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Ege Tıp Bilimleri Dergisi

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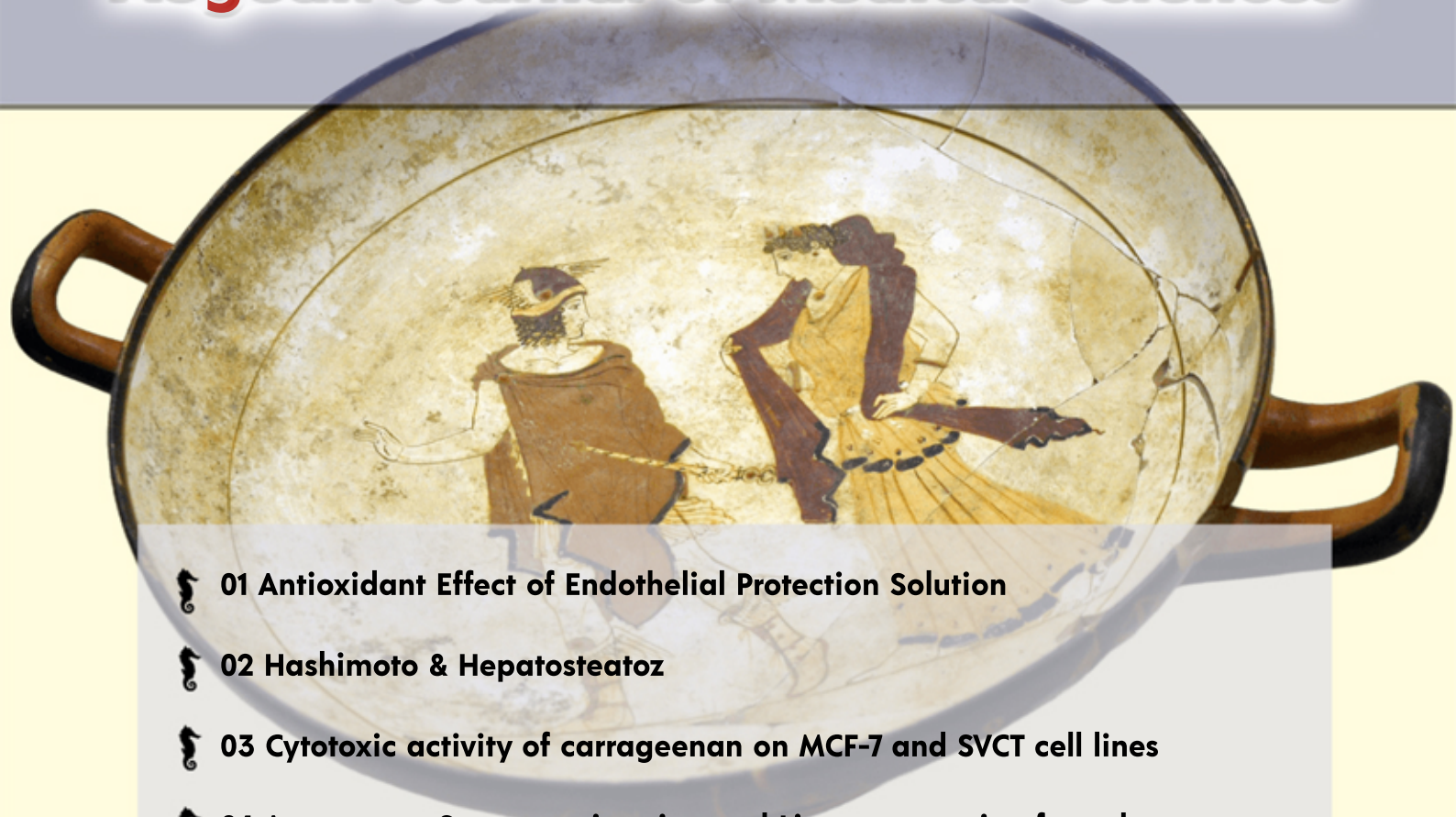

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Derginin Yazı Dili

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Makalenin yayımlanması uygun bulunduktan sonra, tüm yazarlardan "Yayın Hakkı Devir Formu" nu imzalamaları istenir: " Biz aşağıda imzaları bulunan yazarlar, sunduğumuz makalenin orijinal olduğunu; başka bir dergiye yayınlanmak üzere verilmediğini; daha önce yayınlanmadığını; eğer, tümüyle ya da bir bölümü yayınlandı ise yukarıda adı geçen dergide yayınlanabilmesi için gerekli her türlü iznin alındığını ve orijinal telif hakkı devri formu ile birlikte Ege Tıp Bilimleri Dergisi Editörlüğü' ne gönderildiğini garanti ederiz."

Ege Tıp Bilimleri Dergisi, Uluslararası Tıp Dergileri Editörleri Kurulu'nun (International Committee of Medical Journal Editors) "Biyomedikal Dergilere Gönderilen Makalelerin Uyması Gereken Standartlar: Biyomedikal Yayınların Yazımı ve Baskıya Hazırlanması (Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication)" standartlarını kullanmayı kabul etmektedir. "Ege Tıp Bilimleri Dergisi Yazarlara Bilgi" içeriği, bu sürümden yararlanarak hazırlanmıştır. Bu konudaki bilgiye www.icmje.org adresinden ulaşılabilir.

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Ege Tıp Bilimleri Dergisi, deney hayvanları ile yapılan çalışmalarda, genel kabul gören ilgili etik kurallara uyulması zorunluluğunu hatırlatır. Alınmış Etik Kurul Onayı, makale ile birlikte sisteme yüklenmelidir.

Yazar(lar), ticari bağlantı veya çalışma için maddi destek veren kurum varlığında; kullanılan ticari ürün, ilaç, firma vb. ile nasıl bir ilişkisi olduğunu sunum sayfasında Editöre bildirmelidir. Böyle bir durumun yokluğu da yine ayrı bir sayfada belirtilmelidir.

Yazı Türleri

Yazılar, elektronik ortamda http://dergipark.gov.tr/egetbd adresine gönderilir.

Orijinal makaleler: 3000 sözcük sayısını aşmamalı, "Özet (250 sözcükten fazla olmamalı), Giriş, Gereç ve Yöntem, Bulgular, Tartışma, Sonuç, Kaynaklar" bölümlerinden oluşmalıdır.

Olgu Sunumu: "Özet, Giriş, Olgu Sunumu, Tartışma, Kaynaklar" şeklinde düzenlenmelidir. En fazla 1000 sözcük ve 15 kaynak ile sınırlıdır. Sadece bir tablo ya da bir şekil ile desteklenebilir.

Editöre Mektup: yayımlanan metinlerle veya mesleki konularla ilgili olarak 500 sözcüğü aşmayan ve beş kaynak ile bir tablo veya şekil içerecek şekilde yazılabilir. Ayrıca daha önce dergide yayınlanmış metinlerle ilişkili mektuplara cevap hakkı verilir.

Yayın Kurulu'nun daveti üzerine yazılanlar dışında derleme kabul edilmez.

Makalenin Hazırlanması

Dergide yayınlanması istenilen yazı için aşağıdaki kurallara uyulmalıdır.

a) Yazı; iki satır aralıklı olarak, Arial 10 punto ile yazılmalıdır. b) Sayfalar başlık sayfasından başlamak üzere, sağ üst köşesinde numaralandırılmalıdır.

c) Online makale sistemine yüklenen word dosyasının başlık sayfasında (makalenin adını içeren başlık sayfası), yazarlara ait isim ve kurum bilgileri yer almamalıdır.

d) Makale, şu bölümleri içermelidir: Her biri ayrı sayfada yazılmak üzere; Türkçe ve İngilizce Başlık Sayfası, Özet, Abstract, Anahtar Sözcükler, Keywords, Giriş, Gereç ve Yöntem, Bulgular, Tartışma, Sonuç, Açıklamalar (varsa), Kaynaklar, Şekil Alt Yazıları, Tablolar (başlıkları ve açıklamalarıyla beraber), Ekler (varsa).

Yazının Başlığı

Kısa, kolay anlaşılır ve yazının içeriğini tanımlar özellikte olmalıdır.

Özetler

Türkçe (Özet) ve İngilizce (Abstract) olarak yazılmalı, Amaç, Gereç ve Yöntem, Bulgular ve Sonuç (Aim, Materials and Methods, Results, Conclusion) olmak üzere dört bölümden oluşmalı, en fazla 250 sözcük içermelidir. Araştırmanın amacı, yapılan işlemler, gözlemsel ve analitik yöntemler, temel bulgular ve ana sonuçlar belirtilmelidir. Özette kaynak kullanılmamalıdır. Editöre mektup için özet gerekmemektedir.

Anahtar Sözcükler

Türkçe Özet ve İngilizce Abstract bölümünün sonunda, Anahtar Sözcükler ve Keywords başlığı altında, bilimsel yazının ana başlıklarını yakalayan, Index Medicus Medical Subject Headings (MeSH)'e uygun olarak yazılmış en fazla beş anahtar sözcük olmalıdır. Anahtar sözcüklerin, Türkiye Bilim Terimleri'nden (www.bilimterimleri.com) seçilmesine özen gösterilmelidir.

Metin

Yazı metni, yazının türüne göre yukarıda tanımlanan bölümlerden oluşmalıdır. Uygulanan istatistiksel yöntem, Gereç ve Yöntem bölümünde belirtilmelidir.

Kaynaklar

Kaynaklar metinde yer aldıkları sırayla, cümle içinde atıfta bulunulan ad veya özelliği belirten kelimenin hemen bittiği yerde ya da cümle bitiminde noktadan önce parantez içinde Arabik rakamlarla numaralandırılmalıdır. Metinde, tablolarda ve şekil alt yazılarında kaynaklar, parantez içinde Arabik numaralarla nitelendirilir. Sadece tablo veya şekil alt yazılarında kullanılan kaynaklar, tablo ya da şeklin metindeki ilk yer aldığı sıraya uygun olarak numaralandırılmalıdır. Dergi başlıkları, Index Medicus'ta kullanılan tarza uygun olarak kısaltılmalıdır. Kısaltılmış yazar ve dergi adlarından sonra nokta olmamalıdır. Yazar sayısı altı veya daha az olan kaynaklarda tüm yazarların adı yazılmalı, yedi veya daha fazla olan kaynaklarda ise üç yazar adından sonra et al veya ve ark. yazılmalıdır. Kaynak gösterilen derginin sayı ve cilt numarası mutlaka yazılmalıdır.

Kaynaklar, yazının alındığı dilde ve aşağıdaki örneklerde görüldüğü şekilde düzenlenmelidir.

Dergilerdeki Yazılar

Kim CH, Cheon JS, Choi WY, Son KM. The efficacy of mobile application use on recall of surgical risks in nasal bone fracture reduction surgery. Arch Craniofac Surg. 2018; 19: 41-47.

Henüz yayınlanmamış online makale

Kurita GP, Højsted J, Sjøgren P. Tapering off long-term opioid therapy in chronic non-cancer pain patients: a randomized clinical trial. Eur J Pain. 2018 May 13. doi: 10.1002/ejp.1241.

Kitap

Bilgehan H. Klinik Mikrobiyoloji. 2. Baskı. İzmir: Bilgehan Basımevi; 1986:137-40.

Kitap Bölümü

McEwen WK, Goodner IK. Secretion of tears and blinking. In: Davson H (ed). The Eye. Vol. 3, 2nd ed. New York: Academic Press; 1969:34-78.

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Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]. Available from: www.nursingworld.org/AJN/2002/june/wawatch.htm

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Açıklamalar

Varsa finansal kaynaklar, katkı sağlayan kurum, kuruluş ve kişiler bu bölümde belirtilmelidir.

Tablolar

Tablolar metni tamamlayıcı olmalı, metin içerisinde tekrarlanan bilgiler içermemelidir. Metinde yer alma sıralarına göre Arabik sayılarla numaralandırılıp tablonun üstüne kısa ve açıklayıcı bir başlık yazılmalıdır. Tabloda yer alan kısaltmalar, tablonun hemen altında açıklanmalıdır. Dipnotlarda sırasıyla şu semboller kullanılabilir: *, †, ‡, §, ¶.

Şekiller

Şekil, resim, grafik ve fotoğrafların tümü "Şekil" olarak adlandırılmalı ve ayrı birer .jpg veya .gif dosyası olarak (yaklaşık 500x400 piksel, 8 cm eninde ve en az 300 dpi çözünürlükte) sisteme eklenmelidir. Şekiller metin içinde kullanım sıralarına göre Arabik rakamla numaralandırılmalı ve metinde parantez içinde gösterilmelidir.

Şekil Alt Yazıları

Şekil alt yazıları, her biri ayrı bir sayfadan başlayarak, şekillere karşılık gelen Arabik rakamlarla çift aralıklı olarak yazılmalıdır. Şeklin belirli bölümlerini işaret eden sembol, ok veya harfler kullanıldığında bunlar alt yazıda açıklanmalıdır. Başka yerde yayınlanmış olan şekiller kullanıldığında, yazarın bu konuda izin almış olması ve bunu belgelemesi gerekir.

Ölçümler Ve Kısaltmalar

Tüm ölçümler metrik sisteme (Uluslararası Birimler Sistemi, SI) göre yazılmalıdır. Örnek: mg/kg, µg/kg, mL, mL/kg, mL/kg/h, mL/kg/min, L/min, mmHg, vb. Ölçümler ve istatistiksel veriler, cümle başında olmadıkları sürece rakamla belirtilmelidir. Herhangi bir birimi ifade etmeyen ve dokuzdan küçük sayılar yazı ile yazılmalıdır.

Metin içindeki kısaltmalar, ilk kullanıldıkları yerde parantez içinde açıklanmalıdır. Bazı sık kullanılan kısaltmalar; iv, im, pove sc şeklinde yazılabilir.

İlaçların yazımında jenerik isimleri kullanılmalıdır.

İçindekiler

Orijinal Araştırma / Original Investigation
Aegean J Med Sci 2022;2:25 -29

Investigation of the Effects of Endothelial Protection Solution on Oxidative Stress in Saphenous Vein Endothelium in Diabetic Patients undergoing Coronary Bypass

Bypass Uygulanan Diyabetik Hastalarda Endotel Koruma Solüsyonunun Safen Ven Endotelinde Oksidatif Stres Üzerine Etkilerinin İncelenmesi

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ÖZET

AMAÇ: Diyabetik koroner arter hastalarının baypaslarında kullanılan safen ven greftleri normal hasta popülasyonundan daha erken tıkanır. Bu çalışmada, diyabetik hastalarda operasyon sırasında alınan safen ven greftlerinin oksidatif hasara karşı korunmasında endotel koruma solüsyonunun etkileri araştırıldı

GEREÇ VE YÖNTEM: Hastanemizde 2021-2022 yılları arasında koroner bypass ameliyatı olan 50 diyabet hastasının operasyonu sırasında alınan safen ven greftlerinin kalan kısımları üç parçaya bölündü. Her parça salin (grup 1), heparinize otolog kan (grup 2) ve endotel koruma solüsyonu (grup 3) olmak üzere farklı bir solüsyona yerleştirildi. Çözeltilerde 4 saat bekletildikten sonra hücresel ve DNA oksidatif stres belirteçleri araştırıldı.

BULGULAR: Toplam antioksidan kapasite (TAC) açısından gruplar arasında anlamlı fark yoktu (p>0.05). Toplam oksidatif durum (TOS) seviyeleri, endotel solüsyonu olmayan grup 1 ve 2'de (1,57±0,46'ya karşı 1,55±0,19) grup 3'ten (1,31±0,11) anlamlı olarak yüksekti (p<0,05). Ayrıca oksidatif stres indeksi (OSI) grup 3'te anlamlı olarak daha düşüktü (p<0.05). Öte yandan nükleer oksidatif stresi gösteren 8-hidroksi-2'-deoksiguanozin (8-OHdG) düzeyleri de grup 3'te düşük bulundu (p<0.05).

SONUÇ: Sonuçlarımız diyabetik hastalarda kullanılan safen ven greftlerinde endotel solüsyonu kullanımının oksidatif hasarı önlediğini desteklemektedir.

Anahtar Kelimeler: diyabetik koroner arter hastalığı, safen greft, oksidatif stres, endotel koruma solüsyonu

ABSTRACT

OBJECTIVE: Saphenous vein grafts used for bypasses of diabetic coronary artery patients are occluded earlier than the normal patient population. In this study, the effects of endothelial protection solution on the protection of saphenous vein grafts taken during the operation against oxidative damage in diabetic patients were investigated.

MATERIALS AND METHODS: The remaining parts of the saphenous vein grafts removed during the operation of 50 diabetic patients who underwent coronary bypass operation in our hospital between 2021 and 2022 were divided into three pieces. Each piece was placed into a different solution as saline (group 1), heparinized autologous blood (group 2), and endothelial protection solution (group 3). They were kept in solutions for 4 hours and then cellular and DNA oxidative stress markers were investigated.

RESULTS: There was no significant difference between the groups in terms of total antioxidant capacity (TAC) (p>0.05). Total oxidative status (TOS) levels in groups 1 and 2 without endothelial solution (1.57±0.46 vs. 1.55±0.19) were significantly higher than those in-group 3 (1.31±0.11) (p<0.05). Moreover, the oxidative stress index (OSI) was significantly lower in group 3 (p<0.05). On the other hand, 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, which indicate nuclear oxidative stress, were also found to be low in-group 3 (p<0.05).

CONCLUSION: Our results support that the use of endothelial solution on saphenous vein grafts used in diabetic patients prevents oxidative damage.

Keywords: diabetic coronary artery disease, saphenous graft, oxidative stress, endothelial protection solution

INTRODUCTION

In diabetic patients, uncontrolled and high blood sugar damages the vascular endothelium and heart tissue. Therefore, diabetic patients are not only directly at increased risk of coronary artery disease (CAD), but are also more likely to have other conditions that cause CAD (1,2). In addition, when coronary artery bypass graft operation is required in these patients, impaired blood sugar control

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also affects graft patency rates after the operation. Although it is stated in the studies that diabetes does not have a direct effect on the long-term patency rates of the internal mammarian artery (IMA) graft, there are conflicting studies that indicate the long-term patency negatively affected for the saphenous vein (SV) graft due to uncontrolled diabetes-related blood glucose levels (3-5).

It is already known that the mid-long term patency rates of the saphenous vein are worse than other arterial grafts. For this reason, studies on preventing endothelial damage due to various reasons during graft harvesting, and performing more qualified anastomosis during anastomosis have been designed for obtaining longer patency rates (6). It has been reported that even the high pressure applied during harvesting causes endothelial destruction, and the damage results in acute occlusions by causing platelet adherence and thrombosis in the early period. In the advanced period, it has been observed that endothelial damage that occurs during these events, smooth muscle cell migration is stimulated due to mitogenic proteins released from platelets adhering to the intima, resulting in intimal proliferation and hyperplasia (6,7). Considering that the SV endothelium under this risk is much more sensitive in diabetics, it can be said that these patients are at much higher risk in terms of early and late graft patency rates.

This study, it was aimed to investigate the effects of the solutions in which the saphenous vein is kept during the period until the bypass, on the oxidative stress that the SV endothelium is exposed to in diabetic patients who underwent coronary artery bypass graft (CABG).

MATERIAL & METHODS

The study was carried out by using the saphenous vein grafts of 50 diabetic patients who applied to Gaziantep University Cardiovascular Surgery Clinic and underwent bypass surgery in 2021 - 2022. The remaining saphenous vein graft after anastomosis was used for biochemical evaluation. Ethical approval was obtained from the local ethics committee before starting this prospective non-randomized controlled study (Approval No. 2021/352). All study steps were planned and implemented in accordance with the Declaration of Helsinki and good clinical practices.

Each residual saphenous vein graft obtained was divided into three parts and placed in three different solutions as follows:

- Saline (0.9 percent NaCl) solution for four hours (Group 1),
- Heparinized Autologous Blood solution for four hours (Group 2),
- Endothelial Protection Solution (NOESIS®, Noegenix, Ankara, Turkey) for four hours (Group 3).

Then, biochemically cellular and DNA oxidative stress levels were studied with commercially available kits.

Biochemical Analysis

Total Antioxidant Capacity (TAC)

The antioxidant capacity (TAC) of the endothelial extract was evaluated with commercial kits (Rel Assay Diagnostic, Gaziantep, Turkey) as nmolTroloxEquiv/mg protein as previously described (8,9). In this method, which produces hydroxyl radicals based on the Fenton reaction, the antioxidant capacity of the sample against strong radical hydroxyl is evaluated.

Total Oxidative Status (TOS)

The oxidative status (TOS) of the extract obtained from the endothelium was evaluated with commercial kits (Rel Assay Diagnostic, Gaziantep, Turkey) as nmol H2O2 Equiv/mg protein as previously described (8,9). The oxidant stimuli in the sample oxidize the iron ion-o-dianisidine complex to the iron ion and the reaction is potentiated with glycerol to evaluate the oxidative stress level.

Oxidative Stress Index (OSI)

The oxidative stress index (OSI) value over the average of TAC and TOS values is obtained in the Arbitrary Unit (AU) with the following formulation: OSI= (TOS, nmol H2O2 Equiv/mg protein)/(TAS, nmol Trolox Equiv/mg protein/1) x 100 (8,9).

Nuclear and mitochondrial DNA oxidative damage measurement:

OxiSelect™ Oxidative DNA Damage kit [8-hydroxydeoxyguanosine (8-OHdG) assay, Cell Biolabs, Inc. San Diego, CA] and the data obtained were expressed as ng/ml as described in previous reports (10).

