e-ISSN: 2564-6567

ACEM

Archives of Clinical and Experimental Medicine

2019;4(3)

Volume: 4 - Issue: 3

Contents

Case Report

ULAKBİM Dergi Sistemleri (//dergipark.org.tr/en/)

Arch Clin Exp Med 2019;4(3):113-117. e-ISSN: 2564-6567

DOI:10.25000/acem.579968 Araştırma makalesi / Research article

Protective effects of N-acetylcysteine and taurine on oxidative stress induced by chronic acetaldehyde administration in rat liver and brain tissues

Kronik asetaldehit uygulaması ile uyarılan oksidatif streste sıçan karaciğerinde ve beyin dokularında N-asetilsistein ve taurinin koruyucu etkileri

Zeynep Dicle Yıldız [1], Adile Merve Baki [1], Canan Başaran Küçükgergin [1], Pervin Vural [1], Semra Doğru Abbasoğlu [1], Müjdat Uysal [1]

Abstract

Aim: Acetaldehyde (AA) is one of the main products of alcohol metabolism. Exposure to AA can occur through ingestion of several dietary products, inhalation of cigarette smoke/automobile exhausts, or contact with cosmetics. AA accumulation causes oxidative stress. The aim of this study was to investigate the prooxidant/antioxidant status in rats chronically exposed to AA, and to evaluate the effects of N-acetylcysteine (NAC) and taurine (TAU) on prooxidant/antioxidant balance.
Methods: Sprague Dawley rats were divided in the following groups (n=8; each): Control, AA, AA+NAC, AA+TAU. Reactive oxygen species (ROS), diene conjugate (DC), malondialdehyde (MDA), protein carbonyl (PC), ferric reducing antioxidant power (FRAP) and glutathione (GSH) levels as well as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined in liver and brain tissues.
Results: AA treatment in drinking water was detected to induce prooxidant state in both liver and brain of rats. NAC treatment decreased AA-induced prooxidant status in both tissues. Although TAU treatment diminished ROS levels, MDA and PC levels remained unchanged in examined tissues of AA-treated rats. NAC and TAU elevated liver and brain GSH levels in AA-treated rats.
Conclusion: Chronic AA administration has created a prooxidant condition, and NAC/TAU appears to be useful in suppression of the developed oxidative stress.

Keywords: Acetaldehyde, oxidative stress, N-acetylcysteine, taurine, liver, brain.

Öz

Amaç: Asetaldehit (AA), alkol metabolizmasının ana ürünlerinden biridir. AA'e maruz kalma birçok diyet ürününün yenmesi, sigara dumanı/otomobil egzozlarının solunması veya kozmetik ürünlerle temas yoluyla oluşabilir. AA birikimi oksidatif strese neden olur. Bu çalışmanın amacı kronik AA'e maruz kalan sıçanlarda prooksidant/antioksidan durumunu araştırmak ve N-asetil sistein (NAC) ve taurinin (TAU) proksidant/antioksidan dengesi üzerindeki etkilerini değerlendirmektir.
Yöntemler: Sprague Dawley sıçanlar aşağıdaki gruplara ayrıldı (n = 8; her biri): Kontrol, AA, AA+NAC, AA+TAU. Karaciğer ve beyin dokularında reaktif oksijen türleri (ROS), dien konjugatları (DC), malondialdehit (MDA), protein karbonil (PC), ferrik indirgeyici antioksidan güç (FRAP) ve glutatyon (GSH) düzeyleri ve ayrıca süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px) aktiviteleri incelendi.
Bulgular: İçme suyu ile AA uygulanan sıçanların karaciğer ve beyin dokularında prooksidan bir durum oluştuğu saptandı. NAC uygulaması her iki dokuda AA'e bağlı prooksidan durumu azalttı. TAU incelenen dokularda ROS oluşumunu azaltmasına rağmen, MDA ve PC düzeyleri değişmedi. NAC and TAU AA uygulanan sıçanların karaciğer ve beyinlerinde GSH düzeylerini arttırdı.
Sonuç: Kronik AA uygulamasının proksidant bir durum yarattığını, NAC/TAU uygulamalarının AA ile uyarılan oksidatif stresin baskılamada yararlı olabildiği görülmektedir.

Anahtar Kelimeler: Asetaldehit, oksidatif stres, N-asetilsistein, taurin, karaciğer, beyin.

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey.

ZDY: 0000-0002-6518-0538
AMB: 0000-0001-2345-6789
CBK: 0000-0002-1797-5889
PV: 0000-0001-6462-7388
SDA: 0000-0003-3467-9763
MU: 0000-0002-8802-8766

Ethics Committee Approval: The study was approved by the local ethical authority. (Project No: 2013/45)
Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır. (Proje No: 2013/45)

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The present work was supported by the Research Fund of Istanbul University (Project No: 33568 / 42685).
Finansal Destek: Yazarlar bu çalışma için Istanbul Universitesi Arastirma Fonundan finansal destek aldıklarını beyan etmişlerdir (Proje No: 33568 / 42685).

Geliş Tarihi / Received: 20.06.2019
Kabul Tarihi / Accepted: 16.08.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:

Pervin Vural

Adres/Address: Istanbul Faculty of Medicine, Department of Biochemistry, Çapa, 34093, Istanbul, Turkey

e-posta: pervinvural@yahoo.com

Tel/Phone: +90 212 4142188
Fax: +90 212 6215642

Introduction

Acetaldehyde (AA), an organic aldehyde, is a highly reactive compound and the first oxidation product of ethanol metabolism. Ingested ethanol is absorbed from the upper gastrointestinal tract and transported to the liver. Then, it is mainly metabolize into AA by alcohol dehydrogenase-1B and detoxified to acetic acid by aldehyde dehydrogenase-2. Although the liver is the primary site of ethanol oxidation, other organs in gastrointestinal system, heart and brain may also participate in the formation of AA from ethanol [1]. Therefore, many of the toxic effects of ethanol consumption is suggested to be related to AA formation in the liver and other tissues [2, 3].

On the other hand, AA is used as an additive and a flavoring substance in the production of many foods. Therefore, several dietary products such as milk, yogurt, cottage cheese, bread, roasted coffee beans, instant tea, coffee, alcoholic and non-alcoholic beverages contain significant and detectable amounts of AA. AA is widely used in the chemical industry for production of cosmetics, dye, plastics, adhesives, disinfectants and pesticides and is also found in cigarette smoke and automobile exhausts. AA may naturally be formed in small amounts in human body during threonine catabolism [4]. In addition, significant extrahepatic formation of AA takes place in the gastrointestinal system via alcohol dehydrogenase during ethanol oxidation. Therefore, marked amounts of AA may be supplied from dietary sources and generated through extrahepatic metabolism of ethanol [2, 3].

Even though AA is short-lived, prior to its breakdown into acetate, it can cause cellular and tissue injury. Lipid peroxidation, together with the covalent binding of AA to lipids and proteins, is considered a critical process underlying AA-induced toxicity. AA may react with amino, hydroxyl and sulfhydryl groups and modify the structure and function of macromolecules such as DNA, proteins and enzymes [1-3]. In addition, AA induces apoptosis and increases the formation of inflammatory mediators [1, 5, 6]. Findings related to AA toxicity were usually obtained from in vitro experiments with AA [6, 7] or from animals treated with ethanol plus an inhibitor of AA dehydrogenase [8, 9] and transgenic mice [10, 11]. These studies showed that oxidative stress plays an important role in AA-induced toxicity. Therefore, antioxidant therapies may be useful to prevent AA-induced oxidative stress and tissue damage.

N-acetylcysteine (NAC) and taurine (TAU) are well-known sulfur-containing antioxidant molecules [12-15]. NAC is a derivative of cysteine. It acts as free radical scavenger and stimulates GSH synthesis [12, 13]. TAU is important for many physiological functions such as detoxification, membrane stabilization and osmoregulation. It decreases tissue lipid peroxidation by scavenging or quenching ROS or binding free metal ions such as Fe2+ or Cu+ via its sulfonic acid group [14, 15]. Therefore, NAC and TAU treatments were suggested to be useful in oxidative stress-induced pathologies [12-15] such as diabetes mellitus, cancer and various intoxications including ethanol toxicity [16-19], as a result of these properties.

Studies about the effect of in vivo AA treatment are restricted because of its unstable structure and rapid metabolism. In addition, there is no study investigating the effect of in vivo AA treatment on prooxidant-antioxidant balance in hepatic and extrahepatic tissues. Therefore, this study was planned to understand the direct toxic effect of AA from exogeneous sources and the efficacy of NAC and TAU treatments against on AA- toxicity. For this reason, reactive oxygen species formation (ROS), oxidative changes that occur in lipids and proteins as well as antioxidant parameters in liver and brain tissues of rats that were treated chronically with AA along with NAC and TAU were investigated.

Material and methods

Chemicals

AA, NAC, TAU and other chemicals were supplied from Sigma-Aldrich (St. Louis, MO, USA).

Animals and treatments

Male Sprague Dawley rats weighting 240-260 g, were obtained from the Istanbul University Aziz Sancar Institute of Experimental Medicine, Rats were housed in a light- and temperature-controlled room on a 12/12 hours light/dark cycle. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of Istanbul University (Project No: 2013/45).

Animals were divided randomly into four groups. Body weights and drinking and feeding habits were taken into account during experimental period for 8 months.

1) Control (n=8): Rats in this group were fed with a standard pellet lab chow.

2) AA group (n=8): Rats were fed with normal commercial food and treated with AA in the drinking water with increasing concentrations [0.7% (v/v) for the first 4 months, 1.05% for the following 2 months and 1.4% for the last 2 months] to ensure adaptation of rats. The daily consumption of AA was calculated as 400 mg/kg body weight (BW) for 4 months, 600 mg/kg BW for 2 months and 800 mg/kg BW for 2 months. In this period, water containers containing AA were stored in the cold and changed every 2-3 days to prevent evaporation of AA as previously reported [20, 21].

3) AA+NAC group (n=8): Rats were given AA in drinking water and received a normal commercial rat chow containing 1% (w/w) NAC for 8 months. The consumption of NAC was roughly equivalent to 500 mg/kg BW/day.

4) AA+TAU group (n=8): Rats received AA in drinking water and a normal commercial rat chow containing 2.5% (w/w) TAU for 8 months. The consumption of TAU was roughly equivalent to 1.25 g/kg BW/day.

At the end of the treatment period, all rats were anesthetized with sodium thiopental (50 mg/kg, intraperitoneal) and sacrificed by collecting the blood into dry tubes by intracardiac puncture. Serum was obtained by centrifugation at 1,500 × g for 10 min. Liver and brain tissues were rapidly removed, washed in ice-cold saline and stored at -80 ºC until they were needed for analysis. Tissues were homogenized in ice-cold 0.15 M potassium chloride (KCl) (10%; w/v) and homogenates were centrifuged at 600 g for 10 min and obtained postnuclear fractions were used biochemical determinations in tissues. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined in the postmitochondrial fraction of tissues. To obtain this fraction, postnuclear fractions were recentrifuged at 10,000 g for 20 min at 4oC and supernatants were collected. All weight determinations were performed on an EK-i/EW-I scale (A&D Co., Japan).

Determinations in serum

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measurements were performed on Cobas Integra 800 autoanalyzer (Roche Diagnostics, Mannheim, Germany).

Determinations of ROS formation, lipid and protein oxidation products

ROS formation was assayed fluorometrically as described previously [22]. Homogenates were incubated with 100 μM 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA) at 37 °C for 30 min. The fluorescence of 2, 7-dichlorofluorescein

was determined using a microplate fluorometer (Fluoroskan Ascent FL, Thermo Scientific Inc, USA) with an excitation of 485 nm and emission of 538 nm. Results were expressed as relative fluorescence units (RFU).

Lipid peroxidation was determined by measuring dien conjugate (DC) and malondialdehyde (MDA) levels in the liver and brain homogenates [18, 23]. Results were expressed as μmol/g tissue and nmol/g tissue, respectively.

The oxidative protein damage was measured by the quantification of carbonyl groups based on their reaction with 2, 4-dinitrophenylhydrazine (DNPH) to form protein hydrazones. Protein carbonyl (PC) results were calculated from the maximum absorbance (360 nm) using a molar absorption coefficient of 22,000 M−1 cm−1 and expressed as nmol carbonyl per mg protein [24].

Determinations of non-enzymatic and enzymatic antioxidants

Total antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay [25]. In this assay, at low pH, a ferric- tripyridyltriazine (Fe3+-TPTZ) complex is reduced to the ferrous form, which can be monitored by measuring the change in absorbance at 593 nm. Results were expressed as nmol/mg protein. Glutathione (GSH) levels were measured with 5, 5-dithiobis-(2-nitrobenzoate) at 412 nm and were expressed as μmol/g tissue [26].

