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Ege Tıp Bilimleri Dergisi

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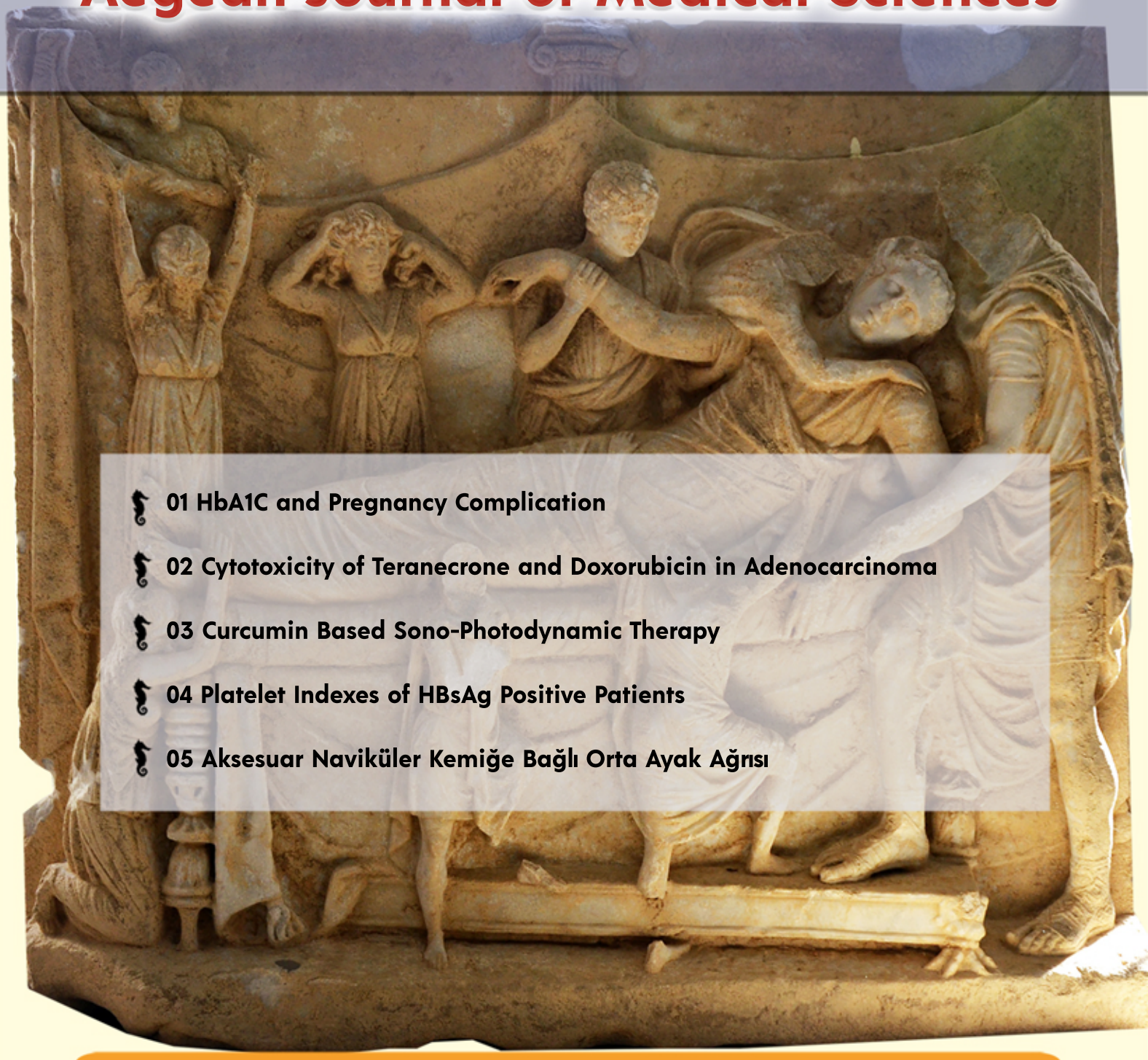

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Derginin Yazı Dili

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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, etik ve bilimsel standartlara uygun makaleleri yayımlar. Makalelerin etik kurallara uygunluğu yazarların sorumluluğundadır. Tüm prospektif çalışmalar için, çalışmanın yapıldığı kurumdan Etik Kurul onayı alınmalı ve yazının içinde belirtilmelidir. Olgu sunumlarında; etik ve yasal kurallar gereği, hastanın mahremiyetinin korunmasına özen gösterilmelidir. Hastaların kimliğini tanımlayıcı bilgiler ve fotoğraflar, hastanın (ya da yasal vasisinin) yazılı bilgilendirilmiş onamı olmadan basılamadığından, "Hastadan (ya da yasal vasisinden) tıbbi verilerinin yayınlanabileceğine ilişkin yazılı onam belgesi alındı" cümlesi, makale metninde yer almalıdır.

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.

İnternet Makalesi

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

Web Sitesi

Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 July 2002]. Available from: www.cancer-pain.org

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;3:68 -78

The Relationship Between Glycosylated Hemoglobin A1c (Hba1c) Levels and Pregnancy Complications in Diabetic Pregnant Women; Retrospective Case-control Study

Diabetik gebelerde glikolize hemoglobin A1c (HbA1c) düzeyi ile gebelik komplikasyonları arasındaki ilişki-Retrospektif vaka-kontrol çalışması

İsa Kaplan[1] Selda Demircan Sezer[2] Mert Küçük[3]

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ÖZET

AMAÇ: Diabetes mellitus (DM) tanılı gebelerde glikolize hemoglobin A1C (HbA1c) düzeyleri ile gebelikte meydana gelebilecek olan komplikasyonlar arasında ilişki olup olmadığını saptamak.

GEREÇ VE YÖNTEM: Çalışmamız retrospektif vaka-kontrol çalışmasıdır. Ocak 2013 ve Aralık 2017 tarihleri arasında Aydın Adnan Menderes Üniversitesi hastanesinde yapılmıştır. Çalışmamız 321 hasta üzerinde yapılmıştır. Hastalarda HbA1C düzeyine göre komplikasyon oranlarına bakılmıştır.

BULGULAR: HbA1c değerleri 66 gebede %4-5,9, 157 gebede %6-7.9 ve 91 gebede HbA1c≥%8'dir. Pregestasyonel DM tanılı gebelerde gestasyonel diabetes mellitus (GDM) tanılı gebelere kıyasla preeklampsi, fetal distres, preterm doğum, omuz distosisi ve yenidoğan hipoglisemisi daha sık saptanmıştır (p<0.05). Tip 1 DM tanılı gebelerde Tip 2 DM tanılı gebelere kıyasla erken doğum tehdidi (EDT), polihidroamnios, hiperbilirübinemi, yenidoğan hipoglisemisi ve yenidoğan kilo kaybı daha sık saptanmıştır (p<0.05). GDMA2 tanılı gebelerde GDMA1 tanılı gebelere kıyasla gestasyonel hipertansiyon (GHT), preeklampsi, EDT ve large for gestational age (LGA) daha sık saptanmıştır (p<0.05). GDMA1 tanılı gebelerde oligohidroamnios daha sık saptanmıştır (p<0.05). HbA1c≥%8 ve HbA1c %6-7,9 olan gebelerde HbA1c değeri normal olan gebelere kıyasla GHT, preeklampsi, EDT, oligohidroamnios, polihidroamnios, LGA, fetal distres, preterm doğum, yenidoğan hipoglisemisi, yenidoğan kilo kaybı, yenidoğan solunum sıkıntısı, respiratuar distres sendromu (RDS), hiperbilirübinemi ve intrauterin ex fetüs daha sık saptanmıştır (p<0.05). GDM tanılı HbA1c>%6 ve HbA1c %5-5,9 olan gebelerde HbA1c %4-4,9 arasında olan gebelere kıyasla GHT, preeklampsi, EDT, polihidroamnios, small for gestational age (SGA), LGA, fetal distres, preterm doğum, yenidoğan hipoglisemisi ve yenidoğan solunum sıkıntısı daha sık saptanmıştır (p<0.05).

SONUÇ: Pregestasyonel DM, Tip 1 DM, GDMA2 ve yüksek HbA1c değeri olan gebelerde obstetrik komplikasyon oranlarında artma olduğu saptanmıştır.

Anahtar Kelimeler: glikolize hemoglobin a1c (hba1c), gebelik komplikasyonu, gestasyonel diabetes mellitus, diyabette gebelik

ABSTRACT

OBJECTIVE: To determine whether there is a relationship between glycosylated hemoglobin A1C (HbA1c) levels and complications that may occur during pregnancy in pregnant women with diabetes mellitus (DM).

MATERIALS AND METHODS: This study was a retrospective case-control study. It was conducted in Aydın Adnan Menderes University hospital between January 2013-December 2017 and performed on 321 patients. Complication rates were evaluated according to the HbA1C level of the patients.

RESULTS: HbA1c values were pregnant women's 4-5.9% in 66, 6-7.9% in 157 HbA1c≥8% in 91. Compared to gestational diabetes mellitus (GDM), preeclampsia, fetal distress, preterm delivery, shoulder dystocia, and neonatal hypoglycemia were found more frequently in pregnant women with pregestational DM (p<0.05). Compared to type 2 DM, threatened premature birth (TPL), polyhydramnios, hyperbilirubinemia, neonatal hypoglycemia, and neonatal weight loss were found more frequently in pregnant women with type 1 DM (p<0.05). Compared to normal HbA1c values in pregnant women, GHT, preeclampsia, TPL, oligohydramnios, polyhydramnios, LGA, fetal distress, preterm birth, neonatal hypoglycemia, neonatal weight loss, neonatal respiratory distress, respiratory distress syndrome (RDS), hyperbilirubinemia and ex fetus were found more frequently in pregnant women with HbA1c≥8% and HbA1c between 6-7.9% (p<0.05). In pregnants with GDM diagnosis with HbA1c>6% and HbA1c between 5-5.9%, GHT, preeclampsia, TPL, polyhydramnios, small for gestational age (SGA), LGA, fetal distress, preterm birth, neonatal hypoglycemia, and neonatal respiratory distress were found more frequently compared to pregnants with HbA1c between 4-4.9%.

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CONCLUSION: Obstetric complication rates increased in pregnant women with pregestational DM, Type 1 DM, GDMA2, and high HbA1c values.

Keywords: glycosylated hemoglobin a1c (hba1c), pregnancy complication, gestational diabetes mellitus, pregnancy in diabetes

INTRODUCTION

Diabetes mellitus (DM) poses a significant problem in pregnancy. Gestational diabetes mellitus (GDM) constitutes approximately 90% of diabetes seen in pregnancy and pregestational DM 10%. Pregestational DM, on the other hand, is divided into Type 1 DM and Type 2 DM. In addition, GDM is divided into two, GDM A1, which requires diet and exercise therapy, and GDM A2, which requires insulin in treatment, according to the need for insulin use (1,2). Glycated hemoglobin A1c (HbA1c) is a crucial parameter for clinicians in the diagnosis and follow-up of DM. The total blood hemoglobin (Hgb) of a normal adult individual consists of 97% hemoglobin A (HbAo), approximately 2.5% HbA2, and approximately 0.5% HbF. Hemoglobin, like many other proteins in the body, undergoes nonenzymatic glycosylation (3). The terms glycosylated hemoglobin (G-Hgb) or HbA1c are used to define Hgb with added glucose as a result of nonenzymatic glycosylation. (3,4). About 4-6% of Hgb in a healthy adult is in the form of HbA1c (4). HbA1c provides information about 8-12 weeks of glycemic control retrospectively. It is an important parameter used in both diagnosis and follow-up of DM. A diagnosis of DM is made if the HbA1c value is 6.5% or more in two different measurements. An HbA1c value of 8% or above indicates that blood sugar regulation is not good in DM. (4,5). Today, it is accepted that HbA1c not only gives information about glycemic control but also indicates the risk of developing complications related to DM and the quality of diabetic care. Thus, HbA1c is very important in obstetric practice, which will give an idea about possible maternal and fetal complications in patients diagnosed with DM during pregnancy (6). If HbA1c values tend to increase in the 3rd trimester during pregnancy, there is an increase in the risk of preeclampsia, macrosomia, and inutero ex fetus (IUEF). Hence, HbA1c is very important to predict possible complications in pregnancy. It is recommended in different publications that the HbA1c value should be below normal before pregnancy to prevent obstetric complications (7,8). For this reason, studies on HbA1c and possible pregnancy complications are vital. The benefits of HbA1c in predicting possible fetal/neonatal and obstetric complications were investigated in our study to contribute to the existing literature.

In our study, we aimed to determine whether there was a relationship between HbA1c levels, which show at least 60 days of retrospective blood sugar regulation, and obstetric complications that might occur during pregnancy in patients diagnosed with pregestational DM and patients diagnosed with GDM.

MATERIAL & METHODS

Research Place and Time

This research was a retrospective case-control study and conducted at Aydın Adnan Menderes University Application and Research Hospital between January 2013 and December 2017.

Population and Sample of the Research

This study was designed on 357 pregnant women who were diagnosed with pregestational DM and GDM and came to their follow-ups. However, 36 patients whose records could not be reached because they did not come to their follow-ups and gave birth in our hospital were not included in the present study. The population of this research consisted of 321 patients who were followed up in Aydın Adnan Menderes University Application and Research Hospital and gave birth. Women over the age of 18 who were diagnosed with DM or GDM before and/or during pregnancy, whose HbA1c values were checked, and who had a singleton pregnancy and gave birth in our hospital were included in the study. Patients under the age of 18, with multiple pregnancies and whose HbA1c values were not checked, were excluded from this study. The HbA1c values of the patients were measured at the end of the 2nd trimester (24-27 weeks of gestation) and the beginning of the 3rd trimester (28-30 weeks of gestation).

Study Design

This study was designed as a single-center, retrospective, multidisciplinary and controlled study. The ages of the patients included in this study, the history of their previous pregnancies, the total number of pregnancies, the number of live births, the number of stillbirths, the types of births, their CVs, drug use during pregnancy, and the total weight gained during pregnancy were recorded with the data collection form.

Weight and height measurement records were obtained from the obstetric follow-up form for each patient with the data collection form. Body mass index (BMI) was calculated as weight (kg)/height2 (m2). The gestational weeks of the patients were calculated by the Negele method according to the first day of the last menstrual period (LMP). In patients who do not know the LMP date and are in doubt, the gestational week was calculated according to the Crown-rump length (CRL) measurements in the first-trimester ultrasonography (USG).

A two-step method was used for the diagnosis of GDM. GDM screening was performed between 24-28 weeks of gestation in patients with no obvious risk of DM. Firstly, a 50 g glucose screening test (OGTT), which does not require fasting, was performed on the patients. Those with 50 gr OGTT 1st hour result of 180 mg/dl and above were accepted as GDM. On the other hand, 100 g or 75 g OGTT tests were performed on patients whose 50 g OGTT 1st hour result was between 140-180 mg/dl. Patients with at least 2 values higher in 100 g OGTT and a single value increase in 75 g OGTT were considered GDM. Patients who were diagnosed with GDM in their previous pregnancy and who had risk factors for overt DM were examined for DM at the beginning of pregnancy.

Patients with a diagnosis of pregestational DM included in this study were in the treatment and follow-up of endocrinology and metabolism diseases. These patients were using insulin and oral antidiabetic. Patients with GDM, on the other hand, receive diet/exercise and insulin therapy.

Premature birth was defined as birth occurring before 37 weeks of gestation. Threatened premature birth (TPL) was defined as pain, bleeding and active amniotic fluid discharge that would require hospitalization between 20-37 weeks of gestation. Intrauterine growth retardation (IUGR) is defined as a fetal weight below the 10th percentile of expected percentile values according to the week of gestation. Small for Gestational Age (SGA) is defined as a fetal weight less than 2500 grams in term deliveries. Large for Gestational Age (LGA) is defined as fetal development above the 90th percentile by the week of gestation.

