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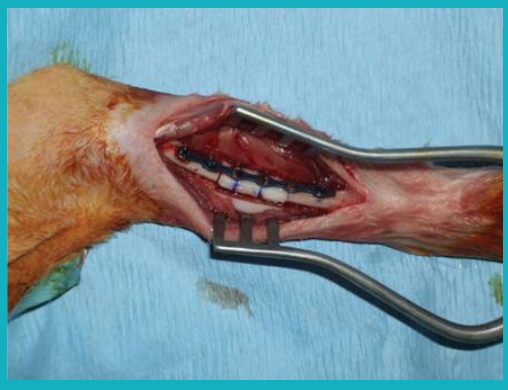

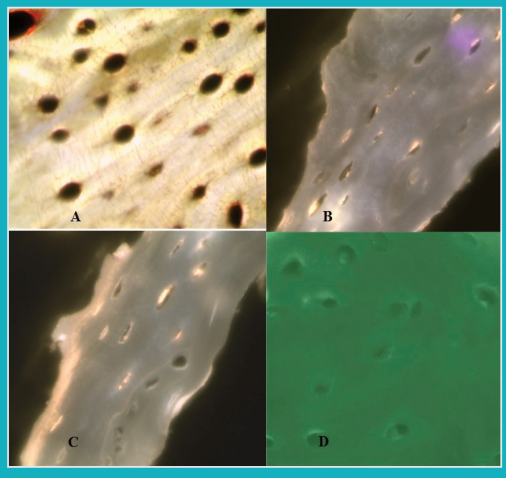

Generation of Bone Tissue Using Adipose Tissue-derived Stem Cells

Ethem GÜNEREN, Emre Gönenç BAYGÖL, Mehmet Veli KARAALTIN1, Halil İbrahim CANTER, Kahraman ÖZTÜRK, Ercüment OVALI, Mustafa Aykut ÖZPUR, Kemalettin YILDIZ, Fatma EYÜBOĞLU; İstanbul, Turkey

A Novel High-performance Liquid Chromatography Method with Fluorescence Detection for the Quantification of Roflumilast in Tablet Formulations

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Evaluation the Early Effects of Compression Stockings on Patient Satisfaction in Acute Proximal Deep Vein Thrombosis

Cemal KOCAASLAN, Mehmet Şenel BADEMCİ, Ahmet ÖZTEKİN, Emine Şeyma DENLİ YALVAÇ, Fatih BAYRAKTAR, Ebuzer AYDIN, İstanbul, Muş, Türkiye

High-performance Liquid Chromatography Analysis of Nebivolol and Amlodipine and Some Related Compounds in Synthetic Mixture

Cem ÖNAL, Kadir ALUÇ, Çiğdem ALUÇ, Armağan ÖNAL; İstanbul, Turkey

Perceived Sources and Levels of Stress Among Turkish Dental Students: A Multi-centre Study

Mustafa DEMİRCİ, Neslihan TEKÇE, Nevin ÇOBANOĞLU, Safa TUNCER, Osman ÜNLÜ, Soner ŞİŞMANOĞLU, Canan BAYDEMİR; İstanbul, Kocaeli, Konya, Türkiye

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The journal Bezmialem Science is an international periodical published in electronic format in accordance with the principles of independent, unbiased, and double-blinded peer-review. Four issues are published per year, in January, April, July and October.

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Manuscripts accepted for publication, not published yet: Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med In press 1997.

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BEZMIALEM VAKIF UNIVERSITY 1845

BEZMİÂLEM science

CONTENTS

Commentary

250 Which Patient with Reflux Should Undergo Surgery?
Prof. Dr. Adem AKÇAKAYA; İstanbul, Turkey

Original Articles

253 A Novel High-performance Liquid Chromatography Method with Fluorescence Detection for the Quantification of Roflumilast in Tablet Formulations
Ulvi MEHDİZADE, Pelin KÖSEOĞLU YILMAZ; İstanbul, Türkiye

259 Evaluation the Early Effects of Compression Stockings on Patient Satisfaction in Acute Proximal Deep Vein Thrombosis
Cemal KOCAASLAN, Mehmet Şenel BADEMCİ, Ahmet ÖZTEKİN, Emine Şeyma DENLİ YALVAÇ, Fatih BAYRAKTAR, Ebuzer AYDIN, İstanbul, Muş, Türkiye

264 High-performance Liquid Chromatography Analysis of Nebivolol and Amlodipine and Some Related Compounds in Synthetic Mixture
Cem ÖNAL, Kadir ALUÇ, Çiğdem ALUÇ, Armağan ÖNAL; İstanbul, Turkey

271 Perceived Sources and Levels of Stress Among Turkish Dental Students: A Multi-centre Study 270
Mustafa DEMİRCİ, Neslihan TEKÇE, Nevin ÇOBANOĞLU, Safa TUNCER, Osman ÜNLÜ, Soner ŞİŞMANOĞLU, Canan BAYDEMİR; İstanbul, Kocaeli, Konya, Türkiye

283 Cross-cultural Adaptation, Reliability and validity of the Turkish Version of the Work Limitations Questionnaire-Short Form
Ramazan ŞAHİN, Seçil ÖZKAN, Mustafa Necmi İLHAN; Ankara, Turkey

290 Effect of Stapler Booster on Post-operative Air Leakage in the Surgical Treatment of Bullous Lung Disease
Cemal KOCAASLAN, Ayten GÜNER AKBIYIK, Ömer Giray İNTEPE, Tamer OKAY; İstanbul, Kırklareli, Turkey

294 Generation of Bone Tissue Using Adipose Tissue-derived Stem Cells
Ethem GÜNEREN, Emre Gönenç BAYGÖL, Mehmet Veli KARAALTIN, Halil İbrahim CANTER, Kahraman ÖZTÜRK, Ercüment OVALI, Mustafa Aykut ÖZPUR, Kemalettin YILDIZ, Fatma EYÜBOĞLU; İstanbul, Turkey

301 Evaluation of Factors Related to Pancreatic Fistula Development in Patients Undergoing Pancreaticoduodenectomy for Periampullary Tumours
Uğur TOPAL, Erdoğan Mütevelli SÖZÜER, Türkmen Bahadır ARIKAN, Mustafa GÖK, Gamze Kübra BOZKURT; Kayseri, Turkey

BEZMIALEM VAKIF UNIVERSITY 1845

BEZMİÂLEM science

CONTENTS

310 An Assessment of the Comfort Level of Cancer Patients with Receiving Chemotheraphy
Aysun CAN, Sevilay HİNTİSTAN; Trabzon, Turkey

317 Needlestick and Sharps Injuries Among Operating Room Nurses, Reasons and Precautions
Mahmut DAĞCI, Yazile YAZICI SAYIN; İstanbul, Turkey

326 Determining the Relationship between Perceived Social Support Levels and Chemotherapy Symptoms in Women with Gynaecological Cancer
Sakine YILMAZ, Gülşen VURAL; Çankırı, Turkey, Lefkoşa, KKTC

334 The Effect of Different Nutrition Education Approaches on Children's Nutritional Status
Burcu ATEŞ ÖZCAN, Aydan ERCAN; İstanbul, Edirne, Turkey

344 Effect of Thermocycle and Bonding Agents on the Bond Strength of Titanium-resin Cements
Gülsüm SAYIN ÖZEL, Ceyda AKIN, Bebek Serra OĞUZ AHMET; İstanbul, Konya, Turkey

351 Optimisation of Centrifugal Speed for PRPBAG®: A Novel Multiple Bag System for Preparing Platelet-Rich Plasma
Güven ÇETİN, Nang Hseng KYIO, Seda TURGUT, Tuba ÖZKAN; İstanbul, Turkey

356 Depression, Anxiety, and Stress Scales 42 (DASS-42) in Dari-Language: Validity and Reliability Study in Adults, Herat, Afghanistan
Nasar Ahmad SHAYAN, Aziz-ur-Rahman NIAZI, Abdul Momen WASEQ, Hilal ÖZCEBE; Herat, Afghanistan, Ankara, Turkey

Case Report

363 Secondary Involvement of the Penis by Non-Hodgkin Lymphoma
Gizem TİMOÇİN YIĞMAN, Abdusselim Adil PEKER, Rasul SHARİFOV, Hüseyin TOPRAK; İstanbul, Turkey

366 A Coin Trapped in Meckel's Diverticulum
Levent CANKORKMAZ; Sivas, Turkey

Review

369 The Golgi Apparatus with the Historical Point of View
Mukaddes EŞREFOĞLU; İstanbul, Turkey

373 Food Allergy in Children
Ayşe GÜNEŞ BAYIR, Büşra CAN, Şeyma EKİNGEN; İstanbul, Turkey

BEZMIALEM VAKIF UNIVERSITY 1845

BEZMİÂLEM science

EDITORIAL

Dear readers,

We are happy to welcome you again in a new issue. With the summer, the relief seen all over the world and the slowdown of the pandemic provided some relief in our country. Even though we did not start our face-to-face meetings fully and our masks were still on, even the feeling of getting closer to normal was good for all of us. The efforts of our Ministry of Health in the supply of the last vaccine have relieved us and we hope that towards the autumn most of our citizens will be vaccinated and we will spend this winter more comfortably. I would like to express my gratitude once again to all our editors, referees and you, our esteemed authors, for the accepted articles and published issues and the point our journal has reached.

We experience the difficulties and advantages of publishing a multi-disciplinary journal together. While the difficult part is to create a balanced issue and to make the branch distribution balanced, I can state that the advantage is the arrival of a large number of articles. We know that every article requires a lot of effort, but we are suffering from not being able to accommodate all articles, and we expect you to understand that our rejection rate is high.

I would like to share with you two developments that make us happy. In addition to our entry into new indexes, we are happy that our citation rates have increased recently. We look forward to your support in this regard.

In this issue, we present many good articles to you. Our eye-catching articles are as follows: “Generation of Bone Tissue Using Adipose Tissue-derived Stem Cells” by Güneren et al., “A Novel High-performance Liquid Chromatography Method with Fluorescence Detection for the Quantification of Roflumilast in Tablet Formulations” by Mehdizade et al., “Evaluation the Early Effects of Compression Stockings on Patient Satisfaction in Acute Proximal Deep Vein Thrombosis” by Kocaaslan et al., “High-performance Liquid Chromatography Analysis of Nebivolol and Amlodipine and Some Related Compounds in Synthetic Mixture” by Önal et al., and “Perceived Sources and Levels of Stress Among Turkish Dental Students: A Multi-centre Study”by Demirci et al.

We also chose the cover art of this issue from the study of Güneren E. et al. which was titled “Generation of Bone Tissue Using Adipose Tissue-derived Stem Cells”. The subject of “tissue engineering”, which is one of the important preoccupations of today’s medicine, is actually one of the important health fields of the future. This will not only be the pinnacle of humanity’s developments in the field of health, but will also attract attention in the future as a commercial investment with great potential of economic return. I present to the attention of our readers this study with the theme of the effect of own adipose tissue-derived stem cells on bone tissue healing, carried out by a team at Bezmialem Vakıf University in our Experimental Research Department.

Dear Readers,

Science is an endless ocean and we scientists are like fishermen navigating this ocean. The contributions of each of you will be instrumental in the discovery of new horizons in this ocean. While we are happy to see great progress in the scientific field in our country, it is important that our work is in university-industry cooperation areas that contribute to daily life and create added value. For this reason, I would like to emphasize once again that your experimental and clinical studies, which we believe will create added value, are a priority for us.

See you in our next issue with a richer content, everything you get your heart desires.

Best regards,

Prof. Dr. Adem AKÇAKAYA
Editor-in-chief

Commentary

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Which Patient with Reflux Should Undergo Surgery?

Hangi Reflü Hastası Ameliyat Olmalı?

Adem AKÇAKAYA

Bezmialem Vakıf University Faculty of Medicine, Department of General Surgery, İstanbul, Turkey

Dear Readers,

In this article, I will talk about reflux disease, its symptoms, diagnosis, treatment methods and which patients should be treated with surgery.

Gastroesophageal reflux disease (GERD) is a common disease in developed and developing societies (1,2). Reflux disease is the reflux of acidic stomach contents into the esophagus. Sometimes, stomach contents may contain pepsin, pancreatic enzymes, bile and foods in addition to acid. This backflow irritates the lining of the esophagus. Most people may experience physiological acid reflux from time to time (2). It is reported that the incidence of reflux in the world is around 20%. In our country, it has been determined that it is slightly more than 25%, although it varies according to the regions. This means that one out of four people has reflux.

Gastroesophageal reflux disease is an important disease that causes significant physical and psychological problems with the disturbing symptoms it causes, and surgical treatment is frequently used in addition to medical treatment in its treatment (3,4). Although symptom control is achieved in a large patient group with the appropriate use of medical treatment, symptom control is not possible at a rate of 30-40% with a significant decrease in quality of life despite medical treatment (5). Although medical treatment is effective in controlling heartburn, it may be insufficient to control regurgitation and respiratory symptoms, especially in patients with GERD accompanied by hiatal hernia. In addition, chronic complications of GERD such as stricture and Barrett's esophagus despite medical treatment, as well as the risk of developing adenocarcinoma on the basis of Barrett's esophagus contribute to the uncertainty in the treatment of the disease.

Although the effectiveness of medical treatment for GERD is known, the side effects of drugs, the cost of long-term drug use and its negative effects on the patient, the decrease in the quality of life of the patients, and the surgical treatment option come to the fore especially in patients in whom drugs fail in symptom control or symptoms recur (3).

Heartburn and regurgitation are the two most important symptoms of GERD, either alone or together. Although these two typical symptoms are seen in almost all patients, it should be kept in mind that irritable bowel syndrome, functional dyspepsia, esophageal hypersensitivity and other somatoform disorders may cause reflux-like complaints. It is known that atypical symptoms such as nausea, belching, slow digestion, early satiety, epigastric pain, bloating, vomiting, precordial catch syndrome, cough, wheezing, chronic rhinosinusitis, hoarseness and hoarse voice are among the complaints of these patients.

The diagnosis can be made based on the anamnesis and findings. However, it is of great importance to make the differential diagnosis of other diseases in this region before the operation. To diagnose and determine the severity of the disease; gastroscopy, pH measurement, contrast radiographs, and manometric studies are performed. The two most important diagnostic tests to confirm the diagnosis of GERD are endoscopy and pH and esophageal impedance monitoring. Manometer is used in the evaluation of disorders such as achalasia and ineffective esophageal motility before surgical treatment rather than diagnosis. In patients with large hiatal hernia, barium examinations are helpful in revealing the anatomy and detecting the short esophagus. It is stated that choosing the appropriate patient and the most appropriate technique with the help of the obtained data, as well as ensuring

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that the surgery to be performed by an experienced surgeon are important for the success of the treatment (3).

Reflux can usually be controlled with lifestyle changes, nutritional support, phytotherapy and supplements. But some people need stronger medications or surgery to relieve symptoms. The aim of treatment in reflux disease is to control the complaints, heal the damage in the esophagus and prevent complications. The course of the disease is variable in each patient and requires a different approach. It is of great importance to decide which patient will be treated with medication, diet and other methods and which patient will be operated on.

In the first stage of reflux treatment; diet regulation, drugs that suppress stomach acid and changes in lifestyle are tried. Raising the head of the bed by 15 cm, sleeping on the left side at night, reducing food consumption that may cause reflux, not eating until at least 3 hours before bedtime, losing excess weight, not wearing tight clothes, reducing smoking, coffee and alcohol consumption are the changes that can be made in lifestyle.

In recent years, some endoscopic treatment methods have been used to strengthen the lower esophageal sphincter in the treatment of reflux. Endoscopic gastroplasty (EndoCinch) and radiofrequency energy application (Stretta method) can be counted among these. Although some centers apply these methods quite successfully, they are not yet included in routine practice.

Before deciding on surgical treatment in a patient with reflux, it is necessary to clearly determine whether the patient needs long-term reflux treatment. Surgical treatment is a method used in approximately 10% of patients with reflux.

Laparoscopic antireflux surgery is recommended for patients with a persistent decrease in quality of life and persistent bothersome symptoms, especially if the disease progresses or symptoms cannot be controlled despite proton pump inhibitor therapy. It is stated that surgical treatment may be preferred more if the following criteria are met: (1,3)

• Typical symptoms of GERD

• Demonstrating the relationship between symptom and reflux

• History longer than one year

• Decreased quality of life

• Response to proton pump inhibitor therapy

• Increasing the proton pump inhibitor treatment dose

• Hiatal hernia

• Presence of esophagitis endoscopically before treatment

• Demonstration of lower esophageal sphincter insufficiency

• Demonstrating acid reflux

In anti-reflux surgery, the valve system where the esophagus meets the stomach is strengthened. The functional antireflux barrier to be created during surgical treatment has 3 important components: Adequate length of the intra-abdominal esophagus, repair of the crura, and fundoplication. In this method, which is called fundoplication in the medical literature, the stomach is wrapped around the esophagus. This wrap can be applied at 180 (Dorr), 270 (Toupet) or 360 (Nissen) degrees.

Although a limited number of randomized controlled studies have shown that partial fundoplication causes less short-term side effects, long-term results are still lacking (6). Therefore, an experienced surgeon working in a high-volume center should decide on his/her own experience and conclusions whether the procedure to be performed will be total or partial fundoplication.

In the Japanese guideline, laparoscopic Toupet fundoplication is recommended as the standard surgical technique for GERD (7). Most frequently, publications compare laparoscopic Nissen fundoplication with posterior partial Toupet hemifundoplication. Their superiority over each other is defined differently in different publications. Therefore, it is an appropriate approach to decide which type of surgery will be used according to the experience and results of the surgeon.

Nissen fundoplication surgery, in which 360-degree wrap is performed in most centers, is considered the gold standard and is the most preferred method. In the meantime, if there is a stomach hernia, this is also repaired. If I don't have any doubts about motility disorder in my patients, I mostly use this method as floopy style.

Total fundoplication surgery was first performed by Rudolp Nissen in 1956. Today, it is widely performed both laparoscopically and floopy style. In the published guidelines, it is especially emphasized that standard procedure steps are necessary for a successful surgery (3).

It is possible to summarize the basic surgical procedures for laparoscopic Nissen fundoplication in line with the consensus of experienced surgeons: (1,7)

1) Opening of the phrenoesophageal ligament from left to right

2) Preservation of the hepatic branch of the anterior vagus,

3) Dissection of both crura

4) Intraabdominal mobilization of the esophagus at least 3 cm

5) Ligation of short gastric vessels for a tension-free wrapping

6) Closing the opening between the crura with non-absorbable sutures from the posterior

7) Forming a 1.5-2 cm wrap, passing the most distal suture through the anterior muscle layer of the esophagus at this stage

8) The use of spark plugs in the wrapping phase (This is not required for every patient, there are opinions that it should not be used)

After the surgery, the patient is discharged within a few days. There may be temporary swallowing difficulties that can last

for several months. Persistent, recurrent or newly developing symptoms after surgical treatment are defined as failure. Primary anti-reflux surgery has an average of 85-90% successful results over five years. Repeat anti-reflux surgery is required in approximately 3-6% of patients. In case of failure, heartburn and regurgitation are the most common symptoms. Dysphagia is the second most common symptom.

As a result; surgery has an important place in the treatment of GERD. Surgical treatment provides sufficient improvement in the majority of patients in the early period. The minimally invasive approach has led to the widespread use of laparoscopic fundoplication surgery. Despite the recent experience, it is still necessary to be meticulous in patient selection, deciding surgery and, making decision of which method to apply. This will directly affect the success of the treatment. It should not be forgotten that it is necessary to communicate with experienced centers and specialist physicians.

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Original Article

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A Novel High-performance Liquid Chromatography Method with Fluorescence Detection for the Quantification of Roflumilast in Tablet Formulations

Roflumilastın Tablet Formülasyonlarda Miktar Tayini için Yeni Bir Floresans Dedeksiyonlu Yüksek Performanslı Sıvı Kromatografisi Yöntemi

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ABSTRACT

Objective: This study aims to develop and validate a novel high-performance liquid chromatography method with fluorescence detection for quantifying roflumilast in tablet formulations.

Methods: Separations were achieved by a C18 analytical column (250x4.6 mm, 5 µm) at 40 ºC. Isocratic elution accompanied by a mobile phase comprising 20% aqueous o-phosphoric acid solution (0.08%) and 80% methanol was applied. The excitation and the emission wavelengths were 290 and 380 nm, respectively.

Results: The linear range was 1.25-10.00 µg/mL. Irbesartan was used as the internal standard. The limits of detection and quantification were 0.07 µg/mL and 0.22 µg/mL, respectively. The precision and accuracy of the method was determined at the concentrations of 1.25, 5.00 and 10.00 µg/mL. The recovery percentage was calculated by the tablet solutions spiked at low, middle and high concentrations. The robustness of the method was tested in terms of flow rate, mobile phase composition and column temperature.

Conclusion: The proposed method was successfully applied for determining roflumilast in tablet formulations with a high precision and accuracy.

Keywords: Fluorescence, HPLC, method development, roflumilast, validation

ÖZ

Amaç: Bu çalışmada roflumilastın tablet formülasyonlarda miktar tayini için yeni bir floresans dedektörlü yüksek performanslı sıvı kromatografisi yönteminin geliştirilmesi amaçlandı.

Yöntemler: Ayırma işlemleri 40 ºC'de bir C18 analitik kolon (250x4,6 mm, 5 µm) ile gerçekleştirildi. Mobil faz olarak %20 sulu o-fosforik asit çözeltisi (%0,08) ve %80 metanol içeren bir sistem kullanıldı ve izokratik elüsyon uygulandı. Eksitasyon ve emisyon dalga boyları sırasıyla 290 ve 380 nm olarak belirlendi.

Bulgular: Doğrusal aralık 1,25-10,00 µg/mL olarak tespit edildi. İç standart olarak irbesartan kullanıldı. Gözlenebilme ve tayin sınırları sırası ile 0,07 µg/mL ve 0,22 µg/mL idi. Yöntemin kesinlik ve doğruluğu 1,25, 5,00 ve 10,00 µg/mL konsantrasyonlarındaki standart çözeltiler ile belirlendi. Yüzde geri kazanım düşük, orta ve yüksek konsantrasyonlarda standart eklenen tablet çözeltilerinin analiz sonuçları ile hesaplandı. Yöntemin sağlamlığı akış hızı, mobil faz bileşimi ve kolon sıcaklığı parametreleri ile incelendi.

Sonuç: Önerilen metot roflumilastın tablet formülasyonlarda yüksek kesinlik ve doğruluk ile tayini için başarılı bir şekilde uygulandı.

Anahtar Sözcükler: Floresans, HPLC, metot geliştirme, roflumilast, validasyon

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Introduction

Chronic obstructive pulmonary disease (COPD) is a health issue causing chronic airflow obstruction that is not fully reversible (1,2). Its risk factors include deficiency of alpha-1 antitrypsin, cigarette smoking, occupational chemical exposure and air pollution with cigarette smoking being the most common one. Pharmacological treatments are successful in reducing symptoms and exacerbations while improving the health status and increasing the exercise tolerance (3).

Roflumilast (RFL) (3-(cyclopropylmethoxy)-N-(3,5-dichloro-4-pyridyl)-4-(difluoromethoxy) benzamide) (Figure 1) is currently an approved selective phosphodiesterase-4 (PDE-4) inhibitor for treating COPD. RFL is available in 500 μg tablets, and the recommended dose is 1 tablet/day. The absolute bioavailability of RFL is 79% following oral administration (4). It is then metabolized by cytochrome p450 (CYP) 3A4 and 1A2 isozymes to its active metabolite-RFL N-oxide (5). Daxas®, with the active pharmaceutical ingredient of RFL, was approved in the European Union in June 2010. It further received Food and Drug Administration's (FDA) approval in the USA in March 2011 (6).

RFL is not an official drug in the British Pharmacopoeia, European Pharmacopoeia and United States Pharmacopoeia yet and has no official monograph (7,8). In contrast, several studies have described the analytical methods for quantifying RFL in pharmaceutical forms in the presence or absence of degradation products (DP). High-performance liquid chromatography (HPLC) is one of the commonly used techniques to achieve this purpose. A previous study developed and validated a reverse phase (RP)-HPLC method with ultraviolet (UV) detection for determining RFL in formulations (6). Belal et al. (7) developed a stability-indicating HPLC method with a diode-array detector (DAD) for determining RFL and achieved the application of the proposed method for analysing the tablet formulation. Pinheiro et al. (8) developed and validated a RP-HPLC method with DAD and corona-charged aerosol detector in line for RFL and its DPs and successfully separated RFL from six DPs. Tan (9) developed and validated an HPLC method for quantifying RFL in the presence of its DPs and related substances to control the drug's purity. Also, a validated stability-indicating high-performance thin-layer chromatography method was applied for determining RFL in tablets (10). Besides chromatographic methods, a different research developed and validated a UV-visible spectrophotometric method for the quantitative determination of RFL in tablet formulation (11). Atmaca and Süslü (12) validated a first-order derivative UV spectrophotometric method for determining RFL in pharmaceutical formulations. Also, Güray (13) reported a new and validated capillary electrophoresis method with UV detection to successfully determine RFL in tablets.

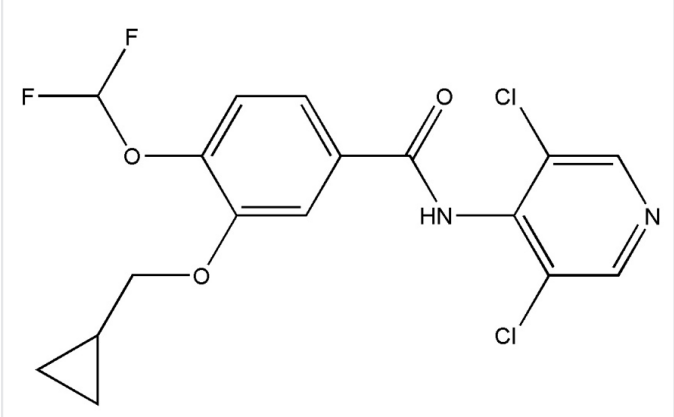

Figure 1. Chemical structure of roflumilast

HPLC with fluorescence detection (FD) may be an alternative for analysing RFL with a considerably higher selectivity than UV detection. The objective of this study is to develop and validate a sensitive and simple method for determining RFL in tablet formulations by HPLC-FD without a derivatisation reaction. To the best of knowledge, our study could be the first report on the determination of RFL by HPLC-FD.

Methods

Chemicals and Solutions

The standards of RFL and irbesartan (IRB) were kindly provided by Abdi İbrahim Pharmaceuticals (İstanbul, Turkey). The HPLC grade methanol (MeOH) was purchased from Isolab (Eschau, Germany) and o-phosphoric acid was purchased from Merck (Darmstadt, Germany). The commercial tablets of DAXAS® containing 500 μg of RFL were analysed.

The stock solutions of RFL and IRB at 100.00 μg/mL were prepared with HPLC grade MeOH and kept at 4 °C protected from daylight. The standard solutions were prepared daily by the dilution of the stock solutions with the mobile phase to desired concentrations.

For the preparation of the tablet solution, ten DAXAS® tablets were weighed individually and the average weight of tablet was calculated (0.2660 g). Then, the tablets were grounded and an amount of powder equal to the average tablet weight was transferred into a 100 mL volumetric flask. In total, 60 mL of MeOH and 2.50 mL of IRB stock solution were added in the flask and the solution was kept in an ultrasonic bath for 30 minutes. Later, the volume was adjusted to 100 mL with MeOH. The final concentrations of RFL and IRB were 5.00 μg/mL and 2.50 μg/mL, respectively. The spiked tablet solutions were prepared by the addition of 1.00, 2.50 and 5.00 mL of the stock RFL solution before fixing the volume to 100 mL.

Appropriate volumes of the stock IRB solution were added to all the solutions for obtaining a final concentration of 2.50 μg/mL. All the solutions were filtered through a 0.45-μm filter before injection into the HPLC-FD system.

Instrument and Analytical Conditions

The analyses were performed by a Shimadzu LC20AT HPLC system with FD (RF20A) (Shimadzu, Kyoto, Japan). The separation was achieved using a GL Sciences Inertsil ODS-3

analytical column (C18, 4.6x250 mm, particle size of 5.0 μm) (GL Sciences Inc., Tokyo, Japan). The data were analysed by the LabSolutions software (version 1.25).

Isocratic elution was applied with a mobile phase system comprising 20% o-phosphoric acid solution (0.08%, pH: 2.3) and 80% methanol. The excitation and the emission wavelengths were 290 and 380 nm, respectively. The flow rate was set to 1.0 mL/min, and the injection volume was 20 μL. The column temperature was adjusted to 40 °C.

Quantification

RFL was identified by comparing the retention time with the one of its standards. The quantification was performed by the internal standard method using IRB as the internal standard.

Validation

The developed method was validated in terms of linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy and robustness.

Linearity

The linearity was determined by a seven-point calibration curve for RFL. The calibration curve was plotted as the analyte's peak area/internal standard's peak area versus concentration with the data of triplicate analyses/day performed in three different days. Calibration equation and r^2 value were calculated using the linear regression analysis based on the least-squares method.

Limits of Detection and Quantitation (LOD and LOQ)

LOD and LOQ were determined as 3.3 and 10 times of the ratio of the standard deviation of the calibration curve to the slope of the calibration curve, respectively.

Precision and Accuracy

Precision was examined as repeatability (intraday) and intermediate precision (interday) with standard solutions at 1.25, 5.00 and 10.00 μg/mL in terms of relative standard deviation relative standard deviation (RSD%). Repeatability was determined by the data of triplicate injections consecutively in one day. Intermediate precision values were calculated in triplicate analytical runs in three different days. Accuracy was determined as the relative mean error (%) with standard solutions at 1.25, 5.00 and 10.00 μg/mL in triplicate analyses.

The original (5.00 μg/mL) and spiked (at 1.00, 2.50 and 5.00 μg/mL) tablet solutions were analysed in triplicates. RSD (%), RME (%) and recovery (%) values were calculated.

Specificity

An injection of only the mobile phase as the sample was performed to check the specificity of the method.

Robustness

The robustness of the method was checked by considering the parameters of the flow rate, the mobile phase composition and the column temperature. The flow rate was varied ±0.1 whereas the others were varied ±1 of the original values and and the RFL concentration of the tablet solution was calculated under these conditions. The results were obtained as RME%.

Results

Selection of HPLC Conditions

Several studies were performed using different types of mobile phase systems comprising mixtures of water, methanol, acetonitrile, o-phosphoric acid and formic acid with various proportions and gradient and isocratic elution. A mobile phase comprising 20% o-phosphoric acid solution (0.08%) and 80% methanol with isocratic elution was selected after considering the system suitability parameters (Table 1). o-Phosphoric acid was incorporated for maintaining the pH of the mobile phase below the pK_a value of RFL (8.74). The excitation and emission wavelengths of RFL in the selected mobile phase were determined by the excitation and emission spectra at 290 and 380 nm, respectively (Figure 2).

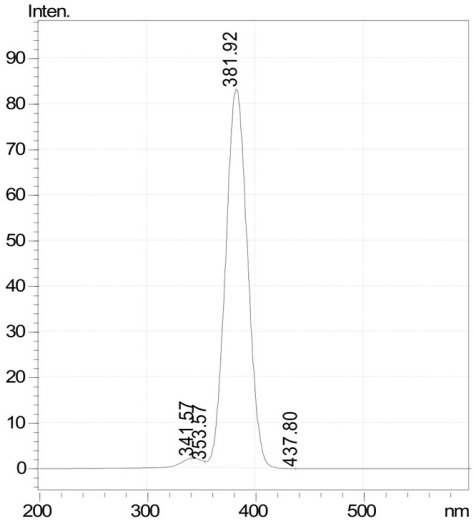

Figure 2. Emission spectrum of roflumilast at excitation wavelength of 290 nm

Table 1. System suitability parameters*

Analyte	t_R (min)	Tailing factor (t)	Resolution (R_s)	Capacity factor (k')	Number of theoretical plates (N)	Height equivalent of a theoretical plate (HETP)
IRB	4.359±0.001	1.242±0.014	-	4.812±0.001	3,272±50	45.86±0.70
RFL	5.801±0.002	1.270±0.002	4.075±0.014	6.094±0.051	3,321±12	45.17±0.16

*Values of the standard solution at 5.00 μg/mL of RFL and 2.50 μg/mL of IRB
$t_R{}^0$ = 1.864±0.009, IRB: Irbesartan, RFL: Roflumilast

Validation

Linearity

A linear relationship was established in the range of 1.25-10.00 µg/mL for RFL by a seven-point calibration curve under the optimised HPLC conditions and the calibration chromatograms were shown in Figure 3. Table 2 presents the regression data of RFL.

Limits of Detection and Quantitation (LOD and LOQ)

LOD and LOQ were calculated as 0.07 and 0.22 µg/mL, respectively, using the data presented in Table 2.

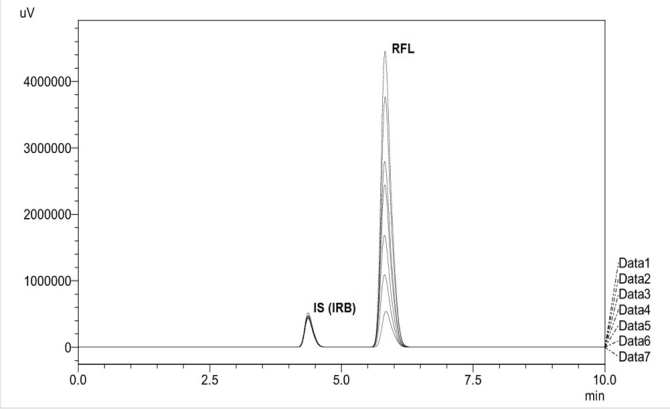

Figure 3. HPLC-FD chromatograms of the calibration standards

HPLC-FD: High-performance liquid chromatography-fluorescence detection

Table 2. Regression analysis results, LOD and LOQ of the proposed method*

Intercept	-0.2013
Standard deviation of the intercept	0.0278
Slope	1.2769
Standard deviation of the slope	0.0047
Coefficient of determination (R2)	0.9992
LOD (µg/mL)	0.07
LOQ (µg/mL)	0.22

*Three replicates/day in three different days, LOD: Limit of detection , LOQ: Limit of quantitation

Precision and Accuracy

Precision and accuracy were analysed at low, middle and high concentrations with standard solutions. The repeatability and the intermediate precision were calculated in terms of RSD% (Table 3) and were ≤0.96. The accuracy was examined in terms of RME% within a range of -0.58 to 0.84%.

Specificity

An injection of the mobile phase indicated that the interference effect was not present under the optimised experimental conditions (Figure 4).

Robustness

The parameters of the flow rate (±0.1), the mobile phase composition and the column temperature (±1) were varied to check the robustness of the method using the tablet solution. The flow rate values of 0.9 mL/min and 1.1 mL/min; the mobile phase compositions of 0.08% o-phosphoric acid:MeOH as 19:81 and 21:79 (v:v); and the column temperatures of 39 °C and 41 °C were examined (Table 4). The flow rate had the highest impact on the results, and the data were significantly different from the ones obtained by the original HPLC conditions (t-test at p=0.05). The variations in the other analysed parameters exerted insignificant effects according to the statistical analyses (t-test at p=0.05).

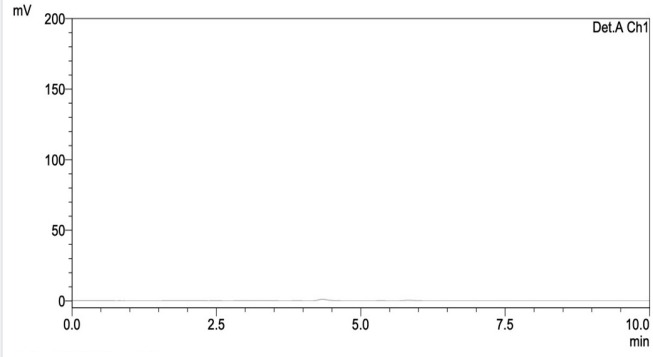

Figure 4. HPLC-FD chromatogram of the mobile phase

HPLC-FD: High-performance liquid chromatography-fluorescence detection

Table 3. Repeatability (interday), intermediate precision (intraday) and accuracy of the proposed method

	Concentration (µg/mL)	Concentration found (µg/mL)*	RSD (%)¥	RME (%)†
Intraday (n=3)	1.25	1.25±0.01	0.71	-0.22
	5.00	5.04±0.02	0.38	0.84
	10.00	10.06±0.01	0.14	0.61
Interday (n=3)	1.25	1.24±0.01	0.96	-0.58
	5.00	5.03±0.02	0.49	0.61
	10.00	10.05±0.01	0.10	0.54

* Mean ± standard deviation, ¥Relative standard deviation percent, †Relative mean error percent, RSD: Relative standard deviation, RME: Relative mean error

Analysis of the Tablet Formulation

The proposed method was applied for quantifying RFL in the tablet formulation of DAXAS® (Figure 5). The original tablet solution and the tablet solutions spiked at 1.00, 2.50 and 5.00 µg/mL were analysed successfully with high precision and accuracy (Table 5). The RSDs were lower than 2%, and the recovery% values were between 99.43 and 101.05%.

Because there was not any official monograph for quantifying RFL, the analyses were performed only by the proposed method.

Discussion

In the proposed work, a new HPLC-FD method was developed and validated for determining RFL in tablets. The validation results were compared with the limitations in the FDA, Reviewer Guidance, Validation of Chromatographic Methods (14). The method provided good system suitability values with t <2, Rs <2, k' >2 and N >2000. In the tablet analysis, the precision values in terms of RSD% were lower than 1 (except tablet solution spiked at 2.50 µg/mL, interday precision, 1.14%) with high recoveries. Selected published studies on the determination of RFL in tablet formulations by HPLC in the literature were compared with the proposed method in Table 6 in terms of retention time, LOD and LOQ (7,15). Unlike this work, the LOD and LOQ were calculated using 3.3 and 10 times of the signal-to-noise ratio in these studies (7,15), but the values could give an idea to examine the results obtained by detection of UV and fluorescence. The linear ranges were different from each other. In the proposed method, it was between 25% and 200% of the concentration of the tablet solution. Barhate and Deosthalee determined the linearity range as 10%-150% of the theoretical test concentration of 150 µg/mL (15), whereas Belal et al. (7) investigated the linear range without considering the concentration of the tablet solution or the test solution. In all the compared methods, the r^2 values were higher than 0.999. Also, in those studies, the external standard method was performed for calibration, but an internal standard was used in this study. The proposed method possessed the shortest retention time. The method developed by Belal et al. (7) was a stability-indicating method. Future studies might be

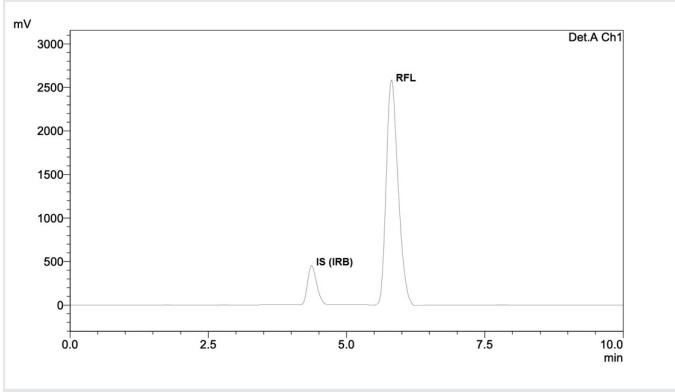

Figure 5. HPLC-FD chromatogram of the tablet solution

HPLC-FD: High-performance liquid chromatography-fluorescence detection

Table 4. Robustness of the proposed method*

Flow rate		Composition of mobile phase		Column temperature	
0.9 mL/min	1.1 mL/min	o-Phosphoric acid 19%: MeOH 81%	o-Phosphoric acid 21%: MeOH 79%	39 °C	41 °C
7.23±0.92	-8.38±0.25	-0.73±0.23	-0.76±0.59	-0.64±0.15	-1.19±0.84

*The studies were performed with the tablet solution and the results were provided in relative mean error % ± standard deviation

Table 5. Analysis results of the original and spiked tablet solutions

Sample	Concentration (µg/mL)	Concentration found (µg/mL)*	RSD%		Recovery %*
			Intraday¥	Interday*	
Original tablet solution	5.00	5.05±0.01	0.20	0.23	101.05±0.23
Tablet solution spiked at 1.00 µg/mL	6.00	6.05±0.03	0.09	0.54	100.78±0.55
Tablet solution spiked at 2.50 µg/mL	7.50	7.50±0.09	0.36	1.14	100.03±1.14
Tablet solution spiked at 5.00 µg/mL	10.00	9.94±0.03	0.29	0.31	99.43±0.31

*Results of triplicate analysis/day in three different days, ¥Results of triplicate analysis in one day

Table 6. Comparison of the proposed method with selected published HPLC methods for determination RFL

Method	t_R (min)	LOD (µg/mL)	LOQ (µg/mL)	Reference
HPLC-DAD	6.24±0.005	0.56	1.87	7
HPLC-UV	8.64	0.02	0.065	15
Proposed method	5.801±0.002	0.07	0.22	

HPLC-DAD: High-performance liquid chromatography-diode array detect, HPLC-UV: High-performance liquid chromatography-diode array detect-ultraviolet, RFL: Roflumilast, LOD: Limit of detection, LOQ: Limit of quantitation

performed to investigate the applicability of the proposed HPLC-FD method for indicating the stability of the drug material.

Conclusion

This study developed and validated a novel HPLC-FD method for determining RFL in tablet formulations. The proposed method has the advantages of simplicity, rapidity and suitable sensitivity. Also, the FD increases the selectivity of the method comparing with the studies performed by HPLC-UV or HPLC-DAD. The method was found to be appropriate for the routine analysis of RFL in tablet formulations in terms of reliability and being easy to perform.

Ethics

Ethics Committee Approval: Ethics committee approval is not required for the study. Analysis was carried out only in tablet formulation.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: P.K.Y., Design: P.K.Y., Analysis or Interpretation: U.M., P.K.Y., Literature Search: U.M., P.K.Y., Writing: P.K.Y.

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Original Article

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Evaluation the Early Effects of Compression Stockings on Patient Satisfaction in Acute Proximal Deep Vein Thrombosis

Akut Proksimal Derin Ven Trombozlarında Medikal Tedavi ile Kullanılan Kompresyon Çoraplarının Hasta Memnuniyetine Erken Dönem Etkilerinin Değerlendirilmesi

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ABSTRACT

Objective: The effective treatment of acute deep vein thrombosis (DVT) has great importance in the prevention of major complications [pulmonary embolism, postthrombotic syndrome (PTS)]. Compression stockings (CS) can also be used to improve the outcome. The aim of this study was to evaluate the effectiveness of CS on PTS prevention in patients with proximal DVT and the effectiveness of the visual analog score (VAS) method to assess patient's satisfaction about pain.

Methods: Between January 2016 and August 2018, 92 patients with proximal acute DVT included in study .They were divided into two groups according to the use of CS. Under knee level and medium pressure computed tomography were used in addition to standard medical treatment. Both groups were followed for 6 months. Baseline, 3rd month, 6th month VAS scores were calculated and also baseline and 6th month visual clinical severity score (VCSS) and Villalta score were calculated.

Results: There was no significant difference between the two groups when VAS scores were compared at baseline and 3rd month follow-up. At the 6th month follow-up, VAS values were significantly lower in favor of Group II, that used CS ($p<0.05$). No statistically significant difference was found between the basal and 6th month results of Villalta and VCSS scores between the groups.

ÖZ

Amaç: Alt extremite proksimal tutulumlu akut derin ven trombozu (DVT) tedavisinin etkinliği, hastalığın önemli komplikasyonlarının [pulmoner emboli, posttrombotik sendrom (PTS)] önlenebilmesinde büyük önem taşımaktadır. Planlanan (medikal ve/veya girişimsel) tedaviye ilaveten kullanılabilen kompresyon çorapları (KÇ) tedavi sonuçlarını etkileyebilmektedir. Bu çalışmada proximal DVT'li hastalarda KÇ kullanımının PTS gelişimi üzerine etkinliği ile aynı semptomatik tedavide ağrıya yönelik hasta memnuniyetinin vizuel analog skor (VAS) yöntemi ile değerlendirilmesinin etkinliği araştırıldı.

Yöntemler: Çalışmaya Ocak 2016 ile Ağustos 2018 tarihleri arasında akut DVT tanılı 92 hasta dahil edildi. Proksimal tutulumu olan 92 hasta kompresyon kullanımına göre 2 gruba ayrıldı. Dizaltı ve orta basınçlı kompresyon çorabı kullanıldı. Her iki grup standart medikal tedaviye ilaveten 6 ay süre ile takip edildi. Bazal, 3. ay, 6. ay VAS skorları ile bazal ve 6. ay visual clinical severity score (VCSS) ve Villalta skorları hesaplandı.

Bulgular: Hastaların bazal ve 3. ay takiplerindeki VAS değerleri karşılaştırıldığında her iki grup arasında anlamlı bir fark saptanmadı. Altıncı ay takiplerinde ise VAS değerleri KÇ kullanan Grup 2 lehine istatistiksel olarak anlamlı şekilde düşük tesbit edildi

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Conclusion: CS has no effect on preventing PTS at the patients with proximal acute DVT but has an impact on reducing pain symptoms. VAS is an effective method to evaluate the effect of CS on patient satisfaction about pain.

Keywords: Acute proximal deep vein thrombosis, compression stockings, visual analog scale

(p<0,05). Gruplar arasında Villalta ve VCSS skorlarının bazal ve 6. ay sonuçları arasında istatistiksel olarak anlamlı bir farklılık tespit edilmedi.

Sonuç: Proksimal tutulumlu alt extremite akut DVT'si olan hastaların tedavisinde kullanılan KÇ'nin PTS gelişimi üzerine etkinliği saptanmamış olup ağrı semptomunun azaltılmasında etkinliği bulunmaktadır. KÇ kullanımının hasta memnuniyetine etkisinin değerlendirilmesinde VAS etkili bir yöntemdir.

Anahtar Sözcükler: Akut proksimal derin ven trombozu, kompresyon çorabı, vizuel analog skala

Introduction

Acute deep vein thrombosis (DVT) is seen with a frequency of 1/1,000 in the general population and is an important cause of mortality and morbidity, especially in the hospitalized patients, when evaluated in terms of the complications it may cause (pulmonary embolism, post thrombotic syndrome) (1). Today, in the treatment of acute DVT with lower extremity proximal involvement (common femoral vein and/or iliac venous involvement), new catheter-based interventions (2) and accompanying medical treatments are important steps in the prevention of post thrombotic syndrome (PTS), which is the most frightening long-term complication of the disease. has been recorded. However, PTS can still develop in 30-50% of all patients (3). In addition to these treatments; compression stocking (CS) is used in acute DVT in order to relieve edema due to stasis and venous hypertension and the associated pain, and to prevent the development of PTS. When current guidelines are examined, routine use of CS is not recommended in preventing the development of PTS after acute DVT (4). Although CS contains difficulties in terms of patient compliance, it is still used in routine practice because it regresses patients' symptoms.

A visual analogue scale (VAS) can be used to numerically express the satisfaction of the patients with DVT with symptomatic acute proximal involvement who used CS in addition to medical treatment, in terms of the reduction of pain in the follow-up (5). VAS is a method that provides patient-based measurement between "no pain" and "the most severe pain" in vertical or horizontal plane of 10 cm length divided into equal parts. In this study, the effectiveness of CS added to the medical treatment of patients with acute proximal DVT on the development of PTS in the early period and the effectiveness of evaluating patient satisfaction with VAS method in the same patient group who were given symptomatic treatment for pain were investigated.

Method

Between January 2016 and August 2018, 151 patients who were admitted to our clinic with a diagnosis of acute DVT and were treated with CS in addition to medical treatment were retrospectively analyzed and 92 were included in the study who met the inclusion criteria. Three patients with active cancer, 10 patients who underwent percutaneous interventional procedure, 14 patients with popliteal and/or distal segment involvement, 4 patients with recurrent DVT, 4 patients who were pregnant, 8 patients below 20 years of age or over 70 years of age, and 16 patients whose follow-up records could not be accessed were excluded from the study. Medium pressure (23-32 mmHg) below-knee CS was given to the patients. Thirty-seven patients who could not comply with CS in the 1st week outpatient controls were followed up with only medical treatment and were identified as Group I. Fifty-five patients who were compliant with the use of CS were named as Group II. The VAS scores of the patients were calculated before the treatment, in the 3rd month and 6th month of the treatment (Figure 1). The Villalta (Figure 2) score and the venous clinical severity score (VCSS) (Figure 3) of the patients in the beginning of the treatment and in the 6th month were calculated. In medical treatment, subcutaneous enoxaparin treatment at a dose of 1 mg/kg/12 hours was started in all patients, followed by oral warfarin at a dose that would provide the effective INR (2-2.5) level, on a patient-based basis. The study was approved by the local ethics committee and all data were evaluated retrospectively before and after treatment. The patients were informed about the treatment to be applied to them, and their consent was obtained.

Statistical Analysis

Baseline, 3rd month and 6th month VAS scores and baseline, 6th month Villalta and VCSS measurements were expressed as mean ± standard deviation. Independent sample t-test was used to compare VAS, Villalta, VCSS scores between Group I and Group II. Descriptive data were presented as mean standard deviation, median (minimum, maximum) or frequency (%). Independent samples t-test and χ^2 test were used to compare groups according to normality test results. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) package program was used for statistical analysis and p<0.05 was considered statistically significant.

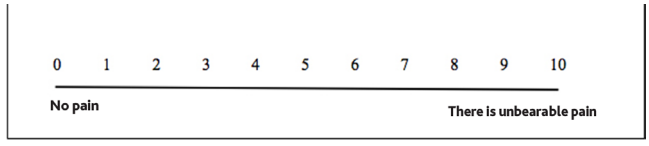

Figure 1. Visual analogue scale

Results

Ninety-two patients who were planned to use CS in the medical treatment of acute DVT were included in the study. The demographic characteristics of all patients are shown in Table 1. The basal, 3rd month and 6th month average VAS scores of the patients before CS treatment were 7, 7 and 5 in Group I, and 7, 6 and 5 in Group II, respectively (Table 2). When the VAS scores at baseline and 3rd month follow-up were compared, no significant difference was observed between the two groups, while a statistically significant difference was found in favor of Group II, which used CS, in terms of VAS values at the 6th month follow-up ($p<0.05$). Basal and 6th month average Villalta scores of the patients before compression therapy were 8 and 6 in Group I, and 7 and 6 in Group II, respectively (Table 2). The basal and 6th month average VCSS scores of the patients before compression therapy were; 6 and 5 in Group I, and 6 and 5 in Group II, respectively. There was no statistically significant difference in terms of the baseline and 6th month Villalta and VCSS scores between the groups.

Discussion

In this study, the early results of the use of medium pressure CS in patients with symptomatic DVT with acute proximal

Symptoms	Clinical findings
Pain	Edema
Cramp	Skin hardness
Feeling of heaviness	Hyperpigmentation
Rash	Redness
Paresthesia	Pain during calf compression
	Venous ectasia
Every symptom /Clinical findings; 0 (no), 1 (light), 2 (middle), 3 (severe)	
Scoring	
0-4	PTS no
5-15	Light-middle PTS
>15 or venous ulcer stenosis	High PTS

Figure 2. Villalta score

Clinical descriptors	No (-)	Light (1)	Middle (2)	High (3)
Pain	No	Rare	Does not restrict daily life	Restricting daily life
Varicose vein	No	Little	Calf and thigh region	Calf and thigh region
Venous edema	No	Foot and wrist	Under the knees	Knee and above
Skin pigmentation	No	Perimalleolar region limited	Common sub 1/3 calf region	Much more common top 1/3 calf region
Inflammation	No	Perimalleolar region limited	Common sub 1/3 calf region	Much more common top 1/3 calf region
Skin hardening	No	Perimalleolar region limited	Common sub 1/3 calf region	Much more common top 1/3 calf region
Number of active venous ulcers	No	1	2	≥3
Venous ulcer duration	No	<3 month	3-12 month	>1 year
Active venous ulcer diameter	No	<2 cm	2-6 cm	>6 cm
Compression therapy	No	Intermittent	Most days	Throughout the treatment

Figure 3. VCSS scoring

Table 1. Demographic characteristics of the patients

	Group I (n=37)	Group II (n=55)	p
Gender (female)	22 (59%)	33 (60%)	0.958[a]
Age	46.33±11.21 (18-72)	48.09±10.31 (18-72)	0.601[b]
Extremity side (left/right)	26 (70.2%)/11 (29.8%)	35 (63.6%)/20 (36.4 %)	0.509[a]
BMI	24.72±11.20 (20-33)	24.73±20-35	0.992 [b]
CEAP category			
C1	7 (18.9%)	8 (14.5%)	
C2	14 (37.8%)	21 (38.1%)	
C3	16 (43.2%)	26 (47.2%)	

Data are presented as mean ± standard deviation (min-max), median (min-max), or n (%).
BMI: Body mass index, CEAP: Comprehensive Classification System for Chronic Venous Disorders, [a]x^2 test, [b]Independent sample t test

involvement were examined. While CS had no effect on the development of PTS, it was found to have a reducing effect on the pain as assessed by VAS. In the follow-up of patients with DVT, many evaluation systems have been used to evaluate both the development of PTS and the regression of symptoms, and VCSS and Villalta scores are the most accepted, valid and up-to-date methods (6).

There are studies showing that the use of CS prevents the development of PTS, which is a long-term complication of acute proximal DVT, as well as there are studies showing that it has no effect on PTS (7,8). In our study, the early 6th month Villalta score results in patients using CS were compared with patients not using CS, and no significant difference was found in terms of preventing the development of PTS.

It has not yet been clarified in the literature whether choosing a higher pressure and high level CS is effective in the development of PTS or in reducing symptoms. Ten Cate-Hoek et al. (9) found no significant difference between different CS pressures and levels in terms of the efficacy, and a regression in symptoms was observed regardless of the pressure and level of CS. Similar results were obtained in studies in which only low-pressure or high-pressure CS was used and the venous filling index was measured (10,11). In our study, we preferred the use of below-knee and medium-pressure CS for each patient in order to increase patient compliance, we did not use higher pressure and high-level stockings.

Study Limitations

When the results of studies using VAS scoring were examined to investigate the effect of CS use on pain symptoms in patients with DVT, it was found that CS especially used in the early period, had positive effects on reducing pain (12). Similarly, in our study, a statistically significant difference was found between the 6th month VAS scores of the patients who used CS and those who did not. In the evaluation of pain satisfaction, VAS can be preferred as a valuable measurement method in DVT with acute proximal involvement. Although it is an advantage that this scoring system is easy to understand and re-applicable, it is a disadvantage that difficulties ocur due to declining cognitive functions, especially in older age groups.

Conclusion

Considering the early results of CS in the treatment of patients with proximal lower extremity DVT, no significant effect was found on the development of PTS. However, in daily practice, they can still be used in symptomatic treatment. Studies that evaluate more patients and include long-term results are needed for efficacy analysis.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee and all data were evaluated retrospectively before and after treatment.