Statistical Analysis

The SPSS ver. 13.0 was used for statistical analysis of obtained data. Continuous and ordinal data were given as mean and standard deviation, and the normal distribution

was evaluated with the Kolmogorov Smirnov test. Comparison of triplicate data was done with the One Way ANOVA test. For the p values obtained from the comparison, less than 0.05 was considered statistically significant.

RESULTS

The mean age of the bypassed diabetic patient population was 62.11±10.53, and 30% (n: 15) of the patients were female.

There was no significant difference between the groups (p=0.34) between the TAC values studied from the saphenous vein tissues. However, TOS levels in groups 1 and 2 without endothelial solution (1.57±0.46 vs. 1.55±0.19) were significantly higher than those in group 3 (1.31±0.11) in which endothelial solution was used (p=0.002). OSI was found to be lower in group 3 as a result of the mean between values (p=0.000). Cellular oxidative stress values between groups are summarized in Table 1.

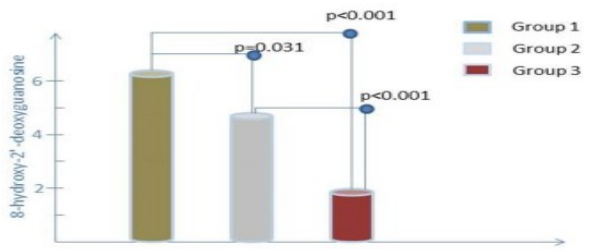
Table 1. *Cellular oxidative stress markers in groups*

Oxidative Markers	Group 1	Group 2	Group 3	P*
TAC** (nmolTroloxEquiv/mg protein)	2.46±0.34	2.41±0.17	2.52±0.31	0.064
TOS*** (nmol H_2O_2 Equiv/mg protein)	1.57±0.46	1.55±0.19	1.31±0.11	0.002
OSI+ (AU)	0.56 ±0.43	0.52±0.33	0.24±0.07	0.000

**Groups were compared with One Way Anova test and p<0.05 was accepted as statistically significant, **TAC: Total antioxidant capacity, ***TOS: Total oxidative status, +OSI: Oxidative stress index*

When groups were evaluated in terms of endothelial oxidative DNA damage, the 8-OHdG levels were found as 6.13±1.7 ng/ml in group 1, 4.81±2.40 ng/ml in group 2, and 1.92±0.67 ng/ml in group 3. When the groups were compared, the most severe endothelial oxidative DNA damage was found in group 1 (saline) (Graph 1).

Graph 1. *The 8-OHdG levels in Groups. Group 1: Saline solution; Group 2: Heparinized blood solution; Group 3: Endothelial protection solution*



DISCUSSION

Our results demonstrated that endothelial protection solution can be beneficial for protecting saphenous vein endothelium against oxidative stress in diabetic patients who underwent coronary bypass. Moreover, our results supported that even a nuclear oxidative stress marker 8-OHdG levels were significantly lower in the endothelial protecting solution applied to harvested saphenous veins.

The processes behind the development of endothelial dysfunction by affecting the endothelial functions in diabetic patients have received increasing attention. Under physiological conditions, the balance between endothelial contraction and relaxation is disrupted in the diabetic patient group, resulting in primary disruption of microcirculation and end-organ damage. Impaired glucose metabolism and associated hyperglycemia have been seen as the main cause of endothelial dysfunction in diabetic patients (11,12). In addition, micro-albuminuria due to end-organ damage, impaired lipid metabolism, and reactive Cu-Zn superoxide dismutase levels caused by acute hyperglycemic attacks are triggering oxidant reactions in diabetes, and creating cumulative oxidative stress on the endothelium that resulting in permanent functional damage (11,12). In Due to endothelial dysfunction, which plays a role in the basic pathogenesis of adverse processes occurring in the diabetic process, it has been emphasized that the protection of the endothelium should be a basic treatment goal (13). Therefore, in this study, the most suitable solution materials that can protect the endothelium by reducing oxidative stress during the period from saphenous harvesting to bypassing were examined.

Ak et al. found intimal fibrosis, endothelial cell vacuolization, and smooth muscle cell damage in the saphenous veins of these patients in their histopathological examination of the LV grafts of diabetic patients who had undergone bypass. However, they reported as a limitation that oxidative stress markers that could cause the pathologies they found in their study were not studied (14). In another report, it was stated that arterial grafts are much more durable than venous grafts in diabetic patients, and it was recommended to use arterial grafts as much as possible for coronary bypass in these patients. However, it has been stated that the use of SV is inevitable in cases where more grafts are required, such as multi-vessel disease, and it is stated that the saphenous vein is more

affected by stress factors that were mentioned in the previous paragraph (15). TAC and TOS measurement and the OSI value obtained by the ratio of these two values are frequently used parameters in the measurement of oxidative stress in tissues and body fluids (16). It has been previously reported that hypoglycemia and hyperglycemia episodes are associated with increased TOS in diabetic patients, and it has been emphasized that this may be due to increased lipid peroxidation measured by TOS/TAC (17). TAC and TOS values were previously evaluated in harvesting SVs and compared in different solutions. That study by Tekin et al. was performed on SVs of patients with or without diabetes, and as a result, it was reported that endothelial protection solution was more protective from oxidative stress than heparinized blood (18). Our study included only diabetic patients and it was determined that the endothelial protection solution protected the vein graft from cellular oxidative stress better than both saline and heparinized blood.

We also investigated the levels of 8-OHdG, a nuclear marker of oxidative stress. 8-OHdG, which occurs metabolically in the oxidative damage of DNA, is a highly sensitive marker for detecting even the smallest nuclear oxidative damage (19). The 8-OHdG levels in urea have also been investigated in diseases caused by occlusive consequences such as vascular dementia and have been accepted as an important indicator of increased oxidative stress. Elevated levels of 8-OHdG in the blood have been associated with an increased risk of atherosclerosis and have been suggested as a predictor of cardiovascular diseases (20,21). In our study, 8-OHdG levels were significantly lower in SVs treated with endothelial protection solution compared to those treated with saline or heparinized blood. This result was interpreted in favor of endothelial protection solution protecting nuclear material against oxidative stress.

In conclusion, our findings supported that endothelial protection solution was more effective than heparinized blood or saline solution in reducing oxidative stress on harvested SV during bypass. In addition, our results suggested that endothelial solution can prevent oxidative stress-induced DNA damage on the SV endothelium.

Limitations of Study: The main limitation of the study is that laboratory analyzes were not confirmed histopathologically. Demonstrating the effects of the obtained data at the cellular level will increase the validity of the results. Another limitation is that the endothelial solution has only been investigated on the saphenous vein. Demonstrating the same results with other bypass grafts will provide more comprehensive results in terms of endothelial protection efficiency.

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Author contribution status; The concept of the study; EH, ÖA design; EH, ÖA literature review; EH, ÖA collecting and processing data; EH, ÖA statistics; EH, ÖA writing phase; EH, ÖA

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Hashimoto Tanılı Hastalarda Non-Alkolik Yağlı Karaciğer Hastalığı Sıklığı ve Hematolojik Parametreler ile İlişkisi

The Frequency of Non-Alcoholic Fatty Liver Disease in Patients with Hashimoto's Diagnosis and Its Relationship with Hematological Parameters

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ÖZET

AMAÇ: Non-alkolik yağlı karaciğer hastalığı (NAYKH), en yaygın karaciğer hastalığıdır ve küresel prevalansının %25 civarında olduğu tahmin edilmektedir. Hipotiroidizm ile NAYKH arasında potansiyel bir ilişki olduğunu gösteren çalışmalar mevcut olsa da, toplum taramalarından çelişkili sonuçlar elde edilmiştir. Platelet/lenfosit oranı (PLO) kardiyovasküler olayların belirleyicisi ve nötrofil/lenfosit oranı (NLO) ise sistemik inflamasyonun bir göstergesidir. Biz de Hashimoto tiroiditi tanısı olanlarda NAYKH sıklığı ve bunun PLO, NLO ile ilişkisini araştırmayı amaçladık.

GEREÇ VE YÖNTEM: En az 6 ay süredir Hashimoto tanısı olan, 18-65 yaş arası, beden kütle indeksi 30 kg/m2'den küçük olan, bilinen başka bir hastalığı olmayan ve TSH düzeyi 10 uIU/mL'nin altında olan toplam 97 birey çalışmaya alındı. Batın ultrasonografi ile NAYKH varlığı ve derecesi ölçüldü.

BULGULAR: Hashimoto tanısı olanlarda NAYKH sıklığı %42,3 olarak belirlendi. Grade 2-3 yağlanması olanlar hem grade 1 yağlanması olanlardan (p<0.001) hem de yağlanması olmayanlardan (p=0.023) anlamlı olarak daha yaşlıydı. Yine grade 1 yağlanması olanlarda olmayanlara göre anlamlı olarak daha yaşlıydı. Glukoz düzeyi grade 2-3 yağlanma olanlarda hem yağlanma olmayanlara (p=0.006) hem de grade 1 yağlanma olanlara göre (p=0.028) anlamlı yüksekti. ALT düzeyi grade 2-3 yağlanma olanlarda olmayanlara göre anlamlı yüksek bulundu (p= 0.013). Beyaz küre sayısı ve C- reaktif protein (CRP) düzeyi yağlanması olmayanlara göre grade 2-3 yağlanma olanlarda daha yüksek bulundu (sırasıyla p=0.011 ve p=0.003). NLO ve PLO oranları ise 3 grup arasında da benzerdi.

SONUÇ: Hashimoto tiroiditi olanlarda NAYKH sıklığı daha önce yapılan çalışmalar ile benzer sıklıkta bulundu. Yağlanma derecesi yaş ile artmaktadır. Ayrıca yağlanma ile metabolik parametreler bozulmakta ve ALT düzeyi artmaktadır. Yağlanma ile beyaz küre ve CRP değerleri artarken, bu durum yeni hematolojik inflamatuvar belirteçlerde gösterilememiştir.

Anahtar Kelimeler: Hashimoto tiroiditi, non-alkolik yağlı karaciğer hastalığı, platelet/lenfosit oranı, nötrofil/lenfosit oranı

ABSTRACT

OBJECTIVE: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease and its global prevalence is currently estimated to be 25%. Although there are studies showing a potential relationship between hypothyroidism and NAFLD, conflicting results have been obtained from population surveys. Platelet/lymphocyte ratio (PLO) is a predictor of cardiovascular events, and neutrophil/lymphocyte ratio (NLR) is an indicator of systemic inflammation. We aimed to investigate the frequency of NAFLD and its relationship with PLR, NLR in patients with Hashimoto's thyroiditis.

MATERIALS AND METHODS: A total of 97 individuals with a diagnosis of Hashimoto's for at least 6 months, aged 18-65, body mass index less than 30 kg/m2, no other known disease, and TSH level below 10 uIU/mL were included in the study and the presence and degree of NAFLD were measured by ultrasonography.

RESULTS: The frequency of NAFLD in patients with Hashimoto diagnosis was 42.3%. Those with grade 2-3 adiposity were significantly older than both those with grade 1 adiposity (p<0.001) and those without adiposity (p=0.023). Again, those with grade 1 adiposity were significantly older than those without. Glucose levels were significantly higher in those with grade 2-3 adiposity than in those without (p=0.006) and those with grade 1 adiposity (p=0.028). ALT level was found to be significantly higher in patients with grade 2-3 adiposity compared to those without (p= 0.013). The white blood cell count and CRP level were found to be higher in those with 2-3 adiposity compared to those without (p=0.011 and p=0.003, respectively). NLR and PLO rates were similar between the 3 groups.

CONCLUSION: The frequency of NAFLD in patients with Hashimoto's thyroiditis was found to be similar to previous studies. The degree of lubrication increases with age. In addition, metabolic parameters deteriorate and ALT level increases with adiposity.

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While white blood cell and CRP values increased with adiposity of liver, this could not be demonstrated in new hematological inflammatory markers.

Keywords: Hashimoto's thyroiditis, non-alcoholic fatty liver disease, platelet/lymphocyte ratio, neutrophil/lymphocyte ratio

GİRİŞ

Kronik lenfositik tiroidit olarak da adlandırılan Hashimoto tiroiditi (HT) en yaygın organa özgü otoimmün bozukluktur ve tiroid hipofonksiyonunun en yaygın nedenidir (1,2). Bu otoimmün olayın tiroid antijenine özgü yardımcı T lenfositlerin aktivasyonu ile başlatıldığına inanılmaktadır ve hastalar çeşitli tiroid fonksiyon durumları ile başvurabilir, ancak çoğu sonunda hipotiroidizme dönüşür (3). Hipotiroidizmde metabolik sendrom prevalansında artış mevcuttur. Hipotiroidili bireylerde vücut metabolizma hızının ve termogenezin azaldığı ve azalan besin alımına rağmen vücut ağırlığının arttığı bilinmektedir (4). Azalan tiroid hormon düzeyi ve fonksiyonlarına bağlı olarak vücudun enerji harcaması da düşmekte ve ağırlık artışı görülmektedir (5). Yapılan kesitsel çalışmalarda da serum tiroid stimülan hormon (TSH) düzeyi ile beden kütle indeksi (BKİ) arasında pozitif ilişki bulunduğu; tiroid fonksiyonlarındaki azalmanın daha yüksek BKİ ve obezite prevalansındaki artış ile ilişkili olduğu gösterilmiştir (6). TSH yüksekliği lipid ve karbonhidrat metabolizmasında bozulma ile ilişkili olup metabolik sendrom gelişimi açısından risk faktörüdür (7).

Alkol kullanımına bağlı olmayan yağlı karaciğer hastalığı (NAYKH), dünya çapında kronik karaciğer hastalığının en yaygın nedenidir (8). Hipotiroidizm ile NAYKH arasında ilişkiyi gösteren çalışmalarda çelişkili sonuçlar elde edilmiş olsa da, geniş toplum taramalarında bu iki durumun birbiri ile ilişkili olabileceği gösterilmiştir (9). Kan sayım parametreleri kullanılarak hesap edilebilen nötrofil lenfosit oranı (NLO) ve trombosit lenfosit oranı (TLO) ise son yıllarda ucuz ve kolay bir yöntem olarak çeşitli kardiyak ve kardiyak olmayan durumlarda kullanılmaya başlanan yeni inflamatuvar belirteçlerdir (10-13). Bu çalışmada HT tanılı hastalarda ultrasonografi ile NAYKH sıklığını ve bu hastalarda NLO ve TLO ile NAYKH arasındaki ilişkiyi araştırmayı amaçladık.

GEREÇ VE YÖNTEM

Çalışmaya en az 6 ay süredir otoimmün belirteç yüksekliği veya ultrasonografi bulguları veya her ikisi ile HT tanısı konulmuş olan, 18-65 yaş arası, bilinen diyabet, hipertansiyon, hiperlipidemi, kalp-damar hastalığı, organ yetmezliği ve romatolojik hastalık gibi başka bir hastalığı olmayan ve tiroid hormon replasmanı alıp almadığına bakılmaksızın TSH düzeyi 10 uIU/mL'nin altında olan bireyler alındı. HT tanısı: Anti-TPO pozitifliği ve ultrasonografi ile desteklenen hastalar Hashimoto kabul edildi. Sonuç olarak çalışmaya 97 HT tanılı hasta dâhil edildi. NAYKH varlığı ve derecesi ultrasonografi ile saptandı. Rutin kan sayımı değerlerine bakılarak NLO, mutlak nötrofil sayısının lenfosit sayısına bölünmesiyle; PLO, trombosit sayısının lenfosit sayısına bölünmesiyle hesaplandı. Ayrıca hastaların yaş, cinsiyet bilgileri ile glukoz, kreatinin ve ALT değerleri kaydedildi. Çalışma protokolü için Harran Üniversitesi Tıp Fakültesi Etik Kurulu tarafından (Evrak tarihi ve sayısı: 18.01.2021-7643) onay alındı.

Veriler ortalama ± standart deviasyon olarak belirtildi. Verilerin dağılımının normal olup olmadığı Kolmogorov-Smirnov testi ile değerlendirildi. Kategorik verileri karşılaştırmak için ki-kare testi yapıldı. Normal dağılıma sahip sürekli değişkenler için, gruplar arasındaki verileri karşılaştırmak için Tek Yönlü ANOVA kullanıldı. Grupların anlamlılığını değerlendirmek için post-hoc test olarak LSD uygulandı. Normal olmayan veriler için Kruskal Wallis testi, gerekli durumlarda Mann Whitney U testi kullanıldı. $p < 0.05$ değeri istatistiksel olarak anlamlı kabul edildi. İstatistiksel analizler için SPSS 22.0 sürümü kullanıldı.

BULGULAR

Hastaların 56'sında (%57,7) NAYKH saptanmaz iken, 22'sinde (%22,7) grade 1, 19'sında (%19,6) ise grade 2-3 yağlanma saptandı; böylece Hashimoto tanısı olanlarda NAYKH sıklığı %42,3 olarak belirlendi. NAYKH olmayan 7 hasta tiroid hormon replasmanı almıyordu (%10,7), diğer tüm hastalar ise ortalama 85,5±42,0 mcg/gün dozunda levotiroksin kullanmaktaydı. Ancak 3 grup arasında tedavi alanların oranı açısından anlamlı fark saptanmadı (p=0.06). Grade 2-3 yağlanması olanlar hem grade 1 yağlanması olanlardan (p<0.001) hem de yağlanması olmayanlardan (p=0.023) anlamlı olarak daha yaşlıydı. Yine grade 1 yağlanması olanlarda olmayanlara göre anlamlı olarak daha yaşlıydı. Glukoz düzeyi grade 2-3 yağlanma olanlarda hem yağlanma olmayanlara (p=0.006) hem de grade 1 yağlanma olanlara göre (p=0.028) anlamlı yüksekti. ALT düzeyi grade 2-3 yağlanma olanlarda olmayanlara göre anlamlı yüksek

bulundu (p= 0.013). Beyaz küre sayısı ve CRP düzeyi yağlanması olmayanlara göre grade 2-3 yağlanma olanlarda daha yüksek bulundu (sırasıyla p=0.011 ve p=0.003). NLO ve PLO oranları ise 3 grup arasında da benzerdi.

Tablo 1. *NAYKH şiddetine göre klinik ve laboratuvar verilerinin karşılaştırılması*

Parametre	NAYKH Yok	NAYKH Grade 1	NAYKH Grade 2-3	P
Yaş (yıl)	36.7±11.9 [a, b]	42.9±11.3[c]	51.2±9.5	<0.001
Cinsiyet (K/E)	51/5	20/2	15/4	0.380
Glukoz (mg/dL)	92.5±11.7[d]	93.7±14.9[e]	103.6±15.3	0.019
Kreatinin (mg/dL)	0.7±0.1	0.7±0.1	0.7±0.1	0.777
ALT (U/L)	22.7±14.6[f]	23.3±9.2	32.1±23.9	0.025
TSH (uIU/mL)	3.5±2.6	4.1±3.0	3.5±2.8	0.699
Beyaz küre ($x10^3$)	6.8±1.8[g]	7.4±2.1	7.9±1.4	0.030
Nötrofil ($x10^3$)	3.9±1.2	4.2±1.4	4.6±0.9	0.051
Lenfosit ($x10^3$)	2.2±0.7	2.4±0.9	2.5±0.6	0.085
CRP (mg/dL)	0.3±0.6[h]	0.5±1.0	0.9±1.2	0.011
NLO	1.9±0.5	1.8±0.6	1.9±0.5	0.855
PLO	147.3±53.4	140.2±42.1	122.1±25.7	0.133

a: *NAYKH yok ile grade 1 arasında p= 0.033* ***b:*** *NAYKH yok ile grade 2-3 arasında p<0.001* ***c:*** *NAYKH grade 1 ile grade 2-3 arasında p=0.023* ***d:*** *NAYKH yok ile grade 2-3 arasında p=0.006* ***e:*** *NAYKH grade 1 ile grade 2-3 arasında p=0.028* ***f:*** *NAYKH yok ile grade 1 arasında p= 0.013* ***g:*** *NAYKH yok ile grade 2-3 arasında p=0.011* ***h:*** *NAYKH yok ile grade 2-3 arasında p=0.003*

TARTIŞMA

Alkol kullanımına bağlı olmayan yağlı karaciğer hastalığı (NAYKH), dünya çapında kronik karaciğer hastalığının en yaygın nedenidir (8). Aşırı alkol alımı veya otoimmün, ilaca bağlı veya viral hepatit gibi diğer karaciğer hastalığı nedenlerinin yokluğunda karaciğer hücrelerinde yağ birikimi (%5) olarak tanımlanır. NAYKH'de histolojik spektrumu basit steatozdan alkolik olmayan steatohepatite (NASH), karaciğer fibrozu ve siroza kadar uzanmaktadır (14). Bu hastalığın Batı ülkelerinde, özellikle metabolik sendrom hastalarında genel popülasyonun %30'unu etkilediği bildirilmektedir (15). Ultrasonografi, klinik uygulamada hepatik lipit birikimini teşhis etmek için önerilen ilk seçenek görüntüleme yöntemi olmaya devam etmektedir (16). Yakın tarihli bir meta-analiz, histolojiye kıyasla orta ila şiddetli yağlı karaciğerin saptanması için ultrasonografinin genel duyarlılığı ve özgüllüğünün %84,8 ve %93,6 olduğunu göstermiştir (17).