Gestational hypertension (GHT) is defined as the detection of blood pressure above 140/90 mmHg at least twice at 6-hour intervals after the 20th week of pregnancy and its return to normal within 12 weeks postpartum. Preeclampsia, on the other hand, has been defined as a progressive multisystemic syndrome that progresses with the addition of organ dysfunctions without proteinuria or proteinuria.

Oligohydramnios was defined as the amniotic fluid index (AFI) value less than 5 cm. Polyhydramnios was defined as an AFI value of 25 cm or more. Intrahepatic cholestasis was defined as increased blood bile acid in the second half of pregnancy, abnormal liver function tests, and widespread itching throughout the body.

Premature rupture of membranes (PROM) was defined as active amniotic fluid discharge before the onset of labor. Placenta previa was defined as the closure of the internal cervical os by the placenta. Placenta accreta was defined as an invasion of the myometrium by chorionic villi.

In addition, fetal distress, shoulder fixation at birth, nerve and bone damage, prenatal examination findings, and neonatal problems were investigated.

The HbA1c values of the patients were measured at the end of the 2nd trimester (between 24-27 weeks of gestation) and the beginning of the 3rd trimester (between 28-30 weeks of gestation).

HbA1c measurements of the patients were made with the Adams HA-8160 (Arkray KDK, Shiga, Japan) HPLC device in the biochemistry laboratory of our hospital. In this method, data are calculated from the peak areas of different hemoglobin fractions as %HbA1c, %HbA1-%HbF and reported as a percentage (%) of total hemoglobin.

In our study, complication rates among pregnant women with pregestational DM-GDM, complication rates among pregnant women with Type 1 DM-Type 2 DM, complication rates between GDMA1-GDMA2 in pregnant women with GDM, and complication rates according to HbA1c levels of pregnants were examined.

Patients were divided into groups based on an 8% HbA1c level, which indicates poor glycemic regulation. Pregnant women with DM were divided into three different groups according to their HbA1c levels 4-5.9%, 6-7.9%, and 8% and above.

In the GDM group, based on the normal range of HbA1c of 4-6%, the patients were divided into three different groups according to their HbA1c levels as being between 4-4.9%, between 5-5.9%, and above 6%.

Ethical Approval

The research protocol was approved by Adnan Menderes University Faculty of Medicine Ethics Committee with the date 07.12.2017, number 53043469-050.04.04 and decision number 2017/1271. The present study was conducted according to the Declaration of Helsinki and informed consent was obtained from the patients.

Statistical Analysis

Categorical variables in our study were expressed as Numbers (N) and Percentages (%). With the Kolmogorov-Smirnov test of normality, it was concluded that the data had a normal distribution. The chi-square test was used to determine the relationship between DM groups and categorical variables. A confidence interval of 95% was determined in the calculations, and P-values of <0.05 were considered statistically significant. SPSS (IBM SPSS for Windows, Ver.24) statistical package program was used for the calculations.

RESULTS

The age of the patients included in this study ranged from 18 to 48 years. We did not have any patients under the age of 18. Given the age distribution of the patients, there were 53 patients (16.5%) between the ages of 18-25, 177 patients (55.1%) between the ages of 25-35, and 91 (28.3%) patients over the age of 35.

When the BMIs of the patients were examined, we did not have any patients with a BMI below 18.5. There were 32 patients (10%) with a BMI of 18.5-24.9, 11 patients (35.2%) with a BMI of 25-29.9, 127 patients (39.6%) with a BMI of 30-39.9, and 49 patients (15.3%) with a BMI of 40 and above.

Seventy three (22.7%) of the patients included in this study had a diagnosis of pregestational DM. 36 (49.3%) of the pregnant women with a diagnosis of pregestational DM had Type 1 DM and 37 (50.7%) had Type 2 DM. Two hundred forty-eight pregnant women (77.3%) were diagnosed with GDM. 31 (42.5%) of the pregnant women diagnosed with pregestational DM had DM between 1-5 years, 30 (41.1%) had 5-10 years, and 12 (16.4%) had DM for 10 years or more.

Of the pregnant women diagnosed with GDM, 126 (50.8%) received diet and exercise therapy. One hundred twenty-two pregnant women (49.2%) diagnosed with GDM received insulin therapy. When the blood sugar follow-ups of the pregnant women were examined, it was seen that the blood sugars of 153 patients (47.7%) were regulated, and the blood sugars of 168 patients (52.3%) were unregulated. When the HbA1c values of the patients are examined, the HbA1c value was between 4-5.9% in 66 (21%) patients, between 6-7.9% in 157 (50%) patients, and 8% and above in 91 (29%) patients.

GHT in 137 patients (42.7%), preeclampsia in 131 patients (40.8%), intrahepatic cholestasis in eight patients (2.5%), placenta accreta in two patients (0.6%), placenta previa in three patients (0.9%), TPL in 80 patients (24.9%), PROM in 17 patients (5.3%), oligohydramnios in 33 patients (10.3%), polyhydramnios in 46 patients (14.3%), IUGR in 42 patients (13.1%), SGA in 10 patients (3.1%), LGA in 55 patients (17.1%) and fetal distress in 102 patients (31.8%) was detected.

28 (8.7%) of the patients were delivered vaginally, and 293 (91.3%) were delivered by cesarean section. Ninety patients (28%) had preterm delivery (<37 weeks), 230 patients (71.7%) had term delivery (38-41 weeks), and one patient (0.3%) had post-term delivery (>42 weeks). Shoulder dystocia was detected in 10 (3.1%) patients at birth. Brachial plexus injury developed in one (0.3%) patient. Obstetrics complications are shown in Table 1.

Table 1. *The incidence of obstetric complications*

	n	%
Preeclampsia	131	40.8
GHT	137	42.7
İntrahepatic Cholestasis	8	2.5
Placenta Accreta	2	0.6
Placenta Previa	3	0.9
TPL	80	24.9
PROM	17	5.3
Oligohydramnios	33	10.3
Polyhydramnios	46	14.3
IUGR	42	13.1
SGA	10	3.1
LGA	55	17.1
Fetal Distress	102	31.8
Preterm Labor	90	28
Post-term Pregnancy	1	0.3
Shoulder Dystocia	10	3.1
Brachial Plexus İnjury	1	0.3
Cesarean Birth	293	91.3
Vaginal Birth	28	8.7

GHT: Gestational Hypertension, TPL: Threatened Preterm Labor, IUGR: Intrauterine Fetal Growth Retardation, SGA: Small For Gestational Age, LGA: Large For Gestational Age, PROM: Premature Rupture of Membranes, n: Number, %: Percent

The development of preeclampsia was 1.39 times, fetal distress 1.49 times, preterm delivery 1.6 times, shoulder dystocia 3.4 times, and neonatal hypoglycemia 1.4 times

more common in pregnant women with pregestational DM compared to pregnant women with GDM. The incidence of existing complications was statistically significant in the pregestational DM group (95% CI, p<0.05). Complication rates between pregestational DM and GDM are given in Table 2.

Table 2. *Complication rates between GDM and Pregestational DM*

	GDM	PDM	P
	n (%)	n (%)	
Preeclampsia	93 (37.5)	38 (52.1)	**0.026***
GHT	104 (41.9)	33 (45.2)	0.620
İntrahepatic Cholestasis	6 (2.4)	2 (2.7)	0.877
Placenta Accreta	2 (0.8)	0 (0)	0.441
Placenta Previa	2 (0.8)	1 (1.4)	0.660
TPL	56 (22.6)	24 (32.9)	0.074
PROM	12 (4.8)	5 (6.8)	0.500
Oligohydramnios	28 (11.3)	5 (6.8)	0.272
Polyhydramnios	32 (12.9)	14 (19.2)	0.179
IUGR	34 (13.7)	8 (11)	0.540
SGA	7 (2.8)	3 (4.1)	0.578
LGA	42 (16.9)	13 (17.8)	0.862
Fetal Distress	71 (28.6)	31 (42.5)	**0.026***
Preterm Birth	61 (24.6)	29 (39.7)	**0.037***
Shoulder Dystocia	5 (2)	5 (6.8)	**0.037***
Brachial Plexus İnjury	1 (0.4)	0 (0)	0.587
Neonatal Hypoglycemia	68 (27.4)	29 (39.7)	**0.044***
Newborn Weight Loss	23 (9.3)	11 (15.1)	0.157
Newborn Respiratory Distress	37 (14.9)	16 (21.9)	0.157
RDS	13 (5.2)	8 (11)	0.083
Hyperbilirubinemia	9 (3.6)	5 (6.8)	0.236

GDM: Gestational Diabetes Mellitus, PDM: Pregestational diabetes Mellitus, DM: Diabetes Mellitus, GHT: Gestational Hypertension, TPL: Threatened Preterm Labor, PROM: Premature Rupture of Membranes, IUGR: Intrauterine Fetal Growth Retardation, SGA: Small For Gestational Age, LGA: Large For Gestational Age, RDS: Respiratory Distress Syndrome, n: Number, %: Percent

**Chi-Square Test, 95% confidence interval was determined in the calculations and P values <0.05 were considered statistically significant.*

TPL was found 2.05 times, polyhydramnios 3.77 times, neonatal hypoglycemia 2.28 times, newborn weight loss 4.62 times, and hyperbilirubinemia five times more common in pregnant women with Type 1 DM compared to pregnant women with Type 2 DM. The incidence of existing complications was statistically significant in the Type1 DM group (95% CI, p<0.05). Complication rates between Type 1 DM and Type 2 DM are given in Table 3.

GHT was found 1.72 times, preeclampsia 1.56 times, TPL 1.85 times, and LGA 2.07 times more in the group using insulin therapy (GDMA2) compared to the group given diet/exercise treatment (GDMA1) in pregnant women with GDM. The incidence of existing complications was statistically significant in the GDMA2 group (95% CI, p<0.05).

Table 3. *Complication rates between Type 1 DM and Type 2 DM*

	Type 1 DM	Type 2 DM	P
	n (%)	n (%)	
Preeclampsia	22 (61.1)	16 (43.2)	0.127
GHT	20 (55.6)	13 (35.1)	0.080
İntrahepatic Cholestasis	2 (5.6)	0 (0)	0.146
Placenta Accreta	0 (0)	0 (0)	-
Placenta Previa	0 (0)	1 (2.7)	0.321
TPL	16 (44.4)	8 (21.6)	**0.038***
PROM	3 (8.3)	2 (5.4)	0.620
Oligohydramnios	4 (11.1)	1 (2.7)	0.155
Polyhydramnios	11 (30.6)	3 (8.1)	**0.015** *
IUGR	5 (13.9)	3 (8.1)	0.429
SGA	1 (2.8)	2 (5.4)	0.572
LGA	9 (25)	4 (10.8)	0.113
Fetal Distress	18 (50)	13 (35.1)	0.119
Preterm Birth	17 (47.2)	12 (32.4)	0.197
shoulder dystocia	4 (11.1)	1 (2.7)	0.155
Brachial Plexus İnjury	0 (0)	0 (0)	-
Neonatal Hypoglycemia	20 (55.6)	9 (24.3)	**0.006***
Newborn Weight Loss	9 (25)	2 (5.4)	**0.019***
Newborn Respiratory Distress	11 (30.6)	5 (13.5)	0.078
RDS	4 (11.1)	4 (10.8)	0.967
Hyperbilirubinemia	5 (13.9)	0 (0)	**0.019***

DM: Diabetes Mellitus, GHT: Gestational Hypertension, TPL: Threatened Preterm Labor, PROM: Premature Rupture of Membranes, IUGR: Intrauterine Fetal Growth Retardation, SGA: Small For Gestational Age, LGA: Large For Gestational Age, RDS: Respiratory Distress Syndrome, n: Number, %: Percent

** Chi-Square Test, 95% confidence interval was determined in the calculations and P values <0.05 were considered statistically significant.*

Oligohydramnios was found 2.4 times more frequently in pregnant women with a diagnosis of GDMA1 compared to pregnant women with a diagnosis of GDMA2. The detection of oligohydramnios in pregnant women with GDMA1 was statistically significant (95% CI, p<0.05). Complication rates between GDMA1 and GDMA2 in patients with GDM according to the treatment method are given in Table 4.

GHT was found 1.8 times, preeclampsia 2.1 times, TPL 3 times, polyhydramnios 2.4 times, LGA 2 times, fetal distress 2.17 times, preterm labor 2.67 times, neonatal hypoglycemia 1.73 times, newborn weight loss 2.09 times, neonatal respiratory distress 2.18 times, respiratory distress syndrome (RDS) 3.62 times and, hyperbilirubinemia 4.82 times more in pregnant women with HbA1c value between 6-7.9% compared to pregnant women with HbA1c value between 4-5.9%. The incidence of existing complications was statistically significant in the patient group with HbA1c values between 6-7.9% (95% CI, p<0.05).

GHT was found 1.84 times, preeclampsia 2.53 times, TPL 4.76 times, oligohydramnios 2.65 times, polyhydramnios 2.65 times, LGA 2.8 times, fetal distress 2.72 times, preterm labor 3.81 times, neonatal hypoglycemia 1.81 times, neonatal weight loss 2.98 times, neonatal respiratory

distress 1.8 times, RDS 1.12 times and hyperbilirubinemia 7.55 times in pregnant women with HbA1c value of 8 and above compared to pregnants with an HbA1c value of 4-5.9%. The incidence of existing complications was statistically significant in the patient group with an HbA1c value of 8% and above (95% CI, p<0.05).

Table 4. *Complication Rates in GDM Patients by Treatment Type*

	Diet/Exercise (GDMA1)	İnsulin (GDMA2)	P
	n (%)	n (%)	
GHT	39(31%)	65(53.3%)	**0.001***
Preeclampsia	37(29.4%)	56(45.9%)	**0.007***
İntrahepatic Cholestasis	3 (2.4%)	3 (2.5%)	0.968
Plasenta Accreta	0(%0)	2(1.6%)	0.149
Plasenta Previa	2(1.6%)	0(%0)	0.162
TPL	20(15.9%)	36(29.5%)	**0.010***
PROM	4 (3.2%)	8 (6.6%)	0.215
Oligohydramnios	20(15.9%)	8 (6.6%)	**0.020***
Polyhydramnios	13(10.3%)	19(15.6%)	0.217
IUGR	21(16.7%)	13(10.7%)	0.169
SGA	3 (2.4%)	4(3.3%)	0.670
LGA	14(11.1%)	28(23%)	**0.013***
Fetal Distress	31(24.6%)	40(32.8%)	0.154
Preterm Birth	29(23%)	32(26.2%)	0.490
Shoulder Dystocia	1 (0.8%)	4(3.3%)	0.164
Brachial Plexus İnjury	0(%0)	1 (0.8%)	0.309
Neonatal Hypoglycemia	31(24.6%)	37(30.3%)	0.312
Newborn Weight Loss	11(8.7%)	12(9.8%)	0.764
Newborn Respiratory Distress	19(15.1%)	18(14.8%)	0.943
RDS	4 (3.2%)	9(7.4%)	0.138
Hyperbilirubinemia	4 (3.2%)	5(4.1%)	0.697

GDM: Gestational Diabetes Mellitus, HbA1c: Glycosylated HemoglobinA1c, GHT: Gestational Hypertension, TPL: Threatened Preterm Labor, PROM: Premature Rupture of Membranes, IUGR: Intrauterine Fetal Growth Retardation, SGA: Small For Gestational Age, LGA Age: Large For Gestational, RDS Respiratory Distress Syndrome, n: Number, %: Percent

** Chi-Square Test, 95% confidence interval was determined in the calculations and P values <0.05 were considered statistically significant.*

IUEF was found 24.46 times more frequently in the patient group with an HbA1c value of 8% and above. A statistically significant correlation was found between the occurrence of IUEF and high HbA1c (95% CI, p<0.05). Complication rates according to HbA1c are given in Table 5.

GHT was found 2.95 times, preeclampsia 4.31 times, TPL 14 times, polyhydramnios 2.01 times, LGA 1.65 times, fetal distress 2.17 times, preterm birth 1.94 times, neonatal hypoglycemia 2.35 times, and neonatal respiratory distress 1.79 times in the patient group with HbA1c value between 5-5.9% in pregnant women diagnosed with GDM, compared to the patient group with an HbA1c value between 4-4.9%. The incidence of existing complications was statistically significant in the patient group with HbA1c values between 5-5.9% (95% CI, p<0.05).

