ISSN 2651-3137 • EISSN 2651-3153

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

Volume: 35 • Number: 1 • March 2019

Highlights

galenos yayınevi

Official Journal of Health Sciences University, Okmeydanı Training and Research Hospital

eurarchmedres.org

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

Publisher Contact
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Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27 **E-mail:** info@galenos.com.tr/yayin@galenos.com.tr **Web:** www.galenos.com.tr
Publisher Certificate Number: 14521

Printing at: Özgün Ofset Ticaret Ltd. Şti.
Yeşilce Mah. Aytekin Sk. No: 21 34418 4. Levent, İstanbul, Turkey **Phone:** +90 (212) 280 00 09 **Certificate Number:** 42419
Printing Date: March 2019 **ISSN:** 2651-3137 **E-ISSN:** 2651-3153
International scientific journal published quarterly.

European Archives of Medical Research

Formerly Okmeydanı Medical Journal

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The target audience of the journal includes researchers, general practitioners and specialists from all fields of medicine.

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The aim of the European Archives of Medical Research is to publish original research papers of the highest scientific and clinical value in all medical fields. Eur Arch Med Res also includes reviews, rare case reports, and letters to the editor that are related to recently published articles.

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All manuscripts should be submitted in 12 point Times New Roman type with 2 line spacing.

Title page: A separate title page should be submitted with all submissions and this page should include:

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- Name(s), affiliations, highest academic degree(s), e-mail addresses, and ORCID IDs of the author(s),
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria.

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Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html).

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Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

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Letters to the Editor: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Letter to the Editor." Readers can also present their comments on the published manuscripts in the form of a "Letter to the Editor." Abstract, Keywords, and Tables, Figures, Images, and other media should not be included. The text should be unstructured. The manuscript that is being commented on must be properly cited within this manuscript.

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables.

Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	40	6	7 or total of 15 images
Review Article	5000	250	80	6	10 or total of 20 images
Case Report	1000	200	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits

should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including

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the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal Article: Stephane A. Management of Congenital Cholesteatoma with Otoendoscopic Surgery: Case Report. Turkiye Klinikleri J Med Sci 2010;30:803-7.

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

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

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

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland;1992. pp.1561-5.

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

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

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dçent Res. 1974. Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

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

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Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

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March 14 Feast of Medicine and The Spirit of Medics

University of Health Sciences is both the first health-themed state university in Turkey and the greatest health university of the world. Our university was founded in the facilities built by Sultan Abdülhamit Han II with the vision, "Transitioning from the past into the future" and "Traditionalist but innovative", and with the mission of founding the first modern medical school, Mektebi Tıbbıyeyi Şahane. Our university conducts educational activities in two cities - Istanbul and Ankara - in Turkey in addition to Somalia and Sudan campuses abroad and provides health education on an international level.

While it first served as a military medical school between 1903-1909, Mektebi Tıbbıyeyi Şahane later became a civil medical school. The military Hospital of Haydarpaşa which was located opposite to these facilities, was linked to the school with an underground tunnel and rail system and used as a training hospital for the students.

After the World War I ended and the Mondros Armistice Agreement was signed in 1918, the Allies invaded İstanbul. While the ships of the invading forces were being deployed in the Marmara Sea, the medical students in Haydarpaşa were watching this scene from the windows of the school with sadness. Their instructor, Dr. Tevfik Salim Saglam turned to the students and told them not to worry, as the army of this nation had never been defeated.

However, the English forces had started to place their soldiers in the official institutions in Istanbul immediately, and had also invaded the buildings of the Mektebi Tıbbiyeyi Sahane. The English soldiers emptied the dormitories of the boarding military students and decided to use that part of the building as their headquarters. They even confiscated the bedsteads of the students. The students converted the loft into a dormitory and moved their mattresses there. Later on, this place was called "Hangar Palace" by the students, as a lot of students could only be accommodated in a very cramped area.

The English also forbade the military students to participate to lessons with their uniforms. As the sons of a nation that had just been out of the war, most of the military medics did not have any clothes to put on other than their uniforms. The students had to attend the classes in their pyjamas. This situation hurt the pride of the prospective military doctors of the soldier-nation.

The medics who were on the front line during the First World War, were uncomfortable with the occupation of their nation and being put in a dishonorable situation by the occupiers. Even though they wanted to resist the pressure that was put on them and to rebel, they chose to proceed cautiously due to the fact that people who raised little objections were being arrested and were being sent into exile by the occupation forces.

The meds who were seeking a way to react without making the situation worse, decided to hold a celebration meeting for the 92nd anniversary of the foundation of the Medicine School which started education in 1827.

The anniversary of the foundation of the School of Medicine had never been celebrated until that time. As occupation forces forbade students to be in groups, it could be only possible for students to come together only with the pretext of such a scientific-based program. On March 14, 1919 the students gathered in the conference hall of Ottoman University with their professors. They invited British, American and French Red Cross representatives and a French general who was in charge of Sanitary İnspection Department. The program started with the speech delivered by Mr. Kemal who was a medical student on a brief history of the school. In his speech, Mr. Kemal also stated the services of the Crimean Azğ Bey who started the medical education in Turkish instead of French and talked about the contributions of the other professors. Then, Dr. Memduh Necdet who studied medicine in the United States, stated that 607 Turkish, 240 Greek, 170 Armenian, 79 Jewish and 11 Serbian and Bulgarian students had graduated from the School of Medicine since its foundation. He also reminded the contributions of the School of Medicine to the First World War with figures. After pointing out the depressive situation in Istanbul, he ended his speech by saying "We have been here, and we will be here... Istanbul belongs to us because our independence is here..." After this statement he received a standing ovation. With this meeting, the medics demonstrated that they could come together under any circumstances. Also, they conveyed this very important message that they would not abandon their country to the occupants. The anniversary of the opening of the School of Medicine continued to be celebrated in the following years. Medical students' fight for independence was not only limited to the Medical Feast. They also played a fundamental role in the independence of the country by supporting the initiation and success of the War of Independence.

Since 1919, the Medical Feast has been celebrated and every year on March 14, all medics continue to come together.

Just like our pioneer colleagues who reacted to the occupation on March 14, 1919 by raising the Turkish flag between the two clock towers of Mekteb-i Tıbbiyye-i Sahane, with the same spirit and the same persistence, we raised our flag once again on the night of July 15, 2016 to resist the treacherous coup attempt. We have shown that medics will not surrender to any occupation and betrayal attempt.

Medics who rebelled and fought against the invasion of Istanbul and homeland, continue to be the guarantee of our independence with the same spirit.

Prof. Dr. Cevdet ERDÖL
RECTOR

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CONTENTS

ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2017.79037
Eur Arch Med Res 2019; 35 (1): 1-5

Investigation of Factors Affecting Postoperative Hemoglobin Decrease in Primary Cesarean Sections

Berna Aslan Çetin, Pınar Kadiroğulları, Pınar Yalçın Bahat, Nadiye Köroğlu, Aysu Akça

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Abstract

Objective: The aim of our study was to evaluate the factors affecting postoperative hemoglobin (Hb) decrease in primary cesarean section patients.

Methods: A total of 560 patients who underwent primary caesarean section between January 2016 and December 2016 were evaluated retrospectively. These patients were divided into two groups according to postoperative Hb values: patients with Hb decrease ≥2 g/dL or <2 g/dL. There were 142 patients with Hb decrease ≥2 g/dL. Preoperative and postoperative 24th hour Hb and hematocrit levels, and factors leading to Hb decrease were evaluated.

Results: Of the 560 pregnant women included in the study, 289 (51.60%) had emergent caesarean sections, while 271 (48.39%) were elective cesarean sections. Demographic data, distribution of cesarean indications and obstetric characteristics were not different between the two groups. Postoperative erythrocyte transfusion requirement was significantly different between the groups ($p<0.001$). The development of atony and additional uterotonic requirement were significantly higher in the group with Hb decrease ≥2 g/dL.

Conclusion: Postoperative Hb decrease is relatively rare in primary cesarean sections. Although severe blood loss and blood product transfusion are rare, caution should be exercised if there is a risk factor in primary cesarean sections.

Keywords: Cesarean delivery, hematocrit decrease, hemoglobin decrease

INTRODUCTION

According to World Health Organization (WHO) data, it is estimated that around 30% of the population and more than half of the pregnant women are anemic worldwide. The prevalence of anemia during pregnancy is reported to be 35-50% (1). Anemia is also an important health problem in our country (2). Hemoglobin (Hb) level below 11.0 g/dL in the first and third trimesters, and below 10.5 g/dL in the second trimester in pregnant women is defined as anemia (3, 4).

There are different claims about the maternal and perinatal effects of anemia during pregnancy. WHO suggests that anemia may contribute to 20% of maternal deaths (1, 5). In addition, maternal anemia has been found to be associated with fetal complications such as intrauterine growth retardation, preterm delivery, low birth weight and maternal complications such as preeclampsia and eclampsia (6-10). Therefore, anemia is an important health problem in terms of female and maternal health. The most common cause of maternal mortality is postpartum hemorrhage and postpartum hemorrhage is the most important cause of morbidity with a rate of 18% in developed and developing countries (5).

Bleeding is one of the most common complications during and after cesarean section and may be a life-threatening event. The need for a blood transfusion for cesarean section is 2.2% in recurrent cesarean and 3.2% in primary cesarean section (10, 11).

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Received: 20.07.2017
Accepted: 24.11.2017

Cite this article as: Çetin BA, Kadiroğulları P, Yalçın Bahat P, Köroğlu N, Akça A. Investigation of Factors Affecting Postoperative Hemoglobin Decrease in Primary Cesarean Sections. Eur Arch Med Res 2018; 35 (1):1-5

The aim of our study was to determine the factors affecting the postoperative Hb decrease in primary cesarean section patients.

METHODS

There were 12.548 births in our hospital between January 2016 and December 2016. Primary cesarean section cases accounted for 1.914 (15.25%) of these deliveries. Pregnant women were standardized as between 18 and 40 years of age, use of iron and/or multivitamin preparation during pregnancy, and cesarean for the first time. Patients with any placental insertion anomaly, caesarean section due to placental detachment, previous cesarean section, maternal connective tissue diseases and pregnancies below 37 weeks were not included in the study. Five hundred and sixty patients who met the criteria and who had complete medical data were retrospectively reviewed. One hundred and forty-two patients with Hb decrease more than 2 g/dL were detected.

According to our hospital protocol, all patients received 10 units of oxytocin in 0.9% sodium chloride 1000 mL following the delivery of the placenta during a caesarean section. Complete blood count was performed 24 hours after surgery. To identify factors associated with severe Hb decrease (≥2 g/dL), data were obtained from our hospital database records. Demographic and obstetric data such as maternal age, gestational week at delivery, cesarean section indication, maternal additional systemic disease, body mass index (BMI), neonatal birth weight, duration of operation and pregnancy complications were recorded.

Statistical Analysis

Statistical analysis was performed using SPSS ver. 16.0 package program (Chicago, IL, USA). In addition to descriptive statistical methods (mean, standard deviation), independent t-test was used for comparison of groups and chi-square test was used for the comparison of qualitative data. Logistic regression analysis was performed to determine the factors affecting Hb decrease. The results were evaluated at $p<0.05$ level.

RESULTS

Of the 560 pregnant women included in the study, 289 (51.60%) had cesarean section for emergency reasons and 271 (48.39%) had elective cesarean section. The distribution of cesarean indications of the two groups is shown in Table 1. While cephalopelvic disproportion was the most common indication in the group with Hb decrease, it was obstructed labor in the group without Hb decrease.

The mean age was found to be 27.90±5.91 in the group with Hb decrease less than 2 g/dL, and 28.85±6.40 in the group with Hb decrease more than 2 g/dL. Maternal BMI and diseases (diabetes, hypertension, thyroid dysfunction) were similar in both groups. The mean gestational week was 38.41±2.18 in the group with Hb decrease less than 2 g/dL and 38.81±1.89 in the other group (Table 2).

Uterotonic was used during labor in 153 patients in the group with Hb decrease less than 2 g/dL and in 75 patients in the group with Hb decrease more than 2 g/dL. Complications such as bladder injury, vascular injury, t incision dehiscence, intraoperative and/or postoperative atony and additional uterotonic use were seen in 104 patients (25%) in the group with Hb decrease less than 2 g/dL and in 76 (49.9%) patients in the group with Hb decrease more than 2 g/dL. Atony and additional uterotonic requirement were significantly higher in the group with more Hb decrease.

Postoperative erythrocyte suspension transfusion was performed in one (0.2%) patient with Hb decrease less than 2 g/dL and in 11 (7.7%) patients with Hb decrease more than 2 g/dL (Table 2). Postoperative erythrocyte transfusion requirement was significantly different between the groups ($p<0.001$).

The risk factors detected in patients with postoperative Hb decrease more than 2 g/dL are shown in Table 3. An infantile head circumference greater than 37 cm was the most important risk factor, followed by maternal additional disease, macrosomic infant, multiple pregnancy, and uterotonic use during labor, respectively.

DISCUSSION

Cesarean section is the most common major obstetric operation. Bleeding is the most common complication during and after cesarean section. According to WHO, iron-deficiency anemia rate

Table 1. The distribution of caesarean indications

	Hgb decrease <2 g/dL (n=418)	Hgb decrease ≥2 g/dL (n=142)	p
Fetal distress	86 (20.57%)	30 (%21.12)	0.54
Obstructed labor	102 (26.79%)	29 (%20.42)	0.38
Cephalopelvic disproportion	108 (24.40%)	35 (%24.64)	0.87
Multiple pregnancy	20 (4.78%)	10 (%7.04)	0.35
Failed induction	25 (5.98%)	2 (%1.40)	0.17
Breech presentation	73 (17.46%)	26 (%18.30)	0.78
LGA infant	9 (2.15%)	7 (%4.92)	0.22
Severe preeclampsia	5 (1.19%)	3 (%2.11)	0.64

Hgb: Hemoglobin, LGA: Large for gestational age

is 14% in Europe and 25% in Turkey (2). In this study, prepartum and postpartum Hb and hematocrit (Hct) levels in pregnant women were examined and the factors causing decrease in standardized mean Hb and Hct levels were determined.

In our study, we aimed to reveal the factors affecting the postoperative Hb decrease in patients who underwent primary cesarean section. There are studies accepting a preoperative and postoperative Hb difference more than 1 g/dL, 2 g/dL or 2.5 g/dL significant (12-14). In our study, we accepted a difference of more than 2 g/dL significant.

In the literature, the rate of Hb decrease after cesarean section is reported as 0.6-1.8%. Although our findings are consistent with the literature, there are not many studies with sufficient data comparing Hb values before and after cesarean section (15-17). Consistent with our findings, previous studies have shown that patients requiring an emergent caesarean section have increased blood loss compared to elective cesarean section and require more blood transfusion (18, 19). While there is no difference between the groups regarding cesarean indications, conditions with increased atony are also risk factors for postoperative Hb decrease.

Table 3. Determination of risk factors in cases with hemoglobin decrease

Risk factors	Odds ratio (95% CI)
Uterotonic use during labor	1.37 (0.38-4.93)
Macrosomic infant	3.69 (0.92-14.86)
Multiple pregnancy	3.00 (0.61-14.85)
Head circumference greater than 37 cm	5.41 (1.49-19.65)
Maternal additional disease	3.85 (1.09-13.66)

Table 2. Demographic data and obstetric outcomes of pregnant women

	Hgb decrease <2 g/dL (n=418)	Hgb decrease ≥2 g/dL (n=142)	p
Age (years)	27.90±5.91	28.85±6.40	0.137
BMI (kg/m^2)	30.36±5.43	31.26±5.10	0.810
Gestational week	38.41±2.18	38.81±1.89	0.134
Maternal additional disease	12 (2.9%)	9 (6.3%)	0.060
Fetal birth weight (gr)	3104.4±679.09	3319.2±677.92	0.687
Apgar at 1 minute	8.26±1.24	8.33±1.15	0.345
Apgar at 5 minute	9.52±0.83	9.64±0.62	***0.036***
Duration of operation (minute)	31.71±8.11	33.50±7.87	0.875
Head circumference (cm)	34.87±2.48	35.46±1.78	0.219
Uterotonic use in labor	153 (36.6%)	75 (52.8%)	***0.001***
Elective cesarean section	201 (48.1%)	70 (49.3%)	0.803
Spinal anesthesia	34 (8.1%)	9 (6.3%)	0.487
Fetal weight >4000 g	37 (8.9%)	22 (15.5%)	***0.026***
Elongation of Kerr incision	51 (12.2%)	26 (18.3%)	0.068
Need for additional suture	46 (11%)	19 (13.4%)	0.445
Vascular injury	18 (4.3%)	10 (7.0%)	0.196
Bladder injury	7 (1.7%)	5 (3.5%)	0.189
T incision	12 (2.9%)	9 (6.3%)	0.060
Atony	27 (6.5%)	19 (13.4%)	0.009
Need for additional uterotonic	40 (9.6%)	28 (19.7%)	0.001
The need for transfusion	1 (0.2%)	11 (7.7%)	<0.001
Preoperative Hgb (g/dL)	11.81±1.46	12.47±1.41	0.607
Preoperative Hct (%)	36.11±3.71	37.26±3.60	0.879
Postoperative Hgb (g/dL)	10.68±1.50	9.77±1.50	0.015
Postoperative Hct (%)	32.50±3.84	29.71±4.04	0.443
Hgb decrease (g/dL)	1.12±0.56	2.69±0.60	0.048
Hct decrease (%)	3.62±2.01	7.54±2.42	0.018

Hgb: Hemoglobin, BMI: Body mass index, Hct: Hematocrit

Other risk factors associated with blood loss and the need for transfusion were general anesthesia, factors associated with uterine atony, increased parity, increase in newborn birth weight, prolonged surgical duration, failed labor induction, prolonged labor, and obstructed labor during the second phase (11, 16, 19, 20). In our study, these criteria were also evaluated, and an infantile head circumference greater than 37 cm was the most important risk factor in patients with Hb decrease more than 2 g/dL, followed by maternal additional disease, macrosomic infant (>4000 g), multiple pregnancy and uterotonic use in labor. These factors are also risk factors for development of atony. In our study, atony rate was significantly higher in the group with Hb decrease more than 2 g/dL. We found no difference between the two groups in terms of anesthesia type and intraoperative complications. This supports the idea that development of atony is an important factor for postoperative Hb decrease.

Studies have shown that visual estimates of peripartum blood loss are often incorrect, and other studies have shown that high blood loss is associated with a false estimate (21-24). Blood transfusion rate after cesarean section is 1.1-7.8% in developed countries and 25% in developing countries. In our study, the rate of blood transfusion was 2.1% (12 patients). In 11 of these patients, Hb decrease was more than 2 g/dL. The other case was the patient who underwent blood transfusion after emergent cesarean section because of low preoperative Hb level.

Study Limitations

The limitations of our study are retrospective design and limited number of patients. Besides, data on additional factors that caused differences in Hb levels, such as maternal smoking status, were lacking.

CONCLUSION

In conclusion, postoperative Hb decrease is relatively rare in primary cesarean sections. Although severe blood loss and blood product transfusion are rare, caution should be exercised if there is a risk factor in primary cesarean sections.

Ethics

Ethics Committee Approval: Retrospoective study.

Informed Consent: Retrospoective study.

Peer-reviw: Externally peer-reviewed.

Authorship Contributions

Concept: B.A.Ç., Design: N.K., Data Collection or Processing: P.Y.B., Analysis or Interpretation: N.K., A.A., Literature Search: P.K., P.Y.B., Writing: B.A.Ç., P.K.

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

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2017.60973
Eur Arch Med Res 2019; 35 (1): 6-10

Prevalence of Pulmonary Thromboembolism in Patients Admitted to Emergency Department

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Abstract

Objective: Pulmonary thromboembolism (PTE) is caused by thrombus originating from deep leg veins and obstructing the pulmonary artery and/or its branches. PTE is a preventable disease with high mortality and morbidity. It may relapse and is sometimes difficult to diagnose. Unnecessary diagnostic tests are applied to many patients with suspected PTE. The aim of this study was to investigate the frequency of PTE in patients presenting with a preliminary diagnosis of PTE to emergency clinic of a tertiary chest diseases hospital.

Methods: The triage forms of all patients who were admitted to the emergency clinic of tertiary chest diseases hospital within one year were examined. Demographic characteristics, risk factors for PTE, examinations and definite diagnosis data were obtained from the automation system of our hospital and evaluated retrospectively.

Results: In 2012, the number of patients admitted to the emergency department was 33,413 and 411 patients (0.12%) were examined with preliminary diagnosis of PTE. After initial evaluation, 292 patients (71%) were hospitalized, 117 patients (28.5%) were called for outpatient clinic follow-up, and two patients were referred to another hospital with non-PTE diagnosis (0.5%). After examinations at outpatient clinic or hospital admission, PTE was detected in 111 patients (27%) and deep vein thrombosis was found in 19 patients (4.6%). While 236 patients (57.4%) were diagnosed as non-PTE, 6 patients (1.5%) died before a definite diagnosis and 39 patients (9.5%) did not attend outpatient clinic examinations. PTE was detected in 16.2% (n=19) of the patients followed in outpatient clinic and in 31.5% (n=92) of the hospitalized patients.

Conclusion: In conclusion, the frequency of suspected PTE was 0.12% in patients admitted to the emergency department and 27% of these patients were diagnosed with definite PTE.