Tiroid hormonları hepatik lipit metabolizmasında önemli bir rol oynamaktadır. Hipotiroidizm azalmış lipoliz ve trigliseritlerden türetilen serbest yağ asitlerinin karaciğer tarafından alınmasında azalma ile ilişkilidir. Ayrıca tiroid hormonları hepatik yağ birikimini değiştirir, adiponektin regülasyonunu etkiler. Böylece tiroid hormonları, adiponektin modülasyonu yoluyla fibrozis gelişimini kontrol edebilir. Bozulmuş adiponektin düzeninin yanı sıra artan leptin ve FGF21 sekresyonu da bu patogenezde rol oynayabilir (18). Aşikâr hipotiroidizm ile ilişkili olarak NAYKH sıklığının arttığı bilinmektedir (19, 20). Bununla birlikte, ötiroid aralığında olup düşük normal tiroid fonksiyonuna sahip olanlarda bu konu açık değildir (21). Bildiğimiz kadarı ile aşikâr hipotiroidisi olmayan Hashimoto hastalarında NAYKH sıklığı literatürde daha önce hiç incelenmemiştir. Biz bu hastalarda NAYKH sıklığını %42,3 olarak bulduk. Bu da Türkiye'de genel toplumda NAYKH sıklığının %48,3 bulunduğu çalışma ile uyumluydu (22). Buna göre aşikâr hipotiroidisi olmayan Hashimoto hastalarında NAYKH sıklığının belirgin artmadığı söylenebilir.

Genel popülasyonda NAYKH prevalansının yaşla birlikte arttığı, 60 yaşına kadar erkeklerde kadınlara göre daha yaygın olduğu bilinmektedir (23). NAYKH metabolik sendromun komponentleri olan tip 2 diyabetes mellitus, dislipidemi veya obezite gibi hastalıklarla yakın ilişkilidir ve bu nedenle NAYKH'nin metabolik sendromun karaciğerde ortaya çıkan yansıması olduğu ileri sürülmektedir (24). ALT'nin ise NAYKH için hem bir tanı aracı hem de hastalığın gerilediğinin bir belirteci olabileceği bulunmuştur (25). Bizim çalışmamızda da Hashimoto tiroiditi olanlarda yağlanma derecesinin yaş ile birlikte arttığı, ayrıca ileri derece yağlanması olanlarda hem metabolik komponentin bir parçası olarak glukozun hem de ALT düzeyinin daha yüksek olduğu bulundu.

NLO ve TLO ise son yıllarda ucuz ve kolay bir yöntem olarak çeşitli kardiyak ve kardiyak olmayan durumlarda kullanılmaya başlanan yeni inflamatuvar belirteçlerdir (14-17). Kara ve ark. yaptığı çalışmada NLO'nun karaciğerdeki inflamasyonun veya fibrozisin şiddeti ile ilişkili olmadığı ve bu nedenle NAYKH'lı hastalarda karaciğer hasarının bir belirteci olarak kullanılamayacağını göstermişlerdir (26). Yi ve ark. ise NLO ile inflamatuvar aktivite ve önemli derecede fibrozis arasında negatif bir korelasyon olduğunu bildirmişlerdir (27). PLO ise bildiğimiz kadarı ile daha önce NAYKH'de çalışılmamıştır. Çalıştığımız popülasyonda hem NLO hem de PLO NAYKH'nin varlığı ve derecesi ile ilişkili bulunmadı.

Çalışmamızın en önemli sınırlayıcı faktörü kan basıncı değerleri, antropometrik ölçümler ve lipit düzeylerine bakılmamış olması ve dolayısıyla metabolik sendromun bir karıştırıcı faktör olarak ortaya sunulmamış olmasıdır. Ayrıca geleneksel inflamasyon belirteci olarak sadece CRP düzeyi dikkate alınmış olup, hem diğer inflamatuvar belirteçler hem de Hashimoto sürecinde artan otoantikor seviyeleri çalışılmamıştır. Ancak bu popülasyonda ilk defa ortaya konulan bu verilerin literatüre katkı sağlayacağını ve sonraki çalışmalara yön vereceğini düşünmekteyiz.

Sonuç olarak, Hashimoto tiroiditi olanlarda NAYKH sıklığı daha önce genel popülasyonda yapılan çalışmalar ile benzer sıklıkta bulunmuştur. NAYKH şiddeti yaş ile artmakta, geleneksel inflamatuvar belirteç seviyeleri yükselmekte ve glukoz ile gösterilen metabolik parametreler bozulmaktadır. Kan sayımından elde edilen inflamatuvar belirteçler ile NAYKH şiddeti arasında ilişki bulunmamıştır. Tek başına Hashimoto tiroiditi varlığı karaciğer yağlanması için bir risk faktörü değildir.

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Cytotoxic Activity of Carrageenan on Malignant MCF-7 Breast Cancer and The Non-Malignant SVCT Breast Epithelial Cell Lines

Karragenan'ın Malign MCF-7 Meme Kanseri ve Malign Olmayan SVCT Meme Epitel Hücre Dizileri Üzerindeki Sitotoksik Aktivitesi

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ÖZET

AMAÇ: Son yapılan çalışmalar deniz alglerinden elde edilen sülfatlanmış polisakkaritlerin birçok biyolojik ve fizyolojik aktivitesi olduğunu göstermektedir. Kırmızı deniz alglerinden elde edilen sülfatlanmış bir polisakkarit olan kappa-karragenanın da çeşitli kanser hücre hatlarında anti-proliferatif etki gösterdiği bilinmektedir. Bu çalışmada kappa-karragenanın, iki farklı hücre hattı, malignant özellikte MCF-7 meme kanseri hücre hattı ve non-malign SVCT meme epiteli hücre hatları üzerindeki sitotoksik etkisi in vitro modelde incelenmiştir.

GEREÇ VE YÖNTEM: Karragenanın 1000 µg/ml başlangıç dozu olacak şekilde ve her hücre için kendi besiyerinde üç farklı konsantrasyonu hazırlanmıştır (Dilution I: 1000 µg/ml; Dilution II: 250 µg/ml; Dilution III: 62,5 µg/ml). Hücreler deney gruplarında belirlenen dozlarda karregenan ile inkübe edilmiştir. Karragenan içermeyen besiyerinde inkübe edilen hücreler de kontrol grubu olarak alınmıştır.

BULGULAR: Hücre canlılığının ölçülmesi için MTT [3-(4,5-dimetiltiyazol-2-il]-2,5-difenil-tetrazolyum bromür] analizi yapılmıştır. Hücre morfolojisi, acridine orange (AO)/propidium iodide (PI) florasan boyama yöntemi ile incelenmiştir. Çalışmamızda elde ettiğimiz sonuçlar, karragenanın hem malign hem de non-malign hücre hatları üzerinde sitotoksik etkiye neden olduğunu göstermektedir.

SONUÇ: Sonuç olarak karragenın malign ve non-malign hücreler üzerinde sitotoksik bir etkisinin olduğunu söylemek mümkündür. Ancak bu etki yüksek karregenan dozları kullanıldığında belirgin olarak görülmekte olup; MCF-7 kanser hücre hattında bu etki daha düşüktür. MCF-7 hücre hattının kültür sırasında ortaya çıkabilecek olası spontan fenotipik ve genotipik değişiklikler de göz önüne alındığında, karreganın anti-tümör etkisinin olabileceği ancak bununla birlikte daha MCF-7 dışında farklı hücre hatları kullanılarak yapılacak çalışmalar ile bu sonuçların desteklenmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Karragenan, sitotoksite, MCF-7, SVCT, MTT

ABSTRACT

OBJECTIVE: Recent studies have shown that sulfated polysaccharides obtained from marine algae have many biological and physiological activities. Kappa-carrageenan, a sulfated polysaccharide obtained from red marine algae, is known to have anti-proliferative effects in various cancer cell lines. In this study, the cytotoxic effect of kappa-carrageenan on two different cell lines, namely the malignant MCF-7 breast cancer cell line and the non-malignant SVCT breast epithelial cell lines, was investigated in an in vitro model.

MATERIALS AND METHODS: Three different concentrations of carrageenan were prepared for each cell in its own medium, with an initial dose of 1000 µg/ml (Dilution I: 1000 µg/ml; Dilution II: 250 µg/ml; Dilution III: 62.5 µg/ml). Cells were incubated with carrageenan at the doses set for each experimental group. Cells were incubated in a carrageenan-free medium comprised the control group.

RESULTS: To measure cell viability, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) analysis was performed. Cell morphology was investigated by the acridine orange (AO)/propidium iodide (PI) fluorescent staining method. The present results indicated that carrageenan caused cytotoxic effects on both malignant and non-malignant cell lines.

CONCLUSION: Considering that the different phenotypic features of the subtypes of the MCF-7 cell line may affect cell viability and cell proliferation, cell selection should be performed very carefully in cytotoxicity studies. We suggest that using the MCF-7 cell line for cytotoxicity experiments needs to contemplate this important phenomenon for further experimental setups.

Keywords: Carrageenan, cytotoxicity, MCF-7, SVCT, MTT

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INTRODUCTION

For the last 30 years, various polysaccharides or protein-polysaccharide components have been isolated from fungi, yeast, some plants, and algae. These polysaccharides draw attention especially due to their immunomodulatory properties and anti-tumor activities (1). Sulfated polysaccharides from marine algae have specific properties that play a role in ionic regulation in cells (1). Recent studies have shown that these sulfated polysaccharides obtained from marine algae have many biological and physiological activities (1).

Carrageenan is the general name given to a group of sulfated polysaccharides obtained from red marine algae (2). Carrageenan, obtained from seaweeds belonging to the Rhodophyceae family, is a 15%-40% sulfated polygalactan molecule with a molecular weight of about 100 kDa (2). There are several types of carrageenan such as λ, κ, ι, ε, and μ, all of which contain 22%-35% sulfate groups. The position and number of ester sulfate groups in the molecule determine the properties and function of the molecule (2). Studies have shown that carrageenan has anti-thrombotic, anti-inflammatory, anti-viral, and anti-tumor activities (3). The molecular structure of carrageenan determines its function (4). Kappa-carrageenan has been shown to have an anti-proliferative effect in various cancer cell lines and an anti-tumor activity in in vivo studies (5, 6, 7).

In this study, the cytotoxic effect of kappa-carrageenan, a type of carrageenan obtained from red algae, on two different cell lines, namely, the malignant MCF-7 breast cancer cell line and non-malignant SVCT cell line were investigated in an in vitro model.

MATERIAL & METHODS

Preparation of cell cultures

MCF-7 and SVCT cell lines were prepared in 96-well culture dishes with an initial cell count of 20,000 cells/200 µl in six replicates. The MCF-7 cell line was incubated in 95% air-5% CO_2 at 37C° for 24 hours under standard culture conditions in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F12 (Biochrom AG, Germany) containing 10% fetal bovine serum (FBS) (Biochrom AG, Germany); and, the SVCT cell line was incubated in 95% air-5% CO_2 at 37C° for 24 hours under standard culture conditions in DMEM/Ham's F12 (Biochrom AG, Germany) containing 10% fetal bovine serum (FBS) (Biochrom AG, Germany), 5 µg/ml hydrocortisone, and 10 µg/ml insulin.

Preparation of the test material and application to cell lines

Carrageenan (Merck, Germany) stock solution was prepared at room temperature as 20 mg/20 ml in serum-free medium and sterilized by passing through a 0.22 µm filter. Based on the studies in the literature (8,9), three different concentrations were prepared for each cell in its own medium with an initial dose of 1000 µg/ml (Dilution I: 1000 µg/ml; Dilution II: 250 µg/ml; Dilution III: 62.5 µg/ml). In the control group, cells were incubated in their own medium not containing carrageenan.

Examination of cell viability

To measure cell viability, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) analysis was performed. MTT method is a standardized procedure assaying cell viability and widely used in cytotoxicity tests. In our previous studies, we used this method to measure cell viability in different in vitro cytotoxicity models (10, 11, 12). This colorimetric method assesses the ability of viable cells to form MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) formazan by the mitochondrial enzyme succinate dehydrogenase. At 24 hours of the incubation of the cells in 96-well plates, the medium was taken out and 12.5 µL of MTT (Sigma-Aldrich, Germany) solution in 100 µL of FBS-free DMEM/F12 was added to each well. Cell culture plates were wrapped with aluminum foil and incubated for 4 hours. At the end of the incubation, the MTT solution was removed and the reaction was stopped by adding 100 µL of isopropyl alcohol (Amresco Inc., USA). Cell viability was measured with an ultraviolet (UV)-visible spectrophotometer (EZ Read 400 Microplate Reader, Biochrom) at a wavelength of 560 nm as the absorbance value.

Assessment of cell morphology

Acridine orange (AO)/propidium iodide (PI) fluorescent staining was used for the assessment of cell morphology. At the 72nd hour of incubation, the medium on the cells was removed and the cells were incubated for 20 seconds by adding AO/PI (Sigma-Aldrich, Germany) (600 mg/ml AO, 600 mg/ml PI, ratio of 1:1) without fixation. The cells were then washed twice with phosphate buffer saline (PBS) (Sigma-

Aldrich, Germany) for 10 seconds. Next, the cells were examined under fluorescence microscopy. Dead cells were evaluated by counting the red cells with fragmented nuclei. The AO/PI-stained cells were observed under a narrow band fluorescein (FITC) filter (520–560 nm) in green color, and the PI-stained cells were observed under a rhodamine filter (510–560 nm) as stained red.

Statistical Analysis

Statistical analyses were performed using the IBM SPSS statistics 23 program. The difference caused by carrageenan on cell viability was analyzed with the Kruskal Wallis test, which is a non-parametric test. The Bonferroni test, which is a post-hoc test, was used for the difference between the groups. Any p-value <0.05 was considered statistically significant.

RESULTS

Assessment of Cell Viability

Cell viability was evaluated for all three dilutions at 72 hours of incubation and compared with the control group. The results are given in Table 1 together with the p values. As seen in Table 1, cell viability was lower in both cell lines compared to the control group at the highest concentration (Dilution I). The difference was not significant in the MCF-7 cell line (p>0.05) but significant in the SVCT cell line (p<0.05). When the MCF-7 and SVCT cell lines were compared with each other for all concentrations, no significant difference in cell viability was observed in all three dilutions (Table 2).

Table 1. *Cell viability in MCF-7 and SVCT cell lines*

Dilutions	MCF-7			SVCT		
	Mean	SD	P	Mean	SD	P
I	0.338	0.025	> 0.05	0.318	0.019	< 0.05
II	0.374	0.036	> 0.05	0.350	0.048	> 0.05
III	0.372	0.031	> 0.05	0.413	0.054	> 0.05
Control	0.358	0.054	-	0.483	0.069	-

SD: Standard deviation p values are given by comparing with the control group

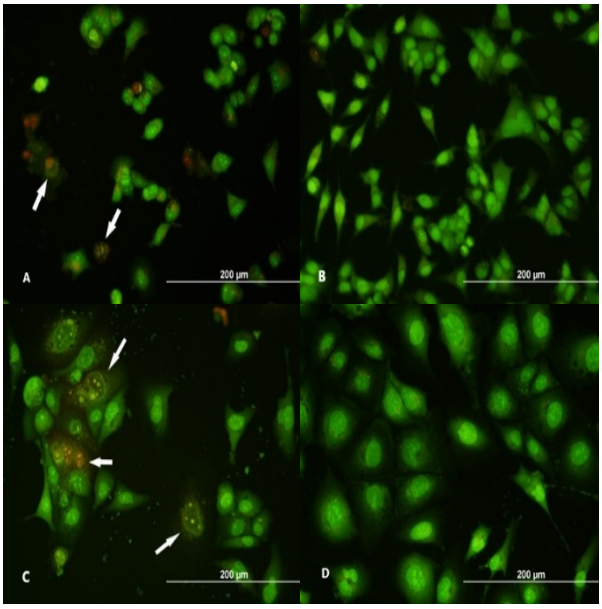
Table 2. *Comparison of cell viability in MCF-7 and SVCT cell lines*

	Dilutions		
	I	II	III
P values (MCF-7 / SVCT)	> 0.05	> 0.05	> 0.05

Assessment of cell morphology

When the cell morphologies were examined, although it was observed dead cell in both cell line, the cell morphology in the SVCT cell line was different than the control group. Furthermore, it was observed that the cells moved away from the epithelial morphology and assumed a more rounded appearance, and at the same time, nuclear condensation became evident. In some cells, DNA fragmentation occurred and the nucleus was stained in yellow-red-orange colors (Figure 1).

Figure 1. *AO/PI staining of cell lines exposed to test material (Dilution I: 1000 µg/ml) and control group at 72 h of incubation.*



(A) *MCF-7 cell line (Dilution I) (20x);* ***(B)*** *MCF-7 cell line (Control) (20x);* ***(C)*** *SVCT cell line (Dilution I) (20x) and* ***(A)*** *SVCT cell line (Control) (20x). (White arrows indicate rounded and degenerated cells).*

DISCUSSION

Cell lines are extremely important in in vitro models for studies on the diagnosis and treatment of breast cancers at the molecular level (13). There are various studies in the literature examining the anti-tumor effects of carrageenan on different cell lines. Luo et al. showed that λ-carrageenan suppressed cell proliferation by altering the tumor microenvironment in B16-F10 melanoma and 4T1 breast cancer cell lines (14). In another study, it was shown that λ-carrageenan suppressed cell proliferation in MDA-MB-231 cells and caused apoptotic cell death (8). The effects of different types of carrageenan on different cell lines are also investigated (15). In a study, lambda carrageenan was shown to have an anti-proliferative effect on SH-SY5Y and MCF-7 breast cancer cell lines, like kappa carrageenan (9). In another study, it was shown that both carrageenan types suppressed cell division in HeLa cell line, and their anti-

tumor effect was emphasized (16). In another study, the effects of low molecular weight lambda and kappa carregenans on cytotoxic and cytokine expression on esophageal cancer cell lines KYSE30 and FLO1 cells were investigated (17).

MCF-7 cell line is a breast cancer cell line with estrogen (+), prolactin (+) and human epidermal growth factor receptor-2 (HER2(+)) features; therefore, it is frequently used in studies on the effects of anti-cancer drugs (18). The MCF-7 cell line was first obtained in 1973 by Dr. By Soule et al. at the Michigan Cancer Foundation (MCF) from a metastatic tumor that developed 7 years later in a woman with breast cancer, and it was named MCF-7 for this reason (13). The SVCT cell line is a human immortalized breast epithelial cell line obtained by creating the immortalized genotype of healthy breast tissue cells with the Simian virus 40 (SV40) (19). The SVCT cell line has the characteristic of non-malignant breast epithelium (20). Therefore, these two cell lines were selected in our study in order to demonstrate the cytotoxic effect of carrageenan on malignant (MCF-7) and non-malignant (SVCT) cells.

In our results, although the cell viability at the highest carrageenan concentration in MCF-7 cells was lower than the control group, this difference was not statistically significant ($p>0.05$). However, in the SVCT cell line, which is obtained from healthy breast tissues, the cytotoxic effect was more pronounced than in the MCF-7 cell line, and cell viability was lower than in the control group, which was a statistically significant difference ($p<0.05$). When the MCF-7 and SVCT cell lines were compared with each other for all concentrations, the difference in cell viability was not statistically significant ($p>0.05$). The MCF-7 cell line is a weakly aggressive non-invasive cell line and shows weak metastatic properties (21, 22). However, the results we obtained in our study showed that, despite this feature, cell viability in the MCF-7 cell line was similar to that of SVCT, a non-malignant mammary epithelial cell line.

When the cell morphologies were examined, although it was seen in both cells, the cell morphology in the SVCT cell line differed compared to the control group; cells moved away from epithelial morphology and became more rounded; nuclear condensation became evident; some cells had DNA fragmentation; and, the nuclei were stained in yellow-red-orange colors. This shows that there is a morphology of the onset of apoptosis in cells. Apoptosis, which is programmed cell death, is seen as a promising approach in anti-cancer drug treatments due to its mechanism of formation (23, 24). In our study, cells with this morphology were observed in both cell types at the highest dose of carrageenan (Dilution I)]. However, in order to make a definitive assessment on this subject, it is inevitable that more detailed studies are required to show the pathway of apoptosis and the signaling mechanisms by which carrageenan acts in this process.

Although MCF-7 cells are mostly of the same phenotype in an in vitro culture, in some cases subtypes of cells with different phenotypes emerge within the same population, and there is a variation in the gene expression of surface receptors and signaling pathways in these cells (13). Differences in cell proliferation also occur in these subtypes of cells, and the likelihood of these phenotypic changes increases with the changes in physicochemical conditions (13). In long-term cultures, these cells show a profile similar to those of breast cancer types that are clinically resistant to anti-estrogen and anti-aromatase treatments. However, the main problem here is that this phenotypic change in subtypes also leads to changes in surface receptors and signaling mechanisms (25). These changes may lead to results that may affect cell viability, proliferation, and morphology in different ways.

In conclusion, the results obtained in the present study showed that carrageenan caused cytotoxic effects on both malignant and non-malignant cell lines. Therefore, carrageenan can be considered a new approach in anti-cancer treatments. On the other hand, not a very effective cytotoxic effect was observed in MCF-7 cancer cells. Considering that the different phenotypic characteristics of the subtypes of this cell line can affect cell viability and cell proliferation, it was concluded that it is extremely important to know the genotypic and phenotypic characteristics of the cells used in in vitro cytotoxicity studies and to determine whether it will undergo a change in the culture in the end.

Etik: Bu çalışmanın etik kurulu alınmıştır.