Informed Consent: The patients were informed about the treatment to be applied to them, and their consent was obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: C.K., M.Ş.B., Concept: C.K., M.Ş.B., E.A., Design: C.K., M.Ş.B., E.A., Data Collection or Processing: C.K., M.Ş.B., A.Ö., E.Ş.D.Y., F.B., Analysis or Interpretation: C.K., M.Ş.B., A.Ö., E.Ş.D.Y., F.B., E.A., Literature Search: C.K., M.Ş.B., Writing: C.K., M.Ş.B.

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Table 2. VAS, Villalta and VCSS scores of the patients

VAS	Group I (n=37)	Group II (n=55)	p
Basal	7.32±1.14	7.28±1.15	0.859
3rd month	7.24±1.19	6.68±1.09	0.232
6th month	5.64±1.07	5.1±1.10	0.021
Villalta score			
Basal	8±2.53	7.89±2.34	0.839
6th month	6.24±2.25	6.17±1.92	0.153
VCSS			
Bazal	6.51±1.46	6.70±1.45	0.546
6th month	5.55±1.05	5.61±0.87	0.717

VAS: Visual analog scale, VCSS: Visual clinical severity score

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Original Article

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High-performance Liquid Chromatography Analysis of Nebivolol and Amlodipine and Some Related Compounds in Synthetic Mixture

Nebivolol ve Amlodipin ve Bazı İlgili Bileşiklerinin Sentetik Karışımlarda HPLC ile Analizi

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ABSTRACT

Objective: This study aimed to develop and validate a method using a high-performance liquid chromatography (HPLC) to perform a quantitative analysis of nebivolol (NEB) and amlodipine (AML) along with some related substances in the synthetic mixture.

Methods: The separation in the described chromatographic system was accomplished using a mobile phase consisting of a mixture of acetate buffer (pH: 4.5) and acetonitrile and a HPLC C18 column (150 mm x 4.6 mm, 2.6 µm) with gradient elution on a consistent flow rate of 1.3 mL/min. Photodiode array detection was carried out at a wavelength of 265 nm. According to The International Conference on Harmonisation guidelines, the drug was exposed to various stress conditions; including photolysis, oxidation, thermal degradation and hydrolysis under acidic, basic and neutral mediums.

Results: Ranges of detection and quantitation limits were determined to be 0.2-10.0 $\mu g.mL^{-1}$ and 0.25-10.0 $\mu g.mL^{-1}$ for NEB and AML, respectively. The relative standard deviation values within and between days precision were determined to be <2%. For all substances, the average recovery values were determined within the range of 98.00%-101.50%.

Conclusion: We conclude that this developed analytical procedure applies to the quality control of drug formulations.

Keywords: Nebivolol, amlodipine, high-performance liquid chromatography, validation, synthetic mixture

ÖZ

Amaç: Bu çalışma, nebivolol (NEB) ve amlodipinin (AML) kantitatif analizini yapmak için yüksek performanslı sıvı kromatografisi (HPLC) kullanarak bir yöntem geliştirmeyi ve validasyonunu amaçlamıştır

Yöntemler: Kromatografik sistemdeki ayırma, asetat tamponu (pH: 4,5) ve asetonitril karışımından oluşan bir mobil faz kullanılarak, 1,3 mL/dakika akış hızında, C18 kolonda (150 mm x 4,6 mm, 2,6 m) gerçekleştirildi. Diyot sıralı dedektör 265 nm dalga boyuna ayarlandı. Uluslararası Uyum Konferansı yönergelerine uygun olarak ilaç, çeşitli stres koşullarına maruz bırakılmıştır. Bunlar; fotoliz, oksidasyon, termal bozunma ve asidik, bazik ve nötr ortamlar altında hidrolizdir.

Bulgular: NBV ve AMV için kantitasyon limit aralıkları sırasıyla 0,2-10,0 g/mL^{-1} ve 0,25-10,0 g/mL^{-1} olarak belirlenmiştir. Gün içi ve günler arası kesinlik bakımından RSD değerlerinin %2'den düşük olduğu belirlenmiştir. Analizlenen tüm maddeler için ortalama geri kazanım değerlerinin %98,00-%101,50 aralığında olduğu belirlenmiştir.

Sonuç: Geliştirilen analitik sürecin ilaç formülasyonlarının kalite kontrolü için uygulanabilir olduğu sonucuna vardık.

Anahtar Sözcükler: Nebivolol, amlodipin, yüksek basınçlı sıvı kromatografisi, validasyon, sentetik karışım

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Introduction

Nebivolol hydrochloride's (NEB) chemical name is (1R)-1-[(2R)-6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl]-2-{[(2R)-2-[(2S)-6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl]-2-hydroxy ethyl]amino}ethan-1-ol hydrochloride. NEB hydrochloride is a third-generation antihypertensive drug, acting as a very specific β1-blocker. The mechanism of action of the drug substance occurs via its β1-receptor blocking activity and nitric oxide potentiation, resulting in its vasodilation effects (1-3).

Amlodipine besylate (AML) is dihydropyridine class calcium channel blocker used via oral route and has the chemical name as follows: 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate; benzenesulfonate (4). AML is used for hypertension management as the first line therapy agent according to therapeutical guidelines based on its suitable adverse event profile and relatively long action. Its mechanism of action involves reducing the calcium ion influx. However, this effect occurs quite selectively on the smooth muscles rather than affecting the cardiac muscle cells. In addition, it does not affect calcium ion mobilisation across the cell membrane, resulting in a reduction in the peripheral vascular resistance and blood pressure (5,6). Concomitant use of NEB and AML for hypertension management is evaluated to be beneficial due to the involvement of mechanisms of action of these two molecules, yielding a synergetic effect in the living organism to lower the blood pressure. Furthermore, lower doses of both molecules are sufficient with this combination therapy.

A literature review was performed and discovered that most of the analytical techniques, employed for determining NEB and AML in the combination formula, were mainly based on spectrophotometry (7-15), high-performance liquid chromatography (HPLC) (16-19) and high-performance thin-layer chromatography (20-22). The quantification of related substances in the combination formula of NEB and AML are individually reported in literature. Based on literature review and current available information, no method which can analyse related substances of simultaneous NEB and AML in combined dosage forms was reported. Related compounds of NEB and AML should be determined in the combination formula of NEB and AML without a need for prior separation for practical reasons. Therefore, our study targeted the development and validation of the developed analytical method to allow quantification of related compounds of NEB and AML in fixed dose combination pharmaceutical dosage forms avoiding any a prior separation procedure.

Some of these related compounds were determined to be possible degradants of AML and NEB in the drug product. This fact, too, shows the need for a method to determine the related compounds of NEB and AML in combined dosage forms without prior separation and supports our aim to develop and validate an analytical method that meets the needs for the combination formula. Method validation for related compounds was accomplished according to the International Conference on Harmonisation (ICH) requirements, by carrying out stress tests for fixed dose combination tablets covering various conditions; photolysis, oxidation, hydrolysis (at different pH mediums) and thermal degradation (23). Results obtained out of the stability testing procedures were anticipated to provide important contributions to properly developed manufacturing processes; contributing to decision-making processes for selecting proper packaging and determining the storage conditions, shelf life of the product and expiration date.

Experimental

Reagents and Solutions

Working standards of AML and NEB and related compounds were obtained from Hetero Drug Ltd. (Telangana Limited, India). Laboratory-prepared tablets were made, containing 10 mg of NEB and 10 mg of AML, Starch maize 50.0 mg, Hydroxypropyl Methyl Cellulose 45.0 mg, Lactose Monohydrate 70.0 mg, Croscarmellose Sodium 15.0 mg, Microcrystalline Cellulose 38.0 mg, Silicon Dioxide 5.0 mg and Magnesium Stearate 2.2 mg per tablet. All used chemical substances and chemical reagents were procured as analytical reagent grade. Ultra pure water was obtained using an aquaMAX™ (Younglin Instrument, Korea) water purification system for HPLC.

Chromatographic Conditions and Instrumentation

Shimadzu HPLC, Binary Pump (Shimadzu, Kyoto, Japan) was used for the chromatographic separation. The HPLC chromatographic system was established using the following parts: SPD M20A photodiode array (PDA) detector, SIL 20AC Autosampler, LC 20AB pump and CTO-10As column oven. Data gathered and derived from chromatographic procedure was processed by the software of the same chromatographic system**.** Separation of substances in chromatographic system was performed on a Phenomenex Kinetex 150 mm x 4.6 mm, 2.6 µ C18 (4.0x100 mm, 3 µm) using a mobile phase consisting

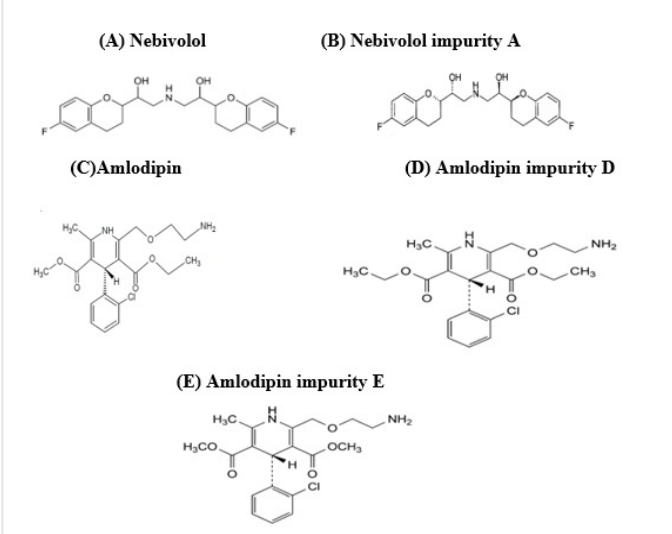

Figure 1. Chemical structures: NEB and AML and some related compounds

NEB: Nebivolol, AML: Amlodipine

of a mixture of acetate buffer (pH: 4.5) and acetonitrile with gradient elution on a consistent flow rate of 1.3 mL/min. Specific wavelength of 265 nm was selected for PDA detection. Temperature Column was kept at 35 °C.

Standard Solution Preparation

Stock solution of 500.0 μg/mL NEB and impurity of NEB A, AML and impurity of AML D and E were formed through dissolution of reference standard materials of the investigated pharmaceutical active ingredients and related compounds in 100 mL of acetonitrile: acetate buffer (pH: 4.5) (50:50, v/v) in a calibrated flask using an ultrasonic bath. Working standard solutions were formed through adequate dilution of the stock solution with the above mentioned solution mixture. Stock solutions were kept at 4 °C and were stable for a month.

Ammonium acetate of 1.5 g was accurately weighed and dissolved in 1,000 mL of water to get 20 mM of solution for the buffer solution preparation. The pH of the final solution was adjusted to 4.5 with acetic acid. It was then filtered with a 0.22 μ filter. The filtered solution was degassed and used as a buffer in the mobile phase.

General Procedures

Construction of the Calibration Curves

Accurately measured aliquots of NEB and AML standard solutions covering the working concentration ranges of 0.20-10.0 μg/mL and 0.25-10.00 μg/mL were prepared, respectively, and then transferred into a series of 10 mL volumetric flasks. Solutions were diluted to the mark with acetonitrile: acetate buffer (pH: 4.5) (50:50, v/v) and mixed well. Related compounds of NEB A, AML D and E stock solutions were formed through diluting 1 mg of related compounds in 1 mL of acetonitrile: acetate buffer (pH: 4.5) (50:50, v/v) and further diluted with the same solvent. Stock solutions were stable when kept at 4 °C in a refrigerator.

Sample Preparation

Fixed dose combination tablets with NEB and AML based on a ratio of 1:1 were prepared in the laboratory. An accurate amount of prepared tablets having equivalent milligrams of active ingredients of 10 mg of NEB and 10 mg of AML (in line with their ratio in the pharmaceutical formulation) was weighed and transferred into a 100 mL volumetric flask, and the volume was completed to final a volume of 100 mL with acetonitrile: acetate buffer (pH: 4.5) (50:50, v/v). Flasks were kept in an ultrasonic bath for 30 min to best dissolve the contents, and then the content was filtered by 0.45 μm membrane polytetrafluoroethylene. The above mentioned procedure was implemented to construct the calibration curves. The quantification of the nominal value of each compound within the content was calculated based on the established calibration curve or the corresponding regression equation.

Validation of Analytical Method

The analytical method development and validation were carried out in line with the ICH guidelines (23). Methods were validated based on the parameters covering quantification, accuracy, precision, specificity, linearity, range and detection limit.

Specificity

Stress degradation investigation was carried out in line with the ICH guidelines Q1A (R2) (23) to show the stability-defining characteristic and specificity of the developed analytical method. Solutions from tablet preparation were investigated under various forced degradation conditions described as follows: under alkaline conditions (1 N sodium hydroxide (NaOH) at ambient temperature in 24 hours), acidic conditions (1N hydrogen chloride (HCl) at ambient temperature in 24 hours), neutral conditions (water at 70 °C in 1 hour) and oxidative conditions (3.0% v/v H_2O_2 at ambient temperature in 3 hours). Samples from alkaline and acidic degradation stress conditions were neutralised using adequate amount of 1 N HCl and 1N NaOH, respectively, and completed to the end volume with the diluent. Thermal stress conditions were created by keeping the investigational medicinal product in heat controlled oven at 80 °C in a week. The pharmaceutical preparation was exposed to ultraviolet lamp for 72 hours to check the photostability. Upon completion of pre-defined time, solutions resulting from stress condition testing were diluted with the methanol: acetate buffer (pH: 4.5) (50:50, v/v), and samples for degradation testing were subjected to analysis by the developed HPLC method as mentioned in the chromatographic condition section. A PDA detector was used to define peak purity concerning peaks resulting from all samples from stress condition testing.

Specificity is the ability of the method to measure the analyte response in the presence of all related substances (NEB A, AML D and E). For specificity determination, all related substances were prepared individually and injected into HPLC to confirm retention times. Later on, solutions of blank, sample and spiked sample (sample spiked with all related substances) were prepared and injected into HPLC to confirm any co-elution with analyte peaks from respective blank and any degradation peaks. From the injections of spiked sample, the known related substance peaks were confirmed to be well separated from each other and without collation, showing that the method is selective and specific. The stability indicating nature of the method was further evaluated by performing forced degradation studies. Stress testing was carried out to identify the likelihood degradation products or to elucidate the inherent stability characteristics of drug substance. In this study, drug was subjected to oxidation, hydrolytic, photolytic, thermal and humidity stress conditions, and the summary of results obtained from forced degradation experiment results is presented in Table 4. Results showed that peaks were found stable in all forced degradation conditions, without interference for related compound peaks from other peaks.

Linearity

The linearity of the method was established for drugs and their related compounds. Drugs solution and their related compounds were formed at five various concentrations within the range of 0.20 and 15.00 μg/mL of concentration of analyte. The regression line was constructed between the peak area and

corresponding analyte concentration based on method of least squares analysis. The slope and Y-intercept values of regression line were calculated.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

LOQ and LOD were calculated as the 10 and 3.3 times of the standard deviation of the peak area divided by the slope of the linearity calibration curve, respectively.

Precision

Method precision and accuracy were determined as within and between the days of precision. The within and between the day precision values were investigated at three different concentrations (n=5) of the analyte during five consecutive days. The relative standard deviation (RSD) was provided as the precision value.

Accuracy

Recoveries by spiking method were used for method accuracy calculation. Standard solutions with known amount (low, medium and high concentrations) were spiked with sample solutions of known amount (0.2 µg/mL). The regression equation of the calibration curve was used to estimate the spiked amount.

Robustness

Investigation of the robustness of the method was performed through deliberate modification of key method conditions like organic phase composition, flow rate of the mobile phase and key method parameters, such as selected wavelength for detection. Investigation of drug solution stability in mobile phase was performed by keeping the drug solution in ambient temperature for one day, 24h.

Results

Chromatographic Conditions

Separation in proposed chromatography system was performed on C18 column (150 mm x 4.6 mm, 2.6 µm) using a gradient elution system. For this procedure, the mobile phase consisting of a mixture of acetate buffer (pH: 4.5) and acetonitrile was formed, and the flow rate was determined as 1.3 mL/min. The wavelength of 265 nm was selected for PDA detection. The column temperature was kept at 35 °C during the procedure. Table 1 presents the gradient elution programme. Figure 2 presents the typical chromatograms.

Five replicates of freshly prepared substances were injected to evaluate the method adequacy for resolution between targeted peaks with high level of repeatability. The chromatogram was analysed based on the following factors: resolution (R), theoretical plates (N), tailing factor, retention time (tR) and symmetry factor (α). The system suitability test results proved that the developed method in this present article comply with the acceptable limits described by the requirements (Table 2).

Method Validation

ICH guidelines were the basis of method validation and optimisation (23). As described in the guideline, parameters consisting precision, accuracy, robustness, specificity, linearity, LOD and LOQ were investigated.

Linearity

The linearity of the method was tested both for the individual drug substances and their related compounds in the combination formula. Five different concentrations of drug solutions and impurity solutions were prepared. The concentration range of the analyte varied from 0.20 to 15.00 µg/mL. The calibration

Table 1. Gradient elution programme

Time	Acetate buffer (pH: 4.5)	Acetonitrile
0	80	20
5	80	20
40	35	65
45	80	20
50	80	20

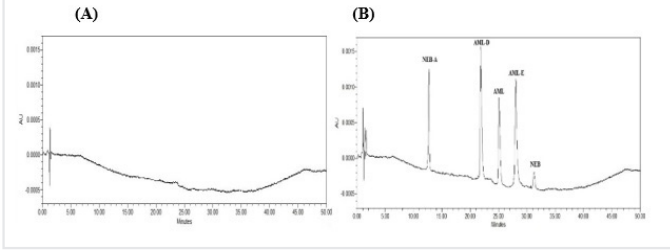

Figure 2. Representative chromatograms of (A) blank, (B) AML 0.2 µg/mL, NEB 0.5 µg/mL and related compounds spiked with 0.5 µg/mL

NEB: Nebivolol, AML: Amlodipine

Table 2. System suitability parameters

Name	(tR) ± SD (min)	Symmetry factor	N ± SD	RSD %	R ± SD	RSD %
NEB	31.094±0.001	1.09	33260±345	1.04	4.93±0.05	1.04
NEB Imp A	12.686±0.001	0.96	23544±158	0.59	5.76±0.03	0.59
AML	25.104±0.002	1.39	36758±419	1.20	6.31±0.08	1.20
AML Imp D	21.881±0.002	1.42	30772±320	1.04	22.19±0.23	1.04
AML Imp E	28.060±0.001	1.47	41048±570	1.34	5.50±0.07	1.34

N: Theoretical plates, R: Resolution, tR: Retention time and RSD: Relative standard deviation obtained from five replicate injections area

curve was plotted by drawing the corresponding impurity peak area against the concentration on the chart. The calculated coefficients of correlation, slopes of the curves, and y-intercepts of the calibration plots were presented. Calibration curves concerning the related substances were determined to be linear within the ranges involved in the study. Correlation coefficients were found to be >0.9990 for all molecules of interest (Table 3).

The LOQ and LOD were measured following a recommended formula (according to ICH Q2 (R1) (23) shown as

LOD = 3.3 SD / slope LOQ = 10 SD/slope (Eq. 1)

where SD is the standard deviation of the intercept and standard deviation of the peak area. The LOD and LOQ values presented in Table 3 prove that the proposed methods are adequately sensitive for determination of these drugs.

Precision

The precision trials were performed by a sequence of analyses of AML and NEB and related compounds for five consecutive days (each n=5). The RSD values were found in the range from 0.59% to 1.34% for intraday precision and from 1.11% to 1.61% for the interday precision. All values were found to be <2%, proving that the method was adequately precise. Results are presented in Table 3.

Specificity

Forced degradation under various experimental conditions was investigated using a starting concentration of 1 mg/mL of the sample. A PDA detector was used to ensure the homogeneity of drug peaks. After subjecting the drug to acid and base hydrolysis and oxidative degradation, the degradation products of NEB, Imp A and AML Imp D were observed. The thermal and water hydrolysis and the photolytic stress study showed that AML was degraded into AML Imp D (Figure 3). These results confirmed that the drug product maintained its stability well when exposed to forced degradation tests (Table 4).

Accuracy

The standard addition technique was used to prove the accuracy of the method. Certain amount (0.2 µg/mL) of pure sample solution were added to three concentration level of the drug standard solutions and related compounds (low, medium and high concentration) and analysed. Percentage recoveries for the drug and related compounds were within the range of 98.00%-101.50%. Results of recovery study were presented in Table 5.

Robustness

The method was evaluated to be robust based on the findings after making intentional changes in the process; including flow rate of the mobile phase (±0.1 mL/min), mobile phase pH

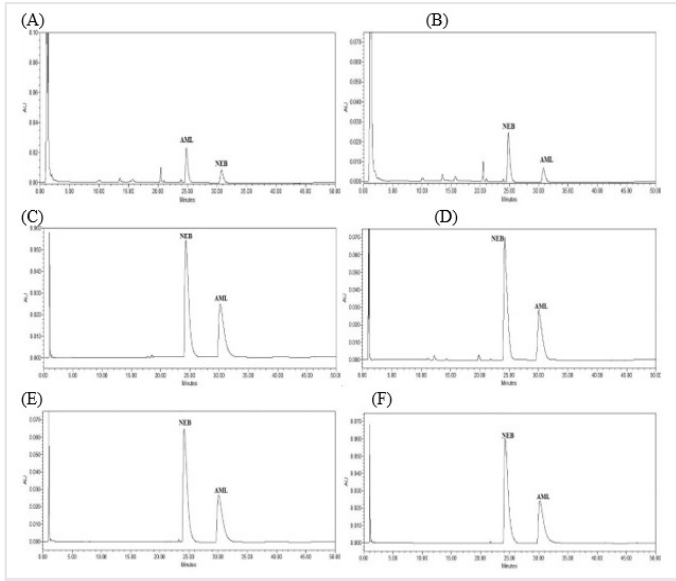

Figure 3. Chromatograms of AML and NEB at synthetic mixture (A) acid-degraded drug, (B) base-degraded drug, (C) water hydrolysis-degraded drug, (D) oxidation-degraded drug, (E) thermal-degraded drug and (F) daylight-degraded drug

NEB: Nebivolol, AML: Amlodipine

Table 3. Regression, precision, limit of detection (LOD) and limit of quantification (LOQ) data

Parameter	NEB	NEB Imp A	AML	AML Imp D	AML Imp E
Slope	1368748	1775624	2450505	3116410	1933305
Intercept	176	-182	-371	-358	-561
Linearity range (µg/mL)	0.20-10.00	0.20-10.00	0.25-10.00	0.30-10.00	0.40-15.00
LOD	0.05	0.05	0.06	0.04	0.03
LOQ	0.17	0.17	0.20	0.13	0.10
Intraday precision (RSD)[a]	1.04	0.59	1.20	1.04	1.34
Interday precision (RSD)[b]	1.13	1.36	1.14	1.61	1.11
[a]n=5					
[b]Results of five different days					

RSD: Relative standard deviation, NEB: Nebivolol, AML: Amlodipine

(4.5±0.1) and column oven temperature (35±2 °C). Some minor variations were observed in the proposed method; however, variables did not significantly affect the outcome. We concluded that the obtained data proved the robustness of the proposed method in this article.

The chemical stability of the stock solutions of the studied compounds has been tested to determine the outcomes of storage for 48 hours at room temperature (25 °C). All compounds were found to be stable in the mobile phase for 48 hours at room temperature, and were also stable in the refrigerator (4 °C). No unexpected peaks were detected in the chromatograms during the stability studies.

Analysis of Synthetic Mixture

The proposed method was implemented for the quantitation of NEB and AML and their related compounds using the synthetic mixture sample. The obtained results are in line with the labelled content for NEB and AML combination formula. Furthermore, the related chemical related compounds were found at levels below the acceptable limit.

Conclusion

In this article, validated HPLC method was presented to determine and quantify NEB, AML, and some related compounds in a synthetic mixture. This method was evaluated for linearity,

Table 4. Forced degradation results

Stress condition	Degradation (%)	Number of impurities	Retention time (min)	Peak purity (Amlodipin)	Peak purity (Nebivolol)
Acidic/1N HCl/RT/24 h	15.5	7	10.038;12.663; 13.537;15.719; 20.489;21.029; 23.927	0.99965	0.99975
Alkaline/1N NaOH/RT/24 h	16.4	7	10.072;12.695; 13.539;15.715; 20.489;21.047; 23.928	0.99981	0.99971
Oxidation/3% H202/3 h	6.5	9	11.158;11.737; 12.244;14.297; 16.541;16.983; 19.282;19.763; 21.822	0.99977	0.99967
Neutral/H2O/70°C/1 h	0.4	6	8.016;17.808; 18.474;19.182; 20.065;21.819	0.99972	0.99962
Photolytic/UV-lamp/72 h	1.3	10	8.731;10.323; 15.963;16415; 16.885;17.651; 18.475;19.190; 20.070;21.737	0.99989	0.99985
Thermal/80°C/1 week	0.9	9	17.419;17.881; 18.225;19.165; 19.554;20.023; 21.206;21.860	0.99990	0.99983

Table 5. Results of recoveries data of AML, NEB and related compounds

Analyte	Amount of drug taken[a] (µg/mL)	Amount of drug spiked[b] (µg/mL)	Total amount found (µg/mL) (Mean ± SD[c])	Recovery (%)	RSD[d]
		0.36	0.53±0.0058	99.6	1.09
Nebivolol A		4.30	4.48±0.0140	99.5	0.31
		6.55	6.72±0.0210	99.5	0.31
		0.21	0.41±0.0053	100.0	1.29
Amlodipine D		4.80	5.13±0.0300	100.6	0.59
		7.30	7.50±0.0430	98.0	0.57
		0.70	0.89±0.0079	98.8	0.89
Amlodipine E	0.2	4.80	4.97±0.0210	99.5	0.42
		7.30	7.46±0.0320	99.5	0.42
		0.70	0.50±0.0053	99.0	1.06
Amlodipine		4.80	5.04±0.0280	101.5	0.55
		7.30	7.56±0.0420	101.5	0.56
		0.65	0.85±0.0077	100.5	0.91
Nebivolol		1.80	2.01±0.0048	101.1	0.24
		2.80	3.02±0.0073	101.1	0.25

[a]synthetic mixture AML/NEB 10:10 mg, [b]Standard solution, [c]Standard deviation, [d]Five independent analyses, NEB: Nebivolol, AML: Amlodipine

precision, accuracy, LOD, LOQ, selectivity, robustness and solution stability. The selectivity of the method is evaluated as per ICH guidelines by carrying out forced degradation tests of NEB and AML combination tablets. Outcomes of these tests demonstrated that the method was stable based on the findings of NEB and AML-related substances. Thereby, we concluded that the developed and validated method proposed in this current article could be widely used in the routine practice to simultaneously determine NEB and AML and their related compounds in the synthetic mixture.

Peer-review: Externally peer review.

Authorship Contributions

Concept: C.Ö., K.A., A.Ö., Design: C.Ö., K.A., A.Ö., Data Collection or Processing: C.Ö., K.A., Ç.A., A.Ö., Analysis or Interpretation: C.Ö., K.A., Ç.A., Literature Search: C.Ö., K.A., Ç.A., A.Ö., Writing: C.Ö., K.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Perceived Sources and Levels of Stress Among Turkish Dental Students: A Multi-centre Study

Türk Diş Hekimliği Öğrencileri Arasında Algılanan Stres Kaynakları ve Düzeyleri: Çok Merkezli Bir Çalışma

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ABSTRACT

Objective: The objective of this study is to examine the perceived sources and levels of stress reported by three different dental faculty students from Turkey. This study also aims to compare stress sources among these students with respect to the role of faculties, specific curricula and institutional differences.

Methods: In total, 1,294 students participated in a survey in 2015. This survey used Modified Dental Environmental Stress questionnaire comprising 47 items that were grouped into 6 categories: academic performance; preclinical and clinical training; patient treatment; faculty administration and education staff; personal life issues; and professional identity after graduation. Responses were recorded on a four-point rating scale ranging from not stressful (1 point) to very stressful (4 points).

Results: The response rate of the study was 63.3% (1,294/2,045). The "concern about failing the course or year due to the inability to finish clinical schoolwork" (clinical score) was found as the highest stress item (mean score =3.57). More than one-third of questionnaire items showed the presence of moderate-to-severe stress with mean scores between 3.57 and 3.06. The preclinical and clinical training category showed the highest mean stress score (3.09) than other

ÖZ

Amaç: Bu çalışmanın amacı Türkiye'de üç farklı diş hekimliği fakültesi öğrencilerinin rapor ettikleri algılanan stres kaynakları ve düzeylerini incelemektir. Ayrıca, öğrenciler arasındaki stres kaynaklarını fakülteler, belirli müfredat ve kurumsal farklılıkların rolü açısından karşılaştırmak amaçlanmıştır.

Yöntemler: Çalışmaya üç diş hekimliği fakültesinden toplam 1.294 öğrenci katılmıştır. Kırk yedi maddeli Modifiye Dental Çevresel Stres anketi kullanılmış ve altı kategoriye ayrılmıştır: akademik performans; klinik öncesi ve klinik eğitim; hasta tedavisi; fakülte yönetimi ve eğitim personeli; kişisel yaşam sorunları; ve mezuniyet sonrası mesleki kimlik. Yanıtlar, stresli olmayan (1 puan) çok stresli (4 puan) olarak dörtlü puan sistemine göre kayıt edilmiştir.

Bulgular: Çalışmanın yanıt oranı %63,3'tür (1.294/2.045). Klinik okul çalışmasının bitirilememesi (klinik puan) nedeniyle dersi veya yılı geçememe korkusu en yüksek stres öğesi (ortalama puan =3,57) olarak bulunmuştur. Anket sorularının üçte birinden fazlası, ortalama puanları 3,57 ile 3,06 arasında olan orta-şiddetli stres göstermiştir. Klinik öncesi ve klinik eğitim kategorisi diğer kategorilere göre en yüksek ortalama stres skorunu (3,09) göstermiştir. Stres maddelerinin çoğunda ve altı stres kategorisinde,

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categories. In most of the stress items and six stress categories, females showed significantly more stress than males. A significant difference was found among at least two or more faculties for each stress category.

Conclusion: The modification of curricula, establishment of student counselling, assignment of student advisors and student-oriented programmes may be considered as stress reduction methods. However, further studies are warranted to examine the success of these methods.

Keywords: Dental education, dental students, stress, stress factors, Turkey

kadınlar erkeklerden daha fazla stres göstermiştir. Her stres kategorisi için en az iki veya daha fazla fakülte arasında anlamlı bir fark bulunmuştur.

Sonuç: Müfredatların değiştirilmesi, öğrenci danışmanlığının oluşturulması, öğrenci danışmanlarının atanması ve öğrenci odaklı programlar stres azaltma yöntemleri olarak düşünülebilir. Ancak, bu yöntemlerin başarısını incelemek için daha fazla çalışma yapılması gerekmektedir.

Anahtar Sözcükler: Diş hekimliği eğitimi, diş hekimliği öğrencileri, stres, stres faktörleri, Türkiye

Introduction

In simple terms, stress is the amount of strain caused by a task that might be perceived positively or negatively and can be adaptive or debilitating. Stress, which is a negative response to anxiety, is yet the sole aspect of stress that has been assessed regarding academic achievement among dental students. Anxiety has been shown to predict reduced performance (1). Largely because of the exhausting nature of the training, dental students frequently feel a great deal of stress during this period (2). The primary causes of stress include academic-based course work and exams, clinical care and personal and faculty-associated factors. The main outcomes of stress were reported to affect academic achievement, psycho-emotional well-being and physical health, which are displayed through behaviours including smoking and alcohol consumption (2).

A few studies have compared stress levels among different faculties, each using alternative teaching styles in line with their respective cultures (3). A study reported the presence of great disparity in how students' perceived stressors, depending on their institution. This disparity was associated with personal and education-related factors (4). In a cross-cultural comparative study of students from Singapore and the United States of America, Yap et al. (5) reported that stressors were principally associated with different areas of their academic courses. Varying degrees of stress appeared to result from course-related factors or the students' immediate surroundings (3). Additionally, differences were reported between the stress levels of preclinical and clinical students; in fact, stressors for the latter were similar, more stress was felt by preclinical students, and sex was again significant (6).

Most stress studies involving dental students used modified versions of the Dental Environmental Stress questionnaire (DES) (7). The 25-item DES questionnaire by Garbee et al. (7) is used to identify the potential areas of stress in dental school education. Stress items are grouped into the following seven stress composite categories of related questions: faculty and administration; academics; manual skills; financial obligations; patient care; personal problems; and family. The mean stress score for each category was calculated by taking the average score of each item in the category. The response to each item is rated from 1 (not stressful) to 4 (very stressful), with a fifth possible response being "not pertinent" (2,7). The DES has been translated into multiple languages and adapted by various cultures, thus making it appropriate for national and international studies (1,4,6,8-13).

Turkey is undergoing a rapid transition from Eastern to Western attitudes, values and lifestyles. The changes increasingly reflect Western values of independence, autonomy and competition (14,15). All these factors and stressors associated with dental environments could affect the stress levels of students. The primary objective of the study is to assess the perceived sources and levels of stress in a large population of Turkish dental students. The study was conducted on students from three different dental faculties in three different cities. Thus, the secondary objective of this study is to establish differences in the sources of stress across diverse dental student populations as well as investigate the impact of specific differences among the institutions.

Methods

The İstanbul University Faculty of Dentistry Clinical Research Ethics Committee approved the study protocol. Informed verbal consent was provided by each volunteer, and they did not receive any compensation for participation in this study. The Ethics Committee also approved the consent procedure. The entire study was conducted in full accordance with the guidelines of the World Medical Association Declaration of Helsinki.

This study was conducted in three dental faculties located in İstanbul University Faculty of Dentistry) (DF1), Konya Selçuk University Faculty of Dentistry) (DF2) and Kocaeli University Faculty of Dentistry) (DF3). In these three faculties, dental education is taught as a five-year curriculum. The first year focusses on basic science and dental introductory courses. The second year focusses on laboratory and preclinical training dental science courses. In the third, fourth and fifth years, students undergo clinical training. However, some curriculum differences are present among dental faculties. Especially, the difference is about clinical training in the third year. In some courses, students attend clinical training as an observer. In some courses, students participate in clinical training by treating patients. Also, in DF3, students must undergo integrated clinical training in the fifth year.

All first- to fifth-year dental students were invited to participate in the study from the three dental faculties. In total, 1,294 students in the 2014-2015 academic year enrolled in this study. The questionnaire was administered from April 2015 to June 2015. Questionnaires were given to students when they attended a lecture and a researcher explained the purpose of the survey. Participation was completely voluntary and all responses remained anonymous (16). A total of 15 minutes were allotted for completing the questionnaire. Students were asked not to communicate, talk or comment about the items of questionnaire while filling out the questionnaire (17). The respondents were requested to note their sex at the top of the questionnaire.

Modified versions of the DES compiled by Murphy et al. (10) and Westerman et al. (18) were used as the original templates for our study. The 47 items of the questionnaire used in this study were appropriate for the Turkish dental education system. Previous studies have also used some of these items (6,10-12,17-21). Therefore, the validity and reliability of the questionnaire were not determined. Each response was rated on a Likert-type scale with a four-point rating scale ranging from not stressful (1 point) to very stressful (4 points) (2,7,10,20).

To establish a theoretical framework and clarity in the assessment of the research questions, the 47 items of the questionnaire were grouped into 6 categories (4,10-12). Each category was derived from the factor analyses of DES scales reported in earlier studies (1,4,7,8,10,12,20,22). The categories were as follows: 1) academic performance; 2) preclinical and clinical training; 3) patient treatment; 4) faculty administration and education staff (professors, instructors or clinical supervisors); 5) personal life issues; and 6) professional identity after graduation (10,13,20).

Statistical Analysis

The statistical software SSPS for Windows version 20.0 was used for statistical analysis (SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics were used to present the stress scores (mean and standard deviation) for each questionnaire item, six stress categories, and the highest stress item in each category and preclinical and clinical years' stress items of DES (10). The Kolmogorov-Smirnov test was used to test the normality of data distribution. Student's t-test, one-way analysis of variance and Tukey' post hoc test were used to compare the scores for each questionnaire item with respect to sex, academic year and overall scores of all five years. These tests were also used to detect differences among the previously defined parameters.

Results

Demographic Profile

Table 1 presents the distribution of students by academic year and sex. A total of 1,294 of 2,045 undergraduate students from the 3 faculties completed the questionnaires with a total response rate of 63.3%. The number of dental students who answered the questionnaires was 554 for the dental faculty in the İstanbul University, 441 for Selçuk University and 299 for Kocaeli University; the response rates were 53.8%, 75.1% and 69.9%, respectively.

Perceived Stress Items

Table 2 presents the stress scores for each item according to the academic year of the students and overall scores of five years. The concern about failing the course or year due to the inability to finish clinical school work (clinical score) was the highest stress item (mean score =3.57). In total, 20 of the 47 (43%) questionnaire items had significant differences ($p<0.05$) among the study years of students.

Highest Stress Items in Six Categories by Sex and Academic Year of Students

Table 3 presents the highest items of six stress categories for males, females and overall scores in the preclinical (first and second years) and clinical years (third, fourth and fifth years). In the academic performance category, the highest stress items were different for males and females in each year, except the fourth year. According to the overall means, in preclinical years, the highest stress items were different for the first and second year. Also, only third-year students showed different highest items than fourth- and fifth-year students in clinical years. In the preclinical and clinical training category, the highest stress items were different in clinical years for the fourth and fifth years with respect to sex. In the patient treatment category, the highest stress item was "patients being late or breaking their appointments" (Item 17) in all the clinical years. In the faculty administration and education staff (professors, instructors or clinical supervisors) category, the highest stress item was different for males and females in the second year. The fourth year showed a different highest stress item than that observed in third- and fifth-year students. In the personal life issues category, the "lack of time for relaxation or leisure activities" (Item 32) was the highest stress item for all preclinical and clinical years. In the professional identity after graduation category, the highest stress item was the "fear of not having the possibility to pursue a post-graduate programme" (Item 46) for all preclinical and clinical years with respect to sex and overall means.

Table 1. Sample distribution by year of students and sex

Year of study	Total	Total		N	Gender		Response rate (%)
		Males	Females		Males	Females	
First year	446	198	248	278	102	176	62.3
Second year	443	178	265	308	121	187	69.5
Third year	406	157	249	259	113	146	63.8
Fourth year	373	159	214	262	110	152	70.2
Fifth year	377	181	196	187	83	104	49.6
Total	2,045	873	1,172	1,294	529	765	63.3

Table 2. Distribution of mean stress scores, standard deviation and overall means by the year of students and significance among years

	Question no	First year	Second year	Third year	Fourth year	Fifth year	Overall	Significance
Academic performance								
Amount of assigned coursework	1	3.28±0.79	3.55±0.73	3.48±0.78	3.48±0.74	3.25±0.86	3.42±0.78	<0.001[a,b,c,g,i,j]
Difficulty of assigned coursework	2	3.37±0.73	3.51±0.67	3.41±0.76	3.36±0.75	3.25±0.79	3.39±0.74	0.003[g]
Examinations and grades	3	3.41±0.72	3.49±0.74	3.33±0.78	3.37±0.72	3.25±0.80	3.38±0.75	0.007[g]
Competition with classmates	4	1.84±0.94	2.15±1.04	2.24±1.09	2.06±1.04	2.07± 1.00	2.07±1.03	<0.001[a,b]
Lack of confidence to be a successful dental student	5	2.37±1.00	2.44±0.97	2.53±1.03	2.27±1.02	2.18±0.98	2.37±1.00	0.002[g,h,i]
Fear of failing course, year or a licensing exam and behaviour of parents in case of failure	6	2.09±1.02	2.14±1.08	2.41±1.12	2.27±1.09	2.10±1.07	2.20±1.08	0.003[b,e,i]
Lack of time to do assigned laboratory, preclinical or clinical school work	7	3.06±0.94	3.23±0.90	3.58±0.73	3.63±0.66	3.26±0.85	3.35±0.86	<0,001[b,c,e,f,i,j]
Preclinical and clinical training								
Difficulty in learning precision manual skills required for clinical, preclinical and laboratory work	8	3.07±0.97	2.93±0.91	2.98±0.97	2.82±0.98	2.9±0.99	2.95±0.96	0.047[c]
Responsibilities for comprehensive patient care and treatment	9	-	-	3.07±0.84	3.07±0.85	3.04±0.89	3.06±0.86	0.898
Difficulty in learning clinical procedures and protocols	10	-	-	2.90±0.91	2.80±0.86	2.79±0.94	2.83±0.90	0.358
Transition to the preclinic to clinic and facing patient treatment	11	-	-	3.01± 0.91	2.85±0.90	2.91±0.99	2.92±0.93	0.152
Fear of failing course or year because of the inability to finish clinical school work (clinical credit)	12	-	-	3.69±0.67	3.57±0.70	3.42±0.87	3.57±0.75	<0.001[i,j]
Lack of confidence in own decision making during clinical school work	13	-	-	2.73±0.96	2.60±0.96	2.53±0.89	2.63±0.95	0.069
Fear of unable to catch up if getting behind the clinical work or course	14	-	-	3.19±0.85	3.18±0.83	3.14±0.94	3.18±0.87	0.839
Lack of adequate or sufficient number of patients for clinical exams or clinical work	15	-	-	3.45±0.78	3.31±0.85	3.20±0.92	3.33±0.85	0.008[i]
Cooperation with dental laboratory due to timing or faulty working	16	-	-	3.43±0.74	3.59±0.74	3.48±0.77	3.50±0.75	0.052
Patient treatment								
Patients being late or breaking their appointments	17	-	-	3.12±0.83	3.00±0.83	3.04±0.92	3.05±0.86	0.249
Negative or uncomplimentary attitudes of patients or patient management	18	-	-	2.61±0.88	2.60±0.86	2.66±0.91	2.62±0.88	0.742
Lack of communication or cooperation with patients	19	-	-	2.51±0.89	2.45±0.91	2.37±0.89	2.45±0.90	0.271
Working on patients with dirty mouths	20	-	-	2.68±0.94	2.63±0.91	2.74±0.92	2.68±0.93	0.440
Faculty administration and education staff (professors, instructors or clinical supervisors)								
Atmosphere created by professors or clinical supervisors	21	-	-	3.31±0.80	3.30±0.76	3.22±0.84	3.28±0.80	0.446
Criticisms from professor or clinical supervisors in front of patients	22	-	-	3.41±0.81	3.28±0.86	3.31±0.86	3.33±0.84	0.206
Inconsistency of feedback on your clinical work among different professor or instructors	23	-	-	3.29±0.79	3.24±0.78	3.16±0.88	3.24±0.82	0.234

Table 2. continued

Inadequate knowledge and/or clinical experience of the instructors in terms of evaluation of your clinical work (inadequate educational aspects of instructors),	24	-	-	2.76±0.93	2.66±0.94	2.79±0.90	2.73±0.93	0.306
Lack of adequate professors, clinical supervisors or instructors in the clinics	25	-	-	2.67±0.98	2.61±0.099	2.72±0.94	2.66±0.97	0.508
Absence of a professor, clinical supervisor or instructor in clinic when assistance is needed	26	-	-	3.03±0.88	3.10±0.89	3.06±0.99	3.06±0.91	0.662
Rules and regulations of the faculty	27	2.78±0.98	3.08±0.86	3.21±0.85	3.34±0.81	3.03±0.93	3.09±0.91	<0,001[a,b,c,d,f,j]
Attitudes of staff, clinical supervisor or instructor towards female students	28	2.13±1.13	2.31±1.06	2.38±1.08	2.44±1.09	2.49±1.03	2.34±1.09	0.003[c,d]
Lack of time to do assigned clinical work or shortage of allocated clinical time	29	-	-	3.26±0.82	3.23±0.87	3.05±0.88	3.19±0.86	0.022[i]
Expectation of dental faculty or the difference between reality of faculty and expectations	30	2.91±0.92	3.03±0.91	3.21±0.84	3.26±0.88	3.14±0.90	3.10±0.90	<0,001[b,c,d,f]
The faculty facilities/environment are not sufficient for social, cultural and sports activities	31	3.02±0.98	3.08±0.95	3.11±0.97	3.13±0.99	2.94±1.04	3.06±0.98	0.243
Personal life issues								
Lack of time for relaxation or leisure activities	32	3.16±0.89	3.24±0.91	3.47±0.79	3.45±0.80	3.17±0.85	3.30±0.86	<0,001[b,c,e,f,i,j]
Difficulty in making friends	33	1.77±0.95	1.89±1.07	1.70±0.92	1.56±0.89	1.85±0.89	1.75±0.96	<0,001[f,j]
Relationship between members of the opposite sex	34	1.87±1.02	1.94±1.08	1.86±0.99	1.73±0.95	1.88±0.94	1.86±1.00	0.132
Difficulty of adaptation to faculty environment	35	2.23±0.98	2.26±1.04	2.20±0.99	2.02±1.01	2.33±0.97	2.20±1.00	0.011[f,j]
Neglect for personal life	36	2.74±1.00	2.78±1.08	2.88±1.07	2.86±1.07	2.89±0.98	2.83±1.05	0.394
Moving away from home or town	37	2.55±1.21	2.36±1.13	2.44±1.14	2.35±1.14	2.41±1.06	2.42±1.14	0.246
Worried about compatibility of dentistry to personality	38	2.36±1.11	2.43±1.09	2.35±1.13	2.35±1.04	2.34±1.06	2.37±1.09	0.862
Difficult home/hostel environment to study, rest or fun	39	2.45±1.11	2.46±1.12	2.30±1.09	2.29±1.07	2.10±0.97	2.34±1.09	0.002[d,g]
Lack of financial resources for personal life expenses	40	2.53±1.13	2.58±1.11	2.70 ± 1.06	2.57±1.07	2.61±1.04	2.60±1.09	0.459
Forced postponement of marriage, engagement or having children	41	1.89±1.09	2.09±1.15	2.08±1.15	2.05±1.12	2.12±1.04	2.04±1.12	0.143
Having dual role of wife/husband/ mother/father/dental student	42	1.67±1.03	2.03±1.15	1.89±1.11	1.76±1.05	1.87±1.03	1.85±1.09	<0,001[a,f]
Personal physical health problems due to chronic disease, drugs, alcohol, etc.	43	1.83±1.06	2.16±1.13	1.99±1.06	1.92±0.99	2.07±1.11	2.00±1.07	0.003[a]
Working while studying	44	1.95±1.09	2.16±1.12	2.10±1.11	2.05±1.04	2.18±1.12	2.08±1.10	0.112
Professional identity after graduation								
Lack of confidence in self to be a successful dentist after graduation	45	2.47±0.97	2.37±1.03	2.42±1.01	2.37±1.02	2.48±0.96	2.42±1.00	0.590
Fear of not having possibility to pursue a post-graduate programme	46	2.80±0.98	2.79±0.98	2.92±0.97	2.92±0.97	2.97±0.90	2.87±0.97	0.134
Fear of unemployment after graduation	47	2.25±1.06	2.25±1.05	2.29±1.05	2.23±1.04	2.30±1.05	2.26±1.05	0.926

Lower case letters denote significant difference between year of students: a: 1-2, b: 1-3, c: 1-4, d: 1-5, e: 2-3, f :2-4, g: 2-5, h: 3-4, i: 3-5, j: 4-5

Table 3. Means and standard deviations for six stress categories with the highest stress item by year of student or preclinical and clinical years

Six stress categories	Preclinical years		Clinical years		
	First year (N=278)	Second year (N=308)	Third year (N=259)	Fourth year (N=262)	Fifth year (N=187)
Academic performance					
Highest stress item					
Male	Examinations and grades (3.39±0.75)	Examinations and grades (3.48±0.77)	Lack of time to do assigned laboratory, preclinical or clinical school work (3.50±0.80)	Lack of time to do assigned laboratory, preclinical or clinical school work (3.58±0.68)	Lack of time to do assigned laboratory, preclinical or clinical school work (3.19±0.85)
Female	-Difficulty of assigned the coursework (3.43±0.69) -Examinations and grades (3.43±0.70)	Amount of assigned coursework (3.60±0.67)	Amount of assigned coursework (3.68±0.59)	Lack of time to do assigned laboratory, preclinical or clinical school work (3.67±0.64)	Examinations and grades (3.38±0.73)
Overall	Examinations and grades (3.41±0.72)	Amount of assigned coursework (3.55±0.73)	Lack of time to do assigned laboratory, preclinical or clinical school work (3.58±0.73)	Lack of time to do assigned laboratory, preclinical or clinical school work (3.63±0.66)	Lack of time to do assigned laboratory, preclinical or clinical school work (3.26±0.85)
Preclinical and clinical training					
Highest stress item					
Male	Difficulty in learning precision manual skills required for clinical, preclinical and laboratory work (2.90±1.00)	Difficulty in learning precision manual skills required for clinical, preclinical and laboratory work (2.79±0.89)	Fear of failing course or year because of the inability to finish clinical school work (clinical score) (3.52±0.81)	Fear of failing course or year because of the inability to finish clinical school work (clinical score) (3.48±0.71)	Cooperation with dental laboratory due to timing or faulty working (3.37±0.76)
Female	Difficulty in learning precision manual skills required for clinical, preclinical and laboratory work (3.17±0.94)	Difficulty in learning precision manual skills required for clinical, preclinical and laboratory work (3.02±0.92)	Fear of failing course or year because of the inability to finish clinical school work (clinical score) (3.82±0.51)	Cooperation with dental laboratory due to timing or faulty working (3.72±0.60)	Fear of failing course or year because of the inability to finish clinical school work (clinical score) (3.57±0.76)
Overall	Difficulty in learning precision manual skills required for clinical, preclinical and laboratory work (3.07±0.97)	Difficulty in learning precision manual skills required for clinical, preclinical and laboratory work (2.93±0.91)	Fear of failing course or year because of the inability to finish clinical school work (clinical score) (3.69±0.67)	Cooperation with dental laboratory due to timing or faulty working (3.59±0.74)	Cooperation with dental laboratory due to timing or faulty working (3.48±0.77)
Patient treatment					
Highest stress item					
Male	–	–	Patients being late or breaking their appointments (2.88±0.85)	Patients being late or breaking their appointments (2.80±0.83)	Patients being late or breaking their appointments (2.87±0.93)
Female	–	–	Patients being late or breaking their appointments (3.30±0.77)	Patients being late or breaking their appointments (3.14±0.80)	Patients being late or breaking their appointments (3.17±0.90)
Overall	–	–	Patients being late or breaking their appointments (3.12±0.83)	Patients being late or breaking their appointments (3.00±0.83)	Patients being late or breaking their appointments (3.04±0.92)

Table 2. continued

Faculty administration and education staff (professors, instructors or clinical supervisors)					
Highest stress item					
Male	The faculty facilities/ environment are not sufficient for social, cultural and sports activities (2.96±1.05)	Rules and regulations of the faculty (3.07±0.88)	Criticisms from professor or clinical supervisors in front of patients (3.30±0.85)	Rules and regulations of the faculty (3.17±0.90)	Criticisms from professor or clinical supervisors in front of patients (3.24±0.86)
Female	The faculty facilities/ environment are not sufficient for social, cultural and sports activities (3.05±0.93)	- Expectation of dental faculty or the difference between reality of faculty and expectations (3.10±0.89) - The faculty facilities/ environment are not sufficient for social, cultural and sports activities (3.10±0.96)	Criticisms from professor or clinical supervisors in front of patients (3.49±0.76)	Rules and regulations of the faculty (3.45±0.73)	Criticisms from professor or clinical supervisors in front of patients (3.37±0.85)
Overall	The faculty facilities/ environment are not sufficient for social, cultural and sports activities (3.02±0.98)	Rules and regulations of the faculty (3.08±0.86) - The faculty facilities/ environment are not sufficient for social, cultural and sports activities (3.08±0.95)	Criticisms from professor or clinical supervisors in front of patients (3.41±0.81)	Rules and regulations of the faculty (3.34±0.81)	Criticisms from professor or clinical supervisors in front of patients (3.31±0.86)
Personal life issues					
Highest stress item					
Male	Lack of time for relaxation or leisure activities (3.02±0.97)	Lack of time for relaxation or leisure activities (3.23±0.92)	Lack of time for relaxation or leisure activities (3.29±0.89)	Lack of time for relaxation or leisure activities (3.36±0.84)	Lack of time for relaxation or leisure activities (3.02±0.86)
Female	Lack of time for relaxation or leisure activities (3.24±0.83)	Lack of time for relaxation or leisure activities (3.25±0.91)	Lack of time for relaxation or leisure activities (3.60±0.67)	Lack of time for relaxation or leisure activities (3.51±0.77)	Lack of time for relaxation or leisure activities (3.28±0.83)
Overall	Lack of time for relaxation or leisure activities (3.16±0.89)	Lack of time for relaxation or leisure activities (3.24±0.91)	Lack of time for relaxation or leisure activities (3.47±0.79)	Lack of time for relaxation or leisure activities (3.45±0.80)	Lack of time for relaxation or leisure activities (3.17±0.85)
Professional identity after graduation					
Highest stress item					
Male	Fear of not having possibility to pursue a post-graduate programme (2.70±1.01)	Fear of not having possibility to pursue a post-graduate programme (2.70±1.01)	Fear of not having possibility to pursue a post-graduate programme (2.86±0.93)	Fear of not having possibility to pursue a post-graduate programme (2.74±1.03)	Fear of not having possibility to pursue a post-graduate programme (2.80±0.91)
Female	Fear of not having possibility to pursue a post-graduate programme (2.86±0.96)	Fear of not having possibility to pursue a post-graduate programme (2.84±0.96)	Fear of not having possibility to pursue a post-graduate programme (2.96±1.00)	Fear of not having possibility to pursue a post-graduate programme (3.05±0.90)	Fear of not having possibility to pursue a post-graduate programme (3.12±0.86)
Overall	Fear of not having possibility to pursue a post-graduate programme (2.80±0.98)	Fear of not having possibility to pursue a post-graduate programme (2.79±0.98)	Fear of not having possibility to pursue a post-graduate programme (2.92±0.97)	Fear of not having possibility to pursue a post-graduate programme (2.92±0.97)	Fear of not having possibility to pursue a post-graduate programme (2.97±0.90)

Six Stress Categories with Respect to Sex, Year, Overall Scores by Year and Overall Scores of All Five Years

Table 4 shows the means and standard deviations for the six stress categories with respect to sex, year, overall scores by academic year of students and the overall scores of all five years. The preclinical and clinical training category showed the highest stress mean score (3.09) as compared with the other categories. Except for the categories of patient treatment and professional identity after graduation, there were significant differences among the study years with respect to males or females and the overall means in each stress category. There was a significant difference between males and females with respect to the year of students, except in first and second academic years in academic performance, faculty administration and education staff and personal life issues categories, as well as third and fifth years in the personal life issues category. With regard to the overall mean of males and females of all five years, a significant difference was observed between the sexes in each category. Also, a significant difference was determined among the six categories for the overall means of all five years.