Keywords: Diagnosis, emergency department, pulmonary thromboembolism

INTRODUCTION

Pulmonary thromboembolism (PTE) is caused by thrombus originating from deep leg veins and obstructing the pulmonary artery and/or its branches. PTE is a preventable disease with high mortality and morbidity. It may relapse and is sometimes difficult to diagnose. The average annual incidence of venous thromboembolism (VTE) is 23-269/100.000 and it increases with age. The risk of VTE is 10 times higher after 80 years of age than at the age of 45-50 years (1-4).

Emergency physicians and chest disease specialists often demand some tests more than necessary to exclude the diagnosis of PTE. Although D-dimer test is widely used, false positive rate is high. In addition, when the pulmonary computed tomography (CT) angiography is used more than necessary in the emergency department, it has high cost and many other risks such as radiation and contrast-induced acute kidney failure (5).

Although pulmonary CT angiography is a very effective method in the diagnosis of thromboembolic lesions located in the main and lobar pulmonary artery branches, its sensitivity in

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Received: 28.09.2016
Accepted: 10.11.2017

Cite this article as: Çiftaslan Gökşenoğlu N, Aksoy E, Bodur Y, Tokgöz Akyıl F, Sevim T. Prevalence of Pulmonary Thromboembolism in Patients Admitting to Emergency Department. Eur Arch Med Res 2019; 35 (1):6-10

the diagnosis of thrombi affecting subsegmental branches is limited. The main reasons for this limitation are the performed examination, the quality of the examination and the experience of the radiologist (6).

In this study, the tests conducted in patients admitted to the emergency clinic of a tertiary chest diseases hospital with suspected PTE and the prevalence of definite PTE diagnosis among these patients were retrospectively evaluated.

METHODS

In this study, the patients who were admitted to the emergency department with suspected PTE between January-December 2012 were examined. The prevalence of PTE was investigated retrospectively.

Our hospital is a tertiary chest diseases hospital with 605 beds. The patients are referred from hospitals inside and outside İstanbul or they can be transported directly from their houses by 112 ambulance service. Patients admitted to the emergency department of our hospital are evaluated by a chest disease specialist and a resident. After the initial intervention and the necessary examinations, patients are prescribed and sent home or referred to the outpatient clinic for further examination. The patients with indication for hospitalization are hospitalized in the service or respiratory intensive care unit. If there is a suspected disease other than chest diseases, the patient is referred to the related outpatient clinic.

Complete blood count, routine biochemistry tests, ELISA D-dimer, arterial blood gas (ABG), electrocardiogram (ECG) and posteroanterior chest X-ray can be done in the emergency setting. The lower extremity venous Doppler ultrasonography (USG) can be performed at daytime outpatient clinic settings. During the study, pulmonary CT angiography and ventilation-perfusion (V/P) scintigraphy tests were not performed in our hospital and patients were referred to contracted centers.

A majority of patients with suspected PTE after initial evaluation and examination are hospitalized and treated. During the hospitalization, further examination and imaging procedures are done by contacting with the contracted centers. Some patients are treated in the intensive care unit if needed. Patients whose general condition is good and who do not need hospitalization or who do not accept hospitalization are referred to the outpatient clinic, and further procedures and examinations are planned from there. If the patients do not have the possibility of being evaluated in the outpatient clinic on the same day, PTE treatment is started with low molecular weight heparin at the outpatient clinic.

In this study, triage forms of the patients who applied to our hospital between 01.01.2012-31.12.2012 were examined. The demographic characteristics of the patients, risk factors for PTE, examinations and definite diagnosis data were obtained from the triage forms and the automation system of our hospital and evaluated retrospectively. ABG and D-dimer values on admission were recorded. The values of patients with and without PTE were compared. Bilateral lower extremity venous Doppler USG was performed for the diagnosis of deep vein thrombosis, which is a risk factor for PTE. ABG and D-dimer values in patients with and without deep vein thrombosis (DVT) were compared.

RESULTS

In 2012, the number of patients admitted to the emergency department was 33.413. During this period, 411 patients (0.12%) were examined for suspected PTE. While 50 (12%) of these 411 patients with suspected PTE were referred from another health institution, 361 patients (88%) presented with various complaints and were evaluated as suspected PTE by emergency physicians. Two hundred and forty-one patients (59%) were female and 170 (41%) were male, and the mean age was 62±18 (17-103) years. Risk factors for PTE were detected in 241 patients (55%). These risk factors include advanced age, major surgical intervention, long-term travel history, presence of malignancy, previous embolism, and oral contraceptive use.

As a laboratory evaluation, all patients underwent chest X-ray and oxygen saturation measurement with pulse oximetry. ABG and D-dimer values were checked in 226 patients (55%) and 276 patients (67%), respectively (Table 1 and Table 2). After the initial evaluation, 292 patients (71%) were hospitalized, 117 patients (28.5%) were called for outpatient clinic follow-up, and two patients were referred to another hospital with non-PTE diagnosis (0.5%) (Table 3).

After examinations at outpatient clinic or hospital admission, PTE was detected in 111 patients (27%) and deep DVT was found in 19 patients (4.6%). While 234 (57.4%) patients were diagnosed as non-PTE, 6 patients (1.5%) died before a definite diagnosis and 39 patients (9.5%) did not attend outpatient clinic examinations. Within this group, there were also patients who were planned to be examined after hospitalization and their results were also planned to be controlled in the outpatient clinic after discharge. Since these patients did not attend to outpatient clinic for showing their results, their diagnosis was not finalized.

PTE was detected in 16.2% (n=19) of the patients followed in outpatient clinic and in 31.5% (n=92) of the hospitalized patients. Massive PTE was found in 1.8% (n=2) of 111 diagnosed patients. These patients were monitored in the intensive care unit and diagnosed by echocardiography.

The mean length of hospitalization was 9.8±4.6 days (1-27 days). For diagnosis in hospitalized patients, 126 bilateral lower extremity venous Doppler USG (thrombus in 27.8%), 100 CT angiography (thrombus findings in 58%) and 39 V/Q scintigraphy (33.3% diagnostic for PTE) were performed.

DISCUSSION

The annual incidence of VTE is 23-269/100.000 (1) and it is known to increase with age. While the incidence of VTE was 1/10.000 in the 4th decade of life, it increases rapidly after 45 years of age and reaches 5-6/1000 around 80 years of age (7). With the new imaging methods, the number of patients examined for PTE and the rate of diagnosis increase (8). Particularly, the widespread use of CT angiography plays an important role in this increase. The effect of increase in diagnosis rates on patient prognosis is controversial. Wiener et al. (9) reported that initiation of treatment to a larger number of patients with the development of diagnostic methods did not significantly reduce mortality. In addition, pulmonary CT angiography increases the length of stay in the emergency room and increases the exposure to radiation. Besides, contrast-induced acute kidney failure can also be seen.

In our study, the prevalence of PTE was 0.12% in patients admitted to the emergency department of our hospital, and 27% of these patients were diagnosed with definite PTE. In other words, the incidence of PTE in our study was 332/10.000. Compared to the studies performed in our country, the number of patients admitted with a preliminary diagnosis of PTE and the number of patients diagnosed with definite PTE within one year are very high in our study (10, 11). Since our hospital is an emergency service clinic where only respiratory diseases are evaluated, the incidence of PTE is expected to be higher. Some of our patients have been sent from other health institutions, including patients with new-onset PTE during hospitalization in the internal medicine clinics of other hospitals or after surgery. Three hundred and sixty-one patients (81%) admitted themselves with various respiratory symptoms such as dyspnea and chest pain. This finding shows that PTE is an important diagnosis in patients admitting to the emergency clinic of tertiary chest

Table 3. Distribution of diagnosis of 411 patients with suspected PTE after pre-evaluation in emergency department

	n (%)
Inpatient follow-up (n=292)	
PTE	92 (31.5%)
DVT	19 (6.5%)
Non-PTE	170 (58.2%)
Dead	6 (2)
Other*	5 (1)
Outpatient clinic follow-up (n=117)	
PTE	19 (16%)
DVT	0
Non-PTE	64 (55%)
Other**	34 (29%)
Transfer to another hospital (n=2)	2 (0.5%)

*The patient whose diagnosis is not definite because he/she did not attend outpatient clinic controls after discharge. **Patients who did not attend to outpatient clinic controls

PTE: Pulmonary thromboembolism, DVT: Deep vein thrombosis

Table 1. Arterial blood gas values of patients with pulmonary thromboembolism

Arterial blood gas	PTE n (%)	DVT n (%)	Non-PTE n (%)	Others n (%)	Total
Normal	2 (15.4%)	0	10 (76.9%)	1 (7.7%)	13
Hypocapnic	10 (25.6%)	2 (5.1%)	24 (61.5%)	3 (7.7%)	39
Hypoxic	6 (14.6%)	3 (7.3%)	30 (73.2%)	2 (4.8%)	41
Hypoxic hypocapnic	45 (34.1%)	4 (3%)	72 (54.5%)	11 (8.3%)	132
Total	63 (28%)	9 (4%)	136 (60.4%)	17 (7.6%)	225

PTE: Pulmonary thromboembolism, DVT: Deep vein thrombosis

Table 2. D-dimer levels of patients with preliminary pulmonary thromboembolism diagnosis

D-dimer	PTE n (%)	DVT n (%)	Non-PTE n (%)	Others n (%)	Total
Normal	0	0	19 (73.1%)	7 (26.9%)	26
High	59 (23.7%)	16 (6.4%)	145 (58.2%)	29 (11.6%)	249
Total	63 (28%)	16 (5.8%)	164 (59.6%)	36 (13.1%)	275

PTE: Pulmonary thromboembolism, DVT: Deep vein thrombosis

diseases hospitals and that it should be considered in the differential diagnosis.

In our study, the data of the patients admitted to emergency room with a preliminary PTE diagnosis were evaluated and it was found that most of the patients with suspected embolism were hospitalized (71% were hospitalized and 28.5% were called to the outpatient clinic control). According to the results, 31.5% of the patients hospitalized with suspected PTE and 16.2% of the patients called to the outpatient clinic control were diagnosed with definite PTE. An important result was that 29% of the patients who were called to the outpatient clinic for further examination did not attend follow up. These patients were also likely to have PTE. Therefore, we think that it is important to inform the patients about the importance of the suspected disease and the importance of coming to the outpatient clinics. Difficulties in working conditions and high number of patients in emergency departments play a role in failure to do these disclosures as required.

There are several studies evaluating clinical and laboratory tests for diagnosing a disease in emergency departments, some parameters and scoring systems come to the fore. In the emergency department of our hospital, first of all, a clinical risk assessment is made by parameters such as age, symptom, physical examination findings, risk factors for VTE, ABG findings, then CT angiography, V/P scintigraphy or Doppler USG is planned regarding D-dimer level and chest radiography. However, there are deficiencies in the processing of these clinical evaluation results in triage forms as clinical risk scores. Therefore, the diagnostic value of clinical scoring systems has not been discussed in our study.

In our clinical practice, the most important parameter leading to suspected PTE after symptoms is the presence of risk factors for PTE. If there is any risk factor in a patient with chest pain and shortness of breath, PTE is immediately taken into differential diagnoses. In this study, risk factor was found in 55% of patients with suspected PTE. The prevalence of DVT, which is an important risk factor for PTE, is 56/100.000 in all patients who applied to the emergency department in our study. In our study, the most common risk factors for DVT were immobilization and advanced age. Other risk factors were history of malignancy, surgery and DVT.

Another parameter used in clinical evaluation is ABG findings. Although ABG findings are not diagnostic alone, it may lead to suspicion of PTE. In our study, 63 out of 225 patients who were evaluated with ABG were diagnosed with PTE. Only 2 patients had normal ABG values. While the most common blood gas measurement in PTE was hypoxic, hypocapnic blood gas values, it was found that these values could also be seen in patients diagnosed with non-PTE. The main consideration in the diagnosis of PTE is that most of the patients with normal blood gas have been diagnosed with a non-PTE.

D-dimer is a test used with more negative predictive value in the diagnosis of PTE. It can be positive in many cases other than PTE. In practice, it is recommended to switch to diagnostic methods such as CT angiography without D-dimer in patients with high clinical scoring. In our study, no PTE was detected in any of the patients with normal/low D-dimer level. The level of D-dimer was not normal in any of the patients diagnosed with PTE, but it was higher than normal in patients diagnosed with DVT. In contrast, 58% of 249 patients with high D-dimer had PTE and non-DVT diagnoses.

Study Limitations

In our study, CT angiography was performed in 100 patients with suspected PTE and thrombus was found in 58% of the patients. In other patients, PTE was diagnosed or excluded by other methods. At that time, the availability of CT angiography in emergency conditions in our hospital may have limited the use of this examination.

CONCLUSION

In this study, the prevalence of PTE and clinical approach in the emergency clinic of a tertiary chest diseases hospital were evaluated. The symptoms and signs of PTE can be confused with many respiratory diseases. PTE is an important disease with high rates of morbidity and mortality. The clinical suspicion of emergency physicians and a wide differential diagnosis approach are vital for rapid diagnosis and treatment.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ç.G., Concept: T.S., Design: T.S., Data Collection or Processing: Y.B., Analysis or Interpretation: E.A., Literature Search: Y.B., Writing: F.T.A.

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

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2017.94830
Eur Arch Med Res 2019; 35 (1): 11-4

The Effect of Pre-treatment Intraretinal Cyst Diameter on Visual and Anatomical Outcomes after Intravitreal Ranibizumab Treatment in Cystoid Macular Edema Secondary to Branch Retinal Vein Occlusion

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Abstract

Objective: To investigate the effect of the largest intraretinal cyst diameter in the macula on visual and anatomical outcomes after intravitreal ranibizumab (IVR) treatment in cystoid macular edema (CME) secondary to branch retinal vein occlusion (BRVO).

Methods: Forty-two eyes of 42 patients who underwent IVR treatment for CME secondary to BRVO were studied retrospectively. Patients were evaluated after three-monthly IVR injection. The pre-treatment and post-treatment spectral domain optical coherence tomography parameters and best-corrected visual acuity (BCVA) were recorded. The effect of pre-treatment intraretinal cyst diameter on post-treatment central macular thickness (CMT) and BCVA was investigated.

Results: There were 27 (64.3%) men and 15 (35.7%) women in the study. The mean age was 59.5±9.6 years. The mean pre-treatment CMT was 485±160 µm and the mean pre-treatment BCVA was 0.84±0.55 logMAR. After 3 doses of IVR, CMT gain was 201±168 µm and BCVA gain was 0.41±0.43 logMAR. The mean pre-treatment cyst diameter was 241±121 µm. Although there was a low degree positive correlation between pre-treatment cyst diameter and CMT gain, this correlation was not statistically significant (r=0.245, p=0.059). Although there was a low degree negative correlation between pre-treatment cyst diameter and BCVA, this correlation was not statistically significant (r=-0.145, p=0.184).

Conclusion: There was no statistically significant correlation between pre-treatment cyst diameter and CMT and BCVA gain in CME treatment secondary to BRVO.

Keywords: Branch retinal vein occlusion, intraretinal cyst diameter, macular edema, optical coherence tomography

INTRODUCTION

Retinal vein occlusion is the most common retinal vascular pathology after diabetic retinopathy (1). Retinal vein occlusions are divided into two as central retinal vein occlusions and branch retinal vein occlusions (BRVOs). Vascular pressure during the arteriovenous transition, degenerative changes in veins and hypercoagulability are the major pathophysiological events that cause BRVO. These events increase vascular endothelial growth factor (VEGF) secretion and increase vascular permeability and thus cause macular edema (2, 3). Macular edema is the most common cause of visual loss in BRVO (4, 5). Laser photocoagulation, intravitreal steroid injection and intravitreal anti-VEGF injection are used for the treatment of macular edema (6, 7). The same results cannot be obtained from all patients despite anti-VEGF therapy. This may be due to the fact that the pathophysiology of the disease cannot be fully elucidated or it may be due to the pre-treatment factors related to the patient.

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Received: 12.09.2017
Accepted: 18.12.2017

Cite this article as: Bayat AH, Çakır A, Bölükbaşı S, Erden B, Şehirli ŞG, Elçioğlu MN. The Effect of Pre-treatment Intraretinal Cyst Diameter on Visual and Anatomical Outcomes after Intravitreal Ranibizumab Treatment in Cystoid Macular Edema Secondary to Branch Retinal Vein Occlusion. Eur Arch Med Res 2019;35 (1):11-14

The aim of this study was to investigate the effect of pre-treatment cyst diameter on visual and anatomic outcome in cystoid macular edema (CME) secondary to BRVO.

METHODS

The data of 42 patients, who were admitted to Okmeydanı Training and Research Hospital Retina Outpatient Clinic between January 2015 and January 2017 and who were treated with intravitreal ranibizumab (IVR), were evaluated retrospectively. The study was conducted in accordance with the Declaration of Helsinki and the ethics committee approval was obtained from the Ethics Committee of Okmeydanı Training and Research Hospital.

All of the patients received three-monthly IVR treatment. Patients with cystic changes due to intraretinal fluid accumulation in any retinal layer detected by spectral domain optical coherence tomography (SD-OCT) were included in the study with the diagnosis of CME (Figure 1).

Data regarding age, gender, pre-treatment and post-treatment best-corrected visual acuity (BCVA) and central macular thickness (CMT) were recorded. Pre-treatment CMT and intraretinal foveal cyst diameters obtained from SD-OCT images were noted. Cyst diameters were measured as the vertical diameter of the largest intraretinal cyst in the SD-OCT sections by two different ophthalmologists. The cyst diameter was measured manually using the caliper in the device (Figure 2). The mean values of these measurements were accepted as data. There were no statistically significant differences between the measurements of two ophthalmologists.

All patients underwent routine ophthalmologic examination before the treatment and at the first month after three-monthly treatment. Biomicroscopic examination, indirect ophthalmoscopy, BCVA measurement, intraocular pressure measurement with Goldmann applanation tonometry, and SD-OCT (Heidelberg engineering, Heidelberg, Germany) were performed respectively. Best-corrected visual acuity was determined with Snellen chart and converted to "the logarithm of the minimal angle of resolution (logMAR)" unit for statistical analysis.

Patients with glaucoma, other macular and/or retinal disease, neurological and systemic diseases that cause vision loss and patients who did not received three-monthly IVR treatment were not included in the study.

Statistical Analysis

Data were analyzed using SPSS for Windows 21 software (IBM Corp., Armonk, NY, USA). Descriptive statistics were shown as mean ± standard deviation (smallest-largest) for continuous variables and categorical variables as number of cases and percentage (%). A multivariate linear regression analysis was performed to examine the effect of pre-treatment findings on BCVA and CMT gains. Significance was evaluated at $p<0.05$. After the study, post-hoc power analysis was performed with G*Power 3.1 (Heinrich-Heine-Universität, Düsseldorf, Germany) and the power of the study was 99.9%.

RESULTS

Forty-two eyes of 42 patients diagnosed as having CME secondary to BRVO were included in this study. Of these patients, 27 were male (64.3%) and 15 were female (35.7%). The mean age was 59.5±9.6 years. The mean follow-up period was 5.7±0.7 months. The mean CMT was 485±160 µm. The pre-treatment BCVA was calculated as 0.84±0.55 logMAR. After 3 doses of IVR, CMT and BCVA gains were 201±168 µm and 0.41±0.43

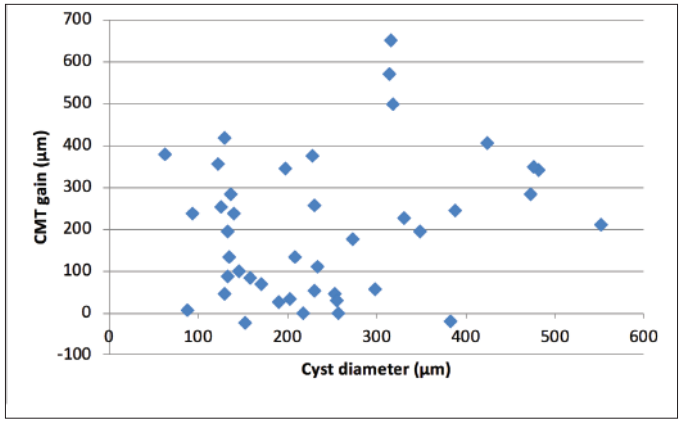

Figure 1. The relationship between pre-treatment cyst diameter and CMT gain (scatter-plot) shows no significant trend in distribution
CMT: Central macular thickness

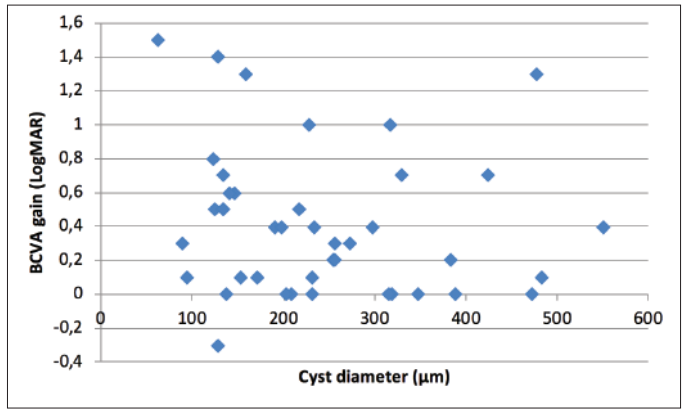

Figure 2. The relationship between pre-treatment cyst diameter and BCVA gain (scatter-plot) shows no significant trend in distribution
BCVA: Best-corrected visual acuity

logMAR, respectively. The mean pre-treatment cyst diameter was 241±121 µm.