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Orijinal Araştırma / Original Investigation
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Obtaining Linear Regression Formulas Depending On Upper Arm Length For Estimating Stature

Boy Tahmini İçin Üst Kol Uzunluğuna Bağlı Lineer Regresyon Formüllerinin Elde Edilmesi

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ÖZET

AMAÇ: Çalışmamızda genç erişkin kadın ve erkek bireylerin üst kol uzunluğu değerlerinden boy tahmini için lineer regresyon formülleri elde edilmesi ve sağ ve sol taraf asimetrisinin regresyon formüllerine etkisinin incelenmesi amaçlanmıştır.

GEREÇ VE YÖNTEM: Çalışma, sağlıklı 18-36 yaş aralığında, 70 genç erişkin gönüllü üzerinde (35 erkek, 35 kadın) gerçekleştirildi. Üst kol uzunluğu, akromiyondan olekranona olan mesafe ölçülerek elde edildi. Asimetri ve cinsiyetler arası farklılıklar analiz edildi. Boy tahmini için cinsiyetlere göre lineer regresyon analizi ile regresyon formülleri oluşturuldu.

BULGULAR: Boy tahmini için gerekli olan lineer regresyon formülleri elde edildi. Üst kol uzunluğuna dayalı lineer regresyon formüllerinden standart tahmin hatasının (SEE) en küçük değeri kadın sağ koluna aitti. SEE değerleri kadınlarda sağ kol için 5.22882 ve sol kol için 5.4979; erkeklerde sağ kol için 6.62943 ve sol kol için 6.60019 olarak bulundu. Her iki cinsiyette de kol uzunluğu ile boy arasında orta düzeyde pozitif bir ilişki bulundu. Pearson korelasyon katsayısı kadınlarda sağ taraf için r=500, sol taraf için r=413, erkekler için sağ taraf için r= 487, sol taraf için r=494, p<0.05 idi.

SONUÇ: Erkeklerde kol uzunluğunda boy tahmini için elde edilen formüllerde SEE değerleri kadınlardakinden daha yüksek bulunmuştur. Regresyon analizine göre, kadınlardaki üst kol uzunluğu değerleri, erkeklerle kıyaslandığında daha iyi bir boy tahmini sonucu vermektedir. Kadınlarda sağ ve sol taraf üst kol uzunlukları arasında asimetri bulunmaktaydı. Sağ tarafın regresyon denkleminin daha güvenilir olduğu tespit edildi.

Anahtar Kelimeler: asimetri, boy tahmini, regresyon denklemi, regresyon formülü, üst kol uzunluğu

ABSTRACT

OBJECTIVE: In our study, it was aimed to obtain linear regression formulas for estimating stature from upper arm length values of young adult female and male individuals and to examine the effect of right-left side asymmetry on regression formulas.

MATERIALS AND METHODS: The study was carried out on 70 healthy young adult volunteers (35 males, 35 females) aged 18-36 years. Upper arm length was obtained by measuring the distance from the acromion to the olecranon. Asymmetry and gender differences analyzed. For the estimation of stature, regression formulas were created by simple linear regression analysis separately according to the genders.

RESULTS: The linear regression formulas required for the stature calculation were obtained. Among the linear regression formulas based on upper arm length, the Standard Error of the Estimate (SEE) value was the lowest in the female upper right arm. SEE values for women were 5.22882 for the right upper arm and 5.4979 for the left upper arm; in men it was 6.62943 for the right upper arm and 6.60019 for the left upper arm. A moderately positive correlation was found between arm length and stature in both genders.

CONCLUSION: In the formulas obtained for the estimation of arm length in men, the SEE value was found to be higher than in women. According to the regression analysis, arm length values in women give a better estimation of stature compared to men. In women, asymmetry was detected between the right and left upper arm lengths. The regression equation of the right side was found to be more reliable.

Keywords: asymmetry, stature estimation, regression equation, regression formula, upper arm length

INTRODUCTION

In cases where bodily integrity is lost due to natural disasters, terrorist attacks, murder, etc., one of the most important points for forensic doctors and law enforcement officers in identifying the victim is to calculate the stature of the person (1). Formulas used for estimating stature from body parts differ according to populations (2). Many body parts such as hand length, foot length, radius and ulna lengths, and metatarsals have been used in different societies for stature estimation (1-5).

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Generally, stature estimation can be made from bone collections. However, it is not always possible to obtain bone collections. For this reason, regression formulas are created for estimating stature with anthropometric measurements. Above all, the average stature and body proportions of the population change over time. It has been reported that there has been an increase in the average stature of the world population in the last hundred years (5). For this reason, it seems important to update the formulas used for stature estimation over time. There are right and left side differences and asymmetry between some parts of the human body (6). Therefore, it is important to consider the existence of asymmetry in the creation of these formulas. In our study, it was aimed to examine the asymmetry between the upper arm lengths of young adult men and women and to derive simple linear regression formulas for stature estimation.

MATERIAL & METHODS

Upper arm length was measured as the distance between the acromion and the olecranon. Measurements were made on both right and left arms. Stature was measured from the vertex point of the skull in a standing person leaning against a vertical wall. Measurements were made with a tape measure. Study approval was obtained from Uşak University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee with the decision numbered 07.04.2021/13.

Statistical analysis

IBM SPSS 26 program was used for statistical analysis. Descriptive statistical analysis (median, minimum, maximum, standard deviation) was performed. The distribution of the obtained data was evaluated using the Shapiro–Wilk test. The relationship between stature and arm length was evaluated with Pearson correlation. Right and left side differences were analyzed with the paired-t test, and the analysis of gender differences was performed with the independent samples-t test. For the estimation of stature, regression formulas were created by simple linear regression analysis separately according to the genders.

RESULTS

The average stature of the volunteers participating in the study; It was 179.11 cm in men and 164.45 cm in women. The mean right upper arm length was 36.43 cm in men and 33.37 cm in women. The mean left upper arm length was 36.48 cm in men and 33.66 cm in women. The measurement values obtained from men were found to be greater than women's, the difference was statistically significant ($p<0.01$).

When the right and left upper arm lengths were compared, there was no significant difference in men $p=0.745$. In females, a significant difference was found between the lengths of the right and left sides, $p=0.01$. Other descriptive statistical values are given in Table 1.

Table 1: *Descriptive statistics and comparison of variables for genders.*

		n	Mean	Min	Max	SD
F	RUAL *	35	33.37**	30.00	38.90	1.91
	LUAL *	35	33.66**	30.00	38.00	2.03
	Stature*	35	164.11	150.00	180.00	5.95
M	RUAL *	35	36.43	32.50	41.10	2.40
	LUAL *	35	36.48	32.60	44.20	2.64
	Stature*	35	179.11	164.00	195.00	7.48

F: *female,* ***M:*** *male* ***SD:*** *Standard Deviation* ***RUAL:*** *Right upper arm lenght,* ***LUAL:*** *Left upper arm lenght* *******$p<0.01$ *difference for genders,* ********$p=0.01$ *difference for sides* ***n:*** *number* ***Min:*** *Minimum* ***Max:*** *Maximum*

In the study, the linear regression formulas necessary to calculate the stature estimation were obtained by using the upper arm length values (Table 2).

Table 2: *Pearson's correlation analyses*

Genders	Variables		RUAL	LUAL	Stature
M, n=35	RUAL	r	1	.944**	.487**
		p		0.000	0.003
	LUAL	r	.944**	1	.494**
		p	0.000		0.003
	Stature	r	.487**	.494**	1
		p	0.003	0.003	
F, n=35	RUAL	r	1	.952**	.500**
		p		0.000	0.002
	LUAL	r	.952**	1	.413*
		p	0.000		0.014
	Stature	r	.500**	.413*	1
		p	0.002	0.014	

F: *female,* ***M:*** *male* ***SD:*** *Standard Deviation* ***RUAL:*** *Right upper arm lenght,* ***LUAL:*** *Left upper arm lenght* *******$p<0.01$ *difference for genders, * Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).*

Among the linear regression formulas based on upper arm length (female, male, right and left sides), the Standard Error of Estimate (SEE) value of the female right upper arm formula was the smallest. SEE values were SEE:5.22882 for female right upper arm and SEE:5.4979 for female left upper arm; SEE:6.62943 for male right upper arm, and

SEE:6.60019 for male left upper arm. A moderate positive correlation was found between upper arm length and stature in both genders. Pearson correlation coefficient for women was r=500 for the right side, r=413 for the left side (p<0.01 and p<0.05, respectively), r=487 for the right side for men, r=494 for the left side, p<0.01 (Table 3).

Table 3: Linear regression formulas

	Linear regression formulas	SEE	R Square
F	Stature = 1.559 x RUAL + 112.072	5.2288	0.25
	Stature = 1.210 x LUAL + 123.381	5.4979	0.171
M	Stature = 1.518 x RUAL + 123.811	6.62943	0.237
	Stature = 1.399 x LUAL + 128.093	6.60018	0.244

***F:** female, **M:** male **SD:** Standard Deviation **RUAL:** Right upper arm lenght, **LUAL:** Left upper arm length **SEE:** Standard Error of Estimate*

DISCUSSION

Stature estimation is one of the four important determinants in determining the identity information of the corpse from human remains. Anatomical sections where anthropometric measurements are made can be affected by many factors such as racial characteristics, gender, geographical and regional characteristics, sportive life, lifestyle, nutrition styles. Therefore, every society has to obtain regression equations suitable for their genetic and environmental characteristics.

In a study conducted in a young population in Iran (mean upper arm length is 33.72cm in men, 30.12cm in women), they found the SEE value of 4.52 in the regression equation obtained from the arm length values of men. They did not report a regression equation since there was no statistical significance between arm length and stature in women (7). In our study, regression equations were obtained for both sexes. The SEE value of the equation obtained from men was 6.62 for the right arm and 6.60 for the left arm. In addition, a moderate positive correlation was found between arm length and stature in women in our study (r=500, p<0.01), and SEE values were found to be 5.22 for the right arm and 5.46 for the left arm in the regression equations obtained.

Shah T et al. They created regression equations for estimating stature from foot length and upper extremity lengths in two groups, Hindu group (n=80) and Muslim group (n=80) in India. In the equations obtained from the upper extremity length in the Muslim group, they reported the SEE value as 6.9193 in men and 3.5586 in women. In the same study, they found the SEE value as 4.1724 in men and 4.2382 in women in the regression equation obtained from the Hindu group. The SEE value obtained in men in the Muslim group is greater than the SEE value in our study (8).

Özaslan et al. in a study they conducted, they found the R square value for the estimation of stature based on upper arm length as 0.20 in men and 0.43 in women. In our results, however, the regression equations obtained from the upper arm length do not have a superiority in terms of women and men. In our study, the R square values of the equations for estimating stature from upper arm length were close to each other in men and women (0.24 in men and 0.25 in women) (9). In addition, in our study, it was concluded that there was asymmetry in terms of right and left upper arm lengths in women, and this affected the success of the regression formulas.

In a study conducted in Sudan (mean upper arm length is 31.65 cm in males and 28.90 cm in females), the regression equations obtained from the upper arm length seem to be more successful than our equations in both genders. The SEE value of the equation obtained by Altayeb A.A in estimating stature from the upper arm in their study in Sudan was 4.48 in men and 4.40 in women; The R square value was reported as 0.490 for men and 0.414 for women. In addition, the correlation values between upper arm length and stature were found to be higher than our correlation values (r=0.698 for men and r=0.643 for women). This difference is probably due to the racial characteristics of the two different societies (10).

In a study conducted in men in northern India, it was reported that the difference between right and left upper arm lengths was statistically significant and this asymmetry may be significant (p<0.01). For this reason, they emphasized the importance of firstly distinguishing the right and left sides of a body part and then using the regression formula of the appropriate side (6). In our study, there was no asymmetry for the right and left side in terms of upper arm length in men. However, it was determined that there was an asymmetry between the right and left sides in women. It was determined that the regression equation obtained from the right side was more successful than the equation obtained from the left side in women. In women, the SEE and R squared values of the right-side regression equation were 5.2288 and 0.250, respectively.

While on the left side, SSE value was 5.4949 and R square value was 0.171.

CONCLUSION

The estimation of the stature of individuals is very important for units such as archeology, forensic medicine and law enforcement. While using the regression equations obtained from the lengths of the body parts, the characteristics of that society gain importance. In addition, regression formulas obtained from the other side should not be used for body parts with right and left side asymmetry in the human body.

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Orijinal Araştırma / Original Investigation
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Chromosomal Anomalies in Bone Marrow Samples of Patients Diagnosed With Hematological Cancer: FISH Results of 109 Cases from One Center

Hematolojik Kanser Tanılı Hastaların Kemik İliği Örneklerindeki Kromozomal Anomaliler: Tek Merkezden 109 Olgunun FISH Sonuçları

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ÖZET

AMAÇ: Hematolojik kanserler, kemik iliği, kan ve lenf düğümlerini etkileyen genellikle yapısal ve sayısal kromozom anomalileri ile ilişkili bir neoplazma grubudur. Hematolojik kanserlerde kemik iliği incelemesi, hastalığın tanısı ve prognozu hakkında aydınlatıcı ve yönlendirici bir role sahiptir. Çalışmamızda, hematolojik kanser tanılı hastalarda belirli genetik düzensizliklerin Floresan In Situ Hibridizasyon (FISH) yöntemi kullanılarak elde edilen sonuçlarının retrospektif olarak değerlendirilmesi amaçlanmıştır.

GEREÇ VE YÖNTEM: Çalışmamızda,1 Ocak 2021-30 Kasım 2021 tarihleri aralığında, Dicle Üniversitesi Tıp Fakültesi Tıbbi Biyoloji ve Genetik Anabilim Dalı'na, Hematoloji anabilimdalı ve diğer kliniklerden yönlendirilen hematolojik kanser ön tanılı 109 hastanın (KML n=14, AML n=40, KLL n=6, ALL n=27, multipl miyelom (n=22) kemik iliği örneklerinden FISH yöntemi kullanılarak elde edilen sonuçları yaş, cinsiyet ve hastalık dağılımı açısından retrospektif olarak incelenmiştir. Çalışma grubuna alınan hastaların tümü kromozomal anomaliler, sayısal ve yapısal değişlikler ya da dengeli translokasyon varlığı açısından değerlendirilmiştir.

BULGULAR: Olguların 47'si kadın 62'si erkek hasta olup yaş ortalaması 48±21,6 olarak tespit edilmiştir. Olguların incelenmesi sonucunda, AML için bilinen; t(15;17), monozomi 7 ve trizomi 8, KML için t(9;22); KLL de del(13q14) ve del(17p13), multipl miyelom için t(11;14)(q13;q32), ALL için (t[9;22]) (bcr-abl) ve t(4;11) yapısal ve sayısal kromozom anomalileri tespit edildi. Ayrıca grupların karşılaştırılmasında kadın ve erkekler arasında anlamlı fark bulunamamıştır.

SONUÇ: FISH yöntemi kullanılarak hematolojik kanser ön tanısı almış hastalarımızla yaptığımız bu retrospektif çalışmada, hastalıklarla ilişkili prognostik olarak önemli anomaliler ile ilgili sonuçlarımızı değerlendirdik. Hastalıkların tanılarının hızlı ve doğru olarak tespitinin, hastalığın tedavi planlaması ve seyrinin ön görüsü açısından çok önemlidir. Sonuç olarak bizim çalışma sonuçlarımıza göre kısıtlı ve zor şartlarda alınan kemik iliklerinden hücre elde etmenin zorluğu göz önüne alındığında FISH yönteminin interfaz hücrelerinde bile çalışma imkânı sağlaması nedeniyle kanser genetiğinde kullanılan güçlü ve etkili bir teknik olduğu kanaatine varılmıştır.

Anahtar Kelimeler: hematolojik kanser, FISH, kemik iliği, kromozomal anomaliler

ABSTRACT

OBJECTIVE: Hematological cancers are a group of neoplasms that affect the bone marrow, blood and lymph nodes, usually associated with structural and numerical chromosomal abnormalities. Bone marrow examination in hematological cancers has an illuminating and guiding role in the diagnosis and prognosis of the disease. In our study, it was aimed to retrospectively evaluate the results obtained by using the Fluorescent In Situ Hybridization (FISH) method of certain genetic disorders in patients with a diagnosis of hematological cancer.

MATERIALS AND METHODS: In our study, between January 1, 2021 and November 30, 2021 The results obtained from bone marrow samples of 109 patients (KML n=14, AML n=40, KLL n=6, ALL n=27, M.Myelom n=22) with pre-diagnosis of hematological cancer, who were referred to Dicle University Faculty of Medicine, Department of Medical Biology and Genetics, Department of Hematology and other clinics, were analyzed retrospectively in terms of age, gender, and disease distribution. All of the patients included in the study group were evaluated in terms of chromosomal anomalies, numerical and structural changes, or presence of balanced translocation.

RESULTS: There were 47 female patients and 62 male patients, and the mean age was 48±21.6 years. As a result of the examination of the cases, known for AML; t(15;17), monosomy 7 and trisomy 8, t(9;22) for CML; Del(13q14) and del(17p13) for CLL, t(11;14)(q13;q32) for M. Myeloma, (t[9;22]) (bcr-abl) and t(4;11) for ALL structural and numerical chromosomal anomalies were detected. In addition, in the comparison of the groups, no significant difference was found between the materials participating in the study, male and female.

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CONCLUSION: In this study, which we conducted with our patients who were prediagnosed with hematological cancer with the FISH method, we evaluated our results regarding the prognostically important anomalies associated with the diseases. Rapid and accurate diagnosis of diseases is very important in terms of treatment planning and prognosis of the course of the disease. Considering the difficulty of obtaining cells from bone marrow taken under limited and difficult conditions according to our study results, it was concluded that the FISH method is a powerful and effective technique used in cancer genetics, since it provides the opportunity to work even in interphase cells.

Keywords: hematological cancer, FISH, bone marrow, chromosomal abnormalities

INTRODUCTION

Bone marrow is the nutritious spongy tissue found in the cavities inside long flat bones such as the sternum and hip bones. There are two kinds of bone marrow: red bone marrow and yellow bone marrow. Both types of bone marrow contain blood vessels. There are two types of stem cells in the bone marrow: Hematopoietic and mesenchymal. This process, which consists of the production process of various erythrocytes and pluripotent stem cells, is known as hematopoiesis. The pluripotency of hematopoietic stem cells can be any cell type in the bloodstream. Under the influence of tissue and hormonal factors, in parallel with the differentiation or maturation process, these cells turn into the cells we know in the bloodstream(1).

Hematological cancers are neoplasms composed of cells originating from the bone marrow. . Most of these malignancies contain structural or numerical chromosomal abnormalities. The first anomaly detected in humans in this regard is the Philadelphia chromosome (2). After the philadelphia chromosome, which is an significant marker of chronic myloid leukemia (CML), the value of translocation, deletion and inversion of different chromosomes in the diagnosis of diseases and evaluation of prognosis in different types of hematological cancers has been understood.

Bone marrow examination is a valuable examination that is mostly performed in peripheral blood smear samples to show the cellular features of the bone marrow, tumors and participation of hematological diseases in suspected cases. For the first time in 1890, German pathologist David Paul Von Hansmann defined nuclear and mitotic structure irregularities in cancer biopsy materials and reported that these findings may be important in the development of cancer(3).

FISH applications, developed as a complement to classical cytogenetic methods, have an important place in cancer studies. The FISH technique is applied to metaphases (metaphase FISH-mFISH) or interphase cells (interphase FISH-iFISH) depending on the situation(4).

In this study, we retrospectively analyzed the results of bone marrow materials of patients diagnosed with hematological malignancy from various clinics who applied to Dicle University Faculty of Medicine, Department of Medical Biology and Genetics between 01/01/2021 and 30/11/2021, and analyzed using the FISH technique. Our aim is to evaluate the detailed examination and analysis of the prevalence of abnormalities in our patient population with the implementation of the FISH method.

MATERIAL & METHODS

A total of 109 patients with the diagnosis of hematological malignancy were included in this retrospective study, from the Department of Hematology, Department of Pediatrics, Hematology, and other clinics, between January 1, 2021 and November 30, 2021, to Dicle University Faculty of Medicine, Department of Medical Biology and Genetics.

FISH analysis results of bone marrow samples of 14 patients in the CML patient group (3 women and 11 men), 27 patients in the ALL patient group (12 women and 15 men), 40 patients in the AML patient group (17 women and 23 men), 6 patients in the CLL patient group (2 women and 4 men), 22 patients in the Multiple Myeloma patient group (13 women and 9 men) and 109 patients in total were evaluated retrospectively(table I). Considering the genetic analysis results of the patients, the FISH method was applied with different FISH probes (table 2) in the samples.

The data of all patients were searched through the closed registry system of the Department of Medical Biology and Genetics. Age, gender and prediagnosis of hematological malignancy of the patients were recorded. Using this information, a database was created in the excel program in computer environment. Cases with missing data were excluded from the study.

Ethics committee approval was obtained for this study from the Dicle University Clinical Research Ethics Committee at the meeting dated 21.01.2022/15

Statistical Evaluation

The data were recorded in the SPSS (Statistical Packages of Social Sciences, SPSS for Windows, Version 10.0, Inc, Chicago, IC, USA) program. Error controls, tables and statistical analyzes were also performed in this program, the relationship between the parameters obtained from the age, gender and diseases of the individuals in the patient groups was made according to the correlation and regression analysis. The comparison of the parameters between the groups was made according to the t test and their arithmetic mean was shown with standard deviation.

RESULTS

Of the 109 patients who participated in our study, 47 were female and 62 were male. The mean age of women was 50±22.02, and the mean age of men was 47±21.3 (table 1). The distribution of the patients participating in the study according to their preliminary diagnoses is CML(n=14), AML(n=40), CLL (n=6), ALL (n=27), M. Myeloma (n=22) (table 2).