Six Stress Categories by Faculty

Table 5 shows the means and standard deviations of the six stress categories for each dental faculty.

A significant difference was found among at least two or more faculties for each category. Also, the DF2 displayed higher mean stress values than the other faculties.

Discussion

Evidence from a systematic review including 124 studies demonstrated that dental students were exposed to a significant amount of stress while training, which was chiefly attributed to the challenging characteristics of the training. Previous studies have indicated the negative effects of increased stress on students' health and well-being (2). Therefore, our aim was to determine the perceived sources and levels of stress in a large population of Turkish dental students across three dental faculties. Also, we aimed to examine the differences in stress sources and levels among faculties to investigate the impact of specific curricular, teaching and institutional differences.

In this study, the overall mean stress scores were considered and the highest stress item changed according to the year of the students. "Examinations and grades" for were highest stress items in the first year, and the "amount of assigned coursework" was the highest stress items in the second year. This was followed by the "difficulty of assigned the coursework" for the first and second years. The first year was mainly dedicated to medical courses and some manual skills and preclinical courses related to prosthetics. Preclinical training and theoretical dental classes are usually taken in the second year. Therefore, the academic environment and dental faculty curriculum are new for first-year students (6,23,24). Silverstein and Kritz-Silverstein (24) found that the DES scores related to class work were high at baseline and increased after one year. Their summations were stress levels resulting from the realisation of the new environment, significant life changes and pressures and anticipation of stressors still to come. In contrast to our results, stress due to academic factors was lower in the second year than in the first year (22). This result might be attributed to the rapid shift in curriculum and subject, to which the new students had difficulty in adjusting. However, in this study, the mean stress of items related to academic performance increased in the second year. This finding may be related to the increase of preclinical work and theoretical dental courses. In accordance with our results, Polychronopoulou and Divaris (12) showed that students in the third year were most affected by acquiring manual skills in the laboratory and preclinical work. The researchers stated that that these students might be overwhelmed by this demanding period. In terms of curriculum, there is a similarity between the second year in our study and the third year in their study. In addition to this result, in the other studies, "examinations and grades" (6,11,21,24), "no time for holiday or reduced holidays" (25), "amount of assigned work" (6), "fear of failing course/year" (6, 11), "fear of facing parents after failure" (23), "financial responsibilities" (24), "inconsistency in feedback on work from different instructors" (24) and "fear of unemployment after graduation" (21) were also reported as the top stress items for the first and second year.

There was a trend of increasing overall mean stress scores related to clinical training, patient treatment and clinical education staff. In line with our findings, it was reported that the mean DES scores had a marked rise in the third year, which is the transition year into clinical training. This rise to moderate stress levels for third-year students suggests that the transition might be challenging for many students (11). Another study with similar findings linked the rise with the additional unfamiliar pressures related to patient care (16). In agreement with our findings, the "fear of failing the course or a year" was found as the top stress item in other study (6,13). Although in line with our findings of the first and second years, another study found examinations and grades were the main stressors for all students (20). However, their mean score (2.86) was much lower than that in our study (3.41).

Elani et al. (8) also found that the chief stressors for all dental students were "examinations and grades" and "workload". In contrast, the "amount of assigned work" (7), "examinations" (7), "treatment grades" (11), "completion of clinical requirements" (21), "lack of time for holiday or reduced holidays" (25) were reported as top stress items for third-year students. In our study, the highest stress item "lack of time to do assigned clinical school work" for fourth-year students may related to increased assigned clinical work (clinical credit). In agreement with our findings, Naidu et al. (11) observed that the "shortage of allocated clinical time" was the highest stress item for fourth-year students. They stated that fourth-year students might be more focused on finishing their clinical requirements. Morse and Dravo (21) observed that fourth-year students were the most stressed about learning clinical procedures because the most advanced techniques were learned and put into practice in this year. Also, in another study performed on Turkish dental

Table 4. Means and standard deviations for six stress categories with sex, overall means by year of students, and overall scores of all five years, and statistical differences between males and females, and between the overall scores of each category by year of students

Six stress categories	First year (N=278)	Second year (N=308)	Third year (N=259)	Fourth year (N=262)	Fifth year (N=187)	Overall (N=1,294)	Significance difference between years
1. Academic performance							
Male	2.78±1.05	2.88±1.07	2.88±1.06	2.83±1.08	2.68±1.06	2.82±1.07	P=0.003[g,i]
Female	2.77±1.08	2.96±1.08	3.09±1.05	2.99±1.08	2.84±1.08	2.93±1.08	P<0.001[a,b,c,g,i,j]
Overall	2.78±1.07	2.93±1.08	3.00±1.06	2.92±1.08	2.77±1.07	2.88±1.08	P<0.001[a,b,c,g,i,j]
Significance between both genders	0.884	0.106	<0.001	0.002	0.010	<0.001	
2. Preclinical and clinical training							
Male	2.90±1.00	2.79±0.89	2.93±0.98	2.92±0.94	2.82±0.98	2.89±0.97	P=0.090
Female	3.17±0.94	3.02±0.92	3.34±0.79	3.21±0.88	3.23±0.89	3.25±0.86	P<0.001[e,f,g,h,i]
Overall	3.07±97	2.93±0.91	3.16±0.90	3.09±0.92	3.05±0.95	3.09±0.92	P<0.001[e,f,h,i]
Significance between both genders	0.026	0.026	<0.001	<0.001	<0.001	<0.001	
3. Patient treatment							
Male	–	–	2.49±0.89	2.43±0.89	2.51±0.91	2.48±0.89	P=0.452
Female	–	–	2.91±0.89	2.84±0.87	2.86±0.94	2.87±0.90	P=0.344
Overall			2.73±0.92	2.67±0.90	2.70±0.94	2.70±0.92	P=0.315
Significance between both genders	–	–	<0.001	<0.001	<0.001	<0.001	
4. Faculty administration and education staff (professors, instructors or clinical supervisors)							
Male	2.78±1.06	2.83±1.00	2.96±0.93	2.91±0.97	2.90±0.96	2.90±0.97	P=0.009[b]
Female	2.67±1.06	2.90±1.00	3.14±0.94	3.16±0.93	3.06±0.95	3.04±0.97	P<0.001[a,b,c,d,e,f,g]
Overall	2.71±1.06	2.88±1.00	3.06±0.94	3.05±0.95	2.99±0.95	2.98±0.97	P<0.001[a,b,c,d,e,f,g]
Significance between both genders	0.107	0.255	<0.001	<0.001	<0.001	<0.001	
5. Personal life issues							
Male	2.25±1.14	2.34±1.12	2.28±1.12	2.13±1.10	2.28±1.02	2.26±1.11	P<0.001[f,h,j]
Female	2.22±1.13	2.33 ± 1.17	2.33±1.17	2.29± 1.15	2.31±1.12	2.30±1.15	P=0.010[a,b]
Overall	2.23±1.14	2.34 ± 1.15	2.31±1.15	2.23±1.13	2.29±1.08	2.28±1.13	P<0.001[a,b,f,h]
Significance between both genders	0.563	0.741	0.192	<0.001	0.445	0.028	
6. Professional identity after graduation							

Table 4. continued

Male	2.40±1.05	2.38±1.08	2.45±1.04	2.28±1.04	2.43±1.00	2.38±1.04	P=0.068
Female	2.57±1.02	2.53±1.02	2.61±1.05	2.67±1.03	2.71±1.01	2.61±1.03	P=0.257
Overall	2.51±1.03	2.47±1.05	2.54±1.05	2.50±1.05	2.59±1.01	2.52±1.04	P=0.270
Significance between both genders	0.018	0.030	0.034	<0.001	<0.001	<0.001	
Significance difference among males of six categories	<0.001^{B,D,E,F,H,I,J,K,L,M,N}	<0.001^{B,D,E,F,H,I,J,K,L,M,N}	<0.001^{B,D,E,F,H,I,J,K,M,N}	<0.001^{B,D,E,F,H,I,J,K,M,N}	<0.001^{C,D,E,F,H,I,J,K,M,N}	<0.001^{B,C,D,E,F,H,I,J,K,M,N,O}	
Significance difference among females of six categories	<0.001^{A,B,D,E,F,G,H,I,J,K,L,M,O}	<0.001^{A,B,D,E,F,G,H,I,J,K,L,M,O}	<0.001^{A,B,D,E,F,G,H,I,J,K,L,M,O}	<0.001^{A,B,C,D,E,F,H,I,J,K,M,N,O}	<0.001^{A,C,D,F,G,H,I,J,K,M,N,O}	<0.001^{A,C,D,E,F,G,H,I,J,K,L,M,N,O}	
Significance difference among overall means of six categories	<0.001^{A,B,D,E,F,G,H,I,J,K,L,M,N,O}	<0.001^{B,D,E,F,H,I,J,K,L,M,N,O}	<0.001^{A,B,D,E,F,G,H,I,J,K,L,M,N,O}	<0.001^{A,B,C,D,E,F,H,I,J,K,L,M,N,O}	<0.001^{A,C,D,E,F,H,I,J,K,M,N,O}	<0.001^{A,B,C,D,E,F,G,H,I,J,K,L,M,N,O}	

-Lower case letters denote significant difference between year of students: a: 1-2, b: 1-3, c: 1-4, d: 1-5, e: 2-3, f: 2-4, g: 2-5, h: 3-4, i: 3-5, j: 4-5.
-Capital letters show significant difference between six categories: A: 1-2, B: 1-3, C: 1-4, D:1-5, E: 1-6, F: 2-3, G: 2-4, H: 2-5, I: 2-6, J: 3-4, K: 3-5, L: 3-6, M: 4-5, N: 4-6, O: 5-6.

students, the "completion of clinical requirements" and "fear of failing the year" were found as the highest stress items for the fourth year (6). Different to our findings, "examination and grades" (8), "lack of time for relaxation" (12), "lack of time for holiday or reduced holidays" (25) and "financial resources" (21) were reported as the highest stress items. Compared with another study, in our study, the "cooperation with the dental laboratory due to timing or faulty working" was the highest stress item and the overall mean score (3.48) was much higher than their mean scores, which were between 1.73 and 2.53 (20,21,25). This may be due to the fact that the laboratory workload is excessive and the number of laboratory technicians is insufficient because the students send the prosthetics work to the laboratory to complete their prosthetic credits. In accordance with our findings, it was reported that seniors would naturally be more concerned about the dental laboratory being prompt in the delivery of their cases because they were trying to meet deadlines for completion of graduation requirements (7). In contrast to our findings, "examination and grades" (10), "completion of clinical requirements" (6), "lack of time for holiday or reduced holidays". (25) "fear of failing a course or a year" (11,21), "differences in opinion between clinical staff concerning patient treatment" (20), "fear of facing parents after failure" (23) and "completing clinical requirements" (26) were reported as the highest stress items for fifth- or final-year students.

In our study, there were statistically significant differences among six categories. The preclinical and clinical training category showed the highest mean stress score (3.09). Faculty administration and education staff exhibited the second highest stress mean score (2.98). Difficulties faced in acquiring the precision manual skills needed for preclinical and laboratory work may be the reason for high stress in preclinical years. In clinical years, the transition to the clinic (8), unfamiliar patients care (16) and credit pressure to complete certain numbers and types of clinical cases (8) may be causes of high stress with respect to clinical training. In contrast to our findings, a systematic review reported that for preclinical students, most researchers found that academic factors were the main source of stress (92.5%). For clinical students, again, most studies identified academic factors as the main cause of stress (84.0%), followed by clinical factors (63.6%). Faculty and personal issues contributed less to clinical students' stress levels (38.6% and 11.4%, respectively) (2). However, in accordance with our study, faculty and administration were reported as the highest stress factor in several studies (1,7,23).

In accordance with previous studies, females in our study reported significantly more stress than males in most of the stress items and six stress categories (4,11,13,16,21). One might draw one of a few conclusions: females perceive and experience more stress than males, males are less expressive/private regarding their concerns, or a combination of these or other unknown factors exists (13). It has been reported that that female students might feel more pressure to succeed in a largely male-dominated profession (11,27) and, with that, find the peer pressure and the competitive nature of dental school more stressful (18,27). In contrast to our findings, some studies found that male students

Table 5. Means and standard deviations of six stress categories for the three dental faculties and a statistical comparison of these three faculties for each category

Six stress categories	1. Faculty (N=554)	2. Faculty (N=441)	3. Faculty (N=299)	Significance difference between years
1. Academic performance	2.76±1.02	3.02±1.09	2.92±1.14	<0.001[a,b,c]
2. Preclinical and clinical training	2.97±0.90	3.23±0.91	3.12±0.97	<0.001[a,b,c]
3. Patient treatment	2.64±0.85	2.77±0.95	2.70±0.98	0.006[a]
4. Faculty administration and education staff (professors, instructors or clinical supervisors)	2.91±0.91	3.12±0.98	2.91±1.07	<0.001[a,c]
5. Personal life issues	2.23±1.06	2.35±1.17	2.26±1.20	<0.001[a,c]
6. Professional identity after graduation	2.53±0.99	2.59±1.07	2.38±1.06	<0.001[b,c]

Lower case letters show significant difference between faculties: a: 1-2, b: 1-3, c: 2-3

reported higher stress than female students (20,23). Yet in other studies, no difference was found between male and female students (3,26).

In our study, a significant difference was observed in academic performance and preclinical and clinical training stress categories among the three faculties. Also, a significant difference was found among at least two faculties in other stress categories. Students of the second faculty showed a higher stress than other faculties. This difference may be related to the teaching and curriculum issues of each faculty or external factors such as the socioeconomic or sociocultural environment of the cities in which the faculties were located. It is noteworthy that issues raised by the students regarding their courses pertained to different aspects of the curriculum in each school. There is some evidence showing the limited effects of the external environment on stress in dental students; hence, variations of student stress arise from both course-related factors and their immediate surroundings (3,5).

Study Limitation

This research is limited to three faculties in Turkey's three largest cities. It is not known whether the sources of stress found in the research reflect the local attitudes or are more common. Therefore, the effects of main stressors, especially the curriculum and personal life issues, should be evaluated by conducting studies in more faculties and cities.

Conclusion

The "fear of failing course or year because of the inability to finish clinical school work" was found as the highest stress item (mean score =3.57) for all students in five years. However, the highest stress item changed according to the year of the students. "Examinations and grades" for the first year and "amount of assigned coursework" for the second year were found to be the highest stress items. For the third year, the "fear of failing the course or year because of the inability to finish clinical school work"; for the fourth year, the "lack of time to do assigned laboratory, preclinical or clinical school work"; and for the fifth year, the "cooperation with dental laboratory due to timing or faulty working" were the highest stress items. With respect to the six categories, the preclinical and clinical training category was the main source of stress as perceived by all the students. For most of the stress items and six stress categories, females showed significantly more stress than males. In addition, there was a significant difference in the perceived stress levels among at least two or more faculties in each stress category. Curricula need to be rearranged or modified by considering factors that cause stress. Additionally, establishing a student counselling section, assigning student advisors and student-oriented programme planning may reduce the stress caused by dental education. However, further studies are warranted to examine the success of programmes and curricula that reduce stress.

Ethics

Ethics Committee Approval: Istanbul University Faculty of Dentistry Clinical Research Ethics Committee approved the study protocol. (Protocol Number: 2015/2)

Informed Consent: Informed verbal consent was provided by each volunteer, and they did not receive any compensation for participation in this study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: M.D., S.T., Design: M.D., N.T., N.Ç., S.T., Data Collection or Processing: N.T., N.Ç., O.Ü., S.Ş., C.B., Analysis or Interpretation: M.D., C.B., Literature Search: M.D., S.T., Writing: M.D., S.T.

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Original Article

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Cross-cultural Adaptation, Reliability and validity of the Turkish Version of the Work Limitations Questionnaire-Short Form

İş Limitasyonu Ölçeği Kısa Formu Türkçe Uyarlaması: Geçerlilik ve Güvenilirlik Çalışması

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ABSTRACT

Objective: The physical or emotional difficulties faced by academicians negatively affect their productivity. The aim of this study is to translate and adapt a Turkish version of the Work Limitations Questionnaire-Short Form (WLQ-SF) and investigate its validity and reliability. The WLQ-SF assesses academicians who have suffered from physical or emotional health limitations in the past 2 weeks while working.

Methods: In this study, the mean age of 104 participants who completed the Turkish version of the WLQ-SF was 37.75±9.43 years. The test-retest reliability was evaluated using the WLQ-SF with a 7-day interval. The test-retest reliability assessed through intraclass correlation coefficient (ICC) and Cronbach's alpha (α) were used to determine the construct validity and time-invariant reliability of the scale over time.

Results: The WLQ-SF provided construct validity with 68.62% variance under two factors (Bartlett's test of sphericity value, 407.830; Kaiser-Meyer-Olkin (KMO) value, 0.746; p=0.0001) and was found to be valid. It demonstrated a high test-retest reliability (ICC =0.96) and good internal consistency (Cronbach's alpha =0.83) for all domains. Therefore, the WLQ-SF in the Turkish language is a valid and reliable test.

Conclusion: The Turkish version of the WLQ-SF was found to be valid and reliable for evaluating the effect of physical and emotional health among academicians. It is an important scale to measure the impact of both physical and emotional health at work.

Keywords: Academicians, work limitation, reliability, validity, Turkish

ÖZ

Amaç: Akademisyenlerin karşılaştıkları fiziksel ve duygusal zorluklar onların üretkenliklerini olumsuz olarak etkilemektedir. Çalışmanın amacı İş Limitasyonu Ölçeği-Kısa Formu'nun (İLÖ-KF) Türkçe uyarlaması, geçerlilik ve güvenilirlik çalışmasının yapılmasıdır. İLÖ-KF akademisyenlerin son iki hafta içerisinde yaşadıkları fiziksel veya duygusal sağlık durumlarının, çalışma hayatları üzerine etkisini inceleyen bir ölçektir.

Yöntemler: İLÖ-KF'nin Türkçe versiyonuna katılan katılımcıların yaş ortalaması 37,75±9,43 yıl olarak hesaplanmıştır. İLÖ-KF'nin test-tekrar test güvenilirliği yedi gün arayla tekrarlanarak değerlendirildi. Ölçeğin yapısal geçerlilik ve zamana göre değişmezlik güvenilirliği için test-tekrar test intraklass korelasyon (ICC) yöntemi ile Cronbach alfa testi kullanıldı.

Bulgular: İLÖ-KF iki faktör altında %68,62 varyans ile yapısal geçerliliği sağlamaktadır (Barlett: 407,830; Kaiser-Meyer-Olkin: 0,746, p=0,0001). İLÖ-KF güvenilirliği (ICC =0,96) ve iç tutarlılığı (Cronbach alfa =0,83) oldukça yüksek olarak bulundu. Bundan dolayı İLÖ-KF Türkçe dilinde geçerli ve güvenilir bir testtir.

Sonuç: İLÖ-KF Türkçe uyarlamasının akademisyenlerin fiziksel ve duygusal sağlık durumları üzerinde geçerli ve güvenilir olduğu bulunmuştur. Bu ölçek işyerinde hem fiziksel hem de duygusal sağlığın etkisini ölçmek için önemli bir ölçektir.

Anahtar Sözcükler: Akademisyenler, iş limitasyonu, geçerlilik, güvenilirlik, Türkçe

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Introduction

The concept of work has emerged from social relationships that arise due to the desire to meet the needs of other people (1). Creating or uncovering anything is defined as a process consisting of both physical and mental occupations and labour. Work is an indispensable part of human life that provides an individual with status and value and supports him/her as part of society (2).

The occupational health and safety standards address the conditions of people who are risking their lives and future due to various risk factors and hazards caused by industrialisation and technology. These can be defined as a set of systematic and scientific studies aiming to protect both physical and mental health during the execution of work in the workplace and in an environment free from threats to a person's physical and mental health (3,4). Approximately, 4% of the Turkey's gross national product is spent on occupational accidents and occupational losses of employees (5). Therefore, measures taken to ensure occupational health and safety will eliminate these losses, and the income earned from being able to continue work will be used to develop the country (6). The need to transform the working environment into a safe and healthy place to protect workers from occupational accidents and diseases must be evaluated for every situation. It should be kept in mind that all kinds of expenditures that are made to improve the occupational health and safety may have a share in minimising the possibility of accidents or injuries, decreasing the costs of the products that a business produces and increasing its profit margin (7).

The profession of an academician can be defined as a civil servant working for a fee, a professional employee or a knowledge worker who can develop fiction works to intersect the intellectual paths within the university (8). As academic staff members work within an academic institution, their performance is evaluated in a way that is similar to that of office workers. Although the working environment of academicians appears to be safer when compared with the hazardous workplaces, they may contain many observed and/or unobserved risk factors (9). Academic staff can work in environments that can be affected by many personal and environmental risk factors, including physical, chemical, biological, psychosocial and ergonomic factors, as observed in other professional groups (10). The main problems that may affect the occupational health and safety of academicians are personal factors, such as workload and stress; psychological factors, such as burnout, depression and anxiety; physical factors, such as inappropriate posture, continuous repetitive movements and ergonomic features of the work environment; and environmental factors, such as noise, thermal comfort, lighting and chemicals (11).

The literature has reported that the costs associated with the loss of productivity in a work environment have a significant economic impact, and additional costs due to paying employees for sick leave and time away from work (e.g. vacations) increase this impact (12,13). When these financial factors are considered, various scales are needed to measure the impact of this health loss of employees on the workplace (14,15). Researchers have adapted some of these scales to different languages. The most frequently used scales are the Job Satisfaction Scale, which is used to evaluate employee satisfaction and personal satisfaction problems, and the Minnesota Satisfaction Questionnaire, which is used to examine the effect of internal and external factors on employee job satisfaction (16-18). The Work Limitations Questionnaire (WLQ) is a scale that is frequently preferred because it evaluates the relationship between the physical health and emotional problems of the employees and their lives. This scale is often used to determine employee-related influences (14).

Considering that employees' physical or emotional difficulties negatively affect the productivity of the business, the present study aimed to ensure the validity and reliability of the Dr Debra Learner's WLQ-Short Form (WLQ-SF), when adapted into Turkish, to examine the effect of physical and emotional situations on employees in the past 2 weeks.

Methods

Before beginning the study, permission was obtained from the authors of the original version of the WLQ-SF for its translation and validation in the Turkish language. The study was designed according to the Helsinki Declaration, and all the participants signed the consent form. The study was approved by the Ethics Committee of University.

Study Population

The study was conducted on the academicians working at the Faculty of Health Sciences from March 2019 to June 2019. These academicians were eligible to be part of the study if they met the following participation criteria: 1) Aged between 25 and 65 years; 2) working as a faculty member at a state university; 3) having at least 3 years of academic experience and 4) are literate and willing to join the study. Participants were ineligible if they had any chronic, physical, mental and/or cognitive disease as diagnosed by a medical doctor.

In the calculation of the sample size required for performing the adaptation, reliability and validity analyses, it was envisaged that the number of items in the model should be evaluated 10 times as suggested in the study by Tabachnik et al. (19) to make reliable inferences in multivariate analyses. Therefore, the number of individuals to be included for assessing the 8-item scale was calculated to be 80. It was found that the inclusion of 100 participants with a considered non-response rate of 25 would be sufficient to obtain the necessary working power.

During the study process, 110 participants, who were working as faculty members at various state universities, were included. Of these, six participants were subsequently excluded (four did not come for the test-etest and two requested to be removed from the study). As a result, our final study population consisted of 104 academicians.

Instruments

A socio-demographic form, comprising questions about age, sex, academic experience and title, were filled out by all the participants.

The Work Limitations Questionnaire Short Form (Dr. Debra Learner's WLQ-SF) consisted of five sub-parameters and eight questions, which were selected from the WLQ, developed in 2001. The WLQ consists of 25 items that are used to evaluate the physical and emotional limitations of employees and how these affect their ability to perform the job (14). The WLQ-SF takes 10-15 minutes to complete, and it uses two questions from four dimensions related to the on-the-job work demands: time management, physical demands, mental-interpersonal demands and output demands. The recall period is 2 weeks with response categories capturing the percentage of time an employee has for meeting the respective work demand. Response options include "all of the time (100%)", "most of the time", "some of the time (about 50%)", "a slight bit of the time," "none of the time (0%)" and "does not apply to my job". A six-point Likert scale (1-6) is used for each question, allowing workers to answer items. The two physical demand questions used a reversed scale; therefore, they were reversed scored. To calculate the percentage of time an employee was unable to meet his/her job demands, the responses were converted to percentages and the average was measured to obtain a score in the range of 0-100. Therefore, an index score of 0 represents an employee who is never unable to meet his/her job demands; whereas, a score of 100 represents an employee who is always unable to meet his/her job demands.

Translation and Coss-cultural Adaptation

The WLQ-SF was translated from English to Turkish according to the standard methodology recommended by Beaton et al. (20). In Stage 1, two independent translators whose native language was Turkish did the translation of the WLQ-SF. After the synthesis of the translated versions by two native speakers, the final version of the translation was developed in Stage 2. The final Turkish version of the questionnaire was translated back from Turkish into English again by two native English speakers who could speak Turkish fluently (Stage 3). In Stage 4, this version was compared with the original version to identify any inconsistencies. No inconsistencies were found between the Turkish and the original version. Lastly, the pre-final version of the assessment was piloted in academicians (n=20) to determine the clarity of all of the items and their compatibility for Turkish participants (Stage 5). The aim of cross-cultural adaptation was to make consistency in the construct validity between the original and translated versions of the assessment scale. The WLQ-SF was evaluated twice in 104 participants with a 7-day interval to assess the test–retest reliability.

Statistical Analysis

The Statistical Package for Social Sciences version 23.0 for Windows was used in the analysis of the data collected within the scope of the study. Statistical data were expressed as mean ± standard deviation (X ± SD), median or percentage (%). The one-sample Kolmogorov-Smirnov test was used to determine the suitability of the data for normal distribution.

Construct Validity

The validity of the WLQ-SF scale was analysed by descriptive factor analysis. To evaluate the suitability of the factor analysis model, the following properties were searched: chi-square score greater than 0.05, CMIN/DF value between 3 and 5, cognitive flexibility inventory (CFI) value greater than 0.9, Tucker-Lewis index (TLI) value greater than 0.9, and RMSEA value less than 0.08. In the absence of a model fit, construct validity was evaluated by explanatory factor analysis. The Kaiser-Meye-Olkin (KMO), Bartlett's sphericity ($p<0.05$) and Bartlett's chi-square values were analysed to assess the model fit through explanatory factor analysis.

Reliability

In our study, the test-retest reliability measured through Cronbach's alpha (α) and intraclass correlation coefficient (ICC) were used to assess the reliability of the scale over time. The acceptable value for the calculated coefficient with a Cronbach's alpha ranging from 0 to 1 is considered to be 0.80 and above for a previously developed scale, and 0.70 and above for a newly developed scale is interpreted as acceptable (21-23).

Results

Descriptive Statistics

In this study, 104 academicians completed the test and retest assessments. The mean age of the participants was 37.75±9.43 years (minimum =27 and maximum =64). Table 1 shows the demographic characteristics of participants.

Construct Validity

The descriptive factor analysis model used to evaluate the construct validity of the WLQ-SF was not found to be suitable (CFI =0.62, chi-square: 151.796 and $p<0.05$; CMIN/DF: 7.5; TLI: 0.5; RMSEA: 0.25). In light of these data, explanatory factor analysis was used to assess validity. The WLQ-SF provides construct validity with a variance of 68.62% under two factors (Bartlett's test of sphericity value, 407.830; KMO value, 0.746; $p=0.0001$). Questions 1, 2 and 5-8 were distributed as factor 1 (workload and concentration limitation) and questions 3 and 4 were distributed as factor 2 (physical limitations of the working environment) (Table 2). In addition, there are two factors in our study. The factor 1 covers six questions based on the workload and concentration limitation as a single factor. As the questions of the scale used in our study did not distribute too many factors, rotation was not performed.

Reliability

The Cronbach's alpha was 0.83 for the whole scale, indicating that the scale has high internal consistency. When questions were excluded, the Cronbach's alpha of the scale ranged from 0.70 to 0.83 (Table 3).

Repetition of the form is a reliability analysis that is used in cases where it is possible to reach the same sample again. In this study, the reliability of the WLQ-SF was evaluated using this method. The scale was directed to 104 participants in the first application and re-commissioned to 104 people in the second application. The data obtained from the two applications were tested using the Pearson correlation coefficient. The obtained data were found to be statistically significant (Table 3).

Table 1. Some of the descriptive characteristics and distribution of participants

Characteristics		N (%)
Sex	Female	76 (73.1)
	Male	28 (26.9)
Marital status	Married	82 (78.8)
	Single	15 (14.4)
	Divorced	7 (6.8)
Title	Research assistant (MSc)	25 (24)
	Research assistant (PhD)	18 (17.3)
	Assistant professor	20 (19.2)
	Associate professor	21 (20.3)
	Professor	20 (19.2)

Table 2. Factor analysis of the WLQ-SF

	Factor components	
	Workload and concentration limitation	Physical limitations of the working environment
Question 1	0.797	
Question 2	0.747	
Question 3		0.854
Question 4		0.826
Question 5	0.742	
Question 6	0.669	
Question 7	0.815	
Question 8	0.743	
Eigenvalue	3.709	1.781
Variance (%)	46.36	22.26

Discussion

The Turkish adaptation of the WLQ-SF, which examines the effects of physical and emotional health conditions on working lives, has been found valid and reliable. Many studies have shown that individuals working as academicians have high psychological and emotional fatigue. Therefore, burnout levels are higher than that among other occupational groups (10,24,25). To evaluate these emotional conditions, we used the WLQ-SF with academicians.

The WLQ-SF uses eight questions to examine four main areas: time management, physical demands, mental-interpersonal demands and output demands. The questions are based on two factors: workload and concentration limitation, and physical limitations of the working environment. Questions about workload and concentration limitation are mostly used to evaluate emotional and psychological effects on participants' workloads. Questions about physical limitations of the working environment assess the physical influences on participants. In our study, we examined the emotional and physical job limitations in our participants. The answers suggested that the emotional and physical limitations made it difficult for all of the participants to perform their job duties well. In other words, it was found that the emotional and physical difficulties that might affect the academicians' work were not very high and their average score in all fields was below 40%. The results of the studies performed in some occupational groups with chronic diseases using this test are similar to the results of our study, given the four main areas mentioned in the test (14,26-31).

To determine the construct validity of the WLQ-SF scale, firstly, we evaluated whether the data collected for the study were suitable for factor analysis and whether the sample size was sufficient. The value of KMO test was 0.746 and the result of Bartlett's test of sphericity was found to be statistically significant (<0.001). These results show that the study sample is sufficient for factor analysis (32). In the explanatory factor analysis, the WLQ-SF scale was found to be two-factor in accordance with the literature (30,31). The factor loadings of the scale ranged between 0.669 and 0.854. In the literature, although validity and reliability studies were conducted for the WLQ-SF scale in

Table 3. Item, subscale and scale descriptive and reliability measurements (N=104)

	Mean ± SD	α if item deleted	Item/scale correlation	Cronbach's α	Test-retest reliability (ICC)
WLQ-SF		-	-	0.83	0.960*
Question 1		0.802	0.652	-	
Question 2		0.708	0.603	-	
Time management	38.10±28.85	-	-	0.781	0.985*
Question 3		0.838	0.370	-	
Question 4		0.836	0.374	-	
Physical demands	33.78±15.75	-	-	0.866	0.986*
Question 5		0.808	0.615	-	
Question 6		0.816	0.543	-	
Mental-interpersonal demands	33.89±25.52	-	-	0.763	0.970*
Question 7		0.791	0.736	-	
Question 8		0.806	0.617	-	
Output demands	40.86±28.58	-	-	0.826	0.971*

*Pearson correlation coefficient, r<0.01, SD: Standard deviation

12 other languages, the factor contents of these studies could not be found (33). While evaluating the study of the original WLQ (25 questions) conducted in 2001, we found that the questions examining the four main areas had two-factor structure similar to our study. According to the results of our study, the WLQ-SF is a valid test for the examination of job limitations in employees as academicians.

Internal consistency of the WLQ-SF scale was evaluated with Cronbach's alpha in our study. Just as in the original English and subsequent translations, the WLQ-SF scale has excellent internal consistency in all areas (14,30). As a result of the statistical analysis, the coefficient was found to be 0.83 in this study. This result shows that the Turkish version of the WLQ-SF scale is highly reliable since the Cronbach's alpha is between 0.80 and 1.00.

In this study, Cronbach's alpha was 0.78 for time management, 0.86 for physical demands, 0.76 for mental-interpersonal demands and 0.82 for output demands, which are sub-domains of the WLQ-SF. Considering that the WLQ-SF examines work limitations in these four main areas, it is found that the test is quite reliable in all subheadings. In the original version of the test with 25 questions, the Cronbach's alpha of these four main areas was reported to be 0.88 and above (14). Compared to the results of this study, this difference may be due to the difference in the number of questions between the two tests; however, it may be that the sample group consisted of employees with a chronic disease.

In our study, test–retest results were evaluated with both Pearson correlation coefficient and ICC to test the reliability of the scale. The total and four basic test-retest results were assessed according to the ICC. The results of these analyses indicated that the total of test–retest results and the results of the WLQ-SF scale correlated perfectly between 0.960 and 0.986.

According to the validity and reliability study of the original WLQ (25 question) in its original language by Lerner et al. (34), the ICC was between 0.690 and 0.860. Tamminga et al. (29) showed that the test–retest results of the total and 4 based scores in the validity and reliability study of the Dutch version of the scale were between 0.65 and 0.74 (28). Verhoef et al. (28), in the Dutch version of the 25-question scale, found that the test-retest results based on ICC were between 0.83 and 0.93 (29). Walker et al. (30) in their study using the WLQ-SF found the test–retest reliability based on ICC was between 0.62 and 0.87. When similar studies in the literature using the WLQ were analysed, it was outlined that the results of this study were similar to the results in both the original language and other languages but were statistically more reliable. In this respect, the results of this study might contribute significantly to the literature.

The WLQ-SF is an important scale that may be preferred in studies regarding detection and prevention of job productivity loss, which is one of the important issues of occupational health and safety. The WLQ-SF is a scale that can be used in different occupations. Therefore, we suggest that future studies with the WLQ-SF in individuals with various professions may contribute to the literature.

Study Limitations

The study was conducted only on academicians, which is one of the limitations of our study. Another limitation is that most of the academicians included in this study were working in the faculty of health sciences. If academicians from other faculties were also included, the results could have been stronger or more generalisable.

Conclusion

The Turkish version of the WLQ-SF was found to be a valid and reliable test for evaluating the effect of physical and emotional health in academicians. The WLQ-SF is an important scale to measure the impact of both physical and emotional health on work. It is also more useful than other scales as it is short, understandable and practical. The WLQ-SF has a two-factor structure, which is in accordance with the literature. Since the factor loadings of the WLQ-SF are between 0.669 and 0.854, the scale was found to be valid. The internal consistency of the WLQ-SF was found to be high. The test-retest analysis indicated that the invariance of the scale over time was also high. This study is an important and major contribution to the literature as it provides evidence of the validity, reliability and cross-cultural adaptation of the Turkish version of the WLQ-SF in academicians. The WLQ-SF is a shorter and timesaving alternative to the long form version. Our study provides support for using the WLQ-SF when more comprehensive measures, such as the long form version, are not feasible. Further studies are needed to examine the validity and reliability of the WLQ-SF in different employee samples and work environments.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of University.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: R.Ş., S.Ö., M.N.İ., Design: R.Ş., S.Ö.,M.N.İ., Data Collection or Processing: R.Ş., Analysis or Interpretation: R.Ş., S.Ö.,M.N.İ., Literature Search: R.Ş., Writing: R.Ş

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Effect of Stapler Booster on Post-operative Air Leakage in the Surgical Treatment of Bullous Lung Disease

Büllöz Akciğer Hastalığının Cerrahi Tedavisinde Stapler Güçlendirici Kullanmanın Postoperatif Hava Kaçağı Üzerine Etkisi

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ABSTRACT

Objective: Prolonged air leakage is a major complication of operations for bullous lung disease. This study aimed to investigate the effect of using buttressed staple lines on post-operative air leakage in patients who underwent bullous lung disease (BLD) surgery by comparing the use and non-use of buttressed staple lines.

Methods: This single-institution study included patients who underwent thoracotomy because of BLD between 2007 and 2015. Patients were retrospectively reviewed for demographic variables, operation technique, buttressed staple line usage and type and post-operative air leakage duration.

Results: We analysed 49 patients [44 (89%) men], with a mean age of 59 years. Of patients, 97.9% (n=48) had wedge resection, and 2.1% (n=1) had lobectomy. In 44% (n=22), a buttressed staple line was used. Autografts (bulla wall) were used as buttressed staple lines in 54.5% (n=12) and synthetic polytetrafluoroethylene grafts in 45.5% (n=10) of cases. The mean post-operative air leakage duration was 7±3 days in the buttressed staple line group and 8±6 days in cases without booster. Prolonged air leakage was seen in 3 patients (27%) in the buttressed staple line group and 11 (25%) in the no booster group. There was no difference between the presence and absence of booster and between organic and synthetic graft usage.

ÖZ

Amaç: Uzamış hava kaçağı büllöz akciğer hastalığı (BAH) operasyonlarının majör komplikasyonlarından biridir. Bu çalışma büllöz akciğer hastalığı nedeniyle opere edilen hastalarda stapler güçlendirici kullanımının post operatif hava kaçağı üzerine olan etkisini araştırmak amacıyla planlandı.

Yöntemler: Tek merkezli yapılan bu çalışmada 2007-2015 yılları arasında BAH nedeniyle opere edilen olgular retrospektif olarak incelendi. Hastalar yaş, cinsiyet, komorbidite (kronik obstrüktif akciğer hastalığı, koroner arter hastalığı), sigara öyküsü, operasyon tekniği, stapler güçlendirici kullanımı ve çeşidi ile postoperatif hava kaçağının süresine bakılarak değerlendirildi.

Bulgular: Kırk dokuz hasta retrospektif olarak analiz edildi. Hastaların yaş ortalaması 59 idi (4879). Kayıtlı hastaların 44'ü erkekti (%89). Operasyonların %97'si (n=48) Wedge rezeksiyon ve %2'si (n=1) lobektomi idi. Operasyonların %44'ünde (n=22), stapler güçlendirici kullanılmıştı. Kullanılan stapler güçlendiricilerinin %54,5'i (n=12) otogreft (bül duvarı) ve %45,5'i (n=10) sentetik politetrafloroetilen greft idi. Stapler güçlendirici kullanılan hastalarda post operatif hava kaçağının ortalama süresi 7±3 gün (1-19 gün), kullanılmayanlarda ise 8±6 (1-25 gün) idi. Uzamış hava kaçağı stapler güçlendirici kullanılanlarda %27 (n=3), kullanılmayanlarda ise %25 (n=11) olarak saptandı. Ayrıca yapılan ikili karşılaştırmalarda eşlik eden kronik obstrüktif akciğer hastalığının hastanede kalış süresini uzattığı tespit edildi.

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Conclusion: We concluded that the use of buttressed staple lines (autograft or synthetic graft) did not create a statistically significant difference in post-operative air leakage duration when compared with patients without booster.

Keywords: Lung diseases, lung injury, lung volume measurements

Sonuç: Çalışmamızda büllöz akciğer hastalarının cerrahi tedavisinde stapler güçlendirici (otogreft veya sentetik greft) kullanımının postoperatif hava kaçağı süresi üzerine istatistiksel olarak anlamlı bir fark yaratmadığı sonucuna ulaşılmıştır.

Anahtar Sözcükler: Akciğer hasarı, akciğer hastalıkları, akciğer volum ölçümleri

Introduction

Emphysema, smoking and fibrotic diseases of the lungs are the most common etiologies of bullous lung disease (BLD) and are usually seen in men of the middle-age group (1,2). The most common complication of BLD is pneumothorax caused by the rupture of bullae into the pleural cavity (3). Wedge resection, bullectomy, bullae ligation and anatomic ligation should be performed for the surgical removal of bullous areas in the lung via thoracotomy, median sternotomy and video-assisted thoracoscopic surgery.

The most frequently preferred surgical approach for resecting the bullous area is wedge resection, which should be achieved with a buttressed or non-buttressed stapler device. Prolonged air leakage is a major complication of these surgical procedures (4-6). The use of bullae wall, bovine pericardium, polytetrafluoroethylene (PTFE) as a buttressed line and the resected lung is supposed to decrease air leakage and hospitalisation duration after wedge resection; however, the benefit of creating a buttressed line for preventing post-operative air leak is still controversial (7,8). This study aimed to investigate the effect of using bullae wall as autograft and PTFE as a buttress on air leakage and hospitalisation duration for lung volume reduction surgery (LVRS) in cases with BLD and to compare the outcomes of these buttressed ones between each other and with a non-buttressed staple line.

Methods

This single-institution study included patients who underwent thoracotomy for BLD between 2007 and 2015. We retrospectively analysed 62 patients' data, and 49 of them were included in the study. The study received approval from the scientific and ethical study board of the hospital (Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital 08/05/2015 - 5918), and informed consent was obtained from the patients according to guidelines of the Helsinki Declaration.

All operations were performed via thoracotomy. Age, gender, chronic obstructive pulmonary disease (COPD) and coronary artery disease (CAD) existence, smoking history, operation type, operation technique, post-operative air leakage duration, hospitalisation duration and other complications were documented. The use of buttressed lines during the operations was grouped as none (Group I) or used (Group II). Then, Group II was further subdivided as follows: bullae wall/autograft (Group IIa) and PTFE (Group IIb). The effects of using bullae wall and PTFE as a buttress on air leakage and hospitalisation duration for surgical treatment of BLD were investigated. The outcomes of Groups I and II were compared, and a subgroup analysis was applied for Group II.

For statistical analyses, the Number Cruncher Statistical System 2007 (Kaysville, Utah, USA) software was used. To evaluate the study data, descriptive statistical methods (mean, standard deviation, median, frequency, minimum and maximum) were used. Mann-Whitney U test was used in the two-group comparison of parameters without normal distribution. To compare qualitative data, Fisher's exact test and Continuity Correction test (Yates corrected chi-square) were used. Significance was evaluated as $p<0.05$.

Results

The mean age of the patients was 59 (range; 48-79) years, and 44 (89.9%) out of 49 were men. Of patients, 31 (63%) had a smoking history, 25 (51%) had COPD and 12 (24%) had CAD. Right-sided thoracotomy was performed in 33 (67.3%) patients. Of patients, 48 (97.9%) had wedge resection and 1 (2.1%) lobectomy procedure. Stapler booster was used in 22 (44%) patients: 6 (27.2%) bullae wall and 16 (72.7%) PTFE graft (Table 1). Post-operative complications were reported in 19 (38.7%) patients. The most frequent complication was prolonged air leakage and detected in 17 (34.6%) patients. The mean post-operative air leakage durations were 8±5 (range; 1-25) days for all groups, 8±6 days for Group I, 7±3 days for Group IIa and 7±3 days for Group IIb.

Prolonged air leakage was noted in 33.3% (n=9) of cases in Group I and 31.8% (n=7) in Group II. There was no significant difference between Groups I and II (p=0.843) and between Groups IIa and IIb (p=0.843; Table 2).

The mean hospitalisation duration was 10±7 (range; 3-40) days in the dual comparisons performed, and hospital stay duration was not different between the groups. Only the presence of COPD as concomitant comorbidity was found to increase hospitalisation duration significantly (p=0.007; Table 3).

Discussion

Air leakage is a major complication seen in those who are operated because of BLD (6), with a prevalence of 37% in the literature (9), which is a score high enough to be considered. Therefore, we evaluated the effect of buttressed staple lines on post-operative air leakage.

Study Limitations

Our study found that the use of buttressed (autograft or PTFE) staple lines in the surgical treatment of BLD did not affect post-operative air leakage and hospitalisation duration. We also reported that the presence of COPD increases hospitalisation duration.

In the National Emphysema Treatment Trial (NETT), there was no difference between cases in which a buttressed line was used or not in LVRS for post-operative prolonged air leakage and hospitalisation duration. The effect of using a buttressed staple line on prolonged air leakage was also investigated in the NETT in which 580 patients underwent LVRS (8). NETT investigators recorded the materials used for staple line buttressing as bovine pericardium and PTFE, and no buttressing materials were used in less than 5% of patients. They found no difference between patients with and without supported stapler lines for post-operative air leakage. In this study, it was stated that air leak was accompanied by LVRS in 90% of patients and often caused long hospitalisation duration. In contrast, other studies have shown that a supporting stapler line considerably reduces the length of post-operative air leakage, but this has not reduced hospitalisation duration. It was argued that the occurrence and duration of air leaks are dependent on the characteristics of the disease of patients and are not for specific surgical techniques. In contrast, there are studies showing that a supporting stapler line considerably reduces the length of post-operative air leakage, but this has not reduced hospitalisation duration (7,8).

There are many comparative studies on the effects of using a buttressed staple line on post-operative air leaks (10,11). Stammberger et al.(11) examined 65 patients who underwent LVRS and found that buttressing the staple line with bovine pericardium significantly shortened air leak duration and drainage time (12).

Drahush et al. (13) developed a standardised approach for air leak reduction (STAR) after lung resection. The following three independent factors were reported to reduce air leaks in this study: fissureless operative technique, staple line buttressing and protocol-driven chest tube management. A remarkable decrease in post-operative prolonged air leak was recorded in 475 patients using STAR for pulmonary resection.

Conclusion

In the literature, the effect of using a buttressed staple line

Table 1. Localisation, buttressed staple line types and operation methods for all cases

		n	%
Localisation (n=49)	**Right**	33	67.3
	Left	16	32.7
Buttressed staple line type (n=22)	**Bullae wall/ autograft**	6	27.2
	PTFE	16	72.8
Operation (n=49)	**Wedge**	48	97.9
	Lobectomy	1	2.1

PTFE: Polytetrafluoroethylene

Table 2. The effect of using a buttressed staple line and its type on air leakage duration

		Air leakage duration (days)		p
Min - max			Mean ± SD	
Buttressed staple line	**No (n=27)**	1-25	8±6	0.843
	Yes (n=22)	1-19	7±3	
Auto graft	**Yes (n=6)**	1-19	7±3	0.843
PTFE graft	**Yes (n=16)**	1-19	7±3	

PTFE: Polytetrafluoroethylene, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 3. The effect of concomitant CAD and presence of COPD on hospitalisation duration

		Hospitalisation duration (days)		p
Median			Mean ± SD	
CAD	**Yes (n=12)**	5-32 (7)	11±9.1	0.930
COPD	**Yes (n=25)**	7-40 (24)	23.2±148	0.007*

CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, SD: Standard deviation

on post-operative air leakage and hospitalisation duration is controversial. Our study showed that the use of a buttressed staple line (autograft or synthetic graft) did not achieve a significant difference in post-operative air leakage duration when compared with patients without booster. Randomised prospective studies with more patients are needed.

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Ethics

Ethics Committee Approval: Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital 08/05/2015- 5918.

Informed Consent: Informed consent was obtained from the patients according to guidelines of the Helsinki Declaration.

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Surgical and Medical Practices: A.G.B., Ö.G.İ., Concept: A.G.B., C.K., T.O., Design: A.G.B., Ö.G.İ., C.K., T.O., Data Collection or Processing: A.G.B., Ö.G.İ., Analysis or Interpretation: A.G.B., C.K., T.O., Literature Search: A.G.B., Ö.G.İ., Writing: A.G.B., Ö.G.İ., C.K., T.O.

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Original Article

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Generation of Bone Tissue Using Adipose Tissue-derived Stem Cells

Adipoz Kökenli Kök Hücre Kullanımıyla Kemik Doku Üretimi

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ABSTRACT

Objective: Bone grafts and even bone substitutes do not meet all of the requirements of bony reconstructions. The aim of this study was to generate bone tissue from autologous adipose tissue-derived mesenchymal stem cells (ATDMSCs) and decellularised bone allografts.

Methods: A 1.5 cm bone defect developed in the middle third of the rabbit's ulna. Reconstructions were carried out using miniplate and screws and interpositional autogenous bone grafts according to the designs of the groups: (1) No touch, (2) cryopreserved, (3) decellularised and (4) ATDMSCs-implanted decellularised bones. Before implantation, ATDMSCs in the last group were labelled with Q-dot and identified microscopically.

Results: Graft recovery and irregular callus formation were observed in the first, second and forth groups. In the first group, the organisation of Haversian systems, the structure of the lacunae and the presence of canaliculi ossiums were observed; in the second group, approximately 40% of the Haversian canals contained blood vessels, and canaliculi ossiums in the form of thin filaments were found in 90% of the microscopically examined areas; in the third group, most Haversian canals were empty, most osteocyte canals were devoid of cells, and canaliculi ossiums were absent; in the fourth

ÖZ

Amaç: Kemik doku onarımda altın standart olan otojen kemik greftleri her zaman tüm gereksinimleri karşılayamamaktadır. Bu çalışmanın amacı, otojen yağ doku kaynaklı mezenkimal kök hücrelerin (ATDMSC) ve hücresizleştirilmiş kemik allo greftlerinin kombinasyonunu kullanarak kemik dokusu üretmektir.

Yöntemler: Tavşan ulnasının 1/3 orta segmentinde oluşturulan 1,5 cm'lik bir kemik defektine, grup tasarımlarına göre, gruplar; 1: kontrol; 2: krio ile muamele edilmiş kemik grefti; 3: hücresizleştirilmiş kemik grefti; 4: ATDMSC implante edilmiş hücresizleştirilmiş kemik grefti olacak şekilde mini plak ve vida ile osteosentez yapıldı.

Bulgular: Birinci, ikinci ve dördüncü grupta greft iyileşmesi ve düzensiz kallus oluşumu gözlendi. Birinci grupta, haversian sistemlerinin organizasyonu, lakunların yapısı ve canaliculi ossiumların varlığı gözlendi; İkinci grupta, havers kanallarının yaklaşık %40'ı kan damarları içeriyordu ve mikroskobik olarak incelenen alanların %90'ında ince filamentler şeklinde kanalikül ossiumları vardı; Üçüncü grupta, havers kanallarının çoğu boştu, osteosid kanallarının çoğu hücre içermiyordu ve orada kanaliküller ossiumu yoktu; dördüncü grupta, havers kanallarının bir kısmı kan damarları içermekteydi ve hücresizleştirmeye bağlı olarak

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group, some of the Haversian canals contained blood vessels, and there were partly lacunae containing cells due to decellularisation, whereas in approximately 50% of the examined microscope areas, the presence of canaliculi ossiums with evidence of mesenchymal stem cells differentiated into osteocytes was demonstrated by Q-dot traced cells.

Conclusion: In this study, the establishment of a proper niche environment for adipose-derived mesenchymal cells promotes their development into osteogenic cells.

Keywords: Tissue, stem cell, adipose, mesenchyme, regeneration

hücreleri içeren kısmen lakazlar mevcutken, incelenen mikroskop alanlarının yaklaşık %50'sinde, osteositlere farklılaşan mezenkimal kök hücrelerin varlığı ile canaliculi ossium varlığı moleküler testler ile kanıtlandı.

Sonuç: ATDMSC ile uygun bir niş ortamının oluşturulması halinde kemik greftinin yaşayabilirliğinde artış sağlandı ve bunun ATDMSC'lerin osteojenik hücrelere dönüşüm yoluyla olduğu gösterildi.

Anahtar Sözcükler: Doku, kök hücre, yağ, mezenkimal, rejenerasyon

Introduction

Autogenous bone grafting is the gold standard in the repair of bony defects. Infection, nonunion, the need for prolonged immobilisation and recovery, donor area shortage and morbidity and the difficulty of three-dimensional shaping, in addition to suboptimal results, continue to be challenging problems (1-3).

Allografts have advantages such as the lack of donor area morbidity, successful long-term results than autogenous bone grafts and a shorter operation period. However, allografts have disadvantages such as potential rejection, the risk of infectious disease transmission, the need for a high-tech infrastructure and the associated high costs (4,5).

Tissue engineering studies and the use of stem cells to enhance viability, osseointegration and ossification via cell migration into artificial and/or alloplastic demineralised bone matrices or decellularised tissues are promising (6-10).

The aim of our study was to regenerate bone tissue using cultured adipose tissue-derived mesenchymal stem cells (ATDMSCs) integrated in a decellularised bone matrix.

Methods

The present study was approved by the Local Institutional Animal Research Ethics Committee. Moreover, 24 rabbits weighing 3,500-4,000 g were used. The procedures were carried out under general anaesthesia. Fat tissue samples were taken from the inguinal region of an animal that was not used in the study due to the animals being from an inbred animal pool. Furthermore, Q-tracker cell labelling kits were used to culture, characterise and label ATDMSCs. Bone grafts were decellularised and then were incubated with ATDMSCs.

ATDMSCs' Culture

The fat tissue was washed in foetal bovine serum (FBS). The washing procedure was repeated until all blood and connective tissue particles were removed and minced into small pieces. By centrifuging the resultant digested material at 800 g, adipocytes and free oil were separated from the stromovascular components. The cells were suspended and plated into 150-cm^2 culture flasks in Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM-LG) (SH3002101; HyClone) containing 10% FBS (SV3016003; HyClone) and 1% penicillin. The culture medium was changed every 3 days, and the cells were grown to 70% confluence.

Passage 1: When the cells reached 70% confluence, the medium was removed from the flasks. The flasks were then filled with 10 mL of trypsin/ethylenediaminetetraacetic acid solution and incubated in an incubator for 5 min. After trypsin reaction was neutralised with 1 mL of FBS, the cells were collected in a tube and centrifuged with FBS at 400 g. The cells were resuspended in fresh complete medium before being seeded in flasks.

Passage 2: When the cells reached 70% confluence, the medium was removed from the flasks. The cells were then trypsinised and resuspended in fresh complete medium. Following that, a 1 mL sample was taken for analysis.

Characterisation of Mesenchymal Stem Cells

After passage 2, the cells were incubated for 45 min at room temperature with 1 μg of R-phycoerythrin (PE)-, fluorescein isothiocyanate and allophycocyanin -conjugated antibodies and isotype-matched control immunoglobulin Gs. After incubation and washing, the samples were analysed for mesenchymal stem cells with CD45, HLA-DR, CD34, CD90, CD105 and CD73 antibodies using a flow cytometer (FACSCalibur®).

Cell Labelling

Stem cells were labelled using Q-tracker cell labelling kits (Invitrogen®), which are designed for loading cells grown in culture with highly fluorescent Q-dot nanocrystals. Moreover, Q-dot nanocrystals target the cytoplasm of living cells. Components A and B were pre-mixed in a tube and incubated at room temperature for 5 min. The tube was filled with fresh complete medium and centrifuged for 30 s. After that, the cells were added and incubated at 37 °C for 45-60 min before washing twice with complete growth medium. Moreover, flow cytometry was used to visualise labelled live cells using appropriate filters.