Multivariate linear regression analysis was performed by using the "Backward" method to compare the factors that could have a prognostic and predictive effect on the BCVA and CMT gains. The relationships between age, gender, pre-treatment cyst diameter, BCVA and CMT and BCVA and CMT gains were investigated.

The CMT gain was statistically significantly correlated with pre-treatment CMT and BCVA values (r=0.879 and p<0.001, r=0.589 and p<0.001, respectively) (Table 1). Although there was a positive correlation between the pre-treatment cyst diameter and CMT gain, this correlation was not statistically significant (r=0.245, p=0.059) (Table 1). The relationship between pre-treatment cyst diameter and CMT gain is shown in Figure 1. As can be seen, there is no trend in this distribution (Figure 1). Pre-treatment BCVA and CMT values were significantly related with BCVA gain (r=0.440, p=0.002 and r=0.340, p=0.014, respectively) (Table 1). Although there was a negative correlation between pre-treatment cyst diameter and BCVA gain, this correlation was not statistically significant (r=-0.145, p=0.180) (Table 1). This relationship is shown in figure 2 and no trend was observed in this distribution.

DISCUSSION

In recent literature, there are many studies that have demonstrated the efficacy of ranibizumab in the treatment of CME due to BRVO (8-12). But as seen in these studies, all patients do not give the same response to this treatment. This suggests that there are other factors affecting the success of the treatment, as well as the pathophysiological process of the disease, which is not fully elucidated. There are some publications investigating this situation in the current literature (13, 14).

According to the results of this study, patients with high pre-treatment CMTs had higher CMT gains. In their study, Ach T et al. (15) also found that pre-treatment CMT was a good prognostic factor in response to anti-VEGF treatment. In this study, it was also found that the patients with low BCVA had more CMT gain. The fact that patients with increased pre-treatment CMT had low pre-treatment BCVA would explain this situation. Indeed, Kriechbaum et al. (16) support this finding.

Table 1. Regression coefficients and p values of parameters affecting CMT and BCVA gains

	CMT gain		BCVA gain	
	Beta coefficient	**p**	**Beta coefficient**	**p**
Pre-treatment cyst diameter	0.245	0.059	0.145	0.180
Pre-treatment CMT	0.879	***<0.001***	0.340	***0.014***
Pre-treatment BCVA	0.589	***<0.001***	0.440	***0.002***

CMT: Central macular thickness, BCVA: Best-corrected visual acuity

Some previous studies have shown that patients with low pre-treatment BCVA have lower post-treatment BCVA (17). In contrast, patients with lower pre-treatment BCVA were found to have higher visual gain in this study. The study by Gesine B. Jaissle et al. (13) supports this finding. This can be explained by the "ceiling effect" in the literature. "Ceiling effect" is the situation that explains that patients with lower pre-treatment values will have more gain as a result of reaching the maximum value.

In their study, Groneberg T et al. (18) found that the pre-treatment cyst diameter was associated with visual gain. They showed that small cysts had more letter gain. In contrast to Groneberg T et al. (18), we could not find a statistically significant relationship between pre-treatment cyst diameter and BCVA and CMT gains in this study.

CONCLUSION

In conclusion, pre-treatment BCVA and CMT can be used as a prognostic factor for visual gain and anatomic success in the treatment of CME secondary to BRVO. In contrast, we believe that the use of pre-treatment cyst diameter as a prognostic factor is not appropriate.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.H.B., A.Ç., S.B., B.E., Ş.C.Ş., M.N.E., Concept: A.H.B., Design: B.E., M.N.E., Data Collection or Processing: A.H.B., A.Ç., Ş.C.Ş., Analysis or Interpretation: A.Ç., Literature Search: A.H.B., S.B., Writing: A.H.B.

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

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.13471
Eur Arch Med Res 2019; 35 (1): 15-18

The Evaluation of Macular Thickness by Optical Coherence Tomography in Autoimmune Diseases

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Abstract

Objective: The aim of this study was to evaluate macular thickness by using optical coherence tomography (OCT) in autoimmune diseases where no ocular clinical findings were observed.

Methods: In our study, we evaluated both eyes of 34 patients with rheumatoid arthritis (RA), 31 patients with systemic lupus erythematosus (SLE) and 21 healthy volunteers. Central macular thickness and nasal, temporal, superior and inferior macular thicknesses within 3 mm area of macula were measured in all patients using RTVue-100 Spectral-Domain OCT (Optovue Inc., Fremont, CA). The measurements were compared using one-way ANOVA test in SPSS 21.0 program. Comparisons were considered significant when $p<0.05$.

Results: There was no statistical significant difference in central macular thickness and nasal, temporal, superior and inferior macular thicknesses within 3 mm area of macula between RA and SLE patients, and age- and gender-matched healthy volunteers (central; p=0.583, nasal; p=0.220, temporal; p=0.303, superior; p=0.466, inferior; p=0.698).

Discussion: Our study demonstrates that macular thickness in RA and SLE patients without ocular findings do not differ with healthy individuals.

Keywords: Autoimmune disease, macula, optical coherence tomography, rheumatoid arthritis, systemic lupus erythematosus

INTRODUCTION

Autoimmune rheumatologic diseases are major causes of morbidity that disrupt the quality of life with multiple organ involvement. Among these diseases, rheumatoid arthritis (RA) is the most common and is characterized by peripheral joint and extra-articular systemic involvement (1, 2). Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by involvement of the eye, skin, kidney, joints and brain (3, 4). The etiology of both autoimmune diseases is not fully understood (5, 6). Keratoconjunctivitis sicca, episcleritis and scleritis are defined ocular manifestations of RA (7, 8). The ocular manifestations of SLE include keratoconjunctivitis sicca, optic nerve, retinal and choroidal vascular changes, recurrent stromal infiltrations, peripheral ulcerative keratitis, and corneal involvement with corneal erosions (9, 10).

Retinal vascular involvement is seen in both autoimmune diseases. The association between ocular complications and retinal vascular involvement has been shown in RA patients (11-14). It has also been shown that the level of endothelin-1, which is a vasoconstrictor agent in RA patients, is associated with extra-articular findings of the disease (15). Retinopathy in SLE patients has been shown to be due to vascular stenosis secondary to leukocyte and immune complex accumulation in retinal vascular endothelial area (16, 17). In these patients, retinal and choroidal vascular stenosis, which may occur due to vasoconstrictor agents and immune complex accumulations in the chronic phase, can

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Received: 06.03.2017
Accepted: 28.01.2018

Cite this article as: Duru Z, Altunel O. The Evaluation of Macular Thickness by Optical Coherence Tomography in Autoimmune Diseases. Eur Arch Med Res 2019; 35 (1):15-18

be seen without any ocular clinical findings (16, 17). There is limited number of studies evaluating the affected macular area due to ischemia caused by stenosis in the retinal and choroidal vascular area in patients with no ocular clinical findings. Optical coherence tomography (OCT) provides objective information in the evaluation of retinal layer thicknesses (18). In our study, it was aimed to evaluate macular areas by OCT in autoimmune diseases without ocular clinical findings.

METHODS

Thirty-four patients with RA and 31 patients with SLE who were followed by physical therapy and rehabilitation clinic and 21 healthy volunteers were included in the study. Following approval of the Institutional Review Board, the study was conducted in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from all participants. The patients included in the study were in remission with hydroxychloroquine treatment and had no active rheumatological clinical findings.

Patients with glaucoma diagnosis, ocular trauma and history of intraocular surgery, choroidal neovascularization or other macular diseases affecting visual acuity, retinopathy due to chloroquine toxicity, patients whose spherical equivalence (D) value measured by autorefractometer is greater than -3 or +3, and patients with severe cataracts and corneal disease reducing the quality of OCT imaging were excluded from the study. Patients with active rheumatologic complaints and clinical signs in at least one year were excluded from the study. Patients with diabetes mellitus, essential hypertension, coronary artery disease and histories of corticosteroid use in the last two years due to their side effects were excluded from the study. Full ophthalmologic examination including corrected visual acuity, measurement of intraocular pressure with Goldmann applanation tonometry, detection of refraction disorder with autorefractometer, biomicroscopic examination and dilated fundus examination was performed in all groups. The patients had no active or previous anterior and posterior segment ocular signs. All OCT scans were performed by the same experienced OCT technician at the same time interval (from 10:00 to 12:00) using the RTVue-100 Spectral Domain (Optovue Inc., Fremont, CA). Central macular thickness and nasal, temporal, superior and inferior macular thicknesses within 3 mm area were evaluated in all patients.

Statistical Analysis

Statistical analysis was performed by SPSS for Windows Version 21.0 (Chicago, Illinois) package program. In our study, power analysis was done in the sample size calculation. The effect size was 0.498 and the sample size required for the confidence level of 95% was calculated as 40 eyes in each group (19). Continuous numerical variables were expressed as mean ± standard deviation and categorical data as numbers and percentages. Histogram, Q-Q plot and Shapiro-Wilk test were used as tests of normality for continuous numerical variables. Chi-square test was used to compare the qualitative data between the groups. Macular thickness was compared with one-way ANOVA between all groups. The significance level was taken as $p<0.05$.

RESULTS

In our study, both eyes of 34 RA patients, 31 SLE patients and 21 healthy volunteers were evaluated. The demographic characteristics and follow-up periods of the patients are shown in Table 1 and no significant difference was found between the groups. Table 2 shows macular thickness of all patients measured from the central macular area and from the nasal, temporal, superior and inferior areas within the 3 mm area. No significant difference was observed between RA patients, SLE patients and healthy volunteers.

DISCUSSION

Autoimmunity related cytokines, immune complexes, T cell activation and oxidative events play a role in the pathogenesis of immune diseases (20, 21). Vascular structures and choroidal layer in the vascular network is of importance in the provision of the metabolic needs of the retina (22). As a result of cellular activity, retinal damage occurs especially in the retinal vascular endothelial area due to immune complex deposits and recurrent vasculitis episodes. In a study in which 60 RA patients without

Table 1. Demographic characteristics and follow-up times

	Systemic lupus erythematosus	Rheumatoid arthritis	Healthy volunteers	p
Gender				
Female	29 (93.5%)	32 (94.1%)	19 (90.5%)	0.751*
Male	2 (6.5%)	2 (5.9%)	2 (9.5%)	
Age (years)				
Mean ± SD	46.3±12.5	47.2±7.7	45.5±8.3	0.816**
Min-max	21-70	25-64	29-59	
Follow-up period (years)				
Mean ± SD	6.6±6.5	7.7±6.2	-	
Min-max	1-25	1-30	-	

*Chi-square test, **One-way ANOVA test, SD: Standard deviation, Min: Minimum, Max: Maximum

clinical and ophthalmoscopic examination showed no signs of inflammation, patients were evaluated by fluorescein angiography imaging and 18% of patients had vasculitis in retinal vessels. Histopathologically, retinal vascular stenosis secondary to immunocomplex accumulation in retinal vascular endothelial areas has been shown in eyes of patients with SLE (16, 17). In addition, the levels of endothelin-1, a potent vasoconstrictor peptide, have been shown to increase in RA patients (15). Endothelin-1 may cause secondary vascular circulation disorder in RA and some other autoimmune diseases and may cause ischemia in the choroid and optic nerve.

Our study was designed with the idea that there may be changes in macular thickness in different retinal areas due to circulatory disorder and ischemia due to vascular damage. In the literature, choroidal layer in which autoimmune diseases can be clearly seen is examined. Duru et al. (23) and Altınkaynak et al. (24) showed that the choroidal layer was thinner in RA and SLE patients than in the normal population. This was attributed to vascular stenosis and vasculitis caused by immune complex deposits accumulated in the vascular space. However, the authors could not prove choroidal ischemia secondary to vascular stenosis with structural thinning in the choroidal layer. Therefore, they discussed the necessity of performing fundus fluorescein angiography and/or indocyanine angiography examinations to evaluate choroidal perfusion along with OCT to evaluate especially external retinal layer and full macular thickness. In our study, the presence of choroidal and retinal vascular ischemia was evaluated by examining the macular thickness in the central macula and all quadrants in the 3 mm area, and there was no significant difference with healthy group. Therefore, it can be said that there is no clinically significant choroidal and retinal vascular ischemia that can affect macular thickness in RA and SLE patients. This situation can be explained by the prevention of recurrent vasculitis attacks by treatment and control of immune deposit in patients enrolled in this study. The limitations of our study were the lack of evaluation of external and internal retinal layer thicknesses, the lack of fluorescein angiography and indocyanine angiography, and the limited number of patients.

In our study, retinal damage due to hydroxychloroquine used by RA and SLE patients was also evaluated. Hydroxychloroquine sulfate is an antimalarial drug that is frequently used in the treatment of autoimmune diseases such as SLE and RA. When histologically examined, chloroquine was found to accumulate in melanin-containing tissues (25). It was observed that the first changes with these accumulations began in ganglion cells and then caused damage in the outer segments of the photoreceptors. In the following periods, rod and con losses are followed by migration of retinal pigment epithelium to outer retinal layers. In the following period, "Bull's eye maculopathy", which is characterized by decreased visual acuity and central visual field damage, may occur (26). There was no evidence of chloroquine

Table 2. Comparison of central macular thickness and nasal, temporal, superior and inferior macular thickness within 3 mm area

	Systemic lupus erythematosus	Rheumatoid arthritis	Healthy volunteers	p*
Central macular thickness (µm)				
Mean ± SD	248.4±19.2	251.3±23.4	247.1±22.1	0.583
Min-Max	212-289	214-315	213-307	
Superior macular thickness (µm)				
Mean ± SD	322.7±15.9	319.2±20.2	319.6±13.5	0.466
Min-max	280-354	240-357	285-344	
Temporal macular thickness (µm)				
Mean ± SD	309.1±13.0	301.1±22.2	304.5±11.8	0.303
Min-max	282-352	200-337	267-328	
Inferior macular thickness (µm)				
Mean ± SD	317.9±19.1	315.9±21.1	318.7±12.8	0.698
Min-max	231-350	242-351	278-346	
Nasal macular thickness (µm)				
Mean ± SD	322.1±15.0	320.6±17.1	316.3±18.4	0.220
Min-max	286-352	272-358	233-349	

*One-way ANOVA test SD: Standard deviation, Min: Minimum, Max: Maximum

toxicity in OCT scans in our study. Due to the fact that all of our patients were under the treatment of hydroxychloroquine, it can be concluded that the use of hydroxychloroquine did not cause a change in the thickness of the macula.

CONCLUSION

Our study showed that there was no change in macular thickness in RA and SLE patients who were in remission with hydroxychloroquine treatment and who had no ocular clinical findings. We believe that this study may be a guide for future studies.

Ethics

Ethics Committee Approval: The study was approved by the Institutional Review Board, the study was conducted in accordance with the principles of the Helsinki Declaration.

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.D., Concept: Z.D., Design: O.A., Data Collection or Processing: O.A., Analysis or Interpretation: Z.D., Literature Search: O.A., Writing: O.A.

Conflict of Interest: The authors have no conflicts of interest to declared.

Financial Disclosure: The authors declared that this study has received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2017.99267
Eur Arch Med Res 2019; 35 (1): 19-22

Incidence of Unlicensed and Off-Label Prescription in the Treatment of Urological Cancers in Turkey: Assessment of Legislative and Regulatory Policy

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Abstract

Objective: Turkish Ministry of Health defines "off-label" drug use as the use of licensed pharmaceutical products at doses outside the approved indication and the use of unlicensed medicinal products that are imported for the purpose of individual treatment. The aim of this study was to evaluate the use of off-label or unlicensed drugs in urological cancers in order to understand the perspective of Turkey within this area of healthcare provisions.

Methods: This study involved metastatic bladder cancer patients (n=105) receiving paclitaxel or other off-label drugs, metastatic renal cell cancer patients (n=194) receiving sorafenib, sunitinib or other off-label drugs, and metastatic testicular cancer patients (n=44) receiving paclitaxel, gemcitabine or other off-label drugs in Turkey. A computer search was performed using patient-based database of Turkish Medicines and Medical Devices Agency (TITCK).

Results: According to data from TITCK database, the number of applications for off-label drug use was 86 for metastatic bladder cancer, 136 for metastatic renal cell cancer and 44 for metastatic testicular cancer. The approval rates for off-label drugs were 90.47% for metastatic bladder cancer, 62.88% for metastatic renal cell cancer and 88.63% for metastatic testicular cancer. University hospitals made the majority of applications for metastatic bladder cancer (79.89%), metastatic renal cell cancer (64.76%) and metastatic testicular cancer (79.89%). The most preferred off-label drugs for bladder, renal and testicular cancers were paclitaxel (84.04%), sorafenib (68.42%) and paclitaxel (43.24%), respectively.

Conclusion: Off-label drug use in urological cancers is increasing in Turkey. If the use of off-label drugs increases in parallel to the use of off-label urology drugs, it is necessary to define new ways of evaluating applications.

Keywords: Bladder cancer, off-label prescription, renal cell cancer, testicular cancer

INTRODUCTION

A large number of licensed medications are routinely used for unapproved indications or dosages, routes of administration, or age groups. This kind of use that is not described in the package insert is called "off-label use". This term includes unlicensed, unregistered or "compassionate use" (1). Off-label use is prescribing pharmaceuticals for an unapproved indication or in an age group outside of an approved indication for use, dosage, or route of administration (2).

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Received: 03.10.2017
Accepted: 22.12.2017

Cite this article as: Tanyeri MH, Koçkaya G, Büyükokuroğlu ME, Tanyeri P, Vural İM, Kerman Saim. Incidence of Unlicensed and off-Label Prescription in the Treatment of Urological Cancers in Turkey: Assessment of Legislative and Regulatory Policy. Eur Arch Med Res 2019; 35 (1):19-22.

Eur Arch Med Res
2019; 35 (1): 19-22

In Turkey, physicians may prescribe off-label or unlicensed drugs under the control of Turkish Medicines and Medical Devices Agency (TITCK) (http://www.titck.gov.tr). TITCK evaluates off-label and unlicensed drug use for each patient through off-label application procedures. A physician who wants to prescribe an off-label or unlicensed pharmaceutical has to apply to TITCK for patient-based approval. TITCK evaluates each application based on published scientific evidence and academic consultants. If TITCK approves off-label or unlicensed prescriptions, the cost of the drugs subject to these prescriptions will be reimbursed by Turkish Social Security Institution (SGK) (http://www.sgk.gov.tr).

The aim of this study was to evaluate the use of off-label or unlicensed drugs in urological cancers to understand Turkey's perspective within this area of healthcare provisions. In addition, we hope that the results of this study will help to update the guidelines and determine pharmaceuticals and off-label indications in urological cancers, such as metastatic bladder cancer, renal cell cancer and testis cancer.

METHODS

This study involved metastatic bladder cancer patients (n=105) receiving paclitaxel or other off-label drugs, metastatic renal cell cancer patients (n=194) receiving sorafenib, sunitinib or other off-label drugs, and metastatic testicular cancer patients (n=44) receiving paclitaxel, gemcitabine or other off-label drugs in Turkey between May 2008-May 2011. Informed consent was obtained from these patients in accordance with the legislation on the use of off-label drugs. These are available in the records of Ministry of Health.

The TITCK database was used to examine off-label drug use in the treatment of urological cancers. In the analysis, the off-label use of drugs during the urological cancer therapy were evaluated to provide an understanding of Turkey's perspective within this area of healthcare provisions. Outcomes were evaluated based on the application status, approval period, locational applications, hospital category and drug status. In addition, it was aimed to help update the guidelines and determine pharmaceuticals and off-label indications for no-need to approval process of off-label indications in urological cancer pharmaceuticals.

Statistical Analysis

Discriminant analysis was conducted with Microsoft Office Excel 2011 program.

RESULTS

The data obtained from TITCK database between May 2008-May. 2011 showed that 86 applications were submitted for off-label metastatic bladder cancer drug use, 136 applications were submitted for off-label metastatic renal cell cancer drug use and 44 applications were submitted for off-label metastatic testicular cancer drug use. The mean age was 64.62±19.42 years for metastatic bladder cancer, 60.52±19.22 years for metastatic renal cell cancer and 39.77±34.66 years for testicular cancer (Figure 1).

Ninety-five (90.47%) off-label drug applications were approved for metastatic bladder cancer, 122 (62.88%) for metastatic renal cell cancer and 39 (88.63%) for metastatic testicular cancer (Figure 2). Also, these applications were concluded in a 12-week period for all cancers. However, the procedure may be longer if the application requirements are acceptable.

Most of the applications for metastatic bladder cancer (79.89%) were made by university hospitals, followed by training and research hospitals (28.57%) and private hospitals (6.66 %). This distribution was similar for renal and testicular cancers (Figure 3).