Table 5. *Complication rates by HbA1c*

HbA1c	Between %4-5.9 n (%)	Between %6-7.9 n (%)	%8 and Above n (%)	P
GHT	77 (34.5)	44 (63.8)	14 (63.6)	**0.001***
Preeclampsia	68 (30.5)	44 (63.8)	17 (77.3)	**0.001***
İntrahepatic Cholestasis	4 (1.8)	3 (4.3)	1 (4.5)	0.414
Placenta Accreta	1 (0.4)	1 (1.4)	0 (0)	0.611
Placenta Previa	3 (1.3)	0 (0)	0 (0)	0.539
TPL	32 (14.3)	37 (47.8)	15 (68.2)	**0.001***
PROM	11 (4.9)	5 (7.2)	1 (4.5)	0.746
Oligohydramnios	23 (10.3)	4 (5.8)	6 (27.3)	**0.016***
Polyhydramnios	23 (10.3)	17 (24.6)	6 (27.3)	**0.003***
IUGR	29 (13)	12 (17.4)	1 (4.5)	0.291
SGA	6 (2.7)	3 (4.3)	1 (4.5)	0.737
LGA	23 (13)	18 (26.1)	8 (36.4)	**0.002***
Fetal Distress	52 (23.3)	35 (50.7)	14 (63.6)	**0.001***
Preterm Birth	40 (17.9)	33 (47.8)	15 (68.2)	**0.001***
Shoulder Dystocia	4 (1.8)	5 (7.2)	1 (4.5)	0.073
Brachial Plexus İnjury	0 (0)	1 (1.4)	0 (0)	0.168
Neonatal Hypoglycemia	56 (25.1)	30 (43.5)	10 (45.5)	**0.004***
Newborn Weight Loss	17 (7.6)	11 (15.9)	5 (22.7)	**0.022***
Newborn Respiratory Distress	28 (12.6)	19 (27.5)	5 (22.7)	**0.010***
RDS	9 (4)	10 (14.5)	1 (4.5)	**0.007***
Hyperbilirubinemia	4 (1.8)	6 (8.7)	3 (13.6)	**0.003***
Ex Fetüs	3 (1.3)	1 (1.5)	7 (31.8)	**0.001***

HbA1c: Glycosylated HemoglobinA1c, GHT: Gestational Hypertension, TPL: Threatened Preterm Labor, PROM: Premature Rupture of Membranes, IUGR: Intrauterine Fetal Growth Retardation, SGA: Small For Gestational Age, LGA: Large For Gestational Age, RDS: Respiratory Distress Syndrome, n: Number, %: Percent

** Chi-Square Test, 95% confidence interval was determined in the calculations and P values <0.05 were considered statistically significant.*

GHT was found 4.05 times, preeclampsia 7.02 times, TPL 34.7 times, polyhydramnios 3.87 times, SGA 1.06 times, LGA 3.5 times, fetal distress 4.37 times, preterm birth 4.6 times, neonatal hypoglycemia 3.32 times, and neonatal respiratory distress 3.37 times İn the patient group with an HbA1c value of 6% and above in pregnant women diagnosed with GDM, compared to the patient group with an HbA1c value between 4-4.9%. The incidence of existing complications was statistically significant in the group of patients with an HbA1c value of 6% and above (95% CI, p<0.05). Complication rates according to HbA1c in patients with GDM are given in Table 6.

Table 6. *Complication rates according to HbA1c in GDM Patients*

HbA1c	**Between %4-4.9**	**Between %5-5.9**	**%6 and Above**	
	n (%)	**n (%)**	**n (%)**	**P**
GHT	10 (15.9)	63 (47)	29 (64.4)	**0.001***
Preeclampsia	6 (9.5)	55 (41)	30 (66.7)	**0.001***
Intrahepatic Cholestasis	3 (4.8)	1 (0.7)	2 (4.4)	0.154
Placenta Accreta	0 (0)	1 (0.7)	1 (2.2)	0.448
Placenta Previa	1 (1.6)	1 (0.7)	0 (0)	0.660
TPL	1 (1.6)	30 (22.4)	25 (55.6)	**0.001***
PROM	1 (1.6)	9 (6.7)	2 (4.4)	0.298
Oligohydramnios	6 (9.5)	16 (11.9)	6 (13.3)	0.814
Polyhydramnios	4 (6.3)	17 (12.7)	11 (24.4)	**0.023***
IUGR	6 (9.5)	20 (14.9)	8 (17.8)	0.434
SGA	4 (6.3)	0 (0)	3 (6.7)	**0.011***
LGA	6 (9.5)	21 (15.7)	15 (33.3)	**0.004***
Fetal Distress	8 (12.7)	37 (27.6)	25 (55.6)	**0.001***
Preterm Birth	7 (11.1)	29 (21.6)	23(51.1)	**0.001***
Shoulder Dystocia	1 (1.6)	1 (0.7)	3 (6.7)	0.052
Brachial Plexus Injury	0 (0)	0 (0)	1 (2.2)	0.111
Neonatal Hypoglycemia	8 (12.7)	40 (29.9)	19 (42.2)	**0.002***
Newborn Weight Loss	3 (4.8)	14 (10.4)	5 (11.1)	0.377
Newborn Respiratory Distress	5 (7.9)	19 (14.2)	12 (26.7)	**0.025***
RDS	2 (3.2)	6 (4.5)	4 (8.9)	0.374
Hyperbilirubinemia	2 (3.2)	2 (1.5)	4 (8.9)	0.056
Ex Fetus	1 (1.6)	2 (1.5)	3 (6.8)	0.350

GDM: Gestational Diabetes Mellitus, HbA1c: Glycosylated HemoglobinA1c, GHT: Gestational Hypertension, TPL: Threatened Preterm Labor, PROM: Premature Rupture of Membranes, IUGR: Intrauterine Fetal Growth Retardation, SGA: Small For Gestational Age, LGA Age: Large For Gestational, RDS Respiratory Distress Syndrome, n: Number, %: Percent

** Chi-Square Test, 95% confidence interval was determined in the calculations and P values <0.05 were considered statistically significant.*

GHT was found 4.05 times, preeclampsia 7.02 times, TPL 34.7 times, polyhydramnios 3.87 times, SGA 1.06 times, LGA 3.5 times, fetal distress 4.37 times, preterm birth 4.6 times, neonatal hypoglycemia 3.32 times, and neonatal respiratory distress 3.37 times in the patient group with an HbA1c value of 6% and above in pregnant women diagnosed with GDM, compared to the patient group with an HbA1c value between 4-4.9%. The incidence of existing complications was statistically significant in the group of patients with an HbA1c value of 6% and above (95% CI, $p<0.05$). Complication rates according to HbA1c in patients with GDM are given in Table 6.

DISCUSSION

Tight glycemic control is essential to minimize maternal and fetal morbidity and mortality in pregnancies complicated with diabetes. HbA1c is a useful parameter in the metabolic control of DM, in addition to home blood glucose measurement, which may not always reflect the true average blood glucose level. High HbA1c levels are associated with increased obstetric complications during pregnancy (9).

In our study, the relationship between the HbA1c value measured between 24-30 weeks of pregnancy and obstetric complications was investigated. This study aimed to identify patients who might be at risk according to HbA1c, predict and manage complications early. In the normal population, an average of 90% of pregnant women with DM is GDM and 10% are pregestational DM (10).

Emma L.J. et al. found the rate of GDM at 28.8% in their study on 466 women in Australia (11). Our study was conducted on 321 pregnant women and we have a 77.3% GDM and 22.7% pregestational DM rate. In our study, the rate of preeclampsia was 52.1% in patients with pregestational DM, and this rate was 61.1% in the Type 1 DM patient group and 43.2% in the Type 2 DM patient group.

Murphy HR et al. found the rate of preeclampsia to be 7.8% in patients with Type 1 DM and 5.2% in patients with Type 2 DM in a study they conducted in 2011 in patients with Type 1 DM and Type 2 DM (12). In our study, the rate of preeclampsia was 37.5% in patients with GDM. In a study conducted by Bodmer-Roy et al. on patients with GDM in Canada between 2008 and 2010, the rate of preeclampsia was 6.5%. Again, in the study conducted by Bodmer-Roy et al., the rate of preeclampsia was 2.7% in patients in the control group (13). In the HAPO study, the rate of development of preeclampsia was 4.8% in pregnant women diagnosed with GDM (2). The reason for the high rate of preeclampsia in our study is the fact that we are a tertiary center.

GHT and preeclampsia were found in 63.8% of pregnant women whose HbA1c values were between 6-7.9%. 63.6% GHT and 77.3% preeclampsia were found in pregnant women with an HbA1c value of 8% and above. In the pregnant women diagnosed with GDM, on the other hand, 47% of GHT and 41% of preeclampsia were found in the patient group whose HbA1c value was between 5 and 5.9%.

In the patient group with an HbA1c value of 6% and above in pregnant women with GDM, 64.4% of GHT and 66.7% of preeclampsia were detected. Sibai reported that preeclampsia rates varying between 9-66% were found in a study conducted in England on patients with pregestational DM. It was reported that this rate increased as the severity of DM increased according to White's classification, and the highest rate was seen in women with pregestational DM and nephropathy. Again, Sibai reported that as the severity of DM increases in women with Type 1 DM, the rates of preeclampsia and adverse neonatal outcomes increase (14).

Holmes et al. investigated the relationship between glycemic control, preeclampsia, and GHT in women with Type 1 DM in their study in 2011. This is a prospective study conducted on 749 pregnant women with Type 1 DM. They found preeclampsia in 17% of the patients and GHT in 11%. They found that women who developed preeclampsia had significantly higher HbA1c values before and during pregnancy compared to women who did not develop preeclampsia ($P<0.05$). Patients with HbA1c≥8.0% in early pregnancy were associated with a significantly increased risk of preeclampsia (Odds ratio 3.68 [95% CI 1.17-11.6]) compared with patients with optimal control (15).

Lapolla et al. conducted a 33-centered study in Italy between 1999-2003. They found the rates of TPL, GHT, and preeclampsia to be significantly higher in patients with HbA1c value of 8% and above compared to the control group. In addition, they found the rates of stillbirth and neonatal death in pregnant women with DM higher than in the normal population (16). Yin B. et al. found the rate of preeclampsia to be 1.7% in a recent study conducted on 8585 women between 2018 and 2019. Again, in this study, it was reported that there was an increased risk of preeclampsia in patients with an HbA1c value between 5.5-5.9% (17). Temple R. et al. conducted a study in which they examined the relationship between monthly HbA1c level, pre-pregnancy care, parity, duration of DM, microvascular complications, and maternal age to determine the risk of preeclampsia in 290 pregnant women with Type 1 DM. In this study, it was determined that week 24th-week HbA1c levels of patients who developed preeclampsia increased significantly compared to patients who did not develop preeclampsia (18).

Odsæter IH. et al. found a significant relationship between first-trimester HbA1c values and the development of preeclampsia (19). Maresh MJ. et al. found an increased risk of preeclampsia at HbA1c values of 6.0-6.4% (42-47 mmol/mol) during pregnancy (20). In our study, a significant relationship was found between high HbA1c levels and the development of GHT and preeclampsia. Current literature information supports our study.

In our study, polyhydramnios was found at a rate of 30.6% in pregnant women with Type 1 DM and 8.1% in pregnant women with Type 2 DM. Polyhydramnios was found at a rate of 24.6% in the patient group with an HbA1c value between 6-7.9% and at 27.3% in the patient group with an HbA1c value of 8% and above. Polyhydramnios was found at a rate of 12.7% in the patient group with an HbA1c value between 5-5.9% in pregnant women diagnosed with GDM and 24.4% in the patient group with an HbA1c value of 6 and above. Idris N. et al. found the incidence of polyhydramnios as 18.8% in a study on polyhydramnios in pregnant women with pregestational DM between 1996 and 2006 at the Maternal-Fetal Medicine Department of Mater Mothers Hospital. The HbA1c values of patients with polyhydramnios were significantly higher, and it was found in patients with poor glycemic control (21). Deniz K. et al., who investigated the relationship between HbA1c and the development of polyhydramnios, found the incidence of polyhydramnios as 2.9%. In this study, they stated that HbA1c was a positive independent predictor for AFI and that AFI value at 32-34 weeks of gestation was associated with mid-pregnancy HbA1c level (22).

In our study, no relationship was found between PROM and DM. The study by Sun B. et al. found the rate of PROM development to be higher in patients with GDM and IGT compared to the normal population (23).

In our study, the incidence of preterm labor was higher in patients with high HbA1c values compared to patients with normal HbA1c values. Murphy HR et al. reported the rate of preterm birth as 37.1% in pregnant women with Type 1 DM and 17.5% in pregnant women with Type 2 DM (12). Ho Yi-Ran et al. conducted a prospective study on 1989 pregnant women. As a result of this study, they reported that high HbA1c levels (7% and above) lead to an increase in the risk of GHT, preeclampsia, premature birth, increased need for neonatal intensive care, low birth weight, and macrosomia (24).

In a study conducted by Barbry F et al. on 4383 women between 2011 and 2018, they found high preterm birth rates in pregnant women with HbA1c>5.9% (25). In our study, a higher rate of preterm delivery was found in pregestational DM patients, which is consistent with the literature.

In our study, SGA was found at a rate of 6.7% in the group with an HbA1c value of 6% and above in pregnant women diagnosed with GDM. Pedersen et al. reported that 10 pregnant women with HbA1c of 8.9% and above had fetuses smaller than normal (26).

Fetal distress was found at a rate of 50.7% in the patient group with an HbA1c value between 6-7.9% and in 63.6% in the patient group with an HbA1c value of 8% or more. Fetal distress was found at a rate of 27.6% in the patient group with an HbA1c value between 5 and 5.9% in pregnant women with GDM and 55.6% in the patient group with an HbA1c value of 6% and above. In a study conducted by Teramo K. et al. on 145 pregnant women, they found the 3rd-trimester average HbA1c values of pregnant women with DM who had fetal heart rate (FHR) abnormalities to be 7.63±0.87%. This value was significantly higher than pregnant women with DM who were included in the study and had normal FHR records (P<0.02) (27). In our study, fetal distress was detected in pregnant women with high HbA1c levels, which is consistent with the literature.

LGA was found at a rate of 26.1% in the patient group with an HbA1c value between 6-7.9% and in 36.4% in the patient group with an HbA1c value of 8% or more. In the pregnant women diagnosed with GDM, 15.7% of the patients with HbA1c values between 5 and 5.9%, and 33.3% of the patients with HbA1c values of 6% and above were found to have LGA. Murphy HR et al. found the LGA rate as 52.9% in pregnant women with Type 1 DM and 37.6% in pregnant women with Type 2 DM (12). The study conducted by Emma L. J. et al. showed that there is an increase in LGA risk in pregnant women with HbA1c≥5.6% (≥38 mmol/mol) at early gestational weeks (11). Lemaitre M. et al. retrospectively analyzed the birth records of 678 pregnant women at Lille Hospital between 1997 and 2019. In this study, the mean HbA1c of the patients before pregnancy was 7.2% (55 mmol/mol). Consistent with Lemaitre M. et al.'s findings, in the present study, LGA was found in 361 (56%), SGA in 29 (4.5%), and preterm delivery (76.1%) in 504 patients. In this study by Lemaitre M. et al., it was stated that high HbA1c was associated with maternal and fetal complications (28).