SOD activity was assayed by its ability to increase the effect of riboflavin-sensitized photooxidation of o-dianisidine and results were given as U/mg protein [27]. GSH-Px activity was measured using cumene hydroperoxide as substrate and results were expressed as nmol/min/mg protein [28]. Protein levels were determined using bicinchoninic acid [29].

Statistical analysis

All statistical analyses were performed with IBM SPSS statistics for Windows (version 21; SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± SEM. Experimental groups were compared using Kruskal–Wallis (post hoc Mann-Whitney U) tests. A p value < 0.05 was considered to be statistically significant.

Results

There were no significant differences in final body weights and liver and brain weights between groups. However, body weight gain during 8 months was found to decrease in comparison with the control group. Liver index (liver weight/BW) increased in treated groups. Chronically AA administration with or without NAC and TAU did not cause significant changes in serum ALT and AST activities (data not shown).

Figure 1 demonstrates the changes in ROS, DC, MDA and PC levels. According to this;

a) AA treatment resulted in significant increases in ROS formation in the liver and brain ($p<0.01$, $p<0.001$). NAC and TAU caused significant decreases in AA-induced ROS formation in the liver ($p<0.001$, $p<0.001$) and brain ($p<0.001$, $p<0.001$) tissues.

b) Hepatic ($p<0.05$) and brain MDA levels increased due to AA treatment, but increases in brain MDA levels were not significant. NAC treatment decreased MDA ($p<0.05$) levels in brain, but not in liver of AA-treated rats. However, there were no changes in hepatic and brain MDA levels of AA-treated rats due to TAU treatment.

c) DC levels in liver and brain tissues remained unchanged.

d) Liver PC levels remained unchanged, but brain PC ($p<0.001$) levels were found to increase in AA-treated rats. NAC treatment diminished liver ($p<0.01$) and brain PC ($p<0.05$) levels in AA-treated rats as compared to AA group, but these levels did not alter due to TAU treatment.

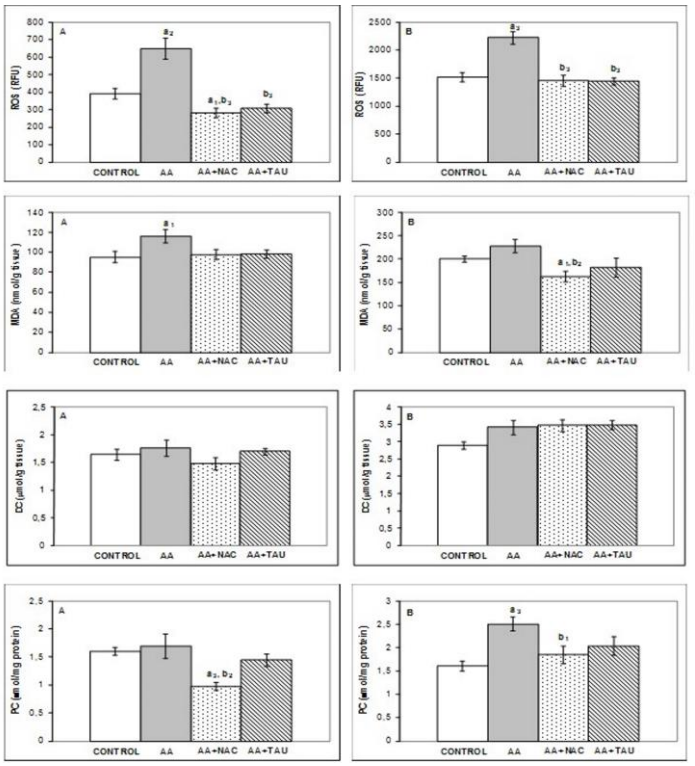

Figure 1: The effects of N-acetylcysteine (NAC) and taurine (TAU) on liver (A) and brain (B) reactive oxygen species (ROS), malondialdehyde (MDA), dien conjugate (DC), and protein carbonyl (PC) levels (Mean ± SEM, n=8 each) a1 $p<0.05$; a2 $p<0.01$; a3 $p<0.001$ according to control group; b1 $p<0.05$; b2 $p<0.01$; b3 $p<0.001$ according to AA group.

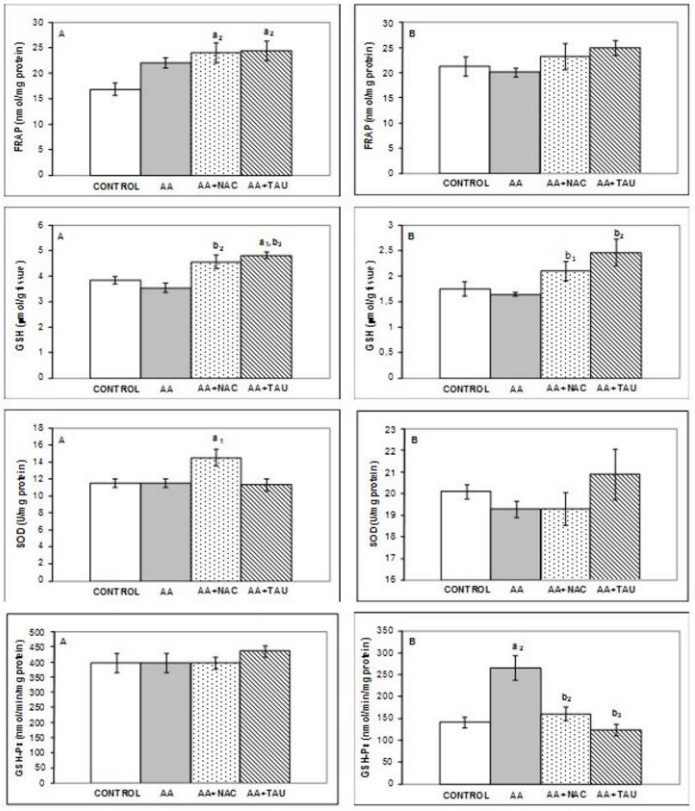

Figure 2: The effects of N-acetylcysteine (NAC) and taurine (TAU) on liver (A) and brain (B) ferric reducing antioxidant power (FRAP), glutathione (GSH) levels, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities (Mean ±SEM, n= 8 each)

a1 p<0.05; a2 p<0.01 according to control group; b1 p<0.05; b2 p<0.01; b3 p<0.001 according to AA group.

Figure 2 demonstrates the changes in antioxidant parameters. According to this;

a) Hepatic and brain FRAP levels were detected not to alter in AA-treated rats. Hepatic FRAP values were higher in AA+NAC (p<0.01) and AA+TAU (p<0.01) groups than control group.

b) There was no significant changes in hepatic and brain GSH levels of AA-treated rats as compared to controls. However, NAC and TAU treatments significantly increased hepatic (p<0.01, p<0.001) and brain (p<0.05, p<0.01) GSH levels as compared to AA group.

c) There were no changes in liver and brain SOD activities following AA treatment. In AA+NAC group, liver SOD (p<0.05) activity increased as compared to control values.

d) Liver GSH-Px activity did not alter in groups. However, significant increase in brain GSH-Px (p<0.01) activity was found in AA-treated rats. NAC and TAU treatments decreased brain GSH-Px (p<0.01, p<0.001) activity in AA-treated rats.

Discussion

Several factors including oxidative stress are implicated in AA-induced toxicity [5, 6]. It has been demonstrated that AA leads to accumulation of ROS through induction of aldehyde oxidase and xanthine oxidase and decreases GSH levels isolated hepatocytes [6, 30]. AA was detected to be 10-30 times more toxic than ethanol, when their LD50 doses were compared [31]. The increasing effect of AA on lipid peroxidation was reported to be more prominent than ethanol [8, 32]. Indeed, lipid peroxide levels were found to increase in hepatic mitochondrial and microsomal fractions in acute ethanol- (5 g/kg; i.p.) and AA- (500 mg/kg; i.p.) treated rats and the effect of AA was more marked in the mitochondrial fraction [33]. Increased hepatic lipid peroxide levels was also detected after acute AA (0.3 g/kg; per oral) administration [34]. Increases in serum ALT, AST and γ-glutamyl transpeptidase activities and decreases in free and protein- bound sulfhydryl groups in plasma, liver and brain were detected in rats after 4 weeks of AA (0.25 g/kg/day, per oral) treatment [35]. Some investigators have also reported that administration of AA for 11 weeks in drinking water was reported to cause steatosis, inflammation and protein-AA adducts in the liver [20, 21]. These authors have pointed that AA delivered via the digestive tract was to be more hepatotoxic than AA formed during ethanol oxidation within the liver. When AA is given in drinking water, it accumulates in the gastrointestinal tract, and even though some amounts are metabolized to acetate by intestinal bacteria, significant amounts of AA arrive to the liver via vena porta, and join to systemic circulation. Therefore, it has been reported that the use of AA in drinking water may be a suitable method to investigate the toxic effects of AA with extrahepatic origin [20, 21].

For this reason, in the current study the effect of long-term AA treatment in drinking water on prooxidant and antioxidant balance were investigated in liver and brain tissues of rats. AA treatment was detected to induce prooxidant state in both liver and brain of rats without any change in antioxidant parameters. Only, brain GSH-Px activity was found to increase significantly in AA-treated rats. This increase may be related to adaptive change against oxidative stress and may prevent further increases in prooxidant status in the brain. The results reported here agree with previous in vitro [6, 30] and in vivo [33, 34] studies showing that AA produces a prooxidant state in rats.

In the literature, there are some in vitro studies investigating the effect of NAC and TAU on AA-toxicity [36-38]. NAC was reported to prevent AA-related toxicity in embryo cell cultures [36]. It has been demonstrated that AA caused increases in ROS generation, mitochondrial dysfunction and apoptosis in cell cultures and that AA-induced toxic effects were averted by NAC [37]. TAU may also have a beneficial effect against aldehyde toxicity by forming conjugates with these compounds such as glucose, AA and MDA via its amino group [38]. However, there is no published in vivo study investigating the effects of NAC and TAU on the direct toxicity of AA.

In the present study, NAC treatment decreased AA-induced prooxidant status in both liver and brain. Although TAU treatment diminished ROS levels, MDA and PC levels remained unchanged in examined tissues of AA-treated rats. NAC and TAU elevated liver and brain GSH levels in AA-treated rats. These results can be attributed to their direct antioxidant properties such as scavenging free radicals and chelating metals together with their stimulating effect on GSH synthesis. Limitation of this study was the lack of histopathological analysis.

In conclusion, our results may indicate that NAC and TAU were found to decrease prooxidant status generated by in vivo AA-treatment and that NAC was more effective than TAU.

Acknowledgement

The present work was supported by the Research Fund of Istanbul University (Project No: 33568 / 42685).

References

1. Guo R, Ren J. Alcohol and acetaldehyde in public health: From marvel to menace. Int J Environ Res Public Health. 2010;7:1285-301.
2. Mizumoto A, Ohashi S, Hirohashi K, Amanuma Y, Matsuda T, Muto M. Molecular mechanisms of acetaldehyde-mediated carcinogenesis in squamous epithelium. Int J Mol Sci. 2017;18:1943.
3. Peana AT, Sánchez-Catalán MJ, Hipólito L, Rosas M, Porru S, Bennardini F, et al. Mystic acetaldehyde: The Never-Ending Story on Alcoholism. Front Behav Neurosci. 2017;11: 81.
4. Correa M, Salamone JD, Segovia KN, Pardo M, Longoni R, Spina L, et al. Piecing together the puzzle of acetaldehyde as a neuroactive agent. Neurosci Biobehav Rev. 2012;36:404-30.
5. Setshedi M, Wands JR, de la Monte SM. Acetaldehyde adducts in alcoholic liver disease. Oxid Med Cell Longev. 2010;3:178-85.
6. Yan T, Zhao Y, Zhang X, Lin X. Astaxanthin inhibits acetaldehyde-induced cytotoxicity in SH-SY5Y cells by modulating Akt/CREB and p38MAPK/ERK signaling pathways. Mar Drugs. 2016;14:56.
7. Tamura M, Ito H, Matsui H, Hyodo I. Acetaldehyde is an oxidative stressor for gastric epithelial cells. J Clin Biochem Nutr. 2014;55:26-31.
8. Stege TE, Hanby JD, Di Luzio NR. Acetaldehyde-induced hepatic lipid peroxidation. In: Seixas FA (ed). Currents in Alcoholism, Vol.1, p. 139. Grune and Stratton, New York 1977.
9. Niemelä O, Parkkila S, Koll M, Preedy VR. Generation of protein adducts with malondialdehyde and acetaldehyde in muscles with predominantly type I or type II fibers in rats exposed to ethanol and the acetaldehyde dehydrogenase inhibitor cyanamide. Am J Clin Nutr. 2002;76:668-74.
10. Aberle II NS, Run J. Experimental assessment of the role of acetaldehyde in alcoholic cardiomyopathy. Biol Proced Online. 2003;5:1-12.
11. Kwon HJ, Won YS, Park O, Chang B, Duryee MJ, Thiele GE, et al. Aldehyde dehydrogenase 2 deficiency ameliorates alcoholic fatty liver but worsens liver inflammation and fibrosis in mice. Hepatology. 2014;60,146-57.
12. Samuni Y, Goldstein S, Dean OM, Berk M. The chemistry and biological activities of N-acetylcysteine. Biochim et Biophys Acta. 2013;1830:4117-29.
13. De Andrade KQ, Moura FA, dos Santos JM, de Araújo OR, de Farias Santos JC, Goulart MO. Oxidative stress and inflammation in hepatic diseases: therapeutic possibilities of N-acetylcysteine. Int J Mol Sci. 2015;16:30269-308.