The most preferred off-label drugs for bladder, renal and testicular cancers were paclitaxel (84.04%), sorafenib (68.42%)

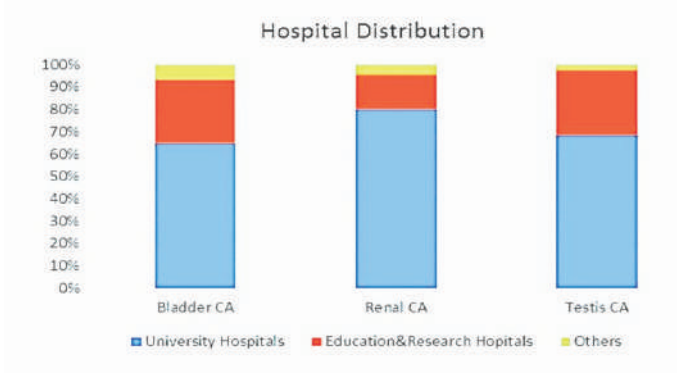

Figure 1. Hospital distribution
CA: Cancer

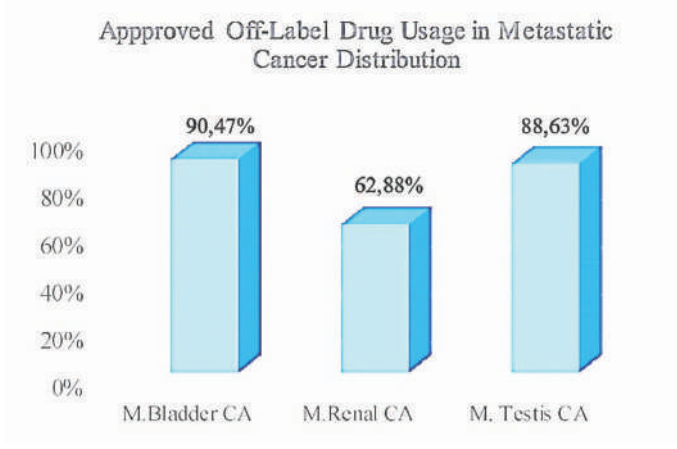

Figure 2. Approved off-label drug usage in metastatic cancer distribution
CA: Cancer

and paclitaxel (43.24%), respectively (Table 1).

DISCUSSION

The principles underlying the use of unlicensed drugs are the same as those of off-label drugs. In general, off-label use is not recommended, but there are legal procedures in many countries' laws and regulations after applying all normal treatment protocols (1). There may be cases when a physician has used all of the normal treatment options, and off-label and/or unlicensed medicinal products may be the last option (3). Off-label drug use is preferred in many countries (4, 5). One of the studies reported that 55% of prescriptions were licensed, 19% were unlicensed, and 26% were licensed pharmaceuticals used through off-label policies. In fact, unlicensed preparations were used in pediatric patients (6). Off-label drug use is also public policy in Turkey in such that off-label use may lead to reimbursement restrictions. Off-label drug use is defined by the Turkish Ministry of Health as the use of licensed pharmaceutical products at doses outside of or exceeding the scope of the approved indication and the use of unlicensed medicinal products that are imported for the purpose of individual treatment. Therefore, off-label use covers both licensed and unlicensed products (7).

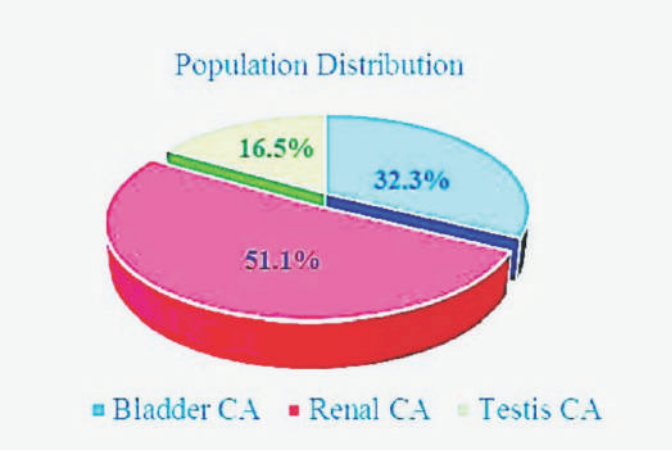

Figure 3. Population distribution
CA: Cancer

Table 1. The most preferable off-label drug medications

CA	Medication	%
Bladder CA	Paclitaxel	84.04%
	Docetaxel	6.38%
	Irinotecan	4.25%
	Vinblastine	2.12%
	IVIG	2.12%
	Topotecan	1.06%
Renal CA	Sorafenib	68.42%
	Sunitinib	21.92%
	Temsirolimus	2.63%
	Bevacixumab	1.75%
	Ibandronic acid	1.75%
	Everolimus	1.75%
	Paklitaxel	0.87%
	Gemcitabin	0.87%
Testis CA	Paklitaxel	43.24%
	Gemcitabin	37.83%
	Paklitaxel+gemcitabin	16.21%
	Gemcitabin+dosetaxel	2.70%

CA: Cancer, IVIG: Immunoglobulin therapy

When an unlicensed drug is approved by TITCK, the Turkish Pharmacists' Association is then responsible for importing (3). TITCK also publishes guidelines for using pharmaceuticals without patient-by-patient based approval process. If a pharmaceutical is mentioned in these guidelines for use in an off-label indication not yet approved, physicians can prescribe it. The pharmaceutical will then be reimbursed by SGK in the off-label indication without approval process. This indication is mentioned as "no-need to approval process of off-label indications" in the guideline. No-need to approve process helps to increase the efficiency of off-label use and decrease the workload of TITCK.

The use of off-label drugs in Oncology has been estimated to reach 50%, or even more (2, 3, 8). In pediatrics, the off-label issue is more common, especially in pediatric oncology (3, 8). In general, the use of these drugs, although off-label, are fully "evidence-based" and therefore falls within the scope. Nonetheless, the off-label use of drugs may be considered illegal. In practice, off-label uses are often "tolerated" under restrictions, in spite of the size of the phenomenon, especially in some medical fields (3, 9).

Unlicensed or off-label drug use is controlled by the Ministry of Health in Turkey. Oncology drugs are imported for such uses. It was reported that unlicensed or off-label medicine use has been rising over the past few years in Turkey (3). On the other hand, off-label drug use is a rising issue in medicine. A PubMed search for "off-label drug use" demonstrates that the majority of the articles were published in recent years (10-14). While the number of published articles was 35 in 2000, it was 368 in 2014 (14).

CONCLUSION

Off-label urology drug use is increasing in Turkey as the off-label use of other oncologic drugs (3). Debates about the use of expensive cancer drugs should consider post-marketing

assessments. The longer duration of off-label drug use in clinical practice and the high rates of off-label drug use are encouraging for new clinical trials. If off-label drug use increases in parallel with the use of off-label urology drugs, new ways of assessing applications should be defined.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Informed consent was obtained from these patients in accordance with the legislation on the use of off-label drugs.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.K., Concept: M.H.T., Design: M.H.T., Data Collection or Processing: P.T., G.K., İ.M.V., Analysis or Interpretation: M.E.B., Literature Search: M.H.T., G.K., Writing: M.H.T., P.T., M.E.B.

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

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.62681
Eur Arch Med Res 2019; 35 (1): 23-26

Evaluation of Erectile Function in Men with Lower Urinary System Symptoms

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Abstract

Objective: The aim of this study was to investigate the possible relationship between erectile dysfunction (ED) and lower urinary tract symptoms (LUTS).

Methods: Sixty-two patients with LUTS due to benign prostatic hyperplasia (BPH) were included in the study. Fifty-eight healthy male patients were included in the study as control group. Ages, comorbidities, body mass index and previous surgeries of the patients were determined. The patients were asked to complete the International Prostate Symptom Score and the International Index of Erectile Function (IIEF-5) questionnaires.

Results: The mean age was 61.41 (41-78) years in the patients with LUTS and 62.34 (40-81) years in the control group. There was no statistically significant difference between the two groups in terms of age. It was observed that LUTS severity increased significantly with age ($p<0.05$). When all subjects were evaluated, there was a statistically significant relationship between LUTS and ED. When the IIEF-5 score less than 22 is accepted as ED, ED was present in 59.06% of patients with LUTS and 29.1% in asymptomatic patients. In addition, the rate of ED was significantly increased with age, as expected ($p<0.05$).

Conclusion: The main finding in our study was that LUTS was independently associated with ED. Because of the relationship between LUTS/BPH and male sexual dysfunction, patients presenting with any of these conditions should be routinely screened for other conditions. A better understanding of the molecular pathways beneath this relationship can help to better define clinical trials.

Keywords: Benign prostatic hyperplasia, erectile dysfunction, IIEF, International Prostate Symptom score, lower urinary tract symptoms

INTRODUCTION

The prevalence of sexual problems in the general population has increased in recent years and erectile dysfunction (ED) is one of the most common types of sexual dysfunction in men worldwide (1, 2). Studies have shown that sexual functions and ED are important factors on quality of life (3-5). ED can affect functions in all aspects of life, including emotional, social, sexual, recreational and intellectual areas (5).

In recent years, studies showing the relationship between lower urinary tract symptoms (LUTS) and ED are noteworthy. Although the mechanisms underlying the association between LUTS and ED in men with benign prostatic hyperplasia (BPH) have not been fully elucidated, various pathophysiological theories have been proposed in the literature and the possible linkages between these pathways are still being investigated (6). The pathophysiology of LUTS and its underlying mechanisms is not yet fully understood (7), however, the presence of concomitant ED or vice versa in many patients with LUTS suggests that its pathophysiological mechanisms may be similar to ED (8). Men with LUTS have one or more physiological diagnoses, some comorbidities and/or risk factors. Nearly 70% of men with LUTS/BPH have ED. Recent treatment trends for LUTS/BPH include the use of various pharmacological agents, such as

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Received: 22.01.2018
Accepted: 05.03.2018

Cite this article as: Dursun M, Beşiroğlu H. Evaluation of Erectile Function in Men with Lower Urinary System Symptoms. Eur Arch Med Res 2019; 35 (1):23-26

tadalafil, a phosphodiesterase-5 (PDE-5) inhibitors (9). Recent epidemiological studies have shown that there is a relationship between LUTS/BPH and ED independent of comorbidities such as age and hypertension, diabetes, dyslipidemia and coronary heart disease (10, 11). In contrast, in some other studies, no association was found between LUTS and ED (12-14). On the other hand, the medical or surgical treatments in patients with LUTS/BPH can significantly affect erectile functions, or similarly, PDE inhibitors used for ED treatment may also improve LUTS (15, 16). Because of these relationships, we aimed to evaluate the possible relationship between LUTS and ED in patients admitted to urology outpatient clinic with LUTS.

METHODS

Sixty-two male patients who applied to urology outpatient clinic due to BPH-related LUTS were included in the study as patient group. Fifty-eight healthy men who admitted for other reasons were included in the study as control group. Ages, comorbidities, body mass index (BMI) and previous surgeries of the patients were determined. Smokers, alcohol and drug addicts, patients with previous pelvic or lumbar surgery/trauma, patients with Peyronie's disease and antidepressant drug use were excluded from the study. In addition, those who were treated for LUTS or prostate disease within the last 6 months were excluded from the study. Informed consent form was obtained from all participants. Institutional review board approval was obtained from the local ethical committee.

The patients were asked to complete the International Prostate Symptom Score (IPSS) and the International Index of Erectile Function (IIEF-5) questionnaires. LUTS was rated according to the IPSS scoring system (0: normal, symptom score less than of equal to 7: mild, symptom score range 8-19: moderate, symptom score range 20-35: severe). ED was classified into five categories based on the scores as suggested by Rosen et al. (11): no ED (IIEF-5 score 22-25), mild (IIEF-5 score 17-21), moderate (IIEF-5 score 8-16) and severe (IIEF-5 score <8).

Statistical Analysis

Data were analyzed using SPSS 14.0. Chi-square and Student's t-test were used to compare the data. P value less than 0.05 was considered statistically significant.

RESULTS

A total of 120 men between the ages of 40 and 81 were included in the study. The study group consisted of 62 patients who were admitted with LUTS (patient group) and 58 patients who applied for other reasons (control group). The mean age was 61.41 (41-78) years in the patients with LUTS and 62.34 (40-81) years in the control group. There was no statistically significant difference between the two groups in terms of age. When BMI was evaluated, the mean BMI was found to be 29.11±2.87 in the patient group and 27.27±2.97 in the control group. BMI was significantly higher in men with LUTS than those without LUTS ($p<0.05$). Regarding IPSS scores, the mean total IPSS score was 18.2 (5-35) in patients with LUTS. Of 62 patients, 18 had mild, 24 had moderate and 20 had severe symptoms. It was observed that LUTS severity increased significantly with age ($p<0.05$).

When all subjects were evaluated, there was a statistically significant relationship between LUTS and ED. When the IIEF-5 score less than 22 is accepted as ED, ED was present in 59.6% of patients with LUTS and 29.1% in asymptomatic patients. When IIEF-5 scores were compared between the two groups, IIEF-5 scores were found to be significantly lower in the patient group. The mean IIEF-5 was 15.8±1.38 in the patient group and 21.8±1.56 in the control group. IIEF-5 scores were negatively correlated with the severity of LUTS ($p<0.05$). In addition, the rate of ED was significantly increased with age, as expected ($p<0.05$). All results are summarized in Tables 1 and 2.

Table 1. All general data of individuals

	Patients with LUTS n=62	Patients without LUTS n=58	p
Age, mean	61.41 (41-78)	62.34 (40-81)	0.81
BMI, mean ± SD	29.11±2.87	27.27±2.97	<0.05
HT, n (%)	13 (20.9%)	11 (18.9%)	0.86
DM, n (%)	18 (29%)	10 (17%)	<0.05
IPSS, mean 0-7, mild (n, %) 8-19, moderate (n, %) 20-35, severe (n, %)	18.2 (5-35) 18 (29%) 24 (38.7%) 20 (32.3%)		

LUTS: Lower urinary tract symptoms, BMI: Body mass index, SD: Standard deviation, HT: Hypertension; DM: Diabetes mellitus, IPSS: International Prostate Symptom Score

Table 2. Comparison of erectile dysfunction rates in the patient and control groups

	Patients with LUTS n=62	Patients without LUTS n=58	p
ED, n (%)	37 (59.6)	17 (29.3)	<0.05
IIEF, mean ± SD	15.8±1.38	21.8±1.56	<0.05

LUTS: Lower urinary tract symptoms, ED: Erectile dysfunction, IIEF: International Index of Erectile Function

DISCUSSION

Sexuality is an indispensable factor in quality of life, thus, the sexual life of elderly individuals has prolonged with the emergence of oral therapies developed for ED. ED is a complex situation with risk factors such as old age, comorbid conditions, some drugs, obesity, lifestyle behaviors, alcohol and tobacco use. As a result of this complex situation, the relationship between ED and LUTS has not been fully elucidated. There are strong epidemiological findings showing a significant relationship between LUTS and sexual dysfunction in elderly men in the world, regardless of age and other comorbidities (17-19).

In this study, we also evaluated the relationship between LUTS and ED. The main finding of our study was that LUTS was independently associated with ED. We have tried to determine the prevalence and predictors of ED in patients with and without LUTS. In this study, the prevalence of ED was significantly higher in patients with LUTS than those without LUTS (58.4% vs. 29.1%, respectively). The high ED prevalence we found in LUTS is similar to that of the Cologne male survey study with 8000 individuals in Germany. In this study, ED prevalence was 72% in men with LUTS (20). Moreover, the higher prevalence in this study suggests that the majority of men with LUTS in this country have ED findings, but that they do not seek medical advice. It is known that the severity of ED and LUTS increases with age. There are several studies showing this finding and our study is consistent with these studies (21, 22). The multinational survey of the aging male study conducted with approximately 14.000 men in the United States and six European countries has showed that ED is strongly associated with LUTS (23). This study reported that most men over 50 years of age were sexually active, but the number of sexual intercourse decreased with increased LUTS severity (up to 7.5 per month for men in their 60s, up to 3.2 per month for men aged 80 years). ED was reported by approximately 49% of the study cohort and the IIEF score was strongly correlated with LUTS severity.

The relationship between LUTS/BPH and male sexual dysfunction has been explained by various pathophysiological mechanisms. These include autonomic hyperactivity, changes in the Rho/Rho kinase pathway, microvascular dysfunction, endothelial [nitric oxide (NO) synthase/NO] dysfunction, pelvic ischemia and age-related hormone imbalances (21). The role of NO in the prostate and penis has been extensively studied, particularly following the development of PDE-5 inhibitors, the first-line treatment for ED. The role of NO in erection is known. NO has been shown to be present in prostate tissue and be effective in smooth muscle tone (24). On the other hand, although PDE5-inhibitors showed significant improvement in both LUTS and ED in men with BPH, these treatments did not affect the urinary flow rate. Therefore, the mechanisms of action of these drugs on LUTS are not fully understood. On the other hand, metabolic syndrome and its components have been shown to be associated with the development of LUTS and ED in various clinical trials (25, 26). This is considered to be one of the reasons for the common pathophysiology and strong relationship. In addition, insulin resistance and developing secondary hyperinsulinemia have been shown to play a role in the pathogenesis of LUTS as well as ED in these studies.

Because of the strong association between LUTS/BPH and male sexual dysfunction, patients presenting with any of these conditions should be routinely screened and questioned for the other disease. In addition, patients with LUTS/BPH should be monitored for treatment-related sexual consequences, as sexual side effects may occur in patients undergoing medical and surgical treatment for LUTS/BPH (16, 21, 27, 28). Beyond the IPSS and IIEF evaluation, it is important to evaluate additional diseases such as the metabolic syndrome and the use of drugs associated with these diseases. It is also important to give advice on lifestyle changes in patients with both disorders (29). Sexual functions should always be evaluated before starting LUTS/BPH treatment. If sexual dysfunction is detected, lifestyle changes such as weight loss and increasing physical activity should be planned. Given the need for medication or surgical treatment for LUTS/BPH, the possible effects of these therapies on ED should be discussed with patients.

CONCLUSION

In conclusion, LUTS and ED are age-related disorders that are quite common. Although no coincidental relationship was found, various epidemiological studies and potential common biological mechanisms confirm the existence of this relationship. Considering that many elderly men do not ask for help with their sexual problems and physicians do not often question their sexual life, it is recommended that men presenting with LUTS should be evaluated for sexual dysfunction and ED. Similarly, patients presenting with ED should also be evaluated for LUTS. In clinical practice, it should be noted that the presence of concomitant ED in the management of LUTS and some of the current medical treatments of LUTS/BPH may have positive or negative effects on sexual functions. A better understanding of the molecular pathways behind this relationship as a result of clinical trials can help to identify new possible targets and develop new treatment approaches to manage both disorders.

Ethics

Ethics Committee Approval: The study was approved by the Malatya Clinical Research Ethics Committee (Approval no: 2018/24).

Informed Consent: Informed consent form was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.03779
Eur Arch Med Res 2019; 35 (1): 27-32

Assessment of the Effect of Fatty Infiltration on Hepatic FDG Uptake

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Abstract

Objective: In fluorodeoxyglucose (FDG) positron emission tomography (PET)/computerized tomography (CT) studies, physiological liver FDG uptake has been used as a reference in the evaluation of FDG uptake in pathological processes, even in malignancies. There is an ongoing debate on the effect of liver attenuation and FDG uptake in the liver. We aimed to evaluate the possible effect of fatty infiltration on the standardized uptake value (SUV) of the liver.

Methods: A total of 88 patients were included in this study. Subjects were divided into 2 groups by calculating the Hounsfield unit (HU) of the liver from the unenhanced CT part of the PET/CT study and comparing it with that of the spleen. Fatty liver group included 42 patients (26 female, 16 male) with a mean age of 59.6±11.6 years, while control group consisted of 46 patients (22 female, 24 male) with a mean age of 60.2±11 years. The patients with a mean liver attenuation value (in HU) equal and greater than that of spleen were enrolled in the control group, while the patients with a mean liver attenuation value lower than the spleen were included in the fatty liver group. A subset of patients in the fatty liver group with difference in attenuation between the liver and spleen of more than 10 HU (HUS-HUL >10) were evaluated separately. Age, weight, history of diabetes mellitus (DM) and chemotherapy, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, simultaneous blood glucose levels during PET scan and the elapsed time between FDG injection and the beginning of PET scan were recorded.

Results: The average SUVmean and SUVmax values were 2.7±0.7 and 3.6±0.9 in the fatty liver group, 2.8±0.7 and 3.8±1 in the HUS-HUL >10 group and 3.3±0.6 and 4.4±0.9 in the control group, respectively. The average SUVmean and SUVmax values of the fatty liver group and HUS-HUL >10 group were significantly different from the control group ($p<0.05$). The patients in the fatty liver group had higher ALT ($p=0.025$) and glucose levels ($p=0.001$), weight ($p=0.001$), and DM rate ($p=0.002$) compared to the control group.

Conclusion: Hepatic steatosis causes a statistically significant decrease in SUVmean and SUVmax values in liver. Therefore, we must be careful when using the liver as an internal reference organ

Keywords: Fluorine 18-fluorodeoxyglucose, hepatosteatosis, positron emission tomography, standardized uptake value

INTRODUCTION

The use of standardized uptake values (SUVs) in determining malignant nature of lesions, aggressiveness of malignancies and therapy response in clinical fluorine 18 (^{18}F)-fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computerized tomography (CT) oncology imaging is increasing. SUV is a semiquantitative measurement that corresponds to measured activity normalized for body weight/surface area and injected dose. The formula for calculating SUV is region of interest (ROI) activity (mCi/mL) x body weight (g)/injected dose (mCi). Although the application of SUV eliminates some degree of uncertainty in patient size and the amount of injected FDG, it is still liable to many weaknesses that can cause misleading results. SUV, rather than being an absolute value in characterization of lesions, is a proportional value without units, so to measure the amount of tumoral FDG uptake, there is always a need for a region in

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Received: 11.06.2018
Accepted: 20.06.2018

Cite this article as: Özülker T, Özülker F. Assessment of the Effect of Fatty Infiltration on Hepatic FDG Uptake. Eur Arch Med Res 2019; 35 (1):27-32.

the body supposed to have normal FDG uptake. The liver has long been used as a reference organ for this purpose (1-4). If the ^{18}F-FDG uptake in the target lesion is greater than the liver in terms of SUV, the hypermetabolic focus is considered abnormal.

