronger bond compared to other sealers (1). This bond has been observed to occur by binding to the amino groups in dentinal collagen (13). Additionally, different irrigation solutions can affect the adhesion of the sealer, which in turn affects the collagen structure, the wettability of the dentin, and root fracture resistance (14, 15).

Fracture strength is the maximum stress that a dental structure or material can withstand before breaking and is considered the best measure of tooth strength (16). This study completed the root canal fillings with the single cone technique to eliminate the effect the root canal filling technique has on the fracture strength values. EDTA showed higher values than the other groups, so the null hypothesis of this study has been rejected. While EDTA showed numerically higher fracture strength values than CHX, no statistically significant difference was found between them. Stelzer et al. showed CHX to have no impact on bond strength in either of the gutta-percha/AH Plus root canals when comparing EDTA and NaOCl as an irrigation solution (17). The current study supports these findings. The reason why the fracture strength value of Irritrol was significantly lower than the other groups in this study may have been due to the interaction of EDTA and CHXs in the solution and the production of some white precipitate (18). This white precipitate may have blocked the dentinal tubules or prevented the sealer from penetrating the tubules, thus resulting in less adhesion. All specimens in the present study included mandibular premolar teeth and were grouped according to similarity of dimensions. The specimens were standardized to 13 mm, and the root canals were enlarged with the same instruments and techniques. However, the strength of the roots could still be affected by uncontrollable physiological variations, such as the unknown age of the patient and dentin thickness (19, 20). An increase in the mineral content (inorganic part) of hard tissues occurs with age, as well as a decrease in collagen content (organic part). Fracture strength and elasticity depend on the amount of collagen in the hard tissue (21). Furthermore, the mechanical properties of obturated roots may vary based on the amount of dentine remaining around the root canal after the different irrigation techniques. As such, these could be limitations for this study.

Küçük et al. examined the dentin tubular penetration activities of Irritrol, CHX, and QMIX, with Irritrol showing similar effects to CHX (18). Other studies have reported Irritrol and CHX to have similar dentin tubule penetration effects when performing the final irrigation with PIPS (22). Another study reported Irritrol, EDTA, and QMIX to provide similar root canal sealer adhesion (23). The current study found binding the root canal sealer to the dentin tubule to be highly influenced by Irritrol. The fracture strength value is thought to be lower in the Irritrol group compare to the other groups due to the unknown amount of EDTA in the Irritrol solution.

The need exists for comprehensive studies to evaluate the solutions' effectiveness at removing the smear layer after using

irrigation activation techniques, as well as their interactions with root canal sealers and their differing contents.

## CONCLUSION

Under the experimental terms of the current in vitro study, the fracture strength of Irritrol, which is combined with EDTA and CHX, has been shown to have a significantly lower fracture strength value than the other two groups.

**Ethics Committee Approval:** This study was approved by Ankara Yıldırım Beyazıt University (AYBU) Health Science Ethics Committee (Approval No. 2022-1231 dated December 8, 2022).

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Journal Article: Blasco V, Colavolpe JC, Antonini F, Zieleskiewicz L, Nafati C, Albanèse J, et al. Long-term outcome in kidney recipients from donor treated with hydroxyethylstarch 130/0.4 and hydroxyethylstarch 200/0.6. *Br J Anaesth* 2015;115(5):797-8.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. *Infectious Diseases*. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. *Martindale the Complete Drug Reference*. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. *Functional reconstructive nasal surgery*. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengissson S, Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. *MEDINFO 92. Proceedings of the 7th World Congress on Medical*

Informatics; 1992 Sept 6-10; Geneva, Switzerland.  
Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerine Beden Kitle İndeksleri Kan Lipidleri Arasındaki İlişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: <http://www.cdc.gov/ncidod/EID/cid.htm>.

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