14. Schaffer S, Kim HW. Effects and mechanisms of taurine as a therapeutic agent. Biomol Therap. 2018;26:225-41.
15. Miyazaki T, Matsuzaki Y. Taurine and liver diseases: a focus on the heterogeneous protective properties of taurine. Amino Acids. 2014;46:101-10.
16. Ronis MJ, Butura A, Sampey BP, Shankar K, Prior RL, Korourian S, et al. Effects of N-acetylcysteine on ethanol-induced hepatotoxicity in rats fed via total enteral nutrition. Free Rad Biol Med. 2005;39:619-30.
17. Caro AA, Bell M, Ejiofor S, Zurcher G, Petersen DR, Ronis MJ. N-acetylcysteine inhibits the up-regulation of mitochondrial biogenesis genes in livers from rats fed ethanol chronically. Alcohol Clin Exp Res. 2014;38:2896-906.
18. Balkan J, Kanbağlı Ö, Aykaç-Toker G, Uysal M. Taurine treatment reduces hepatic lipids and oxidative stress in chronically ethanol-treated rats. Biol Pharm Bull. 2002;25:1231-3.
19. Pushpakiran G, Mahalaksmi K, Anuradha CV. Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissues. Amino Acids. 2004;27:91-6.
20. Matysiak-Budnik T, Jokelainen K, Kärkkäinen P, Mäkisalo H, Ohisalo J, Salaspuro M. Hepatotoxicity and absorption of extrahepatic acetaldehyde in rats. J Pathol. 1996;178:469-74.
21. Jokelainen K, Parkkila S, Salaspuro M, Niemelä O. Covalent adducts of proteins with acetaldehyde in liver as a results of acetaldehyde administration in drinking water. J Hepatol. 2000;33:926-32.
22. Wang H, Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic Biol Med. 1999;27:612-6.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-8.
24. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994;233:357-63.
25. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70-6.
26. Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. J Lab Clin Med. 1963;61:882-8.
27. Mylroie AA, Collins H, Umbles C, Kyle J. Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharmacol. 1986;82:512-20.
28. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158-69.
29. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Anal Biochem. 1985;150:76-85.
30. Farfán Labonne BE, Gutiérrez M, Gómez-Quiroz LE, Konigsberg Fainstein M, Bucio L, Souza V, et al. Acetaldehyde-induced mitochondrial dysfunction sensitizes hepatocytes to oxidative damage. Cell Biol Toxicol. 2009;25:599-609.
31. Ren J, Wold LE. Mechanisms of alcoholic heart disease. Ther Adv Cardiovasc Dis. 2008;2:497-506.
32. Stege TE. Acetaldehyde-induced hepatic lipid peroxidation in isolated hepatocytes. Res Com Chem Pathol Pharmacol. 1982;36:287-97.
33. Uysal M, Özdemirler G, Kutalp G, Oz H. Mitochondrial and microsomal lipid peroxidasyon in rat liver after acute acetaldehyde and ethanol intoxication. J Appl Toxicol. 1989;9:155-8.
34. Videla LA, Fernández V, de Marinis A. Liver lipoperoxidative pressure and glutathione status following acetaldehyde and aliphatic alcohols pretreatments in the rat. Biochem Biophys Res Commun. 1987;104:965-70.
35. Farbiszewski R, Holownia A, Chwiecko M. The changes in sulfhydryl compounds in plasma, liver and brain after acute and chronic ethanol administration in rats. Drug Alcohol Depend. 1987;20:129-33.
36. Menegola E, Broccia ML, Prati M, Ricolfi R, Giavini E.Glutathione and N-acetylcysteine protection against acetaldehyde embryotoxicity in rat embryos developing in vitro. Toxicology In Vitro 1995; 9: 633-641.
37. Seo JB, Gowda GAN, Koh D. Apoptotic Damage of Pancreatic Ductal Epithelia by Alcohol and Its Rescue by an Antioxidant. PLoS One. 2013;8:e81893.
38. Ogasawara M, Nakamura T, Koyama I, Nemoto M, Yoshida T. Reactivity of taurine with aldehydes and its physiological role. Adv Exp Med Biol. 1994;359:71-8.

Arch Clin Exp Med 2019;4(3):118-121. e-ISSN: 2564-6567

DOI:10.25000/acem.586513 Araştırma makalesi / Research article

Does the measurement of the size of the first trimester subchorionic hematoma by 2D and 3D ultrasonographic techniques have any effect on adverse pregnancy outcomes?

Birinci trimester subkoryonik hematom boyutunun 2B ve 3B ultrason ölçüm tekniği ile ölçümünün olumsuz gebelik sonuçlarına etkisi var mıdır?

Sibel Özler [1], Başak Gümüş Güler [2]

Abstract

Aim: We aimed to evaluate whether the measurement of subchorionic hematoma (SCH) size with 2D and 3D ultrasonography affects adverse pregnancy outcomes.
Methods: One hundred fifty-eight pregnant patients having SCH were enrolled in the study. The diagnosis of SCH was made by 2D and 3D ultra-sonographic methods in the first trimester, between 6th and 14th gestational weeks. Patients having SCH were determined with adverse pregnancy outcomes such as miscarriage, intrauterine fetal death (IUFD), and preterm labor (PL). Logistic regression analyses were applied for the relationship of miscarriage, IUFD, PL, and SCH.
Results: There were no statistically significant differences for body mass index, 2-D hematoma sizes, 3-D hematoma sizes, and pregnancy outcomes between the groups. Miscarriage/IUFD rate was 4.6%, PL rate was 6.9%, and the term delivery rate was 88.5% in the primiparas having SCH. Miscarriage/IUFD rate was 7%, PL rate was 3.5%, and the term delivery rate was 89.5% in the multiparas having SCH. No significant association was observed between 2D and 3D hematoma sizes and IUFD and PL. In the logistic regression model, SCH ≥ 500 cm3 was found to be a risk factor associated with PL, not regarding the measurement technique (OR:1.008, 95% CI: 1.002-1.012, p=0.006).
Conclusion: We determined that SCH size increases the risk of PL. We observed no effect of diagnosis and follow-up of SCH, by 2D and 3D ultrasonography techniques on adverse pregnancy outcomes such as miscarriage, IUFD, and PL.

Keywords: Subchorionic hematoma, three dimensional, two-dimensional, ultrasound, adverse pregnancy outcome.

Öz

Amaç: Subkoryonik hematom (SKH) boyutunun 2B ve 3B ultrason ile ölçülmesinin olumsuz gebelik sonuçlarına etkisini değerlendirmeyi amaçladık.
Yöntemler: Yüz elli sekiz SKH olan gebe çalışmaya alındı. SKH tanısı ilk trimesterde, 6. ve 14. gebelik haftaları arasında, 2 ve 3 boyutlu ultra-sonografik yöntemlerle konuldu. SKH olan gebelerin düşük, intrauterin fetal ölüm [IUFÖ] ve preterm doğum [PD] gibi olumsuz gebelik sonuçları belirlendi. SKH ile düşük, intrauterin fetal ölüm ve erken doğum arasındaki ilişkiyi belirlemek için lojistik regresyon analizleri yapıldı.
Bulgular: Primipar ve multipar gebeler arasında vücut kitle indexi, 2B ultrasonografik hematom boyutları, 3B ultrasonografik hematom boyutları ve gebelik sonuçları açısından istatistiksel olarak anlamlı bir fark yoktu. SKH olan primiparlarda düşük/ IUFÖ hızı %4,6, PD hızı %6,9 ve miad doğum hızı %88,5 idi. SKH olan multiparlarda ise düşük/ IUFÖ hızı %7, PD hızı %3,5 ve miad doğum hızı %89,5 idi. 2B ve 3B hematom büyüklüğü ile IUFÖ ve PD arasında anlamlı bir ilişki gözlenmedi. Lojistik regresyon modelinde ölçüm tekniğinden bağımsız olarak SKH'un ≥500 cm3 olması erken doğumla ilişkili risk faktörü olarak bulundu (OR:1,008, %95 CI: 1,002-1,012, p=0,006).
Sonuç: SKH boyutunun PD riskini artırdığını belirledik. SKH'un teşhis ve takibinin 2B ve 3B ultrasonografik ölçüm ile yapılmasının düşük, IUFÖ ve PD gibi olumsuz gebelik sonuçlarına etkisinin olmadığını gördük.

Anahtar Kelimeler: Subkoryonik hematom, 3 boyutlu, 2 boyutlu, ultrason, olumsuz gebelik sonuçları.

[1] **Selcuk University Faculty of Medicine, Department of Perinatology, Konya, Turkey.**

[2] **Istinye University, Department of Health Sciences, Istanbul, Turkey.**

SÖ: 0000-0003-4577-8185
BGG: 0000-0002-0182-6774

Ethics Committee Approval: The study was approved by the local ethical authority (05.04.2019/004).
Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır (05.04.2019/004).

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Geliş Tarihi / Received: 03.07.2019
Kabul Tarihi / Accepted: 16.08.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:

Sibel Özler

Adress/Adres: Kozagaç Mah. Cakılkuyu Sok. No:4/1 Meram/Konya/Turkey.

Phone: +90 545 797 02 57

E-mail: sibelozler@gmail.com

Atıf yazım şekli: Özler S, Gümüş Güler B. Does the measurement of the size of the first trimester subchorionic hematoma by 2D and 3D ultrasonographic techniques have any effect on adverse pregnancy outcomes? Arch Clin Exp Med. 2019;4(3):118-121.
How to cite:

Introduction

Vaginal bleeding is observed approximately in one-fourth of all first- trimester pregnancies [1]. Subchorionic bleeding and hematoma are the leading cause of bleeding in the first trimester, and their incidence is 0.5-22 % [2]. Although many risk factors are leading to subchorionic hematoma (SCH), the etiopathogenesis is still unclear. SCH may be observed with symptoms like pelvic pain and vaginal bleeding, but can also be asymptomatic [3]. SCH has been associated with increased risk of adverse pregnancy outcomes; such as abruption, intrauterine fetal death (IUFD) [3], preterm premature rupture of membranes (PPROM), preterm labor (PL), preeclampsia, and fetal growth restriction (FGR) [4]. SCH is generally observed as an anechoic or hypoechoic area in between the fetal membrane and uterine wall in the first trimester, by ultrasonography [5].

Conventional two- dimensional (2D) ultrasonography technique is commonly used in the evaluation of placenta and the fetus in the pregnancy period [6]. Recently three- dimensional (3D) ultrasonography has been preferred in the assessment of placental and fetal pathologies. 3D imaging modalities are used in the evaluation of volumes of liquids, mass, tissues, and organs. Technically in the 3D method, the borders of the area to be measured are detected, and results are calculated automatically. However in 2D choice, the dimensions of the object to be measured are lined manually in 3 planes, and mathematical methods calculate the volume. In a 2D way, miss-calculation is observed in 2 % of the measurements of hematomas and/ or liquids having unclear borders [7].

We aimed to determine whether any significant difference in SCH measurement by 2D and 3D techniques and to observe if any of these techniques or SCH, regardless from the measurement technique, were associated with adverse pregnancy outcomes in the first trimester.

Material and methods

A prospective observational study was carried out between March 2017 and March 2019. We calculated the SCH areas by 2D and 3D ultrasonographical techniques in the first trimester. One hundred fifty-eight patients (87 primiparas and 71 multiparas), who were diagnosed to have SCH, were recruited consecutively from the Perinatology Department of Konya Education and Research Hospital. The study protocol was performed according to the principles of the Declaration of Helsinki and approved by the local ethical committee of our hospital (Approval date/number:05.04.2019/004).

The diagnosis of SCH is a frequent finding on routine obstetric ultrasonography. The determination of SCH was made by the presence of a hypoechoic or anechoic crescent area behind the gestational sac on ultrasonography, regardless of whether there is pelvic pain and/or vaginal bleeding in the first trimester between 6th and 14th gestational weeks. The age of the patients varied from 18 to 44 years.

Patients were excluded if any of the following criteria were present: multiple pregnancy, habitual aborts, use of anticoagulants, history of in vitro fertilization, excessive obesity, uterine anomaly, fetal chromosomal aneuploidy and/ or malformations.