Fatty liver disease reflects a wide spectrum of conditions characterized histologically by excessive accumulation of triglycerides and cholesterols within the cytoplasm of hepatocytes. Fatty infiltration of the liver is further subdivided as alcoholic fatty liver disease or non-alcoholic fatty liver disease (NAFLD). NAFLD is the most common chronic liver disease in the developed countries with an estimated prevalence of 20%-30% in adult populations (5, 6). NAFLD includes two pathological entities as simple steatosis and non-alcoholic steatohepatitis (NASH). NASH is a more serious condition that can eventually progress to cirrhosis and promote hepatocellular carcinoma (7).

Several studies have been conducted to investigate the possible effect of fatty infiltration on liver SUVs (8-15). Some of these studies have reported no correlation between low attenuation due to high fat content of liver and ^{18}F-FDG uptake (10, 13). In one study, FDG PET showed a significant negative correlation between the severity of fatty liver and SUVmax of the liver (15). Increased FDG uptake due to steatohepatitis was also reported in other studies (9, 14, 16).

These contradictory results have led us to investigate the relation between fatty infiltration of the liver and FDG uptake in terms of SUVmax and SUVmean values.

METHODS

Patients

FDG PET/CT examinations performed at our institution between September 2016 and April 2017 were evaluated retrospectively by investigating the patients' medical charts. Due to being retrospective, study approval by the clinical research ethics committee is waived, but the study was approved by the local institutional review board. A total of 88 patients were enrolled in this study. Patients with liver metastasis or previous liver disease that could effect hepatic uptake were not included in this study. Subjects were divided into 2 groups by calculating the Hounsfield unit (HU) of the liver from the unenhanced CT part of the PET/CT study and comparing it with that of the spleen. The fatty liver group included 42 patients (26 female, 16 male) with a mean age of 59.6±11.6 years. The control group consisted of 46 patients (22 female, 24 male) with a mean age of 60.2±11 years. The patients with a mean liver attenuation value (in Hounsfield units) equal and greater than that of spleen were enrolled in the control group, while the patients with a mean liver attenuation value lower than the spleen were included in the fatty liver group. A subset of patients in the fatty liver group with difference in attenuation between the liver and spleen of more than 10 HU (HUS-HUL >10) were evaluated separately. Age, weight, history of diabetes mellitus (DM) and chemotherapy, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, simultaneous blood glucose levels during PET scan and the elapsed time between FDG injection and the beginning of PET scan were recorded. Primary malignancies of the patients were lung cancer in 12 patients (13.6%) colorectal cancer in eight patients (9%), breast cancer in 24 patients (27.2%), bladder cancer in six patients (6.8%), head and neck cancer in six patients (6.8%), sarcoma in five patients (5.6%), gynecological malignancies in 14 patients (15.9%), skin cancer in five patients (5.6%), neuroendocrine tumor in one patient (1.1%), cancer of unknown primary in four patients (4.5%), gastrointestinal stromal tumor in one patient (1.1%), multiple myeloma in one patient (1.1%) and thyroid cancer in one patient (1.1%).

Imaging

PET/CT images were obtained using an integrated PET/CT scanner consisting of a full-ring HI-REZ LSO PET and a six-slice CT scanner (Siemens Biograph 6, Chicago, IL). Patients were instructed to fast for at least 6 hours before ^{18}F-FDG injection. Blood glucose levels were measured before the study and ^{18}F-FDG was injected only when the blood glucose level was below 11.11 mmoL/L. Patients were injected with 296-555 MBq ^{18}F-FDG based on body weight. After waiting 50 minutes relaxed in a semi reclining chair, the patients were visualized using an integrated PET/CT scanner. The CT part of the study was performed without an intravenous contrast medium to define only anatomical landmarks and make attenuation correction on the PET images. The CT scan was performed first with the following parameters: 50 mAs, 140 kV and 5-mm section thickness. Whole body CT was performed in the craniocaudal direction. The images were obtained with the arms of the patients raised to avoid false increase in liver FDG uptake due to beam-hardening effects.

Measurement of SUV and HU values

A 2-cm diameter ROI was placed over the right lobe of the liver. Same ROIs were plotted on the PET and CT scans of the liver avoiding any lesions, biliary, vascular structures and artifacts. For each ROI, SUVmean and SUVmax of the liver were measured with the formula "ROI activity (mCi/mL) x body weight/injected dose (mCi)". Mean attenuation value (HUmean) were also measured from the ROI plotted on CT part of the study (Figure 1).

Statistical Analysis

IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA) program was used for statistical analysis. The normality of the parameters was evaluated by the Shapiro-Wilk test. Student's t-test or Mann-Whitney U test was used for comparison between two groups, where appropriate. Regarding SUVmean and SUVmax values, comparison between the control group and the fatty liver disease group was done using Student's t-test. Fisher's exact chi-square test and Continuity (Yates) correction were used for the comparison of qualitative data of gender, DM and chemotherapy status, gamma-glutamyl transferase, AST and ALT elevation, elapsed time and glucose levels. Significance was assessed at $p<0.05$.

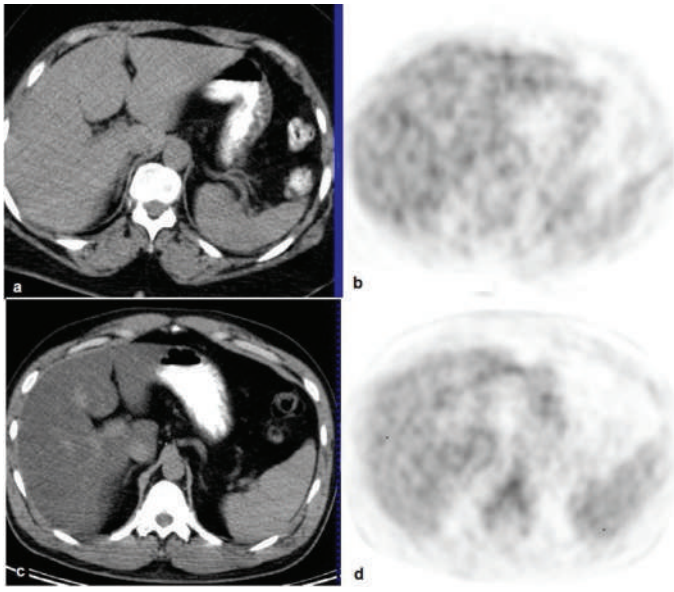

Figure 1. (a, b) Axial computerized tomography and pozitron emisyon tomografi sections of a patient from the control group, (c, d) and fatty liver group

RESULTS

Average liver SUVmax and SUVmean were significantly lower in patients with fatty liver compared to the control group ($p<0.05$). Average spleen SUVmax and Spleen SUVmean were also significantly lower in patients with fatty liver compared to the control group ($p<0.05$) (Table 1). In addition, the average liver SUVmax and SUVmean were significantly lower in patients in the subset of fatty liver group (HUS-HUL >10) compared to the control group ($p<0.05$) (Table 2). The comparison of data of fatty liver and control groups in terms of clinical parameters are presented in Table 3. The fatty liver group showed a significantly higher mean body weight (84.95±13.76 kg) than the control group (74.45±14.28 kg). There were 16 patients (38.1%) with DM in the fatty liver group, while there were four diabetic patients (8.7%) in the control group. Serum ALT values were significantly higher in the fatty liver group than the control group. Serum glucose levels were also higher in the fatty liver group (115.74±33.11) than the control group (91.63±14.4).

Table 1. Evaluation of groups

	Patients with fatty liver (n=42)	Controls (n=46)	p
	Mean ± SD	Mean ± SD	
Liver SUVmax	3.61±0.97	4.41±0.94	0.001*
Liver SUVmean	2.70±0.70	3.34±0.66	0.001*
Liver mean HU	36.43±9.63	57.08±6.36	0.001*
Spleen SUVmean	2.29±0.63	2.62±0.48	0.008*
Spleen SUVmax	2.93±0.76	3.27±0.66	0.028*
Spleen mean HU	47.29±5.59	40.46±9.16	0.001*

Student t-test p<0.05* SD: Standard deviation, Max: Maksimum, SUV: Standardized uptake value, HU: Hounsfield unit

Table 2. Evaluation of groups

	Patients with severe fatty liver (n=23)	Control (n=46)	p
	Mean ± SD	Mean ± SD	
Liver SUVmax	3.84±1.10	4.41±0.94	0.028*
Liver SUVmean	2.87±0.79	3.34±0.66	0.010*
Liver mean HU	32.08±10.39	57.08±6.36	0.001*

*Student t-test** p<0.05 SD: Standard deviation, Max: Maksimum, SUV: Standardized uptake value, HU: Hounsfield unit

DISCUSSION

Non-invasive methods that distinguish benign lesions from malignancies have always been searched. FDG PET has been utilized for this purpose depending on the fact that malignant lesions generally have a higher glucose consumption rate and thus higher FDG uptake. Initially, semiquantitative SUV measurements, as an indicator of amount of FDG uptake, seemed to be a robust method in the characterization of malignant lesions and regarded by some authors as "metabolic biopsy" (17, 18). However, in clinical practice, there have been numerous limitations, such as partial volume and spillover effects, attenuation correction, reconstruction method and parameters for scanner type, count noise bias effect, elapsed time between radiotracer injection and imaging, competing transport effects and body size (19). Therefore, it is not realistic to rely on a certain static SUVmax threshold to distinguish benign lesions from malignant ones. The qualitative visual interpretation of 18F-FDG uptake by using liver as a reference standard became a common practice to overcome this shortcoming. Fatty liver disease, which means accumulation of fat in the form of triglycerides and cholesterol in the liver cells, theoretically might cause a decrease in the uptake of FDG in hepatocytes. This potential decline in FDG has significant clinical implications as a result of misinterpretation of FDG-positive lesions, as it shows itself as a reduction in SUVmax compared to normal livers, therefore, we tried to assess whether liver ^{18}FDG uptake was affected by hepatosteatosis. In our study, we found that average liver SUVmax and SUVmean of patients with fatty liver were significantly lower than the control group ($p<0.05$). In addition, average liver SUVmax and SUVmean in the subset of fatty liver group (HUS-HUL >10) were significantly lower than the control group ($p<0.05$). In the literature, conflicting results have been reported by several studies investigating the relationship between hepatic steatosis and hepatic FDG uptake. One of the oldest studies conducted for this purpose by Qazi et al. (20) reported that liver SUVmax/spleen SUVmax ratio of the fatty liver group was significantly lower than that of the control group (1.1 vs 1.4, $p=0.002$). There were limitations for this preliminary report, such as the relatively small number of subjects enrolled in the study and measurement of SUVmax instead of SUVmean which may give rise to less reliable results in the evaluation of a large organ like liver. In their prospective case-control study, Abikhzer et al. (11) analyzed the effect of fatty infiltration on hepatic metabolic activity in 37 patients. The authors found that patients with hepatic steatosis had significantly lower hepatic metabolic activity in terms of SUVmax measurements compared with control subjects, when the SUV is corrected for lean body mass and not for body weight. Even though the results were statistically significant, the degree of the change in SUVmax values was not found satisfactory by authors to be accepted as clinically significant.

Lin et al. (15) reported that hepatic steatosis had a significant negative effect on hepatic metabolic activity as measured by SUVmax. They retrospectively analyzed ^{18}F-FDG PET studies of 173 patients who were investigated for non-oncological diseases. They divided the patients into four groups according to the ultrasonography findings: no fatty liver, mild, moderate and severe. The mean SUVmax of liver in subjects with no, mild, moderate

Table 3. Evaluation of clinical parameters of groups

	Patients with fatty liver (n=42)	Controls (n=46)	p
Age: mean ± SD	59.69±11.61	60.22±11.04	[1]0.828
Gender, n (%)			
Female	26 (61.9%)	22 (47.8%)	[2]0.267
Male	16 (38.1%)	24 (52.2%)	
Diabetes mellitus, n (%)	16 (38.1%)	4 (8.7%)	[2]0.002*
ALT elevation, n (%)	7 (16.7%)	1 (2.2%)	[3]0.025*
AST elevation, n (%)	2 (4.8%)	0 (0%)	[3]0.225
GGT, n (%)	7 (16.7%)	5 (10.9%)	[2]0.631
Chemotherapy, n (%)	12 (28.6 %)	13 (28.3%)	[2]1.000
Elapsed time: mean ± SD	70.48±15.94 (65)	76.72±17.35 (70)	[4]0.049*
Glucose: mean ± SD	115.74±33.11 (109)	91.63±14.40 (89.5)	[4]0.001*
Weight: mean ± SD	84.95±13.76	74.45±14.28	[1]0.001*

[1]Student t-test [2]Continuity (Yates) correction [3]Fisher's exact Test [4]Mann-Whitney U test

**p<0.05 SD:* Standard deviation, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase

and severe fatty liver were 3.13±0.49, 3.08±0.45, 3.01±0.44, and 2.43±0.27, respectively. Differences in SUVmax were statistically significant. They concluded that the liver could not be used as a comparator of increased FDG activity in the lesions of patients with fatty liver disease. These findings are in accordance with our results, indicating a negative relationship between SUVmax and HU values. However, there are also other reports in the literature that contradict our findings. Pak et al. (21) retrospectively analyzed FDG PET/CT studies of 96 consecutive patients who were screened for cancer and found no significant difference in liver SUVmean and SUVmax between controls and fatty liver group.

Dostbil et al. (13) assessed the relationship between fatty infiltration of liver and hepatic metabolic activity in 79 patients with hepatosteatosis on ^{18}FDG PET/CT. The control group in the study included 77 patients with a mean liver HU value greater than mean spleen HU value and the patient group included 79 patients in whom the mean liver HUvalue was lower than or equal to the mean spleen HU value. The authors further divided the patient group into subsets according to their degree of hepatic steatosis. There was no statistically significant difference between the mean and maximum liver SUVs in patients with fatty liver disease and the control group. Abele and Fung (10) conducted a study to evaluate the association between diffuse fatty infiltration and average FDG uptake, with the assumption that hepatocyte expansion due to fat accumulation may lead to a decrease in SUVmean. The average SUVmean for the control group was 2.18±0.36 and this value was not significantly different for the groups of fatty liver disease (2.03±36) and more strictly defined subset of fatty liver disease (2.07±0.24) groups.

Some authors described a controversial increase in liver SUVmean values in patients with fatty liver. Liu et al. (12) reported a positive relationship between liver SUVmean and fatty infiltration when the severity was mild to moderate, while there is a negative effect when more severe. They also noted that FDG uptake of liver gradually increase in patients as the body mass index (BMI) increases from underweight to overweight, but a decrease in SUVmean values occur when the patient is obese.

High levels of ^{18}F-FDG uptake in inflammatory cells are well known, and this has led to use of FDG PET as a potential imaging modality in infectious diseases. Keramida et al. (14) reported that FDG uptake in the liver is increased in NASH due to irreversible uptake in inflammatory cells superimposed on reversible hepatocyte uptake. Bural et al. (22) compared hepatic SUVs and hepatic metabolic volumetric products (HMVP) among patients with diffuse hepatic steatosis and control subjects with normal liver. They found an increase in HMVP as a result of increased hepatic metabolic activity likely related to the inflammatory process in diffuse hepatic steatosis. Increased FDG uptake in the liver with high fat content could be accounted for the increased activity of Kupffer cells, a kind of macrophage that acts by engulfing FDG (23). This accumulation of FDG uptake at focal hepatic steatosis can cause a diagnostic dilemma in imaging by mimicking metastasis (24, 25). Conversely, focal fat spared area in a liver with diffuse fatty infiltration can demonstrate focal FDG uptake masquerading as liver metastases, probably when steatosis is not accompanied with inflammation (26, 27)

In our study, we found a statistically significant difference between the body weight ($p<0.001$), serum ALT levels (0.025), DM status (0.002), and glucose levels ($p<0.001$) of the patients with fatty liver and the control group. There may be a positive correlation between serum liver enzyme levels and SUVs of liver on FDG PET that can affect diagnostic sensitivity of hepatic malignant or infectious lesions on FDG PET (28). Patients with fatty liver disease have higher AST and ALT levels (21). Although patients in the fatty liver group in our study had higher serum enzyme levels, we could not detect any positive relation between SUVs and ALT and AST levels. It is known that BMI levels are higher in patients with fatty liver than in normal patients (13, 21). In our study, we could not calculate BMI of patients since we did not get their height values, but mean body weight of the patients with hepatic steatosis were significantly higher. All of the subjects in the patient and control groups had oncological diseases and there was not any statistically significant difference between the two groups in terms of chemotherapy history. Lin et al. (8) found that age had a significant and positive effect on both the maximum and mean SUVs of the liver in FDG PET imaging. In our study, the mean ages of the patients were not significantly different between the two groups.

Interestingly, average spleen SUVmean and SUVmax of patients with fatty liver were also significantly lower than the control group ($p<0.05$) in our study. This issue needs to be clarified by additional studies.

We preferred to rely on unenhanced CT part of the PET CT in the diagnosis of fatty liver, as assessment of liver attenuation by use of unenhanced CT represents an objective and non-invasive mean for detection of asymptomatic hepatic steatosis (29, 30). The diagnosis could be done with biopsy and histopathology and this can be a limitation of our study.

CONCLUSION

Contrary to most studies reported in the literature, hepatic steatosis causes a statistically significant decrease in SUVmean and SUVmax values in liver, unless it is associated with

inflammatory conditions as in NASH. Therefore, we must be careful while using the liver as an internal reference organ.

Ethics

Ethics Committee Approval: Due to being retrospective, study approval by the clinical research ethics committee is waived, but the study was approved by the local institutional review board.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Author Contributions

Surgical and Medical Practices: T.Ö., F.Ö., Concept: T.Ö., F.Ö., Design: T.Ö., F.Ö., Data Collection or Processing: T.Ö., F.Ö., Analysis or Interpretation: T.Ö., F.Ö., Literature Search: T.Ö., Writing: T.Ö., F.Ö.

Conflict of Interest: The authors have no conflicts of interest to declared.

Financial Disclosure: The authors declared that this study has received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.43531
Eur Arch Med Res 2019; 35 (1): 33-37

Clinical Results of Platelet-Rich Plasma Injection in the Treatment of Androgenetic Alopecia

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Abstract

Introduction: Platelet-rich plasma (PRP) represents a small volume of plasma in which the platelet concentration in normal blood is increased to optimum values. Its effect depends on the growth factors released from platelets. Although it has been investigated and used in many areas of plastic surgery, PRP injection has recently attracted interest in the treatment of hair loss. In this study, we aimed to evaluate the efficacy of adjuvant therapy in patients with male and female alopecia by establishing a standard protocol for the preparation and application of PRP.

Methods: Fifteen patients with mild to moderate androgenetic alopecia (Ludwig alopecia score I-II for women and Hamilton-Norwood score 1-4 for men) who had not received topical or systemic treatment for alopecia in the last 6 months were included in the study. Eleven of the patients were male and 4 were female. The mean age was 41 years (27-55). PRP was prepared using a single spin method with high acceleration force and low centrifugation time, and three sessions were performed with an interval of 3-4 weeks. Patient satisfaction was assessed by visual analogue scale (VAS). The assessments on day 0, 1st month, 2nd month and 6th month were evaluated retrospectively.

Results: Hair pull test was positive in all patients (100%) before the treatment and the mean number of hair loss was 6.7. After the third treatment session, the hair pull test was negative in 13 patients (86.6%) with a mean of number of 2.5 hair loss. An improvement in hair density and quality was demonstrated with macroscopic photographs taken in a standard manner before and after the procedure. The mean VAS value decreased from 6.8 to 2.1 after the procedure.

Discussion: PRP injections, which are prepared in a simple way without using a commercial kit, are an effective treatment option in alopecia treatment with high patient satisfaction and low cost.

Keywords: Alopecia, androgenetic alopecia, platelet, platelet-rich plasma

INTRODUCTION

Alopecia is divided into two main groups as cicatricial and non-cicatricial. Androgenetic alopecia (AGA) is in the non-cicatricial group and is the most common type of hair loss in both men and women (1, 2). It is an inherited, androgen-dependent disorder characterized by progressive hair loss. However, genetic predisposition is very important to better determine the onset, severity and form of the disease. The hair follicles in the affected scalp area undergo a gradual transformation that causes shrinkage (miniaturization) due to the shortened anagen phase. With each hair cycle, anagen becomes shorter and telogen remains constant (2, 3). As it is thought that hair is an important feature in the self-image of the person, the quality of life of patients diagnosed with AGA may be affected and it may cause emotional stress and decrease in self-esteem (3, 4).