Table 1: *Distribution of patients*

	PD (% value)	Age (AVG, %)	Gender (%) F/M
ALL	26(%23,9)	33.0±23.4	42.3/57.7
AML	40(%36,7)	46.5±19.2	45/55
CLL	7(%6,4)	52.5±13.6	28.6/71.4
CML	14(%12.8)	52.0±16.0	36.4/63.6
MM	22(%20,2)	68.6±9.7	56/44

PD: Patient Distribution F: female, M: male

The median age was 33.0±23.4 years in 12 (42.3%) female and 15 (57.7%) male patients diagnosed with Acute Lymphocytic Leukemia and aged 2-77 years. In the FISH study; Of the 27 interphase nuclei examined, 18 were normal, and chromosomal anomaly was detected in at least one sample type in 9 of them. Translocation specific to 8q24, 9q34, 12p13 and 4q21 regions, in FISH study using dual fusion probe, 2 of 15 male patients had 4q21, 2 had 8q24.9q34 and 12p13, 2 had 9q34.1(ABL1)/22q11.2(BCR) regions-specific translocation was observed, while 9 of them did not. Translocation specific to the 9q34.1(ABL1)/22q11.2(BCR) region was observed in 3 of 12 female patients, while no translocation was observed in 9 of them. In the iFISH study using the 13q14 region-specific deletion probe, it was found that the 13q14.3(DLEU2) region was deletion in 2 female patients. In the FISH study using the 11q23.3(MLL) region-specific rearrangement and deletion probe, 11q23.3(MLL)(trisomy7) was observed in 1 male patient and deletion of the 3'MLL region (11q23.3) in 1 male patient. In the FISH study using centromeric probes specific to 7p11 and 8p11 regions, 8p11 (monosomy8) was observed in 1 male patient, 7p11 (trisomy7) in 1 male patient, and both 7p11 and 8p11 (tetrasomy 7.8 tetraploidy) in 1 male patient.

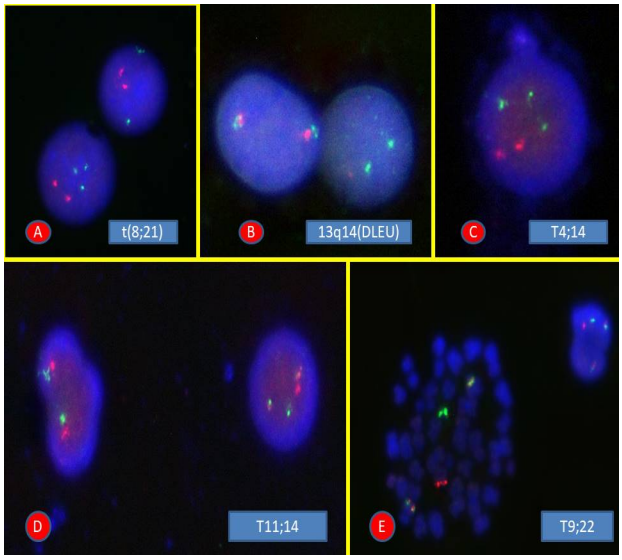
Table 2: *Site-specific centromeric FISH probes used in interphase cells obtained from uncultured bone marrow from patients*

Diseases	Region-Specific Centromeric Probes in Interphase Cells
Acute Lymphocytıc Leukemıa (ALL) (Probe used: ALL Panel / Diagen)	**A)** 8q24.21(MYC)/14q32.33(IGH), 9q34.1(ABL1)/22q11.2(BCR), 12p13.2(TEL)/21q22.12(AML) and 4q21.3-4q22.1)(AFF1)/11q23.3(MLL) region-specific translocation, dual fusion; **B)** 13q14.3(DLEU2)/13q34(LAMP1) region-specific deletion; **C)** 11q23.3(MLL) region-specific reorganization; **D)** 7p11.1-q11.1(D7Z1) ve 8p11.1-q11.1(D8Z2)
Acute Myeloid Leukemia (AML) (Probe used: AML Panel / Diagen)	**A)** 15q24.1(PML)/17q21.1(RARA), 8q21.3(ETO)/21q22.12(AML1) and 16p13.11(MYH11)/16q22.1(CBFB) region-specific translocation, dual fusion; **B)** 5q31.2(EGR1), 7q22.1(RELN)/7q31.2(TES) ve 17p13.1(P53) region-specific deletion; **C)** 11q23.3(MLL)
CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) (Probe used: CLL / Diagen)	**A)** 11q13.3(CCND1)/14q32.33(IGH) and 14q32.33(IGH)/18q21.33(BCL2) region-specific translocation, dual fusion; **B)** 17p13.1(P53) and 13q14.3(DLEU2)/13q34(LAMP1) region-specific deletion; **C)** 12p11.1-q11.1(D12Z3)
Chronic Myeloid Leukemia (CML) (Probe used: BCR-ABL t(9;22)/Diagen)	**A)** 9q34.1(ABL1)/22q11.2(BCR) site-specific translocation, dual fusion probe
Multiple Myeloma (MM) (Probe used: MM Panel/Diagen, Cytocell)	**A)** 4p16.3(FGFR3)/14q32.33(IGH) and 11q13.3(CCND1)/14q32.33(IGH) region-specific translocation, dual fusion; **B)** 17p13.1(P53) and 13q14.3(DLEU2)/13q34(LAMP1) region-specific deletion; **C)** 7p11.1-q11.1(D7Z1) ve 8p11.1-q11.1(D8Z2)

The median age was 46.5±19.2 in 17 (45%) female and 23 (55%) male patients diagnosed with Acute Myeloid Leukemia and aged between 21-87 years. In the FISH study; While 1 of 40 interphase nuclei could not be found to be

evaluated, results were obtained in 39 of them. According to these results, 27 were normal and 12 were anomaly. Translocation specific to 15q24, 8q21 and 16p13 regions, in FISH study using dual fusion probes, specific translocations to regions 15q24.1(PML)/17q21.1(RARA) were observed in 1 male patient, 8q21.3(ETO)/21q22.12(AML1) in 4 male and 2 female patients (fig.1A) ,16p13.11(MYH11)/16q22.1(CBFB) in 1 male and 1 female patient In the iFISH study using deletion probes specific to 5q31 , 7q22 and 17p13 regions, deletion of 7q22.1(RELN)/7q31.2(TES) region was detected in 4 male patients, while deletion specific to 5q31.2(EGR1) and 17p13.1(P53) regions was detected (fig 2A). 11q23.3(MLL)(trisomy7) was observed in 1 male patient as a result of iFISH examinations using the 11q23.3(MLL) region-specific rearrangement probe.

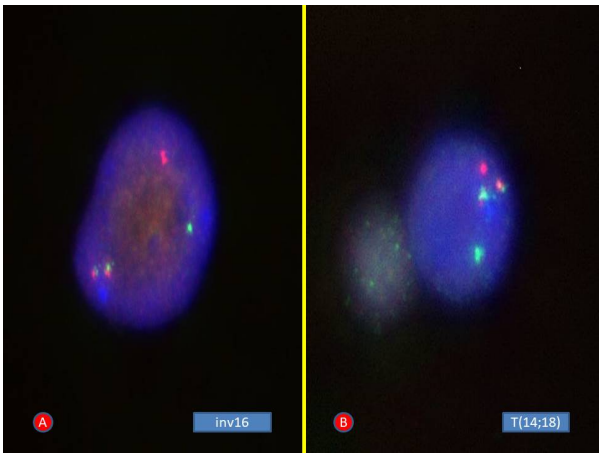
Figure 1: *FISH with different types of probes and partial metaphases.*



A) *t(8;21) Signal pattern (3 green, 2 red signals) consistent with the trisomy of region 8q21.(ETO) was detected.* ***B)*** *Signal pattern consistent with the deletion of the 13q14.3(DLEU2) region was detected* ***C)*** *Signal pattern consistent with the rearrangement of region 14q32.33(IGH) was detected in 33% of region t(4;14).* ***D)*** *Typical double fusion hybridization appearance specific to t(11;14)(q13.3;q32) IGH/CCND1 translocation was detected.* ***E)*** *t(9;22)(q34.1;q11.2)ABL1/BCR translocation was detected*

The median age was 52.5±13.6 years in 2 (28.6%) female and 4 (71.4%) male patients diagnosed with Chronic Lymphocytic Leukemia, aged 36-71 years. In the FISH study, 2 of the 6 interphase nuclei examined were normal and 4 had chromosomal structure anomaly. Translocation specific to 11q13, 14q32, 18q21 regions, in FISH study using dual fusion probe, 14q32(IGH) region-specific translocation was observed in 1 of 4 male patients, and 18q21 region-specific translocation was observed in 1, while no translocation was observed in 2 female patients(fig 2B). In the iFISH study using deletion probes specific to 17p13, 13q14 regions, deletion of 13q14.3(DLEU2) region was detected in 1 of 2 female patients and 2 of 4 male patients (fig 1B). No deletion of the 17p13 region was observed in the analyzed cells. As a result of iFISH examinations using 12p11 region-specific centromeric probes, trisomy 12 was not detected in any of the 6 cases.

Figure 2: *FISH with different types of probes and partial metaphases*



A) *A double fusion hybridization was detected, which may be compatible with the inv(16)(p13.11;q22.1) region.* ***B)*** *Typical double fusion hybridization appearance specific to t(14;18)(q32.33;q21.33) translocation was detected.*

The median age was 52.0±16.0 years in 3 (36.4%) female and 11 (63.6%) male patients, aged 25-79 years, diagnosed with Chronic Myeloid Leukemia. In the FISH study, 5 of the 14 interphase nuclei examined were normal and 9 were translocation.

Translocation specific to 9q34.1(ABL1)/22q11.2(BCR) regions, in FISH study using dual fusion probe, typical double fusion hybridization appearance specific to t(9;22)(q34.1;q11.2)ABL1/BCR translocation was detected in 2 of 3 female patients and 7 of 11 male patients in total 9 people (fig 1E), No translocation was observed in 1 female and 4 male patients.

The median age was 68.6±9.7 years in 13 (56%) female and 9 (44%) male patients diagnosed with Multiple Myeloma, aged between 53 and 83. In the FISH study, 1 of 22 interphase nuclei could not be found to be evaluated, while results were obtained in 21 of them. According to these results, 15 normal and 6 chromosome number and structural anomalies were observed. Translocation and

trisomy11 specific to 11q13, 14q32 regions were detected in 4 female patients in the iFISH study using dual fusion probes(fig 1C-1D). No deletions were detected in 17p13 and 13q14 gene regions. Trisomy 7 was detected in 3 female patients in the iFISH study using centromeric probes specific to 7p11 and 8p11 regions. Cells analyzed from 1 female patient were evaluated for hyperdiploidy, with trisomy 17 in 11% and trisomy 7 in 9%. No chromosomal abnormality was found in any of the 9 male patients examined.

DISCUSSION

Hematological cancers are more common in men, In our study, 47 (43.1%) of 109 cases with hematological malignancy were female and 62 (56.9%) were male, and the M/F ratio was found to be approximately 1.32. In a large retrospective study of 5013 patients with hematological cancer, 69.2% were male (n = 3468) and 30.8% were female (n = 1545) patients, and the M/F ratio was found to be 2.2 The high number of male patients in hematological cancer cases in our study is consistent with the literature.(5) In our study, in accordance with the literature, the most common AML patient group was observed, while the least CLL patient group was detected. Accordingly, while the ALL patient group consists of younger patients, Multiple Myeloma draws attention as a disease of advanced age.

Acute Myeloblastic Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) are the two main groups of acute leukemias. Chronic Myelocytic Leukemia (CML) and Chronic Lymphocytic Leukemia (CLL) are classified as chronic leukemias. Multiple Myeloma (MM); It is a disease characterized by an increase in plasma cells in the bone marrow, the presence of M serum proteins secreted by these cells in the serum and/or urine, and lytic bone lesions (6).

Determination of chromosome aberrations in hematological cancers originating from bone marrow is valuable in terms of diagnosis and prognosis. Fluorescence In Situ Hybridization technique is accepted as a fast, easy and reliable method for detecting such changes. While chromosomal anomalies of 2000 and 3000 kbase can be detected by classical cytogenetic methods, it is possible to detect regions of 0.5 kb by FISH method (7). In the FISH method, the nucleic acid sequence with a complementary label to the target DNA/RNA molecule is called a "probe". There are 35 specific (locus-specific and/or translocation-specific) probes used for diagnosis in hematological cancer diseases, as well as all chromosome, telomere and α-satellite probes specific to 24 chromosomes. These probes are available in single and/or double color probe sets according to their usage patterns and features; they can be marked with three and/or five different colors(8). One of the disadvantages of the technique is that it allows the disease-specific analysis of only special panels to be performed in the FISH technique and that other aberrations cannot be detected at the same time(7). There are many literature reports stating that the results obtained with the FISH technique are more sensitive and specific than the classical cytogenetic method.

Özbey et al. compared chromosomal abnormalities in CML patients with cytogenetic and FISH techniques, interphase Dual-FISH (D-FISH) is an effective technique that is reliable and gives results in a short time, in detecting BCR/ABL reorganizations in patients with CML at the time of diagnosis, They reported that D-FISH could be considered as the first test to be used in the diagnosis of patients with Ph(+) CML (9) .In our study, typical double fusion hybridization appearance specific to the 9q34.1(ABL1)/22q11.2(BCR) region-specific translocation was detected in the majority of patients who underwent bone marrow iFISH with a preliminary diagnosis of CML.

More than 50 recurrent chromosomal anomalies have been detected in acute myeloid leukemia (AML). These specific cytogenetic translocations that can be diagnosed by FISH analysis; t(8;21)(q22;q22) (AML1/ETO) AML-M2, t(15;17)(q22;q11-12) (PML/RARα)AML-M3/M3V, inv(16)(p13q22) or t(16;16)(p13;q22) (CBFβ/MYH11) AML-M4/M4eo, 11q23 translocations (MLL) have been reported(10). Cytogenetic changes that can be diagnosed by FISH are mostly observed in the 5th and/or 7th chromosomes. 15-20% of all AML patients have a complex karyotype consisting of multiple numerical and structural chromosomal abnormalities. Mostly, 5q-, -7 and/or 3q-anomalies are primary anomalies(8). However, the karyotype was found to be normal in the majority of patients diagnosed with AML. Therefore, it should be kept in mind that the FISH technique is limited in the diagnosis of AML(11). Chromosomal number and structural anomalies were not observed in 27 of 40 patients with prediagnosis of AML included in our study, while

chromosomal anomaly was observed in at least one sample type compatible with the literature in the other 12 cases.

Chromosomal abnormalities that are effective in prognosis and treatment in acute lymphocytic leukemia (ALL) have been reported. Monosomy 7 and trisomy 8 have been reported as a rare (0.5%) primary chromosomal anomaly in ALL with isolated numerical anomaly in a single chromosome without structural or numerical anomaly(12). More than 40 recurrent structural chromosome rearrangements have been reported in adult ALL cases, and their incidence is less than 1%. Isochromosomes are unique among all structural chromosomal abnormalities, It creates a combination of loss and gain of genetic material. The isochromosomes observed in ALL are i(7q), i(9q) and i(17q)(13). BCR/ABL oncogene The most frequently observed (20-30%) translocation in adult ALL is t(9;22)(q34;q11), that is, Philadelphia (Ph) translocation. This translocation is characterized by a poor prognosis in ALL. Additional chromosome abrasions are observed in 41-86% of Ph-positive ALL cases. The most frequently observed ones are 9p anomalies, hyperdiploid karyotype and monosomy 7, respectively (14). MLL-fusion gene The second most common structural chromosomal abnormality is translocation t(4;11)(q21;q23) with 3-7%. Translocation t(12;21) TEL-AML1 is the most common translocation observed in pediatric ALL. Translocation is found in 25% of children diagnosed with Pre B ALL(15). In our study, chromosomal number and structural anomalies were not observed in 18 of 27 patients with a preliminary diagnosis of ALL, while chromosomal anomalies were observed in at least one sample type in the other 9 cases. In parallel with the literature, 8p11(monosomy8) chromosome number anomaly was observed in a male patient, 7p11(trisomy7) in a male patient, and both 7p11 and 8p11(tetrasomy 7,8 tetraploidy) in a male patient.

Interphase FISH analysis becomes very valuable in patients with chronic lymphocytic leukemia (CLL) since classical cytogenetic studies cannot yield results. FISH can reveal aberrations in 80% of CLL patients. FISH probes help us to show long arm deletions of chromosome 13, long arm deletions of chromosome 11, short arm deletions of chromosome 17, and presence or absence of trisomy 12, as well as IGH (14q32), BCL6(3q27), and rearrangements of chromosome 6. It can also be used to determine (13). Although the number of patients admitted to our study with a pre-diagnosis of CLL was less than expected, it was noted that mostly chromosomal anomaly was observed in accordance with the literature.

Since spontaneous mitotic activity is low in myeloma cells in Multiple Myeloma (MM) patients, classical cytogenetic techniques may be insufficient. In these cases, FISH is an effective screening tool. The most important chromosomal abnormalities are chromosome 13 abnormalities and immunoglobulin chain locus rearrangements on chromosome 14q32. Different probe options are available for the detection of chromosome 13 and IGH (14q32) rearrangements, and diagnosis can be made with FISH. Apart from this, FISH analysis can be performed to determine numerical anomalies for chromosomes 9, 11, 15(16).

Unlike the literature, in our patient group, results were obtained in 21 of the patients who applied with the pre-diagnosis of multiple myeloma, but no results were obtained in one sample.While chromosomal number and structural anomalies were not observed in 15 of 21 patients, chromosomal anomalies were observed in at least one sample type in the other 6 cases.

Genetic studies continue to occupy a large place in the diagnosis, treatment and follow-up of prognosis of many cancer types, especially in hematological malignancies. In genetic studies, bone marrow cytogenetic examination is more suitable for screening, and the FISH technique is a complementary and alternative method for differential diagnosis and prognosis in patients with pre-diagnosis. In particular, the FISH method is a powerful technique to reveal gene translocations, amplifications, chromosomal aneuploidies and deletions. The main advantage of this technique is that the FISH method also provides the opportunity to work in interphase cells, because there is no need to prepare metaphase cells as in the metaphase FISH study. The main disadvantage of the FISH technique is that it only makes an examination by suspecting a certain abnormality, and the necessity of finding a specific probe for each investigated abnormality is the limitation of this technique.

CONCLUSION

In this study, we evaluated the results obtained using the FISH method from bone marrow samples of our patients with prediagnosis of ALL, AML, CLL, CML and M. Myeloma,

which were analyzed retrospectively. Considering the difficulty of obtaining cells from the bone marrow according to the results of this study, we concluded that the FISH method is a powerful and effective technique used in cancer genetics, since it provides the opportunity to work even in interphase cells.

As a result, according to both our study results and the results of different study groups, it was concluded that the FISH method is an effective and preferred technique in investigating a specific chromosomal disorder, clarifying the diagnosis of diseases and prognosis. We also use this technique frequently in our clinic. We believe that studies on these techniques and probes should be supported.

Etik: Bu çalışmanın etik kurulu alınmıştır. 21.01.2022/15

Ethics committee approval had been taken. 21.01.2022/15

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Author contribution status; The concept of the study; LGÇ, MB, ST, DO, MB,İY, design; LGÇ, MB, ST, DO, MB,İY, literature review; LGÇ, MB, ST, DO, MB,İY, collecting and processing data; LGÇ, MB, ST, DO, MB,İY, statistics; LGÇ, MB, ST, DO, MB,İY, writing phase; LGÇ, MB, ST, DO, MB,İY.

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Pediatrik Obezite ile İlişkili Anahtar Genlerin ve Yolakların Tanımlanması

Identification of Key Genes and Pathways Associated With Pediatric Obesity

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ÖZET

AMAÇ: Bu çalışmada, pediatrik obezite ile ilişkili olası moleküler mekanizmaları araştırmak için obezite tanısı konmuş çocuklarda, obezitede rol oynayan potansiyel anahtar genleri ve yolakları belirlemek için biyoinformatik analiz yapılmıştır.

GEREÇ VE YÖNTEM: Biyoinformatik analiz için GEO veri tabanından pediatrik obezite verilerine ait GSE9624 ve GSE139400 erişim numarasına sahip ekspresyon verileri seçilmiştir. GSE9624 veri seti 14 obez ve 13 obez olmayan çocuktan, GSE139400 veri seti ise 5 Obez ve 5 normal kilolu çocuklardan alınan adipoz doku ve kan örnekleri örnekler ile çalışılmıştır. Obez çocuklardan alınan örneklerle normal kilolu çocuklardan alınan örnekler farklı şekilde ifade edilen genleri (DEG) bulmak için GEO2R ile analiz edildi. DEG'ler için GO ve KEGG zenginleştirme analizleri gerçekleştirilmiştir. Cytoscape yazılımıyla bir protein-protein etkileşimi (PPI) ağı oluşturuldu ve obezite ile ilişkili önemli genler belirlendi.

BULGULAR: GEO2R ile analiz sonucunda P-değeri <0.05 ve log2FC⩾0 veya ⩽0 olan DEG'ler seçildi. GSE9624 veri setinde obezite grubunda kontrol grubuna göre 1933 genin ifadesi artarken 1462 genin ifadesi azalmıştır. GSE139400 veri setinde ise obezite grubunda kontrol grubuna göre 725 genin ifadesi artarken 1372 genin ifadesi azalmıştır. Her iki veri setinde ortak olarak ifadesi artan 89, ifadesi azalan 161 DEG tanımlanmıştır. İfadesi değişen genlerin lipit ve ateroskleroz, Doğal öldürücü hücre aracılı sitotoksisite, mTOR sinyal yolağı ve Paratiroid hormon sentezi, salgılanması ve etkisi yolaklarında toplandığı görülmüştür. Ayrıca, ALB, RHOA, KRAS, CREB1, GNAQ, GATA3, CXCR3, EZR, SERPINA1, GNAO1 genleri en önemli genler arasında çıkmıştır.