The medical history and the physical examination of the patients were recorded. Body mass index (BMI) was calculated in kilograms/square meter (kg/m2) [8]. 2D and 3D scan were performed abdominally by using Samsung HS70A (Samsung Medision Diagnostic Ultrasound System, United States). The size of the gestational sac, crown-rump length (CRL) and fetal heartbeat were recorded, and places of hematomas were described their sites as being subchorionic if they were located between the chorion and the uterine wall, external to the chorionic leave. The longest transverse (A), sagittal (B), and horizontal (C) diameters of the hematomas were measured by Grayscale ultrasonography, and volumes were calculated by using the formula $0.625 \times A \times B \times C$ [9]. SCH area was drawn manually on 3D ultrasound and automatically determined according to the ellipsoid formula defined as volume measurement method on ultrasound. (Ellipsoid: The volume is calculated by using the length of the main and side axes. (4/3 x PI x Main/2 x (Side/2)^2) [10]. SCH with a bleeding area higher than fifty percent of the gestational sac was considered large size [11].

3D volume measurement was performed by using the same device and recorded. The follow up of the patients was repeated every two weeks or monthly, which was decided according to the dimensions of the hematoma. The occurrence of miscarriage, IUFD, and PL were followed up and recorded. The adverse pregnancy outcomes were defined as spontaneous miscarriage (designated as a fetal loss at less than 20 or 24 weeks of gestation), IUFD (defined as fetal death at the first trimester of gestation), and PL (defined as delivery before 36 or 37 weeks of gestation) [12, 13].

Statistical analysis

Therefore sample size calculation by G-power analysis and 136 participants was calculated to detect an anticipated effect size of 0.3 for the regression equation, at a power level of 0.95 ($\beta = 0.95$) and a probability level of 0.05 ($\alpha = 0.05$). Data analysis was performed by using SPSS for Windows, version 22 (SPSS Inc., Chicago, IL, United States). The Kolmogorov Smirnov test was used to test whether or not continuous variables were normally distributed. The Levene test evaluated the homogeneity of variances. Continuous variables were demonstrated as mean ± standard deviation (SD). The groups were divided into two; as SCH <500 cm3 and SCH ≥ 500 cm3. Student's t-test compared mean variations between the groups. We used the Mann-Whitney U test for non-parametric groups. For data not normally distributed, median with data range [minimum to maximum] were used. Pearson's chi-square test analyzed nominal data. Multivariate logistic regression analysis was used to determine if there there is a relationship between 2D hematoma size, 3D hematoma size, and IUFD, PL. Spearman's rho correlation analyses were used to calculate degrees of association between SCH ≥ 500 cm3, IUFD, and PD. A p-value < 0.05 was considered as significant.

Results

Forty-three (27.21%) of the patients had a SCH size ≥ 500 cm3, and 115 (72.79%) had SCH <500 cm3 in the study. 19 (23.45%) of primiparous patients and 24 (33.80%) of multiparous patients had SCH ≥ 500 cm3 (Table 1). When patients were having SCH < 500 cm3, and the ones having SCH ≥ 500 cm3 were compared, no significant difference in pregnancy outcomes (IUFD and PL) were observed (Table 2).

Term delivery rate was 88.5%, PL rate was 6.9%, and miscarriage /IUFD rate was 4.6% in the primiparas having SCH. Term delivery rate was 89.5%, miscarriage /IUFD rate was 7%, and PL rate was 3.5% in the multiparas having SCH (Table 3).

No significant association was observed in between hematoma size measured by 2D and 3D techniques and IUFD and PL, in the logistic regression model (Table 4).

In the logistic regression model, only SCH ≥ 500 cm 3 was found to be an independent risk factor for PL (OR:1.008, 95% CI: 1.002-1.012, p=.006). SCH size was not associated with IUFD (Table 5).

SCH ≥ 500 cm3 and PL were significantly positively correlated (r=.144, p=.046) (Table 6).

Table 1: Baseline characteristics and ultrasonographic subchorionic hematoma size of groups in the first trimester of pregnancy.

	SCH < 500 cm^3	SCH ≥ 500 cm^3	p
n β	115 (72.79)	43 (27.21)	
Age (year) ¥	27.30±6.57	25.95 ± 5.72	0.124
BMI (kg/m^2) ¥	31.26±14.23	30.58±17.43	0.683
Primipara β	62 (76.55)	19 (23.45)	0.141
Multipara β	47 (66.2)	24 (33.80)	

β: n(%),¥: mean±standard deviation, BMI: Body mass index.

Table 2. Pregnancy outcomes according to hematoma size in the first trimester of pregnancy.

	SCH < 500 cm^3	SCH ≥ 500 cm^3	p
n β	115 (72.79)	43 (27.21)	
Abortus/IUFD ¥	2.7	4.2	0.285
Preterm delivery ¥	1.9	2.7	
Term delivery ¥	65.3	23.3	

β: n(%), ¥: %, IUFD: Intrauterine fetal demise.

Table 3. Baseline characteristics and ultrasonographic data of groups having a subchorionic hematoma in the first trimester of pregnancy.

		Primipara (n=87)	Multipara (n=71)	p
Age (year) ¥		23.62±5.53	28.40±6.09	0.001
BMI (kg/m^2) ¥		30.14±11.02	29.85±13.14	0.754
2-dimensional hematoma size (mm^3) μ		2017.50 (585-19,250)	4060.00 (500-18,480)	0.285
3-dimensional hematoma size (mm^3) μ		195.43 (4.18-1,838.78)	229.85 (4.18-2,572.44)	0.264
Pregnancy outcome	Abortus/IUFD β	4 (4.6)	5 (7.04)	0.373
	Preterm delivery β	6 (6.9)	3 (4.22)	
	Term delivery β	77 (88.5)	63 (88.73)	

β: n(%),¥: mean±standard deviation, μ: mean (range), BMI; Body mass index, IUFD; Intrauterine fetal demise.

Table 4. Logistic regression analysis for the relationship between 2D hematoma size, 3D hematoma size, and IUFD and Preterm delivery groups.

	IUFD		Preterm Delivery	
	OR [Cl%95]	p	OR [Cl%95]	p
Age	1.051 [0.981-0.126]	0.161	1.043 [0.958-1.136]	0.329
2-dimensional hematoma size	1.003 [1.000-1.005]	0.248	0.923 [0.018-4.713]	0.968
3-dimensional hematoma size	1.001 [1.000-1.002]	0.129	1.001 [1.000-1.002]	0.247

OR: odds ratio, IUFD: Intrauterine fetal demise.

Table 5. Logistic regression analysis for the relationship between hematoma size, and IUFD and preterm delivery groups.

		IUFD		Preterm delivery	
		OR [Cl%95]	P	OR [Cl%95]	P
Age		1.051 [0.981-1.126]	0.161	1.043 [0.958-1.136]	0.329
SCH cm^3	<500	0.561 [0.208-1.511]	0.253	0.499 [0.153-1.632]	0.251
SCH cm^3	≥500	1.004 [1.000-1.008]	0.074	1.008 [1.002-1.012]	0.006

OR: odds ratio, IUFD: Intrauterine fetal demise, SCH: Subchoronic hematoma.

Table 6. Correlation analysis between SCH ≥ 500 cm3, IUFD, and Preterm delivery.

	SCH ≥ 500 cm^3	
	r	p
IUFD	0.117	0.547
Preterm delivery	0.144	0.046

R: Correlation coefficient, IUFD: Intrauterine fetal demise, SCH: Subchorionic hematoma.

Discussion

We observed no significant differences in miscarriage, IUFD, and PL rates in the patients having large-sized SCH. There are controversial results in the literature when SCH and adverse gestational outcome relation is in concern [15]. Hashem et al. [15] reported an increased risk of miscarriage, PL, IUGR, abruption, low birth weight, cesarean section rate, low Apgar score at 1 and 5 min, and need for NICU admission in the patients having SCH when they were compared to the ones having no SCH. They observed that the size of SCH was only correlated to miscarriage. Maso et al. [16] noticed increased miscarriage risk, especially before the 9th gestational week, in the patients having SCH in the first trimester. Nagy et al. [11] observed increased PL risk in SCH patients when compared to healthy controls. However, in another study, no relation was observed between PL risk and size of SCH [17]. Johns et al. [18] reported that there was no relation of SCH with first-trimester miscarriage risk and PPROM.

We observed no significant difference in miscarriage, IUFD, or PL rates when the size of SCH was in concern. However, regression analyses of our data revealed a significant relation between SCH size and PL. The low number of patients in our study may be the cause of indifference between the groups.

The extent of SCH is considered by comparing the longest linear dimension of the SCH with the size of the gestational sac in the first trimester. If the ratio is less than 20% the SCH is classified as small; if it is between 20-50% SCH is medium; and if it is more than 50%, SCH is large [11]. Studies are demonstrating that large SCH are related to adverse gestational outcome [19]. Ball et al. [20] showed the relation of first-trimester SCH with miscarriage, PL, PPROM, abruption of placenta, low birth weight, and stillbirth. Tuuli et al. [5] reported the relation of SCH with early and late pregnancy loss, PPROM and abruption of the placenta in their meta-analysis of cohort and case-control studies which were conducted in the years between 1981- 2010. However, Li et al. [21] evaluated the case-control and cohort studies of the years 2000- 2015 in their meta-analyses and observed that SCH caused an increased risk of spontaneous miscarriage but was not related to PL in ongoing pregnancies. Peixoto et al. [22] reported increased miscarriage risk in the patients having SCH in the first trimester. Avi et al. [23] reported that there was not a significant relationship between the 2D measurement of SCH, vaginal bleeding, and adverse pregnancy outcome.

Hata et al. [24] suggested the help of 3D method in the evaluation of placental thickness, SCH, and chorangioma to 2D and Doppler sonography. They observed that 3D imaging was more comfortable to apply in hematomas having irregular

borders. The use of 3D technology has been increasing in recent years. Sharma et al. [25] compared the estimation of fetal weights measured both by 2D and 3D technologies and observed that the 3D method was more accurate. Becsek et al. [26] reported a more accurate measurement of CRL by 3D technique when compared to the 2D approach. Sadek et al. [27] observed higher sensitivity in the diagnosis of the low-lying placenta with 3D transvaginal ultrasonography technique. There has not been any exact result in the evaluation of SCH in the first trimester when the 3D and the 2D methods are concerned; because there are only case reports. We suggest the conduction of prospective studies, including more patients having SCH to compare the values of two methods. We believe that to confirm the effect of SCH size on adverse pregnancy outcomes, large population-based controlled studies are needed.

We did not observe any significant results in the prediction of IUFD or PL when we compared the 3D and 2D measurements of SCH in the first trimester, in primiparas and multiparas. On the other hand, we found out that SCH size was correlated to PL.

As a result, SCH observed in the first trimester did not affect the adverse pregnancy outcome in primiparas and multiparas. Although 3D measurement of SCH is more accurate, it does not enhance the prediction of IUFD or PL when compared to 2D size. We think that the primary importance belongs to the diagnosis and follow up of SCH, rather than the technique of measurement.