Drug therapies approved by the US Food and Drug Administration (FDA) for the treatment of androgenetic alopecia are limited to topical minoxidil and oral finasteride. They can be used in single or combined form. These treatments have limited efficacy, and they also have topical and systemic side effects. Therefore,

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Received: 12.07.2018
Accepted: 07.09.2018

Cite this article as: Özer K, Çolak Ö. Clinical Results of Platelet-Rich Plasma Injection in the Treatment of Androgenetic Alopecia. Eur Arch Med Res 2019; 35 (1):33-37.

effective treatment modalities with fewer side effects were investigated. Recently, new approaches such as the application of platelet-rich plasma (PRP) have been introduced and some studies have shown promising results (3, 5).

PRP represents the autologous concentration of human platelets in a small plasma volume, in which the normal blood platelet concentration is optimized (at a minimum of 2 times baseline concentration to a maximum of 4 to 6 times baseline concentration). When platelet alpha granules are activated, they secrete numerous growth factors (GFs) that stimulate cell proliferation, differentiation and angiogenesis. The regenerative potential of PRP depends on these GFs. GFs act on the convex part of the follicle to which they are attached to their respective receptors in the stem cells, thus stimulating neovascularization by stimulating new follicle development (4-6).

Although there is no general consensus about the preparation, dosage, frequency and route of administration for PRP, there is also no reliable method for evaluating the results. The aim of this study was to establish a standardized protocol for the preparation and application of PRP in patients diagnosed with male and female AGA and to evaluate the effectiveness of the treatment.

METHODS

This study was approved by the Ethics Committee of University of Health Sciences, Ankara Training and Research Hospital Hospital and informed consent forms were signed by all patients. Fifteen patients who were followed-up for AGA since January 2016 and who had at least six months follow-up after three doses of PRP were included in the study. The assessments on day 0, 1st month, 2nd month and 6th month were evaluated retrospectively. All patients were in good general health and had mild to moderate AGA (Ludwig alopecia score I-II for women and Hamilton-Norwood score 1-4 for men). Eleven patients were male and four were female, and the mean age of patients over 18 years was 41 (27-55) years (Table 1). Patients who received topical or systemic AGA treatment in the last 6 months, those who had immunodeficiency due to malignancy, chemotherapy, steroid treatment, those who had dermatological diseases that would affect scalp, pregnant or nursing women, patients with autoimmune disease and hematological disease or those receiving anticoagulation treatment were not included in the study. Patients were instructed not to wash their hairs up to 2 days before the treatment and to stop smoking 2 weeks ago. Before the procedure, following conditions were ruled out by following tests: a) anemia by complete blood count, serum iron, serum ferritin and total iron binding capacity, folic acid and Vitamin B12, b) thyroid dysfunction by free T3, free T4 and TSH, c) syphilis by venereal disease research laboratory, d) polycystic ovarian syndrome by dehydroepiandrosterone sulfate, testosterone, androstenedione, prolactin, follicle-stimulating hormone and luteinizing hormone.

Platelet-rich Plasma Protocol

Approximately 11 mL of whole blood was added to 4 acid citrate dextrose-A (ACD-A) tubes in a ratio of 1:9. They were centrifuged at 1650 g for 5 min at room temperature. After centrifugation, the upper 1/3rd of the supernatant (platelet-poor plasma) and the middle layer were transferred and collected in different injectors. The remaining lower 1/3rd plasma (about 1 mL) and the buffy-coat portion with precipitated platelets were obtained as PRP. Thus, a total of 5-6 mL of effective PRP from 45 mL of whole blood was transferred to 1 mL of insulin injectors.

PRP infiltration to the affected scalp area was performed using Nappage technique (0.1 cc/cm^2, multiple small injection in linear pattern at intervals of 1 cm) with 4 mm mesotherapy needles. Each patient received PRP at a total of 3 doses with an interval of 3-4 weeks. All patients were called for control at one-week intervals.

All patients underwent hair pull test at Day 0, 1st month, 2nd month and 6th month and results were noted (T1, pre-treatment; T2, 1st month; T3, 2nd month; T4, 6th month). Hair pull test was done by the same physician, by pulling a small amount of hair (approx. 100 pieces at the same time), and the number of hair loss along with the area of hair loss (to pull from the same area in the control) were recorded. The normal value is 1 to 3 hairs. More than 3 are considered positive. Anterior, lateral, posterior and vertex images were photographed in each evaluation to determine hair growth, volume, quality and fullness. Patients were asked to evaluate satisfaction on a 10 cm visual analog scale (VAS) before and 6 months after the procedure. This scale includes scales with facial images rated from 0 to 10. 0 demonstrates maximum satisfaction and 10 demonstrates the worst satisfaction (Figure 1).

Statistical Analysis

SPSS version 20.0 (SPSS, Chicago, IL, USA) was used for data analysis. Kolmogorov-Smirnov test was used to assess the normality of the data. Paired t-test was used for normal distribution and Wilcoxon signed rank test was used for non-normal distribution.

RESULTS

Fifteen patients, who completed post-procedure 6th month evaluation, were analyzed retrospectively. Three patients had a history of topical 5% minoxidil lotion use due to hair loss, but no drug use was present within the 6 month period prior to PRP treatment.

Before the treatment, the hair pull test was positive in all patients (100%) and the mean number of hair loss was 6.7±2.1. After three sessions, the hair pull test was negative in 13 patients (86.6%) and the mean number of hair loss was 1.6±1.5 ($p<0.0001$). Patients noted a significant decrease in hair loss between the first and third injection (Figure 2). There was a general improvement in the hair density and quality that was demonstrated by the photos of the alopecic region taken from four different aspects at equal distance before and after the procedure (Figures 3, 4).

Patients were evaluated in terms of satisfaction on VAS before and 6 months after the procedure. While the mean pre-procedure VAS satisfaction score was 6.9±1.8, this value was found to decrease to 2.1±1.4 after the procedure ($p<0.0001$). Table 1 shows the hair pull test and satisfaction scores of all patients before and 6 months after the treatment. All patients reported improvement in hair quality and thickness after the procedure, and 70% reported an increase in hair density. Patients had no other side effects except spot bleeding, mild pain, and rash. There was no ecchymosis, infection or increase in hair loss in any patient.

DISCUSSION

Androgenetic alopecia is the most common hair disorder without satisfactory treatment and has a significant impact on psychological stress and is associated with low self-esteem. Current therapeutic strategies in the treatment of androgenetic alopecia, which is characterized by shorter anagen phase and shrunken hair follicles in the follicular unit, aim cellular proliferation and differentiation during the hair cycle (7).

The follicles in the scalp are found as a composite follicular unit. These composite follicular units contain primary follicles and several secondary follicles. Miniaturization starts first in secondary follicles. Initially, arrector pili muscle lost its connection with secondary follicles regressed in several follicular units. At this stage, the muscle is still attached to the primary follicle. The miniaturization continues progressively, and the connection between the muscle and the secondary follicles is lost. This leads to a reduction in hair density. Then the primary follicles are also affected from miniaturization and their connection with muscle is finally lost. Baldness occurs when all follicular units are miniaturized. The same miniaturization pattern and loss of muscle connection progressively continue until all follicular units are affected (8-10).

Androgenetic alopecia is a disorder associated with hormonal and genetic factors, and the treatment is mainly focused on hormonal blockade because the latter cannot be altered.

Table 1. Demographic characteristics of patients and summary of clinical data

Case	Gender	Age	Alopecia stage	VAS score (pre-procedure)	VAS score (post-procedure)	Hair pull test (pre-procedure)	Hair pull test (post-procedure)	Previous Treatment
1	M	33	II	6	4	5	2	-
2	M	42	III	8	4	10	3	5% Minoxidil
3	M	27	II	6	2	4	0	-
4	F	50	I	4	0	5	0	-
5	M	36	I	4	2	4	0	-
6	F	43	II	6	4	9	4	-
7	M	31	IV	8	2	10	3	5% Minoxidil
8	M	28	II	6	2	7	1	-
9	M	37	III	10	2	8	2	5% Minoxidil
10	F	41	II	8	2	6	1	-
11	M	45	III	8	2	8	1	-
12	F	51	II	6	2	5	1	-
13	M	44	II	8	0	6	0	-
14	M	52	II	6	0	5	1	-
15	M	55	IV	10	4	9	5	-

VAS: Visual analogue scale, M: Male, F: Female

Increased dihydrotestosterone (DHT), androgen receptors and caspase levels cause cellular apoptosis and hair loss (11). DHT plays a role in the inhibition of adenylyl cyclase (AMP-c) and thus in the inhibition of hair follicle growth, resulting in anagen phase shortening. All these mechanisms result in the formation of vellus or miniature hair together, resulting in total loss of hair if this stimulus persists (5, 12).

Treatment methods are limited to FDA-approved minoxidil and finasteride (single or combined use) and hair transplantation. They have various side effects such as hypertrichosis, birth defects in children when given to women of childbearing age, decreased libido and prolonged impotence. Low patient compliance and satisfaction, topical and systemic side effects have led to the search for new treatment options (1, 13). Therefore, PRP, which has been shown to be effective in many areas other than plastic surgery and has very few side effects, has attracted attention. Case reports and several patient series have been published on the use of PRP in androgenetic alopecia especially after 2012, and there has been a significant increase in these studies especially in the last two years. The basic idea behind PRP injection is to offer high concentration GFs in the scalp hoping to stimulate hair growth (6).

PRP GFs stimulate the growth of existing miniature hair follicles without interfering with the hormonal pathways of AGA, thus emphasizing the importance of combined therapies to achieve optimal results. GFs are known to activate the proliferative phase, differentiation of hair and stem cells and produce new follicular units. PRP increases cell survival by prolonging the anagen phase of the hair growth cycle and inhibiting apoptosis through increased expression of fibroblast growth factor (FGF-7). It has also been shown to increase perifollicular vascular plexus by an increase in vascular endothelial growth factor and platelet derived growth factor levels with angiogenic potential. PRP-induced activation of anti-apoptotic regulators such as Bcl-2 protein and Akt signal also prolongs the survival of dermal papilla cells during the hair cycle (6, 7, 14). Therefore, it is a powerful tool for the treatment of androgenetic alopecia.

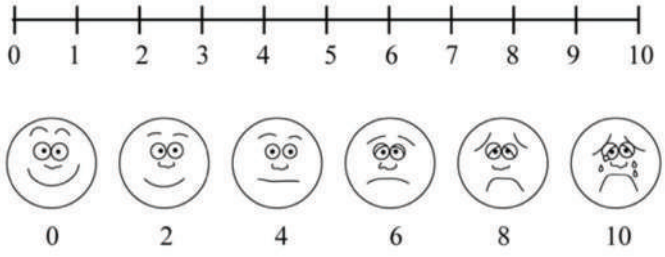

Figure 1. Patient satisfaction scale

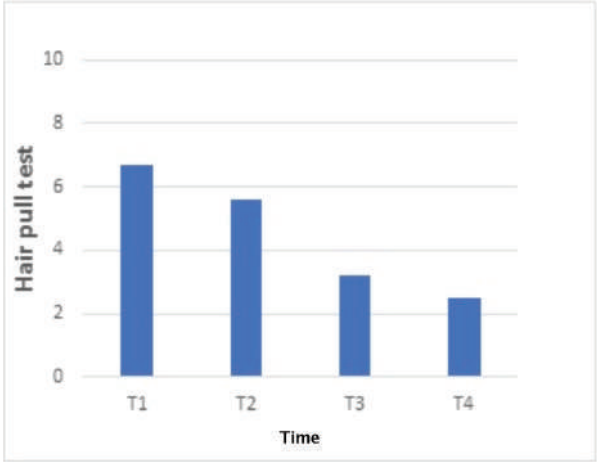

Figure 2. Mean number of hair loss after hair pull test

In our study, PRP was prepared by a single centrifuge method where the blood cells were manually separated into layers and the kit was not used. In our PRP preparation protocol that supports the study of Perez et al. (15), optimum platelet concentration was achieved with low centrifugation time at high acceleration force. Thus, the proliferation of dermal papillary cells is increased and apoptosis is prevented by effectively prepared PRP.

Patients with low-grade alopecia (according to the Norwood-Hamilton and Ludwig scale) had better results than those with advanced alopecia. In our study, hair pull test was found to be positive in 86% of the patients at the 6th month after 3 sessions. This result is comparable to the results of Khatu et al.

Figure 3. Baseline, 1st month and 6th month photographs of a 45-year-old female patient after 3 sessions

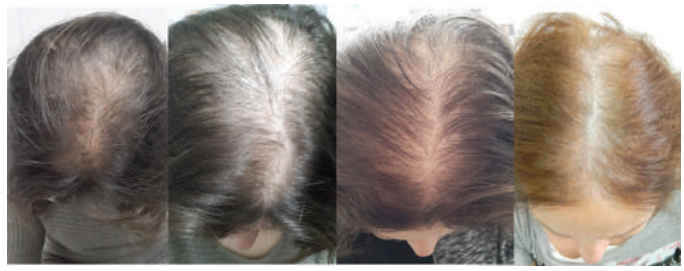

Figure 4. Baseline, 1st month and 6th month photographs of a 51-year-old female patient

(6) reporting negative hair pull test in 81% of patients after 4 sessions with an interval of 2 weeks and the results of Besti et al. (16) reporting negative hair pull test in all patients (after the 3rd session) after 5 sessions with an interval of 2 weeks. In addition, Gkini et al. (4) reported negative hair pull test after 3 PRP sessions with an interval of 3 weeks, but they emphasized an increase in the number of hair loss in the 6th month follow-up and applied a booster at 6th months.

The hair evaluation methods we used in our study were not objective methods, but, before each treatment and after each session, it was standardized for each patient. Although hair pull test was carried out in a standard fashion by two evaluators, it is a subjective assessment. Macroscopic photographs give an overview of hair growth, quality and hair density. The TrichoScan assessment, which is a more objective method that should be applied to a shaved part of the patient's scalp, could not be performed, as most patients, especially women, did not consent its application. The VAS score is a subjective method, as it is filled by the patients. However, a suitable method for measuring hair growth over time is not available in a repeatable, economic and non-invasive manner, and these methods have provided a relatively reasonable assessment of the results after treatment. There are some limitations in this study such as small sample size and short follow-up time to assess long-term efficacy of treatment. Therefore, further studies with longer follow-up periods and larger sample groups are needed.

CONCLUSION

In conclusion, PRP injection, which provides optimal platelet concentration with an effective and easy preparation protocol without using commercial kit, is a simple, low cost and satisfactory treatment option in androgenetic alopecia.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Informed consent forms were signed by all patients.

Peer-review: Externally peer-reviewed.

Author Contributions

Surgical and Medical Practices: K.Ö., Ö.Ç., Concept: K.Ö., Ö.Ç., Design: K.Ö., Ö.Ç., Data Collection or Processing: K.Ö., Ö.Ç., Analysis or Interpretation: K.Ö., Ö.Ç., Literature Search: K.Ö., Ö.Ç., Writing: K.Ö., Ö.Ç.

Conflict of Interest: The authors have no conflicts of interest to declared.

Financial Disclosure: The authors declared that this study has received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.48243
Eur Arch Med Res 2019; 35 (1): 38-42

Does the Timing of Episiotomy Repair Influence the Incidence of Postpartum Hemorrhage? A Randomized Controlled Study

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Abstract

Objective: To evaluate the effect of the timing of episiotomy repair on the incidence of postpartum hemorrhage.

Methods: This randomized controlled trial included 307 pregnant women who delivered vaginally and underwent mediolateral episiotomy in a tertiary-care hospital. In Group I, the repair of the episiotomy was started while the placenta was still inside. In Group II, spontaneous delivery of the placenta was waited, and then the repair was initiated. The primary outcome was the incidence of postpartum hemorrhage. Secondary outcomes were the mean blood loss, postpartum 24th hour hemoglobin (Hb) and hematocrit (Hct) levels, mean Hb and Hct change, and the need for transfusion.

Results: The rate of postpartum hemorrhage (>500 mL) did not differ significantly between the two groups (5.2% in Group I vs. 6.5% in Group II, p=0.62). The mean blood loss did not differ significantly between the two groups (206±120 mL in Group I vs. 210±134 mL in Group II, p=0.76). There was no statistical difference between the two groups regarding postpartum Hb and Hct levels, mean Hb and Hct change, and the need for transfusion.

Discussion: Timing of episiotomy repair has no effect on the incidence of postpartum hemorrhage in a tertiary hospital.

Keywords: Episiotomy, postpartum hemorrhage, timing of episiotomy repair

INTRODUCTION

International Federation of Gynecology and Obstetrics described episiotomy as a surgical incision made in the perineum and vagina by a trained obstetrician to enlarge the vaginal opening (1). For many years, episiotomy has been thought to prevent deep tears that may occur during delivery and provides better healing than natural tears and has been used routinely in nulliparous patients (2). In the Cochrane meta-analysis published in 2000, these ideas were found to be inaccurate and a 'selective' episiotomy was recommended instead of routine episiotomy (3). After this meta-analysis, episiotomy rates have decreased in many countries (4-6). It is recommended that the rate of episiotomy should not exceed 10% in vaginal deliveries (7). Episiotomy has been identified as a risk factor for postpartum hemorrhage (8-10).

Postpartum hemorrhage complicates 0.5-1% of vaginal deliveries and remains the most important cause of maternal mortality in the world (11, 12). The third stage of labor begins after the birth of the fetus and ends with the separation of the placenta. Postpartum hemorrhage is often seen at this stage. Therefore, proper management of the third stage of labor plays a critical role in preventing postpartum hemorrhage. Uterine atony is the most common cause of postpartum hemorrhage and constitutes 60-80% of cases (13). Studies to prevent postpartum hemorrhage

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Received: 25.04.2018
Accepted: 19.09.2018

Cite this article as: Yıldırım D, Bafali İO, Ateş MC, Erdem B, Köroğlu N, Karaaslan O. Does the Timing of Episiotomy Repair Influence the Incidence of Postpartum Hemorrhage? A Randomized Controlled Study. Eur Arch Med Res 2019; 35 (1):38-42.

have often focused on the prevention of atony. Episiotomy, if administered, contributes to the loss of blood in the third stage of labor. Considering that postpartum hemorrhage is defined as blood loss of 500 mL or more in vaginal births, the importance of timing of episiotomy repair can be better understood.

The aim of this randomized controlled study was to investigate the effect of the timing of episiotomy repair on the incidence of postpartum hemorrhage.

METHODS

This randomized controlled trial was conducted at İstanbul Kanuni Sultan Süleyman Training and Research Hospital between February 2015 and November 2016. The study was approved by the Local Ethics Committee (no. 5896) and was performed in accordance with the 1975 Helsinki Declaration, which was revised in 2000.

Inclusion criteria were as follows: gestational age between 36-42 weeks, singleton pregnancy, cephalic presentation, estimated birth weight between 2500-4500 grams, maternal age between 18-40 years, parity between 0-5 and episiotomy.

Exclusion criteria were as follows: acute fetal distress, persistent hypertension (>140/90 mm Hg), placenta previa, ablatio placenta, bleeding due to any reason during birth or pregnancy, previous caesarean section, uterine scar, postpartum hemorrhage in previous pregnancies, polyhydramnios, chorioamnionitis, abnormal placentation, coagulation defects, application of vacuum or forceps, hemoglobin (Hb) <8 gr/dL, anticoagulant or tocolytic use in pregnancy, multiple pregnancy, uterine malformation, deep vaginal or cervical laceration, no need for episiotomy, manual removal of the placenta, vaginal hematoma and postpartum atony.

Power analysis was performed before the study. In a previous study, a Hb decrease of 1.62 gr/dL was detected during delivery. Given this value, it was determined that at least 150 patients should be taken in both groups in order to achieve a 20% decrease in blood loss compared to the control group with an 80% power and a p value less than 0.05. Initially, 443 pregnant women were included in the study. Informed consent was obtained from pregnant women who were admitted for labor and agreed to participate in the study. Standard forms were prepared for both groups and half of them were labeled as “placenta inside”, and the other half was labeled as “placenta outside”. The prepared forms were put in opaque envelopes. When the pregnant woman was taken to the maternity table, one of the envelopes was pulled, and the randomization was achieved.

Mediolateral episiotomy was performed to all pregnant women included in the study. In case of clinical necessity, superficial perineal anesthesia was performed with 4 mL Jetocaine ampoule (ADEKA®, lidocaine HCl 40 mg/2 mL, epinephrine 0.025 mg/2 mL) and episiotomy was performed on the right mediolateral region at an angle of 45 degrees at the level of 7-8 o’clock during the crowning of the head of the fetus. After the fetus was born, the pulsation of the umbilical cord was expected to weaken and was then clamped. Sterile disposable collection bags (Brass V Shape, Ekin-Turkey) were placed to the delivery table in order to determine the amount of hemorrhage. After amniotic fluid drainage with delivery, the zipper of the bag was opened and left open for one hour. In group I (placenta inside group), the episiotomy repair was started after the fetus was born while the placenta was not separated. If the placenta showed signs of separation while the repair was in progress, the placenta was removed, and the repair was resumed. In group II (placenta outside group), the placenta was expected to separate spontaneously, and then the repair was initiated. During placental separation, excessively bleeding vaginal vessels were clamped. In both groups, controlled cord traction or any other procedure was not performed to separate the placenta. The vagina was sutured with size 1 polyglactin 910 (Ethicon® Vicryl Rapid™) by continuous locking technique. The perineal muscles and subcutaneous tissue were sutured by interrupted suture technique. The skin was sutured with size 0 vicryl (Doğsan® Pegelak® Rapid) by interrupted suture technique. Ten IU of oxytocin was administered intramuscularly after the separation of the placenta in both groups. The third stage of birth was similarly managed in other respects.