Cordero L et al. conducted a study on neonatal problems in 530 pregnant women with GDM and pregestational DM. Considering the results of 530 newborns in this study, 76 (14%) were born before 34 weeks of gestation, 115 (22%) were born between 34-37 weeks of gestation, and 339 (64%) were born at term. Again, 233 (47%) infants were reported to be admitted to the neonatal intensive care unit due to RDS, prematurity, hypoglycemia, or congenital malformation. In addition, hypoglycemia was found in 137 (27%) newborns, and RDS of varying severity was found in 182 (34%) newborns in this study. Polycythemia was found in 5%, hyperbilirubinemia in 25%, hypocalcemia in 4%, LGA in 36%, and SGA in 2% of newborns (29). The most common metabolic complications seen in infants of mothers with DM are listed as hypoglycemia, hypocalcemia, and hypomagnesemia (30). In our study, preeclampsia, fetal distress, preterm delivery, shoulder dystocia, and neonatal hypoglycemia were found more frequently in pregnant women with pregestational DM. TPL, polyhydramnios, hyperbilirubinemia, neonatal hypoglycemia, and neonatal weight loss were found more frequently in pregnant women with type 1 DM.

GHT, preeclampsia, TPL, oligohydramnios, polyhydramnios, LGA, fetal distress, preterm delivery, neonatal hypoglycemia, neonatal weight loss, respiratory distress, RDS, hyperbilirubinemia, and IUEF were found more frequently in pregnant women with an HbA1c value of 8% and above. GHT, preeclampsia, TPL, polyhydramnios, SGA, LGA, fetal distress, preterm delivery, neonatal hypoglycemia, and neonatal respiratory distress were found more frequently in pregnant women with GDM diagnosis and high HbA1c values. Our present findings are compatible with the literature information.

CONCLUSION

As a result of our study, it was observed that there was an increase in the rates of existing complications in pregnant women with high HbA1c levels. In conclusion, HbA1c is an important DM follow-up and treatment indicator in pregnancy. It is useful to be careful about complications in patients with high HbA1c. If we look at the additional results of our study, the complication rates are higher in patients with pregestational DM. In patients with a diagnosis of pregestational DM, the rates of existing complications were

higher in patients with a diagnosis of Type 1 DM. HbA1c should be reduced to acceptable ranges before pregnancy in patients with a diagnosis of pregestational DM and at the earliest time in patients with GDM. It should not be forgotten that a high HbA1c value indicates possible complications.

Limitations of the Study

As a result of our study, it was determined that there was an increased complication rate in pregnant women with high HbA1c. In our study, we have obtained data that may be useful in managing pregnant women with DM. Our retrospective study was a single-center study. It was also conducted in a small population and a tertiary center. If all these reasons are considered, prospective, multicenter studies in larger populations are needed.

Etik: Bu çalışmanın etik kurulu alınmıştır.

Ethics committee approval had been taken.

Yazar katkı durumu; Çalışmanın konsepti; İK, SDS, MK, dizaynı; İK, SDS, MK, Literatür taraması; İK, SDS, MK, verilerin toplanması ve işlenmesi; İK, SDS, MK, istatistik; İK, SDS, MK, yazım aşaması; İK, SDS, MK,

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

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Orijinal Araştırma / Original Investigation
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Cytotoxic Effect of Teranecrone and Doxorubicin on Human Colorectal Adenocarcinoma (CaCo-2) Cell Line

İnsan Kolorektal Adenokarsinom (CaCo-2) Hücre Hattında Teranekron ve Doksorubisinin Sitotoksik Etkisi

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ÖZET

AMAÇ: Bu çalışmada veteriner hekimlikte sıklıkla kullanılan teranekronun insan kolorektal adenokarsinomu üzerinde antineoplastik etkinliğinin değerlendirilmesi amaçlandı.

GEREÇ VE YÖNTEM: Çalışmada etkinliğini değerlendirmek için teranekron (TRN) ve pozitif kontrol olarak da antineoplastik ilaç olan doksorubisin kullanıldı. İnsan kolorektal hücre hattı CaCo-2 üzerinde teranekronun sitotoksik etkinliği araştırıldı. Canlılık oranları belirlenmesinde MTT proliferasyonu testi uygulandı. İstatistiksel analizler için ise ANOVA Tukey testi kullanıldı ve anlamlılık düzeyi P<0,01 olarak kabul edildi.

BULGULAR: Canlılık testleri yaptığımızda teranekronun tek olarak uygulandığı gruplarda teranekronun doza bağlı olarak toksisitesinin arttığı görüldü. 10 µM konsantrasyonda CaCo-2 canlılığı %94 iken 100 µM'lık konsantrasyonda canlılık oranı % 79 a düştü. Sadece pozitif kontrol olarak uyguladığımız Dox 40 µM konsantrasyonunda canlılık %78,72 Dox 40 µM + TRN 100 µM uygulandığı grupta ise canlılık oranı %73 olarak belirlendi. Dox+TRN'nin birlikte uygulandığı gruplarda TRN 50 µM dozundan itibaren toksik etki oranı konsantrasyona bağlı olarak artmaya başladı.

SONUÇ: teranekronun CaCo-2 hücre hattı üzerinde yapılan canlılık testi sonucunda doza bağlı olarak TRN'nin sitotoksik etkinliğinin arttığı belirlendi. teranekron etkinliği antineoplastik ilaç olarak kullanılan doksorubisin ile karşılaştırıldığında ise 75 µM TRN'nin 40 µM Dox ile aynı etki ortay çıkarttığı belirlendi. Yan etki profilleri düşünülecek olursa daha az yan etki gösteren TRN insan kolorektal kanser tedavisinde alternatif bir tedavi olma potansiyeli olduğu görülmektedir. TRN'nin antikanser tedavisi kullanılmasının tavsiye edilmesi için daha detaylı çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: CaCo-2, doksorubisin, insan kolorektal adenokarsinom, teranekron,

ABSTRACT

OBJECTIVE: In this study, it was aimed to evaluate the antineoplastic efficacy of teranecron, which is frequently used in veterinary medicine, on human colorectal adenocarcinoma.

MATERIALS AND METHODS: Teranecron (TRN) was used to evaluate its effectiveness in the study, and the antineoplastic drug doxorubicin was used as a positive control. The cytotoxic activity of teranecron on the human colorectal cell line CaCo-2 was investigated. MTT proliferation test was used to determine the viability rates. ANOVA Tukey test was used for statistical analysis and the level of significance was accepted as P<0.01.

RESULTS: When we performed viability tests, it was observed that the toxicity of teranecron increased in a dose-dependent manner in the groups in which teranecron was administered alone. While the viability of CaCo-2 at 10 µM concentration was 94%, the viability rate at 100 µM concentration decreased to 79%. The viability was determined as 78.72% in the Dox 40 µM concentration, which we applied only as a positive control, and 73% in the group in which Dox 40 µM + TRN 100 µM was applied. In the groups where Dox+TRN was administered together, the toxic effect ratio started to increase depending on the concentration, starting from the TRN 50 µM dose.

CONCLUSION: As a result of the viability test of teranecron on the CaCo-2 cell line, it was determined that the cytotoxic activity of TRN increased in a dose-dependent manner. When teranechron efficacy was compared with doxorubicin used as an antineoplastic drug, it was determined that 75 µM TRN had the same effect as 40 µM Dox. Considering the side-effect profiles, TRN with fewer side effects seems to have the potential to be an alternative treatment for human colorectal cancer. More detailed studies are needed to recommend the use of TRN as an anticancer therapy.

Keywords: CaCo-2, doxorubicin, human colorectal adenocarcinoma, teranecrone,

INTRODUCTION

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Homeopathic treatment is among the alternative medicine applications. The main principles of this treatment are 'Similar things can be treated with similar ones' and 'When a substance is given to healthy people, whichever disease it causes similar symptoms in those people, sick people who actually have that disease can be treated with this active substance' (1) . Due to its low side-effect profile, homeopathy has been increasing in popularity since the end of the 18th century, when it was first defined, and studies on homeopathic treatment are increasing in every field (2). There are many studies investigating the effects of homeopathic treatment in human colorectal adenocarcinoma (3-5).

Teranechron® (TRN) is a homepoitic substance used in veterinary medicine. It is an alcohol extract of the spider venom of the Tarantula cubensis species (6). TRN has a supportive effect in the treatment of inflammation and necrotic processes (7). It also has anti-edema effects (8). It has been reported that breast trauma and eczema are also successfully treated with TRN (8).

Doxorubicin (Dox), which is an anthracycline class antineoplastic, is an antibiotic with strong cytotoxicity and a topoisomerase-II enzyme inhibitor that is frequently prescribed in cancer treatment (9). Dox is widely used to treat children and adults in cancer types such as breast and ovarian cancer, leukemia, and lymphoma (10).

Human colorectal cancer (also called rectal cancer or colon cancer) is one of the most common cancer deaths worldwide, regardless of gender (11). Although clinical trials conducted around the world have tried many new strategic treatments to overcome the limitations of conventional cancer therapy, this type of cancer is difficult to treat and many cases are fatal (12). The human colorectal cancer cell line (CaCo-2) is frequently used in many studies on this type of cancer.

In our study, the CaCo-2 cell line was used. The effects of TRN and Dox on this cell line were evaluated. MTT proliferation and viability test were used to evaluate the results.

MATERIAL & METHODS

Reagents

The materials used in the study are respectively; The alcoholic extract of Tarantula cubensis was obtained from Theranekron Richter Pharma (Wels, Austria), doxorubicin hydrochloride from Koçak Farma (Istanbul, Turkey), and the human colorectal adenocarcinoma cell line was obtained from ATCC (Manassas, VA, USA).

Cell culture model

CaCo-2 cells grown in 25 mL flasks were seeded into 96-well flasks with 5000 cells per well. At least 8 wells were used for each dose and substance. The CaCo-2 cell line planted in 96 flasks was incubated in an incubator at 37°C containing 5% CO2 (13). The experiment was started when cells filled 80% of these flask wells. Concentrations used in the experiment were added at 10, 25, 50, 75, 100 µM doses for TRN. Dox 40 µM (This dose has been adjusted according to the approximate IC50 dose specified in the literature. (14)) was used as positive control and only medium was used in the wells used as negative control. Again, in the TRN+Dox combination, the CaCo-2 cell line was added to the wells planted at the dose of TRN 10, 25, 50, 75 and 100 µM and Dox 40 µM. Each of the given doses was homogenized separately in 1000 µL medium and applied to the cells as 100 µL per well. Only 100 µL of medium was added to the negative control group.

MTT Test

MTT proliferation assay was used to determine cell viability. Based on this MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test, it reacts by forming purple colored formazan crystals in the medium depending on mitochondrial reductase enzyme activity. After 24 hours of drug application, the medium in the 96-well plate is removed from the wells. After the medium was removed, 10 µL of the prepared MTT solution was added to each well and the total volume was completed to 100 µL with the medium. After this treatment, it was incubated for 4 hours for the formation of formazene crystals. After 4 hours of incubation, the solution and medium mixture was removed from the wells. To dissolve the formazan crystals, DMSO was added to the wells and when the formazan crystals were completely dissolved, reading was made with a Microplate Reader at 450 nm wavelength (15).

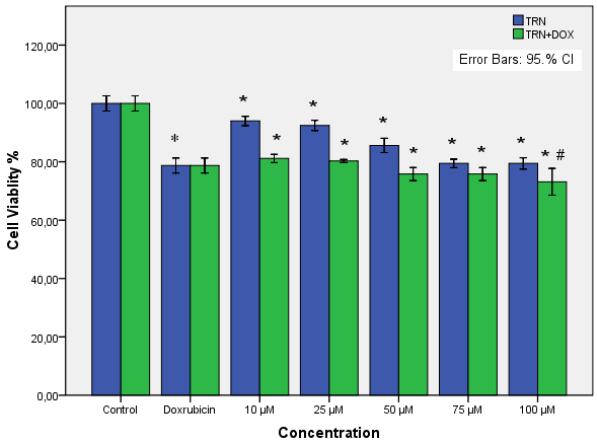
Statistical analysis

SPSS version 17 was used for statistical analysis. One-way analysis of variance (ANOVA) was used in the analysis of the data and Tukey test was performed. The results obtained were proportional to the negative control group and determined as a percentage. Percentages were given as mean ± standard deviation. $P<0.001$ was considered significant in the analyses .

RESULTS

When we evaluated the results of MTT analysis using the human colorectal adenocarcinoma (CaCo-2) cell line, TRN increased cell death depending on the concentration. While the viability was 94±1.5% at 10 µM, the viability decreased to 79.5±1.5% at the 100 µM concentration. This result showed that TRN decreased cell viability depending on the concentration. Another application was the application of the anticancer drug doxorubicin (Dox) together with TRN. Cell viability was measured as 78.72±2.77% with Dox applied as a positive control. Viability rates were also measured with the concentrations applied together with TRN at 5 different concentrations of 10-100 µM. There was no positive effect on cell viability of Dox applied alone and Dox applied together with TRN at concentrations of 10-75 µM. There was only difference in administration of 100 µM TRN+Dox. While there was 79.45±1.54% viability in the 100 µM application of TRN, the viability decreased to 73.14±3.69% in the TRN+Dox 100 µM application, and the viability was 78.72±2% in the Dox application alone. was 77 (Figure. 1). While only Dox (40 µM) and TRN 100 µM alone application decreased viability at a similar rate, this rate increased even more in TRN+Dox 100 µM application. However, it is not possible for us to make any comments about whether there is any summative effect between the two drugs. As a result, both substances have a toxic effect on the COCA-2 cell line. This effect is statistically significant (P<0.001). However, the efficiency is not very high, approximately 20% cell death has been observed.

Figure 1. *Results of CaCo-2 Cell line MTT analysis.*



*The doses applied only Teranecrone and the concentrations where TRN was applied together with Trenakron and Doxorubicin were indicated with TRN+Dox. The concentration of doxorubicin was 40 µM and its concentration was kept constant in all groups administered Dox. * P<0.01 compared to control, # P<0.01 according to doxorubicin was considered significant.*

DISCUSSION

In our study, the effects of Teranectone and doxorubicin on CaCo-2 human colorectal adenocarcinoma cell line were evaluated by viability test. In this study, the cytotoxic effect of teranecrone mono and docrubicin on the CaCo-2 cell line was investigated for the first time.

Teranecron is used in different types of cancer in veterinary medicine and has been investigated in many in vivo and in vitro studies. Animal studies in benign tumors have shown that teranecron may be effective in proliferative diseases. It has been reported that it is effective in achieving complete remission in skin papillomatosis in teranechronous cattle (16), and it is more effective in nipple papillomatosis than levamisole used in the treatment of papillomatosis (17). In another study, in canine oral papillomatosis in which surgical and/or systemic chemotherapeutics were used in the treatment, TRN achieved complete remission in all cases after 5 weeks of treatment (18). It has also been reported that TRN is effective in canine breast cancer (19, 20).

There are also in vitro studies using different cancer cell lines. In particular, many studies have been conducted to investigate cell death mechanisms. It has been reported that 6 hours of TRN administration increases apoptosis in MCF-7 cells (21). In a study using human breast tissue cancer cell line (MCF-7), human head and neck cancer cell line (HN-5) and human embryonic kidney cell line (HEK293) as healthy control, the cytotoxic effects of TRN were evaluated and dose-dependent cell line was determined. It has been reported to reduce proliferation (22). In this study, it was shown that the cytotoxic effect of TRN on cancer cell lines (especially MCF-7) is greater than that of the normal cell line, HEK293. In addition, DNA fragmentation was detected in these cancer cell lines (HN-5, MCF-7), but DNA fragmentation was not observed in the negative control group (HEK293). In order to explain the mechanism of apoptosis, caspase-3 level and activity were evaluated. As a result, it has been reported that the level of caspase-3 increases in all cells treated with TRN and that caspase-3 activity increases more in cancer cells compared to normal cells and induces apoptosis (22). It has been reported that TRN endogenous, extrinsic and endoplasmic reticulum-mediated signaling pathways induce apoptosis by oxidative stress and exert cytotoxic effects against MCF-7, A549 and Saos-2 cell lines (6).