References

1. Mallin M, Dawson M, Schroeder E, Hatch B, Jackson I, Ahern M, et al. Prospective outcomes of pregnant ED patients with documented fetal cardiac activity on ultrasound. Am J Emerg Med. 2012;30:472-5.
2. Seki H, Kuromaki K, Takeda S, Kinoshita K. Persistent subchorionic hematoma with clinical symptoms until delivery. Int J Gynecol Obstet. 1998;63:123–8.
3. Maso G, D'Ottavio G, De Seta F, Sartore A, Piccoli M, Mandruzzato G. First-trimester intrauterine hematoma and outcome of pregnancy. Obstet Gynecol. 2005;105:339–44.
4. Weiss JL, Malone FD, Vidaver J, Ball RH, Nyberg DA, Comstock CH, et al. Threatened miscarriage: A risk factor for poor pregnancy outcome, a population-based screening study. Am J Obstet Gynecol. 2004;190:745–50.
5. Tuuli MG, Norman SM, Odibo AO, Macones GA, Cahill AG. Perinatal Outcomes in Women With Subchorionic Hematoma. Obstet Gynecol. 2011;117:1205-12.
6. Brown DL, DiSalvo DN, Frates MC, Davidson KM, Genest DR: Placental surface cysts detected on sonography: Histologic and clinical correlation. J Ultrasound Med. 2002;21:641–6.
7. Riccabona M, Nelson TR, Pretorius DH, Davidson TE. In vivo three-dimensional sonographic measurements of organ volume: validation in the urinary bladder. J Ultrasound Med. 1996; 15:627-632.
8. Keys A, Fidanza F, Karvonen MJ, Kimura N, Taylor HL. Indices of relative weight and obesity. J Chron Dis. 1972;25:329–43.
9. Hartnell GG, Kiely EA, Williams G, Gibson, RN. Real-time ultrasound measurement of bladder volume: a comparative study of three methods.Br J Radiol. 1987;60:1063-5.
10. Finch W, Johnston R, Shaida N, Winterbottom A, Wiseman O. Measuring stone volume - three-dimensional software reconstruction or an ellipsoid algebra formula? BJU Int. 2014;113:610-4.
11. Nagy S, Bush M, Stone J, Lapinski RH, Gardo S. Clinical significance of subchorionic and retroplacental hematomas detected in the first trimester of pregnancy. Obstet Gynecol. 2003;102:94–100.
12. Zarko Alfirevic, Tamara Stampalija, Nancy Medley. Cervical stitch (cerclage) for preventing preterm birth in singleton pregnancy. Cochrane Database Syst Rev. 2017 Jun;2017(6):CD008991.
13. Committee on Obstetric Practice Society for Maternal-Fetal Medicine. Medically Indicated Late-Preterm and Early-Term Deliveries. Obstet Gynecol. 2019;133:400-3.
14. Xiang L, Wei Z, Cao Y. Symptoms of an intrauterine hematoma associated with pregnancy complications: a systematic review. PLoS One. 2014;9:e111676.
15. Hashem A, Sarsam SD. The Impact of Incidental Ultrasound Finding of Subchorionic and Retroplacental Hematoma in Early Pregnancy. J Obstet Gynaecol India. 2019;69:43-9.
16. Maso G, D'Ottavio G, De Seta F, Sartore A, Piccoli M, Mandruzzato G. First-trimester intrauterine hematoma and outcome of pregnancy. Obstet Gynecol. 2005;105:339-44.
17. Pedersen JF, Mantoni M. Large intrauterine haematomata in threatened miscarriage. Frequency and clinical consequences. Br J Obstet Gynaecol. 1990;97:552–3.
18. Johns J, Hyett J, Jauniaux E. Obstetric outcome after threatened miscarriage with and without a hematoma on ultrasound. Obstet Gynecol. 2003;102:483-7.
19. Ozkaya E, Altay M, Gelisen O. Significance of subchorionic hemorrhage and pregnancy outcome in threatened miscarriage to predict miscarriage, preterm, and intrauterine growth restriction. J Obstet Gynecol. 2011;31:210–212.
20. Ball RH, Ade CM, Schoenborn JA, Crane JP. Significance of ultrasonographically detected subchorionic hemorrhages. Am J Obstet Gynecol. 1996;174:996-1002.
21. Li Q, Zhu J, Hua K. Effects of the subchorionic hematoma on pregnancy outcome: a meta-analysis. Zhonghua Yi XueZaZhi. 2016;96:1383-5.
22. Peixoto AB, Caldas TMRDC, Petrini CG, Romero ACP Júnior LEB, Martins WP Araujo Júnior E. The impact of the first-trimester intrauterine hematoma on adverse perinatal outcomes. Ultrasonography. 2018;37:330-6.
23. Ben-Haroush A, Yogev Y, Mashiach R, Meizner I.Pregnancy outcome of threatened miscarriage with subchorionic hematoma: possible benefit of bed-rest?. Isr Med Assoc J. 2003;5:422-4.
24. Hata T, Kanenishi K, Inubashiri E, Tanaka H, Senoh D, Manabe A, Miyake K, et al. Three-dimensional sonographic features of placental abnormalities. Gynecol Obstet Invest. 2004;57:61-5.
25. Sharma KA, Das D, Dadhwal V, Deka D, Singhal S, Vanamail P. Two-dimensional fetal biometry versus three-dimensional fractional thigh volume for ultrasonographic prediction of birth weight. Int J Gynecol Obstet. 2019;145:47-53.
26. Becsek A, Tzanidakis N, Blanco M, Bollwein H. Transrectal three-dimensional fetal volumetry and crown-rump length measurement during early gestation in mares: Intra- and inter-observer reliability and agreement. Theriogenology. 2019;126:266-71.
27. Sadek SM, Ahmad RA, Atia H, Abdullah AG. Towards a more accurate measurement of edge to os distance in low-lying placenta using transvaginal ultrasound: an innovative technique. BMC Pregnancy Childbirth. 2018;18:472.

Arch Clin Exp Med 2019;4(3):122-126. e-ISSN: 2564-6567

DOI:10.25000/acem.568773 Araştırma makalesi / Research article

Determination of galectin-3, hepsin (Tmprss 1) and thyroid transcription factor-1 levels in thyroid cancer patients: A prospective case-control study

Tiroit kanserli hastalarda galektin-3, hepsin (Tmprss1) ve tiroit transkripsiyon faktör-1 seviyelerinin belirlenmesi: Bir prospektif vaka-kontrol çalışması

Ufuk Memiş [1], Erdem Karadeniz [1], Müfide Nuran Akçay [1], Nurinnisa Öztürk [2]

Abstract

Aim: This study aimed to the efficiacy of usage galectin-3, hepsin, and thyroid transcription factor-1 (TTF-1) levels as markers for the differentiation between patients with malignant and benign thyroid nodules.
Methods: The study was done prospectively between January 2018 and April 2018 in our clinic, in patients who were diagnosed with thyroid nodules and scheduled for surgery. Galectin-3, hepsin, and TTF-1 levels were measured in the preoperative serum of the benign and malignant groups. The measured levels were evaluated statistically to determine whether there was any significant difference between the malignant and benign groups.
Results: When the levels of galectin-3, hepsin, and TTF-1 in the malignant and benign groups were compared, there was a statistically significant difference in TTF-1 levels (p=0.038). However, no significant difference was found in hepsin and galectin-3. When the macropapillary and micropapillary types were compared within the malignant patient group, there was a significant difference between the galectin-3 levels (p = 0.009), but no difference was found between hepsin and TTF-1.
Conclusions: It was seen that the decrease of galectin-3 levels in thyroid papillary carcinoma could be effective in the transformation from microcarcinoma to macrocarcinoma, and TTF-1 could be an important marker for the differentiation between benign and malignant thyroid nodules.

Keywords: Galectin-3, hepsin, TTF-1, thyroid malignancy, cancer.

Öz

Amaç: Çalışmada; galektin-3, hepsin ve tiroit transkripsiyon faktör-1 (TTF-1)'in kan değerlerinin tiroit nodüllerinde malign ve benign ayrımındaki tanısal değerini araştırmayı amaçladık.
Yöntemler: Çalışma kliniğimizde Ocak 2018 ile Nisan 2018 tarihleri arasında tiroit nodülü nedeniyle operasyon planlanan hastalarda prospektif olarak yürütüldü. Benign ve malign grupların ameliyat öncesi elde edilen serumlarında galektin-3, hepsin ve TTF-1 seviyeleri açısından farklılık olup olmadığı istatistiksel olarak araştırıldı.
Bulgular: Malign ve benign grupta ölçülen galektin-3, hepsin ve TTF-1 seviyeleri karşılaştırıldığında; TTF-1 seviyelerinde istatistiksel olarak anlamlı fark bulunurken (p=0,038), hepsin ve galektin-3'te anlamlı fark tespit edilmedi. Malign hasta grubunda makropapiller ve mikropapiller tip karşılaştırıldığında galektin-3 seviyeleri arasından anlamlı bir fark tespit edilirken (p=0,009), hepsin ve TTF-1 arasında fark tespit edilmedi.
Sonuç: Tiroid papiller karsinomunda galectin-3 seviyesinin düşmesinin mikro karsinomdan makro karsinoma dönüşümde etkili olabileceği, TTF-1' in tiroid nodüllerinde benign ve malign ayrımında önemli bir belirteç olabileceği görüldü.

Anahtar Kelimeler: Galektin-3, hepsin, TTF-1, tiroid malignitesi, kanser.

[1] Ataturk University, Faculty of Medicine, Department of General Surgery, Erzurum, Turkey.
[2] Ataturk University, Faculty of Medicine, Department of Biochemistry, Erzurum, Turkey.

UM: 0000-0003-3393-3301
EK: 0000-0001-6319-1754
MNA: 0000-0001-8470-1741
NÖ: 0000-0002-7746-2700

Ethics Committee Approval: The study wass approved by the local ethical authority (15.02.2018/24).
Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır (15.02.2018/24)

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The present work was supported by the Scientific Research Projects Coordination Unit of Atatürk University (Project No: TTU-2018-6644).
Finansal Destek: Yazarlar bu çalışma için Ataturk Universitesi Arastirma Fonundan finansal destek aldıklarını beyan etmişlerdir (Proje No: TTU-2018-6644).

Geliş Tarihi / Received: 22.05.2019
Kabul Tarihi / Accepted: 28.08.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:
Erdem Karadeniz

Adres/Address: Dept of General Surgery, Faculty of Medicine, Ataturk University, 25040, Erzurum, Turkey.

e-posta: erdem7600@hotmail.com.tr
Tel/Phone: +905053543394

Introduction

The incidence of thyroid cancer has increased compared to previous years. The annual incidence of thyroid malignancies in the United States increased from 4.9 in 100,000 in 1975 up to 14.3 in 2009. Although the mortality rate in thyroid cancers varies between 8–20%, it is higher than other endocrine organ tumors [1].

Thyroid nodules are very common within the community. When evaluated by neck ultrasonography (US), thyroid nodules are found in 17-67% of the adult population [2]. Although most thyroid nodules are benign, recently the incidence of thyroid cancer is increasing dramatically [3]. Malignancy rate in thyroid nodules is between 5% and 20% [4]. Patient history, physical examination, serum thyroid Stimulating hormone (TSH) levels, neck USG and fine-needle aspiration biopsy (FNAB) constitute the standard nodule evaluation protocol. FNAB is a safe, fast, and cost-effective diagnostic tool and accepted as the gold standard for the evaluation of thyroid nodules [2]. According to the results of the FNAB, there is a gray zone such as in the Bethesda category 3 and category 4 patient groups, where although the majority of the nodules are benign in the postoperative pathology results, the surgical option is preferred because the benign-malignant distinction of the nodule is not clear. New diagnostic markers are needed to avoid unnecessary surgery especially in patients of this intermediate category.

Galectins are proteins classified as part of the lectin group and act on the cell surface and cytoplasm. Galectin-3, takes part in the intercellular relationship, cell-to-matrix relationship, cell growth, and regulation of neoplastic transformation. There are new studies suggesting that galectin-3 is a marker of malignancy in thyroid cancers, specifically in papillary thyroid cancer [5].

Type 2 transmembrane epithelial serum proteases are a newly identified subgroup of the serine protease family consisting of 17 proteolytic enzymes. Hepsin is a subtype of the type 2 transmembrane epithelial serine proteases. In vitro studies have shown that hepsin is essential for cell growth, maintenance of normal morphology of the cell, and cell motility and development [6]. Serine proteases have been shown to play a role in tumor development and metastasis [7].

Thyroid transcription factors regulate genes that code the proteins related to thyroid hormone syntheses such as thyroglobulin, thyroid peroxidase, thyrotropin receptor, the sodium- iodide symporter, and those that affect the development of the thyroid at the embryological period [8]. Thyroid transcription factor (TTF-1) has been shown to exist in the human thyroid, lung, and brain cells and may be a diagnostic and prognostic marker in lung cancers [9].

In this study; we aimed to evaluate the diagnostic value of blood galectin-3, hepsin, and TTF-1 levels in the differentiation of malignant and benign thyroid nodules.

Material and methods

Study design

This study was carried out prospectively with patients who were scheduled for operation due to the presence of thyroid nodules between January 2018 and April 2018 at our clinic. The study was approved by the University Ethics Committee on the 15th of February 2018 (No:24). The study was conducted in accordance with the Declaration of Helsinki. The patients were interviewed and informed about the study face-to-face, informed consent was taken from the patients who accepted to participate in the study. A total of 120 volunteers, who accepted to participate in the study, were operated. The operations were performed by two surgeons experienced in head and neck surgery. Patients with a history of thyroid drugs, thyroid cancer, or thyroid surgery and radiotheraphy (RT) in the neck region were excluded from the study. After the rejection criteria were applied, 77 patients remained—30 patients were randomly selected from 31 patients who had malignant postoperative pathology, and 30 patients were randomly selected as a control group from 46 patients who had benign postoperative pathology. Malignant and benign groups were compared in terms of galectin-3, hepsin, and TFF-1 levels.

Biochemical analysis

An extra tube of blood was collected in addition to the routine from the patients who consented to participate in the study. The samples were then centrifuged and stored at -80°C in the laboratory of the Biochemistry Department. From the plasma samples previously-stored at -80°C, serum galectin-3 (Catalogue no: 201-12-1952), hepsin (Catalogue no: 201-12-3942), and TTF-1 (Catalogue no: 201-12-5744) levels were measured by ELISA methods using a commercial ELİSA kit (Sunredbio, Waltham, MA, USA) and an automatic ELISA reader (Dynex Magellan Biosciences Chantilly, USA) according to the kit insert. The detection range of galectin-3, hepsin, and TTF-1 kit were 0.2–60 ng/mL, 0.78–50 ng/mL, and 0.05–12 ng/mL, respectively.