Flow Cytometry Analysis of Labelled ATDMSCs

Flow cytometry was used to analyse labelled and unlabelled cells using the appropriate filter (FL2) for red emission. Furthermore, 98.7% of the ATDMSCs were labelled, while 0% were not.

Bone Decellularisation

The bone graft, kept at 2 °C-8 °C, was washed in PBS containing 1% povidone iodine. In order to remove the povidone iodine, it was washed twice with PBS. The washed graft was decellularised for 24 hours in 1% SDS and 1% Triton X-100. The graft was cleared of SDS and Triton X-100 after being washed in a physiological saline solution containing antibiotics and DNase for 24 hours. Further, the graft was kept at 2 °C-8 °C in a container fluid with 1% antibiotics until it was surgically adapted or loaded with cells.

Preparation of Biobone

ATDMSCs were cultured on the outside and inside of the bone graft and were grown in a low glucose DMEM containing 10% PBS and 1% antibiotics. After medium and cell replenishment on the third day of culture and after its addition to the bone graft containing ATDMSCs and bone meal on the fifth day, it was cultured for another day before implantation.

Groups

Groups were formed as (n=6) (1) no touch, (2) cryopreserved, (3) decellularised and (4) ATDMSCs-implanted decellularised bones used as grafts.

Surgical Procedures

After cleaning, the rabbit was placed in a supine position, and both forearms were shaved up to the proximal of the elbow and covered with a sterile polyvinylpyrrolidone iodine (polyvidon-iodine) 10% (Batticon®, Adeka İlaç Sanayi ve Ticaret A.Ş., TR) solution. Skin flaps were elevated after a 5 cm incision was performed on the dorsal side of the right forearm. By dissecting the forearm muscles, the ulna was exposed through the subperiosteal plane. Then, at the middle, one-third of the ulna was resected to form a full-thickness defect.

The first group consisted of autografts (Figure 1). In the second group, the resected fragments were stored at -80 °C for 24 hours before being preserved as a bone graft. In the third group, the defect was reconstructed using decellularised bone grafts prepared from the other leg of the rabbit. In the fourth group, the defect was again reconstructed with a decellularised graft and implanted with previously cultured adipose tissue-derived mesenchymal stem cells prepared from the other leg. In all groups, the prepared bone grafts were secured with six-hole plate and four screws. After wound was closed, a splint was placed on the forearm.

Before the rabbits were sacrificed at the end of the sixth week of study, all groups were subjected to a perfusion test with India ink.

Evaluation

Following decalcification, specimens were stained with hematoxylin and eosin (H&E) for a general morphological evaluation. The second series of sections taken for histomorphological evaluations, on the other hand, were stained with Schmorl's picro-thionin stain. The stained sections were examined with an Olympus BX51 photomicroscope and photographed with an Olympus DP72 camera. For histopathological scoring, bone tissues in each section were examined with x400 zoom in at least three similar areas. A semiquantitative scoring was carried out: (1) the number of empty osteocyte lacunae, (2) the presence of stained canaliculi ossium in each area by dividing the microscope area in four, and (3) the number of empty Haversian canals were evaluated. Furthermore, two experienced histologists performed the histopathological scoring blindly.

Statistical Analysis

An ANOVA test was used to compare more than two groups, taking into account the number of samples and the distribution of data within the groups. In addition, the post hoc Tukey test was used to compare the groups to one another. In the measurements that were taken, there is a 95% confidence interval. For the analysis, the MedCalc statistical software (MedCalc Software, Acacialaan 22, B-8400 Ostend, Belgium) was used, and $p<0.05$ was considered statistically significant.

Results

During the macroscopic examination, graft recovery and irregular callus formation on the plate-screws were observed in all groups. In the third group, graft recovery is lower than in others.

The Results of Bone Staining with India Ink Injection

According to the flow cytometry analysis of labelled ATDMSCs for red emission, 98.7% were labelled.

All grafts from the first group were stained. Moreover, four of the six grafts from the second and fourth groups, as well as three of the six grafts from the third group, were stained.

Microscopic Analysis

In the first group, osteocytes in regular morphology, canaliculi ossium and Haversian canals together with Haversian systems were observed (Figure 2A); in the second group, Haversian systems similar to the first group were observed, but there was

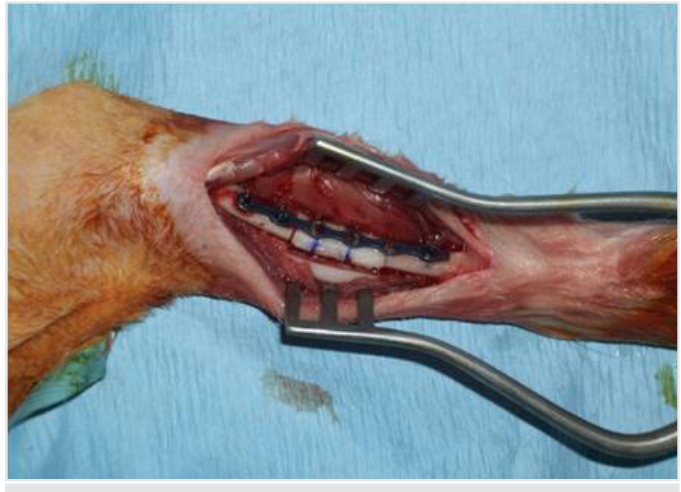


Figure 1. Interpositional bone graft fixation with plate and screws and some polypropylene sutures

an increase in the presence of empty osteocyte lacunae (Figure 2C). In the third group, most of the osteocyte lacunae within the structure of Haversian systems were empty, with only a few lacunae filled with osteocyte (Figure 2E); in the fourth group, the osteocyte lacunae within the structure of Haversian systems were empty, but the number of lacunae filled with cells was increased (Figure 2G).

Schmorl's picro-thionin stained specimens in all groups, lacunae containing osteocyte were observed in fusiform and in brown, canaliculi ossium were observed as thin brown filaments between the lacunae, and the blood vessels within the Haversian canals were observed in various shades of brown.

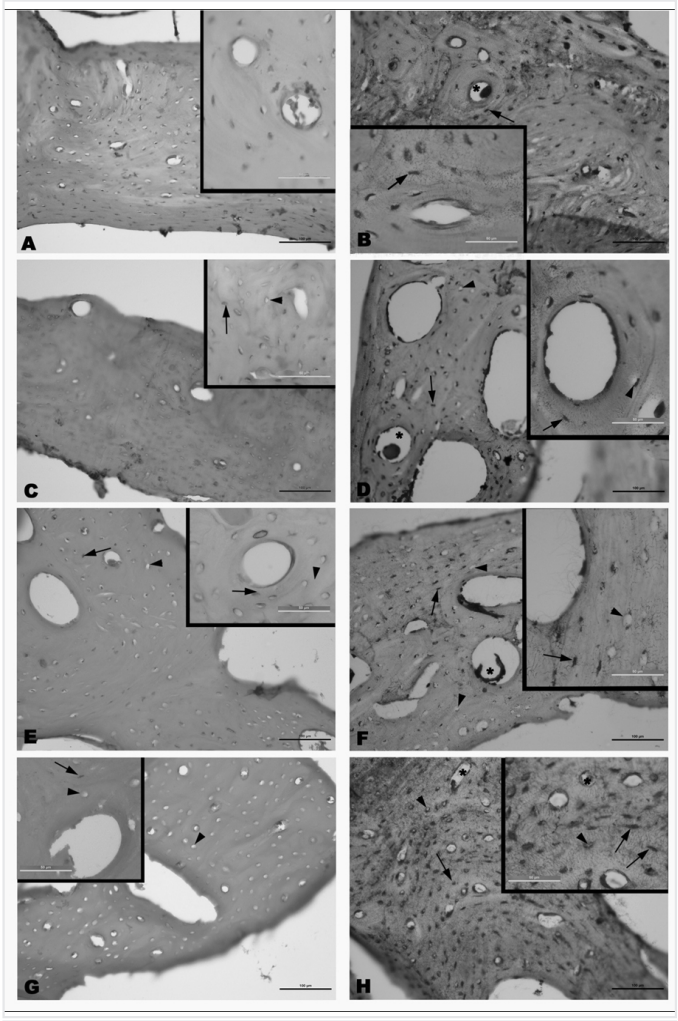


Figure 2. On H&E and Schmorl's picro-thionin stained specimens: 2A, regular osteocytes, canaliculi ossium with Haversian systems; 2C, an increase in the presence of empty osteocyte lacunae; 2E, most of the osteocyte lacunae were empty; 2G, osteocyte lacunae were empty; 2 B, Haversian systems, the lacunae and the presence of canaliculi ossium; 2D, near 40% of the Haversian canals contained blood vessels; 2F, the Haversian canals were empty, the osteocyte canals did not contain any cells and no canaliculi ossium; 2G, the Haversian canals contained blood vessels, empty osteocyte lacunae

In the bone tissue samples from the first group, the organisation of Haversian systems, the structure of the lacunae and the presence of canaliculi ossium were all observed to be in normal morphology (Figure 2B); in the second group, approximately 40% of the Haversian canals contained blood vessels, the number of osteocyte canals observed to be empty increased in comparison to the control group, and canaliculi ossium in the form of thin filaments were found in 90% of microscope areas (Figure 2D); in the third group, most of the Haversian canals were empty, most of the osteocyte canals did not contain any cells, and there were no canaliculi ossium in most of the microscope areas (Figure 2F); in the fourth group, it was discovered that some of the Haversian canals contained blood vessels, that the number of osteocyte lacunae observed to be empty was high once more, but that there were partly lacunae containing cells. In approximately 50% of the examined microscope areas, canaliculi ossium was observed (Figure 2H).

ATDMSCs labelled with Q-dot were identified microscopically in histologic samples from the fourth group. These microscopic views were compared to histologic samples from the third group that had not been marked with Q-dot and others (Figures 3).

Statistical Results

Tukey test was used to compare each of the groups. Moreover, Tukey test shows that the fullness of lacunae in groups 1 and 3-4 is significantly different ($p<0.05$). The fullness of lacunae also differs significantly ($p<0.05$) between groups 2 and 4. The fullness of canaliculi differed significantly between groups 1 and 3-4. The fullness of canaliculi in group 4 is 47% and in group 3

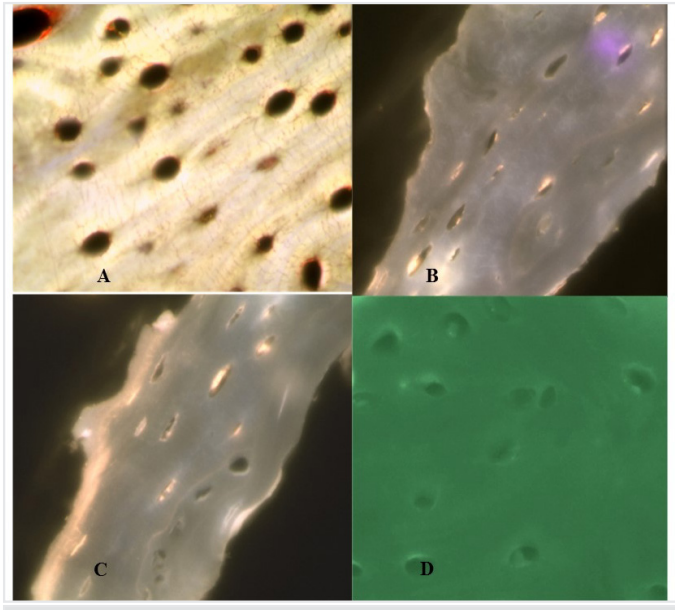


Figure 3. ATDMSCs marked with Q-dot were microscopically identified in histologic samples of fourth group. 3A, Preimplant view of Q-dot nanocrystals; 3B-C, postimplant view of Q-dot nanocrystals, showing the cytoplasm of living cells; 3D, postimplant view of the third group, with no Q-dot nanocrystals

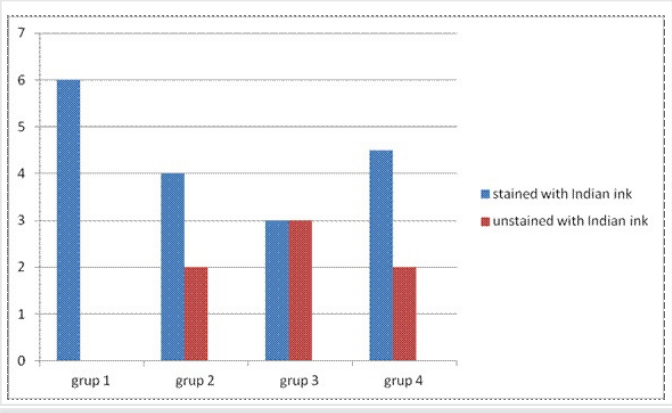
is 20%, but there is no statistically significant difference (p>0.05) (Graphs 1 and 2).

Discussion

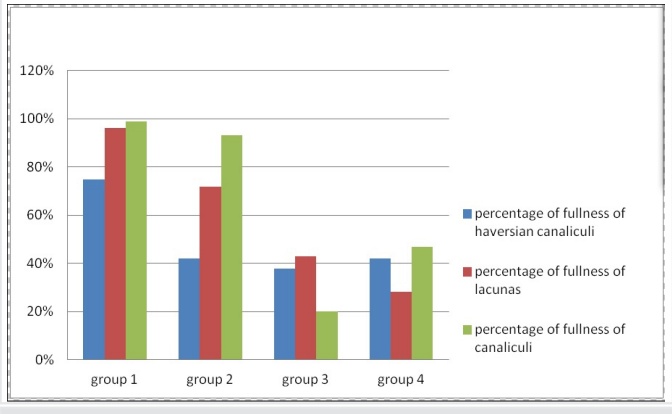
The bone graft serves as a template on the recipient area on for recovery (osteoconduction) and leads to the formation of a new bone tissue by inducing osteoprogenitor cells in the host tissue (osteoinduction). It takes at least 3 weeks to start forming new bone tissue (4-6).

Although autogenous bone grafts are the gold standard, they have some disadvantages, including limited availability, the creation of a new operative field, prolonged operative time, donor area morbidity, additional cost, resorption risk and difficulty in shaping (1-3).

Allografts, which come in cortical, cancellous and demineralised bone matrix forms, have advantages such as not causing donor area morbidity, having successful long-term results when compared to autogenous bone grafts and requiring no additional operative time. However, allografts also have disadvantages such as the risk of rejection, the risk of infectious disease contagion, the risk of resorption and the need for a high-tech infrastructure with correspondingly high costs (4,5).



Graphic 1. The number of the grafts that were stained or unstained with Indian ink



Graphic 2. The percentage of fullness of Haversian canaliculi, fullness of lacunae, and fullness of canaliculi

Alternative bone grafts include synthetic ceramics, silicate-based materials and polymers, which do not cause donor area morbidity and do not pose a risk of disease transmission. Hydroxyapatite made of synthetic ceramics, bioactive glass made of silicate-based materials and polymethyl methacrylate made of polymers are materials that are preferred in cosmetic and reconstructive facial surgery (3-5). However, besides their costs, these substances cannot provide adequate stability for the repair of defects in big and load-bearing bones due to their fragile structure, so their use is limited to the craniofacial zone (4,6).

Adult stem cells, such as ATDMSCs and bone marrow-derived stem cells (BMDSCs), hold tremendous promise in regenerative medicine (10). Traditional treatments for degenerative skeletal defects and wounds include allograft, autograft and synthetic implant applications, but these are inadequate for some defects (11). However, complications of these techniques include donor area morbidity, inadequate tissue provision, the emergence of immunogenicity and implant loss (12-14). The use of autologous ATDMSCs in the treatment of tissue replacements reduces the immune response and provides more sources than BMDSCs. In order to form a tissue engineering structure specific to the tissue, two important components must be present: (1) tissue-specific cells and (2) a biocompatible, mechanically suitable tissue scaffold to which the cells can stick and form an extracellular matrix have been used by researchers in the treatment of tendon, cartilage and bone repair (15-19), which encourages regeneration in the defect area. The cells cultivated scaffolds as a result of tissue engineering. As the use of ATDMSCs and other stem cell types in tissue engineering studies increases, so do studies on improving the tissue scaffold medium. In order to discuss the use of ATDMSCs in the formation of bone tissue-equivalent tissue, it is important to know the differences between BMDSC and ATDMSCs.

Most ATDMSC tissue engineering studies up to this point have been basically derived from BMDSCs studies, so excluding BMDSCs would be a huge mistake. Until now, the majority of stem cell studies have relied on BMDSCs (20). However, because the source of these cells is limited, their extensive commercial use is limited, and the studies focus on ATDMSCs. In contrast to bone marrow, fat tissue is more plentiful and easily accessible. ATDMSCs and BMDSCs have comparable cell yield, cell expansion, growth kinetics and differentiation (21).

Obtaining a large number of ATDMSCs and a high cell yield is much easier than isolating BMDSCs. While some studies show that ATDMSCs, like BMDSCs, undergo osteogenic, myogenic, adipogenic and chondrogenic transformations, the results of other studies have led to increased discussions about the differentiation potential of ATDMSCs (21-25). While some researchers claim that there is no difference in the differentiation of ATDMSCs and BMDSCs (26), others have reported that ATDMSC differentiation is inadequate in comparison to BMDSC differentiation (27-31). Aust et al.

(21) compared the differentiation potential of BMDSCs to that of ATDMSCs and found no difference in their ability for chemically induced differentiation. Similarly, Hattori et al. (24) found that BMDSCs and ATDMSCs cultured in osteogenic medium produce similar results in terms of calcium phosphate accumulation and osteocalcin release. According to Im et al. (29) and Mehlhorn et al. (27), these two cell types do not have the same ability for osteogenic and chondrogenic differentiation. After 2 and 3 weeks of tracking, Im et al. (29) revealed that BMDSCs are clearly superior to ATDMSCs in terms of mineralisation and alkaline phosphatase activity and have similar results in chondrogenic markers. While drawing attention to the concerns that ATDMSCs have lower stem cell potential than BMDSCs in different studies, it is important to note that there is no agreement on the data. Considering their differences in surface markers that they express, medium requirements for differentiation (31) and genes that they are influenced by during differentiation, ATDMSCs are not less potent than BMDSCs, but they behave differently. For recent specific cell populations, there are obvious differences in gene expression profiles between ATDMSCs and BMDSCs (27). Despite evidence supporting the natural differences between ATDMSCs and BMDSCs, there is a need for further studies on cell behaviours before they can be used effectively in tissue engineering practices.

The reason for the overall superior results in the bone autograft group compared to the allograft ATDMSC group could be due to the decellularisation method, which might have damaged the ultrastructure of the bone scaffold, impairing the optimal environment for osteogenesis (32,33).

Study Limitations

In the examination of the samples from the third group, where reconstructed bones were applied as graft after decellularisation, and from the fourth group, where bones were similarly applied as graft with ATDMSC cultivation after reconstruction and decellularisation, the fact that the fourth group showed a more positive result in terms of fullness of canaliculus can be interpreted as a positive effect on recovery caused by stem cells through regeneration.

The presence of Q-dot stained cells in the lacunae in the specimens of group four strongly suggests that these cells have been developed from the mesenchymal stem cells that were loaded on the decellularised bone grafts. This finding indicates the ability of the ATDMSCs to develop into osteogenic cells that can promote tissue regeneration and perhaps may serve as the foundation for future bone tissue engineering studies.

Conclusion

In an optimal stem cell niche environment, ATDMSCs have a high potential for osteogenic tissue formation. Now that the osteogenic effect of stem cells has been proven, we believe that focusing studies in bone tissue engineering on tissue scaffolds as well as bone decellularisation methods will yield more positive results in clinical practices.

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Ethics

Ethics Committee Approval: The present study was approved by the Local Institutional Animal Research Ethics Committee.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: E.G., E.G.B., M.V.K., H.İ.C., K.Ö., E.O., M.A.Ö., K.Y., F.E., Design: E.G., E.G.B., M.V.K., H.İ.C., K.Ö., E.O., M.A.Ö., K.Y., F.E., Data Collection or Processing: E.G., E.G.B., M.V.K., H.İ.C., K.Ö., E.O., M.A.Ö., K.Y., F.E., Analysis or Interpretation: E.G., E.G.B., M.V.K., H.İ.C., K.Ö., E.O., M.A.Ö., K.Y., F.E., Literature Search: E.G., E.G.B., M.V.K., H.İ.C., K.Ö., E.O., M.A.Ö., K.Y., F.E., Writing: E.G., E.G.B., M.V.K., H.İ.C., K.Ö., E.O., M.A.Ö., K.Y., F.E.

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Original Article

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Evaluation of Factors Related to Pancreatic Fistula Development in Patients Undergoing Pancreaticoduodenectomy for Periampullary Tumours

Periampullar Tümör Nedeniyle Pankreatikoduodenektomi Uygulanan Hastalarda Pankreatik Fistülle İlişkili Faktörlerin Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to investigate the factors associated with pancreatic fistula (PF) development after pancreaticoduodenectomy (PD) at our clinic.

Methods: Patients who underwent PD for periampullary tumours between 2010 and 2019 were included in the study and categorised into Group I (with PF) and Group II (without PF). The demographic and clinical characteristics, laboratory parameters, tumour characteristics and post-operative results were compared between the groups. Risk factors for PF were analysed by univariate analysis and multivariate logistic regression analysis.

Results: In total, 155 patients participated in the study (Group I: n=31; Group II: n=124). The rate of PF was 20%. The two groups were comparable with regard to sex (p=0.348) and age (64.8 vs 66.9 years, p=0.916). Compared with Group II, Group I had a higher number of metastatic lymph nodes (1.61 vs 0.87, p=0.041), a higher number of post-operative complications (58.1% vs 21.8%, p=0.000) and a longer duration of post-operative hospital stay (25.25 vs 16.43 days, p=0.000). Haemoglobin (p=0.493) and albumin (p=0.698) levels were similar between the groups. Total survival duration was shorter in Group I (23.9 vs 38.18 months, p=0.024). In multivariate analyses, being ≥65 years (p=0.040), tumour localisation (p=0.021), lymph node stage (p=0.008) and

ÖZ

Amaç: Bu çalışmada kliniğimizde pankreatikoduodenektomi (PD) sonrası gelişen pankreatik fistül (PF) ile ilişkili faktörleri araştırmayı amaçladık.

Yöntemler: 2010-2019 yılları arasında periampullar bölge tümörü nedeniyle PD, uygulanan hastalar çalışmaya dahil edili. Grup 1 (PF var) ve Grup 2 (PF yok) olmak üzere iki grup oluşturuldu. Gruplarda hastaların demografik ve klinik özellikleri, labrotuvar parametreleri, tümöre ait özellikler, postoperatif sonuçlar ortalama sağkalımları karşılaştırıldı. Pankreas fistülü (PF) için risk faktörleri tek değişkenli analiz ve çok değişkenli lojistik regresyon analizi ile analiz edildi.

Bulgular: Çalışmamıza 155 hasta katıldı. PF oranımız %20 olarak bulduk. Buna göre Grup 1: 31 Grup 2: 124 hastadan oluşuyordu. Gruplarda cinsiyet benzer özellikteydi (p=0,348). Gruplarda yaşlar benzer (64,8; 66,9, p=0,916). Metastastik lenf nodu sayısı Grup 1'de 2'ye oranla daha yüksek (1,61; 0,87, p=0,041) Post-operatif komplikayon Grup 1'de yüksek (%58,1; %21,8 p=0,000). Postoperatif yatış süresi Grup 1'de daha uzundu (25,25 vs 16,43 gün p=0,000). Gruplarda hemoglobin p=0,492, albumin p=0,698 benzer. Toplam sağkalım süresi Grup 1'de daha kısa (23,9 ay; 38,18 ay p=0,024). Çok değişkenli analizlerde >65 yaş p=0,040, Tümör

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tumour diameter ≥2 (p=0.021) were the independent risk factors for developing pancreatic fistula.

Conclusion: In our study, tumour diameter, patient age and lymph node status were associated with PF development. The development of PF reduced expected survival. We believe that identifying the pre-operative, intraoperative and post-operative factors related to PF formation may help decrease its development.

Keywords: Pancreatic fistula, pancreaticoduodenectomy, prognosis

lokalizasyon p=0,021 lenf nodu evresi p=0,008. Tümör çapının >2 p=0,021 pankreatik fistül için bağımsız risk faktörüydü.

Sonuç: Çalışmamızda tümör çapı hasta yaşı ve lenf nodu durumu PF gelişmesi ile ilişkiliydi. PF gelişimi beklenen sağ kalımı azaltmıştı. Preoperatif, intraoperatif ve postoperatif dönemdeki etkenlerin ortaya konulması ile PF oluşumunun azalabileceğini düşünmekteyiz.

Anahtar Sözcükler: Pankreatik fistül, pankreotikoduodenektomi, prognoz

Introduction

Pancreaticoduodenectomy (PD) is currently the standard method for the treatment of benign and malignant tumours of the periampullary region (pancreatic head, ampulla, duodenum and distal choledochus) (1-3). Although the results of PD have greatly improved with advances in surgical techniques and perioperative management, this procedure remains one of the most complex abdominal operations and results in high post-operative morbidity rates of 30%-40% (4).

Pancreatic fistula (PF) is the most common and severe complication following PD. Despite the advances and technical changes developed to prevent PF, the incidence of this terrible complication still ranges from 3% to 45% (5). PF can not only prolong hospital stay and increase the cost of treatment but also increase the risk of premature mortality after surgery and cause other complications (4,6,7). Till date, the risk factors of PF have been extensively studied to obtain recommendations for its prevention and treatment; in addition, many studies have investigated the correlation between PF and perioperative variables, but the results are not consistent (8,9).

Determining the factors related to PF development will help prevent and manage this feared complication. The literature has demonstrated that many pre-operative, perioperative and post-operative factors, such as sex, age, hyperbilirubinemia, duration of surgery, intraoperative blood loss, pancreatojejunal anastomotic technique, pancreatic duct size, use of somatostatin and surgeon experience, are related to PF development after PD (10,11).

In this study, we aimed to investigate the factors related to PF development after PD in our clinic during a 10-year period and to discuss our findings in the light of the literature.

Methods

We enrolled 172 patients who underwent PD for periampullary tumours (ampulla, distal choledochus, pancreas head and duodenum) between January 2010 and January 2019. Patients aged <18 years, whose records could not be obtained and whose pathological diagnosis was not adenocarcinoma (n=17) were excluded from the study. Finally, 155 patients were included in the study. Patient data were obtained retrospectively from electronic records, patient files, anaesthesia follow-up forms and nurse observation forms. Owing to the retrospective design of the study, an ethics committee approval was not received.

PF was defined as any measurable volume of drain fluid appearing on or after the 3rd post-operative day, with an amylase content three times higher than the upper normal serum value (12). Patients were divided into two groups: Group I included patients with PF and Group II included those without PF. Demographic and clinical data such as sex; age; presence of comorbidities; American Society of Anesthesiologists (ASA) score; tumour localisation; laboratory parameters such as complete blood count, albumin, bilirubin and tumour markers [carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9)]; pathological features (e.g. tumour differentiation, stage, diameter, number of dissected and metastatic lymph nodes and presence of positive lymph nodes) and post-operative follow-up data (e.g. the presence of non-PF complications, duration of post-operative hospital stay, 30-day post-operative survival, local recurrence status, current clinical status, cause of exitus and total survival time) were analysed. In addition, independent risk factors were evaluated using univariate and multivariate analyses.

Post-operative complications were defined as wound infection, evisceration, intraabdominal abscess, intraabdominal haemorrhage and anastomosis leakage. Tumour-node-metastasis 2010 and 2016 systems was used for tumour staging.

All patients were evaluated at the Hepatobiliary Tumour Council at our centre before the operation. In patients with severe hyperbilirubinemia, pre-operative biliary drainage was performed via percutaneous transhepatic cholangiography or endoscopic retrograde cholangiopancreatography.

Operation Details

Following laparotomy, the choledoch was incised just above the cystic duct choledoch junction (until the negative surgical margin was reached). Subsequently, the pancreas was rotated to reach the posterior and was cut from the superior mesenteric vein border. From the proximal end of the pylorus, the distal stomach was freed, rotated and cut. The Treitz ligament was then freed and incised at approximately 10 cm from the distal end of the jejunum. Ductojejunal anastomosis between the pancreatic duct and jejunum was completed using 5/0 PDS and 3/0 silk sutures in the end-to-side manner. To the proximal end of this anastomosis, biliary tract-jejunum anastomosis was performed-

first on the posterior wall using 4/0 or 5/0 PDS sutures and then on the anterior wall using 4/0 or 5/0 PDS single sutures. Lymph node dissection was advanced from the lymphatic tissue in the hepatoduodenal ligament to the level of the celiac trunk. Subsequently, a drain was placed in the subhepatic region.

Statistical Analysis

SPSS 23.0 (IBM Corp., Armonk, N.Y., USA) package programme was used for statistically analysing data. Categorical measurements were summarised as numbers and percentages, and continuous measurements were summarised as mean, deviation and minimum and maximum values. Pearson's chi-squared test was used to compare categorical variables. While comparing the continuous measurements between the groups, the distributions were assessed and binary variables identified were analysed using independent Student t-test. Cox regression was used for multivariate analyses. Kaplan-Meier analysis and log rank tests were used for survival analyses. Statistical significance was set at 0.05 in all tests.

Results

For the 155 patients who participated in our study, the PF rate was 20%. Group I (with PF) consisted of 31 and Group II (without PF) consisted of 124 patients. Male sex was dominant in both the groups (61.3% vs 66.9%, p=0.348). Both groups were comparable in terms of the mean age (64.6 vs 64.8 years, p=0.916). At least one comorbidity was noted in 41.9% patients in Group I and 52.4% patients in Group II. The most common ASA score was ASA I in both the groups (51.6% vs 42.7%, p=0.589). Choledochus localisation was more common in Group I than in Group II (32.3% vs 12.1%, p=0.001). The demographic and clinical features of the patients are shown in Table 1.

The following laboratory parameters were similar between the groups: white blood cell count (p=0.885), neutrophil count (p=0.671), lymphocyte count (p=0.494), platelet count (p=0.900), haemoglobin level (p=0.492), albumin level (p=0.698), total bilirubin (p=0.891), CEA level (p=0.499) and CA19-9 level (p=0.223). Laboratory parameters of patients in both groups are shown in Table 2.

Poorly differentiated tumours were dominant in both Group I and Group II (61.3% vs 49.2%, p=0.349). Stage T3 tumours were the most common in both the groups (41.9% vs 50.8%, p=0.079). Lymph node stage and positivity were higher in Group I (58.1% vs 38.7%, p=0.041). However, tumour diameter was similar between the groups (1.94 cm vs 2.36 cm, p=0.070). The characteristics of tumours in both groups are shown in Table 3.

The incidence of post-operative complications other than PF was higher in Group I (58.1% vs 21.8%, p=0.000); so was the length of hospital stay (25.25 vs 16.43 days, p=0.000). Post-operative 30-day mortality was similar between the groups (19.4% vs 9.7%, p=0.119). However, the rate of local recurrence was higher in Group I (45.2% vs 37.9, p=0.295). In terms of the cause, mortality caused by sepsis was higher in Group I (22.6% vs 10.5%, p=0.028). For both groups, post-operative follow-up results are shown in Table 4. The average survival duration was shorter in Group I (23.9 months vs 38.18 months, p=0.024), as shown in Table 5 and Graphic 1.

The univariate analysis demonstrated that tumour localisation (p=0.021) was the independent risk factor of PF. However, in multivariate analyses, the independent risk factors of PF were age ≥65 years [hazard ratio (HR) (95% confidence interval (CI)] =2.182 (1.034-4.602), p=0.040), lymph node stage [HR (95% CI) =2.739 (1.304-5.753), p=0.008], presence of post-operative complications [HR (95% CI) =0.275 (0.133-0.567), p=0.001] and tumour diameter >2 [HR (95% CI) =0.423 (0.204-0.879), p=0.021]. The results of the multivariate analysis are shown in Table 6.

Table 1. Characteristics of patients

		Group 1 (n=31) n (%)	Group 2 (n=124) n (%)	p
Sex	Male	19 (61.3)	83 (66.9)	0.348
	Female	12 (38.7)	41 (33.1)	
Age		64.61±11.55 (22-91)	64.87±14.12 (22-93)	0.916
Comorbidities	Yes	13 (41.9)	65 (52.4)	0.200
	No	18 (58.1)	59 (47.6)	
ASA score	1	16 (51.6)	53 (42.7)	0.589
	2	12 (38.7)	52 (41.9)	
	3	3 (9.7)	19 (15.3)	
Tumour localisation	Ampulla	12 (38.7)	52 (41.9)	0.001*
	Duodenum	2 (6.5)	0 (0.0)	
	Choledochus	10 (32.3)	15 (12.1)	
	Pancreas	7 (22.6)	57 (46.0)	

ASA: American Society of Anesthesiologists

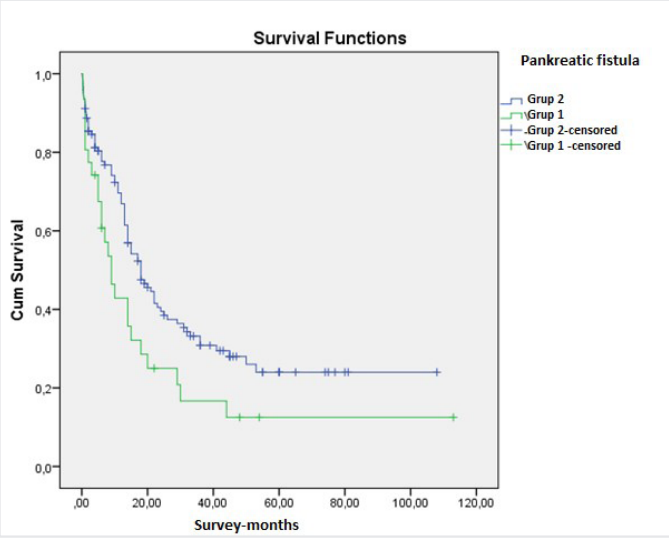
Table 2. Laboratory parameters

	Group 1 (n=31) Mean ± SD (Minimum-maximum)	Group 2 (n=124) Mean ± SD (Minimum-maximum)	
WBC (mm^3)	8285.48±2542.45 (3600-14410)	8202.01±2933.08 (1430-18390)	0.885
Neutrophil count (mm^3)	5785.16±2620.98 (1110-12310)	55868.48±2515.54 (840-15000)	0.671
Lymphocyte count (mm^3)	1627.74±785.51 (470-4490)	1728.22±714.73 (460-4630)	0.494
Platelet count (mm^3)	299.74±120.17 (104-684)	297.10±99.53 (92-690)	0.900
Haemoglobin (g/dL)	12.77±1.59 (9.8-15.6)	12.52±1.87 (7.4-17.7)	0.492
Albumin (g/dL)	3.48±0.80 (1.4-4.6)	3.53±0.68 (1.8-4.9)	0.698
Total bilirubin (mg/dL)	5.96±7.24 (0.2-29)	5.77±6.80 (0.1-34)	0.891
Pre-operative CEA (ng/mL)	5.81±15.57 (0.01-87.4)	4.30±9.71 (0-81.6)	0.499
Pre-operative CA19-9 (U/mL)	325.55±633.30 (2-2704)	671.96±1540.92 (1-9683)	0.223

WBC: White blood cell, SD: Standard deviation, CEA: Carcinoembryonic antigen, CA19-9: Carbohydrate antigen 19-9

Table 3. Characteristics of tumours

		Group 1 (n=31) n (%)	Group 2 (n=124) n (%)	p
Differentiation	Well	7 (22.6)	28 (22.6)	0.348
	Moderate	5 (16.1)	35 (28.2)	
	Poor	19 (61.3)	61 (49.2)	
T	T1	6 (19.4)	8 (6.5)	0.079
	T2	12 (38.7)	53 (42.7)	
	T3	13 (41.9)	63 (50.8)	
N	N0	13 (41.9)	76 (61.3)	**0.041***
	N1	18 (58.1)	48 (38.7)	
Number of total dissected lymph nodes (minimum-maximum)		10.48±8.08 (1-42)	10.45±5.71 (1-29)	0.982
Metastatic lymph node number (minimum-maximum)		1.61±2.38 (0-11)	0.87±1.58 (0-9)	**0.041***
Lymph node involvement	Negative	13 (41.9)	76 (61.3)	**0.040***
	Positive	18 (58.1)	48 (38.7)	
Tumour diameter		1.94±1.16 (0.7-5.0)	2.36±1.15 (0.4-6.5)	0.070



Graphic 1. Total survival duration of patients according to pancreatic fistula

Discussion

PD is one of the more complex abdominal surgical techniques and is associated with several post-operative complications. The most important complication that can develop following PD is a PF. The incidence of fistula formation after PD is much higher than that after other gastrointestinal operations and ranges from 3% to 45%. In addition, PF might lead to the development of other major complications (5,13).

The prognosis and aggressiveness of periampullary tumours vary according to tumour localisation. Overall, pancreatic head carcinoma is thought to have the worst prognosis among all periampullary tumour types (14). Reportedly, there are conflicting data regarding the effect of tumour localisation on PF development. Chen et al. (3) found that PF was associated with tumour localisation in periampullary tumours [odds ratio (OR) =3.00, p=0.029]. In contrast, Schmidt et al. (15) demonstrated that tumour localisation in the periampullary region was not associated with PF development. In addition, they reported that lymph node status was not a risk factor for

Table 4. Post-operative outcomes

		Group 1 (n=31) n (%)	Group 2 (n=124) n (%)	p
Post-operative complications other than pancreatic fistula	Yes	18 (58.1)	27 (21.8)	**0.000***
	No	13 (41.9)	97 (78.2)	
Length of hospital stay (minimum–maximum)		25.25±13.41 (6-56)	16.43±8.33 (5-43)	**0.000***
Post-operative 30-day mortality	Yes	6 (19.4)	12 (9.7)	0.119
	No	25 (80.6)	112 (90.3)	
90-day reoperation	Yes	3 (9.7)	6 (4.8)	0.258
	No	28 (90.3)	118 (95.2)	
Local recurrence	Yes	14 (45.2)	47 (37.9)	0.295
	No	17 (54.8)	77 (62.1)	
Current situation	Ex	25 (80.6)	80 (64.5)	0.063
	Alive	6 (19.4)	44 (35.5)	
Cause of death	None	7 (22.6)	54 (43.5)	**0.028***
	Cardiac causes	7 (22.6)	12 (9.7)	
	Sepsis	7 (22.6)	13 (10.5)	
	Tumour-related causes	10 (32.2)	45 (36.3)	

Table 5. Total survival duration according to pancreatic fistula groups

Group	Mean [Mean ± SD (minimum-maximum)]	Median [Mean ± SD (minimum-maximum)]	p
1	23.90±6.77 (10.62-37.18)	9.0±1.93 (5.20-12.79)	**0.024***
2	38.18±4.16 (30.01-46.35)	18.0±2.67 (12.76-23.23)	

SD: Standard deviation

Table 6. Univariate and multivariate analyses of factors associated pancreatic fistula in periampullary tumours

Measurements		Univariate	Multivariate	
		p	HR (95% Cl)	p
Age group (years)	<65	**0.036**	1.00	**0.040***
	≥65		2.182 (1.034-4.602)	
Sex	Male	0.893	1.00	0.862
	Female		1.053 (0.500-2.215)	
Localisation	Ampulla	**0.021***	1.00	**0.011***
	Duodenum		5.767 (1.276-26.074)	**0.023***
	Choledochus		2.011 (0.869-4.656)	0.103
	Pancreas		0.566 (0.212-1.513)	0.257
Differentiation	Well	0.765	1.00	0.192
	Moderate		1.135 (0.910-1.276)	0.213
	Poor		1.231 (0.876-1.652)	0.672
T	T1	0.444	1.00	0.391
	T2		0.535 (0.188-1.519)	0.240
	T3		0.494 (0.175-1.394)	0.183
N	N0	**0.007***	1.00	**0.008***
	N1		2.739 (1.304-5.753)	
Post-operative complication	Yes	**0.001***	1.00	**0.001***
	No		0.275 (0.133-0.567)	
Local recurrence	Yes	0.751	1.00	0.750
	No		0.887 (0.424-1.856)	
ASA score	1	0.928	1.00	0.932
	2		0.963 (0.449-2.065)	0.922
	3		0.787 (0.226-2.740)	0.707
Tumour diameter	Below 2	**0.020***	1.00	**0.021***
	2 and above		0.423 (0.204-0.879)	
Haemoglobin (g/dL)	Below 12	0.153	1.00	0.166
	12 and above		1.740 (0.794-3.813)	
Albumin (g/dL)	Below 3.5	0.963	1.00	0.963
	3.5 and above		0.983 (0.477-2.025)	
Bilirubin (mg/dL)	Below 5	0.525	1.00	0.522
	5 and above		1.277 (0.604-2.698)	

ASA: American society of Anesthiologists, HR: Hazard ratio, CI: Confidence interval

PF. In our study, tumour localisation was associated with PF and was an independent risk factor for PF development. In addition, PF was more common in patients with choledochal tumours. PF rate varied based on lymph node stage, tumour diameter, tumour differentiation and tumour localisation. We additionally noted that parallel to these factors, lymph node positivity and the number of metastatic lymph nodes were higher in the PF group. Lymph node positivity was an independent risk factor of PF development [HR (95% CI) =2.739 (1.304-5.753), p=0.008].

Several studies have indicated that a small diameter of the pancreatic duct (≤3 mm) is a risk factor for post-operative PF. When performing PD, surgeons should consider this risk factor and achieve a satisfactory pancreatic anastomosis to reduce PF formation (8,10,16,17). Notably, the debate on the relationship between tumour diameter and PF development is still ongoing. In their study, Polanco et al. (18) observed a smaller tumour diameter in the PF group (2.1 vs 2.9 cm, p=0.02; OR =0.594, 95% CI: 0.383-0.922, p=0.002). However, in another study, tumour diameters were similar in groups with and without PF (3.2 vs 3.1 cm, p>0.05) (15). We noted a similar result in our study. Although the tumour diameters were similar in the groups with and without PF (1.94 cm vs 2.36 cm, p=0.070), a tumour diameter of >2 cm was an independent risk factor for PF development.

The effect of intraoperative variables on PF development has been previously discussed in the literature. The type of

anastomosis performed during operation has also been cited as a predictor of PF (15,19,20). Schmidt et al. (15) reported that PJ invagination performed after PD resulted in a lower incidence of PF than Wirsung-jejunostomy (WJ). In addition, Bartoli et al. (20) reported that the incidence of PF development after WJ was the lowest compared to that after other anastomoses. Reportedly, soft pancreatic tissue is a strong risk factor for the development of PF (19). In the study by Sert et al. (19), PF was observed in 18 patients (75%) with soft pancreatic tissue, with the texture of pancreatic tissue being significantly associated with PF development (p<0.001). In the present study, we attempted to perform the same procedure in all patients. For this reason, we could not compare the details of operation because the surgical techniques were similar in both the groups.

Pancreatic duct stenting during anastomosis formation has been discussed in the literature (21,22). This was examined in a randomised trial by Winter et al. (22); they randomised 238 patients undergoing PD with or without internal pancreatic duct stent, with the endpoint being postoperative pancreatic fistula (POPF) development. The authors concluded that internal pancreatic duct stenting did not alter the incidence of POPF. Pancreatic duct drainage was also examined with external stents. In a study by Poon et al. (23), 120 patients undergoing PD with PJ duct-to-mucosa anastomosis were prospectively randomised to an external stent or no-stent group. Patients in the stent group had a significantly lower PF rate than those in the no-stent group (6.7% vs 20%, p=0.032) (23). In our routine practice, we use internal stents. In the present study, we used the same application in all patients; therefore, we could not evaluate the effect of stents.

In studies where the pancreatic duct is blocked with biological substances, the results have been reported to be very successful. For example, a group of authors have suggested that a possible anastomotic leak could be treated with fibrin glue around the anastomosis during surgery (24). However, we did not use these methods in our patients.

Despite the controversy regarding the preventive and therapeutic value of abdominal drains after pancreatic resection, several studies have highlighted the importance of drainage analysis for the prediction of POPF (25,26). In our study, we placed prophylactic abdominal drains in all patients. We believe that these abdominal drains contributed in the prediction of the incidence of PF development.

Reportedly, PF is associated with increased morbidity, mortality and longer hospital stay as well as additional cost. As pancreatic fluid is an enzymatically active and aggressive substance, it causes erosions in the surrounding tissue and may affect the intestinal, bile duct or vascular walls. PF has been associated with other non-fistula complications, particularly delayed gastric emptying, ileus, wound infection, intraabdominal abscess, pancreatitis, bleeding and sepsis. It has also been associated with significantly increased hospital costs and the rate of reoperation and admission to hospital (10,27).

In their series, Schmidt et al. (15) found that sepsis (21% vs 5%, p<0.001) and other infection-related complications were higher in the group with PF than in the group without PF. Similarly, Chen et al. (3) reported higher rates of post-operative haemorrhage in the group with PF development (33% vs 1.3, p=0.000). In both these studies, the length of hospital stay was longer in the PF group. Similarly, in our study, the incidence of post-operative complications other than PF was higher in the PF group than in the other group (58.1% vs 21.8%, p=0.000). Accordingly, the duration of hospitalisation in the PF group increased by 9 days compared to that in the other group. Prolonged hospitalisation might result in additional medical costs and a decrease in the quality of patient care. In our study, the post-operative complication rate was higher in the PF group, and the presence of post-operative complications other than PF was an independent risk factor for PF development. From this point of view, PF might increase post-operative complications, which may play a role in the development of PF.

PF development increases post-operative mortality through the complications it causes (28). In addition, post-operative complications in patients with cancer delay their oncological treatment and sometimes make treatment impossible. Accordingly, PF development is expected to increase post-operative mortality rates and decrease long-term survival. In our study, although the 30-day and 90-day mortality rates were higher in the PF group, the difference was not statistically significant. Regarding the causes of mortality in patients who developed PF, we noted that septic complications were more common (22.6% vs 10.5%, p=0.024). In our study, PF significantly reduced long-term survival (23 vs 38 months, p=0.024).

Study Limitations

Our study is limited by the small number of patients, the single-centre nature and the operative variables and features of the pancreatic tissue that were not adequately evaluated. However, we believe that our study will provide detailed data on PF to the literature.

Conclusion

In conclusion, we found that localisation, stage and diameter of the tumour were related to PF development. In addition, the development of PF contributed to post-operative complications, which consequently prolonged hospital stay. PF also significantly shortened long-term survival. Thus, PF formation after PD poses a great threat to patients' life and health. Therefore, an early estimation of PF development and the investigation of related risk factors are of great importance in preventing PF and its complications.

Ethics

Ethics Committee Approval: Owing to the retrospective design of the study, an ethics committee approval was not received.

Informed Consent: Due to the retrospective design of the study, patient consent was not obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: E.M.S., T.B.A., M.G., G.K.B., Concept: U.T., E.M.S., T.B.A., M.G., Design: U.T., E.M.S., Data Collection or Processing: U.T., E.M.S., M.G., G.K.B., Analysis or Interpretation: EM.S., T.B.A., Literature Search: U.T., T.B.A., M.G., G.K.B., Writing: U.T., T.B.A.

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Original Article

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An Assessment of the Comfort Level of Cancer Patients with Receiving Chemotheraphy

Kemoterapi Alan Kanser Hastalarının Konfor Düzeylerinin Değerlendirilmesi

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ABSTRACT

Objective: The study aimed on determining the comfort level of cancer patients with receiving chemotheraphy and to evaluate the factors that have an impact on this.

Methods: The study was conducted with 213 patients who received cancer chemotherapy. It was designed as a cross-sectional and descriptive model. A Patient Information Form and General Comfort Questionnaire was administered to patients in person. For the data assessment, frequency, percentages, the t test, one-way variance analysis, the Mann-Whitney U test, Kruskal -Wallis H test, and the Dunnet T3 Post Hoc test were employed.

Results: The average General Comfort Questionnaire score for patients was 2.93±0.35. Patients psychospiritual comfort was at the highest degree (39.72±5.87), while environmental comfort was at the lowest degree (35.58±6.45). Patients comfort level was significantly affected by sex, age and profession ($p<0.05$), whereas it was not affected significantly by marital status, educational status, person(s) with whom they lived cancer type, duration of cancer ($p>0.05$).

Conclusion: Patients in the study had moderate comfort levels. There is increased recognition that cancer patients comfort needs should be explored, and practices that will enhance their comfort level should be promoted.

Keywords: Cancer survivors, chemotherapy, oncology nursing, patient comfort

ÖZ

Amaç: Bu çalışma kemoterapi alan kanser hastalarının konfor düzeyini ve bunu etkileyen faktörleri değerlendirmek amacı ile yapıldı.

Yöntemler: Çalışma kanser kemoterapisi alan 213 hasta ile gerçekleştirildi. Çalışma, kesitsel ve tanımlayıcı tipte yapıldı. Hastalara yüz yüze görüşme tekniği ile Hasta Bilgi Formu ve Genel Konfor Ölçeği uygulandı. Verilerin değerlendirilmesinde frekans, yüzde, t testi, tek yönlü varyans analizi, Mann-Whitney U testi, Kruskal-Wallis H testi, Dunnet T3 Post Hoc testi analizi testleri kullanıldı.

Bulgular: Hastaların Genel Konfor Ölçeği puan ortalaması 2,93±0,35'tir. Hastaların psikospritüel konfor boyutu en yüksek düzeyde (39,72±5,87) iken çevresel konfor boyutu en düşük düzeydedir (35,58±6,45). Hastaların konfor düzeyini cinsiyet, yaş ve meslek anlamlı olarak etkilerken ($p<0,05$); medeni durum, eğitim düzeyi, evde birlikte yaşanan kişiler, kanser türü ve kanser süresi anlamlı olarak etkilememiştir ($p>0,05$).

Sonuç: Hastaların konfor düzeyi orta düzeydedir. Kanser hastalarının konfor gereksinimleri belirlenerek konfor düzeylerini artırabilecek uygulamaların yapılması önerilir.

Anahtar Sözcükler: Kanser hastaları, kemoterapi, kanser hemşireliği, hasta konforu

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Introduction

Cancer, characterized with impairment in programmed cell death and excessive cell proliferation, is a chronic disease with challenges in diagnosis, treatment, and care processes (1,2). The World Health Organization has reported that there are 18.1 million new cancer cases throughout the world each year, and cancer-related mortality was 9.6 million in 2018 (1). In Turkey, 20.7% of the deaths in 2014 were caused by cancer (2).

With early diagnosis and advancements in treatment and care, patients' lifespan with cancer has been extended. These positive developments have now brought a focus on the possibilities for patients' longer and improved quality of life (3,4). A diagnosis of cancer often evokes many emotional, psychological, and behavioral responses. These can include guilt, abandonment, anxiety, pain, grief, and thoughts of dying, which can trigger many symptoms that negatively affect a person's comfort and well-being (5,6). One of the major weapons used in the treatment of cancer is the use of chemotherapy. Although it can produce many positive outcomes, this treatment also causes a great number of negative side effects. Many cancer patients experience nausea, emesis, pain, diarrhea, alopecia, fatigue, and sleeplessness. Other side effects include psychological problems such as feelings of despair, depression, anger, anxiety, fear, hopelessness, vulnerability, and loss of control (3,5,7). The social problems that many patients confront are those of isolation, role changes, loss of work, difficult interpersonal relations, and decreased interest in their social environment (8).

The term "comfort," defined as "lack of pain, distress, worry and uneasiness," has been analyzed by Katharina Kolcaba. Kolcaba defines comfort as "the immediate state of experience of solving problems and thus having comfort by being strengthened through answering the human needs for relief and ease and transcendence in physical, psychospiritual, environmental, and sociocultural contexts" (9-11). Cancer patients with a large number of physical, psychosocial, socio-cultural, and environmental problems often receive palliative care services (12,13). Palliative care is said to focus on eight elements: evaluations and consultations, tests, care practices, pharmacotherapy, diet, activity-environment safety, patient and family education, and discharge planning, which intend to increase patient comfort when managing their disease (14). With cancer treatment, palliative care is often part of comfort care, specifically providing psychosocial and spiritual support and symptom control (13,14).

Other goals for comfort care are to offer cancer patients encouragement, hope, feelings of control over their life, and to enhance their decision-making skills (9,15). Comfort care, which is an inseparable part and basis of nursing services, is a therapeutic nursing practice. Although each patient has different comfort needs, all comfort dimensions may affect each other negatively or positively. Therefore, comfort care, emphasizing individual needs and a holistic approach, is both a desired outcome and an indication of productivity (10,16). By assessing cancer patients' comfort levels, physical needs psychospiritual needs, sociocultural needs and environmental needs, health care personnel will be able to determine ways in which to maximize their patients' comfort as they go through a rigorous and difficult life crisis of ill health (6,12). In one study, it was found that the patient's comfort increased as a result of the attempt to cope with the symptoms of chemotherapy (17). In this way, considering all aspects of the individual's needs can help in the planning, decision making, and implementation of the best nursing interventions possible. A main goal would be to keep the patient's maximum comfort level in focus (11,18).

In Turkey, few studies have been conducted which assess the comfort levels of cancer patients (6,17). For this reason, we believe that this research, which was the first study on comfort done with cancer patients living in the Eastern Black Sea region of Turkey, will make a significant contribution to the existing literature. The study focused on determining the comfort level of cancer patients during their personal and stressful experiences of diagnosis and treatment and to help inform future nursing interventions on how to best comfort oncology patients to improve their quality of life during chemotherapy treatments. The aim of the study was to determine the comfort level of cancer patients and to evaluate the factors that have an impact on this.

Methods

Study Design

This cross-sectional and descriptive study was performed to determine comfort levels of patients in the ambulatory chemotherapy unit of one Hospital of University.

The Population and Sample

The study population was drawn from patients who received cancer chemotherapy during that time, n=689 patients in the hospital's medical records between 1 August - 31 December, 2015. The research sample was then calculated to n=247 participants, with a 95% confidence interval and a 5% margin of error nonreference prevalence of comfort of 50% being adopted using Open Epi Programs (19). The 247 participants were taken by simple random sampling technique. These patients were recruited mechanically a list of patients from the hospital's medical records who received chemotherapy between 1 August-31 December 2015, and accessing this list was approved by the ethics committee. In the end, the study was conducted with 213 patients after 34 patients dropped out for various reasons like giving up the treatment, moving from the city to another place, interrupting the forms. To be eligible patients needed to know about their cancer diagnosis, regardless of type of cancer and time since diagnosis. Also, they needed to be: 1) receiving chemotherapy; 2) not receiving palliative or hospice care; 3) be over ≥18 years old; 4) have no psychiatric disorders which needed treatment; 5) have no other chronic disease needing continuous treatment; 6) have person/place/time orientation; 7) be able to verbally communicate; and 8) agree to participate in the study. Patients would be excluded if they had a cancer diagnosis before the age of 18.