In our study, it was determined that the cytotoxic effect of TRN increased depending on the dose. However, this cytotoxic effect was not seen more than the toxic effect of Dox. The 40 µM concentration of Dox and the 75 and 100 µM concentrations of TRN induced cell death at similar rates. This ratio was found to be approximately 79% for Dox, TRN 75 and 100 µM concentrations. In the doses where TRN+Dox was applied together, the viability percentages were found to be 75%, 75% and 73%, respectively, at the doses where TRN was applied as 50 µM, 75 µM and 100 µM. It was observed that TRN increased cell death in a dose-dependent manner, especially after a concentration of 50 µM, at doses co-administered with Dox (Figure 1).

CONCLUSION

As a result, TRN was applied to CaCo-2 cell lines in 5 different concentrations between 10-100 µM and its cytotoxic effect was evaluated by MTT proliferation test. It was observed that the toxic effect of TRN increased depending on the dose. This cytotoxic effect increased at the doses where TRN was administered together with Dox. However, the available data were not sufficient to make a sound assessment of whether there was a synergistic effect at the doses where the two substances were applied together. There are limitations in our study. This study was evaluated with a single analysis parameter. More detailed and detailed molecular mechanisms need to be evaluated. The lack of these detailed tests is a limitation of our study. It may be useful to investigate the synergistic effect in future studies. In addition, detailed research on TRN may be beneficial, especially in cancer treatment. TRN alone has potential as an alternative treatment to Dox for human colorectal adenocarcinoma due to the side-effect profiles seen in antineoplastic drugs.

Etik: Bu çalışmanın etik kurulu alınmıştır.

Ethics committee approval had been taken.

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

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Sono-photodynamic Therapy-a New Method in the Treatment of Cutaneous Leishmaniasis: an in Vitro Study

Sonofotodinamik Tedavi - Kutanöz Leishmaniasis Tedavisinde Yeni Bir Yöntem: Bir in vitro Çalışma

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ÖZET

AMAÇ: Bu çalışmada, kurkumin aracılı sonodinamik (SDT), fotodinamik (FDT) ve sonofotodinamik (SFDT) tedavilerin Leishmania tropica (L. tropica) promastigotlarına karşı olası bir mekanizma ile anti-leishmanial etkinliğini araştırmayı amaçladık. SFDT, sonodinamik ve fotodinamik tedavileri birleştiren Leishmaniasis tedavisine yeni bir yaklaşımdır. Kurkumin, uzun yıllardır tıbbi durumlarda da kullanılan doğal bir anti-inflamatuvar ajandır. Kurkumin, bu çalışmada PDT, SDT, SPDT'nin L. tropica promastigotları üzerindeki etkisini karşılaştırmak için hem sonosensitizer hem de fotosensitizör olarak kullanılmıştır.

GEREÇ VE YÖNTEM: Hücreler, farklı konsantrasyonlarda (0.25, 1,4,16 ve 64μM) kurkumin ile bir saat süreyle inkübe edildi, 1 MHz frekansında 3 W/cm^2 yoğunluklu ultrasona ve/veya 30 dakika süreyle 1.32 J/cm^2 ışık ışımasına maruz bırakıldı. Aynı zamanda parazit hücreleri sadece ultrason ve ışık ile ve her ikisi de kurkumin varlığında veya yokluğunda SFDT için maruz bırakıldı. Hücre canlılığını değerlendirmek için XTT ve morfolojik değişiklikleri belirlemek için Giemsa boyama kullanıldı.

BULGULAR: Kurkumin ve ultrason, kurkumin ve ışık, kurkumin aracılı ultrason ve ışık kombinasyonu ile L. tropica promastigot canlılığının kontrol, ultrason-kontrol ve ışık kontrol grubuna göre azaldığı bulundu. En büyük azalmanın SPDT grubunda olduğu tespit edildi. Giemsa boyama bulguları, kurkumin aracılı SDT, PDT ve SPDT'nin L. tropica promastigotlarında atipik çeşitli morfolojik değişikliklere neden olduğunu göstermiştir. Bu sonuçlar ile SPDT'nin L. tropica promastigotları üzerinde diğer tedavilerden daha etkili olduğu bulunmuştur.

SONUÇ: Kurkumin aracılı SPDT, L. tropica promastigotları için umut verici bir yaklaşım sağlayabilir.

Anahtar Kelimeler: Leishmania tropica, kurkumin, sonodinamik tedavi, fotodinamik tedavi, sono-fotodinamik tedavi

ABSTRACT

OBJECTİVE: In this study, we aimed to examine of anti-leishmanial effect of curcumin-mediated sonodynamic (SDT), photodynamic (PDT), and sonophotodynamic (SPDT) therapies with a potential mechanism against the Leishmania tropica (L. tropica) promastigotes. SPDT is a new treatment modality for Leishmaniasis that combines photodynamic and sonodynamic therapies. Curcumin is a natural anti-inflammatory agent that has been used for treating medical conditions for many years. Curcumin was used in this study both as a sonosensitizer and photosensitizer to compare the effect of PDT, SDT, SPDT on L. tropica promastigotes.

MATERIALS AND METHODS: The cells were incubated with different concentrations (0.25, 1,4,16 and 64μM) of curcumin for 1 hour, were exposed to 3 W/cm^2 intensity ultrasound for 1MHz frequency and/or subjected to 1.32 J/cm^2 light irradiation for 30 minutes. Also, parasite cells were exposed for SPDT with ultrasound and light only and both in the presence or absence of curcumin. XTT was used to evaluate cell viability and Giemsa staining was used to determine morphological changes.

RESULTS: With the combination of curcumin and ultrasound, curcumin and light, curcumin mediated ultrasound and light, L. tropica promastigote viability was found to be decreased compared to the control, ultrasound-control and light-control group. The greatest reduction was found in the SPDT group. Giemsa staining findings showed that curcumin-mediated SDT, PDT and SPDT induced several morphological alterations in L. tropica promastigotes atypical. These results showed that SPDT is more effective than other therapies on L. tropica promastigotes

CONCLUSIONS: Curcumin-mediated SPDT may provide a promising approach for L. tropica promastigotes.

Key Words: Leishmania Tropica, curcumin, sonodynamic therapy, photodynamic therapy, sono-photodynamic therapy.

INTRODUCTION

Cutaneous leishmaniasis (CL) is a significant public health problem characterized by various skin lesions. Cutaneous leishmaniasis exhibits an increasing trend in Turkey as well as in the rest of the world due to different local and global factors (1,2). Treatment of CL involves topical therapy, systemic drug therapy, and intralesional therapy, alone or in combination, depending on the location, number,

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severity of the lesion, the type of leishmania, and the immune response (3). Meglumine antimonate compounds are used as the first-line treatment in Turkey. However, studies have reported increased resistance to meglumine antimonate. In addition, there are other drugs used in treatment such as amphotericin B, which is associated with side effects, liposomal amphotericin B, which has low side effects but it is expensive, and miltefosine, which is known to exert teratogenic effects in pregnant women (4,5). Today, treatment methods vary depending on the clinical picture, immune response, and the type of Leishmania spp. There is still no standard treatment scheme for side effects in the treatment of Leishmaniasis. An ongoing search is in question for alternative treatment and, therefore, alternative anti-leishmanial therapies based on physical mechanisms such as ultrasound and light are being further looked into.

Sound and light have both been used as sources of energy for minimally-invasive treatment in clinics, such as correction of refractive errors, interstitial laser thermotherapy, and high-intensity focused ultrasound (HIFU) ablation of tumors (6,7).

Photodynamic therapy (PDT) is used as a minimally invasive treatment for many diseases such as keratoconus (8), age-related macular degeneration (9), malignant diseases (10), acne, non-melanoma skin cancer, chronic ulcers, and cutaneous leishmaniasis (11,12). PDT requires the presence of three basic components: light, O_2, and a photosensitizer. An alternative treatment method targets cell death with apoptosis or necrosis using a photosensitizer activated in the presence of light and molecular oxygen to produce reactive oxygen radicals. However, the penetration depth of light in the skin is approximately 1.5, 1.8, 2.2, and 2.4 mm for wavelengths of 600, 660, 750, and 850 nm, respectively (13). It places a serious limitation on traditional Photodynamic Therapy in terms of transmitting sufficient energy to deep targets. To eliminate this limitation, researchers have investigated the possibility of photosensitizer modification. Ultrasound has the ability to deliver to a much greater depth in biological tissues and has also been found to stimulate the same types of photosensitizers and to produce similar therapeutic effects with PDT. Sonodynamic therapy (SDT) is a treatment method based on killing tumor cells by triggering the overproduction of reactive oxygen species activated by the combination of ultrasound and sonosensitizer. Because of the inherent characteristics of ultrasound, SDT is an effective therapy for deep-seated tumors (14-16). Ultrasound itself can also induce cell death in localized region with the help of sonosensitizers through thermal effects and mechanical stress. Such therapeutic properties of ultrasound may synergistic effects by enabling combined therapy. For sensitizers that respond to both light and ultrasound stimulation, the effect of sono-photodynamic therapy (SPDT) has proven to be even superior to single-source irradiation (17,18). However, despite that, SDT and PDT are frequently considered individual therapies; it is recommended that combined SPDT may have even more clinical translation potential with its lowered incident energy levels.

Curcumin, which is obtained from turmeric (Curcuma longa), is a safe, non-toxic natural polyphenol with anti-inflammatory and antioxidant properties, and is being used in food coloring, and traditional medicine (19). Curcumin is used as a medicine for the treatment of inflammatory diseases for many years. It is used as a sensitizer in photo-sonodynamic therapy (20,21). In addition, several studies have reported that curcumin exerts anti-bacterial, anti-fungal and antitrypanosomal activity while exerting antiparasitic activity against Leishmania spp (22-25).

In this study, it was aimed to determine the efficacy of curcumin-mediated sonodynamic, photodynamic and sono-photodynamic therapy, which acts as a sonophotosensitizer on *L. tropica* promastigotes, in terms of cell viability and morphology.

MATERIAL & METHODS

Parasites: *L. tropica* promastigotes were obtained from Prof. Dr. Hatice ERTABAKLAR from Aydın Adnan Menderes University, Faculty of Medicine, Department of Parasitology. Promastigotes were stored in liquid nitrogen until used. *L. tropica* promastigotes removed from liquid nitrogen tank were dissolved in a 37°C hot water bath for 2 minutes and then maintained in RPMI-1640 (10% FBS + 1% penicillin/streptomycin + 1% gentamicin). Parasites were incubated at 26 °C and a new medium was added to the flasks every three days. Promastigotes were counted on the hemocytometer and promastigotes suspension was prepared as $1x10^7$ promastigotes/ml.

Curcumin Preparation: Curcumin (Thermo Fisher Scientific, UK) was used as both a sonosensitizer and photosensitizer in this study. The stock solution of curcumin was prepared

in 2% DMSO. Curcumin concentrations of 0.25, 1, 4, 16, and 64 µM were prepared.

Study groups:

Control Group: No Curcumin, No ultrasound and No light

Curcumin Group: Parasite samples with $1x10^7$ promastigotes/ml were exposed to curcumin for one hour with no ultrasound and light

Curcumin + SDT Group: Parasite samples with $1x10^7$ promastigotes/ml were exposed to curcumin for one hour and then free curcumin was removed and samples were exposed to ultrasound with a density of 3 W/cm 2 at a frequency of 1MHz at a distance of 5 cm for 5 minutes (50% duty cycle).

Curcumin + PDT Group: Parasite samples with $1x10^7$ promastigotes/ml were exposed to curcumin for one hour and then free curcumin was removed and exposed to blue light for 30 minutes at a distance of 10 cm.

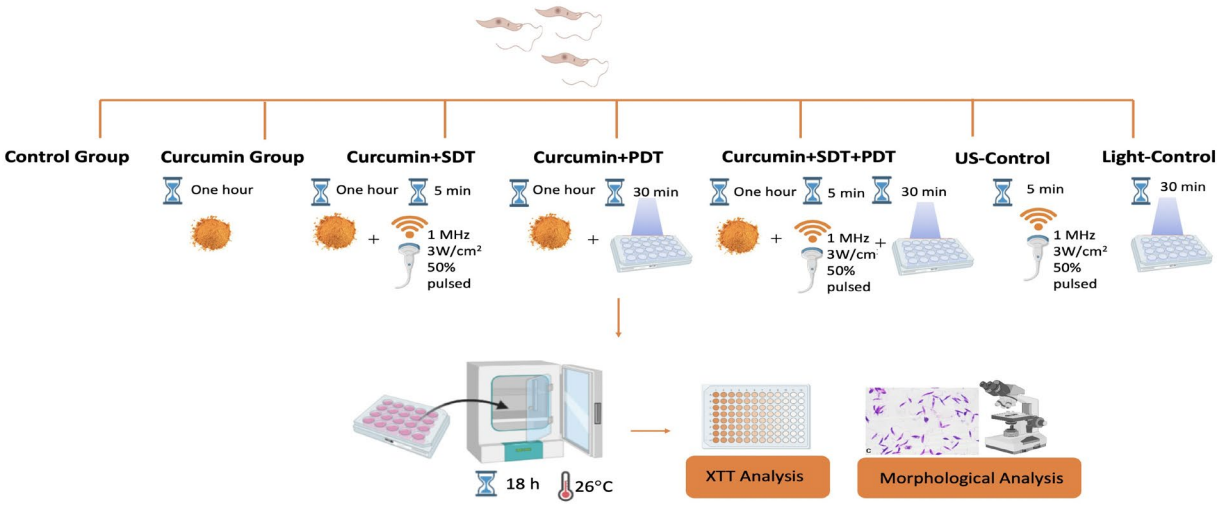
Curcumin + SPDT Group: Parasite samples with $1x10^7$ promastigotes/ml were exposed to curcumin for one hour and then free curcumin was removed and samples were exposed to ultrasound at a distance of 5 cm with a density of 3 W/cm^2 at a frequency of 1MHz for 5 minutes (50% duty cycle) and exposed to blue light for 30 minutes at a distance of 10 cm.

Ultrasound Group: Samples were exposed to ultrasound at 1MHz frequency, 3 W/cm^2 intensity at a distance of 5 cm for 5 minutes (50% duty cycle).

Light Group: Samples were exposed to blue light for 30 minutes at a distance of 10 cm.

Determination of the Efficacy of Curcumin-mediated SDT, PDT and SPDT in vitro : Experimental setup for curcumin-mediated SDT, PDT and SPDT is shown in Figure 1. The light source was an LED (O'melon Omega Led) system containing 287 units (in a three panel) that emit blue light, with 450 nm wavelength. The light output was measured by a power meter (Newport, USA) and delivered an irradiance of 0.73 mW/cm^2 and a fluence of 1.32 J/cm^2 at 30 min. The LED system was chosen because curcumin exhibits maximum absorbance at 435 nm light but also absorbs components at longer wavelengths in the blue light ranging 400 to 500 nm. BTL 4710 Sono dual-frequency ultrasound therapy device (BTL, CZ) was used to apply ultrasound to the *L. tropica* promastigotes. After *L. tropica* promastigotes were exposed to curcumin at different doses for one hour, the samples were centrifuged at 1500 rpm for five minutes and free curcumin was removed from the medium. For SDT therapy, ultrasound was applied to the cells in fluid using a frequency of 1 MHz from a distance of 5 cm at an intensity of 3 W/cm^2 and 50% pulse. For PDT therapy, cells were exposed to blue light in the dark for 30 minutes. For SPDT therapy, cells were exposed to light for 30 minutes following 5 minutes of ultrasound application. Then, fresh medium was added to the samples and incubated at 26 °C for 18 hours.

Figure 1. *Experimental setup for curcumin-mediated SDT, PDT and SPDT*



Analysis of cell viability of promastigotes of *L. tropica* by the XTT cell proliferation test: XTT is a water-soluble tetrazolium salt that, if degraded by the dehydrogenase enzyme in viable cell mitochondria, is converted into a soluble formazan. The concentration of the orange color formed by formazan is metabolically proportional to the number of viable cells. Samples were assessed by a spectrophotometric method using XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl] -2H-tetrazolium-5-caboxanilide salt) solution in 96 microplates. Percent cell viability was calculated according to the following formula measured in each well.