Statistical analysis

Statistical analysis of the study was performed with the SPSS for Windows 22.00 statistical software package, and the power analysis was done using the Sample Size Power Analysis Calculation version 3.1.2 program. As a result of the power analysis, a Type I error of 0.05 and power of 0.80, a minimal sample size of 18 patients was required to achieve a significant difference between the two means. Therefore, the experimental study with thirty patients in each group showed an effect size of 0.967. The Kolmogorov–Smirnov and Shapiro–Wilk tests were applied in order to determine whether the data showed a normal distribution and nonparametric tests were applied, as the data did not conform to a normal distribution. Data were analyzed by the Chi-square analysis and Mann-–Whitney U test. Statistical significance was accepted as $p < 0.05$.

Results

Of the 30 patients in the study (malignant) group, seven (23.3%) were male and 23 (76.7%) were female. The mean age was 45.4±9.2 years. Seven (23.3%) patients had a family history of thyroid carcinoma. In preoperative US, 5 (16.7%) patients had a unilateral nodule, and 25 (83.3%) patients had bilateral nodules. Total thyroidectomy was performed in 27 (90%) patients, and lobectomy was performed in 3 (10%) patients. The histopathological type of the malignant group was papillary cancer; 18 (60%) were of the micropapillary type, and 12 (40%) were of the macropapillary type cancer. Of the 30 patients in the control (benign) group, 8 (26.7%) were male, and 22 (73.3%) were female. The mean age was 46.43±10 years. Four (13.3%) patients had a family history of thyroid carcinoma. Preoperatively, 12 (40%) patients had unilateral, and 18 (60%) patients had bilateral nodules. Total thyroidectomy was performed in 16 (53.3%) patients, and lobectomy was performed in 14 (46.7%) patients. A comparison of the characteristics of the patients in the malignant and benign patients in terms of their characteristics is given in Table 1.

Table 1. Comparison of the characteristics of the patients in the malignant and benign groups.

Parameter		Benign (n=30)	Malignant (n=30)	p
Age ¥		46.43±10	45.40±9.2	0.679
Gender β	Male	8 (26.7)	7 (23.3)	0.766
	Female	22 (73.3)	23 (76.7)	
History of thyroid cancer in family β	Present	4 (13.3)	7 (23.3)	0.317
	Absent	26 (86.7)	23 (76.7)	
US nodule β	Unilateral nodule	12 (40.0)	5 (16.7)	0.045
	Bilateral nodule	18 (60.0)	25 (83.3)	
Operation β	Total thyroidectomy	16 (53.3)	27 (90.0)	0.002
	Lobectomy	14 (46.7)	3 (10.0)	

β: n (%), ¥: mean±standard deviation

The mean of the TFF-1 levels in the malignant patient group was 6.02 ± 3.19 ng/ml, and the median was 35.17 ng/ml, the mean in the benign patient group was 4.54 ± 2.54 ng/ml, and the median was 25.83 ng/ml, and the difference was significant (p=0.038) (Table 2) (Figure 1).

Table 2. Comparison of Galectin-3, hepsin (TMPRSS1) and thyroid transcription factor-1 (TTF-1) levels in patients with malignant and benign groups.

Parameter	Benign (n=30)	Malignant (n=30)	p
Galektin-3 (ng/ml) μ	13.61±8.21 (26.53)	19.10±12.99 (34.47)	0.079
Hepsin (TMPRSS1)(ng/ml) μ	18.31±11.32 (28.67)	24.66±19.93 (32.33)	0.416
Thyroid transcription factor-1 (ng/ml) μ	4.54±2.54 (25.83)	6.02±3.19 (35.17)	0.038

μ: mean±standard deviation (median)

The malignant patient group were grouped as macropapillary and micropapillary according to their pathologies and the galectin-3, hepsin, and TTF-1 levels of the patients were compared. The mean and the median of the galectin-3 levels was 13.55±11.04 ng/ml and 10.33 ng/ml, and in the patients were 22.81±13.15 ng/ml and the median was 18.94 ng/ml in the macropapillary and micropapillary patients, respectively, and the difference was significant (p=0.009) (Table 3) (Figure 2).

Table 3. Comparison of Galectin-3, Hepsin (TMPRSS1) and thyroid transcription factor-1 levels in patients with macropapillary and micropapillary thyroid cancers in malignant groups

Parameter	Macropapillary (n=12)	Micropapillary (n=18)	p
Galektin-3 (ng/ml) μ	13.55±11.04 (11.33)	22.81±13.15 (18.94)	0.009
Hepsin (TMPRSS1) (ng/ml) μ	19.76±18.25 (13.17)	27.92±20.83 (17.06)	0.236
Thyroid transcription factor-1 (ng/ml) μ	5.81±3.12 (15.42)	6.17±3.33 (15.56)	0.966

μ: mean±standard deviation (median)

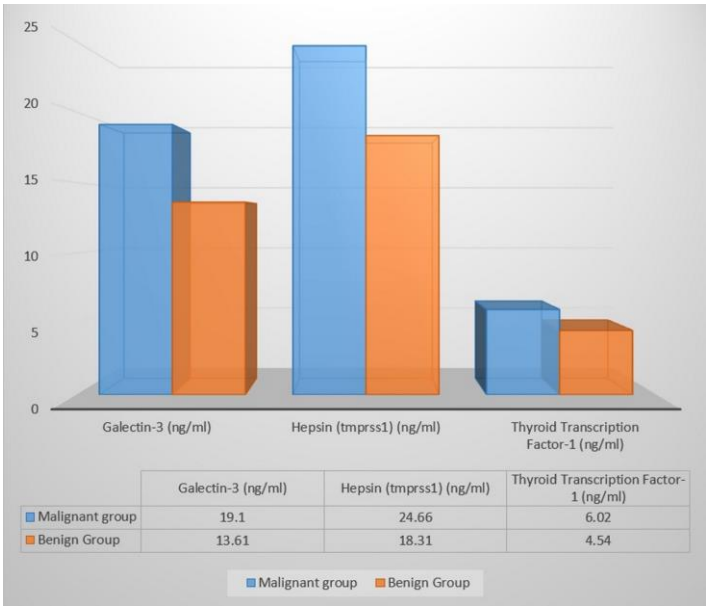

Figure 1. Galectin-3, hepsin (TMPRSS1) and thyroid transcription factor-1 (TTF-1) level in patients with malignant and benign patients.

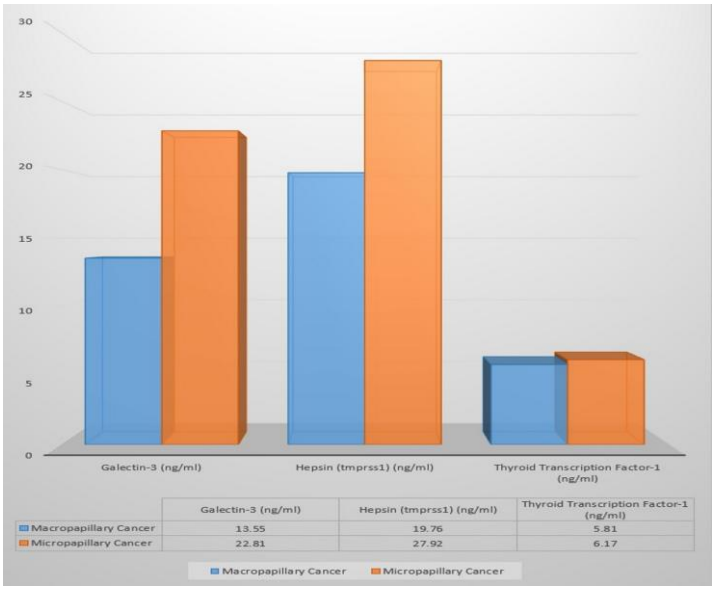

Figure 2. Galectin-3, hepsin (TMPRSS1) and thyroid transcription factor-1 levels of patients with macropapillary and micropapillary in malignant groups.

Discussion

A thyroid nodule could be described as a separate lesion that occurs in the thyroid gland and differs radiologically from the normal thyroid parenchyma. The main clinical problem in thyroid nodules is to distinguish malignancies. FNAB is golden standard for its safety, rapidity, and cost-effectivity in the evaluation of thyroid nodules. FNAB is widely used by many specialists and the false negativity rate is below 5%. Combining it with US reduces the rate of false negatives and nondiagnostic cytology rates [2, 10]. The results of the FNAB are considered as one of the most important determinants of the decision for surgery or medical treatment. According to the Bethesda classification, FNAB results separated the groups into nondiagnostic (category 1), Benign (category 2), Atypia of underdetermined Significance (AUS)/Follicular Lesion of underdetermined Significance (FLUS) (category 3), Follicular neoplasm or suspicious for a Follicular Neoplasm (category 4),

suspicious for malignancy (category 5), and malignant (category 6). Especially in the Bethesda category 3 and category 4, patient groups are in the gray zone.

Although most of the postoperative pathology results are benign, the surgical option is applied because the benign-malignant distinction of the nodule cannot be made clear. New diagnostic markers are needed to avoid unnecessary surgery especially for patients in this intermediate category.

Galectin-3 is a beta-galactocyte binding polypeptide. It is a member of the lectin family and plays an important role in some biological processes. It plays a role in regulating cell-cell and cell-matrix interactions, adhesion, migration, and damaged cell repair. It also plays a role in inflammation and neoplastic transformation [5]. Many studies have shown that galectin-3 is a reliable diagnostic marker with high sensitivity in the preoperative diagnosis of thyroid carcinomas. In a meta-analysis of 39 studies by De Matos et al. [11], the sensitivity and specificity of galectin-3 in detecting malign thyroid lesions was 82% and 81%, respectively. Galectin 3 has a high 81.9 % sensitivity and 92.3% specificity in papillary thyroid carcinoma, reported to be the most effective single marker for differentiating benign from malignant tumors [12]. In our study, although the whole malignant group consisted of papillary carcinoma, serum levels of galectin-3 were higher in the malignant group consistent with other studies. But there was no statistically significant difference found between the malign and benign group that could further be explained by the low number of cases in the study. In a study by Kawachi and Ark et al. [13], papillary thyroid carcinoma patients who metastasized to the lymph nodes had more galectin-3 expression than the non-metastatic patients. However, they found that galectin-3 expression in the lymph nodes metastasized from papillary carcinoma was lower than the primary thyroid lesion. With the help of these results, the decrease in galectin-3 levels in the progression of papillary thyroid carcinoma reported could contribute to cell release and metastasis from the primary tumor [13]. In our study, there were no metastasized patients, when papillary thyroid carcinoma in the malignant group were separated into micro (<1 cm) and macro (>1 cm) papillary carcinoma according to the tumor size, galectin-3 level was higher in the micropapillary group compared to the macropapillary group and this difference was statistically significant. This result suggested that the decrease of galectin-3 level in papillary carcinoma may be effective in the microcarcinoma transformation to macrocarcinoma. Based on our study and the study by Kawachi et al. [13], in the later the stages of the disease in papillary thyroid carcinoma, the more the decrease in galectin-3 levels may be an indicator for metastasis and poor prognosis.

Hepsin is a type II transmembrane serine protease frequently overexpressed in most tissues and different tumors. Studies suggest that hepsin is required for the growth and maintenance of normal morphology, as well as for cell motility and development, initiation of blood coagulation, and pro-inflammatory immune response [6]. Recent studies on different malignant diseases revealed that hepsin expression increased in malignant tissues, and hepsin may have a prognostic and therapeutic value for some malignant diseases. In a study by Stephan et al. [14], they found that hepsin expression in malignant prostate tissue was higher than normal prostate tissue. In 53% of patients, it was more than 10 times higher. They also reported that high hepsin expression was associated with the degree of disease and may be a prognostic marker. Xing et al. [15] reported that the expression of hepsin in cancerous breast tissue was increased. The overexpression of Hepsin was associated with tumor stage, lymph node metastasis, and inhibition of hepsin expression could, therefore, be of the therapeutic importance. In a study by El-Rebey et al. [16], in 27 patients with endometrial cancer and 18 patients with endometrial hyperplasia, hepsin expression was higher in cancer patients and hepsin was reported to be associated with tumor grade and size. In a study by Tanimoto et al. [17], the effect of hepsin serine protease in patients with ovarian carcinoma showed that hepsin expression increased in patients with ovarian carcinoma. In a study by Zhang et al. [18] on patients with gastric carcinoma, high hepsin expression was associated with poor prognosis.

In our study, no significant difference was found between the thyroid malignant and benign patients in terms of hepsin expression. There is no record in the literature on hepsin in thyroid malignancy, so a comparison could not be made. There is a need for additional studies on thyroid malignancies for such a protein like hepsin that would also be important in the diagnosis and prognosis of many malignancies.