Data Collection Forms

Patient Information Form

A researcher designed the Patient Information Form by surveying the relevant studies on patients receiving cancer chemotherapy (12,15,17). The form included seven questions concerning patients (i.e., sex, age, educational status, profession, marital status, cancer type, duration of cancer).

General Comfort Questionnaire (GCQ)

The GCQ was developed by Kolcaba in 1992 (18). The Turkish adaptation of the GCQ was done by Kuguoglu and Karabacak in 2008 (20), and its Cronbach alpha was found to be 0.85. In the current study, the Cronbach alpha of the GCQ was found as 0.87. It was created according to 12-cell grid called the taxonomic structure involving four dimensions (physical, psychospiritual, environmental, sociocultural) and three levels (relief, ease, transcendence) of theoretical components of comfort which were used to determine patients' needs, evaluate the nursing implementations for increasing comfort levels, and to achieve the expected results about increasing comfort. All items were scored on a Likert scale ranging from 1 (strongly disagree) to 4 (strongly agree). This scale consists 24 positive and 24 negative items (Table 1). The lowest possible GCQ total score was 48 points, average score 1, and the highest total score was 192 points, average score 4. The average score is determined by the total score, dividing the number of items. It is suggested that GCQ can be evaluated and used on both total score and average score in both research and practice (9,10,20).

According to studies regarding comfort levels conducted by Kolcaba, the concept of "comfort" has a holistic structure, and therefore should be assessed as a whole. Although nurses know intuitively what comfort is and what nursing interventions are necessary to enhance it, nurses and researchers currently utilize measures of discomfort that designate a neutral sense of comfort as being the absence of a specific discomfort (12). Integration of comfort to nursing care and comfort measurements of patients determines unmet comfort needs allows bundling of interventions in a single patient interaction and aids in creating measures of holistic comfort for documentation (18). Comfort remains a substantive need throughout life, and as such, should be considered an indispensable constituent of holistic nursing care.

Measures

The data were gathered using the Patient Information Form and the General Comfort Questionnaire (GCQ) by one researcher. Patients who agreed to participate in the study and met the inclusion criteria were asked to sign an informed consent form. Patient Information Form and GCQ were administered face-to-face by the researcher at a suitable time while patients were receiving chemotherapy at the Ambulatory Chemotherapy Unit. The patients were given the option of completing the Patient Information Form and GCQ with or without the researcher's assistance. Data collection took nearly 30 minutes, and the patients were given the chance to ask any question related to the study.

Data Analysis

All analyses were performed using the Statistical Package for the Social Sciences for Windows IBM SPSS Statistics 22.0. Skewness, Kurtosis, Kolmogorov-Smirnow, and Shapiro-Wilk values were used to determine whether the sample followed a normal distribution. For the data analyses, frequency, percentages, the t-test, one-way variance analysis, the Mann-Whitney U test, the Kruskal-Wallis H test, and the Dunnet T3 Post Hoc test were used. Results were considered significant at $p<0.05$, and the confidence interval was set at 95%.

Ethical Considerations

The protocol of the study was approved by the ethical review boards at the authors' institution and the hospital. In order to conduct the study, written permissions were obtained from

Table 1. 12-cell called the taxonomic structure of GCQ

Subscales of comfort	Comfort levels of subscales		
	Relief (16 items)	Ease (17 items)	Transcendence (15 items)
Physical (12 items) †Total score 12-48 ‡Average score 1-4	14¶,19¶,48¶,25¶	1§,36§,20¶,28¶	15§,29§,5¶,6¶
Psychospiritual (13 items) †Total score 13-52 ‡Average score 1-4	44§,46§,22¶,40¶	2§,7§,31§,38§,24¶	9§,17§,41¶,45¶
Environmental (13 items) †Total score 13-52 ‡Average score 1-4	3§,27§,12¶,34¶	11§,47§,32¶,42¶	30§,33§,18¶,21¶,35¶
Sociocultural (10 items) †Total score 10-40 ‡Average score 1-4	37§,8¶,13¶,26¶	4§,23§,43§,39¶	10§,16§

Both of "†" and "‡" have the same meaning in GCQ; §Positive items of GCQ; ¶Negative items of GCQ
GCQ: General Comfort Questionnaire

the hospital. The study was granted ethical clearance by the Institutional Review Board of the University (IRB no: 2015-24237859-388) and was undertaken in compliance with the Helsinki Declaration. Permission was obtained to use the questionnaire from Kuguoglu and Karabacak (20) adapting in Turkish of the GCQ. In addition, verbal and written consent was obtained from each patient prior to the study.

Results

A total of 213 patients were taking cancer chemotherapy were included in the study. When the descriptive characteristics of the patients were examined, it was determined that their 59.2% female, 43.7% 50-64 years, 55.8% literate/primary school, 46.5% housewives, and 85.9% married. Besides, when the disease information of the patients was looked at, it seemed that their type of cancer 36.6% breast cancer, 31.5% gastrointestinal system cancer, and the durations of cancer 52.1% one year and less.

Patients' average GCQ score and total GCQ score are in Table 2. Patients' average GCQ score was found to be 2.93±0.35, whereas their total GCQ score was 140.63±16.86.

Table 3 presents the cross-sectional and descriptive statistics patients' average GCQ, and subscale scores according to taxonomic structure. Patients' total scores for the GCQ subscales were physical subscale 34.80±5.33, psychospiritual subscale 39.72±5.87, environmental subscale 35.58±6.45, sociocultural subscale 30.53±3.56; relief 47.39±5.92, ease 49.57±6.85 transcendence 43.67±6.28. Patients' total scores for the GCQ subscales were the highest is the psychospiritual subscale, and the lowest is the environmental subscale.

Table 4 shows the GCQ mean scores according to some characteristics. It was found that there were statistically significant differences between patients' GCQ and sex (p=0.036), age (p=0.028), and profession (p=0.002). However, no statistically significant differences were found between the GCQ and marital status, educational status, type of cancer, and duration of cancer (p>0.05).

Discussion

Our results showed that the patients' average comfort level was above 2 out of 4 levels of comfort. This means the patients feel fairly comfortable. Our results were in agreement with a similar study, which was conducted with breast cancer patients receiving chemotherapy, found the comfort level higher than 2 (15). Other studies which used derivates Kolcaba's GCQ with similar scales and questions, reported similar results with this study (6,12,17).

Amoung the subtypes of comfort, the psychospiritual comfort dimension is found the highest. Besides, the data in the current study showed that the level of patients' comfort was transcendence level which was the top level of comfort. It was considered that they were able to cope with their fears, and overcome bothersome symptoms more effectively during the cancer treatment process. A similar result by Bilgic and Acaroglu (17) reported that cancer patients' psychospiritual comfort dimension were found to be at

Table 2. Patients' total score and average score of GCQ (n=213)

GCQ	Mean ± SD	Min - max
‡Total score (48-192)	140.63±16.86	92-177
†Average score (1-4)	2.93±0.35	2.29-3.42

GCQ: General Comfort Questionnaire, SD: Standard deviation, Both of "†" and "‡" have the same meaning in GCQ, Min: Minimum, Max: Maximum

Table 3. Patients' average GCQ and subscale scores according to taxonomic structure (n=213)

	Comfort levels			
	Relief	Ease	Transcendence	Total score
Subscales of GCQ	Mean ± SD (Min - max)	Mean ± SD (Min - max)	Mean ± SD (Min - max)	Mean ± SD (Min - max)
Physical	10.73±1.94 (5-14)	11.47±2.52 (4-16)	12.61±2.19 (7-16)	34.80±5.33 (21-44)
Psychospiritual	12.46±2.51 (6-16)	14.37±2.35 (7-20)	12.90±2.07 (8-16)	39.72±5.87 (23-52)
Environmental	12.23±1.97 (7-16)	11.65±2.29 (5-16)	11.70±3.78 (5-20)	35.58±6.45 (21-49)
Sociocultural	11.97±2.24 (4-16)	12.08±1.97 (7-16)	6.47±1.34 (2-8)	30.53±3.56 (18-39)
In all subdimensions score	47.39±5.92 (30-60)	49.57±6.85 (27-65)	43.67±6.28 (28-59)	

GCQ=General Comfort Questionnaire, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 4. Patients' average GCQ scores according to demographic characteristics (n=213)

Characteristics	n (%)	Mean ± SD	Test value	P
Gender				
Female	126 (59.2)	2.89±0.36	T: 2.116	0.036*
Male	87 (40.8)	2.99±0.31		
Age				
35 years and ↓	14 (6.6)	2.84±0.40	F: 9.092	0.028*
36-49 years	70 (32.8)	2.88±0.37		
50-64 years	93 (43.7)	2.92±0.32		
65 years and ↑	36 (16.9)	3.08±0.31		
Educational level				
Illiterate	17 (8.0)	2.79±0.28		
Literate/Primary school	119 (55.8)	2.93±0.34	F: 4.795	0.187
High school	43 (20.2)	2.91±0.37		
University	34 (16.0)	3.01±0.35		
Profession				
Housewife	99 (46.5)	2.84±0.37	F: 12.962	0.002*
Retired	61 (28.6)	3.05±0.33		
Self-employed	33 (15.5)	2.91±0.29		
Government employee	20 (9.4)	3.02±0.24		
Marital Status				
Married	183 (85.9)	2.93±0.35	T: 0.268	0.789
Single	30 (14.1)	2.91±0.30		
Type of Cancer				
Breast cancer	78 (36.6)	2.90±0.37	F: 2.962	0.564
Gastrointestinal system cancer	67 (31.5)	2.99±0.29		
Genitourinary system cancer	42 (19.7)	2.90±0.36		
Lung cancer	17 (8.0)	2.96±0.38		
Unknown primary	9 (4.2)	2.86±0.44		
Duration of xancer				
1 year and ↓	111 (52.1)	2.92±0.33	F: 3.965	0.265
1-5 years	74 (34.7)	2.93±0.36		
6-10 years	20 (9.4)	2.87±0.39		
11 years and ↑	8 (3.8)	3.15±0.31		

*p<0.05, GCQ: General Comfort Questionnaire, SD: Standard deviation

the highest. A previous research study shows that cancer patients emphasize that they use spiritual coping methods such as faith, prayer, and that these methods enhance their coping skills (21).

The sociocultural comfort dimension was found as comfortable which was almost the same as psychospiritual comfort dimension of patients. This study obtained a level of transcendence in the cancer patients' sociocultural comfort dimension. It is also found similar results in study with patients' sociocultural comfort score which was the best comfortable and feeling the best amongst other sub-dimensions (12). Sociocultural comfort of patients is the highest compared to other subdimensions and transcendence level they probably feel more loved, remembered, belonged (22). Korean patients with cancer was found to have eight attributes: "acceptance, self-control, goals of life, change in belief, and positive attitude, supportive relationships, therapeutic environment, and reliance on faith." (23).

In the current study, patients' physical dimension was found to be moderate. In line with the current study, a study done with cancer patients, identified the physical comfort level as moderate (17). Because patients' GCQ physical comfort levels were above 2 out of 4 levels of comfort and transcendence, we concluded that patients did not have serious difficulties in coping with physical symptoms; they may not feel uncomfortable and, able to handle cancer' these symptoms.

The current study showed that the participants' environmental comfort dimension was at the most uncomfortable area. However, the patients are best comfortable at the relief level in terms of environmental comfort. It was thought that filling the scale in hospital conditions which may adversely affect the patient's comfort could affect the comfort of patients. Similarly, another patient study found that the environmental comfort subscale was the worst of all other comfort subscales (17). The study of Chen and Cheng (24) detected a decrease in the environmental comfort subscale because hospital conditions are different from conditions at home. The patients who were included in Kim and Kwon (12) study were determined that the highest level of environmental comfort respectively the patients who were followed at home, day care chemotheraphy unit, outpatient clinic and inpatient unit.

The current study found that male patients' were considerably higher comfortable than that of female patients. It is guessed that women's greater responsibilities in their lives outside work may explain their feeling less comfortable. Similarly, in a study when asked to indicate the actual side effects experienced during chemotherapy, women being significantly more affected than men. In the same study, women generally reporting more uncomfortable than men (25).

This current study also found that as age increased, so did aged ≥65 years was considerably higher comfortable than for other age groups. Interestingly, although functioning and symptoms differed significantly with age, they were not related to comfort (12). Indeed, the older a person is when they are first diagnosed with cancer, the more they seem able to adapt to their new health situation, and developed more effective coping skills, and have feeling more comfortable (26). However, different studies done with cancer patients did not determine a significant correlation between age and comfort level (15,21).

One study has revealed that cancer patients' comfort level may be elevated because they have been able to maintain their professional life and social responsibilities (3). In the current study, patients who were government employees, and retired employees were significantly higher comfortable than the housewives. This may possibly suggest that patients who were government employees with a regular income, job satisfaction, and social responsibilities demonstrated greater comfort, and were thus able to cope with diseases, and health crises more effectively.

The GCQ is an effective measurement tool that determines the comfort level of cancer patients. Nurses should administer the GCQ at any time points in the patients' chemotherapy. The GCQ needs more research to increase its usability in practice. However, the results of this study may increase awareness among individuals in the occupational healthcare field about the associations between GCQ, and health, and work-related variables. In particular, emphasis should be put on development of assessment, and monitoring tools for use in everyday clinical practice.

Study Limitations

The limitation of the study was that only patients hospitalized in the ambulatory chemotherapy unit of Hospital of University were included in the study. The results of this study are therefore directed at patients receiving treatment in this unit. The results of this study cannot be generalized to all patients.

Conclusion

The results of the current study indicated that cancer patients' comfort level was above the average. The psychospiritual comfort aspect was at the best degree, and sex, age, and profession affected the comfort level. In order to provide the highest level of nursing care for cancer patients, their comfort needs must first be determined before a care plan can be properly implemented. Nurses are responsible for planning, and executing the interventions which can increase patients' comfort. Accordingly, nurses should cooperate with the family to determine the comfort levels of patients during treatment, and to control the negative factors that may arise. Factors that decreasing and increasing comfort in the treatment process of chemotherapy patients should be identified. Patients who will be receiving outpatient chemotherapy should undergo an examination with comfort scale before, during, and after their therapy.

Ethics

Ethics Committee Approval: In order to conduct the study, written permissions were obtained from the hospital. The study was granted ethical clearance by the Institutional Review Board of the University (IRB no: 2015-24237859-388).

Informed Consent: Patient Information Form and GCQ were administered face-to-face by the researcher at a suitable time while patients were receiving chemotherapy at the Ambulatory Chemotherapy Unit.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: A.C., S.H., Design: A.C., S.H., Data Collection or Processing: A.C., S.H., Analysis or Interpretation: A.C., S.H., Literature Search: A.C., S.H., Writing: A.C., S.H.

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Original Article

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Needlestick and Sharps Injuries Among Operating Room Nurses, Reasons and Precautions

Ameliyathane Hemşirelerinin Kesici-Delici Aletler ile Yaralanma Durumu, Nedenleri ve Önlemleri

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ABSTRACT

Objective: This study was carried out to determine the needlestick injuries (NSI) of the operating room nurses with penetrating and sharp tools, the causes of the injury and the precautions taken to prevent the injury.

Methods: A descriptive and cross-sectional study was conducted with the participation of 463 volunteer nurses from 27 different hospitals in Istanbul, Turkey. After obtaining the ethics committee, institutions and volunteer permissions of the study, the data were collected with two data collection forms formed in line with the literature knowledge and expert opinions. The data were analyzed using IBM SPSS 22.0 program and $p<0.05$ was significance value.

Results: Participants were 18-61 years old, (mean: 35.9±0.84), 63.7% had bachelor's degrees and above, 80.8% of operating room nurses were graduated from nursing program. 68.9% of the participants reported that they had lived at least once during their working life. There were significantly different for NSI ($p<0.05$) women, married and had low educational levels, working in university hospitals with deep invasive intervention and using protective equipment. Participants reported that 54.8% of the injuries were performed in the right hand and the primary cause was exchanging instruments between nurse and surgeon, the secondary was assisting surgeon. Most of the participants (37.1%) were injured by the suture material. The most common NSI encountered operating theaters were pediatric surgery, cardiovascular surgery, thoracic surgery and general surgery.

ÖZ

Amaç: Bu araştırma, ameliyathane hemşirelerinin delici kesici aletlerle yaralanma (DKAY) durumunu, yaralanma nedenlerini ve yaralanmadan korunmak için aldıkları önlemleri belirlemek amacıyla yapıldı.

Yöntemler: Tanımlayıcı ve kesitsel nitelikteki araştırma, Türkiye'nin İstanbul ilinin 27 farklı hastanesindeki 463 gönüllü hemşirenin katılımı ile yapıldı. Araştırmanın verileri, etik kurul, kurum ve gönüllü izinleri alındıktan sonra literatür bilgisi ve uzman görüşleri doğrultusunda oluşturulan farklı iki veri toplama formu ile toplandı. Veri analizinde IBM SPSS 22.0 programı kullanılarak anlamlılık $p<0,05$ düzeyinde değerlendirildi.

Bulgular: Katılımcıların, 18-61 yaş (ortalama: 35,9±0,840) arasında, %63,7'sinin lisans ve lisansüstü eğitim düzeyinde olduğu, %80,8'inin hemşire unvanı ile ameliyathane hemşiresi olduğu belirlendi. Katılımcıların %68,9'u çalışma hayatları boyunca en az bir kez DKAY yaşadığını bildirdi. DKAY; kadın, evli ve eğitim düzeyi düşük, devlete bağlı üniversite hastanelerinde çalışan, koruyucu ekipman olanağı yeterli sağlanmayan/kullanmayan katılımcılarda daha fazlaydı, fark istatistiksel olarak anlamlıydı ($p<0,05$). Katılımcılar DKAY'nin nedeninin çoğunlukla cerrahi alet alışverişi/yardım edici davranışlar sırasında yaşanabildiğini bildirdi. DKAY yaşayan katılımcıların sağ elden yaralandıkları (%54,8), en fazla yaralanmanın %37,1 ile sutür materyalinden kaynaklandığı bulundu. Yaralanmanın en fazla yaşandığı ameliyathaneler sırasıyla çocuk cerrahisi, kardiyovasküler cerrahi, göğüs cerrahisi ve genel cerrahiydi.

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Conclusion: The results of the research show that NSI is an important problem related to healthcare workers' safety in operating theaters.

Keywords: Operating room, operating room nurses, needlestick injuries, risk factors, prevention

Sonuç: Araştırma sonuçları, DKAY'nin ameliyathane sağlık çalışanlarının güvenliğini etkileyecek kadar önemli bir sorun olduğunu göstermektedir.

Anahtar Sözcükler: Ameliyathane, ameliyathane hemşireleri, delici kesici alet yaralanmaları, risk faktörleri, önlem

Introduction

Needlestick injury (NSI) is among the leading occupational accidents and risks that healthcare workers are exposed to (1). Such injuries are seen as an important problem that most of the health workers face at least once during their professional life (2). NSI is considered as a risk factor especially for the surgical team in terms of employee health (3). Operating rooms within the hospital are the most risky places for NSI. Studies have shown that the health workers most exposed to NSI are nurses and physicians (4-6). It has been reported that NSI can be experienced for many reasons, especially the intensity of the intraoperative process, stress and factors related to the employees (7).

The Centers for Disease Control and Prevention pointed out that contact with blood and body fluids and its effects were mostly seen in nurses (1,8). In the 2018 report of the EPINet™ database prepared for the surveillance of NSI and blood-borne infections, it was reported that NSI occured mostly in operating rooms and in nurses among all healthcare workers (4,9). According to the data of the European Agency for Safety and Health at Work, approximately 1 million injuries occur in healthcare workers each year in Europe caused by injectors alone (10). Research emphasizes that the measures to be taken and the adoption of safety practices can reduce the NSI by 80% (11,12).

Although it is known that protective measures are necessary in the researches, it is reported that infectious diseases continue to be transmitted under the name of "work accident". When the studies conducted in this area are examined, it is seen that there are suggestions that it should be questioned whether the rate of NSI of nurses decreases in the face of developing science and technology (13-17).

The present study was carried out to determine the conditions of operating room nurses that caused NSI, the precautions they took to prevent injury, and to draw attention to the importance of this issue in the light of the literature.

Within the scope of the research purpose, answers were sought to the following questions;

1. What is the prevalence of NSI among operating room nurses?

2. Is NSI related to the sociodemographic characteristics of operating room nurses?

3. Is NSI related to the working conditions of operating room nurses?

4. What are the precautions taken regarding NSI in the conditions where the operating room nurses work?

Methods

The Universe and Sample of the Research

The "general universe" of the study, which was planned as a descriptive and cross-sectional study, was represented by the operating room nurses of 220 hospitals in İstanbul, including university, state, educational research and private hospitals (18). Among these hospitals, the "study universe" of the research was chosen. In the literature, attention is drawn to the risk of NSI in healthcare workers in training and research hospitals (19). Therefore, the "study universe" was composed of operating room nurses of training and research hospitals, state hospitals and university hospitals (n=1266). Since the presented research was a master's thesis, it had to be completed within a certain time, so the research was limited to the Istanbul European region. Thus, the study population consisted of 570 operating room nurses working in 27 hospitals. Since it was aimed to reach the whole research universe, no sample calculation was made. While collecting the data, nurses who were in surgery (n=25; 4.38%), who did not want to participate in the study (n=47; 8.24%) or who were sick (n=35; 6.13%) were excluded from the study. The research was completed with a participation rate of 81.2%. Thus, a total of 463 nurses participated in the study.

Data Collection Tools

Data were collected with a questionnaire developed in line with the literature (20-23) and expert opinions. Survey form included 42 closed questions that determined the socio-demographic characteristics of the operating room nurses, their situations and reasons for NSI, and their and their institutions' approaches to the measures taken to prevent NSI. In order to evaluate the clarity of the questions, a preliminary study was carried out with approximately 10% of the sample number (n=47). After the preliminary study, the questions in the data collection form were rearranged and it was decided to use the revised form.

Data Collection

Methods based on "self-report" were used in data collection.

After the necessary permissions were completed, the operating rooms of the hospitals where the research would be conducted were visited according to a pre-planned schedule (between 23.01.2017 and 28.08.2017). Information about the research was given by interviewing with the operating room nurse in charge of each hospital about the questionnaire forms, the

importance of the study to be conducted and ethical permissions. Questionnaires and voluntary consent forms were delivered to the nurses. A second visit was made by c ontacting the nurse in charge within 10-15 days to collect the forms distributed by the researcher.

Evaluation of Data

Data were analyzed in IBM SPSS 22.0 package program. Statistical significance of the data was analyzed with Pearson Chi-Square Test, Pearson Correlation Test, Fisher's Exact test, t-Test, and f-test (ANOVA) according to the distribution status of the data. Significance was evaluated at the 95% confidence level and at the $p<0.05$ level.

Ethical Considerations

The study was approved by the Bezmialem Vakıf University Non-interventional Clinical Research Ethics Committee (29.11.2016 8/93). Institutional permissions were taken from the rectors of university hospitals, and from the general secretariats of the public hospitals to which they were affiliated. Voluntary participation consent was obtained from the participants in writing.

Results

In this section, there are introductory and comparative findings obtained with the aim of assessing the participants' NSI status.

In the present study, it was determined that 80.8% of the participants were female, 19.2% were male, 57% were married, 42.8% were between 29-39 years old (mean age 35±0.840), and 63.7% had undergraduate or higher education level. It was determined that 80.8% of the operating room nurses graduated from a nursing program, 41.7% worked in training and research hospitals, and 53.6% had 7 years or more of operating room nursing experience. In addition, it was determined that 68.9% of the participants reported that they had experienced NSI (Table 1).

Table 2 shows some of the characteristics of the participants about NSI. Of the participants 28.3% stated that they experienced NSI during the exchange of surgical instruments, 16.3% during assisting the surgeons, and 15.3% during collecting and counting of surgical instruments. It was reported that injuries were most common in the hand (96.8%), with 54.8% of the injuries occurring in the right hand. In 37.7% of the nurses suture material and in 28.2% scalpel were shown as the instrument causing injury.

When the socio-demographic characteristics of the participants were compared with NSI, it was found that female participants were more likely to encounter NSI than male participants ($p=0.007$), and that married participants were more likely to encounter NSI than singles ($p=0.002$). Although the frequency of NSI increased with increasing age, there was no statistically significant difference ($p=0.607$). As the level of education increased, frequency of NSI also increased and the difference was statistically significant ($p=0.016$) (Table 3).

Table 4 shows the comparison of the characteristics of the participants with NSI. In the operating room nurses of state university hospitals, NSI was less than the others, but the difference was not statistically significant ($p=0.938$). Although the nurses working in the pediatric surgery (81.3%), thoracic surgery and cardiovascular surgery (78.2%), and general surgery (77.1%) operating rooms reported more NSI compared to the other operating rooms, the difference was not statistically significant ($p=0.651$).

It was observed that as the working years as an operating room nurse, the duration of working hours in a shift, and the average time spent in surgery increased, the frequency of NSI increased ($p=0.01$), and working in a mixed shift system was significantly related with NSI ($p=0.001$).

NSI was less in healthcare workers (n=58) employed as operating room nurses than in nurses. However, this difference was not statistically significant ($p=0.235$).

Table 5 shows the comparison of the participants' NSI status according to the security measures taken. Difficulty in finding protective equipment showed an insignificant relationship with the NSI status ($p=0.887$). However, as the use of protective equipment increased, it was observed that the NSI decreased and the difference was statistically significant ($p=0.001$). When the precautions the participants took against NSI were examined, it was determined that the practices such as the use of double gloves and control of patient serology did not make a difference for participanst with and without NSI ($p<0.05$).

Discussion

In the present study, the fact that the majority of the participants are young, married, had a high level of education and were working in the operating room for a long time suggested that this group consisted of dynamic and high-conscious individuals with a regular family life. It could be concluded that the presented study was similar to the studies published in the national and international literature in terms of socio-demographic characteristics (2,24-29). In addition, the fact that the majority of the participants were state hospital employees might be related to the fact that the participants preferred institutions with more personal rights, or it might be due to the selection of samples.

According to OECD (Organization for Economic Co-operation and Development) 2018 data, the number of nurses providing care to every 1000 citizens was 17.8 in Norway, 17.2 in Switzerland, 14.8 in Iceland, while it was 2.1 in Turkey, which was far behind the OECD average (30). In the presented study, the fact that most of the nurses worked in random units and mixed-shift system in the operating room suggested that nurses worked under hard conditions according to OECD recommendations and their conditions regarding employee safety were risky. In the study conducted by Kan (31), it was reported that 29.4% of the operating room nurses were distracted at the 4th hour, that the distraction peaked at the 6th hour, and that there was a relationship between prolonged surgeries and NSI in 94.1% of the operating

Table 1. Descriptive characteristics of the participants (n=463)

Characteristics		n	%
Gender	Female	374	80.8
	Male	89	19.2
Age*	18-28	169	36.5
	29-39	198	42.8
	40 or above	96	20.7
Marital status	Married	264	57.0
	Single	199	43.0
Education	High school or undergraduate	168	36.3
	Graduate or postgraduate	295	63.7
Occupation	Nurse	374	80.8
	Participants other than nurses **	89	19.2
Institution	Training and research hospital	193	41.7
	Foundation affiliated university hospital	107	23.1
	State university hospital	98	21.2
	Public hospital	65	14.0
Deparment***	Gynecological surgery and urology	118	14.3
	Ear nose throat and plastic surgery	115	14.0
	General surgery	105	12.7
	Orthopedics	99	12.0
	Working in all departments	92	11.2
	Thoracic surgery and cardiovascular surgery	87	10.6
	Neurosurgery	54	6.6
	Pediatric surgery	48	5.8
	Other****	39	12.8
Shift	Day shift only	180	38.9
	Mixed day or night	283	61.1
Average working time per shift	8-10 hours	344	74.3
	11-13 hours	82	17.6
	14 hours or above	37	8.1
Average length of stay in a surgery	1-2 hours	76	16.4
	3-4 hours	221	47.7
	5-6 hours	117	25.3
	6 hours or above	49	10.6
Operating room nursing experience	1-6 years	215	46.5
	7-12 years	99	21.4
	13 years or above	149	32.2
Needlestick injury status	Yes	319	68.9
	No	144	31.1

*The oldest age is **61**, the mean age is **35.924±0.840**
**Operating room technician (n=33), emergency medical technician (n=22), health officer (n=14), paramedic (n=5), perfusionist (n=1), laboratory technician (n=3), prosthesis-orthotics Technician (n=2), midwife (n=2), not specifying the professional title (n=7)
***Participants gave more than one answer.
****Emergency surgery (n=39), ophthalmology operating room (n=39), supervisor (n=27), robotic surgery (n=1)

room nurses (31). The NSI rates of nurses in the present study (Table 1) were similar to some developing countries, suggesting that operating room nurses expected solutions for similar risks internationally (32). Zhang et al. (33) reported the rate of NSI as 84.6% in China, Kasatpibal et al. (2) 71.5% in Thailand, Yazar et al. (34) 65.8%, and Benli et al. (35) 77.9% (33). It is

Table 2. Some characteristics of the participants about needlestick injury

Needlestick injury characteristics		n	%
Causes of injury*	While exchanging drilling-cutting tool	279	28.3
	While assisting the surgeon	161	16.3
	During the collection and counting of instruments	151	15.3
	When using an injector	117	11.9
	In emergency surgeries/events	105	10.7
	While preparing the surgery table	56	5.7
	During the use of the Sharp-Box	49	5.0
	While controlling medical wastes in surgical instrument loss	27	2.7
	During washing and packaging of surgical instruments	22	2.2
	Other (responsible nurse, robotic surgery…)	18	1.8
Injured organ*	Right hand	256	54.8
	Left hand	196	42.0
	Other (arms, head, neck, trunk, eye …)	15	3.2
Injuring instrument*	Suture material	245	37.7
	Lancet	183	28.2
	Injector/IV catheter	144	22.2
	Other**	78	12.0
Provision of protective equipment	Always having trouble	62	13.4
	Sometimes can't find	180	38.9
	Available whenever	221	47.7
The use of protective equipment in surgeries	Always	209	45.1
	Sometimes	88	19.0
	Never	13	2.8
	Only in infected cases	153	33.0
Use of double gloves during surgery	In all surgeries	166	35.9
	Not used	188	40.0
	Used during preoperative skin asepsis	45	9.7
	Other***	64	13.8
Checking patient serology before surgery	Always	284	61.3
	Sometimes	143	30.9
	Never	36	7.8

* Participants gave more than one answer.
**It shows injury with Kirschner wire (n=4), laparoscopic trocar guide (n=2), chainsaw (n=1), root cannula needle (n=1), drain guide (n=1), (n=69) did not report in writing what he/she was injured with.
***Infected cases (n=44), Orthopedic surgery (n=20)

controversial that gender is a risk factor for NSI (32-36). Afridi et al. (36) in Pakistan compared the gender of participants (35.8% male and 64.2% female) in terms of NSI and reported that gender did not show a significant difference in terms of NSI. In the present study, the reason for the significantly higher NSI in females was the lower number of male participants. With the increase in age, it is expected that the NSI will decrease as the experience, education level and knowledge increase. Educational level may not be a determining risk factor for NSI, as it does not always indicate skill level in clinical practice. However, education can provide awareness of the problem and accelerate finding solution by organizing against the problem (37). In the study presented, it is believed that trained and experienced nurses were more likely to face DKAY because they participated in operations more due to their age and faced risk (Table 3).

In the present study, when the causes of NSI were examined, it was thought that the "hands-free" technique was not used effectively, especially in the operating rooms, as the participants reported that they were injured by instruments with stinging and cutting

Table 3. Comparison of participants' socio-demographic characteristics with needlestick injury status (n=463)

Socio-demographic characteristics		Needlestick injury status				
		Yes			No	
		n	%	p	n	%
Gender	Female	268	71.7	0.007	106	28.3
	Male	51	57.3		38	42.7
Age	18-28 years	112	66.3	0.607	57	33.7
	29-39 years	138	69.7		60	30.3
	40 years or above	69	71.9		27	28.1
Marital status	Married	197	74.6	0.002	67	25.4
	Single	122	61.3		77	38.7
Education	High school or undergraduate	107	63.6	0.016	61	36.4
	Graduate or postgraduate	212	68.9		83	31.1

Table 4. Comparison of the participants' working life characteristics with needlestick injury status (n=463)

Working life characteristics		Needlestick injury status				
		Yes			No	
		n	%	p	n	%
Institution	Training and research hospital	135	69.9	0.938	58	30.1
	Foundation affiliated university hospital	74	69.2		33	30.8
	State university hospital	65	66.3		33	33.7
	Public hospital	45	69.2		20	30.8
Department	Gynecological surgery and urology	86	72.9	0.651	32	27.1
	Ear nose throat and plastic surgery	85	73.9		30	26.1
	General surgery	81	77.1		24	22.9
	Orthopedics	71	71.7		28	28.3
	Working in all departments	67	72.8		25	27.2
	Thoracic surgery and cardiovascular surgery	68	78.2		19	21.8
	Neurosurgery	41	75.9		13	24.1
	Pediatric surgery	39	81.3		9	18.7
	Other	67	63.2		39	36.8
Operating room nursing experience	1-6 years	134	62.3	0.01	81	37.7
	7-12 years	70	70.7		29	29.3
	13 years or above	115	77.2		34	22.8
Shift	Day shift only	109	60.6	0.001	71	39.4
	Mixed day or night	210	74.2		73	25.8
Average working time per shift	8-10 hours	235	68.3	0.367	109	31.7
	11-13 hours	84	70.6		35	29.4
Average length of stay in a surgery	1-2 hours	49	64.5	0.222	27	35.5
	3-4 hours	146	66.1		75	33.9
	5-6 hours	86	73.5		31	26.5
	6 hours or above	38	77.6		11	22.4
Occupation	Nurse	261	69.8	0.235	113	30.2
	Participants other than nurses	58	65.2		31	34.8

Table 5. Comparison of the participants' needlestick injury status according to the security measures taken (n=463)

Security measures		Needlestick injury status				
		Yes			No	
		n	%	p	n	%
Provision of protective equipment	Always having trouble	44	71.0	0.887	18	29.0
	Sometimes can't find	122	67.8		58	32.2
	Available whenever	153	69.2		68	30.8
The use of protective equipment in surgeries	Always	126	60.3	0.001	83	39.7
	Sometimes	59	67.0		29	33.0
	Never	9	69.2		4	30.8
Use of double gloves during surgery	Only in infected cases	125	81.7	0.296	28	18.3
	In all surgeries	111	66.9		55	33.1
	Not used	137	72.9		51	27.1
	Used during preoperative skin asepsis before surgery	32	71.1		13	28.9
	In orthopedic surgeries and infected cases	39	60.9		25	39.1
Checking patient serology before surgery	Always	193	68.0	0.743	91	32.0
	Sometimes	102	71.3		41	28.7
	Never	24	66.7		12	33.3

properties while exchanging surgical instruments and helping the surgeon. Penetrating tools used in the operating room are generally capable of causing injuries such as cutting, puncturing, and scratching (37,38). However, Stringer et al. (39) reported that the injury rate was 1.2% in surgeries using the "hands-free technique" and 4.0% in surgeries that did not use. In another study, Stringer et al. (40) reported that the "hands-free technique" and the visual materials used in the training of this technique were effective in reducing the NSI. Jagger and Perry (41) reported that the use of the same technique was effective in their study. Considering the studies on the properties of the injurious agent; Kürtünlü (42) showed that suture material (41.5%) and scalpel (38.4%), Mohammad (43) injector needles (45%) and surgical sutures (30%), Lakbala et al. (27) sutures (59%), Hajipour (44) sutures (43.4%), and Wada et al. (26) sutures (48.3%) were the most common causes of injury. The fact that the majority of the injured participants in the present study experienced injury during pediatric and cardiac surgery operations might be related to the physical properties of the instruments used in these operations and the surgical technique. In these surgeries, small suture materials that force hand manipulation are used or deep invasive procedures are performed.

It has been suggested that while double-layered and different colored indicator gloves used in surgery reduce the risk of NSI, wearing single-colored standard gloves on each other creates an unnecessary sense of security (45). Makama et al. (46) reported that 15.2% of the gloves worn in single layer, 27.5% of the outer part of the gloves worn in double layer and 1.2% of the inner part of the gloves worn in double layer were not damaged. Demircay et al. (47) reported that the outer part of 18.4% and the inner part of 8.4% of the double-layered gloves were not damaged. Guo et al. (48) reported that 11.3% of the outer part of gloves that were worn double layer were torn while the inner part was intact, and that 8.9% of the gloves that were worn in one layer were torn (46-48). In the presented study, it was seen that double layer gloves were used in a rate which could not be underestimated. This situation might lead healthcare workers to more risky interventions by feeling more secure and might increase the rates of NSI.

According to the findings of the study presented, the nurses were unable to realize standard protection measures related to NSI and they reported that they had difficulties in finding equipment to protect against NSI. This might be due to the characteristics of the hospitals where the participants worked, because while private hospitals had to live on their own capital, state hospitals might have difficulty in supplying materials due to excessive patient load. In a study conducted by Özenir (49) in a private hospital in Turkey, it was reported that 79% of nurses had difficulty in accessing protective equipment.

Study Limitations

During the study, it was learned that different occupational groups (emergency medical technician, paramedic, midwife, perfusionist, prosthesis-orthotics technician, operating room technician) were employed under the name of operating room nurse in some institutions where the study was conducted (19.2%). Since these non-nurses were employed as operating room nurses, it was thought that it would be unethical to exclude

them from the sample, as they were considered to be at risk in terms of NSI. Discussing the results of these healthcare workers, who did not have nursing education, regarding NSI may have limited the observation of the nurses' injury status.

Unfortunately, many healthcare graduates are employed as nurses in hospitals for various reasons, although they do not graduate from nursing schools. In this case, examining and presenting these health workers' NSI separately may have correct results in many respects.

Conclusion

According to the findings of the study, the operating room nurses encounter a significant number of NSI. The conditions related to the institution where they work/employ rather than their sociodemographic characteristics play a prominent role in NSI. The fact that the majority of NSI is preventable indicates that institutional policies should change. It is recommended to investigate the causes and consequences of institutional sanctions in order to achieve the expected goals with experienced, highly educated nurses employed in the operating room.

Ethics

Ethics Committee Approval: The study was approved by the Bezmialem Vakıf University Non-interventional Clinical Research Ethics Committee (29.11.2016 8/93).

Informed Consent: Voluntary participation consent was obtained from the participants in writing.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: M.D., Y.Y.S., Design: M.D., Y.Y.S., Data Collection or Processing: M.D., Analysis or Interpretation: M.D., Y.Y.S., Literature Search: M.D., Y.Y.S., Writing: M.D., Y.Y.S.,

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Original Article

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Determining the Relationship between Perceived Social Support Levels and Chemotherapy Symptoms in Women with Gynaecological Cancer

Jinekolojik Kanserli Kadınlarda Algılanan Sosyal Destek Düzeyleri ve Kemoterapi Semptomları Arasındaki İlişkinin Belirlenmesi

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ABSTRACT

Objective: Patients experience uncertainty and fear about what chemotherapy will bring as a cancer treatment. This uncertainty and fear often increase their need for social support. Social support, regardless of whether it is provided by family, peers or health staff, plays a crucial role in coping with chemotherapy. Social support functions as a buffer against conditions such chemotherapy symptoms. It also helps patients cope with stress caused by chemotherapy symptoms and increase their quality of life. This research aimed to determine the relationship between perceived social support levels and chemotherapy symptoms in women with gynaecological cancer.

Methods: The study was planned descriptively. The sample comprised 148 patients who were admitted to two public hospitals. Data were collected using the Chemotherapy Symptom Assessment Scale, the Multidimensional Scale of Perceived Social Support (MDSPSP) and a descriptive feature form. Data were analysed using the SPSS software programs.

Results: There was a significant relationship detected between MDSPSP and skin, nail, throat, mouth and weight complaints, sleeplessness, anxiety and pessimistic feeling.

Conclusion: In this study, although social support was associated with chemotherapy-related symptoms (throat and mouth, skin and nails and feeling of tension), there was no significant relationship

ÖZ

Amaç: Kanser tedavilerinden biri olan kemoterapinin neler getireceği konusunda hastalar belirsizlik ve korku yaşamaktadırlar. Bu belirsizlik ve korku, sıklıkla hastaların sosyal destek gereksinimini artırmaktadır. Sosyal desteğin, aile, arkadaş yada sağlık personelinden gelmiş olması önemsenmeksizin, kemoterapiyle daha etkin baş etmede önemli rol oynadığı düşünülmektedir. Sosyal destek, kemoterapinin semptomları gibi olumsuz durumlara karşı bir tampon işlevi görmektedir. Sosyal destek, kemoterapinin neden olduğu stres ile başa çıkmasına ve bunun yanı sıra yaşam kalitelerini de artırmaya yardımcı olmaktadır. Araştırma, jinekolojik kanserli kadınlarda algılanan sosyal destek düzeyleri ve kemoterapi semptomları arasındaki ilişkinin belirlenmesi amacıyla yapılmıştır.

Yöntemler: Çalışma tanımlayıcı olarak planlandı. Örneklem, iki devlet hastanesine başvuran 148 hastayı içermektedir. Veriler Kemoterapi Semptom Değerlendirme Ölçeği, Çok Boyutlu Algılanan Sosyal Destek Ölçeği (ÇBASD) ve bir tanımlayıcı özellikler formu kullanılarak toplanmıştır. Veriler SPSS yazılım programları kullanılarak analiz edilmiştir.

Bulgular: Deri ve tırnaklar, ağız, boğaz, kilo, uyuma güçlüğü, endişeli veya sıkıntılı hissetme, karamsar veya üzüntülü hissetme ile ilgili problemler ile ÇBASD ölçeği arasında anlamlı bir ilişki bulunmuştur ($p<0,05$).

Sonuç: Bu çalışmada sosyal desteğin kemoterapiye ilişkin bazı semptomlar (boğaz ve ağız, deri ve tırnaklar ve gerginlik hissi) ile anlamlı ilişkisi bulunurken, bazı semptomları (nefes darlığı, ishal,

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with other symptoms (shortness of breath, diarrhoea, constipation, infection signs, alopecia and numbness in the hands or feet). It was concluded that social support status was an important factor for experiencing physical and psychological symptoms in Turkey.

Keywords: Gynaecological cancers, social support, chemotherapy

kabızlık, enfeksiyon belirtileri, alopesi, ellerde veya ayaklarda uyuşma) etkilemediği saptanmıştır. Sosyal destek durumunun ülkemizde kanser hastalarında fiziksel ve psikolojik semptom yaşamada önemli bir faktör olduğu sonucuna varılmıştır.

Anahtar Sözcükler: Jinekolojik kanserler, sosyal destek, kemoterapi

Introduction

Gynaecological cancers constitute 18% of cancers diagnosed in women (1). According to Pınar et al. (2), 16% of women die because of cancer-related reasons, and gynaecological cancer comes after breast cancer with the highest global mortality rates associated with cancer. In 2015, the American Cancer Society reported that among women, endometrial cancer ranked fourth as the most frequently diagnosed cancer and ovarian cancer accounted for 5% of cancer deaths (3). Similarly, the Department of Cancer Control announced that endometrial, ovarian and cervical cancers ranked fourth, seventh and ninth, respectively, as the most frequently diagnosed cancers in women in Turkey in 2014 (4).

Today, the nature of cancer is well understood, and treatment methods are developing rapidly. However, the toxic side effects of treatment are commonly seen. Although the positive effects of chemotherapy, such as increasing the life expectancy of patients, are known, it also has toxic qualities that cause undesirable side effects, including nausea, vomiting, loss of appetite and hair loss (5-7). Additionally, Bektaş and Akdemir (8) reported that patients who receive chemotherapy show depression and anxiety disorders that cause difficulties in coping with chemotherapy. The quality of life of patients is affected by disturbances in physical, emotional, social and economic status. Decreased quality of life increases the occurrences of depression, anxiety and social isolation, as well as similar psychological issues (9). Social support from family members, relatives and peers, in addition to others in the social circle, should be available to individuals who go through hardships or feel tense (10-13). Social support is known to influence physical and mental well-being and adaptation to cancer. Social support decreases harmful influences of negative life events on physical and mental well-being by buffering the individual and his or her stress. It also protects individuals from the negative effects of stress and is crucial for supplying a sense of belonging and self-esteem and helping them regain their health and feel good (14,15). Sufficient social support and coping with the psychological, social and mental problems associated with gynaecological cancer require additional supportive approaches to treatment and care (16,17). As a result, social support, regardless of whether it is provided by family, peers or medical staff, is crucial for effectively coping with cancer and its treatment. Although there are many studies on chemotherapy and its symptoms in the field of nursing, there are a limited number of studies that determine the relationship between chemotherapy symptoms and social support. In our country, no study has determined the relationship between chemotherapy symptoms and social support in gynaecological cancers. In this respect, the results of the study are valuable, and it is anticipated to make an important contribution to the nursing literature.

In addition to systematically evaluating the side effects of women with gynaecological cancer, social and psychological needs should also be considered. Nurses who care for cancer patients should be aware of the psychosocial needs of patients in addition to their physical needs and follow collaborative approaches that aim to adequately meet patients' needs.

This research aimed to determine the relationship between perceived social support levels and chemotherapy symptoms in women with gynaecological cancer and advise medical staff in future consultations.

Methods

Design and Setting

This descriptive design study was conducted at the outpatient chemotherapy unit of two public hospitals in Ankara. This research aimed to determine the relationship between perceived social support levels and chemotherapy symptoms in women with gynaecological cancer. The total population in the two public hospitals consisted of 605 patients with gynaecological cancer who received chemotherapy in 2008.

Sample Size

The following formula was used to determine the sample size for the research: the number of questions in the scales x number of answer options. For the Multidimensional Scale of Perceived Social Support (MDSPSP) and Chemotherapy Symptom Assessment Scale (C-SAS), 84 (12 questions x 7 answer options) and 96 (24 questions x 4 answer options) patients, respectively, were identified as the maximum number of patients among the sample sizes calculated. The C-SAS scale had 24 questions with four multiple options each and was thus equal to 96 patients. It is envisaged to include at least 1.5 times the number of patients determined for the sample size. The total sample size was calculated as follows: 96x1.5=144 patients (6). Quota sampling method was applied for sample selection, and voluntary sampling was conducted.

Participants and Procedures

Participants were 148 patients who were admitted to the two public hospitals in 2010. Participants were native Turkish-speaking patients who were diagnosed with non-metastatic gynaecological cancer and were receiving chemotherapy for the first time. Moreover, they have taken chemotherapy four times over time and had undergone a surgical procedure. All patients

were at least 18 years old and volunteered to participate. The study excluded patients who were disabled or had hearing, communication or psychiatric problems.

Research Question

What is the relationship of perceived social support on chemotherapy side effects?

Instruments

Data were collected using C-SAS, MDSPSP and a descriptive feature form developed by the researchers**.**

A descriptive feature form was used to gather sociodemographic information (age, education, marital status, living arrangements, income rate, residence area (village, town or city), position relative to the hospital (in the same place or not), transportation from and to the hospital, health insurance and job) and disease characteristics of the patients (diagnosis, disease duration, knowledge about the disease, information on drug treatment, experiencing treatment-related problems, taken preventions against the symptoms or not, someone supporting during illness and who was the most supportive during illness).

MDSPSP was developed to measure perceived social support (18). The scale's reliability and validity for use in Turkey were established by Eker et al. (18) in 2001. The highest and lowest scores that can be earned on the scale are 84 and 12, respectively. Higher scores indicate that there is relatively more social support (18). The revised MDSPSP of Eker, Arkar and Yaldız (18) was internally consistent, with a Cronbach's alpha of 0.92.

C-SAS was developed in England where its validity and reliability were initially established (19). In Turkey, a similar investigation was conducted by Aslan et al. (6) in 2003. Scores increased with the intensity and severity of symptoms. The Cronbach's alpha values for the internal consistency of the three C-SAS subscales are as follows: frequency of symptoms (α=0.67), intensity of symptoms (α=0.80) and severity of symptoms (α=0.82) (6).

The Implementation of Data Collection Tools

The researcher introduced herself before starting the data collection and gave information about the purpose of the research to the cancer patients. Data collection tools were applied during a face-to-face session by the researcher. Interviews to collect data lasted for just about ten min.

Statistical Analyses

Data collected through questionnaires and scales were transferred to a computer. The appropriateness of the normal distribution of scale scores was analysed using the Shapiro-Wilk test. All scores were not normally distributed. Number and percentage were used for categorical data and median and interquartile range for scale scores. MDSPSP scores were presented as mean ± standard deviation. Relationship between diagnosis and some patient information were investigated using the chi-square test. The relationship between the two scale scores were analysed using the Spearman rank correlation coefficient. MS Excel 2003 and SPSS for Windows version 15.0 package programme were used for all statistical analyses and calculations. A p value of <0.05 was considered to be statistically significant.

Ethical Issues

Before completing the questionnaires, the participants gave their consent in written form. Patients were under no obligation, financial or otherwise, to participate. Ethical approval was provided by the clinical research ethics committee in Ankara, and written approval was received from the surgeon generals of two public hospitals (B.10.4.1SM.4.06.00.22/2933 and B.10.4.1SM.4.06.00.09/773).

Results

Sociodemographic Characteristics

The mean age of the participants was 52.7±7.8 years. Of participants, 75% were married, 49.3% lived with their husbands and children, 42.5% did not have any education and 81.8% had medium-sized incomes. Furthermore, 68.3% lived in urban areas and 90.5% had social insurance (Table 1).

Distribution of Women's Cancer and Some of the Properties of Chemotherapy

In this study, 52.7% of participants were receiving chemotherapy four times, and 62.8% were diagnosed with ovarian cancer. Of participants, 77.7% declared that they had been informed about their disease. About two-thirds (67.6%) had been informed about chemotherapy medicines, and 66.2% had been informed about the possible side effects of medicines. Of participants, 55.4% had no prior history of discomfort with chemotherapy, whereas 81.8% of those who had previous discomfort were taking precautions (breathing exercise, regular diet, using herbal tea and some herbal foods, using a wig or hat, etc.). More than half (52.7%) of the sample reported that their husbands gave the maximum amount of social support, and 97.3% of the participants had at least one individual providing social support during the process (Table 2).

The Relationship Between Perceived Social Support and Chemotherapy Side Effects

Surprisingly, there was a significant positive correlation between social support from the family and skin- and nail-related problems such as dryness and itchiness (r=0.442, p=0.045), indicating that those with high social support from their family experienced more problems with their skin and nails. However, a weak positive correlation was observed between social support from a special person and throat- and mouth-related problems (r=0.38, p=0.024). Furthermore, there was a weak negative correlation between social support from a special person and weight changes (r=-0.254, p=0.026; Table 3).

There was a weak positive correlation between social support from a special person and total average score (r=0.393, p=0.002) as well as falling asleep difficulties (r=0.362, p=0.005). Perhaps patients who had falling asleep difficulties eventually got more social support from a special person, as reflected by their higher total social support score. Also, a weak significant positive

Table 1. Distribution of sociodemographic characteristics of patients (n=148)

Sociodemographic characteristics	n	%
Age (years)		
49 and below	51	34.5
50-55	35	23.6
56 and above	62	41.9
Marital status		
Married	111	75.0
Not married*	37	25.0
Education status		
Illiterate	40	27.0
Literate	23	15.5
Primary school	47	31.8
Middle school	10	6.8
High school and older	28	18.9
People who live together		
Alone	14	9.5
Spouse and children	73	49.3
Other (brother/sister, friend, relative, etc.)	61	41.2
Income rate		
Good	11	7.4
Middle	121	81.8
Low	16	10.8
Area of residence		
Rural	47	31.7
Urban	101	68.3
Social security		
Yes	134	90.5
No	14	9.5
Job		
Officer	4	2.7
Working at home (housewife)	118	79.7
Retired	12	8.1
Farmer	9	6.1
Other (self-employed, worker, unemployed, etc.)	5	3.4

*Single, widowed or divorced

correlation indicated that patients who reported greater feelings of tension or anxiety on the C-SAS also had higher MDSPSP scores from family (r=0.261, p=0.016) and friends (r=0.288, p=0.008) as well as a higher total score. A similar positive correlation was also found between pessimism and upset on the C-SAS and social support. Specifically, patients who experienced pessimism and upset received more social support from family (r=0.222, p=0.048) and friends (r=0.285, p=0.010) and also had higher overall total scores on the MDSPSP (r=0.349, p=0.002; Table 4).

Table 2. Distribution of patients' diseases and chemotherapy-related properties **(n=148)**

Properties of diseases and chemotherapy	n	%
Number of cures		
4	78	52.7
5	29	19.6
6	41	27.7
Cancer types		
Endometrial cancer	30	20.3
Ovarian cancer	93	62.8
Others*	25	16.9
Information on the disease		
Received	115	77.7
Did not receive	33	22.3
Information on medicines of chemotherapy		
Received	100	67.6
Did not receive	48	32.4
Information on possible side effects of medicines involved		
Received	98	66.2
Did not receive	50	33.8
Any history of disturbances with former cures of chemotherapy		
Present	66	44.6
Absent	82	55.4
Precautions for possible side effects (n=66)		
Taken	54	81.8
Not taken	12	18.2
Social support source		
Yes	144	97.3
No	4	2.7
Supporting individuals (n=144)**		
Partner	78	52.7
Sister/brother	22	14.9
Friend	5	3.4
Child	77	52.0
Others (relatives, neighbours, etc.)	15	10.1
Total	197	130.4

*Cervical cancer (n=23), vaginal cancer (n=1) and uterine tubal cancer (n =1)
**Since it can be more than one supporter, n folded.

Additionally, there was no statistically significant relationship between the other C-SAS symptoms and MDSPSP subgroups or total scores (p>0.05; Tables 3 and 4).

Discussion

In this study, 77.7% of our patients were knowledgeable of their disease. It was determined that 67.6% of the patients had knowledge about chemotherapy, 66.2% knew the side effects of chemotherapy and 44.6% had previously experienced problems in chemotherapy treatment (Table 2). According to the research of Pınar et al. (20), patients who receive information about their treatment have a higher quality of life and better overall well-being. The study of Özyurt (14) reported that 41% of the patients' emphasised that being informed was beneficial. Having knowledge about chemotherapy and knowing what to do and how to deal with any problems can provide comfort to patients. In our study, 18.2% of patients who experienced chemotherapy side effects did not take precautions, whereas 81.8% took precautions (Table 2). According to the research of Kayış (21), 11.1% of the patients who experienced chemotherapy side effects did not take precautions, and 68.9% took precautions. These results show that the majority of patients receiving chemotherapy take precautions against the side effects of treatment. However, when the proportion of patients not taking precautions was examined, the result suggested that some patients are not adequately helped to deal with side effects or the nursing services provided to these patients are not used effectively. It is thought that nurses should give more consultancy support to patients who do not take precautions.