Cell Viability % = (Sample OD value- Blank OD value) / (Control OD value- Blank OD value) X 100

Giemsa Staining: Three alliquotes were prepared for each of the study groups, and some Leishmania samples were taken from each group and spread on the slides to dry. Methanol was placed on the slides for fixation of dried cells, and the Giemsa staining solution was dripped onto the slides and let sit for 20 minutes. Followingly, the slides were rinsed under tap water and dried, then immersion oil was dripped on them and cell morphologies were examined under the light microscope with x100 magnification.

Statistical Analysis

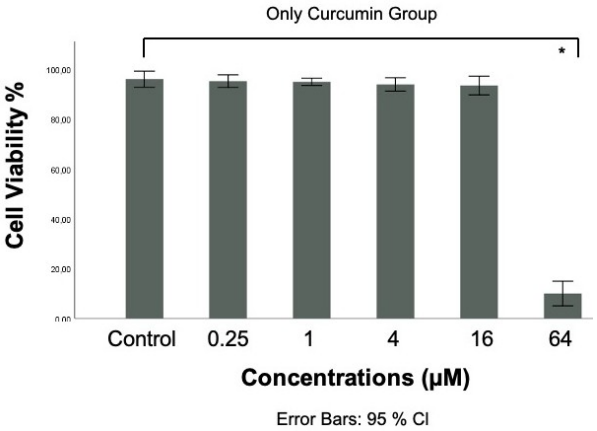
Statistical analyzes were performed using the SPSS 25.0 program. Group comparisons were made using one-way ANOVA of variance followed by Tukey post-hoc test . The values were considered statistically significant when the p value was ≤ 0.05. The degree of signifcance was symbolized by an asterisks (*) for the comparison of all groups with respect to the control group; by a daggers (†) for the comparison of SDT and PDT treated group with respect to SPDT treated group.

RESULTS

The cytotoxicity of curcumin-mediated treatments on L. tropica promastigotes

At 64 μM concentration of curcumin, cell viability decreased by 9.9% (p<0.001). No significant difference was found in the concentrations of 0.25, 1, 4 and 16μM of curcumin compared to the control group. The results of cell viability (XTT assay) showed that curcumin had no cytotoxic effects (all values >90%) and the values of cell viability ranged from 91.2% to 98.4%. Since curcumin alone affects *L. tropica* promastigotes, the 64 μM concentration of curcumin was excluded from the study.No effect of curcumin application on *L. tropica* promastigotes was detected at concentrations of 0.25, 1, 4 and 16μM (Figure 2).

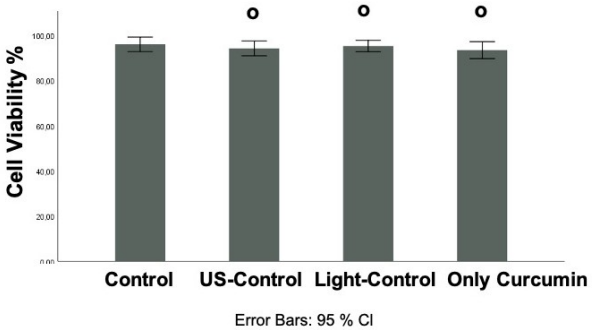
Figure 2. *% survival rate of L. tropica promastigotes treated with curcumin alone.*



Results are presented as means + SD; n = 3 (denotes significant alterations in comparison to the control group, *p<0.001, Error bars 95% confidence interval)*

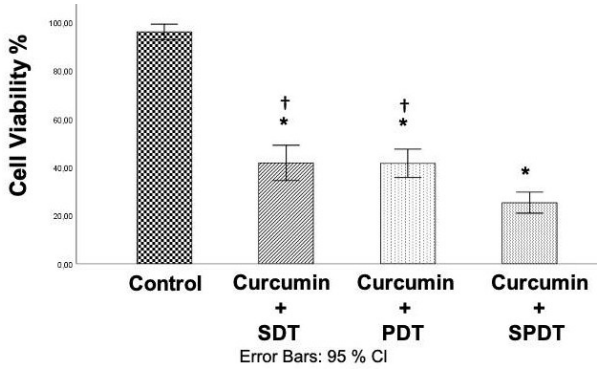
According to the XTT results of the curcumin group alone, the curcumin concentration of the treatment groups was chosen as the medium dose of 4 μM. The cytotoxicity effect of curcumin mediated sonodynamic, photodynamic, and sonophotodynamic therapies were detected by XTT assay, and results were shown in Figure 3 and Figure 4. As shown in Figure 3, the cytotoxic effect of curcumin alone (p=0.149), light alone (p=0.897) and, ultrasound alone (p=0.383) were not observed significantly on *L. tropica* promastigotes. Besides, after sonodynamic, photodynamic and sonophotodynamic therapies, the cell viability was detected at 41.7 ± 2.93%, 41.6± 2.35%, 25.2 ± 1.76% respectively. These results showed that SPDT is more effective than SDT and PDT on *L. tropica* promastigotes (Figure 4).

Figure 3. *The cytotoxic efficiency of untreated control groups*



(Control, Ultrasound-Control, Light Control and Curcumin Control). Results are presented as means + SD; n = 3 (°p> 0.5, Error bars 95% confidence interval)

Figure 4. *The cytotoxicity effect of curcumin mediated sonodynamic, photodynamic, and sonophotodynamic therapies.*

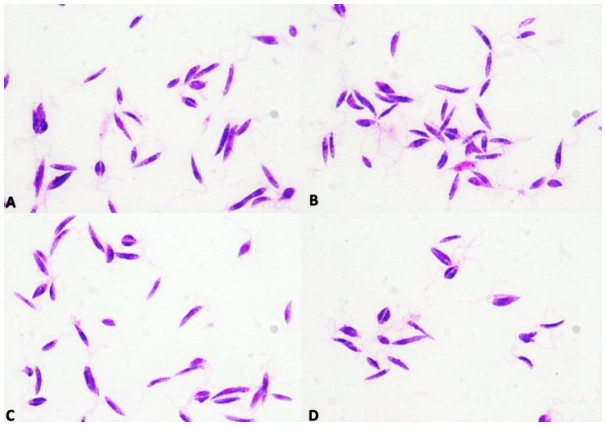


Results are presented as means + SD; n = 3 (denotes significant alterations in comparison to the control group, ***p<0.001; † denotes significant alterations in comparison to the SPDT group, Error bars 95% confidence interval).*

Morphological analysis of Leishmania tropica promastigotes

Determine to treatment's role in morphology, Giemsa stain were used and the results were shown in Figure 5 and Figure 6. The morphological analysis of *L. tropica* promastigotes, revealed that the control groups, US-group, light group and the curcumin group showed no morphological changes, maintaining fusiform appearance, with single nucleus, kinetoplast, narrow body and flagellum. Parasites in all groups had typical morphological features (Figure 5A-D).

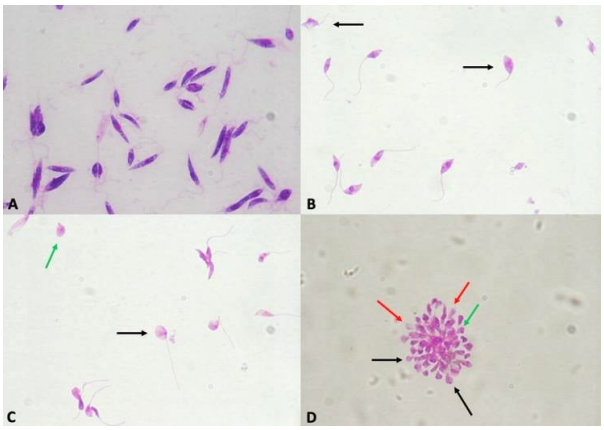
Figure 5. *Morphology of L. tropica promastigotes with Giemsa staining for untreated control groups (x100).*



A. Control B. Ultrasound-Control C. Light-Control D. Curcumin-Control

The damage caused by the action of SDT, PDT and SPDT on the parasites were observed in the morphological analysis presented in Figure 6. Atypical morphological features were increased after treatments. In the SDT group, *L. tropica* promastigotes exhibited alterations like round and altered shape than the control group (Figure 6B). In the PDT group, *L. tropica* promastigotes lost their characteristic morphological features such as fusiform shape and flagellum (Figure 6C). In turn, the morphology of *L. tropica* promastigotes was affected in a much more pronounced way in the SPDT group, since all the cells in the treated group were irregular, round and altered shape, no nucleus, and presenting a absence of the flagellum (Figure 6D).The most increased atypical cells were observed in the SPDT group.

Figure 6. *Morphology of L. tropica promastigotes with Giemsa staining for treatments groups (x 100)*



A. Control B. Curcumin mediated SDT C. Curcumin mediated PDT D. Curcumin mediated Sono-photodynamic therapy. ( ) irregular shape, round structures, ( ) No nucleus, ( ) No flagellum

DISCUSSION

SDT and PDT have been used as individual therapies for many years. There are many published articles on their potential use in treatment. Using light and sound at a certain wavelength and frequency, sonophotodynamic therapy selectively binds to the target cells and activates a light- and sound sensitizer that damages the cells. The basis of SPDT is to stimulate a sensitizer with light and sound to initiate photochemical and sonochemical events that produce cytotoxicity in cells (26). SDT has been widely reported to integrate with PDT due to the fact that most sonosensitizers are also photosensitizers (27).

There are recent studies reporting the use of curcumin as a photosensitizer and sonosensitizer on Leishmaniasis . Four different studies in the literature have focused on testing the efficiency of PDT on Leishmania using curcumin in vitro and the investigated Leishmania species were *L. amazonensis, L. major*, and *L. braziliensis*. However, no studies have been focused on *L. tropica* so far. Marcolino et al. (28) examined the photodynamic therapy of curcumin in *L. braziliensis* and *L. major* promastigotes. Furtermore, cell

viability decreases depending on curcumin concentration and they demonstrated the morphological changes with SEM analyses, and they detected that as the curcumin concentrations increased, irregular flagellum, its shape alters, or there is a shortened or absence of the flagellum. Pinto et al. (29) investigated the photodynamic activity of curcumin on *L. major* and *L. braziliensis* promastigotes. They reported that the morphology of L. major and *L. braziliensis* promastigotes was highly affected by PDT. Maciel et al. (30) reported that curcumin-mediated PDT was effective in inducing the mortality of promastigotes of *L. braziliensis* and *L. amazonensis* in vitro. Pereira et al. (31) reported that curcumin-mediated PDT has the potential to inactivate infected macrophages even at the lowest concentration. A study in the literature, including our group have focused on testing the efficiency of SDT on Leishmania using curcumin in vitro and the investigated Leishmania species was *L. tropica* promastigotes. Caliskan-Ozlem et al. (32) detected that with the combination of curcumin and ultrasound, *L. tropica* promastigote viability was significantly reduced compared to the control group. Giemsa staining findings showed that curcumin-mediated SDT induced morphological changes typical for apoptosis. However, to the best of our knowledge, there are no studies using two therapies in combination for Leishmaniasis. SPDT studies have focused mostly on cancer and antibacterial studies in recent years.

The study of De melo et al.(33), in which the effect of curcumin-mediated SPDT on S. mutans was examined, reported that 55s and 7 minutes of 15J light application and 42 kHz frequency and 5 minutes of ultrasound application with an intensity of 757 mW/cm^2 resulted in a decrease in bacterial viability and that curcumin showed promising results as a sensitizer for sono-photodynamic therapy. Zongfang et al. (34) demonstrated that hematoporphyrin monomethyl ether-mediated SPDT, which they integrated with nanoparticles on E. coli provided higher ROS generation and antibacterial activity compared to PDT alone or SDT alone (1 W cm^2 laser, 2 W cm^2 ultrasound, 10 minutes). Bhavya and Hebbar studied curcumin-mediated SDT, PDT, and SPDT on E. coli and S. aureus. They observed that combined therapy was more effective on E. coli than individual application, while curcumin-mediated PDT exerted greater efficacy than SDT and SPDT on S. aureus (35). The study of Zang et al. (20) on Listeria monocytogenes reported that curcumin-mediated SPDT induced excessive ROS generation whereas combined therapy lead to membrane rupture. Niavarki et al. (36) aimed to compare the relationship between methylene blue-mediated ultrasound and light to inactivate Enterococcus faecalis biofilms formed in root canals. They observed that methylene blue exhibited higher penetration depth when applied with ultrasound and light. Pourhajibagher et al. (37) evaluated the efficacy of PDT, SDT and SPDT mediated by nanoparticles-indocyanine green (CNPs-ICG) against bacterial biofilms on the surfaces of titanium dental implants. They showed that SPDT was more effective than SDT and PDT in reducing bacterial biofilm and was as effective as chlorhexidine, which was used as a standard.

Sono-photodynamic therapy was developed to overcome problems with SDT and PDT. The mechanism of action of sono-photodynamic therapy stems from the benefits of both light source and ultrasound energy. As a biological therapeutic approach, ultrasound energy can produce microbubbles, and their oscillations have the ability to increase the permeability of photosensitizers. This increased permeability leads to increased cellular uptake of molecules, therapeutic agents and nanoparticles (38). Therefore, SPDT can be a promising treatment method for eradicating *L. tropica* parasites and causing cell damage. SPDT is largely used for the treatment of cancer cells (39, 40), and there is no information on the use of SPDT in parasites. Also, there are few studies investigating possible suitable sensitizers for SPDT.

In this study, it was observed that curcumin-mediated SDT, PDT and SPDT cause a decrease in the proliferation of *L. tropica* promastigotes, a more effective result was obtained with SPDT than SDT and PDT, and changes occurred in cell morphology following treatments. Compared with the control group, our results showed that SPDT (~3.8-fold decrease, $p < 0.001$) showed a greater antileishmanial effect than PDT (~2.3-fold decrease, $p < 0.001$) and SDT (~2.3-fold decrease, $p < 0.001$).

This study has a main limitation which is an in vitro-based study of curcumin-mediated SPDT against *L. tropica* promastigotes. There are no intracellular amastigotes or experimental animal models. Yet, our study has some strengths as it enabled the evaluation of a treatment regime in a specific *L. tropica* promastigotes by curcumin. The results obtained in this study are further important in the determination of Leishmaniasis-specific treatment

which may lead to the discovery of novel therapeutics based on SPDT.

In conclusion, the cytotoxicity and changes in cell morphology (round, no flagellum, large, nucleus and kinetoplast-free structures) observed following curcumin-mediated SPDT therapy offer significant potential as an inexpensive, non-toxic, and non-invasive treatment for *L. tropica* promastigotes. In this approach, the fact that each component (curcumin, ultrasound, and light) has been previously used safely in humans will provide a significant opportunity for clinical applications. We anticipate a higher number of prospective studies will focus on the use of curcumin-mediated SPDT in biomedical applications in line with the promising results in various fields and the interesting insights curcumin-mediated SPDT offers. Nevertheless, further in situ and in vivo studies are needed to verify these results in a clinical setting.

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Since the methodological structure of the study is a "cell culture study", it does not require ethics committee approval in accordance with the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research on Humans".

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

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

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Evaluation of Platelet Indexes of HBsAg Positive Patients

HBsAg Pozitif Hastaların Trombosit İndekslerinin Değerlendirilmesi

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ÖZET

AMAÇ: Hepatit B virüsü, kronik karaciğer inflamasyonunun en önemli nedenidir. Trombositlerin, kronik inflamasyon durumlarında inflamatuvar tetikleyici olarak önemli fonksiyonlarının olduğunu gösteren çalışmalar son yıllarda artmıştır. Trombosit (PLT) indeksleri olarak ifade edilen; trombosit hacmi (MPV), trombosit dağılım genişliği (PDW) ve trombosit yüzdesi (PCT) değerleri; trombositlerin fonksiyonu ve aktivasyonu hakkında bilgi veren önemli belirteçlerdendir. Bu çalışmada; HBsAg pozitif ve HBsAg negatif hastalarda; PLT, MPV, PDW, PCT parametrelerinin değerlendirilmesi amaçlanmıştır.