Tissue-specific transcription factors play a crucial role in regulating expression of tissue-specific genes. In this way, it controls the function, homeostasis, and differentiation of the tissue in which expression is made. Three different thyroid specific transcription factors are critical for thyroid function: NKX2-1 (TTF-1), FOXE1 (TTF-2), and PAX8. They regulate genes of thyroglobulin, thyroid peroxidase, thyrotropin receptor, and the sodium/iodide simporter, which synthesizes proteins critical for thyroid hormone synthesis. They are also important for thyroid development [19]. TTF-1 transcription factor plays a role in the production of surfactants in lung tissue and thyroglobulin secretion in thyroid glands. In a study by Anagnostou et al. [20] on patients with lung adenocarcinoma, increased TTF-1 expression in stage 1 adenocarcinoma patients was associated with long survival time and TTF-1 could be a prognostic marker in stage 1 adenocarcinoma patients. In a study by Zhang et al. [21] comparing endometrial cancer, endometrial proliferation, and normal endometrial tissue, TTF-1 expression in the tumor tissue was lower than the proliferation tissue and normal endometrial tissue so TTF-1 could be used in the diagnosis of early endometrial cancer. Daisuke et al. [22] reported that TTF-1 was expressed in only 5 (18%) of 28 patients with anaplastic carcinoma. Fenton et al. [23] reported that the recurrence rate was higher in patients with TTF-1 expression in papillary thyroid carcinoma. In a study by Katoh et al. [8], TTF-1 expression was found to be lower in differentiated thyroid carcinomas compared to undifferentiated thyroid carcinomas and TTF-1 may provide information about the functional activity and differentiation of thyroid tumors Tan et al. reported, in a study comparing patients with thyroid papillary carcinoma, follicular carcinoma, and follicular adenoma, that there was no difference in TTF-1 expression between the three groups so that TTF-1 was not diagnostically beneficial. In a study by Ai et al. [25] comparing thyroid papillary carcinoma with healthy people, there was no relationship between TTF-1 and thyroid papillary carcinoma. In our study, TFF-1 level of the patients in the malignant group was higher than the patients in the benign group. In the literature, there are studies reporting that TTF-1 has no diagnostic role in thyroid carcinoma and there are studies indicating that TTF-1 is a prognostic marker in thyroid carcinoma. Although our study shows that TTF-1 may be a diagnostic marker in contrast to previous studies, it is a fact that new studies are needed in this field.

The single-center design of the study and the low number of cases were the key limitations of this work.

In conclusion, although there is a need for new prospective controlled studies in this area; it was seen that the decrease in galectin-3 levels in thyroid papillary carcinoma could be effective in the transformation from microcarcinoma to

macrocarcinoma and TTF-1 could be an important marker in the benign-malign differentiation in thyroid nodules.

Acknowledgement: ELISA kits was provided in commercially available kits as part of the project supported by the Scientific Research Projects Coordination Unit of University (Project No: TTU-2018-6644).

References

1. Davies L, Welch HG. Current thyroid cancer trends in the United States. JAMA otolaryngology-head & neck surgery. 2014;140:317-22.
2. Ogilvie JB, Piatigorsky EJ, Clark OH. Current status of fine needle aspiration for thyroid nodules. Adv Surg. 2006;40:223-38.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62:10-29.
4. Brito JP, Yarur AJ, Prokop LJ, McIver B, Murad MH, Montori VM. Prevalence of thyroid cancer in multinodular goiter versus single nodule: a systematic review and meta-analysis. Thyroid. 2013;23:449-55.
5. Abd-El Raouf SM, Ibrahim TR. Immunohistochemical expression of HBME-1 and galectin-3 in the differential diagnosis of follicular-derived thyroid nodules. Pathol Res Pract. 2014;210:971-8.
6. Zhang C, Zhang M, Song S. Cathepsin D enhances breast cancer invasion and metastasis through promoting hepsin ubiquitin-proteasome degradation. Cancer Lett. 2018;438:105-15.
7. Netzel-Arnett S, Hooper JD, Szabo R, Madison EL, Quigley JP, Bugge TH, et al. Membrane anchored serine proteases: a rapidly expanding group of cell surface proteolytic enzymes with potential roles in cancer. Cancer Metastasis Rev. 2003;22:237-58.
8. Tan A, Etit D, Bayol U, Altinel D, Tan S. Comparison of proliferating cell nuclear antigen, thyroid transcription factor-1, Ki-67, p63, p53 and high-molecular weight cytokeratin expressions in papillary thyroid carcinoma, follicular carcinoma, and follicular adenoma. Ann Diagn Pathol. 2011;15:108-16.
9. Barletta JA, Perner S, Iafrate AJ, Yeap BY, Weir BA, Johnson LA, et al. Clinical significance of TTF-1 protein expression and TTF-1 gene amplification in lung adenocarcinoma. J Cell Mol Med. 2009;13:1977-86.
10. Danese D, Sciacchitano S, Farsetti A, Andreoli M, Pontecorvi A. Diagnostic accuracy of conventional versus sonography-guided fine-needle aspiration biopsy of thyroid nodules. Thyroid. 1998;8:15-21.
11. de Matos LL, Del Giglio AB, Matsubayashi CO, de Lima Farah M, Del Giglio A, da Silva Pinhal MA. Expression of CK-19, galectin-3 and HBME-1 in the differentiation of thyroid lesions: systematic review and diagnostic meta-analysis. Diagn Pathol. 2012;7:97.
12. Wu G, Wang J, Zhou Z, Li T, Tang F. Combined staining for immunohistochemical markers in the diagnosis of papillary thyroid carcinoma: improvement in the sensitivity or specificity? J Int Med Res. 2013;41:975-83.
13. Kawachi K, Matsushita Y, Yonezawa S, Nakano S, Shirao K, Natsugoe S, et al. Galectin-3 expression in various thyroid neoplasms and its possible role in metastasis formation. Hum Pathol. 2000;31:428-33.
14. Stephan C, Yousef GM, Scorilas A, Jung K, Jung M, Kristiansen G, et al. Hepsin is highly over expressed in and a new candidate for a prognostic indicator in prostate cancer. J Urol. 2004;171:187-91.
15. Xing P, Li JG, Jin F, Zhao TT, Liu Q, Dong HT, et al. Clinical and biological significance of hepsin overexpression in breast cancer. J Investig Med. 2011;59:803-10.
16. El-Rebey HS, Kandil MA, Samaka RM, Al-Sharaky DR, El Deeb K. The Role of Hepsin in Endometrial Carcinoma. Appl Immunohistochem Mol Morphol. 2017;25:624-631.
17. Tanimoto H, Yan Y, Clarke J, Korourian S, Shigemasa K, Parmley TH, et al. Hepsin, a cell surface serine protease identified in hepatoma cells, is overexpressed in ovarian cancer. Cancer Res. 1997;57:2884-7.
18. Zhang M, Zhao J, Tang W, Wang Y, Peng P, Li L, et al. High Hepsin expression predicts poor prognosis in Gastric Cancer. Sci Rep. 2016;6:36902.
19. Kimura S. Thyroid-specific transcription factors and their roles in thyroid cancer. J Thyroid Res. 2011;2011:710213.
20. Anagnostou VK, Syrigos KN, Bepler G, Homer RJ, Rimm DL. Thyroid transcription factor 1 is an independent prognostic factor for patients with stage I lung adenocarcinoma. J Clin Oncol. 2009;27:271-8.
21. Zhang HM, Fan TT, Li W, Li XX. Expressions and significances of TTF-1 and PTEN in early endometrial cancer. Eur Rev Med Pharmacol Sci. 2017;21(3 Suppl):20-6.
22. Nonaka D, Tang Y, Chiriboga L, Rivera M, Ghossein R. Diagnostic utility of thyroid transcription factors Pax8 and TTF-2 (FoxE1) in thyroid epithelial neoplasms. Mod Pathol. 2008;21:192-200.
23. Fenton CL, Patel A, Burch HB, Tuttle RM, Francis GL. Nuclear localization of thyroid transcription factor-1 correlates with serum thyrotropin activity and may be increased in differentiated thyroid carcinomas with aggressive clinical course. Ann Clin Lab Sci. 2001;31:245-52.
24. Katoh R, Kawaoi A, Miyagi E, Li X, Suzuki K, Nakamura Y, et al. Thyroid transcription factor-1 in normal, hyperplastic, and neoplastic follicular thyroid cells examined by immunohistochemistry and nonradioactive in situ hybridization. Mod Pathol. 2000;13:570-6.
25. Ai L, Yu Y, Liu X, Wang C, Shi J, Sun H, et al. Are the SNPs of NKX2-1 associated with papillary thyroid carcinoma in the Han population of Northern China? Front Med. 2014;8:113-7.

Arch Clin Exp Med 2019;4(3):127-131. e-ISSN: 2564-6567

DOI:10.25000/acem.601633 Araştırma makalesi / Research article

The effects of mesenchymal stem cells on the IDO, HLA-G and PD-L1 expression of breast tumor cells MDA-MB-231 and MCF-7

Mezenkimal kök hücrelerin, meme tümörü hücreleri MDA-MB-231 ve MCF-7'nin IDO, HLA-G ve PD-L1 ifadeleri üzerine etkileri

Rabia Bilge Özgül Özdemir [1], Alper Tunga Özdemir [2], Cengiz Kırmaz [3], Mehmet İbrahim Tuğlu [4], Özgür Şenol [5], Cenk Serhan Özverel [6], Afig Berdeli [2]

Abstract

Aim: Mesenchymal stem cells (MSCs) are strong immunomodulatory cells and a component of the tumor microenvironment. In this study, we aimed to investigate the effects of MSCs derived from adipose tissue on the expressions of immune evasive molecules indoleamine 2,3-dioxygenase (IDO), human leukocyte antigen G (HLA-G) and programmed death-ligand 1 (PD-L1) of breast tumor cell lines MDA-MB-231 and MCF-7.

Methods: For this purpose, MSCs, MDA-MB-231 and MCF-7 cells were cultured with increased doses of interferon gamma (IFN-g). In another plate, tumor cells were cultured in transwell inserts using the same IFN-g stimulation to evaluate the effect of MSCs. At the end of the culture period, the HLA-G and PD-L1 expression was detected by flow cytometry, and IDO expression by the Luminex method.

Results: We found that in low-dose IFN-g stimulation (10 ng/mL), MSCs led to a significant increase in the HLA-G and PD-L1 expression of MCF-7 cells. On the contrary, at a high dose of IFN-g (50 ng/mL), their expression significantly decreased in both tumor cells. In addition, we observed that the IDO expression of MDA-MB-231 cells was significantly increased in the presence of MSCs, but MCF-7 cells were not affected.

Conclusion: In conclusion, for MDA-MB-231 cells, MSCs may play a protective role because they reduce the expression of HLA-G and PD-L1 that are involved in the suppression of cytotoxic cells and exhaustion of T cells. On the other hand, MSCs may be an important source of high IDO levels, and therefore may negatively affect the antitumor immune response. However, our data should be supported by further studies.

Key words: Mesenchymal stem cells, immune evasion, HLA-G, PD-L1, IDO.

Öz

Amaç: Mezenkimal kök hücreler (MKH) güçlü immünomodülatör hücreleridir ve ayrıca tümör mikroçevresinin bir bileşenidir. Bu çalışmada meme tümör hücre hatları MDA-MB-231 ve MCF-7'nin immün evazif moleküller olan Indoleamine 2,3-dioxygenase (IDO), Human Leukocyte Antigen G (HLA-G) and Programmed Death-Ligand 1 (PD-L1) ifadelerine yağ dokusu kökenli mezenkimal kök hücrelerin etkilerini araştırmayı amaçladık.

Yöntemler: Bu amaçla MKH, MDA-MB-231 ve MCF-7 hücrelerini artan dozlarda interferon gama (IFN-g) ile kültüre edildi. Başka bir kültür kabında MKH'ler ile tümör hücreleri trans-well insertler ve aynı IFN-g uyarımı ile kültür edildi. Kültür süresinin bitiminde HLA-G ve PD-L1 ifadeleri flow-sitmotetri yöntemi ile IDO ifadeleri Luminex yöntemi ile analiz edildi.

Bulgular: Düşük dozlu IFN-g uyarımında (10 ng/mL), MSC'lerin MCF-7 hücrelerinin HLA-G ve PD-L1 ekspresyonunda önemli bir artışa yol açtığını bulduk. Aksine, yüksek doz IFN-g ile (50 ng/mL) bu ifadelerin her iki tümör hücresinde de önemli ölçüde azaldığını gördük. Ek olarak, MDA-MB-231 hücrelerinin IDO ekspresyonunun MSC'lerin varlığında anlamlı şekilde arttığını, ancak MCF-7 hücrelerinin etkilenmediği gördük.