Table 3. Co-relation between MDSPSP subgroups and C-SAS score

	MDSPSP subgroups							
Physical symptoms of C-SAS	Family		Peers		Special person		Sum of the scale	
	r	p	r	p	r	p	r	p
Nausea/vomiting prior to treatment	0.358	0.254	-0.044	0.891	0.338	0.283	0.199	0.536
Nausea after the treatment	0.010	0.922	0.180	0.078	0.037	0.716	0.156	0.127
Vomiting after the treatment	-0.053	0.697	0.082	0.542	-0.080	0.554	0.019	0.890
Constipation	0.032	0.783	0.095	0.415	0.000	1.000	0.043	0.711
Diarrhoea	0.144	0.473	-0.006	0.976	0.007	0.972	0.074	0.714
Aches	0.120	0.309	-0.136	0.249	-0.090	0.443	-0.102	0.386
Shortness of breath	-0.325	0.162	-0.152	0.521	-0.096	0.689	-0.136	0.568
Infection (fever, flu-like symptoms, etc.)	0.251	0.146	0.132	0.451	0.156	0.371	0.156	0.371
Sensation of pricking and lethargy in hands and feet	-0.011	0.910	-0.034	0.732	-0.048	0.631	-0.043	0.668
Problems of skin and nails (dry, itchy, etc.)	0.442	0.045	-0.030	0.897	0.191	0.408	0.166	0.471
Hair loss	0.015	0.885	0.017	0.868	-0.187	0.066	-0.110	0.280
Problems of throat and mouth (sensitivity or dryness)	0.141	0.419	0.191	0.272	0.381	0.024	0.284	0.098
Change in appetite	0.205	0.061	0.122	0.270	0.050	0.648	0.180	0.102
Weight loss or gain	0.030	0.792	-0.070	0.543	-0.254	0.026	-0.110	0.341
Problems of eyes (itchiness, sensitivity or dryness, etc.)	0.369	0.175	0.191	0.496	0.169	0.547	0.359	0.189
Sense of weakness	0.141	0.102	0.127	0.140	0.057	0.506	0.118	0.173
Sense of unusual exhaustion	0.061	0.498	0.081	0.372	0.003	0.974	0.026	0.771

MDSPSP: Multidimensional scale of perceived social support, C-SAS: Chemotherapy symptom assessment scale

Table 4. Co-relation between MDSPSP subgroups and C-SAS score

	MDSPSP subgroups							
Psychosocial symptoms of C-SAS	Family		Peers		Special person		Sum of the scale	
	r	p	r	p	r	p	r	p
Difficulties in falling asleep	0.194	0.141	0.108	0.415	0.393	0.002	0.362	**0.005**
Feeling anxious or tense	0.261	**0.016**	0.288	**0.008**	0.105	0.343	0.305	**0.005**
Feeling pessimistic or upset	0.222	**0.048**	0.285	**0.010**	0.195	0.083	0.349	**0.002**

MDSPSP: Multidimensional scale of perceived social support, C-SAS: Chemotherapy symptom assessment scale

Additionally, more than half of the sample (52.7%) reported that their husbands gave the maximum amount of social support (Table 2). According to the prosecutor's report, the support required by the individual is primarily provided by their immediate surroundings. Dansuk et al. (22) found that women receive more social support from their husbands and children than from any other group of social support providers. Further, Tel and Tel (23) found that women receive more social support from their families than from any other group of social support providers. However, Sammarco and Konecny's (24) research indicated that patients receive social support in the following order: from friends, family and health staff. According to Ayaz et al. (17), women need support from family, friends and medical staff during their treatment as the diagnosis of cancer, duration of treatment and obscurity of the disease cause social isolation. Social support serves to decrease cancer patients' anxiety, helps them cope with the disease and provides positive influences on their quality of life. Although social support could not eliminate the condition completely, it helps patients adapt to chronic physical or psychological conditions and has a positive influence on their mood and abilities to cope with the disease (25,26).

A significant positive correlation was found between social support degree provided by family members on the MDSPSP and skin- and nail-related problems (p=0.045; Table 3). It is thought that patients with physical appearance problems may be offered more social support than their families. There was also a statistically significant relationship, although it is weak (p=0.024), between social support degree provided by a special person on the MDSPSP and mouth- or throat-related problems (Table 3). As a consequence of the higher discomfort level associated with such symptoms, patients may get more support from "a special person" who can be a romantic interest, neighbour or family relative. In addition, throat- and mouth-related problems attract more attention because they cause pain and disability in speaking or prevent proper nourishment, which can further wear patients down. Therefore, under these conditions, it is possible that such patients would receive more support from their special person. Also, chemotherapy is highly likely to cause changes in the physical appearance of patients. Such changes are especially depressing for women. Consequently, some women develop more intimate bonds with their social support givers during treatment, whereas others withdraw from communication. MDSPSP scores and changes were significantly negatively correlated (p=0.026; Table 3). Patients who received less social support had more difficulties with weight. Chemotherapy treatment takes a long time, and there are many symptoms in this process. The long duration of physical symptoms can cause psychosocial problems (25). Therefore, social support is needed to cope with these problems. Social support may sometimes positively and sometimes negatively affect symptoms in this process. As a result, it is possible to expect a better perception of physical health (physical symptoms) in the long term by better understanding of psychological health and quality of life. According to Rustøen and Begnum (27), patients with breast cancer need psychological support and psychological improvements help make the physical condition more positive. There is some evidence that social support is associated with the physical and psychological adjustment of cancer patients to the disease, but a detailed study on these issues could not be reached in the literature review. We can say that there is a need for studies that determine the relationship between perceived social support and side effects seen in patients receiving chemotherapy.

As can be seen in Table 4, there is a weak significant positive correlation between support degree from a special person and both sleeping difficulties and total MDSPSP scores (p=0.002 and 0.005, respectively). Quality of life is highly related to sleeping. Göktalay et al. (28) noted that symptoms of chemotherapy influence the quality of life of patients no matter the intensity of symptoms (pain, fatigue, etc.). Although patients receive social support from their social circles, they still face sleeping difficulties. Even if the social supports cannot remove the stressful situation, they facilitate the adaptation to situation related to physical and psychological health, create positive moral effects and help patients cope with these problems (25). Studies have shown that many psychological methods, such as providing social support, are effective in reducing emotional (difficulty sleeping and stress) and physical symptoms (fatigue, pain, nausea and vomiting and taste change) associated with cancer treatment and enhancing coping skills (29,30).

Beser and Öz (31) pointed out that living with cancer involves learning how to cope with cancer symptoms, dealing with its influences on life and reorganising one's life for a new life standard. Since the course is long and full of side effects, patients may experience hopelessness, despair, anticipation of the worst, social isolation, depression or a sense of exhaustion. Our research indicated an increase in the total scores of MDSPSP (p=0.005) and its subgroups, family (p=0.016) and friends (p=0.008), as well as of the feelings of tension and anxiety on the C-SAS. Similar significant positive correlations were also observed between scores representing support provided by the family (p=0.048) and friends (p=0.010) and total MDSPSP scores (p=0.002) and feelings of pessimism or upset on the C-SAS (Table 4). These correlations were positive but weak. Karabulutlu et al. (32) supported the belief that cancer patients cope with the anxiety, depression and other negative emotions caused by their disease with help from their families and friends. Özdemir et al. (33) acknowledged that patients received 70% of social support from their spouses or children. The social support that patients need to cope with their condition should contribute to their self-esteem and self-confidence and make them feel secure. Unfortunately, social support occasionally conflicts with patients' self-esteem. As a consequence, patients feel insecure about themselves.

There is no statistically significant correlation between other subgroups or MDSPSP total score and C-SAS symptoms (p>0.05; Tables 3, 4). There are many side effects (nausea, vomiting, weakness, insomnia, anxiety, depression and the like) and therapeutic effects of chemotherapy, and these problems are the daily lives of patients functioning in the negative direction. The long duration of physical symptoms causes psychosocial problems. Thus, physical and psychosocial symptoms lead to an increase in patients' social support needs (25,34). In

Turkey, social support is often emphasised in patients receiving chemotherapy, and the findings of research on this subject are insufficient. Therefore, our study focuses on whether there is a relationship between chemotherapy-related symptoms and social support status patients perceive.

Conlusion

In conclusion, despite the limitations caused by the diseases and treatment methods, some important goals of health services have begun to include helping patients feel good, increase healthcare skills, maintaining daily life activities and adapting to the planned treatment programmes. In this regard, nurses as healthcare professionals have great responsibilities. This research showed that patients perceive social support in accordance with the intensity of their symptoms during treatment. The relation is very probably influenced by different cancer types or treatment and symptoms that each patient experiences. To take precautions against symptoms, discovering their causes and frequencies is essential. As a consequence, adaptation to treatment gets easier and patients' quality of life increases. Nurses who spend time with patients during the treatment have a significant responsibility since they also spend time with patients' family and friends.

Furthermore, a detailed study on these issues could not be reached in the literature review. There is a need for studies that determine the relationship between perceived social support and side effects seen in patients receiving chemotherapy. This research consisted of women without hearing or visual impairments who were at least 18 years old and were fluent speakers. It is possible to conduct a similar research without limiting the sample to Turkish speakers or those without visual or hearing defects.

Ethics

Ethics Committee Approval: Ethical approval was provided by the clinical research ethics committee in Ankara, and written approval was received from the surgeon generals of two public hospitals (B.10.4.1SM.4.06.00.22/2933 and B.10.4.1SM.4.06.00.09/773).

Informed Consent: Before completing the questionnaires, the participants gave their consent in written form.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: G.V., S.Y., Design: G.V., S.Y., Data Collection or Processing: G.V., S.Y., Analysis or Interpretation: G.V., S.Y., Literature Search: G.V., S.Y., Writing: G.V., S.Y.

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Original Article

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The Effect of Different Nutrition Education Approaches on Children's Nutritional Status

Farklı Beslenme Eğitimi Yaklaşımlarının Çocukların Beslenme Durumlarına Etkisi

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ABSTRACT

Objective: The study was performed to assess the potential of different educational approaches given to primary school children on their nutritional status.

Methods: The study was conducted with all second and third-grade students studying in an elementary school. First, 2 second and third-grade teachers were given nutrition education by the researcher. These teachers gave a healthy nutrition education to their classes for 2 months as indirect education. During the same period, the researcher gave direct nutrition education to other students. Before all education, a questionnaire and food consumption frequency forms were tested on all students. After waiting for 2 months, the food consumption frequency form applied to the students again.

Results: While the percentage of students' energy from total fat and saturated fat was decreased at the end of the education, the percentages of energy from monounsaturated fatty acids and polyunsaturated fatty acids were increased ($p<0.05$). The decrease in percentages of total fat and saturated fat is more efficient in indirect education ($p<0.05$). Direct nutrition education was more effective in increasing the intake of riboflavin, niacin and vitamin B12, while indirect nutritional education was more effective in increasing the intake of thiamine, A, B6, E and C vitamins ($p<0.05$).

Conclusion: Nutrition education given to children had a positive effect on their h nutritional status. This effect was moderately better through indirect education, and we believe this is due to the fact that students considered their teachers as role models.

Keywords: Child nutrition, nutrition, nutrition education

ÖZ

Amaç: Bu çalışma, ilkokul çocuklarına verilen farklı eğitim yaklaşımlarının çocukların beslenme durumlarına etkisinin belirlenmesi amacı ile yapılmıştır.

Yöntemler: Çalışma, bir ilkokulda okuyan tüm ikinci ve üçüncü sınıf öğrencileri ile gerçekleştirilmiştir. İlk olarak, dört sınıf öğretmeninden ikisine araştırmacı tarafından beslenme eğitimi verilmiştir. Biri 2. sınıf, biri de 3. sınıfla ilgilenen bu öğretmenler, dolaylı eğitim olarak, 2 ay boyunca kendi derslerinde konu ile ilgili sağlıklı beslenme eğitimi vermişlerdir. Aynı dönemde araştırmacı, diğer öğrencilere doğrudan beslenme eğitimi vermiştir. Eğitimlerden önce tüm öğrencilerden anket ve besin tüketim sıklığı formları alınmıştır. İki ay bekledikten sonra öğrencilere tekrar besin tüketim sıklığı formu uygulanmıştır.

Bulgular: Eğitim sonunda, öğrencilerin toplam yağ ve doymuş yağdan alınan enerji yüzdesi azalırken, tekli doymamış yağ asitlerinden ve çoklu doymamış yağ asitlerinden gelen enerji yüzdeleri artmıştır ($p<0,05$). Toplam yağ ve doymuş yağ yüzdelerindeki azalma dolaylı eğitimde daha etkilidir ($p<0,05$). Doğrudan beslenme eğitiminin riboflavin, niasin ve B12 vitamini artışlarında daha etkili olduğu, dolaylı beslenme eğitimi ise tiamin, A, B6, E ve C vitaminlerinin artış oranlarında daha etkili olduğu bulunmuştur ($p<0,05$).

Sonuç: Çocuklara verilen beslenme eğitimi, çocukların her iki durumda da beslenmeleri üzerinde olumlu bir etkiye sahiptir. Öğretmenlerin öğrenciler için rol model olması nedeniyle bu etkinin öğretmen eğitiminde ılımlı derecede daha çok olduğu düşünülmektedir.

Anahtar Sözcükler: Çocuk beslenmesi, beslenme, beslenme eğitimi

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Introduction

Child nutrition directly affects their growth, development and academic performance and is closely related to the state of health in adulthood. Optimal nutrition reduces the risk of developing iron-deficiency anaemia, vitamin D deficiency, obesity, dental caries and chronic diseases such as diabetes, osteoporosis and heart disease (1-3). Nutritional behaviours of individuals were established in childhood (4,5). It is, therefore, necessary to convey the correct information to children through nutrition education in childhood to have a positive impact. A study conducted on children and young adolescents showed that improvement of nutritional knowledge enables children to acquire better eating habits (6). For this purpose, regular nutrition education in primary, secondary and high schools is the best way to convert this information into behaviour (2,7). Nutrition education is a part of applied nutrition which is implemented to improve people's health, raise awareness of individuals about healthy nutrition and lifestyle. By increasing their scientific knowledge, they will probably translate this information into a lifestyle (8). School-based nutrition education aims to promote the welfare of the community through the recognition, adoption and motivation of healthier diet and eating practices by children and possibly by their families and, therefore, by the community (9). Since the risk of many non-communicable diseases is closely related to eating habits, various diet education programmes aiming at establishing healthy eating habits in children are being implemented worldwide (4). This study was planned and conducted to determine and evaluate the effect of nutritional education given to the second and third-grade students of a selected primary school by a dietician directly or indirectly with the class teacher trained by the dietician on the nutritional status of the students.

Methods

Study Design

For this study, the Ethics Committee Approval was obtained from Başkent University Medicine and Health Sciences Research Committee with the decision of 18/09 dated 03/01/2018. The study was conducted with all the second and third-grade students studying at the same school between January 2018 and April 2018, after obtaining the necessary permissions and approval from the Okan College Primary School Directorate. The permissions for the student's attendance to the study were given by the parents. The study included students whose parents allowed their children to participate in the study, read and signed the "Parent Voluntary Consent Form". The students also read and signed the "Student Voluntary Consent Form". Students with any chronic and/or metabolic disease, taking any medication, laxative and/or stool softener, oral supplementation were not included in the study. Two students who could not come to school due to their illnesses were excluded from the study. Two students who were excluded from the study were also given nutritional education, and anthropometric measurements were taken in order not to be separated from their classmates. However, their evaluations were not included in the study.

Participants and Recruitment

There are 2 second and 2 third-grade classes at the primary school. In this study, two groups were randomly selected as 1 second class and 1 third class in each group. Nutrition education is planned to be given directly to one group and indirectly to the other group. Indirect nutritional education (TE) is defined as the education given by the teachers, second and third-grade teachers trained by the researcher and direct nutritional education (DE) is defined when it is given directly to the other students by the researcher. All teachers voluntarily participated in the study. 2 teachers from indirect education were given nutritional education.

Procedures

Before educating the students, two teachers who were in indirect education were given nutrition education twice a week (total 140 minutes) for 70 minutes each. The other two teachers did not receive any nutrition education. Educated teachers transferred their acquired nutritional knowledge to their students through their own Life Science courses. The teachers devoted a total of 1 lesson (35 minutes) per week to related topics for 4 weeks (140 minutes in total). The topics are the same as the direct nutrition education group, which is parallel to the nutrition education that teachers receive from the researcher. Teachers integrated the topics into their lessons and explained them with a visual presentation.

In direct nutrition education, students were given nutritional education by a computer-assisted visual presentation during a course (35 minutes) and a total of 4-course hours (total 140 minutes) by the researcher simultaneous with the indirect nutrition education. The content of nutrition education given in all classes was the same, and the subjects included were as follows: 1- Healthy Nutrition-1: definition and importance of healthy nutrition, adequate and balanced nutrition, food groups. 2- Healthy Nutrition-2: nutrients and their importance for health, the healthy plate, food selection and daily nutrition issues. 3- Healthy Nutrition in Children: the importance of child nutrition, its role in the growth and development, problems in cases of inadequacy and imbalance, adequate and balanced nutrition in children. 4- Misconceptions and Frequently asked questions. Before the educations, a questionnaire including the demographic characteristics of the students and the frequency of nutrient consumption form were applied. At the end of the two month waiting period after the educations, the frequency of food consumption status was re-evaluated in students. The questionnaire form was designed by the researcher based on the review of the literature and the face to face interview method was used in the application of the questionnaire. The questionnaire included questions about the demographic characteristics of students such as age, gender and nutritional habits such as fruit and vegetable consumption of the students. The frequency and amount of food consumption of the students were recorded in the food consumption frequency form containing 49 different food types. The daily energy and nutrients of the students were calculated through the records. These data were analysed using the Computer-Aided Nutrition Programme (Nutrition Information

Systems Package Programme- BEBIS) developed for Turkey. The calculated energy and nutrient data were examined according to the dietary reference intake (DRI) by age and gender (10).

Statistical Analysis

Since the numbers in the groups were sufficient according to the central limit theorem for continuous data, the analyses were performed under the normal distribution approach (11).

Student's t-test was used for the comparison of two independent groups, Paired t-test was used for the comparison of two dependent groups and two-way analysis of variance was used for repetitive measurements from the general linear models to observe the change of independent groups over time. Descriptive statistics are expressed as mean, standard deviation, minimum and maximum values. For the analysis of categorical data, chi-square test was used for independent groups and Fisher Exact test was used when the number of observations less than 5 was above 20%. Marginal homogeneity test was applied for the dependent data with more than two groups, and descriptive statistics were expressed with frequency and percentage. Two ratios were compared for those with significant relationships. Significance level was taken as 0.05. Data were analysed using SPSS 21 package programme.

Results

A total of 70 students consisting of 32 females (45.7%) and 38 males (54.3%) with a mean age of 8.17 years (±0.68) participated in the study. The mean age of the DE group was 8.06 years (±0.56) and the mean age of the TE group was 8.26 years (±0.76). There was no statistically significant difference in age between the two groups (p>0.05). While 15 (46.9%) of the DE group were female and 17 (53.1%) were male, 17 (44.7%) of the TE group were female, and 21 (55.3%) were male. Gender was homogeneous in the education groups (p>0.05). 42 (60.0%) of the students who participated in the study were in the 2nd grade, and 28 (40.0%) of them were 3rd-grade students. There was no statistically significant difference between 1st and 2nd-grade students according to educational groups (p>0.05) (Table 1). While total fat, saturated fat, monounsaturated fatty acids (MUFA) and sodium intake of all students decreased significantly (p<0.05), all other macro and micronutrients, except potassium, showed a significant increase (p<0.05). DE was found to be more effective in riboflavin, niacin and vitamin B12 increase rates, while indirect nutritional education was more effective in increasing the consumption of thiamine, vitamins A, B6, E and C (p<0.05). The amount of vegetables and fruits intake and the number of students consuming the fruit without peeling increased with education. This increase was significantly higher in the indirect nutritional education group than the direct nutrition education group (p<0.05).

Discussion

Childhood is the period when the learning speed is the highest. It is considered as the period in which parents and teachers have important effects on child development. It is thought that all kinds of education given to school children are more effective, permanent and those habits, attitudes and behaviours acquired during childhood are reflected in adulthood. Hence, it is

Table 1. Distribution of students according to various characteristics

	DE X ± SD		TE X ± SD		Total X ± SD		p
Age, year							
Girl	8.13±0.52		8.24±0.83		8.190.69		0.662[a]
Boy	8.00±0.61		8.29±0.72		8.160.68		0.195[a]
Total	8.060.56		8.260.76		8.170.68		0.210[a]
p	0.645[b]		0.627[b]		0.065[b]		
	DE		**TE**		**Total**		**p**
	Number	**%**	**Number**	**%**	**Number**	**%**	
Sex							
Girl	15	46.9	17	44.7	32	45.7	0.858[a]
Boy	17	53.1	21	55.3	38	54.3	
Class level							
2nd	23	71.9	19	50.0	42	60.0	0.063[a]
3rd	9	28.1	19	50.0	28	40.0	
Total	32	100.0	38	100.0	70	100.0	

a: Chi-square test, b: Student's t-test, *: p<0.05, SD: Standard deviation
DE: Nutritional education directly provided by the dietitian, TE: Indirect nutrition education is given to the students by the teacher who has received nutritional education from the researcher

emphasised that nutrition and health education given to school-going children will have far-reaching benefits for society in gaining effective and lasting habits (12-14). A diet rich in fruits and vegetables has many health benefits, such as reducing the risk of cardiovascular disease, stroke and cancer (15). The World Health Organization recommends that children should consume at least 5 (400 g) portions of vegetables and fruits per day (16).

In this study, it was seen that while the consumption of vegetables and fruits before the education was below the recommendations, there was a significant increase at the end of the education and the consumption of the students reached the recommended levels. Both direct and indirect nutritional education had a positive effect. It was determined that indirect nutrition education had a better effect on daily fruit and vegetable consumption (p<0.001) (Table 2). Similarly, in a study lasting 14 months, teachers were educated about healthy nutrition and physical activity and, the effect of teachers on students was examined (17). As a result of the study, there was an increase in the consumption of vegetables and fruits. In another study in which the effect of direct nutrition education given by dietitian and indirect nutrition education given by the teacher was investigated on fruit and vegetable consumption of children, similar to this study, it was observed that both direct and indirect nutrition educations increased the consumption of vegetables and fruits. Indirect nutrition education was more effective than direct nutrition education (13). According to DRI, for children aged 7-9, the recommended daily fibre intake is 25 grams (18). The Turkey-specific dietary guidelines have been reported that 55.9% of children ages 7-10 in Turkey have fibre consumption below requirements (19). These data showed that the average daily fibre intake of students before the training was below the recommended amount (9.67±2.33). At the end of the education, it was observed that the average daily pulp intake of all students (28.99±8.47) exceeded the recommended level. Fibre intake increased both in direct and indirect nutrition education. However, the amount of fibre intake in indirect education was above the recommendations, while it remained below the recommendations in DE (Table 2).

Increasing the daily amounts of consumption of vegetables and fruits helps to increase the amount of daily fibre intake (20). In this study, indeed, the increase in the daily fruit and vegetable consumption helped to increase the daily fibre intake of the students above the recommended level. In addition to providing nutritional diversity through a healthy diet, the ratios of energy from carbohydrates, proteins and fats must be met. According to DRI for children aged 4-18, it is recommended that 50%-60% of daily energy is supplied from carbohydrates, 25%-35% from fats and 10%-20% from proteins (18). In this study, the percentage of total energy from carbohydrates for all students before the education was noted below the recommended level. At the end of the education, the percentages for both groups met the recommendations (p<0.05). Before the education, the percentage of total energy from fat was 40.59%±4.04% for all students. At the end of the educations, the percentages of these students' daily energy intake from fat were reduced to the recommended limits (TE 28.63%±1.00%, DE 31.25%±3.28%), (p<0.001). The percentage of daily energy intake from protein was found to be higher in TE group than n the direct nutrition education group (p<0.001). In healthy nutrition, the type of fat should be carefully examined as well as the ratio of energy from fat. It is recommended that the energy from the total fat taken from the diet is less than 8% for saturated fat, 10%-15% for MUFA and 6%-10% for polyunsaturated fats (PUFA). In total fat intake, the rate of energy from n-6 should be limited to 4%-13% and the rate from n-3 to 1%-2% (21). In this study, it was seen that the percentage of students' dietary energy from saturated fat was higher than the recommended levels in both direct and indirect education. But the percentage values at the end of the educations were significantly lower than previous ones (for all groups p<0.001). The percentage of the energy coming above-recommended level of saturated fat occurs probably, due to students' preference for animal-based foods as protein sources for increasing the protein intake. This, in turn, increased the saturated fat intake. Indirect nutrition education was found to be more effective in reducing the percentage of energy from saturated fat than direct nutrition education (p<0.001). The percentages of students' energy coming from the PUFA were lower than the recommended levels and after the education, and this was increased in all groups. Direct training was found to be more effective in this increase (p<0.05). While the percentage of

Table 2. Meal and vegetable-fruit consumption status of students

	DE		TE		Total		p_t	p_{GLM}
	X ± SD	Min-max	X ± SD	Min-max	X ± SD	Min-max		
Vegetable-fruit portion consumed daily								
Before education	1.37±0.83	0.30-4.00	1.32±0.72	0.40-3.00	1.34±0.77	0.30-4.00	0.787	**<0.001****
After education	4.75±0.67	3.00-6.00	6.79±1.23	5.00-9.00	5.86±1.44	3.00-9.00	**<0.001****	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
The daily amount of fibre intake (g)								
Before education	10.82±2.60	7.10-21.40	8.70±1.53	6.50-12.00	9.67±2.33	6.50-21.40	<0.001**	**<0.001****
After education	22.92±7.96	12.00-44.20	34.11±4.71	26.51-45.10	28.99±8.47	12.00-45.100	<0.001**	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			

p_{pair}: Paired t-test, p_t: Student's t-test, p_{GLM}: Two-way analysis of variance for repeated measurements from general linear models (GLM) *: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

students' daily energy from n-6 was within the recommended levels before and after the education, a significant increase was observed in all groups at the end. While the percentages of energy from n-3 were below the recommendations in all groups before the educations, it was increased to the recommended level at the end (for all groups p<0.001). The ratio of energy from n-3 fatty acids is higher in the direct nutrition education group (1.46%±0.24%) than in TE group (1.34%±0.24%) (p<0.05) (Table 3).

Vitamins and minerals, which are very important for the body, contribute to the growth and development and play an essential role in many biological processes such as energy metabolism, bone formation and preservation, blood production, immune system, maintenance of normal functions of the body cells and prevention of damage (22). In this study, while the levels of vitamin E, thiamine, calcium and iron of nutrition education students given both directly and indirectly were lower than the recommended levels, the levels of the two groups reached the reference values at the end of the education (p<0.001). In both groups, vitamin A, riboflavin, niacin, vitamin B6, vitamin B12, phosphorus, zinc and magnesium levels were found to be within normal ranges before the education and all the values were increased at the end. Regarding the increase in vitamins and minerals at the end of the education, indirect nutrition education was more effective for vitamin E, vitamin A, vitamin C, vitamin B6, thiamine, calcium, iron, phosphorus, magnesium and direct nutrition education was found to be more effective for

Table 3. Average energy, macro and micro nutrients of the students by diet

Energy and nutrients	DE		TE		Total		pt	pGLM
	$\bar{X}$ ±SD	Min-max		Min-max	$\bar{X}$ ±SD	Min-max		
Energy, kkal								
Before education	1465.80±220.73	1269.00-2497.50	1497.20±225.88	1153.5±2019.10	1482.89±222.48	1153.50-2497.5	0.559	0.658
After education	1570.26±248.33	1276.90-2550.50	1616.93±226.42	1260.90-2129.00	1595.60±236.10	1260.90-2550.50	0.414	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Energy from carbohydrates %								
Before education	50.41±3.42	43.00-57.00	46.13±4.22	36.00-53.00	48.09±4.41	36.00-57.00	**<0.001****	**<0.001****
After education	52.19±3.12	43.00-59.00	53.45±1.45	51.00-59.00	52.87±2.43	43.00-59.00	0.041*	
p_{pair}	0.060		**<0.001****		**<0.001****			

p_{pair}: Paired t Test, pt: Student's t Test, pGLM two-way analysis of variance for repeated measurements from general linear models (GLM)
*: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 3. Average energy, macro and micronutrients of the students by diet (cont.)

Energy and nutrients	DE		TE		Total		pt	pGLM
	X ± SD	Min-max	X ± SD	Min-max		Min-max		
Carbohydrate, g								
Before education	171.78±44.91	45.30-318.60	166.49±26.35	118.60-227.00	168.91±35.86	45.30-318.60	0.543	0.021*
After education	200.53±33.10	138.40-326.10	213.16±30.23	159.20-286.00	207.38±31.97	138.4-326.10	0.100	
ppair	**<0.001****		**<0.001****		**<0.001****			
Energy % from protein								
Before education	13.13±1.86	7.00-16.00	13.58±1.75	11.00-19.00	13.37±1.80	7.00-19.00	0.297	0.070
After education	16.50±1.95	13.00-20.00	18.13±0.99	16.00-20.00	17.39±1.71	13.00-20.00	**<0.001****	
ppair	**<0.001****		**<0.001****		**<0.001****			

ppair: Paired t-test, pt: Student's t-test, pGLM: Two-way analysis of variance for repeated measurements from general linear models (GLM)
*: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

riboflavin, niacin, B12 and zinc. In TE group, all vitamin and mineral levels except B12, potassium and zinc were found to be higher at the end. In a study, the nutrient intake of 146 female students aged 10-16 years was examined. It was observed that the average energy, protein, total fat, calcium, iron, vitamin A, riboflavin, niacin and vitamin C intake of the students were lower than the RDA (recommended daily intake level) (23). Nutrition education in children provides a significant positive impact on nutritional knowledge, nutritional behaviours and eating habits (24,25). In a study conducted with 72 female students

Table 3. Average energy, macro and micronutrients of the students by diet (cont.)

Energy and nutrients	DE		TE		Total		pt	pGLM
	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max		
Energy % from FAT								
Before education	40.92±4.44	32.00-49.00	40.32±3.79	31.00-48.00	40.59±4.08	31.00-49.00	0.543	0.087
After education	31.25±3.28	27.00-43.00	28.63±1.00	26.00-30.00	29.83±2.66	26.00-43.00	<0.001*	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Energy % from SFA								
Before education	17.26±3.93	12.04-33.58	20.43±3.75	13.77-33.36	18.98±4.13	12.04-23.58	0.001*	<0.001**
After education	13.21±4.44	6.71-26.47	10.26±1.94	7.41-14.74	11.61±3.61	6.71-16.47	0.001*	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Energy % from MUFA								
Before education	11.98±1.34	9.18-15.53	12.43±1.35	9.20-16.15	12.23±1.35	8.09-18.91	0.160	0.775
After education	10.85±2.13	8.34-18.91	11.16±1.35	8.09-14.19	11.01±1.74	3.25-9.06	0.464	
p_{pair}	0.358		0.219		0.125			

p_{pair}: Paired t t-test pt: Student's t t-test, pGLM: Two-way analysis of variance for repeated measurements from general linear models (GLM) *: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 3. Average energy, macro and micronutrients of the students by diet (cont.)

Energy and nutrients	DE		ÖE		Total		pt	pGLM
	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max		
Energy % from PUFA								
Before education	4.89±1.09	3.25-9.06	4.85±0.62	3.93-6.76	4.87±0.86	3.25-9.06	0.822	0.418
After education	5.63±1.80	3.96-12.81	5.36±0.88	4.38-7.82	5.48±1.37	3.96-12.81	0.428	
p_{pair}	0.005*		0.003*		**<0.001****			
Energy % from n-3								
Before education	0.85±0.38	0.51-2.69	0.94±0.19	0.63-1.47	0.90±0.30	0.51-2.69	0.188	0.715
After education	1.34±0.24	0.83-1.96	1.46±0.24	1.03-1.86	1.41±0.24	0.83-1.96	0.033*	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Energy % from n-6								
Before education	4.11±1.33	2.09-8.06	3.94±0.74	2.57-5.94	4.02±1.05	2.09-8.06	0.509	-
After education	5.03±1.77	2.53-10.11	4.92±1.50	3.13-9.92	4.97±1.62	2.53-10.11	0.778	
ppair	**<0.001****		**<0.001****		**<0.001****			

p_{pair}: Paired t-test, pt: Student's t-test pGLM: Two-way analysis of variance for repeated measurements from general linear models (GLM)
*: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 3. Average energy, macro and micronutrients of the students by diet (cont.)

Dietary vitamin intake	DE		TE		Total		pt	pGLM
	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max		
Vitamin A, mcg RE								
Before education	592.82±151.77	401.00-1000.00	683.97±230.74	413.00-1367.00	642.30±202.46	401.00-1367.00	0.052	0.368
After education	697.31±162.71	469.00-1243.00	840.22±263.57	520.00-1459.50	774.89±233.00	469.00-1459.50	0.007*	
p_{pair}	0.001*		0.002*		<0.001**			
Vitamin E, mg								
Before education	5.46±2.02	2.80-12.20	4.82±1.31	3.30-10.90	5.11±1.69	2.80-12.20	0.128	<0.001**
After education	10.83±4.15	6.20-23.80	14.23±3.12	10.80-20.20	12.68±3.98	6.20-23.80	<0.001**	
p_{pair}	<0.001**		<0.001**		<0.001**			
Thiamine, mg								
Before education	0.53±0.08	0.40-0.70	0.57±0.12	0.40-0.80	0.56±0.20	0.40-0.80	0.106	<0.001**
After education	0.88±0.24	0.50-1.60	1.23±0.20	1.00-1.70	1.07±0.28	0.50-1.70	<0.001**	
p_{pair}	<0.001**		<0.001**		<0.001**			
Riboflavin, mg								
Before education	1.14±0.35	0.40-1.80	1.71±0.47	1.10-2.80	1.45±0.50	0.40-2.80	<0.001**	0.003*
After education	1.68±0.34	1.10-2.50	1.91±0.24	1.50-2.40	1.80±0.31	1.10-2.50	0.003*	
p_{pair}	0.001*		0.007*		<0.001**			

p_{pair}: Paired t-test pt: Student's t-test pGLM: Two-way analysis of variance for repeated measurements from general linear models (GLM), *: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 3. Average energy, macro and micronutrients of the students by diet (cont.)

Dietary vitamin intake	DE		TE		Total		pt	pGLM
	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max		
Niacin, mg								
Before education	8.15±2.01	3.90-11.00	10.90±3.18	7.70-19.00	9.64±3.02	3.90-19.00	**<0.001****	0.248
After education	10.58±3.28	6.90-17.40	12.25±1.99	9.00-15.90	11.49±2.77	6.90-17.40	0.016*	
p_{pair}	**<0.001****		0.036*		**<0.001****			
Vitamin B6, mg								
Before education	0.85±0.21	0.40-1.20	1.08±0.26	0.80-1.70	0.98±0.26	0.40-1.70	**<0.001****	0.024*
After education	1.29±0.31	0.90-2.10	1.69±0.22	1.40-2.20	1.51±0.33	0.90-2.20	**<0.001****	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Vitamin C, mg								
Before education	58.35±18.05	20.30-87.40	68.47±16.13	49.50-109.90	63.84±17.66	20.30-109.90	0.016*	**<0.001****
After education	99.87±30.48	47.10-158.70	145.03±20.46	120.50-190.90	124.38±33.99	47.10-190.90	**<0.001****	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Vitamin B12, mcg								
Before education	1.31±0.30	0.90-2.10	1.22±0.25	0.87-1.70	1.26±0.27	0.87-2.10	0.154	0.385
After education	2.82±0.99	1.40-4.50	2.56±0.50	1.70-4.10	2.68±0.77	1.40-4.5	0.178	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			

ppair: Paired t-test, pt: Student's t-Test, pGLM: Two-way analysis of variance for repeated measurements from general linear models (GLM)
*: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 3. Average energy, macro and micronutrients of the students by diet (cont.)

Dietary mineral intake	DE		TE		Total		pt	pGLM
	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max		
Sodium, mg								
Before education	2375.51±511.67	1356.70-3468.40	2025.97±578.02	1313.80-3610.20	2185.76±572.31	1313.80-3610.20	0.010*	0.009*
After education	1685.88±204.38	1446.00-2456.10	1719.71±101.43	1538.00-2898.00	1704.24±156.76	1446.00-2456.10	0.399	
p_{pair}	**0.001****		0.003*		**<0.001****			
Potassium, mg								
Before education	4363.16±565.66	3199.00-5565.00	4021.71±299.97	3294.00-4744.00	4177.80±470.49	3199.00-5565.00	0.004*	-
After education	4258.65±667.41	3014.80-5206.40	4173.05±632.00	3056.70-5301.00	4212.18±645.10	3014.80-5301.00	0.584	
p_{pair}	0.440		0.171		0.689			
Calcium, mg								
Before education	492.61±107.33	156.60-659.60	539.94±147.41	299.50–884.80	518.30±131.88	156.60-884.80	0.126	0.075
After education	998.70±215.09	562.80-1447.00	1138.56±133.24	860.30-1413.50	1074.62±187.70	562.80-1447.00	0.002*	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Iron, mg								
Before education	7.82±1.14	5.60-10.70	8.43±1.77	6.50-12.80	8.15±1.54	5.60-12.80	0.089	**<0.001****
After education	10.96±3.07	6.30-19.10	14.86±1.88	11.70-19.40	13.08±3.16	6.30-19.40	**<0.001****	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			

p_{pair}: Paired Tt-Testt: Student's t Tt-TestGLM: Two-way analysis of variance for repeated measurements from general linear models (GLM)
*: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

attending primary school, 4-step nutrition education was given to the students, and a questionnaire was tested before and 2 months after the education. As a result, it was seen that students' knowledge, attitudes and behaviours improved positively with education (2). Similarly, another study that provided a nutrition education programme to 64 low-income school children for 13 weeks showed that children had positive behavioural changes after education (26). At the school-going age, students spend long hours in school. They are in constant contact with their teachers, and in the process, students take their teachers as role models. Therefore, it is very important to carry out advanced school-based nutrition education. Besides, whether the teachers are interested in the subject is important regarding their impact on the students (14,27). That is why teacher education curricula should include detailed nutrition and wellness education courses. Thus currently working teachers participating in in-service training programmes organised by nutrition experts can be ensured.

Study Limitations

The difference in teachers' interest in nutrition is the most restrictive factor of the study. The teacher must have an interest in education, the enthusiasm to turn this information into a lifestyle, and the ability to transfer it to students. In our study, although the teachers were thought to be effective in this respect, the results would have been different if they had exerted more effort. Studies in which teachers' nutritional knowledge and behaviour change can be detected will form more precise judgements on this result.

Conclusion

An adequate and balanced diet is very important for children to ensure their growth and development. Diseases that develop as a result of unhealthy diet and poor lifestyle progress over time, affecting their health negatively. Gaining healthy eating habits delays and/or eliminates the risk of developing adulthood diseases. Nutrition is a lifestyle, and it needs to be integrated into life from childhood. Since the experiences gained in childhood are reflected in adulthood, it is necessary to practice and teach children adequate and balanced nutrition and a healthy lifestyle. Therefore, nutritional education programmes for school-age children are needed, and these programmes must be available across the country to see socially positive results.

Table 3. Average energy, macro and micronutrients the students by diet (cont.)

Dietary mineral intake	DE		TE		Total		pt	pGLM
	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max	$\bar{X}$ ±SD	Min-max		
Phosphorus, mg								
Before education	719.47±109.11	353.80-914.70	776.71±163.16	572.40-1095.00	750.54±143.00	353.80-1095.00	0.085	**<0.001****
After education	1334.19±368.34	756.10-2350.70	1718.07±215.82	1360.60-2194.30	1542.58±350.76	756.10-2350.70	**<0.001****	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Zinch, mg								
Before education	7.62±1.31	4.50-10.20	7.99±1.30	6.10-11.90	7.82±1.31	4.50-11.90	0.235	0.017*
After education	10.87±1.04	7.80-12.30	10.29±0.91	8.70-11.90	10.55±1.01	7.80-12.30	0.016*	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			
Magnesium, mg								
Before education	241.80±22.35	141.90-241.00	268.98±45.45	121.20-389.00	256.56±38.96	205.70-389.00	0.002*	**<0.001****
After education	350.09±84.60	220.70-590.70	478.64±60.11	371.70-601.50	419.88±96.51	220.70-601.50	**<0.001****	
p_{pair}	**<0.001****		**<0.001****		**<0.001****			

p_{pair}: Paired t Tt-Testt: Student's t Tt-TestGLM: two-way analysis of variance for repeated measurements from general linear models (GLM)
*: p<0.05, **: p<0.001, SD: Standard deviation, Min: Minimum, Max: Maximum

Schools and teachers have great responsibilities in educating the children with healthy eating and lifestyle habits. School children who spend most of the day at the school also meet their nutritional needs at the school. Meanwhile, they remain under the influence of their fellow students and teachers. Interestingly, 2nd and 3rd-year students from primary school also take their teachers as role models, and it affects their nutrition and lifestyle. For this reason, awareness-raising education should be given to developing a healthy diet and quality lifestyle. Furthermore, education should be organised to create and increase the knowledge of teachers on these issues.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Baskent University Institutional Review Board and Ethics Committee for this study (2018, 18/9).

Informed Consent: Informed consent form has been signed.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: B.A.Ö., Concept: B.A.Ö., A.E., Design: B.A.Ö., A.E., Data Collection or Processing: B.A.Ö., Analysis or Interpretation: B.A.Ö., Literature Search: B.A.Ö., Writing: B.A.Ö., A.E.

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Original Article

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Effect of Thermocycle and Bonding Agents on the Bond Strength of Titanium-resin Cements

Titanyum-rezin Siman Bağlanma Dayanımına Isıl Döngü ile Yaşlandırmanın ve Bonding Ajanlarının Etkisi

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ABSTRACT

Objective: This research aimed to evaluate the effect of different resin cement (RC) types, compare the effect of surface treatments and bonding applications and evaluate the effect of thermal cycling on bond strengths to Titanium (Ti) surfaces.

Methods: A total of 240 Ti discs (10x3 mm) were randomly divided into two groups. Half of the specimens were sandblasted with 110 μm Al_2O_3 particles, whereas the other half had no surface treatments (non-treated). Both sandblasted and non-treated specimens of each surface treatment type were divided into five subgroups, which received one of the following surface conditions and luting self-adhesive resin cement: (a) Panavia SA Cement, (b) Clearfil SE Bond + Panavia SA Cement, (c) RelyX U200, (d) Single Bond Universal + RelyX U200 and (e) MIS Crown Set Cement. A mould with a 4-mm diameter and 2-mm thickness was applied to the central region of the specimens. Each group was divided into subgroups, according to whether performing thermocycling or not. The shear bond tests were conducted at a crosshead speed of 1 mm/min. Data (N) were analysed using one-way analysis of variance and Tukey's honestly significant difference tests ($p<0.05$).

Results: The sandblasted + bonding agent groups provided higher shear bond strength than the non-treated groups for all RC types ($p<0.05$). Sandblasted Clearfil SE Bond + Panavia SA Cement (non-thermocycled) showed the highest values (182.761±41.55), whereas the MIS Cement (17.681±9.33) and Panavia SA Cement

ÖZ

Amaç: Bu çalışmanın amacıfarklı rezin simanların, kumlama yüzey işleminin, bonding ajanlarının ve yaşlandırmanın Titanyum (Ti)-rezin siman (RS) arasındaki makaslama bağlanma dayanımına etkisinin değerlendirilmesidir.

Yöntemler: İki yüz kırk adet Ti disk (10x3 mm) yüksekliğinde olacak şekilde Ti bloklardan kazınarak hazırlandı ve akril reçine içerisine gömüldü. Rastgele olarak 2'ye ayrılan Ti disklerin yarısına 110 μm Al_2O_3 ile kumlama işlemi yapıldı, yarısına herhangi bir yüzey işlemi uygulanmadı. Hem kumlanmış hem de yüzey işlemsiz olan bu iki grup da 5 alt gruba ayrıldı. a) Panavia SA Cement (Kuraray) b) Clearfil SE Bond (Kuraray) + Panavia SA Cement (Kuraray) c) Rely X U-200 (3M-Espe) d) Single bond Universal + Rely X U-200 (3M-Espe) and Mis Crown Set Cement (MIS). RS'ler özel bir kalıpla 4 mm çapında ve 2 mm kalınlıkta olacak şekilde Ti disklerin ortasına yerleştirildi. Sonrasında her grup kendi içerisinde yaşlandırma uygulanıp uygulanmamasına göre 2 alt gruba daha ayrıldı. Makaslama bağlanma dayanımı testleri 1 mm/dk hızla yapıldı. Veriler one-way ANOVA ve Tukey HSD testi kullanılarak istatistiksel olarak analiz edildi.

Bulgular: Gruplar arasında anlamlı bir fark vardır ($p<0,05$). Tüm rezin siman grupları için kumlanmış ve bonding ajan uygulanmış gruplar, yüzey işlemsiz gruplara göre daha yüksek bağlanma dayanımı göstermiştir ($p<0,05$). Kumlama + Clearfil SE Bond (Kuraray) + Panavia SA Cement (yaşlandırma uygulanmamış) grup en yüksek bağlantı değerini (182,761±41,55) gösterirken,

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(15.32±7.38) non-treated (thermocycled) groups had the lowest values.

Conclusion: Sandblasting and bonding agents can improve bond strength. The thermocycling period decreased the bond strength values for all groups.

Keywords: Titanium, resin cement, thermocycle, bond strength, semipermanent cements, methacryloyloxydecyl dihydrogen phosphate

MIS Cement (17,681±9,33) ve Panavia SA Cement (15,32±7,38) yüzey işlemsiz (yaşlandırma uygulanmış) gruplar en düşük değerleri göster-miştir.

Sonuç: Kumlama ve bonding ajan uygulaması bağlanma dayanımı arttırmıştır. Isıl döngü ile yaşlandırma tüm gruplar için bağlantı değerlerini düşürmüştür.

Anahtar Sözcükler: Titanyum, rezin siman, termal siklus, bağlantı dayanmı, semipermanent simanlar, MDP

Introduction

The durability of the connection between the prosthetic superstructure and implant is an essential factor for the longevity of implant-supported fixed denture prostheses (FDPs). This integrity is achieved by either cement or screw retention, which has advantages and disadvantages compared with one another (1). In today's dental practice, the choice of cement versus screw retention of implant-supported FDPs mostly depends on the clinicians' experience and preference with respect to the clinical situation (2).

One advantage of cement-retained implant-supported FDPs is the compensation of improperly placed implants. Especially in the anterior region, it is often impossible to manage aesthetics due to the visibility of the screw access hole (3). Moreover, it is observed that clinicians prefer cemented implant restorations because of their lower complication rate and higher fracture resistance of veneering porcelain (4,5). The situation becomes more challenging if the basic mechanical parameters are not optimum as well, such as reduced abutment/restoration interface and over tapered abutment due to the interarch tooth relations, especially for a single implant-retained crown, which might probably result in decementation, although permanently cemented. However, it is often preferred to make temporary cementation for cement-retained FDPs to maintain retrievability without damage to abutment or implant. However, temporary cementation might result in debonding, especially for restoring reduced abutments because of poor physical properties such as decreased tensile strength and increased solubility (6,7). According to this problem, using permanent cements, including polycarboxylate cement and self-adhesive resins, seems appropriate for cementing fixed implant-supported prostheses due to their high retentive values and lower retention loss risk (8,9). Several studies have also reported the unexpectedly high bonding values of polycarboxylate cement with Titanium (Ti) structures and indicated that some dental cements, including glass ionomer and polycarboxylate, alter the protective Ti oxide layer, resulting in colour changes (10). Even the instructions for using one polycarboxylate cement state that a discoloration effect may result when used with Ti. Thus, it is necessary to use resin cement (RC) for Ti cementation, especially when applying Ti to new areas for aesthetics, such as two-component abutments.

To get the advantages of both cementation types, semipermanent cements that provide the reduction of retention by using petroleum jelly or acrylic with polyurethane resin are advised by various manufacturers, particularly for cementing implant-supported crowns for adequate retention and easy restoration removal (11,12). For both semipermanent and permanent cement types, surface treatments are an important effect for clinical longevity. Several studies about the bond strength of Ti and the effect of different surface treatments were published in the literature (13-15). Micromechanical and chemical bonding effects were compared with these studies (14). Sandblasting with Al_2O_3 particles is the most commonly used method for micromechanical retention promotion and chemical bondings, and it can be achieved by both bonding mechanisms, such as silica coating systems (coJet and Rocatec) and metal primers (16,17). Several surface treatment combinations are possible, so studies investigate the effects of different cementation type protocols. But there are no guidelines on the most appropriate luting procedure between the Ti-RC bond strengths.

Thermocycling can simulate the effect of destructive oral conditions with temperature changes and masticatory forces (18). Therefore, this study aimed to evaluate and compare the effect of surface treatments on shear bond strength (SBS) of two self-adhesive RCs and one semipermanent cement to Ti surfaces before and after thermocycling. The null hypothesis tested was that the RCs tested provide similar bond strength to the non-treated Ti surfaces and the surface modification of Ti enhanced the bond strength values for all types of RCs.

Methods

A total of 240 Ti discs (10 mm in diameter and 3 mm in height) were fabricated and embedded in acrylic resin blocks. A total of 20 groups were planned for this study, with n=12. Firstly, 240 Ti discs were randomly divided into two groups; half of the specimens were sandblasted with 110 μm Al_2O_3 particles, and the other half had no surface treatments (non-treated). Both sandblasted and non-treated specimens were divided into five subgroups, which received one of the following surface conditions and self-adhesive RCs: (a) Panavia SA Cement (Kuraray Noritake Dental Inc., Okayama, Japan), (b) Clearfil SE Bond (Kuraray Noritake Dental Inc.) + Panavia SA Cement, (c) RelyX U200 (3M-Espe, MN, USA), (d) Single Bond Universal + RelyX U200 and (e) MIS Crown Set Cement (MIS, Israel). The materials used in this study are listed in Table 1.

Bonding and Testing Procedures

The Ti discs were ultrasonically cleaned in 96% isopropanol for 3 min, followed by air drying. A mould with a 4 mm diameter and 2 mm thickness was placed on the central area of each of the Ti surfaces. RCs were applied into the moulds with or without using the relevant bonding agents for the determined subgroups, and the cementation procedures were completed according to the manufacturer's instructions (Figure 1). For the bonding groups, a bonding agent was applied for 10 s, air-dried for 5 s and light-cured for 20 s at a 5 mm distance from the sample's surface and at a 1,200 mW/cm intensity (BluePhase curing light, Ivoclar Vivadent, Liechtenstein) for polymerisation. The RC application procedure was the same for all bonding and non-bonding groups, and RC was applied with the same Teflon mould. The samples were light-cured for 5 s with the same distance and light source for initial polymerisation. After gently removing the Teflon mould, each side of the RC cylinders was light-cured for 20 s. The bonding process was performed as recommended by the manufacturers. The bonded specimens were stored in distilled water at 37 °C for 24 h, and subsequently, each group was again divided into two subgroups, according to whether performing thermocycling or not. The thermocycling procedure was set as 5,000 cycles between 5 °C and 55 °C (Thermal Cycler Tester, Dental Teknik, Konya, Turkey); the dwell and transfer times were 30 and 10 s, respectively. The SBS tests were performed using a universal testing machine (TSTM 02500, Elista Ltd., Şti., Istanbul, Turkey) at a 1 mm/min crosshead speed via a knife-edge rod. The failure loads were in *N*. The failure modes were analysed under a stereomicroscope (Olympus SZ40, Olympus Optical Co., Tokyo, Japan) at 40x magnification. The failures were classified as adhesive, cohesive or mixed failure.

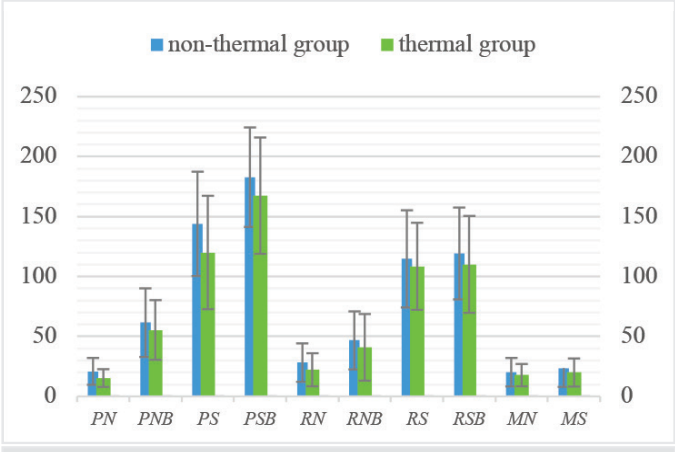

Figure 1. Comparision of the shear bod strength of the groups

Statistical Analysis

Statistical package SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA) was used at a significance level of α=0.05. The Kolmogorov-Smirnov statistical test was performed on the SBS values to evaluate the normal distribution and homogeneity of variances. One-way analysis of variance and Tukey's honestly significant difference test were conducted to determine statistical differences in the SBS values between subgroups, and an independent paired t-test was applied to determine the effect of thermocycling on SBS values.

Results

Table 2 lists the mean, median and standard deviation of the SBS values and summarises the results of the statistical tests that indicated significant differences between groups ($p<0.05$). The differences between the groups are shown in Table 1. Sandblasted and sandblasted + bonding agents provided significantly higher SBS compared with the non-treated and non-treated + bonding agent groups for all RC types ($p<0.05$), except for MIS Crown Set Cement ($p>0.05$). In both non-thermocycled and thermocyled specimens, the sandblasted Clearfil SE Bond + Panavia SA Cement group showed the highest values, whereas the non-treated Panavia SA Cement thermocycled (15.32±7.38) and non-treated MIS Cement thermocycled (17.681±9.33) groups showed the lowest values.

The highest SBS values were recorded for the Panavia sandblasted + bonding agents than the other groups after thermal cycling ($p<0.05$). However, there was a significant effect on applying bonding agents on sandblasted or non-treated surfaces for only Panavia cement for both thermal and non-thermal conditions ($p<0.05$). Additionally, sandblasting alone created higher SBS values for Panavia and RelyX cements compared with applying bonding agents only and non-treated surfaces ($p<0.05$). The SBS values of the sandblasted and sandblasted + bonding agent Panavia and RelyX groups were significantly different from those in the non-treated and non-treated + bonded groups ($p<0.05$).

Also, for the thermocycled groups, the Panavia SA Cement non-treated group showed the lowest SBS values, followed by the MIS Crown Set Cement non-treated, MIS Crown Set Cement sandblasted and RelyX Cement non-treated groups. But there

Table 1. Materials and surface treatments used in this study

	Surface treatments, code	
	Non-treated, N	110 µm Al_2O_3 Sandblasting, S
Cementing procedure	Panavia SA Cement, P	Panavia SA Cement, P
	Clearfil SE Bond (Kuraray) + Panavia SA Cement (Kuraray), PB	Clearfil SE Bond (Kuraray) + Panavia SA Cement (Kuraray), PB
	RelyX U200 (3M-Espe), R	RelyX U200 (3M-Espe), R
	Single Bond Universal + RelyX U200 (3M-Espe), RB	Single Bond Universal + RelyX U200 (3M-Espe), RB
	MK Crown Set Cement (Moredent), M	MK Crown Set Cement (Moredent), M

were no statistically significant differences between these four groups (p>0.05).