SONUÇ: Bu çalışmanın sonuçları, ortaya çıkan yolak ve genlerin pediatrik obezitede önemli bir rolü olabileceğini göstermiştir.

Anahtar Kelimeler: pediatrik obezite, gen ifadesi, biyoinformatik analiz, GEO, mikroarray

ABSTRACT

OBJECTIVE: In this study, it was aimed to identify potential key genes and pathways that play a role in obesity in children diagnosed with obesity in order to investigate possible molecular mechanisms associated with childhood obesity.

MATERIALS AND METHODS: Expression data of pediatric obesity data with accession numbers GSE9624 and GSE139400 were selected from the GEO database for bioinformatics analysis. The GSE9624 dataset was studied with samples from 14 obese and 13 non-obese children, and the GSE139400 dataset was studied with samples of adipose tissue and blood samples from 5 obese and 5 normal-weight children. Samples from obese children and samples from normal-weight children were analyzed by GEO2R to find differentially expressed genes (DEG). GO and KEGG enrichment analyzes were performed for DEGs. A protein-protein interaction (PPI) network was created with the Cytoscape software and important genes associated with obesity were identified.

RESULTS: DEGs with a P-value <0.05 and log2FC⩾0 or ⩽0 as a result of analysis with GEO2R were selected. In the GSE9624 dataset, the expression of 1933 genes increased in the obesity group compared to the control group, while the expression of 1462 genes decreased. In the GSE139400 data set, while the expression of 725 genes increased in the obesity group compared to the control group, the expression of 1372 genes decreased. In both data sets, 89 DEGs with increased expression and 161 DEGs with decreased expression were identified. It has been observed that the genes whose expression changes are collected in Lipid and atherosclerosis, Natural killer cell mediated cytotoxicity, mTOR signaling pathway and Parathyroid hormone synthesis, secretion and effect pathways. In addition, ALB, RHOA, KRAS, CREB1, GNAQ, GATA3, CXCR3, EZR, SERPINA1, GNAO1 genes were among the most important genes.

CONCLUSION: The results of this study showed that emerging pathways and genes may have an important role in childhood obesity.

Keywords: pediatric obesity, gene expression, bioinformatics analysis, GEO, microarray

GİRİŞ

Obezite, aşırı vücut yağının belirli bir dereceye kadar biriktiği ve etkilenen bireylerin yaşam beklentisinin kısaldığı ve sağlık sorunlarının arttığı tıbbi bir durumdur (1). Çocukluk çağı obezitesi gelişmiş ve gelişmekte olan ülkelerde bir sağlık sorunu haline gelmektedir (2, 3).

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Obezite, genetik, davranışsal, sosyal, kültürel, metabolik ve fizyolojik faktörlerin kombinasyonundan kaynaklanmaktadır ve hiperlipidemi, insülin direnci ve hipertansiyon gibi bazı hastalıkların olasılığını artırmaktadır. Obezite, adipoz dokularda bulunan veya bu dokuları işgal eden hem adipositlerde hem de makrofajlarda inflamatuvar yolakların kalıcı aktivasyonu ile bağlantılı olan yağ dokusunda artış ile karakterize edilir (4, 5). Obezite ile ilişkili fizyolojik mekanizmalar derinlemesine araştırılmaktadır (6), ancak obezite ile ilgili bazı anahtar moleküler mekanizmalar tanımlanamamıştır. Obezite yaşamın erken dönemlerinde geliştiğinden, çocuklarda bu bozukluğa yol açan mekanizmaları anlamak önemlidir. Sadece obezitenin karmaşık etiyolojisinin ve ilgili bozuklukların daha iyi anlaşılması, etkin müdahalelerinin yolunu açacaktır (7).

Obezite ile ilgili yeni bilgi ve teorilere rağmen, mevcut araştırma yöntemleri obez hastalar için kişiselleştirilmiş tedaviler sağlamada yetersiz kalmaktadır. Bu nedenle, sonuçları hafifletmek ve dünya çapında artan obez hasta popülasyonunu durdurmak için obezite için spesifik terapötik hedeflere ihtiyaç vardır. Moleküler teknolojinin ve biyoinformatiğin gelişmesiyle birlikte, giderek daha yüksek düzeyde diferansiyel olarak ifade edilen aday genler taranmaktadır. Bu sayede kişiselleştirilmiş tedavi yöntemleri geliştirmemize olanak sağlayacaktır (8).

Obezite ile ilgili genlerin tanımlanması için güçlü araçlar olan DNA mikrodizileri ve RNA dizileme (RNA-seq) teknolojileri, transkript seviyelerini ölçerek diferansiyel gen ekspresyonu profilini çıkarmayı sağlamaktadır. İnsan adipoz dokusu üzerindeki bu yaklaşım, diferansiyel olarak eksprese edilen genler (DEG'ler) açısından zengin olan ve dolayısıyla potansiyel olarak obezitenin patogenezinde yer alan moleküler fonksiyonların ve yolakların belirlenmesine yardımcı olmuştur. Gen ekspresyonu analizi, çeşitli yağ dokusu veya hücrelerinden elde edilen ve gen ekspresyonunun fonksiyonel genomik veri kümelerinin arşivlenmesini, işlenmesini ve alınmasını kolaylaştıran genel bir havuz olan Gene Expression Omnibus (GEO) veri tabanında depolanan nispeten büyük bir veri koleksiyonuna yol açmıştır (9).

Bu çalışmada, GEO veri tabanından obez ve sağlıklı çocuklarda yapılan, 2 çalışmanın gen ekspresyon mikrodizin veri setlerini kullanarak, biyolojik ağlar, gen imzaları ve obezite ile ilişkili yolaklar bağlamında DEG'leri tanımlamak için biyoinformatik analiz gerçekleştirilmiştir. Obezitenin altında yatan moleküler mekanizmaları aydınlatmak için aday genleri ve obezite ilişkili yolakları tanımlayarak tedavi için yeni bilgiler sağlanması amaçlanmıştır. mRNA düzenleyici ağ sisteminin ortaya çıkarılması ile yeni mekanizmaları daha fazla araştırmak için yeni teorik rehberlik sağlayacak ve gen hedefli tedavinin altında yatan biyolojik süreçleri anlamak için yeni bir bakış açısı sağlayacaktır.

GEREÇ VE YÖNTEM

Mikrodizin verileri

Pediatrik obezite ile ilgili tüm birincil gen ekspresyon verilerini belirlemek için PubMed ve GEO veri tabanını kullanarak bir literatür araştırması yapılmıştır. Gen ifadesi veri kümeleri, "pediatrik obezite" arama terimi kullanılarak Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) veri tabanından çocukluk çağı obezite verilerine ait GSE9624 ve GSE139400 erişim numarasına sahip ekspresyon verileri seçilmiştir. Dahil etme kriterleri: (a) mikrodizi gen ekspresyon verilerini içeren veri setleri, (b) çalışmalar hem obez hem de obez olmayan denekleri içermekte ve (c) gen ekspresyon analizi yapılan çalışma olması, olarak belirlenmiştir.

GSE9624 veri seti adipoz doku ve kan örnekleri, 14 obez ve apendiks ameliyatı geçiren 13 obez olmayan çocuktan alınmıştır. GSE139400 veri seti 5 Obezite ve 5 normal kilolu çocuklardan alınan rektus femoristen alınan örneklerle çalışılmıştır. Her iki çalışmada GPL570 [HG-U133_Plus_2] Affymetrix İnsan Genomu U133 Plus 2.0 array kullanılmıştır.

Mikrodizin veri işleme

Her iki veri setindeki vakalar ve kontroller arasındaki diferansiyel olarak ifade edilen genler, GEO veri setindeki iki numune grubunu karşılaştıran çevrimiçi bir araç olan GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r) ile analiz edilmiştir. P-değeri <0.05 ve log2FC≥0 veya ≤0 olan DEG'ler istatistiksel olarak anlamlı kabul edildi. İstatistiksel olarak anlamlı genler venny programı (https://bioinfogp.cnb.csic.es/tools/venny/) ile karşılaştırılarak ortak DEG'ler bulundu ve sonraki analizlere bu genler ile devam edildi.

Farklı ifade edilen genlerin fonksiyonel analizleri

İki veri setinde ortak DEG'lerin rol oynadıkları moleküler yolakları ve biyolojik süreçlerin analizi için DAVID (https://david.ncifcrf.gov/) çevrimiçi aracı kullanılmıştır. Gen Ontolojisi (GO) biyolojik işlemler, hücresel komponentler ve moleküler fonksiyonlarına göre belirlenmiştir. Kyoto Genler

ve Genomlar Ansiklopedisi (KEGG) ile biyolojik yolaklar tespit edilmiştir. Fonksiyon zenginleştirme analizinde göre p<0.05 olan terimleri istatiksel olarak anlamlı kabul edilmiştir.

Protein etkileşim ağ network analizi ve hub genlerin tanımlanması

İlk olarak, ortak DEG'ler, protein-protein etkileşimlerini (PPE) analiz etmek için STRING veri tabanına (https://string-db.org/) aktarıldı ve güven sınırı > 0.4 seçilerek protein etkileşim ağı oluşturuldu. Ağ görselleştirilmesi ve ağın topolojik özelliklerinin analizi Cytoscape yazılımında (Cytoscape v3.9.2) Cytohubba eklentisi kullanılarak yapılarak hub genler tespit edildi. Seçilen proteinler protein etkileşim ağında en fazla etkileşime sahip hub genlerdir.

BULGULAR

DEG analizleri ve ortak DEG'lerin tanımlanması

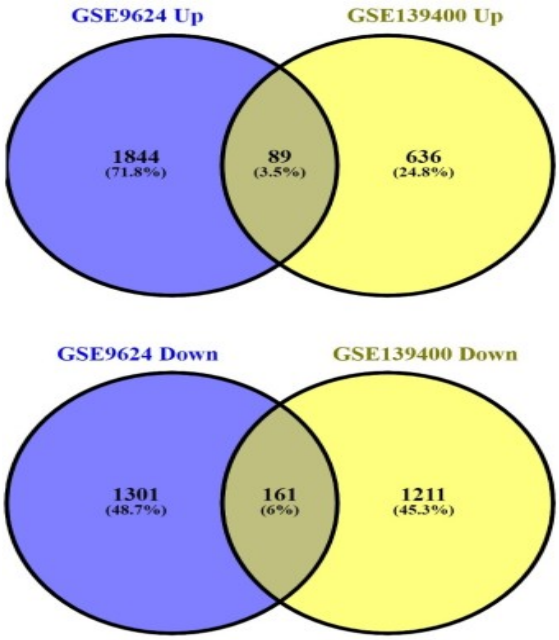
GEO2R ile analiz sonucunda P-değeri <0.05 ve log2FC≥0 veya ≤0 olan DEG'ler seçildi. GSE9624 veri setinde obezite grubunda kontrol grubuna göre 1933 genin ifadesi artarken 1462 genin ifadesi azalmıştır. GSE139400 veri setinde obezite grubunda kontrol grubuna göre 725 genin ifadesi artarken 1372 genin ifadesi azalmıştır. Her iki veri setinde artan ve azalan DEG'ler karşılaştırıldığında, ortak olarak ifadesi artan 89, ifadesi azalan 161 DEG tanımlanmıştır (Şekil 1).

DEG'lerin Gen ontolojisi ve KEGG Yolak Analizi

Çalışmamızda her 2 veri setinde ortak ifade olan DEG'lerin fonksiyonel ve yol zenginleştirme analizleri için DAVID yazılımı kullanılarak GO ve KEGG analizleri yapıldı. GO analizi ifadesi artan ve azalan DEG'lerin 'moleküler fonksiyon (MF)', 'biyolojik süreç (BP)' ve 'hücresel bileşen (CC)' gruplarında çeşitli açılardan fazla miktarda zenginleştiği görülmüştür (p < 0.05). Plazma zarı (CC), DNA bağlama (MF) ve transkripsiyonun pozitif düzenlenmesi, DNA template (BP)'de en fazla genin değiştiği gösterilmiştir. GO terimleri ve zenginleşen genlerin listesi Tablo 1 'te gösterilmiştir.

KEGG yolak analizlerinde ifadesi azalan genlerin lipit ve ateroskleroz, doğal öldürücü (NK) hücre aracılı sitotoksisite, mTOR sinyal yolu, Kompleman ve pıhtılaşma kaskadları, Paratiroid hormon sentezi, salgılanması ve etkisi" başta olmak üzere 10 yolakta anlamlı olarak zenginleştiği, ifadesi artan genlerin ise 3 yolakta zenginleştiği görülmüştür (Tablo 2).

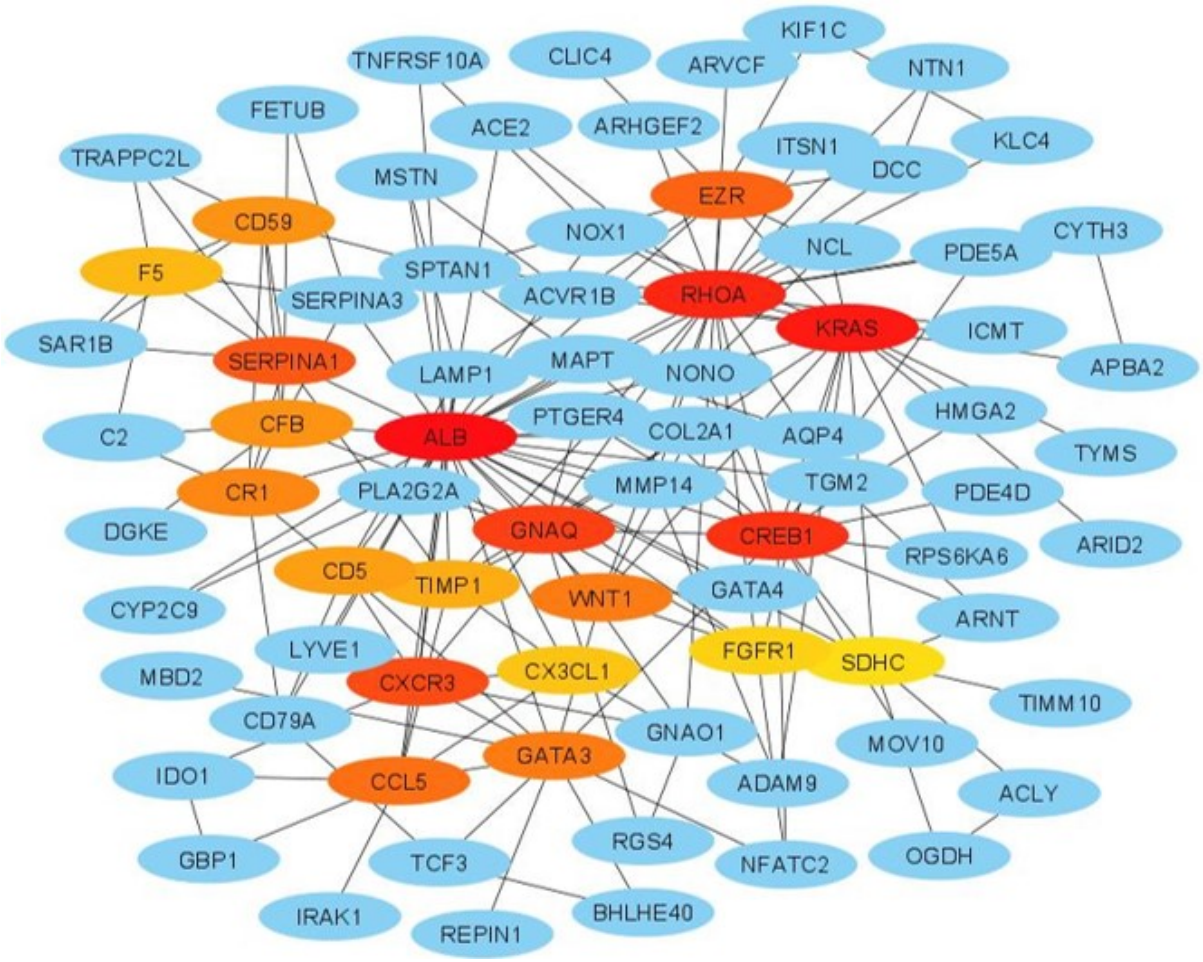
Şekil 1. *GEO veri setlerine ait ortak DEG'lerin Ven diyagramları*



Entegre Protein-Protein Etkileşim Ağı (PPI)

Her 2 veri setinin karşılaştırmasında ortak olarak azalan ve artan genlerin PPI ağ yapısı STRING veri tabanı kullanılarak analiz edildi. STRING analizi sonucu, ifadesi değişen genlerde gen etkileşim ağı 219 düğüm ve 282 kenar içermektedir. Düğümler DEG'leri ve kenarlar DEG'ler arasındaki etkileşimleri göstermektedir (p<0.05) (Şekil 2). STRING analizi sonrasında TIMP1-MMP14'nın en yüksek güven aralığında etkileşim gösterdiği bulunmuştur. Bu genleri analiz etmek için Cytoscape yazılımındaki Network Analyzer kullanıldı ve çekirdek genler, tahmin edilen puanlara göre sıralandı. İlk 10 yüksek dereceli hub düğümü ALB (Albümin), RHOA (Ras Homolog Family Member A), KRAS (KRAS Proto-Oncogene, GTPase), CREB1 (CAMP Responsive Element Binding Protein 1), GNAQ (G Protein Subunit Alpha Q), GATA3 (GATA Binding Protein 3), CXCR3 (C-X-C Motif Chemokine Receptor 3), EZR (Ezrin), SERPINA1 (Serpin Family A Member 1), GNAO1 (G Protein Subunit Alpha O1) genlerdir. Bu genler arasında ALB, RHO ve KRAS en yüksek düğüm derecesi olan genlerdir. Daha sonra, gen etkileşim ağının modüllerini taramak için Cytoscape yazılımında cytoHubba analizi ile hub genler seçildi. Şekil 2'de skoru en yüksek olan 20 gen gösterildi. Hub genler mcc skoruna göre sırasıyla ALB, KRAS, RHOA, CREB1, GNAQ, SERPINA1, EZR, CCL5, WNT1, GATA3, CR1, CFB, CD59, CD5, TIMP1, F5, CX3CL1, FGFR1, SDHC genleri olarak sıralanmıştır. Şekil 2 de proteinlerin birbirleri ile bağlantıları gösterilmiştir. Kırmızıdan sarıya skor azalmaktadır.