GEREÇ VE YÖNTEM: Bu retrospektif çalışmada; Uşak Üniversitesi Tıp Fakültesi Eğitim ve Araştırma Hastanesi Enfeksiyon hastalıkları polikliniğine başvurmuş olan 193 HBsAg pozitif ve 193 HBsAg negatif hastanın sonuçları değerlendirilmiştir. PLT, PDW, PCT ve MPV parametrelerinin değerleri hemogram cihazıyla kullanılarak belirlenmiştir. Gruplar arası karşılaştırmada, student t-testi ve one-way ANOVA testi kullanılmıştır. P<0.05 değeri istatistiksel olarak anlamlı kabul edilmiştir.

BULGULAR: Çalışmamız; HBsAg pozitif hasta grubunda; PLT, PDW, PCT ve MPV değerleri sırası ile 227.36±69.98 103/mm3, % 16.34±1.52, % 0.22±0.62 ve 9.74±1.10 fl olarak belirlenirken, HBsAg negatif hasta grubunda ise değerler sırasıyla; 224.95±67.48 103/mm3, %16.16±1.08, % 0.22±0.62, 9.82±1.20 fl olarak tespit edildi (sırasıyla; p>0.05, p>0.05, p>0.05 ve p>0.05). Bu değerler yönünden HBsAg pozitif ve HBsAg negatif hasta grubu arasında istatistiki olarak anlamlı fark tespit edilememiştir.

Tüm olgularda (n=386) yapılan korelasyon analizinde PLT ile yaş, MPV, PDW arasında oldukça güçlü negatif korelasyon (sırası ile, r=-0.156 p=0.006, r=-0.394 p<0.001, r=-0.467 p< 0.001) belirlenmiş olup, PLT ile PCT arasında ise güçlü bir pozitif korelasyonun (r=0.915 p<0.001) olduğu saptanmıştır.

SONUÇ: Bu çalışmada; trombosit indeksleri yönünden HBsAg pozitif hastalar ile HBsAg negatif hastalar arasında anlamlı fark tespit edilememiştir. Bu konuyla ilgili çalışmaların hepatit B hastalarını farklı klinik evrelere göre gruplandırarak yapılmasını önermekteyiz.

Anahtar Kelimeler: Hepatit B, MPV, PCT, PDW, PLT

ABSTRACT

OBJECTIVE: Hepatitis B virus is the most important cause of chronic liver inflammation. Studies showing that platelets have important functions as an inflammatory trigger in cases of chronic inflammation have increased in recent years. Expressed as platelet (PLT) indices; platelet volume (MPV), platelet distribution width (PDW), and platelet percentage (PCT) values; it is one of the important markers that provide information about the function and activation of platelets. In this study, in HBsAg positive and HBsAg negative patients, it is aimed to evaluate the parameters of PLT, MPV, PDW and PCT.

MATERIALS AND METHODS: In this retrospective study, the results of 193 HBsAg positive and 193 HBsAg negative patients who applied to Infectious Diseases Outpatient Clinic of Uşak University Faculty of Medicine Training and Research Hospital were evaluated. The values of PLT, PDW, PCT and MPV parameters were determined using a hemogram device. Student t-test and one-way ANOVA test were used for comparison between the groups. A value of P<0.05 was considered as statistically significant.

RESULTS: In our study, while PLT, PDW, PCT and MPV values were determined as 227.36±69.98 103/mm3, 16.34±1.52%, 0.22±0.62% and 9.74±1.10 fl, respectively, in the HBsAg positive patient group, the same values were determined as 224.95±67.48 103/mm3, 16.16±1.08%, 0.22±0.62%, 9.82±1.20 fl, respectively, (p>0.05, p>0.05, p>0.05 and p>0.05, respectively) in the HBsAg negative patient group. In terms of these values, no statistically significant difference was found between the HBsAg positive patient group and the HBsAg negative patient group. In the correlation analysis performed in all cases (n=386), it can be said that there is a very strong negative correlation between PLT and age, MPV, and PDW (respectively, r=-0.156 p=0.06, r=-0.394 p<0.01, r=-0.467 p< 0.01). It was also determined that there was a strong positive correlation (r=0.915 p<0.01) between PLT and PCT.

CONCLUSION: In this study, there was no significant difference between HBsAg positive patients and HBsAg negative patients in terms of platelet indices. We recommend that studies on this subject be conducted by grouping hepatitis B patients according to different clinical stages.

Keywords: Hepatitis B, MPV, PCT, PDW, PLT

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INTRODUCTION

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family, known as the smallest enveloped DNA viruses. In addition to its high tissue and species specificity, its unique genomic organization and asymmetric replication mechanism stand out as the characteristic features (1). HBV infections are a global public health problem. It has been reported that 2 billion people worldwide are infected with HBV, approximately 248 million HBV carriers are detected every year, and HBV is among the most important causes of cirrhosis and hepatocellular cancer, with approximately 600,000 people per year (2,3). Chronic hepatitis B is characterized by inflammation and HBV is the main cause of chronic liver inflammation worldwide. Chronic inflammation of the liver occurs through the immune system. Continuous cell death and cell proliferation may increase the frequency of genetic changes and the risk of hepatocellular carcinoma (4).

Platelets, one of the main components of blood, play a role in systemic inflammation, immune modulation, angiogenesis and wound healing, in addition to their well-known roles in hemostasis and thrombosis pathways (5-8). Platelets have an important role in the pathogenesis of disorders associated with local and systemic inflammation. Thrombotic and inflammatory agents released from platelets can trigger disease-specific complications (9). The clinical importance of platelets in HBV-related liver diseases has been demonstrated in many studies. As a result of the close relationship between blood and liver cells in the sinusoids, platelets have been held responsible for the primary contributor to liver inflammation (10). In HBV-related liver diseases, different mechanisms leading to thrombocytopenia have been revealed, including abnormal platelet production and destruction, and platelet-specific glycoprotein levels (11). Recent findings suggest that platelets have both beneficial and harmful effects for the liver. While platelets play an important role in liver regeneration with the critical effect of platelet-derived serotonin, they can also increase liver damage through immune-mediated damage (10).

Platelet indices (TIN) are biological markers that provide information about the morphology and functions of platelets. There are few studies that associate TIN with disease severity and prognosis in critically ill patients (12). Platelet indices consist of mean platelet volume (MPV), platelet distribution width (PDW) and “plateletcrit” (PCT), which is the percentage of platelets in the blood (9). MPV, which reflects platelet size and platelet production rate in the bone marrow, is a frequently-used parameter to evaluate platelet activation and function (13,14). PDW is an another platelet function marker that reflects changes in platelet activation and platelet function. PDW shows the difference in platelet volume and the degree of variation in platelet size (15). In this study, platelet count and platelet indices MPV, PDW and PCT parameters were evaluated in HBsAg positive patients.

MATERIAL & METHODS

In this retrospective study, 193 patients with HBsAg positivity who applied to the infectious diseases outpatient clinic of Uşak Training and Research Hospital between January 2018 and December 2018, and 193 patients with negative HBsAg results, whose complete blood count parameters were examined, were included in this retrospective study. Patients with acute and chronic renal failure, malignancy, hemorrhagic stroke, cerebrovascular disease, diabetes mellitus, sepsis and ischemic stroke were excluded from the study. The data of the individuals included in the study were analyzed retrospectively from the hospital electronic database and the laboratory values at the last admission were recorded. Approval for the study was obtained from the Uşak University Faculty of Medicine Clinical Research Ethics Committee with the document dated 20.03.2019 and numbered 163-04. Hemoglobin, hematocrit, leukocyte count, platelet count, MPV, PDW and PCT parameters values were recorded from whole blood.

The HBsAg test (Abbott Alinity –i System) was studied with the microELISA method. Samples with an absorbance value less than the limit value were considered as negative, and samples with an absorbance value equal to or greater than the limit value were considered as positive. Complete blood count was studied by electrical impedance method in Mindray BC 6800 (MindrayBio-Medical ElectronicsCo. China) after regular daily blood samples were given.

Statistical Analysis

Statistical analyzes were performed using SPSS (Statistics Package for Social Sciences) version 22 software. Data are given as mean ± standard deviation. The conformity of the variables to the normal distribution was examined using visual (histograms and probability graphs) and analytical methods (Kolmogorov-Smirnov / Shapiro Wilk tests). In comparison between groups, Student's t-test for normally

distributed parameters and Mann-Whitney U test for non-normally distributed variables were applied. Spearman correlation test was used when examining the correlations. Cases with a P value below 0.05 were considered as statistically significant.

RESULTS

The study was conducted with a total of 386 individuals aged between 18 and 60 years. The average age of the groups was determined as 47.93±14.02 years in HBsAg positive patients and 45.10±15.92 years in HBsAg negative patients. Demographic characteristics and laboratory data of the patients are shown in Table 1. There was no significant difference between the HBsAg-positive and HBsAg-negative patients in terms of age and gender (p>0.05).

Table 1. *Demographic and laboratory characteristics of HBsAg positive and negative patients.*

	HBsAg positive patients (n=193)	HBsAg negative patients (n=193)	p
Age (year)	47.93±14.02	45.10±15.92	0.380
Gender (f/m)	83/110	99/94	0.213
PLT (10^3/mm^3)	227.36±69.98	224.95±67.48	0.731
PDW (%)	16.34±1.52	16.16±1.08	0.181
PCT (%)	0.22±0.62	0.22±0.62	0.965
MPV (fl)	9.74±1.10	9.82±1.20	0.538
WBC (10^3/mm^3)	6.87±1.90	6.89±1.95	0.947
RBC (10^6/mm^3)	4.86±0.53	4.81±0.54	0.261
HGB (g/dl)	14.33±1.74	14.12±1.71	0.221
HCT (%)	42.84±4.51	42.06±4.6	0.096

PLT*: Platelets* ***PDW****: Platelet distribution width,* ***PCT****: platelet percentage,* ***MPV****: Mean platelet volume* ***WBC****: White blood cell,* ***RBC****: Red blood cell* ***HGB****: Hemoglobin,* ***HCT****: Hematocrit,* ***f/m****: female/male*

In the HBsAg positive patient group, the PLT, PDW, PCT and MPV values were determined as 227.36±69.98 103/mm3, 16.34±1.52 %, 0.22±0.62% and 9.74±1.10 fl, respectively. In the control group, these values were determined as 224.95±67.48 103/mm3, 16.16±1.08%, 0.22±0.62% and 9.82±1.20 fl, respectively with the p>0.05, p>0.05, p>0.05 and p>0.05, in the same order. In terms of these values, no statistically significant difference was found between the HBsAg positive patient group and HBsAg negative patient group.

DISCUSSION

It is known that about two billion people in the world have encountered hepatitis B virus (HBV), and about 400 million people have chronic hepatitis B. It is estimated that 500,000-700,000 people die each year due to HBV infection and/or related complications (16). Hepatitis B virus infection is one of the important causes of mortality and morbidity in terms of the picture and results it creates in our country as well as all over the world (17, 18). The shortening of platelet lifespan in chronic liver diseases increases platelet production in the bone marrow and the release of young platelets into the circulation. It has been reported that interleukin-6, which increases due to inflammation in HBV-infected patients, increases platelet production by stimulating the bone marrow (19). In a study conducted in 11 cases of chronic hepatitis B, it was suggested that MPV and PDW are independent variables that determine the severity of liver fibrosis (20).

In their study where Turhan et al. evaluated MPV in 260 inactive chronic hepatitis B patients, they obtained statistically higher MPV values compared to the control group (21). Similarly, in a study conducted by Ceylan et al.(19), chronic in hepatitis B infection, high MPV values have been detected due to the presence of newly produced platelets, which are larger than the old ones (19).

In their retrospective study conducted with 108 patients in 2019, Çoşkun and Özmen reported that they found an increase in MPV levels and a decrease in PLT levels in the HBsAg positive patient group (22). In a study conducted in Korean hepatitis B patients, they found the MPV level to be higher in patients with chronic hepatitis B and hepatocarcinoma compared to the control group (23).

Karabulut and Namlı in their study conducted with a total of 320 patients in 2015 reported that platelet count and PCT values were statistically lower in the HBsAg positive group than the ones in the HBsAg negative group, but the PDW value was higher in the HBsAg positive group and this difference was statistically significant. In the same study, they stated that there was no statistically significant difference in terms of MPV values in parallel with our study (24).

In hepatitis B virus infections, a strong immune system is very important for an effective fight against the virus. Weak immunity can cause the virus to spread easily and thus develop chronic hepatitis, liver fibrosis and cirrhosis. Determining the level of inflammation in people infected with HBV is important for the follow-up of patients. Therefore, it is important to develop simple, inexpensive

and non-invasive methods to determine the level of chronicity in these patients. PLT, PDW, PCT and MPV parameters are easy and fast methods that can be used for this purpose.

CONCLUSION

In this study, it was determined that there was no significant difference between HBsAg positive patients and HBsAg negative patients in terms of platelet indices. In addition, no significant difference was found in terms of demographic data such as age and gender.

In addition to the retrospective and single-centered nature of our study, the limiting factor is that the parameters showing the acute and chronic infection status of the patients included in the study could not be analyzed. Another limiting factor is the fact that the drugs used by the patients could not be evaluated, since the study was retrospective.

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

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Medial orta ayak ağrısının göz ardı edilen bir nedeni: Aksesuar naviküler kemik

An overlooked cause of medial midfoot pain: the accessory navicular bone

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ÖZET

Aksesuar naviküler kemik, medial ayak ağrısı ve hassasiyetine neden olabilen, ayağın en sık görülen anatomik ve radyolojik varyasyonudur. Genel popülasyondaki prevalansı %4-21 arasında değişmekte olup, ülkemizde yapılan radyolojik temelli çalışmalarda sıklığı %11-25 olarak bildirilmiştir. Aksesuar naviküler kemik genellikle asemptomatiktir ve kadınlarda biraz daha sık görülür. Travma, mekanik yüklenme, ayak bileği burkulması gibi tetikleyici faktörler ağrıya yol açabilir. Semptomatik bazı vakalar naviküler kemik kırığı zannedilerek gereksiz müdahalelerin yapılmasına neden olabilmektedir. Burada, akut medial orta ayak ağrısı ile prezente olan genç kadın bir hastadaki aksesuar naviküler kemik olgusu sunulacaktır.

Anahtar Kelimeler: aksesuar naviküler kemik, ağrı, ayak, manyetik rezonans görüntüleme

ABSTRACT

The accessory navicular bone is the most common anatomical and radiological variation of the foot that can cause medial foot pain and tenderness. Its prevalence in the general population varies between 4-21%, and its frequency has been reported as 11-25% in radiological-based studies conducted in Turkey. The accessory navicular bone is usually asymptomatic and is slightly more common in women than in men. Triggering factors such as trauma, mechanical load, or ankle sprain can cause pain. Some symptomatic cases may be mistaken for navicular bone fractures and cause unnecessary interventions. Here, a case of the accessory navicular bone in a young female patient presenting with acute medial midfoot pain will be presented.

Keywords: accessory navicular bone, pain, foot, magnetic resonance imaging

GİRİŞ

Aksesuar naviküler kemik (ANK), naviküler kemik tüberositesinin ikincil bir kemikleşme merkezinden gelişen konjenital bir anomalidir. Ayağın en sık görülen anatomik ve radyolojik varyasyonudur. Genel popülasyondaki prevalansının %2 ile %14 arasında olduğu tahmin edilmektedir (1). Radyolojik görünümüne göre üç farklı tipi tanımlanmıştır ve en sık görülen tip II'de ANK, esas naviküler kemiğin hemen medialinde fibrokartilajinöz bir yapıyla sinkondral birleşmiş şekilde izlenmektedir (2).