The thermal cycling effect was evaluated and showed that the thermocycling period decreased bond strength values for all groups, but there were no statistically significant differences (p>0.05).

Failure Mode Analysis

All failure types of the sequential sandblasted and sandblasted + bonding agents were seen as adhesive. Adhesive failures were also the predominant failure types in all RC types and both thermal and non-thermal conditions. Mixed and cohesive failure modes of RC were evident in the sandblasted and sandblasted + bonding agent groups of Panavia and RelyX cements for both thermal and non-thermal conditions (Table 3). Microscopic image samples for each failure mode are displayed in Figure 2.

Discussion

The results of this study indicate that RCs that bonded to non-treated Ti surfaces yielded similar SBS values regardless of the cement type and thermocycling procedure. Nevertheless, although the bond strengths of two self-adhesive RCs tested were significantly increased by sandblasting with or without the application of bonding agents, sandblasting did not have any favourable effect on the bond strength of semipermanent cement. Therefore, the null hypothesis was partially accepted. As for the groups with bonding agents solely, no statistical differences were found compared with the non-treated groups

Table 2. Mean and standard deviation of the tested groups

Non-thermocycle.			Sig.	Thermocycle.		Sig.	T-test sig.
Group	Mean	SD		Mean	SD		
PN	20.9[a]	11.2		15.3[a]	7.4		0.117
PNB	61.5[b]	28.6		55.4[b]	24.9		0.554
PS	143.9[c,d]	43.5		120.01[c]	47.3		0.211
PSB	182.8[e]	41.5		167.4[d]	48.5		0.414
RN	28.2[a]	16.03		22.2[a]	13.8		0.294
RNB	46.7[a,b]	24.2		40.9[a,b]	27.8		0.576
RS	114.7[c]	40.5		108.5[c]	36.3		0.603
RSB	119.2[c]	38.3	0.000	110.1[c]	40.4	0.000	0.568
MN	20.2[a]	11.9		17.7[a]	9.3		0.621
MS	23.6[a]	15.7		19.9[a]	11.7		0.500

The same superscripts indicate statistically insignificant difference.
[+]One-way analysis of variance (p<0.05).
[++]Tukey honestly significant difference (p<0.05).
PN, Panavia SA Cement non-treated; PNB, Clearfil SE Bond + Panavia SA Cement non-treated; PS, Panavia SA Cement sandblasting; PSB, Clearfil SE Bond + Panavia SA Cement sandblasting; RN, RelyX U200 non-treated; RNB, Single Bond Universal + RelyX U200 non-treated; RS, RelyX U200 sandblasting; RSB, Single Bond Universal + RelyX U200 sandblasting; MN, MK Crown Set Cement non-treated; MS, MK Crown Set Cement sandblasting.

Table 3. Types of bonding fracture failures for each group

Failure types	Adhesive	Mixed	Cohesive	Adhesive	Mixed	Cohesive
Groups	Non-thermocycle			Thermocycle		
PN	10	2	-	12	-	-
PNB	8	3	1	9	3	-
PS	7	4	1	8	4	-
PSB	5	5	2	6	5	1
RN	12	-	-	12	-	-
RNB	8	4	-	9	3	-
RS	8	4	-	8	4	-
RSB	6	5	1	9	3	
MN	12	-	-	12	-	-
MS	12	-	-	12	-	-

PN, Panavia SA Cement non-treated; PNB, Clearfil SE Bond + Panavia SA Cement non-treated; PS, Panavia SA Cement sandblasting; PSB, Clearfil SE Bond + Panavia SA Cement sandblasting; RN, RelyX U200 non-treated; RNB, Single Bond Universal + RelyX U200 non-treated; RS, RelyX U200 sandblasting; RSB, Single Bond Universal + RelyX U200 sandblasting; MN, MK Crown Set Cement non-treated; MS, MK Crown Set Cement sandblasting.

for RelyX (p>0.05). Meanwhile, the bond strength values of the sandblasted + bonding agent groups were significantly higher than the bonding agent and non-treated groups (p<0.05).

The permanent RCs used in this study (Panavia SA Cement and RelyX U200) are dual-cured RCs applied solely and applied with a universal adhesive that belongs to their manufacturers (Clearfil SE Bond and Single Bond, respectively). This bonding agent and Panavia SA Cement contain the monomer 10-methacryloyloxydecyl dihydrogen phosphate (MDP), which was originally designed to bond to metal oxides, although its use has been extended to oxide ceramics (19). It has been stated that MDP-containing RCs are preferable to obtain a chemical bond between the hydroxyl groups of the passive surface and the phosphate ester group of the MDP (19,20). The other cement used in this study is a semipermanent cement, MIS Crown Set Cement, which is advised by the manufacturer due to its high retention properties and easy restoration removal ability. In our study, the bond strength of MIS Crown Set Cement provided the lowest values even for the sandblasted Ti surfaces.

On the other hand, sandblasting of Ti significantly enhanced the bond strength of Panavia SA Cement and RelyX U200 than that of MIS Cement. Therefore, it is sensible to prefer sandblasting of Ti abutments and using permanent cements with bonding agents including MDP for reduced abutment/restoration interface and over tapered abutments in the existence of limited interarch space. After sandblasting, both the debris and metal oxide layer are removed from Ti alloys. However, immediately afterwards, a thin, stable oxide layer is reproduced, which interacts with the MDP monomer, forming a chemical bond between the dihydrogen phosphate group and metal oxides (18,21). In a study by Koizumi et al. (22), primers containing MDP were determined to be more effective for treating Ti than other primers without MDP. Di Francescantonio et al. (23) stated that the direct application of Panavia F 2.0 to Ti surface without using an adhesive primer provided a favourable bond strength due to the MDP content. Correspondingly, in our study, Panavia samples with or without using a bonding agent (Clearfil SE Bond 2) yielded similar bond strength values as well. But although there were no significant differences between all RC types for the thermocycled non-treated groups (p>0.05), the non-treated thermocycled Panavia SA Cement group showed the lowest values among all groups in our results. This situation can be explained by the fact that the MDP content in Panavia SA Cement is not as high as the MDP content in Clearfil SE Bond, which is defined as the gold standard for MDP content. Nevertheless, the Clearfil SE Bond + Panavia SA Cement sandblasting group showed the highest bond strength among all the other groups both before and after thermocycling.

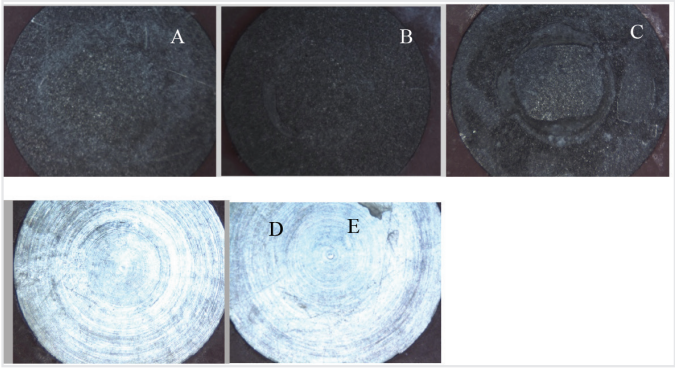

Figure 2. View of failure types; A- Adhesive type for sandblasted surfaces, B-Mixed type for sandblasted surfaces, C- Cohesive type for sandblasted surfaces, D-Adhesive type for non-treated surfaces, E- Mixed type for non-treated surfaces

It is well documented in the literature that sandblasting of Ti surfaces enhanced the bond strength of cemented restorations compared with smooth Ti surfaces (18,24-26). Tsuchimoto et al. (27) also reported that sandblasting of Ti increased micromechanical interlocking between resin and Ti, particularly when combined with adhesive primers (27). Yanagida et al. noted that the primers containing MDP monomer improved the bonding durability of composite resins to air-abraded Ti surfaces even after thermocycling (28). Hon et al. (29) compared the effects of five commercially available silane coupling agents for Ti-RC adhesion and reported that conditions, especially thermocycling, significantly affected adhesion, but the five silane coupling agents provided similar and clinically acceptable adhesions. In our study, we used bonding agents that also included silane for Panavia and RelyX. Bonding agents are commonly used in clinical practice as they are easily accessible for clinicians. Because of that, we wanted to compare if there is an adhesive effect of bonding agents, including metal primers or silane coupling agents, for Ti surfaces. Our results are also similar to Hon et al. (29), in which silane content bonding agents provide acceptable adhesion.

Study Limitations

There were some limitations in this study. Firstly, only sandblasting and machined surfaces were used for comparison because sandblasting is clinically the most common surface treatment method for metals, Ti and zirconia. However, various types of surface treatment methods should be investigated for further studies to understand the effects of surface treatments on bond strength to Ti and select a more proper type of luting cement for clinical applications. Secondly, this study only used bonding agents that contain silane coupling agents, and only Ti surfaces were used as substrates. Therefore, further investigations are needed to compare metal primers and silane agents with bonding agents for abutment surfaces. Also, different kinds of abutment materials such as zirconia, lithium disilicate and polyeterkethonekethone can be evaluated for future studies.

Based on these findings, it might be clinically helpful to modify the Ti abutments by sandblasting procedures and pay attention to MDP contents while making the selection of not only the primer or self-etch bonding agent but also the adhesive luting cement itself.

Conclusion

It can be concluded that there was no effect in respect to bonding to non-treated Ti surfaces among the cements tested before and after thermocycling. Nonetheless, for the sandblasted Ti surfaces, the Panavia SA Cement sandblasting, Clearfil SE Bond + Panavia SA Cement sandblasting, RelyX U200 sandblasting and Single Bond Universal + RelyX U200 sandblasting groups provided higher bond strength than the MIS Crown Set Cement sandblasting group. Therefore, clinicians should prefer a higher retention cement strength with the combination of Ti abutment surface modification for specific cases where weak retention is predicted over single cement-retained crowns.

Ethics

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: C.A., G.S.Ö., Concept: G.S.Ö., C.A., Design: G.S.Ö., C.A., Data Collection or Processing: G.S.Ö., C.A., Analysis or Interpretation: G.S.Ö., B.S.O.A., Literature Search: B.S.O.A., G.S.Ö., Writing: B.S.O.A., G.S.Ö.

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Original Article

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Optimisation of Centrifugal Speed for PRPBAG®: A Novel Multiple Bag System for Preparing Platelet-Rich Plasma

Plateletten Zengin Plazma Eldesinde Yeni Bir Çoklu Torba Sistemi olan PRPBAG® için Santrifüj Hızının Optimizasyonu

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ABSTRACT

Objective: This study aimed to identify the optimal centrifugal speed for the preparation of platelet-rich plasma (PRP) with PRPBAG®, a novel multi-bag system.

Methods: The study included 120 (60 women and 60 men) clinically healthy volunteers aged 18-35 years. Participants were divided into three age-sex matched groups according to the centrifugation speeds as follows: 1600 rpm (n=40), 1800 rpm (n=40) or 2000 rpm (n=40). Whole blood (150 mL) was drawn from the volunteers, and centrifugation was performed for 10 minutes. After the first spin, the erythrocytes precipitated in the first bag, and the supernatant plasma was transferred into the second bag. Plasma centrifugation was performed at 3500 rpm for 15 minutes. The high-quality PRP product precipitated in the second bag. The supernatant platelet-poor plasma was transferred to the third bag and destroyed. Blood cell count of whole blood and PRP product was measured.

Results: The mean white blood cell level of PRP in the 1800 rpm group was higher than the other groups (p=0.027). The mean platelet levels were 1005.22±105.96, 1743.7±145.2 and 1743.7±145.2 103/uL in the 1600, 1800 and 2000 rpm groups, respectively, after centrifugation (p<0.001). Accordingly, the platelet yield (fold increase) was significantly greater in the 1800 rpm group than in the other groups (p<0.001).

Conclusion: This study showed that 1800 rpm for 10 minutes is the optimum first centrifugation speed for preparing PRP with PRPBAG®, as it provides an approximately eightfold increase in platelet concentration in PRPBAG®.

Keywords: Platelet-Rich Plasma, PRPBAG, optimisation

ÖZ

Amaç: Bu çalışmada yeni bir çoklu torba sistemi olan PRPBAG® ile Plateletten Zengin Plazmanın (PRP) hazırlanması için optimum santrifüj hızını belirlenmesi amaçlanmıştır.

Yöntemler: Bu çalısmaya 18-35 yaş arası 120 sağlıklı gönüllü (60 kadın, 60 kadın) dahil edildi. Yaş ve cinsiyet uyumlu olarak katılımcılar santrifuj hızlarına göre 1600 rpm (n=40), 1800 rpm (n=40) veya 2000 rpm (n=40) olarak üç gruba ayrıldı. Gönüllülerden 150 ml tam kan alındı ve 10 dakika santrifüj edildi. İlk santrifüjde birinci torbadaki eritrositler çöktürüldü ve üstte kalan plazma kısmı ikinci torbaya aktarıldı. İkinci torba 3500 rpm'de 15 dakika santrifüj edilerek yüksek kaliteli PRP ürünü ikinci torbada çöktürüldü. Üstte kalan plateletten fakir plazma kısmı üçüncü torbaya aktarıldı ve imha edildi. Tam kan ve elde edilen son PRP ürününden kan sayımları yapıldı.

Bulgular: PRP'nin 1800 rpm gurbunda ortalama lökosit düzeyi diğer gruplara göre yüksekti (p=0,027). Santrifüj sonrası ortalama platelet seviyeleri 1600 rpm grubunda 1005,22±105,96 103/uL, 1800 rpm grubunda 1743,7±145,2 103/uL ve 2000 rpm grubunda 1282,2±105,32 103/uL idi (p<0,001). Buna göre trombosit verimi (kat artışı) 1800 rpm grubunda diğerlerine göre anlamlı olarak daha yüksekti (p<0,001).

Sonuç: Bu çalışmada, PRPBAG® ile elde edilen trombosit konsantrasyonunda yaklaşık 8 kat artış sağladığından 10 dakika süreyle 1800 rpm'de ilk santrifüjleme yapılmasının anlamlı olarak daha verimli ve uygun olduğu gösterilmiştir.

Anahtar Sözcükler: Trombositten zengin plazma, PRPBAG, optimizasyon

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Introduction

Megakaryocytes, the largest cells in the body, are found in the bone marrow. The extensions of megakaryocytes pass into the bloodstream in small pieces to form the body's smallest cells, known as platelets. The primary function of platelets is to prevent blood loss by coagulating damaged blood vessels (1,2). The α-granules of platelets are rich in various growth factors, such as transforming growth factor β, platelet-derived growth factor, insulin-like growth factor, epidermal growth factor and vascular endothelial growth factor that play a crucial role in the acceleration of tissue healing (3,4). Additionally, antimicrobial, anti-inflammatory and analgesic factors could be released by platelets (5). Therefore, the administration of autologous platelets at a supraphysiological concentration could be useful for treatment in many different areas.

Platelet-rich plasma (PRP) is a plasma component obtained by centrifuging whole blood, which contains approximately six to eight times higher platelet concentration than whole blood. This supraphysiological concentration allows the therapeutic use of platelets. PRP is preferred in therapeutic and cosmetic fields, from chronic ulcers to alopecia treatment, because of its positive effects on wound healing (6-8). Recent studies have shown that PRP injections are used in different clinics, including orthopaedic surgery, cardiovascular surgery, neurology, plastic surgery, gynaecology and urology (9-12).

For PRP preparation, blood is taken from a patient and transferred into instruments that contain anticoagulants. Later, it is fractionated by centrifuging. There are more platelets in PRP compared with whole blood. However, there are different separation techniques for PRP preparation, such as centrifugation and filtration (13,14). Also, the volume of blood drawn from the patient in the first place differs. These differences in PRP preparation lead to the obtainment of different platelet amounts. To the best of our knowledge, a standard protocol for the preparation of PRP has not been established yet. Tube methods are widely used to obtain PRP, especially in cosmetics, but they are quite insufficient, especially in patients who need high-quality and high-quantity products. The uncertainty of the use of different methods and high cost of the PRP processing kits limit the widespread clinical use of PRP. In light of these findings, a multi-bag system (PRPBAG®) was invented to simplify modalities and increase the PRP product's quality and quantity economically.

We aim to identify the optimal centrifugal speed for PRP preparation, which is prepared by PRPBAG®, a novel multi-bag system.

Methods

Participants

The study included 120 (60 women and 60 men) clinically healthy volunteers aged between 18 and 35 years. Participants were divided into three age-sex matched groups according to the centrifugation speed as follows: 1600 rpm (n=40), 1800 rpm (n=40) or 2000 rpm (n=40). The range of body mass index (BMI) was 18.5-29.9 kg/m^2, and the groups were also matched for BMI. The local ethics committee approved this study, and informed consent was obtained from all participants individually.

PRPBAG®

PRPBAG® is a multi (three) bag system that was produced with apheresis bag technology. Its oxygen-permeable feature prevents platelets from remaining in the hypoxic environment. Whole blood (50-150 mL) can be drawn depending on the patient's needs, and the process starts with centrifugation. As detailed in the methodology below, centrifugation is performed in the refrigerated centrifuge specially produced for PRPBAG®. The extractor separates the erythrocytes; then, the remaining product is centrifuged to obtain a high-quality PRP product. All three bags in the system are of the same size and can hold a maximum of 200 mL of liquid. The separation process is manual and takes place under the supervision of an experienced operator.

PRPBAG® is a patented multi-bag method and a completely closed, sterile system (Figure 1). All the products used in this method are medical devices authorised for both trade and clinical use by the responsible institution (patent no: TR 2016 10467 B).

Blood Collection and Preparation steps of PRP

Whole blood (150 mL) was directly drawn from the antecubital veins by venipuncture and collected in specially produced PRPBAG. Each PRPBAG® contains 21 mL anticoagulant solution of citrate phosphate dextrose adenine, USP, to preserve the 150 mL of whole blood. Of this blood, 1 mL was used to conduct a complete blood count (CBC) analysis using the Swelab Alfa Plus hematology analyzer.

PRP product was obtained by centrifugation at different spin speeds from blood samples. A double-spin preparation method was applied for all samples using a refrigerated centrifuge specially manufactured for PRPBAG® (Large Capacity Refrigerated Centrifuge, Inovia Technology INO-FBC 5000). The centrifugation processes were started within 1 h of blood collection. A flow chart for the preparation of the PRP product with PRPBAG® was demonstrated in Figure 2.

Centrifugation Process (First Spin)

Collected samples were placed in the hole of the refrigerated centrifuge. Since mutual balance was essential in the centrifuge, the same weight balances were prepared against the blood collected to prepare the PRP product. Whole blood was centrifuged at 1600 (540 x g), 1800 (684 x g) and 2000 (845 x g) rpm for 10 minutes. Depending on the blood component density, the erythrocytes precipitated in the first bag, and the supernatant plasma was transferred into the second bag with a manual plasma extractor. The third bag remained empty after the first spin. The hose of the first bag, which contained erythrocytes, was separated from the other bags with a hose-closing device and was later destroyed. The process was continued with the remaining two bags.

Centrifugation Process (2nd Spin)

The remaining two bags were placed on the hole of the refrigerated centrifuge. The same weight balance was prepared against the opposite level of this hole. The plasma was centrifuged at 3500 rpm (2587 x g) for 15 minutes immediately. The high-quality PRP product precipitated in the second bag. The supernatant platelet-poor plasma was moved into the third bag with a manual plasma extractor, leaving 10-18 mL of high-quality PRP in the second bag. The third bag containing platelet-poor plasma was separated and destroyed with the help of the hose-closing device. As soon as the PRP was produced, a 1 mL aliquot was collected from PRPBAG® for blood cell measurements. Blood cell measurements of PRP were analysed using an automated hematology analyzer (Swelab Alfa Plus). European guideline for the preparation, use and quality assurance of blood components has been considered throughout the process (15).

Statistical Analysis

Statistical analyses were performed using SPSS version 20.0 for Windows (SPSS Inc. Chicago, IL, USA). Values were given as mean ± standard deviation or standard error according to the distribution of parameters. The associations between groups were assessed by Student's t-test or analysis of variance and Mann-Whitney U or Kruskal-Wallis tests, depending on the number of groups and distribution of data. Dependent variables were analysed with a paired t-test. Numeric values were evaluated using the Kruskal-Wallis test, followed by the post-hoc analysis (Bonferroni correction for all pairs). The categorical variables were assessed using the chi-square test. The threshold for significance was $p<0.05$.

The number of volunteers included in the study was 36 in each group with a 95% confidence level and 80% power, and 40 cases were included in each group.

Results

The mean age was 26.88±5.02 years, and the mean BMI was 23.39±3.5 kg/m^2 in the study group. Each spin speed group consisted of 20 female and 20 male and age-BMI matched participants. Table 1 presents the CBC results before the centrifugation of whole blood and the white blood cell (WBC) and red blood cell (RBC) levels from the PRP final product. A comparison of the measurements showed no significant difference in whole blood results among the spin speed groups.

Figure 1. PRPBAG®: a completely closed sterile and multi (three) bag system

On the other hand, the mean WBC level of PRP in the 1800 rpm group (6.5±0.77 10^3/uL) was significantly higher than in the other groups ($p=0.027$). The PRP products contained negligible RBC concentrations.

WBC levels in whole blood (6.95±1.37 10^3/uL) significantly decreased in PRP products (3.98±0.6 10^3/uL) in the 1600 rpm group ($p<0.001$). WBC levels showed no significant difference before and after centrifugation in the other groups.

Platelet counts were evaluated before (whole blood) and after (PRP) centrifugation (Table 2). The mean platelet levels were 1005.22±105.96, 1743.7±145.2 and 1282.2±105.32 10^3/uL in the 1600, 1800 and 2000 rpm groups, respectively, after centrifugation process ($p<0.001$). Accordingly, statistical analyses revealed that the platelet yield (fold increase) was significantly greater in the 1800 rpm group than in the other groups ($p<0.001$).

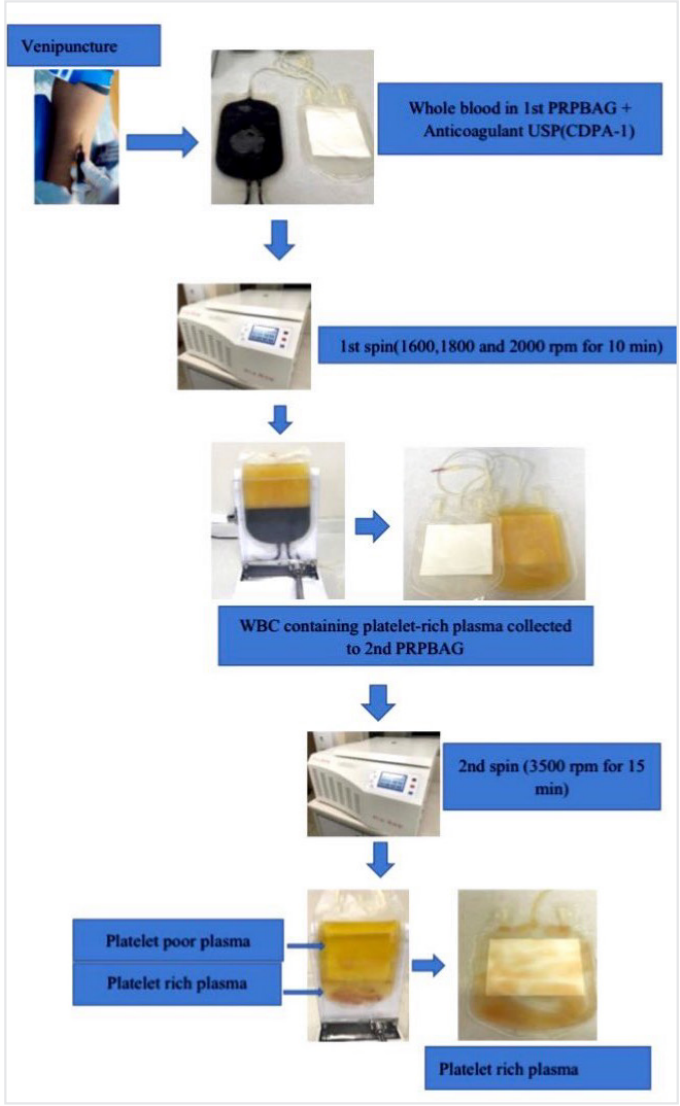

Figure 2. A flow chart for the preparation of platelet-rich plasma product with PRPBAG®

Discussion

The use of PRP products in treatments has become an increasingly popular option; many available commercial kits and PRP preparation methods exist. To the best of our knowledge, bag systems are mostly used for blood product transfusions, whereas tube systems are for PRP production. PRPBAG® system is a novel method for PRP preparation, and this is the first report to compare the first spin speeds to optimise the content of blood cells in PRP produced with PRPBAG® from whole blood. Based on the findings of this study, the optimum first centrifugation speed for preparing PRP with PRPBAG® is 1800 rpm for 10 minutes. The second spin that follows is 3500 rpm for 15 minutes. We suggest this as an optimal centrifugation protocol for PRPBAG®, as this provides the greatest yield, which is approximately an eightfold increase in platelet concentration.

Centrifugation separates RBCs, WBCs, platelet and plasma, depending on the density gradient of these blood components. WBCs (1,077 g/mL) and RBCs (1,100 g/mL) centrifuge faster than platelets (1,058 g/mL) because they are both heavier (15,16). In an experimental animal study, Shin et al. reported that most WBCs and RBCs precipitate in the bag and separate from the supernatant platelets where the platelet yields depend on the duration and speed of centrifugation. They found the platelet yield to be 6.48 ± 0.46 (fold increase) in their optimum protocol. Moreover, they showed that a low centrifugation force and short centrifugation duration could not provide a good separation of platelets, resulting in the low yield of platelets and, therefore, the low recovery rate in animals when the tube method was used for PRP preparation. Prolonged centrifugation force and duration led the platelet yield levels to increase to a certain point; however, the yield started to decrease after the optimum point (17). Consistent with these findings, our results show that the PRP products of the 1800 rpm group reached the best platelet yield at 7.66 ± 3.49 (fold increase), and the WBC levels were higher in the 1800 rpm group than in both the 1600 and 2000 rpm groups. Therefore, the optimum protocol for the second spin in our study was 1800 rpm for 15 minutes.

The role of WBCs in the PRP product is controversial, and few studies have evaluated the benefits of WBCs and the interactions between WBCs and platelets on PRP composition. It is suggested that platelets are the main source of growth factors, and WBCs are the main source of inflammatory cytokines (18). McCarrel et al. (19) pointed out that high WBC levels in PRP contribute to inflammatory cytokine expression, and persistent inflammation results in the inferior repair of damaged tissues. On the other hand, Kobayashi et al. reported that growth factor concentration was related to WBC concentrations. Thus, when the number of leukocytes in PRP increases, the quality of the product increases as well (20). They showed that as they performed double centrifugation (first spin 400 × g for 10 min and second spin 2000 × g for 3 min), the WBCs reached higher levels than single centrifugation in PRP.

Similarly, we performed double centrifugation. However, our study revealed that the WBC levels decreased in the 1600 and 2000 rpm groups, but they were stable in the 1800 rpm group. With these findings, other unmeasured and unknown substances released from WBCs could have contributed to the different effects of PRP. Because of this difference, the preparation method of PRP must be assessed separately, and the optimum conditions must be established, especially in medical treatments. Further studies are needed to clarify the effects of WBCs on PRP in different pathologies.

RBCs are the heaviest components of whole blood, and the first spin of centrifugation is especially important to separate RBCs from the other components. Similar to the literature, our study shows that plasma contains negligible amount of RBCs in all of the spin speed groups, namely, 1600, 1800 and 2000 rpm, after the first spin of the centrifugation process [17, 21].

Study Limitations

There are some limitations to this study. Firstly, since the PRP samples produced in the study were acquired from healthy volunteers, the recovery levels could not be pointed even though the yield values were revealed. Secondly, the number of groups with different centrifugal speeds could be increased but not to compromise the statistically calculated minimum number of patients and compare a large group of people with the drawn 150 mL blood. According to the expected usage results, these optimal speeds possibly predicted the best results that were selected by experienced members of our blood bank centre.

Conclusion

In conclusion, the methods of PRP preparation may directly affect the characteristics of the product. We suggest that the centrifugation initiation with 1800 rpm for 10 minutes is an optimal first spin speed as this method provides an approximately eightfold increase in platelet concentration in PRP products obtained by PRPBAG® system.

Ethics

Ethics Committee Approval: Obtained.

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: , Concept: , Design: , Data Collection or Processing: Analysis or Interpretation: , Literature Search: , Writing:

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Original Article

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Depression, Anxiety, and Stress Scales 42 (DASS-42) in Dari-Language: Validity and Reliability Study in Adults, Herat, Afghanistan

Dari Dilinde Depresyon, Kaygı ve Stres Ölçeği 42 (DASS-42): Yetişkinlerde Geçerlik ve Güvenirlik Çalışması, Herat, Afganistan

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ABSTRACT

Objective: This study aimed to assess the validity and reliability of Depression, Anxiety, and Stress Scales-42 (DASS-42) in Dari, in adult population of Herat province of Afghanistan.

Methods: The Dari-translated version of DASS-42 questionnaire was administered to 1310 non-clinical samples randomly selected in Herat. Internal reliability, exploratory factor analyses, confirmatory factor analyses and Pearson's product moment correlation were assessed to test the validity and reliability of the questionnaire. Data analyses were performed in IBM SPSS Statistics 23.0 and AMOS 23.0.

Results: Internal consistency of the Dari-translated version of DASS-42 questionnaire subscales was high with Cronbach's alpha values of 0.888, 0.866 and 0.833 for depression, anxiety and stress, respectively. Construct validity was further supported with acceptable correlation measures of 0.799, 0.822 and 0.818 for depression, anxiety and stress subscales, respectively, which all were statistically significant ($p<0.05$). Confirmatory factor analysis gave acceptable goodness-of-fit indices.

Conclusion: The Dari-translated version of DASS-42 questionnaire is a reliable and valid assessment tool for identification and measurement of the magnitude of depression, anxiety and stress in the Dari-speaking population of Herat province of Afghanistan.

Keywords: Depression, anxiety, stress, DASS-42, Herat, Afghanistan

ÖZ

Amaç: Bu çalışmanın amacı Depresyon, Kaygı ve Stres Ölçeği-42 (DASS-42) Dari dilinde Afganistan Heart İlinde erişkinler arasında geçerlik ve güvenirliğini değerlendirmektedir.

Yöntemler: Dari diline tercüme edillmiş DASS-42 anketi, Herat'ta yaşayan nüfustan rastgele belirlenen 1.310 kişiye uygulanmıştır. Testin geçerlik ve güvenirlik analizi, iç tutarlılık, açıklayıcı faktör analizi, doğrulayıcı faktör analizi ve Pearson korelasyon analizi ile değerlendirilmiştir. Veri analizi IBM SPSS Statistics 23.0 ve AMOS 23.0 programlarıyla yapılmıştır.

Bulgular: DASS-42 anketi alt ölçeklerinin Dari dili versiyonunun iç tutarlılığı depresyon, anksiyete ve stress için Cronbach alfa değerleri yüksek değerlerde (0,888, 0.866 ve 0.833) bulunmuştur. Depresyon, anksiyete ve stres alt ölçekleri için yapı geçerliliği, kabul edilebilir korelasyon değerlerinde bulunmuştur (0,799, 0,822 ve 0,818); tüm değerler istatistiksel olarak anlamlıdır ($p<0,05$). Doğrulayıcı faktör analizi kabul edilebilir uyum iyiliği indeksine sahiptir.

Sonuç: DASS-42 anketinin Dari dili versiyonu, Afganistan'ın Herat ilinin Dari konuşan nüfusda depresyon, anksiyete ve stress sıklığının ölçülmesi için güvenilir ve geçerli bir değerlendirme aracıdır.

Anahtar Sözcükler: Depresyon, anksiyete, stres, DASS-42, Herat, Afganistan

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Introduction

Depression and anxiety are among the most common mental disorders in human community (1). Depression and anxiety have conceptually and theoretically similarities and sometimes overlapping, especially in the young generation (2). Clark and Watson have developed a depression and anxiety model to specify the specific and common variances of these two illnesses (3). Stress is a biologic response to any intrinsic and external stimulus, the stress can cause changes in brain, and different aspects of the nervous system such as different parts of the brain, immune system functions, cardiovascular system, gastrointestinal system, and endocrine system (4). Furthermore, clinicians and researchers need sensitive and specific instruments to assess the extent of depression, anxiety and stress, for which, several tools have been developed previously. Beck anxiety inventory (BAI) and Beck depression inventory (BDI) have been developed to assess the level of anxiety and depression; however, they do not address the "stress" component as body reactions. To address this, Loviband and Loviband developed "depression, anxiety and stress scale (DASS)" to define, understand and measure the magnitude of these three negative emotional states in adults (5). DASS depression scale assesses the mood, motivation and self-esteem, while the DASS anxiety scale focuses on physiological arousal, fear and panic. DASS stress scale assesses the magnitude of tension and irritability. DASS is able to discriminate between the three negative emotional states as a screening test by researchers and clinicians (6). The original DASS is a 42-item questionnaire in the English language containing 14 questions for each subscale. There is a short version of the 42-item questionnaire called DASS-21, which contains 7 questions for assessing depression, anxiety and stress each. Both questionnaires evaluate depression, anxiety and stress in the community as well as clinical settings.

DASS-42 and DASS-21 are used in English spoken countries including England, the United States, Canada and Australia (7-10). The scales have been translated into different languages. Validity and reliability of translated versions have been assessed for many languages and is underway for others (11-23). Studies conducted in different countries using DASS valid and reliable translations illustrated its internal consistency and validity in both clinical and non-clinical samples in different ethnic groups (12,15,17,20,22). Although DASS has been translated in Persian Language and used on high school students, (23) and non-clinical adults of 18-56 years old, (20) it has not been validated in the Afghan population yet.

The aim of this study is to assess the validity and reliability of the Dari-translated version of the DASS-42 questionnaire in the adult population of Herat province in western Afghanistan. This will serve as a starting point to use DASS for understanding, identifying and measuring the magnitude of depression, anxiety and stress among Afghan people, living in the war-torn country.

Methods

Persian translation of DASS-42 was already present on DASS website, it was downloaded, after a written permission, and used in this study. It is worth noting that despite the fact that although Persian translation was present on the website, it was only validated elsewhere, but not in Afghanistan. A cognitive interview was conducted on 27 participants to both understand the language appropriateness of the questionnaire. The purpose of this study was to validate DASS-42 Dari (Persian) questionnaire in the context of Afghanistan, considering differences in culture and environment.

DASS-42 questionnaire measures the magnitude of depression, anxiety and stress. Each of these three subscales consists of 14 questions answered using 0-3 scale, with 0 meaning "it did not apply to me", and 3 meaning "it applied to me very much". Participants were asked to select one of the four response options for each question. Therefore, for each subscale, the possible score ranged between 0 and 42. Scores considered normal for depression were 0-9, for anxiety 0-7 and for stress 0-14. Scores above these ranges indicated the degree of the three mental illnesses, ranging mild to severe, as stated elsewhere (5).

Data Collection

This was a population-based study conducted in Herat province of Afghanistan. A minimum of 30 individuals were considered for each question, with a further 50 individuals added for a better assessment of the validity and reliability of the Dari-translated version of DASS-42 (24).

Therefore, a total of 1,310 participants aged 18 years and over residing in Herat province were included in the study. Before the initiation of the study, informed consent was obtained from each participant. Participants were native Dari speakers without a known psychological problem. Central Statistics Organization of Herat province was contacted to understand the age- and gender-specific characteristics of the population in each of Herat 15 city suburbs. Participants were selected proportionally randomly in Heart.

A group of 15 professional healthcare workers (physcians, nurses and medical university students) was intensively both theoretically and practically trained for one week to conduct DASS-42 questionnaire by research team in Afghanistan. Although the original format of questionnaires was designed to be self-administered, it was slightly modified to conform an interview-based questionnaire, because of the high illiteracy rate in Afghanistan. During data collection the re-test was done on 251 participants, 2-3 weeks after the initial test.

Data collection was carried out between April and November 2017. Data were screened at the end of each week for consistency and accuracy. Any questionnaire with missing data was excluded from the study.

The methodology and conduct of this research was assessed and approved by the Ethics Committee of the Institutional Review Board and the Research and Development Bureau of Herat University (HU-IRB-032017).

Statistical Analyses

Cronbach's alpha coefficients were used to evaluate the internal reliability for each of the DASS-42 subscales. Cronbach's alpha values of 0.70 and over were considered satisfactory, as described elsewhere (25). Exploratory factor analysis was performed using the principal component analysis with oblimin rotation (Kaiser normalization) for the factor structure. The Kaiser-Meyer-Olkin (KMO) statistic and Bartlett's test of sphericity were carried out to check for sampling suitability and factor analysis. Factor loading greater than 0.32 was considered statistically meaningful, as described elsewhere (26). Item analysis was used to eliminate items which did not represent its own subscale. Confirmatory factor analysis was used to determine the goodness-of-fit of the three construct models of DASS after explanatory factor analysis in Herat population. The following parameters were used to evaluate model fit: Chi-square to df ratio (CMIN/df), the root mean square error of approximation (RMSEA), standardized root mean square residual (SRMR), the goodness-of-fit index (GFI), the adjusted goodness-of-fit index (AGFI), the comparative fit index (CFI), the Tucker-Lewis Index (TLI). The following criteria were used to assess model fit as described elsewhere: CMIN/df <5; Standardized RMR<0.05; RMSEA <0.08; GFI >0.90; AGFI >0.90; CFI >0.95 and TLI >0.95 (27,28). Pearson's product-moment correlations was used for assessing test-retest reliability. The test-retest reliability value of 0.70 or above was considered satisfactory, as described elsewhere (27). Mann-Whitney U test was used for comparison of two independent groups. Median and interquartile range (IQR) values were given as descriptive statistics for quantitative data. Qualitative data were summarized using frequency and percentages. A p value of less than 0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics, Version 23.0; Chicago, IL) and AMOS (Version 23.0)

Results

Of the 1,310 participants in this study, 48.3% were male, 58.4% were married and 33.7% aged 50 years or over. 62.1% of participants were from Tajik ethnicity and 62.6% lived in the urban area. 50.0% of participants were either illiterate or could only read and write. Only 10.4% were either university students or graduates. 57.2% of the participants had no income, while 11.3% earned less than 50 USD per month. Only 6.4% of participants claimed a monthly income of over 300 USD (Table 1).

Item analysis was made for the scale before the factor analysis. First, we determined the items' extreme arithmetic mean and standard deviation. Item 5 (0.45±0.811) in depression subscale, item 20 (0.49±0.81), item 23 (0.47±0.78) and item 30 (0.49±0.81) in anxiety subscale and item 8 (0.64±0.91), item 22 (0.6±0.89) and item 39 (0.77±0.89) in stress subscale were removed from DASS-42. Item 42 was deleted from the depression subscale because its deleted Cronbach's Alpha was bigger than the overall Cronbach's alpha of the subscale (Table 2).

Principal component analysis with oblimin rotation for Exploratory Factor Analyses was performed for the remaining items after item analysis. Item 3 and item 13 were removed from the depression subscale. Because factor loading of item 3 was smaller than 0.30 and item 13 was loaded on the stress subscale. Item 33 and item 9 could not be loaded to any subscale. Furthermore, items 28, 36 and 40 were loaded on depression subscale. Exploratory Factor Analyses with oblimin rotation

Table 1. Characteristics of participants (n=1310) (Herat 2018)

	n	%
Age group		
18-29	317	24.2
30-39	288	22.0
40-49	264	20.2
50-59	216	16.5
60-69	156	11.9
70+	69	5.3
Gender		
Male	633	48.3
Female	677	51.7
Nationality		
Tajik	814	62.1
Poshton	276	21.1
Other (Ozbik, Hazara)	220	16.8
Marital status		
Married	765	58.4
Single	441	33.7
Widowed/divorce	104	7.9
Residence		
Urban	820	62.6
Rural	490	37.4
Education status		
Illiterate	469	35.8
Literate (can read and write)	186	14.2
Primary school	105	8.0
Secondary school	115	8.8
High school	299	22.8
University	130	9.9
Master/Ph.D	6	0.5
Economic status (montly)		
No income	749	57.2
Less than 50 $	148	11.3
50-100 $	174	13.3
100-200 $	126	9.6
200-300$	29	2.2
More than 300 $	84	6.4
Total	1310	100.0

was recalculated for the remaining 27 items. The range of factor loadings (after oblimin rotation) was 0.346 to 0.854. Among depression items, ten loaded on depression factor, ten on stress factor, and only seven on the depression factor. The principal component analysis showed that the three factors together accounted for 49.481% of the variance, KMO =0.963, (Approx. Chi-Square: 1501,61), Bartlett's Test of Sphericity (p<0.001). Results showed that samples in this study were suitable for factor analysis (Table 2).

The internal reliability of the DASS-42 subscales anxiety, depression, stress was assessed using Cronbach's alpha. Alpha was 0.888 for the depression scale, 0.866 for the stress scale, 0.833

Table 2. Results of factor loading, Cronbach's alpha and test-retest for the three dimensions of DASS (n=1310)

Pattern Matrixa

	Component		
	Depression	Stress	Anxiety
DASS-depression			
37 - nothing to be hopeful	0.750		
34 - worthless	0.738		
17 - wasn't worth as a person	0.715		
31 - unable to be enthusiastic	0.692		
21 - wasn't worthwhile	0.685		
38 - life was meaningless	0.672		
24 - couldn't seem enjoyment	0.525		
16 - lost interest about everything	0.523		
10 - to look forward to	0.469		
26 - down hearted & blue	0.346		
DASS-stress			
11- getting upset easily		0.854	
1 - upset		0.750	
18 - feeling touchy		0.743	
27 - irritable		0.719	
14 - getting impatient		0.617	
29 - hard to calm down		0.565	
35 - intolerant		0.508	
12 - using nervous energy		0.507	
6 - over-react		0.494	
32 - difficult to tolerate		0.442	
DASS-anxiety			
4 - breathing difficulty			0.760
2 - dryness of mouth			0.727
7 - feeling of shakiness			0.614
15 - feeling of faintness			0.600
25 - awareness of heart action			0.595
41 - trembling			0.584
19 - perspired			0.454
Extraction Method: Principal Component Analysis. Rotation Method: Oblimin with Kaiser Normalization Kaiser-Meyer-Olkin Measure of Sampling Adequacy: 0.963 (Approx. chi-square: 15019,610), Bartlett's Test of Sphericity (p<0.001)			
Explained of the variance (%)	**38.611**	**6.702**	**4.168**
Cronbach's Alpha	**0.888**	**0.866**	**0.833**
Test-retest (Spearman's rho correlation coefficient)	**0.799***	**0.822***	**0.818***

*: p<0.001

for the anxiety subscales. Subscales have good item-internal consistency (Cronbach's alpha values were higher than 0.70). Spearman's correlation coefficient was used to determine the test-retest reliability. The correlation coefficients were obtained between 0.799 and 0.822. The subscales showed satisfactory test-retest reliability due to a higher than 0.70 correlation (Table 2).

In the validation process of Dari-translated version of DASS-42 questionnaire, in Herat province, 7 questions from depression component, 6 questions from anxiety component and 4 questions from stress component were omitted (Table 3).

Confirmatory factor analysis (CFA) was used to determine the goodness-of-fit of three constructs of DASS, then the factor structure of DASS has been determined by using exploratory factor analysis. Confirmatory factor analysis gave a three-factor structure with acceptable goodness-of-fit indices. Fig 1. shows the confirmatory model (CMIN/df =.59, RMSEA =0.052 (90% CI =0.050-0.055), SRMR =0.0407, GFI =0.918, AGFI =0.904, TLI =0.915 and CFI =0.922).

Table 3. The items excluded from the scale

Items	
DASS depression	
3	No positive feeling
5	Not seem to get going
13	Sad & depression
42	Difficult to work
9	Anxious
20	Scared
23	Difficult in swallowing
28	Panic
30	Feared
36	Terrified
40	Worried
DASS-stress	
8	Difficult to relax
22	Hard to wind down
33	Difficult to tolerate
39	Agidated

Table 4 shows median and inter quartile range for three scales by gender groups. Three scales of DASS were statistically different for male and female. The scores of female were higher compared to male.

Discussion

Quality of life is an important outcome to be considered in chronic disorders such as depression, anxiety and stress. Identification and addressing factors that significantly affect the quality of life is essential in improving the standards of life and well-being. DASS-42 is one of the most common validated

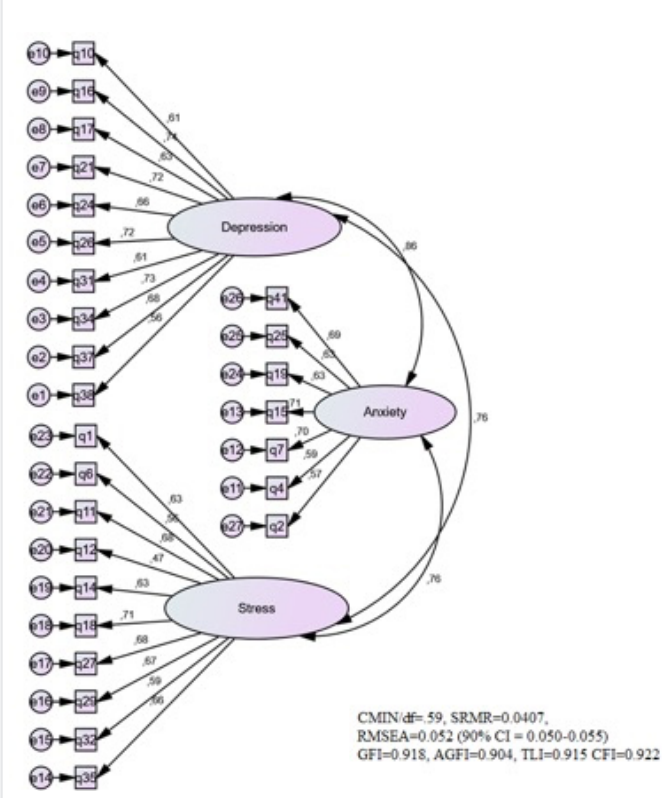

Figure 1. Standardized estimated of the three-dimensions model for DASS obtained from confirmatory factor analysis

Table 4. Comparison of the DASS scores (depression, anxiety, stress) for the Herat population by gender (Herat 2018)

		n	%25	%50	%75	Min	Max	P*
Depression	Male	633	0.00	3.00	9.00	0.00	30.00	**<0.001**
	Female	677	2.00	6.00	12.00	0.00	29.00	
Anxiety	Male	633	1.00	3.00	7.00	0.00	21.00	**0.002**
	Female	677	1.00	4.00	8.00	0.00	19.00	
Stress	Male	633	5.00	9.00	15.00	0.00	30.00	**0.016**
	Female	677	6.00	10.00	15.00	0.00	30.00	

screening test to define depression, anxiety and stress states in many countries (7-23).

We found that the Dari-translated version of DASS-42 questionnaire is a reliable and valid assessment tool for identification and measurement of the magnitude of the three mental illnesses in the adult population living in Herat province of Afghanistan.

Regarding the internal reliability of the DASS-42, the Cronbach's alpha values found in this study were 0.888 for depression, 0.866 for stress and 0.833 for anxiety. The subscales have good item-internal consistency as the internal consistency (Cronbach's alpha) of each subscale were higher than the referred level as 0.70. Spearman's correlation coefficient was found as 0.799 for depression, 0.822 for stress and 0.818 for anxiety, which were satisfactory. However, our values of Cronbach's alpha were lower than original study conducted by Lovibond and Lovibond (Cronbach's alpha was 0.91 for depression, 0.81 for anxiety and 0.89 for stress) (5). Cronbach's alpha values for university students in Turkey were 0.92, 0.86, and 0.88 for depression, anxiety, and stress, respectively;(13) and for high school students in Turkey were 0.91 for depression, 0.84 for anxiety and 0.86 for stress (12). Internal consistency of DASS-42 subscales was high, with Cronbach's alphas of 0.94, 0.88, and 0.93 for depression, anxiety and stress, respectively. Bayani translated DASS-42 into Persian, and validated in undergraduate students aged between 18 to 51 years; the study reported Cronbach's alpha of 0.92 for depression, 0.88 for anxiety and 0.82 for stress in Iran (21). Furthermore, another study in Iran conducted on university students reported Cronbach's alpha of 0.94 for depression, 0.89 for anxiety and 0.92 for stress (22). In a study conducted in undergraduate and master students in Malaysia, Cronbach's alpha values were 0.88 for depression, 0.85 for anxiety and 0.86 for stress (30). Similarly, Rosnani and Ar reported that Cronbach's alpha values for DASS-42 among medical school students, were 0.94, 0.90 and 0.87 for depression, anxiety and stress, respectively (31).

In the validation process of DASS-42 in Dari, 4 questions from depression subscale, 6 questions from anxiety subscale and 4 questions from stress subscale were omitted. Two questions from depression subscale and 7 questions from the anxiety subscale of DASS-42 in Persian validation conducted by Habibi et al. (22) were removed to reach a good exploratory factor analysis. Only 8 items from depression, 4 items from anxiety and 7 items from stress subscales loaded on the original subscales of DASS-42 in the factor analysis of DASS-42 conducted in Malaysia (30). The other Malaysian validation study conducted had also removed 4 items from the original scale to have a good result (31). Asghar also reached to a different scale from the original DASS-42; depression subscale had 18 items, anxiety subscale had 17 items and stress subscale had 7 items in Iran (21). In an attempt to validate DASS-42, Chan et al. (16) removed some items and loaded some items on different subscales of the questionnaire. Bilgel and Bayram (13) also reported that some items were loaded on the different subscales in their study conducted in Turkey. Most of the studies on validation of DASS-42 brought about some changes in the original scales, depending on the differences in cultures and feelings of people for which DASS-42 is translated and validated.

Study Limitations

This study was conducted on native-Dari speakers who resided in Herat city and is therefore valid for the assessment of magnitude of depression, anxiety and stress in said population. More research is yet to be done to validate this questionnaire in all Dari-speaking Afghan population.

Conclusion

The Dari-translated version of DASS-42 questionnaire reported here is a valid and reliable tool for the assessment of depression, anxiety and stress state in the Dari-speaking population of Herat province of Afghanistan. This questionnaire can serve as a baseline for a more comprehensive validation analysis of the magnitude of depression, anxiety and stress state in the Dari-speaking Afghan population as a whole.

Ethics

Ethics Committee Approval: Islamic Republic of Afghanistan Ministry of Higher Education Herat University (date: 12.03.2017).

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: N.A.S., A.R.N., A.M.W., H.Ö., Design: N.A.S., A.R.N., A.M.W., H.Ö., Data Collection or Processing: N.A.S., A.R.N., A.M.W., H.Ö., Analysis or Interpretation: N.A.S., A.R.N., A.M.W., H.Ö., Literature Search: N.A.S., A.R.N., A.M.W., H.Ö., Writing: N.A.S., A.R.N., A.M.W., H.Ö.

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Case Report

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Secondary Involvement of the Penis by Non-Hodgkin Lymphoma

Non-Hodgkin Lenfomanın İnfiltrasyonuna Sekonder Penis Tutulumu

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ABSTRACT

Primary penile lymphoma is extremely rare and there are less than 30 reported cases worldwide. Frequently penile lymphoma is seen as a secondary involvement of the penis by lymphoma, most likely by Non-Hodgkin lymphoma (NHL). Here, we report a case with penile lymphoma caused by previously known NHL.

Keywords: Non-Hodgkin lymphoma, penis, MRI, penile lymphoma

ÖZ

Penil lenfoma oldukça nadirdir. Toplamda 30 olgunun olduğu bildirilmiştir. Penil lenfoma tutulumu genellikle lenfomanın sekonder tutulumu şeklinde karşımıza çıkmaktadır. Genellikle Non-Hodgkin lenfomanın (NHL) sekonder tutulumu şeklindedir. Bilinen NHL tanılı olguda penisin sekonder tutulumunun olduğu olguyu sunacağız.

Anahtar Sözcükler: Non-Hodgkin lenfoma, penis, MRG, penil lenfoma

Introduction

Penile malignant tumours are uncommon. The ultrasonography discloses that penile cancer accounts for around 0.4% of all male malignancies. Primary penile lymphoma is extremely rare (less than 30 cases) (1). The penile lymphoma is often seen as the secondary involvement of the penis, most likely by Non-Hodgkin lymphoma (NHL) (2-4). In most cases, penile lymphoma represents secondary involvement due to haematogenous or lymphatic spread or direct extension from a neighbouring organ (5), such as bladder, lymph nodes and seminal vesicles. For the diagnosis, physical examination and radiological imaging [computed tomography (CT) and magnetic resonance imaging (MRI)] should be carefully undertaken (5). In this case, we report a case with penile lymphoma caused by previously known NHL.

Case Report

A 58-year-old male presented with penis pain and erectile dysfunction; he had a history of NHL for 4 years, and he was getting chemotherapy. His physical examination indicated a dysmorphic penile shaft, and the prostate was hard. The clinician suspected Peyronie's disease. Routine blood, liver and renal function tests were normal. His urine leukocyte level was high [12 high pass filter (HPF); normal 0-5 HPF]. Multiple enlarged inguinal lymph nodes and minimal abdominal tenderness were noted. Abdominal ultrasound showed hepatomegaly, hepatosteatosis and normal configuration of both kidneys. MRI of the pelvis and penis showed isointense on T1 weighted imaging and hypointense on T2 weighted imaging signal changes and homogenous contrast enhancement on post-contrast T1 images

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in the corpus spongiosum of the penis (Figure 1, 5). Diffusion-weighted imaging shows restricted diffusion (Figure 2). Also, the central and peripheral zone of the prostate and left seminal vesicle were involved (Figure 3, 4). Bilateral obturator, external and inguinal lymph nodes were enlarged. With these findings, the diagnosis of penile lymphoma due to the involvement of the penis by NHL was established. And his treatment (systemic chemotherapy), which he had already taken, was continued. After chemotherapy, clinical response was observed. The symptoms of the patients were all reduced.