Şekil 2. *Ayrıntılı protein etkileşimlerini gösteren düzenleyici genlerin protein-protein etkileşimi (PPI) ağı; PPI zenginleştirme p-değeri >0.05*



Tablo 1. *İfadesi artan ve azalan genlerin GO analizi*

İfadesi artan genlerin GO analizi		
GOTERM_BP_DIRECT	Gen Sayısı	Genler
GO:0006661~phosphatidylinositol biosynthetic process	4	DGKE, MTMR3, OCRL, SYNJ2
GO:0007623~circadian rhythm	4	CREB1, NONO, BHLHE40, TYMS
GO:0046856~phosphatidylinositol dephosphorylation	3	MTMR3, OCRL, SYNJ2
GOTERM_CC_DIRECT		
GO:0005886~plasma membrane	56	RAB3B, CLIC4, DGKE, ITSN1, AQP4, SLC4A4, RGS4, GPR173, LAMP1, SLCO2B1, TGM2, CR1, DCC, PDE4D, OCRL, F5, ACLY, MMP14, FRMD8, GPRIN1, RAB35, EZR, IL6ST, CFB, SLC29A2, PTGER4, SDC3, NAIF1, SLC5A12, ACVR1B, CYTH3, CD79A, ARVCF, IRAK1, TMEM204, CXCR3, KCNMB2, PLA2G2A, LSAMP, ATP2B4, SYNJ2, LYVE1, PTPRD, GNAO1, SLC6A6, CYP2C9, NEDD1, RIT1, VNN1, KCNS2, GNAQ, TDP1, APBA2, NOX1, FGFR1, SNTB2
GO:0000785~chromatin	14	BNC2, ARNT, HMGA2, SMARCA2, KLF3, TOX4, MTBP, CREB1, HOXB9, BHLHE40, TCF3, E2F5, SPDEF, TGM2
GO:0090575~RNA polymerase II transcription factor complex	6	HOXB9, CREB1, NONO, ARNT, TCF3, E2F5
GO:0045177~apical part of cell	4	DYNC2LI1, NEDD1, CLIC4, EZR
GOTERM_MF_DIRECT		
GO:0046872~metal ion binding	29	ZNF551, DGKE, MTMR3, BNC2, SAR1B, ITSN1, PRDM15, ACVR1B, GNS, C2, PHF21B, REPIN1, TGM2, ZNF286A, PDE4D, ATP2B4, CDKAL1, KLF3, AGMAT, GNAO1, ACLY, GNAQ, OGDH, ADAM9, PDE5A, ZMYND11, NOX1, NAIP, RNF187
GO:0003924~GTPase activity	8	GNAO1, RAB3B, RGS4, RIT1, SAR1B, GNAQ, RAB35, TGM2
GO:0061629~RNA polymerase II sequence-specific DNA binding transcription factor binding	5	CREB1, BHLHE40, ARNT, HMGA2, TCF3

Tablo 1. İfadesi artan ve azalan genlerin GO analizi **DEVAMI**		
İfadesi azalan genlerin GO analizi		
GOTERM_BP_DIRECT	Gen sayısı	Genler
GO:0045893~positive regulation of transcription, DNA-templated	9	MSTN, RBPMS, MBD2, NFATC2, RFX3, GATA4, GATA3, WNT1, PHF8
GO:0045165~cell fate commitment	4	SOX13, GATA4, GATA3, WNT1
GO:0071356~cellular response to tumor necrosis factor	4	CCL5, GATA3, GBP1, CX3CL1
GO:0048568~embryonic organ development	3	MBD2, GATA3, ARID2
GO:0071347~cellular response to interleukin-1	3	CCL5, GBP1, CX3CL1
GOTERM_CC_DIRECT		
GO:0005886~plasma membrane	26	GABRB3, STAU2, ABHD6, CX3CL1, CBARP, ATXN3, CD59, WNT1, ARID2, GBP1, SPTAN1, KIR2DL5A, MAGI1, GABRA5, HIP1R, PTOV1, NPY1R, TNFRSF10A, PARVB, RHOA, SLC7A4, ACE2, CD5, KRAS, MAPT, CNGB1
GO:0030424~axon	6	STAU2, TAOK2, FEZ2, KIF1C, MAPT, RHOA
GO:0005874~microtubule	5	KLC4, STAU2, KIF1C, MAPT, TPGS2
GOTERM_MF_DIRECT		
GO:0003677~DNA binding	11	MBD2, ALB, SOX13, NFATC2, RFX3, CAMTA1, GATA4, GATA3, MAPT, ARID2, SIM1
GO:0003700~transcription factor activity, sequence-specific DNA binding	6	SOX13, NFATC2, RFX3, GATA4, GATA3, SIM1
GO:0003779~actin binding	5	HIP1R, PARVB, MAPT, SPTAN1, GBP1
GO:0008134~transcription factor binding	4	NFATC2, GATA4, TNFRSF10A, GATA3
GO:0019003~GDP binding	3	KRAS, GBP1, RHOA

Tablo 2. *İfadesi artan ve azalan genlerin KEGG yolakları*

Term	Count	Genes
İfadesi artan genlerin KEGG Yolağı		
hsa05417:Lipid and atherosclerosis	5	CCL5, NFATC2, KRAS, TNFRSF10A, RHOA
hsa04650:Natural killer cell mediated cytotoxicity	4	NFATC2, KRAS, TNFRSF10A, KIR2DL5A
hsa05163:Human cytomegalovirus infection	5	CCL5, NFATC2, KRAS, RHOA, CX3CL1
hsa05022:Pathways of neurodegeneration - multiple diseases	7	KLC4, ATXN3, KRAS, SDHC, MAPT, AMBRA1, WNT1
hsa05010:Alzheimer disease	6	KLC4, KRAS, SDHC, MAPT, AMBRA1, WNT1
hsa04150:mTOR signaling pathway	4	RPS6KA6, KRAS, WNT1, RHOA
İfadesi azalan genlerin KEEG Yolağı		
hsa04610:Complement and coagulation cascades	5	SERPINA1, CR1, CFB, F5, C2
hsa04962:Vasopressin-regulated water reabsorption	4	DYNC2LI1, DCTN5, CREB1, AQP4
hsa04928:Parathyroid hormone synthesis, secretion and action	5	MMP14, CREB1, PDE4D, GNAQ, FGFR1
hsa05130:Pathogenic Escherichia coli infection	6	CYTH3, IRAK1, NCL, ARHGEF2, EZR, NAIP

TARTIŞMA

Çocukluk çağı obezitesi, potansiyel olarak yaşamı tehdit eden sonuçları olan çok sistemli bir hastalıktır (10). Çocukluk çağı obezitesinin patogenezini aydınlatmayı amaçlayan çok sayıda genetik çalışmaya rağmen, bu hastalığın gelişimi ve ilerlemesindeki moleküler mekanizmalar belirsizliğini koruyor. Bu çalışmada, belirgin şekilde ifadesi artmış 3 gen (ALB, KRAS ve RHOA) ve ifadesi azalmış 3 gen (CREB1, GNAQ ve CXCR3) tanımlanmıştır. İfadesi artan genlerin "Lipid ve ateroskleroz (CCL5, NFATC2, KRAS, TNFRSF10A, RHOA), Doğal öldürücü (NK) hücre aracılı sitotoksisite (NFATC2, KRAS, TNFRSF10A, KIR2DL5A), mTOR sinyal yolağı (RPS6KA6, KRAS, WNT1, RHOA) yolaklarında, ifadesi azalan genlerin ise Complement ve koagulasyon kaskadı (SERPINA1, CR1, CFB, F5, C2), Parathyroid hormon sentezi (MMP14, CREB1, PDE4D, GNAQ, FGFR1) yolaklarında, düzenlenmesi dahil olmak üzere çeşitli fonksiyonların bu DEG'ler tarafından önemli ölçüde zenginleştirildiği gözlendi.

Çocukluk çağında lipit yönetimi, genetik lipit bozuklukları ve artan obezite ile ilişkili bulunmuştur (11). Çocukluk çağı obezitesi, tip 2 diyabet, hipertansiyon, ateroskleroz, koroner arter hastalığı ve yetişkinlikte bazı kanser türleri gibi yüksek metabolik ve kardiyovasküler bozukluklar riski taşır (12).

Bizimde sonuçlarımız arasında ifadesi artan genlerin lipit ve ateroskleroz yolağında toplandığı gösterilmiştir. CCL5, NFATC2, KRAS, TNFRSF10A ve RHOA genlerinin ise çalışmamızda ilk defa önemli olduğu ve obezitenin gelişmesinde rol alabilecekleri gösterilmiştir.

Obeziteye, sistemik kronik düşük dereceli inflamasyonun yanı sıra çeşitli doğuştan gelen ve adaptif bağışıklık hücrelerinin işlev bozuklukları eşlik eder. Son bulgular, obez koşullar altında doğal öldürücü (NK) hücrelerin işlevsellik ve fenotipinin bozulduğunu vurgulamaktadır. İn vitro olarak incelenen NK hücrelerinin alt kümelerinde, dağılımında, fenotipinde, sitotoksisitesinde, sitokin salgılanmasında ve sinyalleşme basamaklarında obezite ile ilişkili olduğu gösterilmiştir (13). Bizimde çalışmamızda Doğal öldürücü hücre aracılı sitotoksisite yolağında rol alan NFATC2, KRAS, TNFRSF10A ve KIR2DL5A genlerinin ifadesinin arttığı artığı bulunmuştur.

Çalışmada öne çıkan ifadesi artan genlerin toplandığı diğer bir yolak mTOR sinyal yolağıdır. Yapılan çalışmalarda mTOR sinyal yolağının aktivitesindeki bozulmalar, sayısız malignite, kardiyovasküler hastalıklar, obezite, nörodejeneratif hastalıklar ve metabolik bozukluklarla ilişkilendirilmiştir (14). Bizimde çalışmamızda RPS6KA6, KRAS, WNT1, RHOA genleri ilk defa obezite ile ilişkilendirilmiştir.

Obez ve aşırı kilolu ergenlerde D vitamini eksikliğinin yüksek olduğu bilinmektedir. Yapılan az sayıda çalışma, yüksek D vitamini eksikliğine rağmen obez ergenlerde daha düşük paratiroid hormon (PTH) düzeylerini göstermiştir (15). Serum 25 hidroksivitamin D [25(OH)D] ve PTH düzeylerinin de ters orantılı olduğu bilinmektedir (16). D vitamini eksikliği olanlarda paratiroid hormonunun PTH sentezi ve salgılanması daha yüksektir (17). Hem PTH hem de 25(OH)D, kalsiyum homeostazında önemli roller oynar. Çalışmamızda ifadesi azalan genler arasında MMP14, CREB1, PDE4D, GNAQ, FGFR1 genleri öne çıkmış ve PTH yolağı ile ilişkili bulunmuştur.

Çalışmamızda öne çıkan bir diğer yolak kompleman ve koagülasyon kaskadı yolağı olmuştur. Kompleman sistemi başlangıçta mikrobiyal istilacılara karşı destekleyici bir ilk savunma sistemi olarak görülüyordu. Fakat yapılan son çalışmalarda kompleman sisteminin aktivasyonunun obezite, insülin direnci ve diabetes mellitus dahil olmak üzere metabolik bozuklukların başlaması ve ilerlemesin rolü olduğunu göstermiştir (18). Çalışmamızda SERPINA1, CR1, CFB, F5, C2 genlerinin ifadesinin obez çocuklarda azaldığı görülmüştür.

SONUÇ

Sonuç olarak, bu çalışmanın sonuçları NFATC2, KRAS, TNFRSF10A, RHOA genlerinin çocukluk çağı obezitesi ile ilgili olabileceğini ortaya koymuştur. Mevcut çalışmanın gözlemlerinin, çocukluk çağı obezitesi için moleküler mekanizmaların anlaşılması ve aday terapötik ajanların tanımlanması için etkileri olabilir. Bununla birlikte, bu bilgilerin klinik bir ortamda kullanılması amacıyla aday genleri doğrulamak için daha fazla çalışmaya ihtiyaç vardır. Pediatrik obezitenin endişe verici eğilimi, büyümeyi ve işlevselliği etkileyen metabolik komplikasyonların erken başlangıcını engellemek için en büyük farkındalığı hak etmektedir. Şu anda, çocukluk çağı obezitesinin moleküler mekanizmaları ve ilişkili metabolik komorbiditeler hakkında bilgi sınırlıdır ve pediatrik obezite ilişkili anahtar genler ve yolaklar hakkında daha fazla çalışmaya ihtiyaç vardır

Etik: Etik kurul onayı tüm veriler anonim olduğundan bu çalışma için geçerli ve gerekli değildir..

Ethics committee approval is not applicable and not necessary for this study.

Yazar katkı durumu; Çalışmanın konsepti; AKG, SG, dizaynı; AKG, SG, Literatür taraması; AKG, SG, verilerin toplanması ve işlenmesi; AKG, SG, istatistik; AKG, SG, yazım aşaması; AKG, SG.

Author contribution status; The concept of the study; AKG, SG, design; AKG, SG, literature review; AKG, SG, collecting and processing data; AKG, SG, statistics; AKG, SG, writing phase; AKG, SG.

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Alternative Analytical Methods for Quantification of Galantamine in Pharmaceuticals

Farmasötiklerde Galantamin Miktarının Belirlenmesi için Alternatif Analitik Yöntemler

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ÖZET

AMAÇ: Galantaminin yığın halinde ve farmasötik dozaj formunda miktar tayini için, yeni seçici, hızlı, kesin ve doğru spektrofotometrik ve kromatografik prosedürler geliştirildi ve doğrulandı.

GEREÇ VE YÖNTEM: Kromatografik ayırma, bir Agilent Extend C18 (250x4.6 mm, 5 um) kolonu üzerinde gerçekleştirilmiştir. %0.1 TFA içeren ultra saf su ve %0,1 TFA içeren asetonitril (85/15, v/v) 1,0 mL/dk akış hızında mobil faz olarak kullanıldı. Eluent tespiti, bir UV detektörü kullanılarak 288 nm dalga boyunda gerçekleştirilmiştir. Galantamin ise 288 nm dalga boyunda çözeltilerin absorbansı ölçülerek spektrofotometrik teknikle belirlendi.

BULGULAR: Galantamin için Lambert-Beer grafikleri, 5-30 µg/mL konsantrasyon aralığında doğrusal ilişkiler gösterdi. Her iki teknik de Uluslararası Uyumlaştırma Konferansı (ICH) standartlarını karşılamak için istatistiksel olarak değerlendirilmiş ve doğrulanmıştır ve sonuçlar spektrofotometrik ve sıvı kromatografik yöntemlerin doğrusal, hassas, doğru, sağlam ve RSD değerlerinin %1'den düşük olduğunu gösterdi ve geri kazanım yüzdesi standart sınırlar içindeydi (%98-102). Daha sonra bu analitik yöntemlerin istatistiksel bir karşılaştırması yapıldı. Her iki yöntemin sonuçları da %95 güven aralığında ($p<0.05$) birbirlerine göre farklılık göstermedi ve istatistiksel olarak anlamlı değildi.

SONUÇ: Geliştirilen analitik yöntemlerin doğru, oldukça etkili, güvenilir, hızlı, basit olduğu belirlendi ve farmasötik dozaj formunun yanı sıra yığın halindeki galantaminin rutin analizi için başarıyla kullanılabilir. Kalite kontrol analizi ve temizlik doğrulaması sırasında numune analizi için bile kullanılabilirler.

Anahtar Kelimeler: galantamin, HPLC, UV, yöntem, validasyon

ABSTRACT

OBJECTIVE: For the quantitafication of galantamine in bulk and pharmaceutical dosage form, new selective, quick, precise, and accurate spectrophotometric and chromatographic procedures were developed and validated.

MATERIALS AND METHODS: Chromatographic separation was performed on an Agilent Extend C18 (250×4.6 mm, 5 µm) column. Ultrapure water containing 0.1% TFA and acetonitrile containing 0.1% TFA (85/15, v/v) were used as mobile phase at a flow rate of 1.0 mL/min. Eluent detection was performed at a wavelength of 288 nm using a UV detector. On the other hand, galantamine was determined by spectrophotometric technique by measuring the absorbance of the solutions at a wavelength of 288 nm.

RESULTS: Lambert–Beer plots for galantamine showed linear relationships in the concentration range of 5-30 µg/mL. Both techniques have been statistically evaluated and validated in order to meet the standards of the International Conference on Harmonisation (ICH) and the results showed that spectrophotometric and liquid chromatographic methods were linear, precise, accurate, rugged and robust with RSD values less than 1.00%, and the recovery percentage was within standard limits (98-102%). Then a statistical comparison of these analytical methods was performed. The results of both methods showed no difference and not statistically significant with respect to each other in the 95% confidence interval ($p<0.05$).

CONCLUSION: The developed analytical methods were determined to be accurate, highly effective, reliable, fast, simple, and may be employed successfully for routine analysis of galantamine in the bulk as well as pharmaceutical dosage form. They may even be used for quality control analysis and for sample analysis during cleaning validation.

Keywords: galantamine, HPLC, UV, method, validation

INTRODUCTION

Alzheimer's disease now affects more than 42 million individuals worldwide (1). According to certain recent publications, it is seen in approximately 15% of women over the age of 60 worldwide. In addition, it ranks third among the causes of death due to diseases worldwide, after heart disease and cancer.

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Galantamine is an allosteric nicotinic receptor modulator and reversible acetylcholinesterase inhibitor that reduces cognitive and functional deterioration in mild to severe Alzheimer's disease dementia. Although galantamine cannot completely treat this condition, it can help to slow its course. Furthermore, throughout the early to mid-stages of the illness, it can improve symptoms by inhibiting reversible and competitive acetylcholinesterase. It affects just those parts of the brain with low acetylcholine levels (2).

Several analytical techniques for galantamine amount analysis have been reported using techniques such as spectrophotometry (3, 4) capillary zone electrophoresis (5), micellar electrokinetic chromatography (6, 7), high-performance liquid chromatography UV (8, 9), high-performance liquid chromatography photodiode-array radiometric detection (10, 11), ion-exchange chromatography (12, 13), high-performance liquid chromatography tandem mass spectrometry (14-16), and chiral liquid chromatography method (17). The monograph of galantamine hydrobromide is available in both the European (18) and the United States Pharmacopoeia (19). A validated stability studies including structure elucidation and bioactivity assessment of degradation products have been reported for galantamine hydrobromide (20, 21). CE-Electrospray ionization application has been reported for the impurity profile of Galantamine hydrobromide (22).

Analysis procedures require expensive equipment, organic solvents, and significant volumes of specialized reagents. Some of these techniques are somewhat complicated. The analysis process takes a lengthy time.

In our study covers the development and validation of new spectrophotometric and liquid chromatographic methods for galantamine assay, including the determination and identification of galantamine in pharmaceutical products. These analytical methods are precise, accurate, linear, selective, and robust. Therefore, these newly developed and validated methods would be suitable for quality control laboratories for the analysis of galantamine in pharmaceutical products. The analysis of variance (ANOVA) was used to compare the results of these analytical methods. In addition, their reliability was assessed by concentrating on routine quality control analyses.

MATERIAL & METHODS

Reagents

Galantamine Hydrobromide United States Pharmacopeia (USP) reference standard trifluoroacetic acid (≥99.0percentage), methanol (≥99.9percentage), and acetonitrile (≥99.9percentage) was purchased from Sigma-Aldrich Chemie GmbH (St Louis, MO, USA). Reminyl (Cologno, Monzese, Italy) extended-release capsule (8 mg) was bought from the local pharmacy. Ultrapure water was produced using a Merck Millipore water purification system (Bedford, MA, USA).

Analytical instruments and conditions

HPLC analyzes were performed with an Agilent 1260 system (Palo Alto, CA, USA) consisting of a quaternary pump, autosampler, UV detector, and ChemStation software. An Agilent Extend-C18 (4.6 mm × 250 mm, 5.0 µm) column was used, and maintained at 25 ◦C. Chromatographic detection of eluents was performed at 288 nm using a UV detector. The mobile phase consisted of ultrapure water containing 0.1% TFA and acetonitrile (85/15, v/v) containing 0.1% TFA at a flow rate of 1.0 mL/min. The injection volume was 20µl.

Spectrophotometric analyses were performed on a Shimadzu UV 1800 double beam (Shimadzu, Japan) spectrophotometer, with UV-Probe software and 1 cm quartz cuvette. The standard solutions were scanned in the range of 200-800 nm on the UV spectrophotometer to determine the value of λmax and the measurements were obtained against methanol as a blank. The wavelength of 288 nm was selected for the quantitation of galantamine. The absorbance values of the standard solution series at the wavelength of 288 nm were recorded and it was shown that the absorbance values were proportional to the concentration of standard solutions.

Preparation of sample and standard solutions

Galantamine standard solutions: Galantamine hydrobromide reference standard equivalent to 25 mg of galantamine and 20 ml of methanol into a 50 ml volumetric flask and the total volume was completed to 50 ml with methanol. Thus, a stock standard solution was prepared at a concentration of 500 µg/mL. By diluting the standard stock solution with ultrapure water, six standard solutions were prepared in the concentration range of 5-30 µg/mL.

Galantamine sample solutions: 10 capsule contents were emptied and weighed and ground into fine powder by grinding in a mortar. The powder equivalent to 25 mg of galantamine was weighed accurately, taken into a 50 mL of

volumetric flask. 20 mL of methanol was added to dissolve the galantamine, the volume was diluted to 50 mL with methanol when dissolution was complete. Finally, methanol was added up to the marked line, and shaked homogenously for 15 minutes. This supernatant was filtered through membrane filter (0.45 µm pore size). To a 50 mL volumetric flask, 2 mL of transferred this solution and was added methanol to obtain 20 µg/mL of galantamine solution.

Validation

The optimized chromatographic and spectrophotometric methods are fully validated according to the procedures specified in ICH guidelines Q2(R1) for the validation of analytical methods (23, 24). Validation parameters such as sensitivity, linearity, accuracy, robustness, precision, specificity, system suitability tests, stability studies have been investigated.

Linearity: Stock standard solutions containing 500 µg/mL of galantamine in methanol were prepared, in triplicate. Stock standard solutions were diluted with methanol to six different concentrations containing 5, 10, 15, 20, 25 and 30 µg/mL galantamine. Calibration curves were plotted concentration versus peak area for the chromatographic method, and concentration versus absorbance value for the spectrophotometric method. The acquired data were analyzed using the least squares method.

Precision: The intraday precision was assessed by analyzing capsule samples (n = 6) at the test concentration (20 µg mL-1) using spectrophotometric and high-performance liquids chromatographic methods. Similarly, the precision between days was assessed three days in a row (n = 18). Galantamine contents were determined and the relative standard deviations were calculated.

Accuracy: The accuracy of the methods was evaluated at three levels (80, 100, and 120% of the test concentration) with recovery studies. This was done by analyzing a sample of known concentration and comparing the measured value with the "real" value. A well-characterized sample solution (20 µg/mL of galantamine) was used. Samples were prepared in triplicate for each concentration, analyzed by spectrophotometric and high-performance liquids chromatographic methods, and recovery percentages were calculated.

Specificity: A sample solution (20 µg/mL of galantamine) was prepared using the sample preparation procedure and injected into the chromatographic system to assess the possibility of interfering peaks. For spectrophotometric analysis, the UV spectrum of this sample solution was recorded in the wavelength range of 200-400 nm to assess the existence of potentially interfering bands at 288 nm. Furthermore, the UV spectra recorded by a UV detector were used to assess the spectral purity of galantamine peaks in chromatograms produced with sample solutions.

Detection and quantitation limits: Limit of detection (LOD) and limit of quantification (LOQ) were used to evaluate the sensitivity of the chromatographic and spectrophotometric methods. They were calculated separately depending on standard deviation of the slope and intercept of the calibration curve by using the equations (1) and (2), respectively.

$$LOD = 3.3x\sigma/S \quad (1) \qquad || \qquad LOQ = 10x\sigma/S \quad (2)$$

S: *calibration curve slope and σ: standard deviation of y intercept*

Analysis of marketed formulations

Samples of galantamine raw material and Reminyl® capsules were analyzed by the validated chromatographic and spectrophotometric methods. Before analyzing, the capsule contents were weighed and powdered. The sample solutions for the chromatographic and spectrophotometric analyses were prepared as explained in Section 2.3. Galantamine contents were analyzed by both methods. One Way ANOVA Tukey test was used for statistical results and 0.05 was considered significant.