Hb and hematocrit (Hct) levels were recorded from all pregnant women during the hospitalization. If the placenta did not separate spontaneously within 30 minutes, it was performed manually. Uterine tonus was evaluated every 15 min until the patient left the delivery room. If the vaginal bleeding persisted, the cervix and episiotomy line were re-evaluated for tears that could be overlooked. Episiotomy line of the patients with hematoma was opened and sutured again. Uterine massage and extra uterotonics were used in case of atony. Hb and Hct levels of all patients were recorded on postpartum day 1. Transfusion was performed to women who had Hb levels less than 8 g/dL and had symptoms of anemia.

Statistical Analysis

Jamovi v0.8.6.000 program was used for statistical analysis. Shapiro-Wilk analysis was used to evaluate normality of continuous variables. The data with normal distribution were

analyzed by independent samples t-test and the data with non-normal distribution were analyzed by Mann-Whitney U test. Chi-square test was used for categorical variables and Fisher's exact test was used for appropriate data. P<0.05 was accepted as statistically significant.

RESULTS

Initially, 443 pregnant women were included in the study (Figure 1). Following patients were excluded from the study: 26 patients due to urgent cesarean need, 98 patients due to lack of need for episiotomies and 2 patients due to vacuum application. Three hundred and seventeen pregnant women were randomized into two groups, 160 patients in group I and 157 patients in group II. Six patients in group I and four patients in group II were excluded from the study due to deep vaginal tears, hematoma, atony or manual removal of placenta. So, 154 women in group I and 153 women in group II were included in the study and their results were analyzed.

The clinical characteristics of the study cohort are shown in Table 1. There were no statistically significant differences between the two groups in terms of age, body mass index, gestational week, birth weight, prepartum Hb, augmentation and parity. Prepartum Hct level was significantly lower in group I (p=0.04).

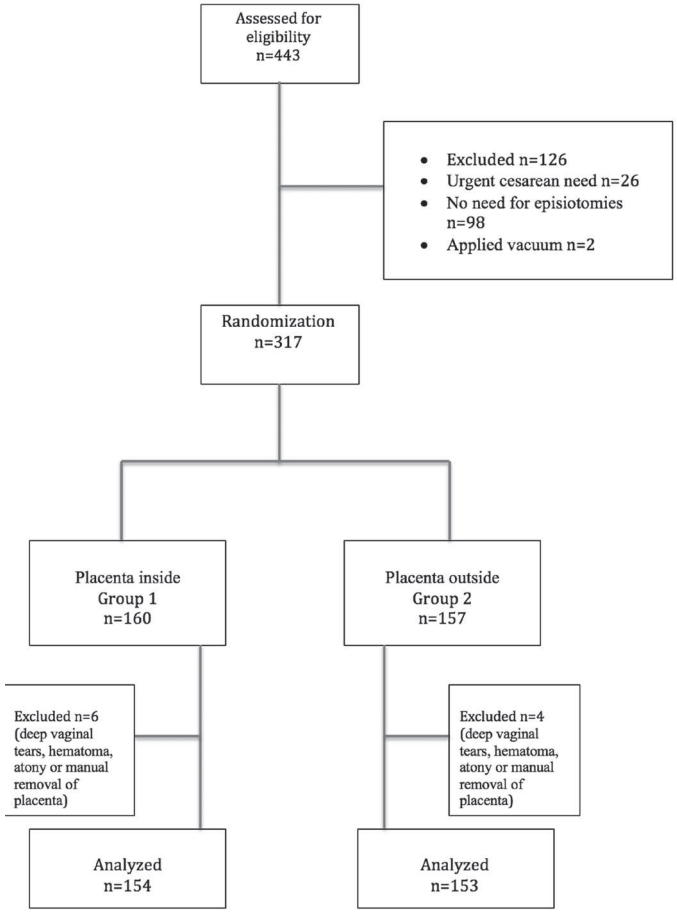

Figure 1. Study flow chart

Table 2 shows the results of the study. No statistically significant difference was found between the two groups in terms of the number of women with blood loss >500 mL [(8/154 (5.2%) in group I vs. 10/153 (6.5%) in group II, p=0.62)]. No statistically significant difference was found between the two groups in terms of mean blood loss (206±120 mL in group I vs. 210±134 mL in group II, p=0.76). There was no statistical difference between

Table 1. Clinical characteristics of groups

		Group I (Placenta inside) n=154	Group II (Placenta outside) n=153	p
Age, years		24.5 (4.5)	24.2 (4.3)	0.55
BMI, kg/m^2		26.9 (2.3)	27.1 (1.9)	0.29
Gestational age, weeks		38.8 (1.2)	38.7 (1.3)	0.67
Birth weight, grams		3205 (354)	3260 (375)	0.19
Prepartum Hb, g/dL		11.6 (1.4)	11.9 (1.5)	0.12
Prepartum Hct, %		35.1 (3.6)	36.3 (3.7)	0.04*
Augmentation, n (%)		75 (49)	72 (47)	0.77
Parity, n (%)				
	Nulliparous	123 (79.9)	129 (84.3)	0.31
	Multiparous	31 (20.1)	24 (15.7)	

All variables were expressed as mean (standard deviation). BMI: Body mass index, Hb: Hemoglobin, Hct: Hematocrit *Statistical significance (p<0.05)

Table 2. The results of the study group

	Group I (Placenta inside) n=154	Group II (Placenta outside) n=153	p
The amount of bleeding, mL	206 (120)	210 (134)	0.76
Blood loss >500 mL, n (%)	8 (5.2)	10 (6.5)	0.62
Postpartum 24th hour Hb, (g/dL)	10.3 (1.4)	10.4 (1.6)	0.12
ΔHb, g/dL	1.31 (0.7)	1.47 (0.9)	0.13
Postpartum 24th hour Hct, %	30.9 (3.6)	31.7 (3.8)	0.08
ΔHct, %	4.21 (2)	4.65 (2.3)	0.06
Transfusion, n (%)	3 (2)	3 (1.9)	0.99

All variables were expressed as mean (standard deviation).

Hb: Hemoglobin, Hct: Hematocrit, ΔHb: Hemoglobin difference; ΔHct: Hematocrit difference

the two groups regarding postpartum Hb and Hct levels, mean peripartum Hb and Hct change, and the need for transfusion.

DISCUSSION

Postpartum hemorrhage is defined as blood loss of 500 mL or more within 24 hours following vaginal delivery. Most of the maternal deaths occur in this critical 24 hours, mainly as a consequence of uterine atony (8). In 2007, the World Health Organization (WHO) recommended the use of active management in the third stage of labor in order to prevent postpartum hemorrhage (14). Active management has three components: administration of uterotonic agent, early cord clamping and controlled traction of the umbilical cord. The use of two of these three components of active management is controversial: early cord clamping leads to low levels of neonatal Hb, and controlled traction of the cord requires experienced practitioners and may cause severe side effects such as inversion. In a multicentre study in 23681 pregnant women, Gülmezoğlu et al. (15) reported that the main component of active management was the use of uterotonic agent and that omission of controlled cord traction did not affect postpartum hemorrhage.

In Cochrane meta-analysis, Westhoff et al. (16) compared the use of different uterotonic agents in the third stage of labor and found that prophylactic oxytocin was superior to the ergot alkaloids in preventing postpartum hemorrhage. WHO published a new guide in 2012 stating that the use of oxytocin (10 IU IV/IM) in the prevention of postpartum hemorrhage should be the first choice, but that the timing of oxytocin administration is optional (17). In a Cochrane meta-analysis comparing the timing of oxytocin administration, Soltani et al. (18) reported that the application of oxytocin before or after the removal of placenta had no effect on the incidence of postpartum hemorrhage (18). In a recent study, this finding was found to be similar in low-risk pregnancies (19).

Studies on postpartum hemorrhage have rightly focused on the prevention of uterine atony, the most common cause. However, there are other factors affecting the amount of postpartum hemorrhage. Episiotomy is estimated to cause extra blood loss of 300-600 mL (2). Mediolateral episiotomies have more blood loss than midline episiotomies (8). In patients undergoing episiotomy, repair is often performed after the placenta has been removed. In theory, suturing the episiotomy without waiting for the placenta removal may reduce blood loss. There is limited number of studies investigating this issue.

Baksu et al. (20) found that the repair of the mediolateral episiotomy with the placenta inside caused a lower Hb and Hct difference than the repair after removal of the placenta. Kelekci et al. (21) found that postpartum Hb levels were significantly lower in women who underwent episiotomy repair while the placenta was inside, however postpartum Hct levels remained similar between the two groups. Özdeğirmenci et al. (22) found no difference in mean blood loss, mean Hb and Hct decrease between the two groups. Dündar et al. (23) found that the mean Hb and Hct differences were similar between the two groups.

Our study showed that the primary outcome parameter, the timing of mediolateral episiotomy repair, has no effect on the incidence of postpartum hemorrhage. There was no difference between the two groups in terms of secondary outcomes, including mean blood loss, mean Hb and Hct changes. The major limitation of our study was that only the data analyst could be blinded. The practitioner could not be blinded because of the different behaviors required for the interventions being tested. The other limitation was the lack of data on the duration of placental separation and additional analgesic requirement. The strength of the study was that it was a randomized, controlled study and that the blood loss was calculated not only by peripartum Hb and Hct changes, but also by collection bags.

CONCLUSION

Timing of episiotomy repair has no effect on the incidence of postpartum hemorrhage in a tertiary-care hospital. In our country, approximately 1.3 million births occur annually and mediolateral episiotomy is performed frequently. It is reasonable to avoid routine episiotomy to prevent blood loss due to episiotomy. In cases where episiotomy is performed, the timing of repair can be left to the choice of the practitioner. Randomized controlled trials are needed to better clarify the subject.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee (no. 5896) and was performed in accordance with the 1975 Helsinki Declaration, which was revised in 2000.

Informed Consent: Informed consent was obtained from pregnant women who were admitted for labor and agreed to participate in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.Y., İ.O.B., M.C.A., O.K., Concept: D.Y., Design: D.Y., Data Collection or Processing: İ.O.B., M.C.A.,

O.K., Analysis or Interpretation: İ.O.B., B.E., N.K., Literature Search: D.Y., İ.O.B., M.C.A., Writing: D.Y., İ.O.B., M.C.A., B.E., N.K., O.K.

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

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2018.09719
Eur Arch Med Res 2019; 35 (1): 43-8

Palmar Fibromatosis: an Analysis of 25 Cases

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Abstract

Objective: Dupuytren's contracture (DC) is a benign tumor that results in nodular or fibrous band-like thickening of the palmar fascia and leads to palmar and digital contractures. Although they are benign lesions, post-operative recurrence is frequent. In the present study, we retrospectively analyzed clinicopathological findings of 25 cases that underwent surgery for DC. We also aimed to determine the relationship between relapse and Ki-67 proliferation index and smooth muscle actin (SMA) staining intensity in excision materials by immunohistochemical method.

Methods: The demographic characteristics, severity of contracture, treatment type, Ki-67 proliferation index, SMA staining intensity and recurrence were evaluated retrospectively in 25 patients who were operated with the diagnosis of DC at the Department of Orthopedics and Traumatology in Recep Tayyip Erdogan University between 2009 and 2015.

Results: The age range of the patients was 42-75 years (mean age: 55 years), and 6 were female and 19 were male. Fifteen of the lesions were in the right hand, and 10 were in the left hand. All patients underwent extensive palmar fascia excision with regional intravenous anesthesia. Only one patient had recurrence. Ki-67 proliferation index was 1-2% in patients without recurrence, however, >5% nuclear positivity was detected in a patient with recurrence. In addition, strong positive staining for SMA was observed in this patient.

Conclusion: The use of Ki-67 proliferation index and SMA staining intensity to evaluate fibroblastic proliferation in the pathological examination of surgical DC specimens may be valuable in clinical follow-up in terms of recurrence.

Keywords: Dupuytren's contracture, Dupuytren's disease, fasciectomy, palmar contracture

INTRODUCTION

Dupuytren's contracture (DC) was first reported in 1777 by Henry Cline. It was described in detail by Baron Guillaume Dupuytren, a French surgeon, in 1834 (1, 2). DC is a tumor that results in nodular or fibrous band-like thickening of the palmar fascia in the palm and fingers. They are benign in character; however, they may also be locally aggressive. The clinical symptoms are seen in the palms and fingers. It is generally seen as a movement restriction and a painless mass (3, 4). DC can be associated with other fibromatoses including plantar fibromatosis (Ledderhose disease), penile fibromatosis (Peyronie disease), and fibromatosis of the dorsum of the proximal interphalangeal joints (Garrod's nodules or knuckle pads) (5). The etiology is not clear. It extends from the palm towards the fingers in the form of myofibroblast-induced fibrous bands. Over time, palmar and digital flexion contractures occur. It disrupts hand function and affects quality of life (6). Even though it is widely seen among ethnic groups, its prevalence is high in North American men. Moreover, its prevalence increases with age (4). Despite recent research on different treatments (7), the surgical method commonly used in the treatment of contracture is incision and fasciectomy (8, 9). Post-operative recurrence has been reported to vary in a wide range from 0 and 70% (10). Despite different treatment modalities, the likelihood of recurrence is quite high (7). In the literature, there are studies investigating the relationship

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Received: 25.07.2018
Accepted: 06.11.2018

Cite this article as: Balık M.S, Bedir R, Palmar Fibromatosis: An Analysis of 25 Cases. Eur Arch Med Res 2019; 35 (1):43-48.

between the surgical method and the clinic in terms of determining the recurrence of DC (10, 11, 12). For this reason, it is very important to estimate in which cases the recurrence will be observed.

In the present study, it was aimed to investigate the relationship between recurrence and Ki-67 proliferation index, smooth muscle actin (SMA) staining intensity, and clinicopathological findings of cases who had been diagnosed with DC and undergone surgical intervention.

METHODS

After the approval of Recep Tayyip Erdoğan University Ethics Committee (no: 34, date: 12.23.2016), the clinical and pathological records of 25 cases diagnosed with DC in the Department of Pathology between 2009 and 2015 were documented. Written informed consent was obtained from the patients. The hematoxylin-eosin (HE) stained sections were retrospectively analyzed. Age, gender, localization, contracture level, treatment type, and recurrence were determined. In addition, Ki-67 proliferation index and SMA staining intensity were examined.

HE-stained preparations were fixed in formol. The 5 µm-thick cross-sections obtained from the selected paraffin blocks were taken on slides treated with HistoGrip™ (Zymed Laboratories, CA, USA). After deparaffinization, the cross-sections were boiled in citrate buffer (pH=6) in order to extract antigen. In immunohistochemical examination using the streptavidin-biotin method, Ki-67 (MM1, Cod: 801704, pre-prepared) primary antibody and SMA (MM1, Cod: 28330030, pre-prepared) were used, as well as aminoethyl carbazole chromogen used as chromogen. Mayer's hematoxylin was used in contrast staining. Mounting medium (Zymed) was used when closing the slide. Sections were analyzed by Olympus BX50 light microscope (Olympus America, Inc., NY, USA).

Randomly selected tumor sites were examined in Ki-67 scoring. The ratio of the number of positive stained cells to the total number of cells under 5 different magnification zones (40X) for each sample was determined as the labeling index (L1). Only the nuclei that were stained clearly were accepted as positive. The number of mitosis was determined at 10X magnification in a randomly selected microscopic region (x400). SMA staining intensity was divided into 3 groups as mild (+), moderate (++), and intense (+++).

Statistical Analysis

The SPSS 16.0 statistical software package (SPSS Inc, Chicago, Illinois, USA) was used for all calculations. Normality was tested using the Kolmogorov-Smirnov test. Data are presented as median (inter-quartile range, IQR) for non-normally distributed continuous variables. Measurement of central tendency was given as a box-plot graphic. Difference between variables were evaluated by Kruskal-Wallis with post hoc Mann-Whitney U test. Group differences with p value <0.05 were accepted significant.

RESULTS

Twenty-five DC cases were examined. The patients' ages ranged from 42 and 75 years, with a mean age of 55 years. Twenty-four percent of the patients were female and 76% were male. All patients underwent extensive palmar fascia excision under regional intravenous anesthesia. The excised masses were in the form of cords. Fifteen cases (60%) were in the right hand and 10 (40%) were in the left hand. The most frequently observed contracture localization was the 4th and 5th fingers. Seven patients had type-II diabetes mellitus (DM) for more than five years. Table 1 shows the distribution of DC cases by age, gender, type of treatment, localization, Ki-67 proliferation index, and

Table 1. Clinical and histopathological findings

Case no	Age	Gender	Recurrence	Side	Ki-67	SMA
1	49	Female	No	Right	0%	+
2	56	Male	No	Right	1%	+
3	42	Male	No	Left	1%	+
4	75	Female	No	Right	0%	+
5	42	Male	No	Left	1%	+
6	56	Male	No	Right	2%	++
7	62	Male	Yes	Right	5%	+++
8	54	Male	No	Left	1%	+
9	46	Male	No	Right	2%	+
10	46	Male	No	Right	1%	+
11	67	Male	No	Left	2%	+
12	75	Male	No	Left	2%	++
13	63	Male	No	Left	1%	+
14	49	Male	No	Right	1%	+
15	67	Male	No	Right	1%	+
16	75	Male	No	Right	1%	+
17	50	Female	No	Left	2%	+
18	60	Male	No	Left	1%	+
19	50	Male	No	Right	1%	+
20	58	Male	No	Right	1%	+
21	57	Female	No	Right	1%	+
22	45	Male	No	Right	2%	++
23	54	Male	No	Left	1%	+
24	52	Female	No	Left	2%	+
25	45	Female	No	Right	1%	+

SMA: Smooth muscle actin, Ki-67: Proliferation index

SMA staining intensity. The treatment was extensive excision for all contractures, and recurrence was observed only in one patient after approximately one year. All the patients admitted with a painless mass growing slowly on the hand and contracture of the fingers and metacarpophalangeal joint. Bone tissue was normal in the conventional radiological examination of the patients. Mass shadow was observed in soft tissues. In six cases with digital contracture, the clinical problem was trigger finger due to the compression in the A1 pulley. Nodules accompanied fibrous bands in 12 cases. On physical examination of all cases, there was a fibrous band that caused 20-30º flexion contracture in the metacarpophalangeal joint in the hand.

Pathological examination revealed irregularly shaped tissues with localized elastic nodules of different sizes and gray-white color. Microscopic examination revealed fibroblastic proliferation in fascial tissue samples (Figure 1). There was a severe increase in cellularity in four cases, moderate increase in eight cases, mild increase in seven cases, and no increase in six cases. In immunohistochemical analysis, Ki-67 proliferation rates of lesions were generally low (1-2%), but high in only one case (5%) (Figures 2 and 3). SMA staining was intense in one case (4%), moderate in three cases (12%), and mild in 21 cases (84%) (Figure 4). The relationship between Ki-67 proliferation rates and age and gender is presented in Figure 5.

DISCUSSION

Although the etiology of DC is not clear, it has been reported to be associated with age, genetic factors, gender, alcohol and smoking, as well as working (3, 4, 5, 9, 13). The most common site of the disease is palmar fascia. But, the involvement of body parts other than the palm can also be seen (5). Furthermore, it has been reported to be associated with frozen shoulder syndrome due to its fibro-proliferative characteristics (11). However, no involvement was observed in other body regions in our cases.

In terms of gender, the prevalence of DC was 1.5-fold higher in males (14). It is more common especially among 40 year-old males (15). Palmar fascia is the target tissue for androgen activity. The higher number of androgen receptors in DC when compared to normal palmar fascia explains the higher frequency in male gender (16). In our patient group, the dominant gender was male with 76%. However, female/male ratio was higher than the literature. Moreover, the males had more severe contractures than females.