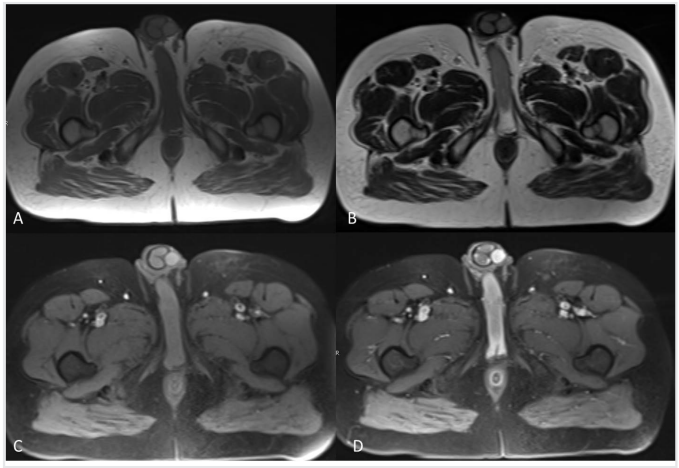

Figure 1. (A) Axial T1 weighted image showed homogeneous isointensity in the corpus spongiosum penis and (B) axial T2 weighted image showed hypointensity of the corpus spongiosum. (C) axial fat sat T1 weighted image and (D) axial fat sat T1 postcontrast image showed homogenous contrast enhancement in the corpus spongiosum of penis

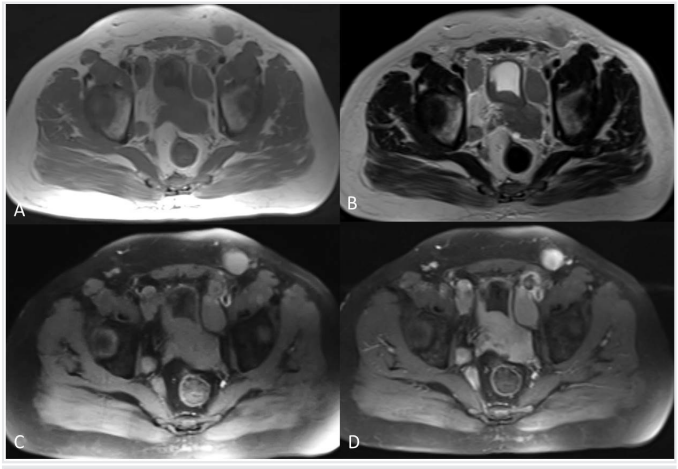

Figure 2. (A) Axial T1 weighted image and (B) axial T2 weighted image showed enlarged bilateral obturator and inguinal lymph nodes. (C) Axial fat sat T1 weighted image and (D) axial fat sat T1 postcontrast image showed homogenous contrast enhancement on this lymph nodes

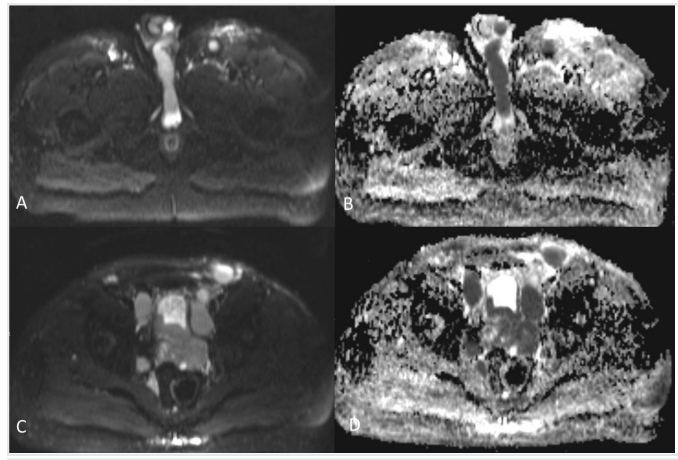

Figure 3. Axial Diffusion weighted images (A-B) in the corpus spongiosum and (C-D) prostate and left seminal vesicle shows diffusion restriction

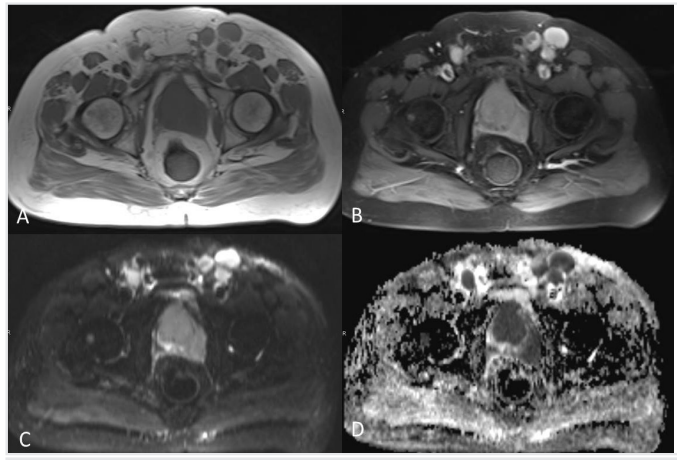

Figure 4. (A) Axial T1 weighted image showed enlarged inguinal lymph nodes and (B) axial fat sat T1 postcontrast image showed homogenous contrast enhancement in this lymph nodes. Axial Diffusion weighted images (C-D) left inguinal lymph nodes, prostate and left seminal vesicle shows diffusion restriction

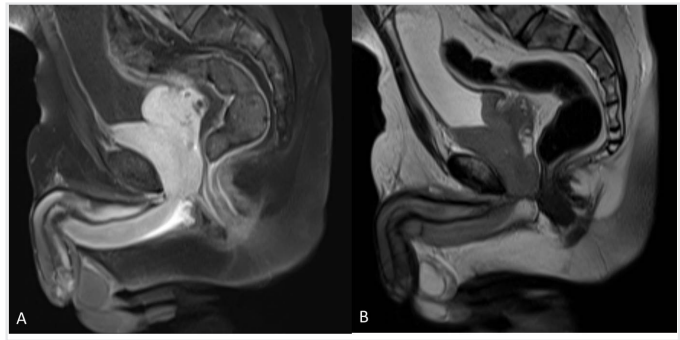

Figure 5. (A) Saggital T1 weighted image showed homogeneous isointensity in the corpus spongiosum of penis and (B) Saggital fat sat T1 postcontrast image showed homogenous contrast enhancement in the corpus spongiosum of penis

Discussion

NHL is generally observed in large, conglomerated lymph nodes. However, it can also occur outside the lymph nodes, called extra-nodal lymphoma. Extra-nodal lymphoma can also be seen in the gastrointestinal tract, liver, spleen, Waldeyer's lymphatic ring, skin, central nervous system, bone, thyroid gland and the urogenital system, although with low probability (7). Primary penile lymphoma is extremely rare, with less than 30 cases reported in the literature. Secondary involvement of the penis can occur due to retrograde spread or to direct extension from the neighbouring organ (5). The affected locations are the penile shaft (most common) and glans penis (4,6). Lymphoma of the penis can present as a nodule, painless mass, plaques or ulcers and results in a dysmorphic appearance of the penile shaft. Painless mass is the most common symptom, followed by the ulcer (1). When the corpus cavernosum of the penis is involved, erectile dysfunction and penile swelling can be seen. Radiological modalities such as CT, MRI and PET CT should be undertaken for treatment and prognosis. MRI of most penile cancers shows superficial, hypointense infiltrative soft-tissue masses on T1- and T2-weighted imaging (2). After intravenous contrast injection, lesions enhance homogeneously. In the present case, after an early consideration of the diagnosis of Peyronie's disease, the final diagnosis of NHL of the penis was confirmed by MRI images (6).

Systemic chemotherapy is the treatment of choice for the second presentation of lymphoma in the penis. Chemotherapy has the clear advantage of obtaining good cosmetic and functional results (1). Radical surgery can also be used, but only after the failure of other modalities like local radiotherapy, immunotherapy and combined modalities (3).

In conclusion, the penile malignant tumour and penile lymphoma are very rare and can be mistaken for other soft-tissue tumours, and hence it poses a significant differential diagnostic challenge (2). If the patient diagnosed with squamous cell carcinoma of the penis, treatment typically consists of radical penectomy (3,8). Excision biopsy is essential to reach the final diagnosis (7). Other differential diagnosis includes vasculitis, trauma nad sexually transmitted disease (1).

MRI plays a vital role in diagnosing penile lymphoma and assessing the local extent of the disease.

Ethics

Informed Consent:

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: G.T.Y., A.A.P., R.S., H.T., Design: G.T.Y., A.A.P., R.S., H.T., Data Collection or Processing: G.T.Y., A.A.P., R.S., H.T., Analysis or Interpretation: G.T.Y., A.A.P., R.S., H.T., Literature Search: G.T.Y., A.A.P., R.S., H.T., Writing: G.T.Y., A.A.P., R.S., H.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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Case Report

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A Coin Trapped in Meckel's Diverticulum

Meckel Divertikülünde Tutsak Bozuk Para

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ABSTRACT

Foreign body ingestion is a common problem among children. Most foreign bodies that have passed the esophagus will pass uneventfully through the intestinal tract. Foreign bodies that remain blocked in the narrower segments of the gastrointestinal tract require intervention. We herein report the case of a child who presented with a coin trapped in Meckel's diverticulum (MD). A 2-year-old boy was brought to our hospital 3 months after ingestion of coin. Physical examination showed no abdominal tenderness. Laboratory was normal. The foreign body appeared to be a coin located in the middle lower quadrant in the abdominal X-ray. Exploratory laparotomy was performed, and Meckel's diverticulum was discovered. The coin was detected in Meckel?'s diverticulum and it wedge resection were performed. After the operation, the patient had an uneventful recovery and started enteral feeding within 5 days. Early treatment of ingested foreign bodies in the gastrointestinal system is important in terms of preventing possible complications. The determination of the exact location of the coin, decision for intervention, and management may be difficult in cases with prolonged lodgment. The diagnosis of MD should be considered when there is a prolonged lodgment of a foreign body in the lower quadrant.

Keywords: Meckel, child, ingestion foregien body

ÖZ

Yabancı cisim yutulması çocuklar arasında yaygın bir sorundur. Özofagusu geçen yabancı cisimlerin çoğu bağırsak sisteminden sorunsuz bir şekilde geçecektir. Gastrointestinal sistemin daha dar segmentlerinde bloke kalan yabancı cisimler müdahale gerektirir. Burada Meckel divertikülünde (MD) sıkışmış bir bozuk para ile başvuran çocuk olgusunu sunuyoruz. İki yaşında erkek çocuk, bozuk para yutmasından 3 ay sonra hastanemize getirildi. Fizik muayenede karın hassasiyeti görülmedi. Laboratuvar normaldi. Yabancı cisim, karın grafisinde orta alt kadranda görünüyordu. Laparotomi yapıldı ve paranın MD'si olduğu saptandı ve divertikül rezeke edildi. Ameliyattan sonra hasta sorunsuz iyileşti ve 5 gün içinde oral beslenmeye başladı. Gastrointestinal sistemde yutulan yabancı cisimlerin erken tedavisi, olası komplikasyonların önlenmesi açısından önemlidir. Yabancı cismin uzun süre aynı yerde kaldığı vakalarda cismin tam yerini, müdahale kararını ve yönetimi belirlemek zor olabilir. Yabancı cismin alt kadranda uzun süre sabit kaldığı durumunda Meckel divertikülü akla gelmelidir.

Anahtar Sözcükler: Meckel, çocuk, yabancı cisim yutma

Introduction

Foreign body ingestion is a common problem in children. Most foreign bodies that cross the esophagus pass through the gastrointestinal tract without any problems. Of foreign bodies 80-90% entering the small intestine are excreted spontaneously in the stool (1). Foreign bodies blocking in narrower segments of the gastrointestinal tract (10-20%) require non-surgical interventions, while approximately ≤1% require surgical intervention (2).

In this case report, a child who were admitted to our hospital with a history of swallowing coin 3 months ago was presented. The

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coin was found to have settled in Meckel's diverticulum (MD) during the exploration. To our knowledge, there are few reports of foreign body stuck in MD in the pediatric literature.

Case Report

A two-year-old boy was admitted to our hospital with a history of swallowing foreign body (coin) 3 months ago. It was learned that the patient was under follow-up in an external center for three months and was referred to our pediatric surgery outpatient clinic because the coin did not move in the control direct radiographs.

No pathology was detected in the physical examination of the patient. Abdominal examination did not reveal tenderness or defense. A radiopaque foreign body (coin) was detected in the middle lower quadrant of the patient's standing direct abdominal X-ray (Figure 1). Laboratory values were evaluated as normal. Since the history of foreign body ingestion was three months ago, the family was informed and the surgery was planned. It was observed that there was no displacement in the control standing direct abdominal X-ray of the patient who was prepared for the operation under elective conditions, and that the coin persisted in the lower abdominal quadrant.

During the exploration under general anesthesia, MD was detected approximately 50 cm proximal to the ileocecal valve. When the exploration was continued, the diagnosis was made by palpating the coin in the diverticulum. The bowel was repaired by performing wedge resection of MD. Macroscopic examination of the resected specimen revealed a coin stuck in MD (Figure 2a, b). The patient, who had an uneventful recovery after the operation, started oral feeding within 5 days and was discharged on the 7th day.

Discussion

Foreign body ingestion is a common problem in the pediatric age group. Early diagnosis and intervention are important because of possible serious complications of swallowed foreign bodies such as mucosal erosion, airway obstruction or intestinal perforation (3). In young children, the time and type of foreign body ingestion cannot always be determined exactly. Although Chauvin et al. (4) have argued that enteroscopy or surgery should be considered for the removal of dangerous foreign objects such as sharp, pointed or elongated objects, batteries or magnets that cross the ligament of Treitz, there is no consensus in the literature. Since the structure of the foreign body is important for the management of the follow-up, the coin must be distinguished from the batteries (2). While straight-edged foreign bodies usually do not cause a serious condition, emergency intervention is required if the foreign body is a sharp object (3). It is difficult to make an intervention decision in cases where the foreign body in the gastrointestinal tract remains in the same localization for a long time. Daily stool observation and abdominal radiographs are required to monitor the progression of foreign bodies, such as coins, that have a lower risk of causing perforation through the gastrointestinal tract. However, the patient's family should

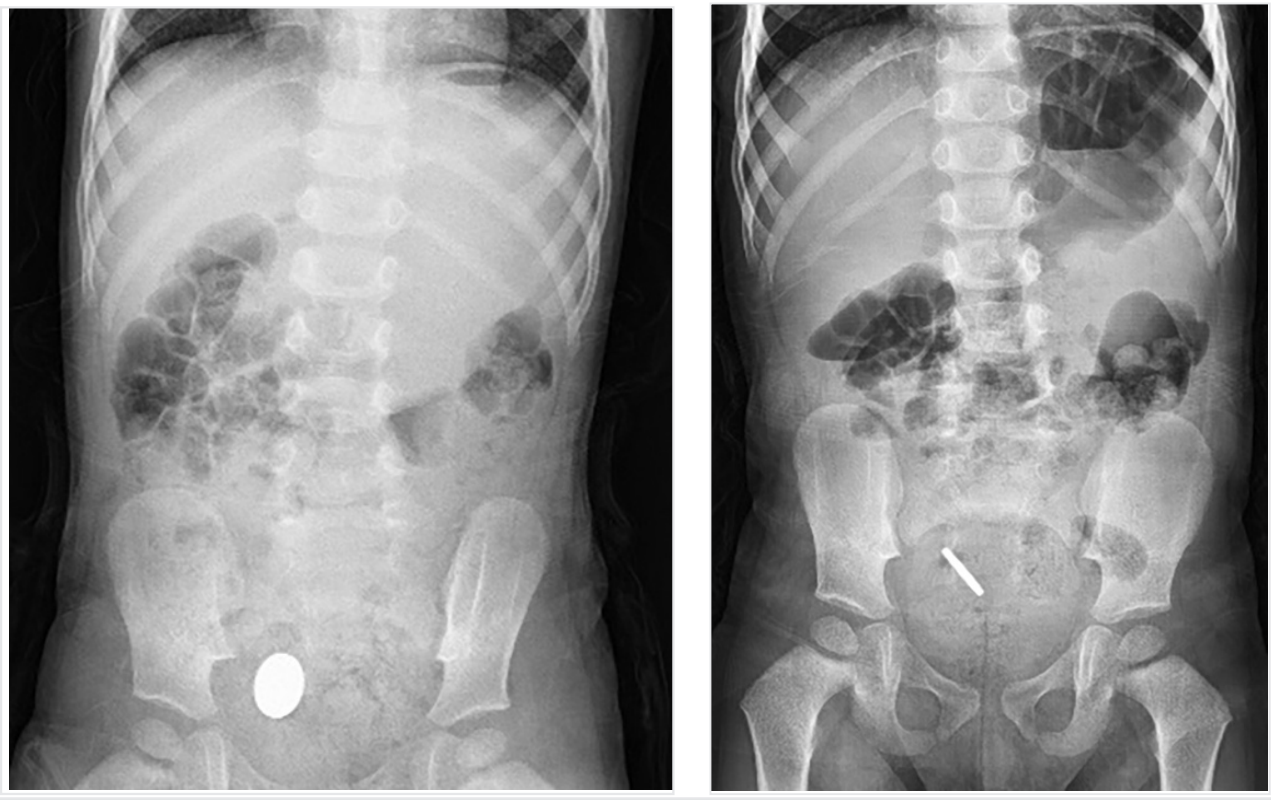

Figure 1. Patient's standing direct abdominal X-ray view showing a coin.

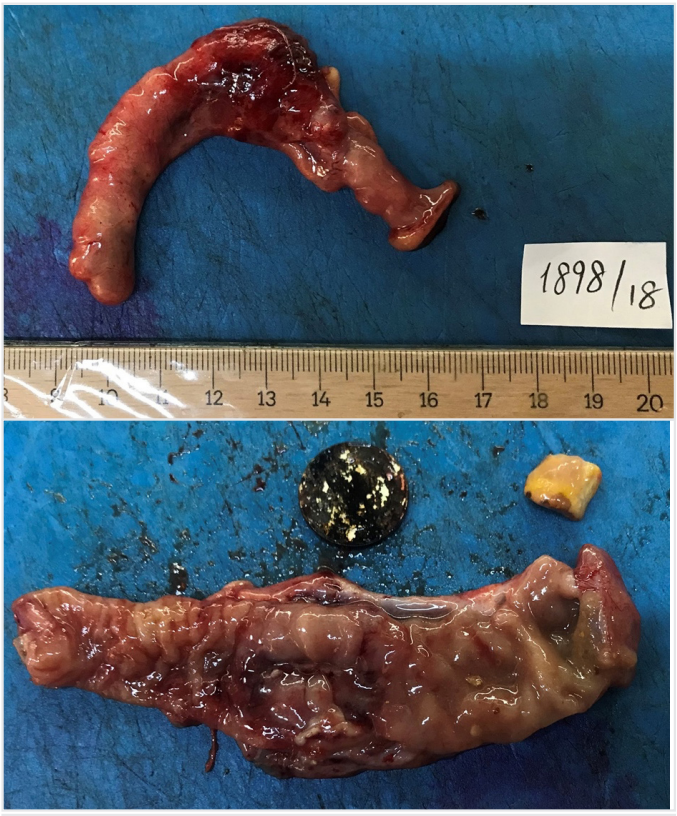

Figure 2a,b. Coin trapped within Meckel's diverticulum. This study was presented as poster at the 6th World Congress of Pediatric Surgery – WOFAPS 2019, 1-3 November 2019, Doha, Qatar.

be aware of the clinical signs of perforation and obstruction in this period (2).

Meckel's diverticulum is a remnant of the omphalomesenteric (vitelline) duct. MD has potential complications such as intestinal obstruction, gastrointestinal bleeding, or inflammation of the diverticulum with or without perforation (5). While the prevalence of MD is between 0.3-2.9% in the general population, the rate found in autopsy studies is 1.2% (5). In the literature, there are several case reports of puncture or occlusion of MD with disc pillars, phytobezoars, fish bones, chicken bones and needles in children and adults (6-9). In the three case reports of coin located in MD, which was detected in the English literature review, two patients were children and one patient was a 25-year-old woman. An adult patient presented with perforation, while the 19-month-old patient presented with abdominal pain and vomiting. Karadeniz Cerit et al. (2) presented their laparoscopic approach to an asymptomatic 10-year-old patient like our patient (10,11).

In particular, it may be difficult to determine the exact location of the foreign body, which does not move for a long time, and to make a follow-up or intervention decision (2). In such cases, it is not easy to find coins with X-ray images. We think that MD is a pathology that should be kept in mind, especially in foreign bodies that do not change their localization for a long time in the lower quadrant.

Ethics

Informed Consent:

Peer-review: Externally peer reviewed.

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

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Review

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The Golgi Apparatus with the Historical Point of View

Tarihsel Bir Bakış Açisi ile Golgi Apparatusu

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ABSTRACT

Scientists were introduced to the Golgi apparatus (GA) in 1898, when it was discovered by Camillo Golgi in 1898 as a "cytoplasmic reticular network". Researchers heard Camillo Golgi's name not only because of the GA, but also because of many definitions such as Golgi silver impregnation techniques, Golgi type I and II cells, Golgi cells of the cerebellum, and Golgi tendon organ. In fact, although the GA beared the name of this scientist, many scientists did numerous studies on the morphological and functional properties of this unique organelle before him, simultaneously with him or after him. Despite the simple technical possibilities of the old times, the scientists, whom we gratefully commemorated, obtained magnificent findings about the GA and presented them to the world of science. In this short article, which was a review, the following historical developments, starting from the discovery of the GA, were summarized.

Keywords: Camillo Golgi, Golgi apparatus, discovery, historical narrative

ÖZ

Bilim insanları Golgi apparatusu (GA) ile Camillo Golgi tarafından 1898 yılında "sitoplazmik retiküler bir ağ" olarak keşfedildiği 1898 yılında tanıştılar. Araştırmacılar Camillo Golgi'nin ismini sadece GA'dan dolayı değil, Golgi gümüş impregnasyon teknikleri, Golgi tip 1 ve 2 hücreler, beyinciğin Golgi hücreleri, Golgi tendon organı gibi pek çok tanımlama ile duydular. Aslında GA bu bilim insanının ismini taşıyor olsa da kendisinden önce, eş zamanlı olarak veya sonra pek çok bilim insanı bu benzersiz organelin morfolojik ve fonksiyonel özellikleri ile ilgili sayısız çalışma yaptılar. Eski zamanların basit teknik olanaklarna rağmen minnetle andığımız bilim insanları, GA ile ilgili muhteşem bulgular elde ederek bilim dünyasına hediye ettiler. Bu derleme niteliğindeki kısa yazıda GA'nın keşfinden başlamak üzere takip eden tarihsel gelişmeler özetlenmiştir.

Anahtar Sözcükler: Camillo Golgi, Golgi apparatus, tarihsel gelişim

Discovery of Golgi Apparatus and Historical Developments

The Golgi apparatus (GA), defined as the "post office of the cell", was first described in history about 100 years ago by the Italian doctor and pathologist Camillo Golgi, who was known for his studies on the nervous system (1844-1926). Professor Golgi was working at the University of Pavia, the oldest and most respected university in Italy, founded in 1361. Camillo Golgi and Spanish Anatomist Ramón y Cajal (1852-1934) shared the Nobel Prize in Physiology and Medicine in 1906 for their separate studies on the anatomy of the nervous system (1). The first award in this field was given to the German physiologist Emil Adolf von Behring, who discovered serum therapy in the development of diphtheria and tetanus vaccines in 1901. Serum therapy was interpreted as opening a new path in the field of Medical Sciences. Emil Adolf von Behring went down in history with the sentence that "a

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victorious weapon was placed in the hands of the doctor against sickness and death" (2).

Camillo Golgi developed a new technique in 1873 that stained neurons and special cells in the nervous system, thus allowing them to be marked. This technique was called the 'black reaction' because it stained the cell bodies and extensions of the nervous system black. With his technique, Golgi determined that the axons he clearly saw formed an uninterrupted network carrying nerve impulses (1). Today, the scientific writings of Camillo Golgi, including original drawings, are exhibited in the History Museum of the University of Pavia. Golgi has studies on various parts of the brain. However, his studies on the cerebellum, olfactory bulb, hippocampus and cerebral cortex are particularly important. For example, Golgi cells, which were named after him, were identified in the cerebellum (3). While Camillo Golgi's work on the nervous system continued, his rival, Santiago Ramo'n y Cajal, was also on the rise in the scientific world. Cajal developed a theory that challenged Golgi. While this theory, known as the "neuron theory", was generally supported by scientists, Golgi's work was criticized (4). These criticisms did not deter Camillo Golgi. While examining the spinal ganglia in 1897, he noticed that there was a cytoplasmic network in their cell bodies, although not in every cell. Later, he detected the same reticulated structure in the cytoplasm of Purkinje cells of the genus Tyto alba owl (barn owl) (3). Yet this new and strange structure was not stained in every cell. Therefore, he shared his observation with his assistant Emilio Veratti (1872-1967). Emilio Veratti (5), who described the sarcoplasmic reticulum in 1902, confirmed its existence by showing this cytoplasmic network in the 4th cranial nerve. He made the first official presentation of this structure, which he described as the "internal reticulated structure", on 19 April 1898 at the Pavia Medical-Surgical Society (6,7). He stated that this newly discovered reticulated structure consisted of anastomosing ribbon-shaped filamentous elements, small plates with a clear center that served as the nodal points of the reticulum, and rounded discs (8). Antonio Pensa (1874-1970), working in the General Pathology and Histology laboratory of Golgi in 1899, detected this organelle in the cells of the adrenal medulla (9). A short time later, 5th year medical student Adelchi Negri (1876-1912) demonstrated the presence of a similar structure in thyroid, epididymis, salivary glands, and ovarian cells besides nerve cells (10). Negri incidentally detected intraneuronal inclusions while examining rabies-infected brains. These inclusions are known as "Negri bodies". (8). Meanwhile, Edoardo Gemelli (1878-1959), one of Golgi's students, showed that a similar structure was found in the cells of the pituitary gland (11). From these observations it became clear that this structure was probably ubiquitous in eukaryotic cell types. Camillo Golgi hypothesized that this cytoplasmic network might be related to secretory function, more broadly to cell nutrition (8). Camillo Golgi changed the technique defined by Ramón y Cajal in 1903 and developed a new technique (12). With the advantages of this new technique, he was able to observe the morphology and localization of the GA during the secretion process in the mucous glands of the frog stomach. Thus, he found that the GA was located in the apical cytoplasm above the nucleus (13). Camillo Golgi tried to explain the physiological role of the GA in gastric and intestinal mucous cells (14). Meanwhile, Giuseppe D'Agata (1927-2011) was investigating this reticular network in the gastric epithelium (15).

In fact, although the GA was defined by being inspired by the work of Camillo Golgi, between 1867 and 1887, various scientists talked about the reticular structures existing in the cell from time to time before or after Camillo Golgi introduced this organelle (3,4,16,17). Perhaps these researchers also observed the GA. Yet all these years the GA was considered almost entirely a specific subject of the University of Pavia. Camillo Golgi and his students published more than 70 articles on this organelle. In these articles, they reported the changes observed in the GA in various developmental, physiological and pathological conditions, as well as the wide variety of cell types they observed (18). Thus, the organelle was found to be highly unstable and variable. Despite all these studies, the authenticity of this organelle was questioned by many researchers in the following years. Scientists defined this structure as an unreal structure that occured due to fixation or metallic impregnation technique (3). For example, George Palade and Albert Claude, scientists of the Rockefeller Institute, who showed similar cytoplasmic structures 20 years later in various cells without applying special staining methods using 40-55% ethanol, suggested based on these observations that GA represented one or more myelin figures that emerged artificially during the preparation of cytological samples (19). These discussions continued even when the GA could not be demonstrated with the first electron microscopic examinations (20). Finally, the GA, which was observed electron microscopically in the mid-1950s, was accepted as a real organelle and gained the respect it deserved (3). Taking into account Palade's suggestion that phosphate-buffered osmium tetroxide should be used, Bethesda National Cancer Institute scientists Albert Dalton and Felix (21) soon demonstrated the detailed electron microscopic structure of the GA in epididymis cells. These researchers described the organelle as a structure in the cytoplasm consisting of folded, smooth-surfaced sacs and numerous vesicles and vacuoles with the staining technique developed by Camillo Golgi (21).

For many years, scientists focused on morphology rather than the function of the GA. It was known almost from the beginning by light microscopic observations that this organelle developed well in secreting cells. However, the role of this organelle in secretion and glycosylation was not elucidated until the 1960s (22). Palade suggested that the GA was associated with the vectorial transport of secretory proteins in exocrine pancreatic cells, and that vesicular transport also occured between the sacs of this organelle (23). Fleischer et al. (24), Morre et al. (25), and Neutra and Leblond (26) emphasized the important role of the GA in glycoprotein synthesis. The results of the autoradiographic studies of Godman and Lane showing the uptake of sulfate into the GA suggested that this organelle had an important role in sulfation and therefore in glycoprotein biosynthesis (27). Between 1967-1975, various researchers conducted numerous studies on the role of the GA in the secretory pathway and vesicular transport

(28-30). In the 1980s, studies were carried out emphasizing the importance of mannose-6 phosphate in the exiting of lysosomal enzymes from the GA and in targeting them (31-34). Between 1981 and 1984, Rothman et al. studied substance transport in the Golgi sacs (35-37). The COPII protein cover was determined by Duden et al., Seratini et al., and Waters et al. in 1991, and by Barlow et al. in 1994 (22).

In parallel with the technological developments, it was possible to reach detailed information about the location of the GA, its morphological, functional and pathological features. Extraordinary new information is being obtained about morphological and functional properties of the GA with the discovery of new genes, the development of advanced technology techniques such as green fluorescent protein based live cell imaging techniques, dynamic live cell imaging techniques, high resolution electron microscopy techniques that can create three-dimensional structure, and correlative microscopy techniques (CLEM) (38,39). In particular, CLEM provides great advantages for obtaining new findings about substance trafficking, targeting and signaling mechanisms in Golgi sacs.

Naming the Golgi Apparatus

The discovery of the detailed morphological features of the GA with the use of electron microscopes caused this organelle to be given various names such as "Golgi body", "Golgi zone", "Golgi substance", and "Golgi net". In 1910, Carlo Besta named this organelle "Golgi apparatus" (40), but this name was officially entered into the scientific literature in 1913, using it in Nusbaum's article (41). The definition of "Golgi complex" entered the scientific literature in 1956 with the study of Dalton and Felix (42). Today, both the names "Golgi apparatus" and "Golgi complex" are used, but the "Golgi apparatus" is mostly preferred. Scientists are accustomed to the word "Golgi" not only because of this organelle, but also because of Golgi's silver implantation techniques, Golgi type I and type II cells, Golgi cells in the cerebellum, and Golgi tendon organ. Especially in recent years, many new terms have entered the literature in parallel with the fact that techniques that provide detailed information and three-dimensional imaging provide new information about the features of this organelle and its region. These are definitions such as "Golgi receptor", "Golgi strip", "Golgi cluster", "Golgi skeleton", "Golgi sac", "Golgi tubule", "Golgi vesicle", and "Golgi vacuole" (43). Although Camillo Golgi's name was mentioned in these common uses, many researchers do not know who this scientist actually was, how, where and under what conditions he lived. This review article was written with respect to Camillo Golgi and the scientists in his team, who provided the recognition of the GA, despite the limited possibilities that could not be compared with today's technological facilities, and other scientists who played very important roles in the recognition of the GA with their research, although their names were not remembered.

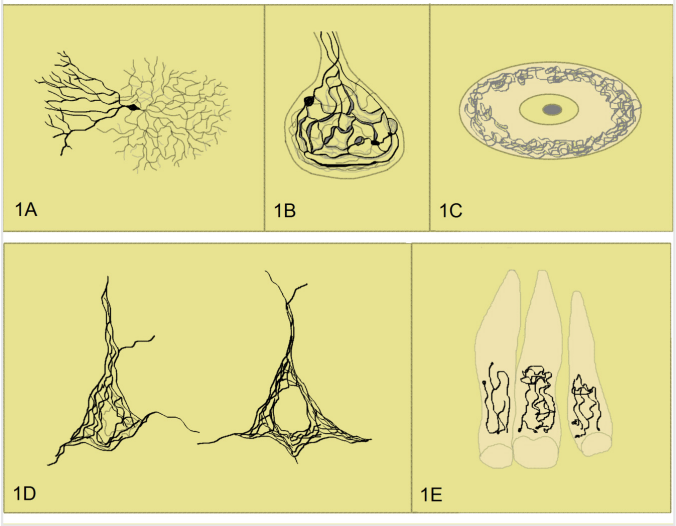

Figure 1. It was drawn by the author, inspired by Camillo Golgi's own drawings from the works of him on display at the University of Pavia Museum. 1 A. Nerve cell stained with black reaction by Camillo Golgi, 1B. First published figure showing the Golgi apparatus in the spinal ganglion cell, 1C. Golgi apparatus, defined as a reticular network in the cytoplasm of the spinal ganglion cell, 1D. Golgi apparatus in nerve cell in mouse cerebral cortex, 1E. Golgi apparatus in frog gastric mucosa cells (the original of this figure probably belongs to Emilio Veratti)

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Review

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Food Allergy in Children

Çocuklarda Besin Alerjisi

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ABSTRACT

Food allergy, which is defined as an inverse immune response to food proteins, appears to increase gradually in children as a result of studies. It affects 6% of children and 3-4% of adults. The role of breast milk in preventing the development of allergies in children is controversial. Allergic reactions caused by food in children cause many symptoms and disorders affecting the gastrointestinal tract, respiratory tract and skin. These symptoms occur by immunoglobulin (Ig)E-mediated or non-IgE-mediated mechanisms. The cornerstone of food allergy treatment is removing foods from the diet by strict elimination method. Along with the genetic tendency, environmental factors can eliminate oral tolerance and cause food allergy. Disease results are affected by the immune system and trigger allergen properties. Foods that cause food allergies are a few, mainly milk, eggs, peanuts, nuts, fish and shellfish. In this review, the level of knowledge about the pathogenesis of the immunological response about most allergens, which are special substances in the protein structure found in foods, has been discussed with studies conducted. Thus, this study will shed light on identifying new immunotherapeutic approaches to allergens.

Keywords: Food allergy, allergy in children, IgE, allergens, breast milk

ÖZ

Besin proteinlerine ters bir immün yanıt olarak tanımlanan besin alerjisi, yapılan çalışmaların sonucunda çocuklarda giderek arttığı görülmektedir. Çocukların %6'sını ve yetişkinlerin %3-4'ünü etkilemektedir. Anne sütünün çocuklarda alerji gelişimini önlemesindeki rolü ise tartışmalıdır. Çocuklarda görülen besin kaynaklı alerjik reaksiyonlar gastrointestinal sistemi, solunum yollarını ve deriyi etkileyen birçok semptom ve bozukluklara yol açmaktadır. Bu semptomlar immünoglobulin (Ig)E aracılı ve IgE aracılı olmayan mekanizmalarla gerçekleşmektedir. Besin alerjisi tedavisinin temel taşı, besinlerin diyetten katı eliminasyon yöntemiyle çıkarılmasıdır. Genetik eğilim ile birlikte çevresel faktörler de oral toleransı ortadan kaldırarak besin alerjisi oluşmasına neden olabilmektedir. Hastalık sonuçları, bağışıklık sistemi ve tetikleyici alerjen özelliklerinden etkilenmektedir. Besin alerjilerine yol açan besinler az sayıda olup başlıcalarını süt, yumurta, yerfıstığı, fındık, balık ve kabuklu deniz ürünleri oluşturmaktadır. Bu derlemede besinlerde bulunan protein yapısındaki özel maddeler olan alerjenlerin çoğu hakkında immünolojik yanıtın patogenezi konusunda bilgi düzeyi yapılan çalışmalarla tartışılmıştır. Böylece, bu çalışma ile alerjenlere karşı yeni immünoterapötik yaklaşımların belirlenmesine ışık tutulacaktır.

Anahtar Sözcükler: Besin alerjisi, çocuklarda alerji, IgE, alerjenler, anne sütü

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Introduction

Food allergy in children aged 2-6 years is a health problem that causes changes in social life and diet, which is seen in approximately 4% of children and families worldwide (1). Food allergy, which is defined as an immune response to food proteins, has become a public health problem as it has been observed to increase in the society as a result of studies (2). While milk and egg are the two most common allergen foods, the most third common allergen food is peanut in Switzerland, wheat in Japan and Germany, and tree nuts in Spain. Based on this situation, it can be said that it differs between countries (1).

Today, the cornerstone of food allergy treatment is the strict elimination of allergen foods from the diet. But; removal of important nutrients such as fish, milk and eggs, which should be included in the nutrition of children aged 2-6, may cause regression in growth-development and nutritional deficiencies in this age group (3).

Allergic reactions due to allergenic foods cause various disorders by affecting the skin and respiratory tract, especially the gastrointestinal system (4). There are 2 types of these reactions that affect immunoglobulin (Ig)E-mediated and non-IgE-mediated immune pathways (5). Along with these factors, genetic and environmental factors that eliminate oral tolerance also contribute to reactions related to food allergy (4).

What is a Food Allergy?

When exposed to a specific food or food group orally or by inhalation, the immune response that is not IgE-mediated, that is IgE-mediated, or that is both, it is called "food allergy" (6). Any protein in food can potentially affect an allergic reaction. The "major allergens" most responsible for these reactions are peanuts, tree nuts, milk, eggs, soy, wheat, shellfish and fish. The progression of food allergy can be very variable. Many individuals with food allergies seen in infancy develop tolerance to these allergenic foods as they get older (7). This situation depends on factors such as food, age of onset of allergy, severity of sensitivity and recognition of allergen components. In general, it is more likely to develop tolerance to allergens in chicken eggs, cow's milk, wheat and soybeans. At the same time, tolerance to allergens such as peanuts, nuts, crustaceans and fish is greater than to buckwheat.

The results of studies on food allergy show that one-fifth of the population reacts negatively to foods. In some countries, it is common in pre-school children and its rate is up to 4-7% (8). Large-scale epidemiological studies in Japan show that the incidence of food allergy is 5-10% in infants, 5% in young children, and 4.5% in school-age children (7). The changing natural history and disease profile of allergic disease in children and epigenetic effects that may cause hereditary changes, may be due to a complex gene-environment interaction in which the allergenic tendency can be changed or reproduced over generations (6). The immaturity of the intestinal barrier and immune system in infants and young children may contribute to the increased prevalence of allergies in the first few years of life.

Allergic reactions may result in various forms, ranging from urticaria to anaphylactic shock, and death (8). Skin reactions are one of the most common symptoms observed in food allergies. It occurs in 92% of patients. Respiratory symptoms constitute 33.6% of these symptoms, mucosal symptoms 28%, digestive symptoms 18.6% and anaphylaxis 10.4% (7).

Food Allergy Mechanisms

In infants and young children, the function of the mucosal barrier, the components of the intestinal barrier and the immune system do not fully reach developmental maturity until 4 years of age (2). Therefore, this maturation may play a role in the incidence of gastrointestinal tract inflammations and food allergies seen in the first years of life. Differences in the digestive capacity of gastritis, including antacid administration, may affect the allergy due to consumed food proteins (9). Urinary lactulose and mannose levels are increased in individuals with food allergies compared to healthy people. All people have low levels of IgE antibodies (10). Persons susceptible to allergic reactions are more likely to produce IgE antibodies that are specific to environmental antigens such as food, dust, and pollen. While an external antigen is digested by antibodies in normal situations, in case of allergy, macrophages cannot fully digest the antigen and the unabsorbed part passes to lymphocytes with RNA-antigen complexity. This complexity creates a series of reactions within lymphocytes, and as a result antibodies are produced. The antibodies produced, on the other hand, reveal clinical allergy symptoms in some special tissues.

Hypersensitivity reactions are classified into four types (10). These four types of reactions occur independently of each other. The first three types produce antibody-dependent reactions, while in the fourth type, T-lymphocytes and macrophages create reaction. It is involved in asthma and food allergy type 1 reactions. Type 1 reactions are known as anaphylactic (sudden) type sensitivity. In the immune response, excessive or inappropriate reactions may occur against the antigen.

Food-specific IgG, IgM, and IgA antibodies are often found to be low in serum in healthy children without food allergy (4). Food allergen-specific IgG antibodies increase in the first months after consuming a food. Even if the individual continues to consume the food allergen, it often subsides. People with various inflammatory bowel disorders often have high levels of food-specific IgG and IgM antibodies. However, there is no evidence that these antibodies are pathogenic. As a result of cross-linking of IgE receptors to mast cells and basophils, the primary and rapid-resulting effect of the allergen is observed in people with allergies (10). These reactions develop due to cross-linking of surface IgE receptors and mast cells and secretion of substances (mediators) in the cell. The most important mediator for the formation of allergic reactions is histamine.

One of the first developments in the pathogenesis of the allergen is the capture and delivery of the allergen by the antigen-containing cells in the airways or intestinal tract mucosal areas and lymph nodes (11). Specific T-cells for active allergens are present in the circulation of allergic patients. The classical way of IgE is to bind to T-cell (12). T cells produce CD40 ligand

(CD40L) in response to an antigenic stimulus and produce T helper 2 (Th2) type cytokines, interleukin-4 (IL-4) or IL-13 to secrete.

Mast cells play a critical role in allergy (11). Triggering of mast cells and release of previously stored mediators from mast cells are important factors in causing vasodilation, edema formation, or bronchoconstriction during an allergic reaction. Basophils have similar properties to mast cells. It is thought that they often play a minor role in allergic reactions. In addition, basophils, like mast cells, respond to stimuli via IgE and it is often accepted that they are involved in the effector phase at the onset of allergic reactions.

Food Types That Cause Allergies

In general, food allergy is seen in 58.1% of all patients (7). It is mostly seen in children under the age of 1, at a rate of 88.1%, and as the age increases, the rate of food allergy decreases. Predominant food allergies show differences according to age groups. For example, in children under the age of 1, chicken eggs (57.6%) are in the first place, cow's milk (24.3%) is in the second place, and wheat is in the third place, while fruit (16.5%) is in the first place in the 4-6 age group, and in the second place is wheat. chicken eggs (15.6%) and peanuts (11.6%) take the third place. In the adult group (≥20 years), wheat (38%) is in the first place, fish (13%) is in the second place and crustaceans (10%) are in the third place.

Many food allergens can cause a reaction when the food is raw, after it has been cooked or even after it has been digested. However, some allergens found in fruits and vegetables cause allergic reactions, especially when these foods are consumed raw (13). Food allergens can also react in case of inhalation of allergy-causing proteins. Cross-reactivity can occur when the allergen in a food has similarity in structure or sequence with a different food allergen or aeroallergen. The likelihood of cross-reactive allergens in clinical allergic reactions is highly variable and depends on the type of food.

Proteins that initiate IgE-mediated immune reactions are food allergens (14). Members of the Solanaceae family may cause severe reactions in susceptible individuals. However, with the exception of eggplant (Solanum melongena L.), food allergies are identified due to the consumption of bell peppers, potatoes and tomatoes. On the other hand, some studies explain allergic reactions to eggplant (15). The incidence of allergic reactions is mainly due to the histamine content. A large number of other allergens have been found and some have been found to be heat stable. However, some allergens are heat sensitive and can be destroyed by cooking.

Egg Allergy

Chicken eggs are one of the most common foods that cause allergies in pediatric patients via IgE (16). There are more than 20 glycoproteins in glair. Some of these have been identified as major allergens: Ovomucoid, ovaalbumin, conalbumin and lysozyme. Fifty eight children with egg allergy were followed for a period between 7 and 86 months (all children under 2 years of age) (17). Thirty-four children (59%) gained tolerance to egg allergy. Kaplan-Meier curves showed that the overall tolerance was 50% at 35 months follow-up. This showed that half of the children were able to tolerate eggs when they were 4 to 4.5 years old. In this process, the best tolerance time was seen as the third year. After the third year, the tolerance decreased. The tolerance ratio was inversely proportional to the IgE antibody concentration in glair. The most common egg allergy symptoms are skin related symptoms (angioedema, urticaria) in 59%, gastrointestinal symptoms (diarrhea, vomiting, abdominal pain, bloody stools) in 21%, eczema in 18%, lower respiratory tract symptoms in 10% (difficulty in breathing, cough, wheezing) and upper respiratory tract symptoms (rhinitis, nasal congestion) in 4% (18).

Milk Allergy

Cow's milk allergy in infants and children is the most common type of allergy and its prevalence is between 2% and 3% (19). The protein in cow's milk causes the body to show an allergic reaction. Generally, cow's milk allergy becomes evident before 6 months and appears with various symptoms that regress until 6 years of age. About 80% of children with cow's milk allergy at the age of 3 can tolerate the allergy. Children with cow's milk allergy are often allergic to more than one milk protein (19). Casein constitutes 80% of cow's milk proteins, and whey constitutes the remaining 20%. Symptoms occur when IgE antibodies bind to mast cells due to proteins found in cow's milk, and mast cell degranulation and release of mediators including histamine. In addition to symptoms such as diarrhea and vomiting, anaphylactic shock may also occur (19). In cases where anaphylactic shock occurs, it is absolutely necessary to seek help from the emergency department, as it should be treated with epinephrine. Symptoms such as flushing of the face, swollen throat, itching, increased respiratory effort, and narrowing of the airways occur.

Children with cow's milk allergy may experience vitamin and mineral deficiencies when starting a dairy-free diet (20). Therefore; in order to have a balanced and adequate diet, it is necessary to get help from a dietitian. In particular, vitamin D has a very important place in bone development in children of this age group. As a result of eliminating cow's milk products from the diet, it may be necessary to take supplements because there may be a deficiency of vitamins and minerals in milk such as vitamin D, riboflavin and calcium. It is known that the cow's milk allergy type with rapid symptoms is IgE-mediated. Urticaria, hives, sore throat, angioedema, coughing, vomiting, shortness of breath and anaphylaxis are the main symptoms (19). Reactions with slower symptoms are related with non-IgE-mediated cow's milk allergy. Its symptoms are diarrhea, hematochezia, colic, and abdominal cramps.

Peanut Allergy

The rate of peanut allergy in children increased by 0.4% in 1997, 0.8% in 2002, and 1.4% in 2008 compared to the previous years (21). The reason for this increase is due to problems such as the extra allergen in the roasted peanut forms, the early exposure to peanuts when the immune system is not fully mature, and the

fact that peanuts are not included in the diet. Peanut allergy can be lifelong, often severe and fatal. The severity and persistence of allergies also increase over time (22). Factors that can lead to a fatal outcome include the diagnosis of asthma, a delay in the administration of epinephrine, and the presence of peanuts at meals (23). Peanut allergy symptoms usually develop a few minutes after consuming a small amount of peanuts. These can affect the skin, cardiovascular, gastrointestinal, genitourinary or respiratory systems. In fatal cases, progressive upper and lower respiratory tract symptoms, hypertension, and arrhythmia typically develop. In one study, 89% of the reactions occur in the skin, 52% in the respiratory tract, and 34% in the gastrointestinal tract. There is no known cure for peanut allergy (24). It is common for children with allergies to accidentally eat peanuts. If a person with a peanut allergy accidentally consumes a peanut-containing food, serious allergic reactions develop. Epinephrine and antihistamines are given to patients as an emergency treatment plan.

Pollen Allergy

Pollen in the environment is becoming a growing problem for people with pollen allergies (25). Allergenic pollens are emitted from anemophile trees. The feeding habits of the bee affect the plant diversity or weather conditions in a particular region. These allergenic pollens can be found in all bee products. It is even known as an important component of honey and can determine the quality and type of honey. Pollen can enter honey in different ways. The amount of pollen may differ according to the type and collection period. Pollen allergens in honey can cause allergic reactions. However, pollen-induced allergy is rarely seen and can cause anaphylactic reactions. Climatic and weather conditions, biological characteristics of plants or environmental conditions are determinants for pollen abundance (25). Therefore, pollen concentrations in honey vary from year to year. Often, pollen concentrations and prolonged pollen season can also increase the severity of symptoms. Mostly, patients are affected by climate change (66%) (26).

Oral Allergy Syndrome

Oral allergy syndrome is a form of food allergy due to uncooked foods, that often causes allergic symptoms limited to the oral mucosa (27). Most IgE epitopes in foods recognized by patients with oral allergy syndrome may be the same as pollen allergens (28). Therefore, pollen allergens may be responsible for the emergence and maintenance of the oral allergy syndrome. In oral allergy syndrome, oral pharyngeal symptoms first appear after consumption of fruits and vegetables (29). Systemic symptoms can be seen in 8.7% of the patients and anaphylactic shock in 1.7%. Localized oral symptoms such as numbness in the lips or mouth, itching, tingling and swelling in the lips, tongue, palate, and pharynx are mostly observed (30). There are various risk factors for oral allergy syndrome (30). Patients with symptomatic pollen allergy have a higher risk of oral allergy syndrome than patients with only pollen sensitivity and who are asymptomatic. Allergen distribution is not the same in all parts of the fruit or vegetable (30). There is much more allergen in the peel of apples and peaches. In a small-scale study; while 40% of patients with apple and pear allergy could tolerate the fleshy part of the fruit, allergy occured when the whole fruit was eaten. Elimination of known allergenic foods from the diet is usually the first treatment that comes to mind for oral allergy syndrome (31). Restricting all other fresh vegetables and fruits in the same plant family or cross-reacting with the same pollen is also considered unnecessary.

Wheat Allergy

One of the most common childhood food allergies is wheat allergy, affecting 0.4% of children (32). Wheat contains four classes of allergenic proteins (33). These are albumins, globulins, gliadin, and glutenins. Gliadin and glutenin make up 85% of wheat protein (34). Symptoms due to IgE-mediated reactions may develop, usually 2 hours after consuming wheat. As a result of digesting the proteins in wheat, bronchial obstruction, urticaria, angioedema, nausea, abdominal pain or systemic anaphylaxis may occur. It is known that the prognosis of IgE-mediated wheat allergy in children is good. Patients with anaphylaxis before the age of 3 and patients with high wheat-specific IgE antibodies seem to be at risk of being more persistent (1). Since it is not necessary to limit the consumption of foods containing barley and rye in the treatment of wheat allergy, it is essential to avoid foods containing wheat, which will be less restricted in wheat allergy than in celiac disease (35). Celiac disease is a lifelong food allergy that is not associated with IgE (36). It is mostly seen in children aged 6-12 years. Besides wheat; there is also sensitivity to gluten in barley and rye. As a result of the toxic effect of gluten in the intestines, the digestive and absorption activities of the intestines are impaired. Diarrhea, frequent defecation, swelling in the abdomen, malnutrition, and as a result, developmental delay occur. The disease is diagnosed by small intestinal mucosal biopsy, blood and stool tests. The treatment of Celiac disease is still not found today. A gluten-free diet should be applied as an elimination diet. Parents should have their child's allergy status checked regularly. Most likely, children will recover from this allergy and may no longer need an elimination diet, a gluten-free diet.

Seafood Allergy

More than one-third of children today show an allergic reaction to fish species (37). A retrospective study among 2999 children with food allergies in a clinic in Australia showed a 5.6% prevalence of fish allergy (38). Tuna and salmon were the most common fish species causing allergy. Another study conducted in Singapore confirmed that 13% of 227 children with food sensitivities had a significant sensitivity to fish. Interestingly, it was observed for the first time in Asian societies that fish consumption started very early, at about 7 months (37). Although heat is thought to increase the allergenicity of some fish allergens, commercial heating procedures used in producing canned fish appear to have a different effect on fish proteins (38). A recent descriptive study from Australia showed that more than 20% of children allergic to tuna and salmon could tolerate canned fish.

Breast Milk and Food Allergy

Due to the increasing rates of allergic disease in developed countries, there is interest in the potential effects of breast milk in modulating immune responses (39). There are different mechanical ways that breast milk can protect against allergic disease. Breast milk contains bioactive factors, regulatory cytokine converting growth factor-β (TGF-β), secretory IgA antibodies and innate immune receptor soluble CD14 (sCD14). TGF-β in breast milk is required for the initiation of IgA production in newborn infants and induces mucosal tolerance to allergens. There are controversial results about the role of infants' consumption of breast milk in the prevention of allergy development (40). This is because there are differences in the composition of breast milk. For example; it is thought that high IgA and TGF-β levels in breast milk provide protection against the development of allergic diseases in some studies, while there is no significant relationship between breast milk TGF-β and atopic risk in others. Breast milk contains various cytokines and chemokines such as TGF-IL, IL-10, IL-4, RANTES, and IL-8 that are involved in allergic reactions (41). A few of these factors differ between allergic and non-allergic mothers. While high IgE levels were detected with breastfeeding in the children of mothers with high IgE levels, low antibody levels were observed in children of mothers with low IgE levels (41).

Diagnostic Methods of Food Allergy

Diagnosing food allergies allows knowing which mechanism is involved and identifying potential food triggers (42). Clinical history, skin tests, or serum-specific IgE tests alone or in combination give results between 50% and 100% (43). The first line tests to assess IgE sensitivity for food allergens are; *in vivo* skin tests and serum-specific IgE tests, but these tests or patient history cannot always accurately diagnose food allergy (44). Endoscopy and biopsy are required to diagnose some clinical findings such as food-borne enteropathies.

Specific IgE: Skin Tests and *In Vitro* Test

It is performed to determine sensitivity to suspected food allergens and to evaluate cross-sensitivity to the relevant food (45). Serum specific IgE or skin prick tests, although not standardized, are cited as scientifically valid tests. Detailed clinical history should guide the selection of tests (46). Skin tests should only be performed by trained healthcare professionals who can interpret results and manage potential adverse reactions. These tests are performed on the forearm or upper back. While performing and interpreting skin tests, there are variables that should be considered such as age, gender, test site and timing. While these tests show high performance in allergens such as eggs, milk, fish, shrimp, nuts, peanuts, they do not have high performance in allergens such as soy and wheat (47). Placebo (single/double) studies can be conducted on difficulties occurring after oral food consumption (48). Especially; the blinded placebo test is considered the gold standard for the diagnosis of food allergy. The double-blind placebo-controlled food challenge is more difficult due to difficulty in sourcing and time consuming (42). In patients with suspected psychological symptoms or atopic eczema, the double-blind placebo-controlled test outperforms the oral nutritional challenge comparison. Certain portions of the food protein recognized by the IgE antibody have been identified for some food allergens (2). In molecular or serological diagnostic tests, specific IgE antibodies can be measured against individual allergenic molecules from foods that have the potential to improve the specificity of the serum IgE test and the specificity of the selected food (49). This can be performed in single test formats or in a micropattern to test multiple purified allergens at the same time. There are a number of tests whose accuracy has not been fully proven in food allergy (50). These tests are kinesiology, iridology, bioresonance, hair analysis, cytotoxic test, IgE and IgG4 determination. Since these tests have not been confirmed so far, their use in the diagnosis of food allergy is not recommended.

Conclusion

Food allergy is now recognized as a problem worldwide, especially in children, and is increasing like other atopic disorders. In recent years, much attention has been paid to research on food allergy. Food allergens cause both acute and chronic diseases and may cause some negative effects on quality of life. Depending on the severity of the allergic reaction, the result can be fatal. Clinical studies have caused changes to be made in early nutrition guidelines for the prevention of food allergy. More information needs to be learned about the immune mechanisms of oral tolerance. New diagnostic tools can be developed to more accurately identify food allergens. In general, clinical trials of potential therapy in children are being conducted. Whether these treatments will cause only short-term desensitization or long-term tolerance is under investigation, and these studies may provide a better understanding of tolerance mechanisms. More specific diagnostic tests and more effective forms of treatment are still needed in this area.

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