Comparative analysis

For commercial formulations, recovery percentages were compared with the F test and t test using both analytical methods.

Stability of solutions

Over a 24-hour period, the reference standard solutions were evaluated for stability. During the stability research, standard solutions were kept at room temperature (25 °C) and shielded from light.

RESULTS

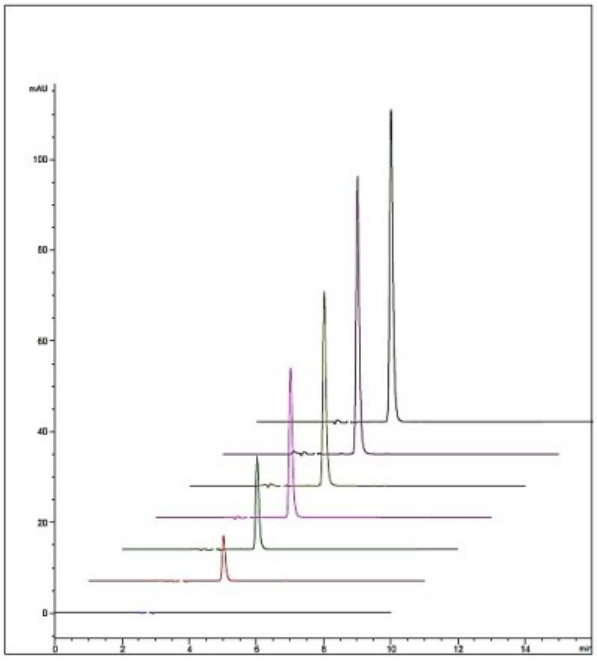
Method development

The tests of mobile phases were carried out with organic solvents, ultrapure water and different pH range at different flow rates (0.8–1.5 mL min-1). The best

chromatographic conditions were achieved using an isocratic mobile phase comprising ultra-pure water containing 0.1% TFA (pH=1.5)-acetonitrile containing 0.1% TFA (85/15, v/v) at a flow rate of 1.0 µg/mL on an Agilent Extend-C18 (4.6 mm × 250 mm, 5.0 µm) that was kept at 25°C.

Eluent was measured using a UV detector at a wavelength of 288 nm. In these chromatographic conditions, the retention time for galantamine was determined to be 4.04 minutes as demonstrated in the chromatogram of Fig. 1.

Figure 1: *Overlay chromatogram obtained for galantamine standard solutions (5-30 µg/mL)*



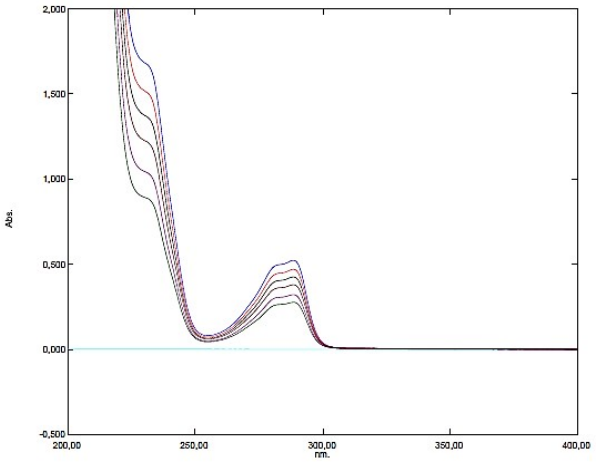
The UV spectrum of in the range of 200-400 nm was evaluated (Fig. 2). Galantmaine has shown sufficient molar absorptivity at a wavelength of 288 nm. The wavelength of 288 nm showed higher selectivity with respect to possible interfering compounds in the samples. For all these reasons, a wavelength of 288 nm was chosen for detection.

Analytical validation

A linear relationship was determined between galantamine concentrations and the responses of both UV and HPLC methods. Regression data analysis are given in Table 1. For both methods, high regression coefficient (r2) values were obtained (>0.999). There was no significant deviation in linearity in the analysis range.

The precision data obtained from the analytical methods are presented in Table 2. R.S.D.% values were less than 2.0 for both analytical methods. This provides good sensitivity in both methods.

Figure 2: *Overlay spectrum of galantamine standard solutions (5-30 µg/mL)*



Accuracy was investigated by means of the standard addition method. The results of the recovery test are presented in Table 2. Both analytical methods exhibited average recoveries close to 100% and showed sufficient accuracy.

Table 1: *The linearity data obtained for galantamine by both methods*

Regression parameters	HPLC	UV
Number of points	6	6
Concentration range (µg mL^{-1})	5-30	5-30
Relative standard error (%)	1.2155	0.0026
Slope ± standard error	14.4340±0.1300	0.0098±0.0003
Intercept ± standard error	-3.2667±0.080	0.2279±0.0040
Regression coefficient (r^2)	0.9999	0.9993

Table 2: *Validation parameters of the developed methods*

Parameters	HPLC	UV
Intra-day precision, (R.S.D. %, *n* = 6)	0.34	0.45
Inter-day precision, (R.S.D. %, *n* = 18)	0.56	0.63
Accuracy, (mean recovery, %, *n* = 9)	99.78	99.25
LOD (µg mL^{-1}) / LOQ (µg mL^{-1})	0.70/2.10	2.10/6.50

Analysis of marketed formulations

The validated chromatographic and spectrophotometric methods were applied to the analysis of galantamine in raw material and Reminyl tablets (Table 3). The ANOVA test revealed that there was no statistically significant difference at a confidence level of 0.05 between the results obtained for the raw material and tablet samples from the different methods. Tukey's multiple comparison test showed that the means obtained by HPLC and UV for raw material analysis

were statistically equivalent ($p < 0.05$). Tukey test for analysis of tablets revealed statistical equivalence ($p < 0.05$) between HPLC and UV averages.

Table 3: *Galantamine contents in raw material and capsule sample (n=6)*

Samples	Galantamine content (%) ± SD	
	HPLC	UV
Raw material	99.82±0.32	99.57±0.46
Capsule (8 mg)	8.02±0.03	7.98±0.05
F-test F calculation/Ftable	0.32/0.54	
t-test t calculation/ttable	1.45/2.78	

SD: Standard Deviation

Statistical comparison of methods

Both methods were statistically compared using the F-test and the t-test. Statistical analyzes have shown that there is no significant difference between the values obtained from the analyzes performed by both methods.

F-value and t-value were calculated and these values were found to be lower than the table values of both methods at the 95% confidence interval. It is clear that both of the proposed methods are applicable for the quantification of galantamine in dosage forms. Table 3 shows the statistical comparison results of both methods.

Stability of standard solutions

The stability of the reference standard solutions over a 24-hour period was examined. Standard solutions were injected into the HPLC system for 8 hours and peak area and the retention time were recorded (Table 4).

Table 4. *Standard solution stability (n=3, 20 µg mL^{-1})*

Time period hours	Peak area	Average Peak area	SD	RSD (%)	Retention time min.	Average Retention time min.	SD	RSD (%)
0	286.5	286.5	0.2	0.053	4.006	4.010	0.005	0.118
	286.3				4.008			
	286.6				4.015			
24	286.3	286.1	0.3	0.092	4.016	4.014	0.005	0.118
	285.8				4.009			
	286.2				4.018			
48	286.4	286.3	0.3	0.107	4.016	4.014	0.006	0.146
	286.6				4.007			
	286.0				4.018			

R.S.D percentages at the end of the 48-hour period were determined as 0.146% for the retention time and 0.107% for the peak area. No significant change was observed in the active metabolite concentration in the standard solution.

DISCUSSION

Within the scope of the chromatographic method development studies for the quantification of galantamine, several preliminary studies have been carried out to optimize the conditions. Various chromatographic methods have been performed to optimize galantamine quantification. In the study, firstly, only ultrapure water was tested and long analysis times were obtained. Optimal conditions were provided by investigating different acetonitrile ratios. Containing 0.1% TFA ultrapure water and acetonitrile (85/15, v/v) were used as the mobile phase to obtain a good galantamine peak symmetry and shape. Finally, this mobile phase provides more powerful theoretical plates (>7.000) and a peak queuing factor (<1.0). Analyzes at 25C° were not only economical, but also had advantages in terms of low column pressure, column efficiency and good chromatographic peak shape (3-6, 8).

To ensure results, tablet samples were analyzed for 60 minutes and no matrix components remained in the column much longer in the specified conditions. However, continuing the analysis for more than 10 minutes will increase the analysis time and cost. No overlapping peaks were observed in the samples injected into the system for analysis for consecutive 10-minute periods. Therefore, the analysis time was determined as 10 minutes.

In the studies of assessing the specificity of the HPLC method, peak purities of more than 99.0% for galantamine were obtained on chromatograms of sample solutions. This showed that other compounds did not elute together with the main peak. No interfering peaks were observed in the retention time of galantamine in the chromatogram obtained with a mixture of tablet excipients (20-22).

No absorption bands were detected at 288 nm in the spectrum obtained with a mixture of tablet excipients in methanol for the UV method. Therefore, the method was shown to be selective for quantifying galantamine at this wavelength.

The LOD and LOQ values for the chromatographic method were calculated as 0.70 and 2.10 µg mL-1, respectively. In spectrophotometric analysis, the absorbance value of galantamine standard solution at a concentration of 0.70 µg mL-1 at a wavelength of 288 nm was measured as 0.2348. Therefore, this concentration is set as the detection limit. The absorbance value of galantamine standard solution at a concentration of 2.10 µg mL-1 at a wavelength of 288 nm was measured as 0.2485. Therefore, this concentration is set as the quantitation limit.

The validation parameters of both analytical methods are presented in Table 2. According to the results obtained, it is proved that the HPLC method is a more sensitive method that allows determining the amount of galantamine at concentrations about four times lower than the UV method.

Potential interactions in raw material studies were not identified in any of the evaluated methods, despite the fact that spectrophotometric analysis assessed degradation products or related chemicals with comparable chemical structures. It was observed that the chromatographic method is the most sensitive and selective method. This method can be successfully applied for the determination of the amount of galantamine. However, the time and expense of analysis cannot be overlooked. The spectrophotometric approach is clearly more cost-effective and needs less analytical time while also being simple to apply. Because galantamine is such a widely used anticholinesterase drug, it is critical to develop and validate simple and reliable procedures to verify the quality of raw materials and pharmaceutical formulations on the market today.

CONCLUSION

The UV spectrophotometric method has advantages over the LC chromatographic method because the UV spectrophotometric method generally does not require detailed processes and procedures as in the LC chromatographic method. UV spectrophotometric method is more economical and consumes less time than LC chromatographic method. However, statistical comparison of both methods shows that the LC chromatographic method is more precise and accurate than the UV spectrophotometric method. HPLC and UV spectrophotometric methods have shown that they are both adequate methods to determine the amount of galantamine in raw materials, tablets and injectable solutions, and the most reliable results have been obtained. No interfering peaks were observed during the retention time of galantamine in the chromatographic method and no interfering absorption bands were observed at 288 nm in the spectrophotometric method. Because HPLC and UV are simple and rapid methods, they can be successfully applied to quality control analysis to quantify and determine the amount of galantamine in marketed formulations. Our study is complementary to the lack of information in the literature to make a comparative evaluation of HPLC and UV spectrophotometric methods in galantamine.

Etik: Bu çalışmada etik kurul onayı gerekmez.

No ethical approval required.

Yazar katkı durumu; Çalışmanın konsepti; NT, İB, dizaynı; NT, İB, Literatür taraması; NT, İB, verilerin toplanması ve işlenmesi; NT, İB, istatistik; NT, İB, yazım aşaması; NT, İB,

Author contribution status; The concept of the study; NT, İB, design; NT, İB, literature review; NT, İB, collecting and processing data; NT, İB, statistics; NT, İB, writing phase; NT, İB,

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Olgu Sunumu / Case Report
Aegean J Med Sci 2022;2:65-67

Successful transcatheter arterial embolization due to massive hemoptysis in a metastatic lung cancer patient with tumoral blood supply from the inferior phrenic artery: A case report

Masif hemoptizi nedeniyle başvuran tümöral besleyici arteri inferior frenik arter olan metastatik akciğer kanserli bir hastada başarılı transkateter arteriyel embolizasyon: Bir olgu sunumu

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ÖZET

İnferior frenik arter kanaması ve buna sekonder masif hemoptizi komplikasyonuyla ilişkili vakalar nadiren bildirilmiştir. 17 yaşında erkek hasta hemoptizi, halsizlik, bilinç bulanıklığı şikayetleri ile hastanemize başvurdu. Hastanın sinoviyal sarkom nedeniyle ameliyat öyküsü ve sağ akciğerde metastatik kitlesi vardı. Yapılan BT Anjiyografi incelemesinde metastatik akciğer kitlesini besleyen inferior frenik arterde hemoraji saptandı. Trans-arteriyel embolizasyon (TAE) ile başarılı bir şekilde tedavi edilen ve embolizasyon sonrası şikayetleri kaybolan frenik arter hasarının neden olduğu nadir bir hemoptizi vakasını sunuyoruz.

Anahtar Kelimeler: inferior frenik arter, transkateter arteriyel embolizasyon, akciğer metastazı, masif hemoptizi

ABSTRACT

Cases of inferior phrenic artery hemorrhage and those complicated by massive hemoptysis have been rarely reported. A 17-year-old man presented to our hospital with a chief complaint of hemoptysis weakness and confusion. The patient had history of surgery for synovial sarcoma. Additionally the patient has metastatic right lung mass. CT Angiography examination revealed hemorrhage in the inferior phrenic artery feeding the metastatic lung mass. We present a rare case of right lower phrenic artery rupture, which was successfully treated using TAE and the patient had no complaints after embolization.

Keywords: inferior phrenic artery, transcatheter arterial embolization, lung metastases, massive hemoptysis

INTRODUCTION

Massive hemoptysis may result from various causes, and the frequency with which these causes occur differs greatly between the Western and the non-Western world. The source of massive hemoptysis is usually the bronchial circulation (90% of cases) rather than the pulmonary circulation . In a minority of cases (5%), massive hemoptysis may originate with the aorta (e.g, aortobronchial fistula, ruptured aortic aneurysm) or the systemic arterial supply to the lungs (1). Hemorrhage due to the inferior phrenic artery is a highly rare situation and may cause massive hemothorax and hemoptysis. To the author's knowledge, there are very few case in the literature that bleeding ınferior phrenic arteries (2-7). This case report describes an hemorrhage to the idiopathic right inferior phrenic artery, which was complicated by massive hemothorax and hemoptysis, and treated with transcatheter arterial embolization (TAE).

CASE

A 17-year-old man admitted to our hospital with a chief complaint of hemoptysis, weakness and confusion. On physical examination, the patient was tachypnea and respiratory sounds were decreased in the right lung. The patient had no history of trauma or smoking. In the examinations performed during the patient's admission to the emergency department, the hemoglobin value was 7.4 (gm/dL), the platelet value was 267,000 (mcL), the hematocrit value was 21.8, the creatinine value was 1.69 mg/dL , the ALT value was 158 (U/L), the AST value was 130 (U/L) and the CRP value was 301.5 (gm/L). The patient was operated on 26 November 2019 for a dedifferentiated synovial sarcoma mass located in the left paravertebral at the thoracolumbar level. The patient had a solitary metastatic mass in the lower lobe of the right lung diagnosed at the earlier of 2022. This metastatic mass was detected in the lower lobe of the right lung in positron emission tomography (PET CT) performed in a different

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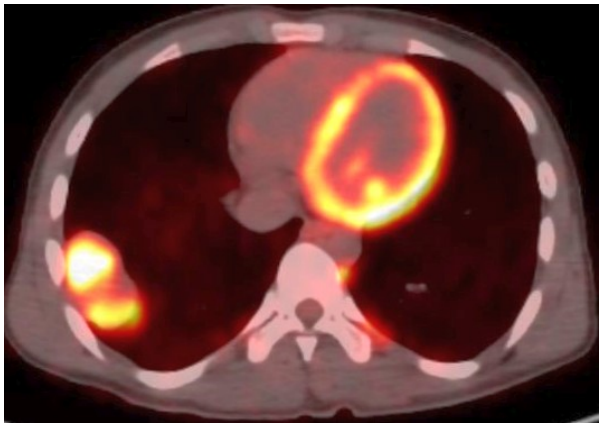
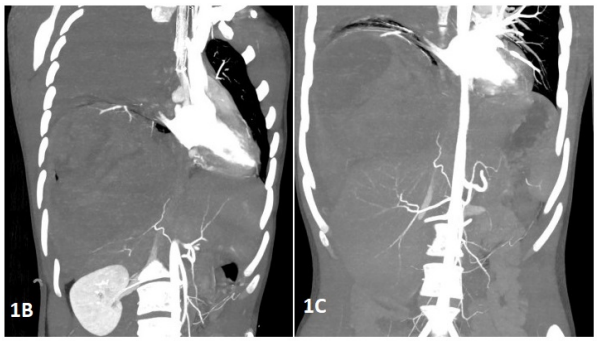
center (Fig 1A). There were no metastatic lesions in either lungs or other systems.

Figure 1A: *PET CT image showing a mass with prominent f-18 fluorodeoxyglucose uptake in the lower lobe of the right lung.*



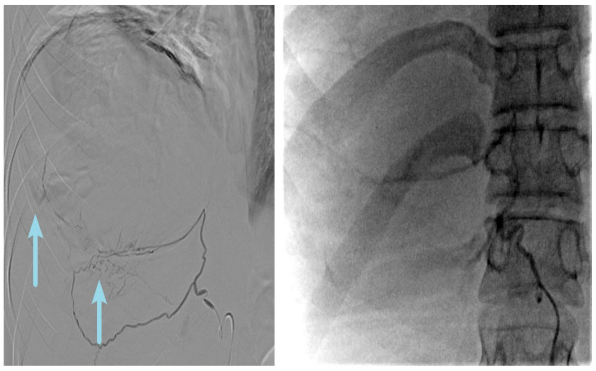
In the pulmonary CT Angiography examination of the patient, active extravasation lower lobe of the right lung and intraparenchymal hematoma in the right hemithorax were observed. In this examination, it was thought that the metastatic mass in the lower lobe of the right lung was bleeding and that its feeder was the right inferior phrenic artery. (Fig 1B and 1C).

Figure 1B and 1C: *CT Angiography image showing massive hemothorax, active extravasation originating inferior phrenic arterial system and tumoral blood supply from the inferior phrenic artery.*



The patient was transferred to the interventional radiology unit for embolization procedure. During the procedure, the patient was intubated together with the anesthesia team. After local asepsis and anesthesia, the right main femoral artery was punctured with a Seldinger needle under US guidance, and a 5F vascular sheath was advanced over a guiding wire. A celiac angiogram was obtained with a cobra catheter. The right phrenic artery was then catheterized. In the angiogram taken, filling and extravasation were detected in the tumoral area. Thereupon, the artery was super selectively catheterized with the microsystem. Embolization was done with Bead Block 300/500 microsphere. Then, since no extravasation was detected, the embolization process was terminated (Fig 1D and 1E). After this process with local asepsis and anesthesia, the right main femoral vein was punctured with a Seldinger needle under US guidance and a 5F vein sheath was advanced. The pulmonary artery was catheterized. Angiograms were obtained from the main pulmonary and right pulmonary artery inferior segments. No active extravasation was detected. Compression was applied by removing the vessel sheaths in the groin. The process took about 4 hours. There were no complications during the procedure. After the procedure, the patient was followed up in the intensive care unit for approximately 1 week. One week after the procedure, the patient underwent right lung lower lobectomy and partial diaphragm repair. Since the operation was performed after the post-embolization procedure, there was no major hemorrhage during the operation. The pathology report of the right lung lower lobectomy material was reported as synovial sarcoma metastasis. A few weeks after the operation, the patient recovered completely and was discharged.

Figure 1D and 1E: *Angiography images showing extravasation in the tumoral area and loss of extraluminal filling after post-embolectomy*



DISCUSSION

In this case report , we present a rare case in which successful transcatheter artery embolization due to massive hemoptysis in a metastatic lung cancer patient with tumoral blood supply from the inferior phrenic artery. The patient with massive hemoptysis had no complaints after embolization.

İnferior phrenic artery hemorrhage feeding the metastatic mass has not been reported, although there are case reports in the literature that caused massive hemoptysis due to blunt trauma, fistula, aneurysm, pseudoaneurysm, and opening of abscess cavitation to the arterial system (2-7). In a series of only 11 cases, the role of the inferior phrenic artery in the interventional treatment of metastatic lung tumor was mentioned (8).

Non-bronchial systemic arteries can be a significant source of massive hemoptysis, especially in patients with pleural involvement caused by an underlying disease. Missing the non-bronchial systemic arteries at initial angiography may result in early recurrent bleeding after successful embolization of the bronchial artery. It should be kept in mind that non-bronchial arterial structures such as intercostal branches of the axillary and subclavian arteries, internal mammarian artery and phrenic artery may cause massive hemoptysis, as in this case.

CONCLUSION

We reported a case in which due to massive hemoptysis in a metastatic lung cancer patient with tumoral blood supply from the inferior phrenic artery was treated using transcatheter arterial embolization and in this case, we considered that Non-bronchial systemic arteries can be a significant source of massive hemoptysis was important.

Etik; Bu yazıda sunulan olgu için sunulan bilgilerin akademik amaçlı kullanımı hakkında detaylı bilgileri de içeren imzalı "Bilgilendirilmiş onam formu" alınmıştır.

Ethics; For the case presented in this article, a signed "informed consent form" was obtained, which includes detailed information about the use of the information presented for academic purposes.

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