DC is a hand pathology that is frequently observed with DM. Its prevalence varies between 16% and 42% (17). The prevalence of DC increases with the duration of DM (18). The formation of contracture at hand is associated with smoking and free radical

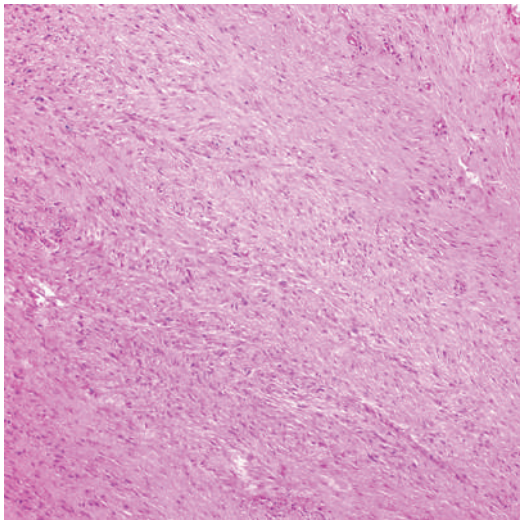

Figure 1. Lesion with proliferation of fibroblasts (H&Ex100)

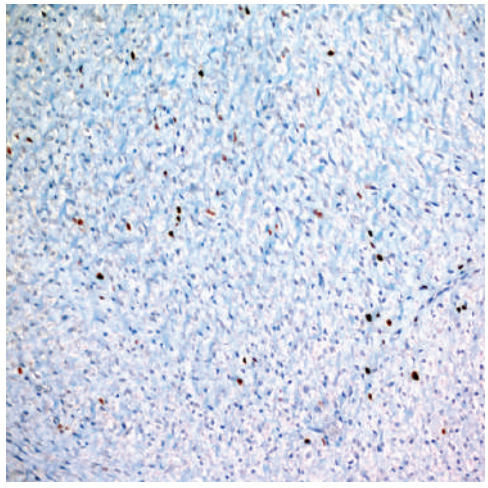

Figure 2. Low Ki-67 proliferation index (x200)

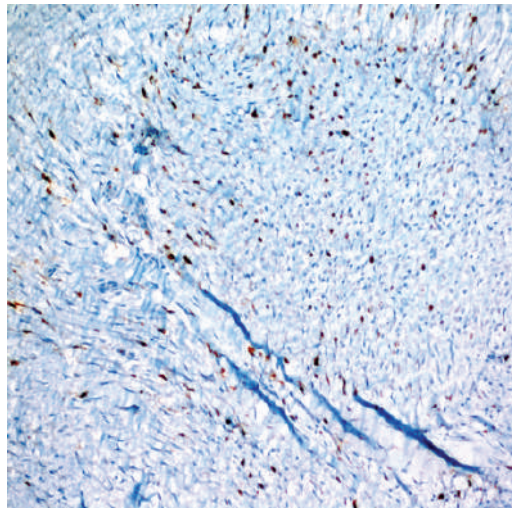

Figure 3. High Ki-67 proliferation index (x200)

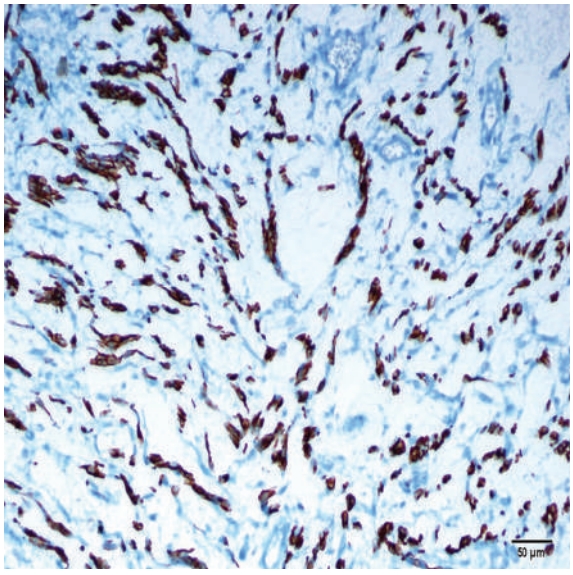

Figure 4. Strong smooth muscle actin positivity (x200)

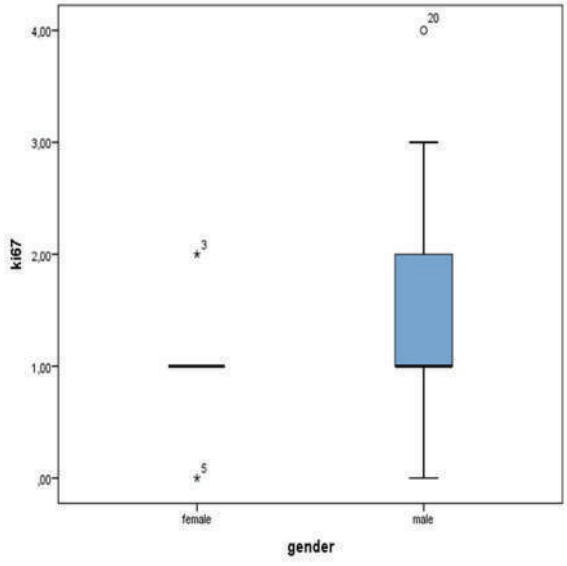

Figure 5. a) Relationship between Ki-67 proliferation rates and gender

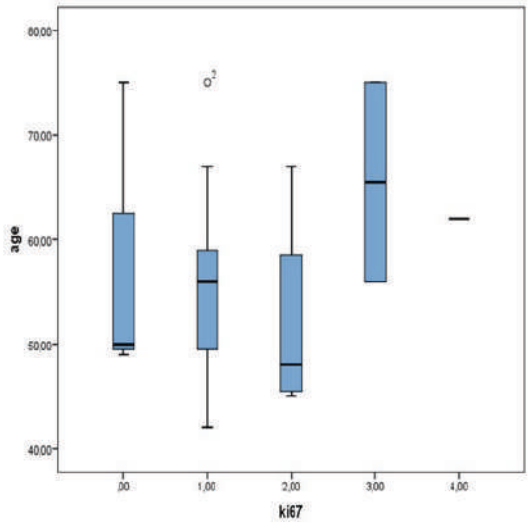

Figure 5. b) Relationship between Ki-67 proliferation rates and gender

(in DM) and local ischemia (13). Twenty-eight percent of our cases had DM. The prevalence was consistent with the literature. However, in all cases, there were nodules accompanying the fibrous bands. But, there was no difference between the duration of DM and DC clinic.

In the histopathology of DC, there are proliferative, involutional, and residual phases. In the proliferative phase, the number of fibroblasts increases. Due to various factors, fibroblasts form myofibroblasts. The myofibroblasts show the characteristics of both smooth muscle cell and fibroblasts. Thus, they have the ability to contract (19). The involutional phase is characterized by cell loss and the number of myofibroblasts decreases (20). In residual phase, reductions are observed in the number of myofibroblasts. Together with mature fibroblasts, also the amount of collagen increases. At this phase, type III collagen increases (21, 22). Moreover, nodules form band-like structure from the mature scar tissue (23). Myofibroblasts play significant role in tissue contraction in DC (24).

DC nodules have a cell density of myofibroblasts that provides fibroblast and smooth muscle cell character. Compared with the cells derived from band, positive SMA staining was found to be higher in majority of nodule cell culture and it corroborates this conclusion (25).

Various factors have been proposed to play role in post-operative clinical recurrence (26, 27). One of these factors is the relationship between myofibroblasts and intracellular microtubules. The presence of myofibroblast in residual tissue can be used to predict the likelihood of recurrence. For this reason, radical excision of the palmar fascia and the involved skin zone with open surgery method and covering the surgery zone with full-thickness skin graft is useful in preventing the contracture (26).

Passive fibrous band fibroblasts may be reactivated as myofibroblasts with the effect of growth factors. This may be a major factor for high post-operative recurrence rates or for progression after injury (27).

Increased post-operative recurrence increased the importance of predicting the recurrence. Recurrence and aggressiveness can be estimated using a high level of increase in cellularity in DC, because the cellularity tends to decrease and the amount of type-III collagen tends to increase as the disease matures. Myofibroblasts decrease and the fibroblasts replace them in the residual phase (28). Significant increase in cellularity was

observed in four of our cases, but the recurrence was seen in only one of these cases.

If DC is aggressive and the proliferative phase is stronger, maturation is delayed. Recurrence of this disease is known to be associated with cellular diversity. The collagen rate is lower in aggressive cases (28). The presence of SMA in myofibroblasts is an indicator of disease activity. It is commonly found in aggressive surgical materials (29). Ki-67 proliferation index, which is a proliferative marker, can be frequently seen at high levels in aggressive cases (28). When our symptomatic cases were operated for 20-30° flexion contracture in the metacarpophalangeal joint, moderate-strong SMA positive staining was observed in three of four cases with high cellularity, and Ki-67 proliferation index was found to be at high level only in one case. The level of Ki-67 proliferation index was low in other cases. Despite the absence of benign lesions, the post-operative recurrence is common. Extensive surgical excision is very important because of the insufficient surgical excision (24). However, the recurrence was observed in our case with >5% Ki-67 proliferation index. Because of the recurrence despite the extensive excision of the palmar fascia, the use of Ki-67 proliferation index and SMA staining intensity in the analysis of the fibroblastic proliferation in the pathological examination of excised materials in DC may be beneficial in the clinical follow-up of the recurrence of the lesion.

The main limitation of the present study is limited number of cases. Moreover, no histopathological comparison could be made between different surgical samples, such as nodules and bands. However, despite all the limitations, we believe that it will contribute to further studies on recurrence in DC.

CONCLUSION

We believe that the use of Ki-67 proliferation index and SMA staining intensity with immunohistochemical staining method in surgical specimens in order to determine the clinical course of DC might be beneficial in estimating the recurrence.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Recep Tayyip Erdoğan University Ethics Committee (no: 34, date: 12.23.2016)

Informed Consent: Written informed consent was obtained from the patients.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.S.B., R.B., Concept: M.S.B., R.B., Design: M.S.B., R.B Data Collection or Processing: M.S.B., R.B., Analysis or Interpretation: M.S.B., R.B., Literature Search: M.S.B., Writing: M.B.S.

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

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/eamr.galenos.2019.47955
Eur Arch Med Res 2019; 35 (1): 49-53

The Relationship Between Smoking in Pregnancy and Oxidative Stress Biomarker Levels in Cord Blood

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Abstract

Objective: Maternal smoking results in an increase in the incidence of complications such as spontaneous abortion, placental abruption, preterm delivery, intrauterine growth restriction, and stillbirth. There are few studies in the literature about smoking-related oxidative stress in pregnant women and this relationship is not clear. The aim of our study was to determine the relationship between oxidative stress and smoking in pregnancy by measuring oxidative stress indicators such as malondialdehyde and protein carbonyl and total antioxidant status in cord blood and to raise awareness about the public health measures that can be taken in this regard.

Methods: A total of 56 pregnant women (24 pregnant smokers and 32 pregnant non-smokers) were included in the study. Pregnant women were divided into two groups as smokers (n=24) and non-smokers (n=32). Then, smokers were divided into 3 groups according to their smoking frequency. Malondialdehyde, protein carbonyl and total antioxidant status levels in cord blood were compared between these groups.

Results: There was no statistically significant difference between groups in terms of malondialdehyde, protein carbonyl levels and total antioxidant capacity ($p>0.05$). In addition, no statistically significant difference was found between smoking subgroups ($p>0.05$).

Discussion: The potential of smoking to produce oxidative stress in the fetus is not clear enough due to the contradictory findings of clinical trials. Our findings make it difficult to establish a relationship between maternal smoking and oxidative stress. The subject should be illuminated by further clinical studies evaluating different oxidants and antioxidant molecules.

Keywords: Cord blood, newborn, oxidative stress, smoking

INTRODUCTION

Oxidative stress shows the serious imbalance between free radical formation and antioxidant defense mechanism. Uncontrolled increased free radicals lead to oxidation of biomolecules such as nucleic acid, protein and lipid, alteration of genetic information, deterioration of protein structure, inhibition of enzyme activity and damage to cell membrane, thus leading to dysfunction of cells, tissues and organs. As a result, many diseases such as cancer and cardiovascular diseases can develop (1, 2). Smoking exacerbates the production of free radicals and increases the oxidative stress significantly *in vivo* (3).

Maternal smoking is the most important modifiable factor associated with adverse pregnancy outcomes (4, 5). Epidemiological studies have reported that 20-30% of pregnant women smoke (6, 7). Thus, significant metabolic and biochemical changes and adaptive responses are observed in both the mother and the fetus, and there is an increase in the incidence of complications such as spontaneous abortion, placental abruption, preterm delivery, intrauterine growth restriction and stillbirth (8, 9). In the literature, there are few studies about smoking-related oxidative stress in pregnant women and this relationship is not clear (10, 11).

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Received: 28.12.2018
Accepted: 08.01.2019

Cite this article as: Dağ H, Dikker. The Relationship Between Smoking in Pregnancy and Oxidative Stress Biomarker Levels in Cord Blood. Eur Arch Med Res 2019; 35 (1):49-53.

The aim of our study was to determine the relationship between oxidative stress and smoking in pregnancy by measuring oxidative stress indicators such as malondialdehyde and protein carbonyl and total antioxidant status in cord blood and to raise awareness about the public health measures that can be taken in this regard.

METHODS

A total of 56 healthy pregnant women, who were between 20-35 years of age and who gave birth at our hospital, were included in the study. Pregnant women were divided into two groups as smokers (n=24) and non-smokers (n=32). Then, smokers were divided into 3 groups according to their smoking frequency. During their pregnancy, pregnant women in group I had <5 cigarettes a day, group II had 6-10 cigarettes a day and pregnant women in group III had >10 cigarettes a day. Detailed medical histories were obtained and following data were recorded into the case report form: maternal birth year, age, weight, occupation, economic status, presence of gestational diabetes mellitus, number of pregnancies, history of chronic disease, alcohol intake, follow-up status, primiparity-multiparity, drugs used, smoking, fetal birth weight, type of delivery, gender and gestational week of the infant. Patients with gestational diabetes mellitus, maternal or fetal history of a genetic or chronic disease, and patients with alcohol intake were excluded from the study.

The umbilical cord blood was collected into blood tubes. Following 20 minutes of coagulation, the blood was centrifuged and stored at -80 degrees in freezer until studied. Then, sera were studied for malondialdehyde (MDA), which is the end product of lipid peroxidation, and protein carbonyl, which is a product of protein oxidation, and for total antioxidant status (TAS), which demonstrates the antioxidant status. MDA levels were determined by the ELISA method (Uscn Life Science Inc. Wuhan, China). The analytical measuring range is 24.6-2000 ng/mL. Protein carbonyl levels were determined by colorimetric kit (Cayman Chemical, USA). The analytical measurement range of the assay is 1-100 nmol/mL. TAS levels were determined by colorimetric kit (Randox, Lot 362601, UK). The analytical measuring range is 1.3-1.77 mmol/L.

The study was approved by the ethics committee of our hospital with the letter dated 23.02.2016 and numbered 430. Our study was conducted in accordance with the principles of the Helsinki Declaration and we obtained informed consent from all pregnant women who participated in the study.

Statistical Analysis

Statistical analysis was performed with MedCalc (MedCalc Software, Broekstraat, Mariakerke, Belgium) program. Normality of continuous variables was investigated by Kolmogorov-Smirnov test. Variables with Gaussian distribution were expressed as mean ± SD and variables with non-Gaussian distribution as median (interquartile range). Student's t-test was used to compare the variables with normal distribution. Chi-square test was used to compare the group ratios. Correlations between variables were analyzed by Spearman's correlation coefficient (rs) or Pearson's correlation coefficient (r). Statistical significance was assessed at $p<0.05$.

RESULTS

The mean age, maternal weight, gestational week, and parental monthly income levels were not significantly different between the two groups. The educational status profiles and primiparity-multiparity were similar in both groups (Table 1). The birth weights, genders, and mean MDA, protein carbonyl and TAS values in cord blood were not statistically different between the two groups (Table 2).

No statistically significant difference was found between smoking sub-groups (Table 3).

Table 1. Demographic information of mothers in non-smoker and non-smoker groups

	Non-smokers (n=32)	Smokers (n=24)	p
Maternal age (years)	26.6±5.6	28.8±4.9	0.1320
Maternal weight (kg)	79.4±9.2	75.5±16.1	0.2980
Primiparity-multiparity (n/n)	9/23	4/20	0.3580
Gestational week	39.3±0.5	39.2±0.5	0.3480
Education level			
Illiterate (n, %) Elementary school (n, %) Secondary school (n, %) High school (n, %) University (n, %)	 2 (6.3%) 12 (37.5%) 10 (31.3%) 5 (15.6%) 3 (9.4%)	 3 (12.5%) 12 (50.0%) 7 (29.2%) 2 (8.3%) 0 (0.0%)	0.4120
Monthly income level (TL)	1717±762	1604±454	0.5220

Table 2. Neonatal demographic information and cord blood analysis results in smoker and non-smoker groups

	Non-smokers (n=32)	Smokers (n=24)	p
Fetal weight (gr)	3332.9±382.7	3222.1±313.6	0.2530
Gender (boys/girls)	14/18	9/15	0.6380
MDA (ng/mL)	30.8±9.2	29.9±11.0	0.7190
TAS (µmol/L)	1.39±0.18	1.42±0.17	0.5820
Protein carbonyl (nmol/mL)	23.3±6.9	23.9±6.2	0.7310

MDA: Malondialdehyde, TAS: Total antioxidant status

Spearman and Pearson correlation analyzes for each of the two groups showed a poor negative correlation between MDA levels and infant weight in the smoker group among maternal and fetal variables (r=-0.428; p=0.037).

DISCUSSION

Increased oxidative stress in smokers can be explained either by increased free radical production or by deteriorated antioxidant defense system due to direct exposure to smoke (12). In a single breath, a smoker exposes more than 1015 free radicals (13). When compared to non-smokers, it has been reported that glutathione peroxidase and superoxide dismutase antioxidant enzyme levels are significantly low in smokers (14, 15) and oxidative stress markers (MDA levels) are higher in smokers (15, 16) during resting. Even similar dietary consumption (total calories, protein, carbohydrate, fat, vitamin E, vitamin C, and some minerals) did not change these results (12). It has been reported that young and smoker individuals have low plasma antioxidant capacity and high lipid peroxidation level compared to non-smokers and it is emphasized that the most contributing factor to negative changes is the number of smoking years (12). In another study, there was a moderate negative correlation between the number of cigarette packs consumed annually and the level of lipid peroxidation (17). In addition, it was found that DNA damage was significantly higher in young, healthy, male smokers compared to non-smoking control group and the level of reduced glutathione, which is a strong component of the cellular antioxidant defense system, was found to be low (18). In our study, MDA and protein carbonyl levels and TAS levels were measured in cord blood in order to determine the oxidative stress status in pregnant women. The levels of MDA, protein carbonyl and TAS were not statistically different between smokers and non-smokers, and between smoking subgroups.

Ermiş et al. (19) did not find significantly different MDA and superoxide dismutase enzyme levels in smokers and their infants compared to non-smokers and passive smokers, but glutathione peroxidase antioxidant enzyme levels were found to be significantly higher in smokers and their infants. This information suggests that antioxidant activity increases for the purpose of compensation in smoking. The MDA results in the study by Ermiş et al. (19) were similar to MDA results in our study. Some researchers reported that serum levels of superoxide dismutase and glutathione peroxidase were higher in non-smokers than in smokers (20, 21). Bolisetty et al. (22) reported that an increase in oxidative stress in the smokers group resulted in lower vitamin E levels. Chelchowska et al. (11) reported high malondialdehyde and low total antioxidant capacity levels in cord blood of smoker pregnant women. However, in this study, they reported low total antioxidant capacity levels by measuring the total radical capture parameters (TRAP) levels instead of TAS. Fayol et al. (23), on the other hand, reported high TRAP levels in passive smokers compared to non-smokers, while there was no difference in active smokers. In contrast to this information, we did not find significantly different TAS levels between the groups. Olympio Rua et al. (10) reported that the levels of reactive oxygen species and advanced oxidation protein products increased in cord blood of smoker pregnant women. In our study, we found that protein carbonyl levels were not statistically different between the groups. To determine protein oxidation, the formation of protein carbonyl, nitrotyrosine and advanced protein oxidation products or the loss of thiol groups can be used (24, 25).

The antioxidant molecules are exogenous and endogenous structures, which neutralize the damage caused by the oxidant molecules by both intracellular and extracellular defense. While extracellular defense includes a variety of molecules such as albumin, bilirubin, transferrin, ceruloplasmin and uric acid, intracellular free radical collecting enzymes provide the main antioxidant defense. These enzymes are superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase, glutathione reductase and cytochrome oxidase (26). As there is an oxidant-antioxidant balance in the body, the organism is not affected from free radicals as long as the oxidative balance is achieved. Therefore, the changes in the tests we measured in our study may be maintained at normal levels with a compensatory effect, and this may be the reason why we could not find any

Table 3. Results of cord blood analysis in smoker subgroups

	Group I (n=11, <5 cigarettes per day)	Group II (n=5, 6-10 cigarettes per day)	Group III (n=8, 10 cigarettes day)	p
MDA (ng/mL)	27.5±11.7	28.8±5.1	33.8±12.5	0.4680
TAS (µmol/L)	1.43±0.12	1.29±0.28	1.48±0.10	0.6730
Protein carbonyl (nmol/mL)	24.5±5.4	18.4±3.2	26.6±6.8	0.0650

MDA: Malondialdehyde, TAS: Total antioxidant status

different results between the groups. Smoking may cause significant changes in the levels of other oxidant and antioxidant molecules that we did not measure in our study. It is stated that it is difficult to explain the oxidative stress with a single marker (27, 28), and that it is necessary to use more than one marker to show the presence of oxidative stress (28). Therefore, it is clear that further studies are needed to measure other oxidant and antioxidant tests. In our study, we also examined the socio-economic status of pregnant women. We did not find any differences between the smoker and non-smoker groups in terms of educational status and income level.

CONCLUSION

The potential of oxidative stress to produce oxidative stress in the fetus is not clear enough due to the contradictory findings of clinical studies. Our findings make it difficult to establish a relationship between maternal smoking and oxidative stress. The subject should be illuminated by further clinical studies evaluating different oxidants and antioxidant molecules.

Ethics

Ethics Committee Approval: The study was approved by the İstanbul Okmeydanı Training and Research Hospital Ethics Committee (Approval no: 2016/430).

Informed Consent: Obtained informed consent from all pregnant women who participated in the study.

Peer-review: Externally peer-reviewed.

Author Contributions

Surgical and Medical Practices: H.D., O.D., Concept: H.D., O.D., Design: H.D., O.D., Data Collection or Processing: H.D., O.D., Analysis or Interpretation: H.D., O.D., Literature Search: H.D., O.D., Writing: H.D., O.D.

Conflict of Interest: The authors have no conflicts of interest to declared.

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

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