Aksesuar naviküler kemik çoğunlukla asemptomatiktir ve kadınlarda biraz daha sık görülür. Direk travma, aşırı sportif aktivite, ayak bileği burkulması, ayakkabı vurması, posterior tibial tendon zorlanması gibi mekanik faktörler nedeniyle semptomatik olabilir. Tipik olarak ayağın orta medial yüzünde hassasiyet, eritemli kemik çıkıntısı ile karakterize mekanik ağrı ile prezente olur. Semptomatik ANK'nin, erişkin bireylerin %0,1'inde görüldüğü bildirilmiştir (3). Bu vakalar, çoğu zaman naviküler kemik kırığı ile karıştırılarak agresif tedavilerin yapılmasına neden olabilmektedir. Gereksiz müdahalelerin engellenmesi ve doğru ayrıcı tanı yapabilmek için ayağın aksesuar kemiklerini tanımak önemlidir. Burada, genç bir kadın hastada, medial ayak ağrısı ayırıcı tanısında göz önünde bulundurulması gereken bir tip II ANK olgusu paylaşılacaktır.

OLGU

27 yaşında kadın hasta, bir haftadır sol ayak tabanında yürümekle artan mekanik karakterde ağrı şikayeti ile Fizik Tedavi ve Rehabilitasyon (FTR) polikliniğimize başvurdu. Belirgin travma öyküsü olmayan hasta, son birkaç haftadır uzun süreli ayakta kaldığını ve uzun mesafe yürüdüğünü belirtmekteydi. Ek hastalık ve ilaç kullanımı olmayan hastanın özgeçmiş sorgusunda; yaklaşık 10 yıl önce, sol ayak bileği burkulması nedeniyle başvurduğu acil serviste ayak kemiğinde kırık olduğu söylenerek bir ay atele alındığı öğrenildi. Fizik muayenesinde; ayak tabanı orta kısmının medialinde, medial malleolün hafif antero-inferiorunda belirgin ağrı ve hassasiyet mevcuttu. Aktif ayak inversiyon sonunda ve pasif eversiyonla ağrısı artmaktaydı. Ayakta ekimoz, şişlik, ısı artışı ve krepitasyon izlenmedi. Periferik

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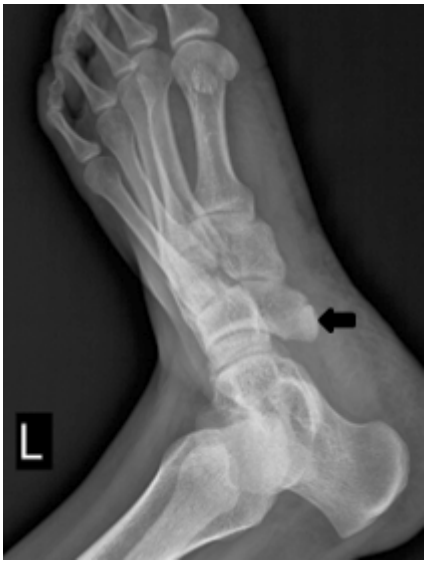
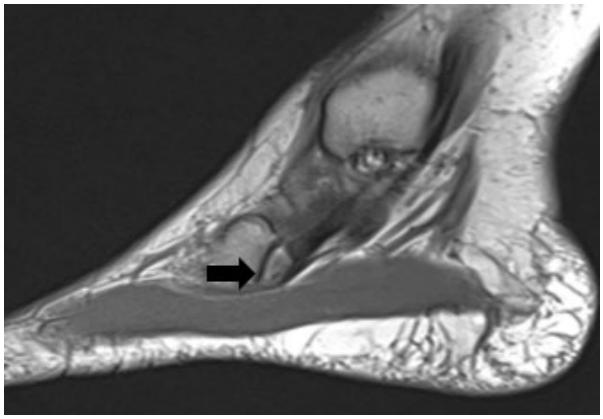
nabızları alınmaktaydı ve tarsal tünel açısından Tinnel testi negatifti. Kas gücü ve eklem hareket açıklığı kaybı yoktu. Pes planus dahil belirgin ayak deformitesi gözlenmedi. Hastanın hemogram, biyokimya, sedimantasyon ve C reaktif proteini (CRP) içeren rutin kan tetkiklerinde patolojik değer saptanmadı. Üç yönlü ayak direk grafisinde, naviküler kemiğin medialinde radyolüsen ince bir hatla ayrılan, sklerotik kemiksel bir yapı izlendi (Resim 1). Ön planda naviküler kemik non-deplase stres kırığı düşünülen hasta alçı-atele alınmak istemediği için hastaya istirahat, elevasyon ve 6-8 saat ara ile parasetamol 500 mg tablet kullanması önerildi. Stres kırığı, avasküler nekroz, posterior tibial tendinit, deltoid ligaman yaralanması gibi patolojilerin ayrıcı tanısı amacıyla sol ayak manyetik rezonans görüntüleme (MRG) istendi. MRG'de aksesuar naviküler kemik izlendi (Resim 2). Ayrıca hem esas naviküler kemikte, hem de ANK'de T2 sekanslarda hiperintens kemik iliği ödemi saptandı (Resim 3).

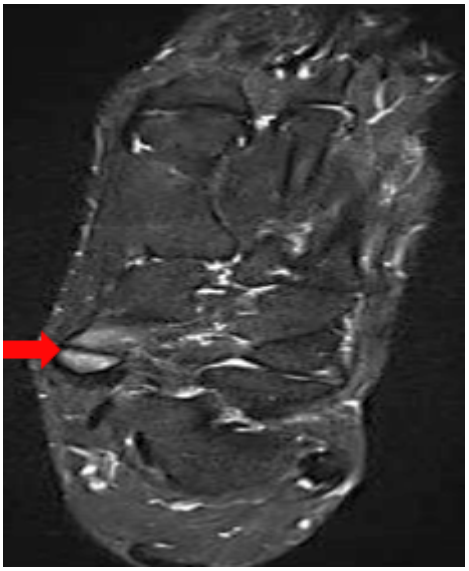
Resim 1. *Ayak oblik grafisinde aksesuar naviküler kemik*



Resim 2. *Sagittal T1 kesitlerde aksesuar naviküler kemik*



Resim 3. *T2 transvers kesitlerde aksesuar kemik ve naviküler kemikte ödem izlenmektedir.*



Hasta mevcut klinik tablo ile semptomatik tip II ANK olarak değerlendirildi. Hastaya istirahat, aktivite ve ayakkabı modifikasyonu (geniş, yumuşak ayakkabı), günde 3 defa 15 dk. lokal buz uygulaması, topikal ve oral non-steroid anti-inflamatuvar ilaçlar (NSAİİ) (ibuprofen 1600 mg/gün, 7 gün) verildi. 2 hafta sonra yapılan poliklinik kontrolünde, hastanın naviküler kemik medialinde basmakla minimal hassasiyet dışında bulgusu yoktu ve yürümekle artan ağrısı tamamen geçmişti. Hastaya tıbbi durumu hakkında ayrıntılı bilgi verildi; semptomatik iyileşme gözlenen hastaya ek bir tedavi planlanmadı ve poliklinik kontrolü önerildi.

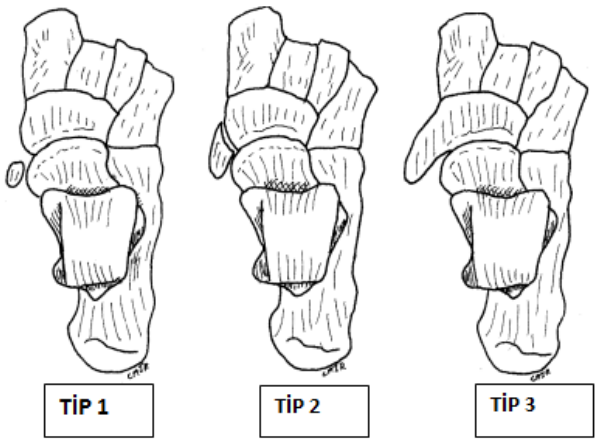
SONUÇ

Ayak ve ayak bileği çevresindeki aksesuar kemikler, tahmin edilenden daha sık mevcut olup genellikle başka amaçlı çekilen radyografilerde insidental olarak gözlenmektedir. Bunları tanımak özellikle kırıklar ile karışması açısından kritik öneme sahiptir. Ayrıca, ayağın aksesuar kemikleri, hastanın semptomlarının doğrudan nedeni de olabilir. Türkiye'de bilgisayarlı tomografi temelli yapılan bir araştırmada, ayakta aksesuar kemik görülme sıklığının % 45,3 ve bilateral görülme sıklığının ise % 9,3 olduğu saptanmıştır (4). Bu çalışmada, literatürle uyumlu şekilde ANK (%24,8) ve os trigonumun (%20,3) en sık izlenen aksesuar kemikler olduğu bildirilmiştir.

Aksesuar naviküler kemik, ayakta en yaygın gözlenen aksesuar kemik olmasına rağmen, çoğunlukla asemptomatik olması nedeniyle tesadüfi olarak

rastlanmaktadır. Bazı olgularda ise ANK ayak ağrısının doğrudan nedenidir (4-5). Literatürde üç tip aksesuar naviküler kemik tanımlanmıştır (6). Tip I (%30); esas naviküler kemik ile posterior tibial tendon arasında seyreden küçük, yuvarlak veya oval bir kemik sesamoidi şeklindedir. Tip II (%50); fibro-kıkırdak sinkondrozu veya psödo-artroz kıkırdağı ile naviküler kemikle 1-3 mm arasında bir tabanla birleşen, 8-12 mm kalınlığında üçgensi bir kemiktir. Tip III (%20) ise; belirgin bir medial naviküler çıktı şeklinde gözlenen ve navikula ile birleşik bir yapıdır (Resim 4).

Resim 4: *Aksesuar naviküler kemik tiplerinin şematik görünüşü*



(https://drrodrigomacedo.com.br/2020/11/18/navicular-acessorio/ sayfasından adapte edilmiştir.)

Hangi tip ANK'nin daha sık görüldüğü yapılmış çalışmalarda farklılık göstermektedir. Aslan S ve ark. ile Huang ve ark. yaptığı çalışmalarda en sık tip I, Coşkun ve ark.'nın çalışmasında en sık tip III ANK saptanmıştır (4, 7-8). Bizim olgumuz ise tip II ANK ile uyumlu görüntüye sahipti.

Aksesuar naviküler kemikle ilişkili ağrının etiyolojisi tartışmalıdır. Posterior tibial tendinit, orta ayağın kemik çıkıntısına bağlı basınç ve inflamasyon ve aksesuar ile esas navikula arasındaki sinkondroza binen mekanik stres gibi birçok teori öne sürülmüştür (9). Posterior tibialis kası medial naviküler kemiğe yapışarak ayağın inversiyon ve plantar fleksiyonu yaptırır. Tip II ANK'si olan birçok olguda, posterior tibialis tendonu aksesuar kemiğe yapışır, bu da normalden daha proksimal bir insersiyona neden olarak tendon üzerindeki gerilimi artırır. Medial ayak ağrısı, tendondaki mikro yırtıklardan veya inter-naviküler eklemin tekrarlayan gerilme hasarından da kaynaklanabilir (10).

Bölgeye özgü direkt minör travmalar nadiren fark edildiği veya hatırlandığı için, klinikte bu sebepten ziyade, kronik biyomekanik yüklenmeler, özellikle iskelet matürasyonunun tamamlanmadığı çocukluk çağındaki bireylerde daha baskın bir ağrı nedeni olarak kabul edilebilir. Bu durum, bir çeşit aşırı kullanım sendromu veya stres kırığına benzetilebilir (9). Bizim olgumuzda da alışılagelmişin dışında mekanik yüklenme sonrası ağrı gelişmiş ve olası stres fraktürünü ekarte edilmek için MRG istenmiştir. Hastanın öyküsünde 10 yıl önce aynı bölgenin fraktür nedeniyle atele alınmış olması, ANK tanısını destekler niteliktedir. Eversiyon yaralanması olmaması ve ayak medialinde ekimoz gözlenmemesi, ayrıcı tanı açısından deltoid ligaman hasarından uzaklaştırmıştır. Ve yine hasta, olası ayrıcı tanılardan olan tibialis posterior tendiniti, tarsal tünel sendromu, pes planovalgus, fleksör hallusis longus tendiniti ve avasküler nekroz açısından klinik ve görüntüleme ile değerlendirilerek bu tanılar ekarte edilmiştir.

Semptomatik ANK'nin primer tedavisi konservatiftir. Bu tedavi temel olarak, ayağın orta medial kısmındaki biyomekanik baskıyı ve inflamasyonu hafifletmeyi içerir. Basıncı azaltıcı ayakkabı modifikasyonu birinci basamak yaklaşım olmalıdır. Medial orta ayakta baskı yapmayan, geniş ve yumuşak ortopedik ayakkabı önerilir. Bizim vakamızda olduğu gibi mekanik yüklenmenin tetiklediği durumlarda aktivitenin sınırlandırılarak istirahat verilmesi oldukça etkili primer tedavidir. Beraberinde posterior tibial tendinitin gözlendiği olgularda kısa bacak ateli veya çıkarılabilir ayak-ayak bileği yürüme ortezleri (air cam walker gibi) kullanılabilir (11). NSAİİ, lokal buz uygulama (3-4 x15 dk), elevasyon, fiziksel tedavi ajanları (ultrason, transkütanöz elektriksel sinir stimülasyonu (TENS), kontrast banyo gibi) ve egzersiz terapisi (eklem hareket açıklığı, güçlendirme, germe ve propriosepsiyon egzersizleri) ile vakaların çoğunluğu iyileşmektedir.

Konservatif tedaviye yanıtsızlık durumunda basit eksizyon, Kidner prosedürü gibi minör cerrahi girişimler gerekebilir (12). Jegal ve ark. semptomatik ANK ağrısı olan sporcuların, genel popülasyona kıyasla konservatif tedaviye daha dirençli olduğunu bildirmiştir (1). Wynn ve ark., yaş ortalaması 12 olan 169 pediatrik semptomatik ANK hastasını retrospektif olarak incelemiştir (13). Hastaların %28'inin konservatif yaklaşımlar ile ortalama 8 aylık takip sonrası tam iyileştiğini; %31'inin ise cerrahi tedavi

gerektirdiğini bildirmiştir. Kalan %41'inde ise, konservatif tedavi ile kısmi semptomatik iyileşme gözlenmiş ve bu hastalara takip önerilmiştir. Bu çalışmada, basit cerrahi prosedürlerle, hastalardan yüksek memnuniyet oranı bildirildiği ve düşük komplikasyon gözlendiği vurgulanmıştır.

Bu vakada, semptomatik tip II ANK tanısı koyduğumuz hastaya istirahat, aktivite ve ayakkabı modifikasyonu (geniş, yumuşak ayakkabı), günde 3 defa 15 dk. buz uygulaması, topikal ve oral NSAİİ verilerek çok kısa sürede semptomatik tam iyileşme kaydedildi. Hastaya ek bir tedavi planlanmadı ve poliklinik takibi önerildi. Literatüre çok az sayıda da olsa tip II ANK'de avasküler nekroz vakaları bildirilmiştir (14). Her ne kadar bu olguda, gece ağrısı ve kötüye gidiş izlenmemiş olsa da; travma öyküsü ve MRG'de kemik iliği ödemi gözlenmesi avasküler nekroz açısından yakın klinik ve görüntüleme takibini gerekli kılmaktadır.

Aksesuar naviküler kemik ayakta yaygın görülen ancak az bilinen anatomik bir varyasyondur. Gereksiz agresif tedavilerin önüne geçmek için, medial ayak ağrısı ile başvuran olguların ayırıcı tanısında öykü, fizik muayene ve görüntüleme ile kolayca tanı konabilen ANK olguları göz ardı edilmemelidir.

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.

Yazar katkı durumu; Çalışmanın konsepti; RY, HK, SK, HY, dizaynı; RY, HK, SK, HY, Literatür taraması; RY, HK, SK, HY, verilerin toplanması ve işlenmesi; RY, HK, SK, HY, yazım aşaması; RY, HK, SK, HY,

Author contribution status; The concept of the study; RY, HK, SK, HY, design; RY, HK, SK, HY, literature review; RY, HK, SK, HY, collecting and processing data; RY, HK, SK, HY, writing phase; RY, HK, SK, HY,

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