Sonuç: Sonuç olarak, MDA-MB-231 hücreleri için MSC'ler, sitotoksik hücrelerin baskılanmasında ve T hücrelerinin tükenmesinde önemli bir rol oynayan HLA-G ve PD-L1 ekspresyonunu azalttığı için koruyucu bir rol oynayabilir. Öte yandan, MSC'ler yüksek IDO seviyeleri için önemli bir kaynak olabilir ve bu nedenle anti-tümör immün yanıtı olumsuz yönde etkileyebilir. Bununla birlikte, verilerimiz diğer çalışmalarla desteklenmelidir.

Anahtar Kelimeler: Mezenkimal kök hücreler, immün kaçınma, HLA-G, PD-L1, IDO.

[1]Manisa City Hospital, Department of Allergy and Clinical Immunology, Manisa, Turkey.
[2]Ege University, Institute of Health Sciences, Department of Stem Cell, Izmir, Turkey.
[3]Manisa Celal Bayar University, School of Medicine, Department of Internal Medicine, Division of Allergy and Clinical Immunology, Manisa, Turkey.
[4]Manisa Celal Bayar University, School of Medicine, Department of Histology and Embryology, Manisa, Turkey.
[5]Ege University, Department of Biotechnology, İzmir, Turkey.
[6]Ege University, Department of Bioengineering, İzmir, Turkey.

RBÖÖ: 0000-0002-8171-3402
ATÖ: 0000-0002-7708-077X
CK: 0000-0001-8873-1681
MİT: 0000-0002-0569-8415
ÖŞ: 0000-0002-1062-3290
CSÖ: 0000-0001-9932-4774
AB: 0000-0001-5627-6100

Ethics Committee Approval: This study was no human or animal study, no ethical approval has been taken.
Etik Kurul Onayı: Bu çalışma insan veya hayvan çalışması olmadığı için etik kurul onayı alınmamıştır.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this project has been supported by Manisa Celal Bayar University, Scientific Research Projects Coordination Unit (Project number 2017-224).
Finansal Destek: Yazarlar bu çalışma için Manisa Celal Bayar Universitesi, Bilimsel Projeler Arastirma Koordinasyon biriminden destek aldıklarını beyan etmişlerdir (2017-224).

Geliş Tarihi / Received: 05.08.2019
Kabul Tarihi / Accepted: 06.11.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:
Alper Tunga Özdemir
Adres/Address: 5522 street No: 25/A/8 Uncubozköy, Yunusemre, Manisa, Turkey.

e-posta: alpertungaozdemir@gmail.com
Tel/Phone: +905052973269
Fax: +902842357652

Introduction

Mesenchymal stem/stromal cells (MSCs) are adult cells that are able to differentiate chondrocytes, osteocyte sand adipocytes [1]. In addition to these regenerative abilities, MSCs are also strong immunomodulatory cells that show their effects in a non-selective and non-specific manner [2]. When MSCs migrate into the inflammatory sites, they start to produce immunosuppressive molecules, such as prostaglandin E2, indoleamine 2,3-dioxygenase (IDO), interleukin 10 (IL-10), transforming growth factor beta (TGF-b), hepatocyte growth factor, and soluble human leukocyte antigen G (HLA-G). These molecules affect almost all immune cells [3]. Adaptive immune cells; i.e., T helper (Th) 1, Th2 and Th17, which are responsible for the formation of allergies, asthma and autoimmune diseases, are efficiently suppressed by MSCs whereas regulatory T cells (Treg), which are involved in self-tolerance development, are stimulated by MSCs [4]. In presence of MSCs, antigen presentation functions of dendritic cells are impaired due to their immature state [5], and the cytotoxic effects of natural killer (NK) cells are suppressed [6]. With their strong immunomodulatory effects, MSCs are used in fatal conditions, such as steroid resistant graft versus host disease or autoimmune disorders; e.g., systemic lupus erythematosus, Crohn's disease, and ulcerative colitis [7].

Breast cancer (BC) is the most common cancer type in women, and several studies have been performed to investigate the interactions between MSCs and BC [8–10]. The mutational loads of tumor cells may lead to vulnerability to immune elimination. Most of these mutations are immunogenic and can easily be detected by cytotoxic lymphocytes (CTLs) [11]. In addition, tumor cells down-regulate the major histocompatibility antigen class I expression, which is an inhibitor of NK cells, and without this inhibition, tumor cells would be destroyed by the activated NK cells [12]. Despite these anti-tumor immune responses, tumor cells can still evade the immune system through the assistance of the microenvironment.

HLA-G it is crucial for fetal tolerance. HLA-G, expressed from trophoblast cells, inhibits immune cells and blocks cytotoxic effects of especially NK and CTL cells by interacting with their receptors; i.e., killer cell immunoglobulin-like receptor 2DL4 and immunoglobulin-like transcript 4 [13,14]. In addition, HLA-G expression is only detected in fetal tissues and almost never found in adult tissues. However, some immune privileged cells continue to express HLA-G, such as dendritic cells, MSCs and cornea [13,15,16]. Other cell surface molecules include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programed death ligand 1 (PD-L1), which are important in the development of self-tolerance in a healthy immune system. However, especially PD-L1 is strongly expressed in some tumor tissues, and interactions with PD-1 results in the suppression of T, NK and dendritic cells [17,18]. PD-1 receptors expressed on various immune cells, such as CD4/CD8 T, B, dendritic and monocyte/macrophage cells. PD-1/PD-L1 interactions lead to the strong inhibition of these cells by the dephosphorylation of proximal signaling molecules and augmentation of PTEN expression [19]. In addition to surface molecules, some important inhibitory molecules are secreted, including IDO, IL-10, and TGF-b. Tryptophan is an essential amino acid for T cells, and IDO is an enzyme that metabolizes tryptophan to kynurenine. IDO enzyme activation leads to relative tryptophan starvation for immune cells, especially T cells [20]. Kynurenine is the ligand for the aryl hydrocarbon receptor of immune cells. This receptor-ligand interaction results in suppressing effector T lymphocytes, and activating Treg cells [21]. In most somatic cells, IDO is expressed in response to IFN-g stimulation, and IDO plays a critical role in the protection of cells from over-reactive immune cells [22]. MSCs are a component of the tumor microenvironment and can contribute to the immune evasion of tumor cells or increase the metastasis rate by accelerating the epithelial-to-mesenchymal transition [9].

Adipose tissue is a good source for MSCs and is the main component of breast tissue. In this respect, breast tumors constitute a valuable model to demonstrate the interactions between BC and MSCs derived from adipose tissue.

In this study, we aimed to investigate the alterations in immune evasive molecules, namely IDO, HLA-G, and PD-L1 of MSCs, invasive BC cells (MDA-MB-231) and non-invasive BC cells (MCF-7) in certain inflammatory conditions by performing in vitro co-culture experiments.

Material and methods

Cell culture

Two commonly used breast cancer cell lines, MDA-MB-231 (ATCC® HTB-26™) and MCF-7 (ATCC® HTB-22™), and MSCs derived from human adipose tissue were purchased from the American Type Culture Collection (ATCC). MDA-MB-231 cells were selected as invasive cells and MCF cells for their non-invasive nature. To culture MDA-MB-231 cells and MSCs, 10% fetal bovine serum (FBS) (Biosera, USA, Cat#FB-1001), 1% penicillin/streptomycin (Biosera, USA, Cat#XC-A4122), and 1% L-glutamine (Biosera, USA, Cat#XC-T1715) containing the DMEM-F12 medium (Biosera, USA, Cat#LM-D1225) were used. MCF-7 cells were cultured using 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine containing the RPMI-1640 medium (Biosera, USA, Cat#LM-R1638). All cells were cultured at 37 °C in a 5% CO2 incubator for passage three, and the cell culture media were renewed every two days with fresh media (Figure 1).

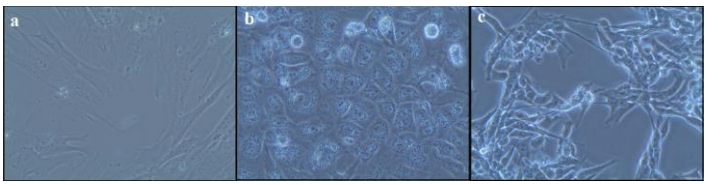

Figure 1: The microscopic images of human adipose tissue-derived mesenchymal stem cells (a), breast tumor cells MCF-7 (b), and MDA-MB-231 (c).

To mimic the inflammatory condition, 1x105 cells of MDA-MB-231, MCF-7 and MSCs were separately cultured in 24-well plates with or without 10 ng/ml and 50 ng/ml human recombinant IFN-g (Reprokine Ltd, Israel Cat# RKP01579) for 24 hours. In another plate, tumor cells were cultured with MSCs in 0.4 µm pore-sized transwell inserts (Corning, USA, Cat# CLS3378) using the same IFN-g stimulation to evaluate the effects of MSCs.

FACS and Luminex analyses

After the incubation period, conditioned medium supernatants were collected for the detection of IDO levels. All cells were detached using a trypsin-EDTA (Biosera, USA, Cat#LM-T1705) solution and collected into individual tubes for flow cytometric analyses. To observe the alterations in the immune suppressor and co-inhibitory molecules, HLA-G (Clone: MEM-G/11; Exbio, Czech Republic) was stained with fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies and PD-L1 (Clone: 29E.2A3; Exbio, Czech Republic) was

stained with phycoerythrin (PE)-labeled monoclonal antibodies. Isotype negative IgG1 FITC (Clone: B-Z1, Diaclone, France) and IgG2a PE (Clone: B-Z2, Diaclone, France) controls were used to eliminate non-specific binding. The IDO levels were measured using an IDO Human ProcartaPlex™ Simplex Luminex kit (Invitrogen, USA Cat# EPX01A-12213-901).

All FACS measurements were performed by a BD Accuri C6 device, and the analyses were undertaken using FlowJo v.10 software (BD Biosciences, USA). After choosing the appropriate cell population, the mean fluorescent intensity (MFI) values were calculated to evaluate the changes in the expression of HLA-G and PD-L1. The IDO levels were detected using a Luminex 200TM (Luminex Corp., USA) device, and the data were analyzed by xPonent v.3.1 software (Luminex Corp., USA). All experiments were performed in triplicate, and the detected values are summarized in Table-1.

Table 1: The results of the HLA-G, PD-L1 and IDO analyses of all experimental groups.

	Unstimulated	IFN-g (10 ng/ml)	IFN-g (50 ng/ml)
	HLA-G (MFI)		
MDA-MB-31	1283.7 ± 32.3	1145 ± 42.0	1220.3 ± 25.8
MSC Co-culture	1355 ± 50.1	1083.66 ± 28.1	1065.3 ± 11.0
MCF-7	2533.3 ± 165.0	2486 ± 104.1	2638.7 ± 63.0
MSC Co-culture	3106.3 ± 82.7	3479.3 ± 109.3	2678.3 ± 102.8
	PD-L1 (MFI)		
MDA-MB-231	173.7 ± 8.5	167.33 ± 6.8	187.3 ± 5.0
MSC Co-culture	161.3 ± 6.5	140.33 ± 4.5	171.7 ± 3.5
MCF-7	181.7 ± 6.5	208.00 ± 13.0	255.3 ± 12.1
MSC Co-culture	183.7 ± 8.1	310.33 ± 19.6	171.7 ± 7.6
	IDO (pg/ml)		
MDA-MB-231	0.01 ± 0.0	0.01 ± 0.0	0.03 ± 0.02
MSC Co-culture	0.023 ± 0.015	0.046 ± 0.030	1.74 ± 0.24
MCF-7	0.05 ± 0.03	0.07 ± 0.02	0.09 ± 0.05
MSC Co-culture	0.03 ± 0.01	0.09 ± 0.045	0.13 ± 0.05

MFI: Median fluorescent intensity

Statistical analysis

GraphPad Prism software v.6 was used for the statistical analyses of the data sets. Descriptive analysis was performed to determine whether the groups were homogeneous. According to the Shapiro-Wilk normality test, the groups with a p of > 0.05 or above were considered to be homogeneously distributed. The data were analyzed using the one-way analysis of variance method, and the results were considered significant when at $p < 0.05$.

Results

The comparative HLA-G and PD-L1 histogram graphs of the FACS analysis of the experimental groups are shown in Figure-2, and the comparison graphs of HLA-G, PD-L1 MFI values and IDO Luminex results are given in Figure-3. The HLA-G MFI values of MCF-7 cells significantly increased in the unstimulated (US) MSC and IFNg-10/MSC co-culture groups compared to the US and IFNg-10 groups (p=0.0004 and p<0.0001 respectively). However, there was no difference between the IFNg-50 and IFNg-50/MSC co-culture groups (p = 0,9972). In addition, the HLA-G MFI values of the IFNg-10/MSC co-culture group was significantly higher than the remaining groups. Among MDA-MB-231 cells, the highest HLA-G MFI value was detected in the US-MSC co-culture group. On the other hand, as a response to 10 ng/ml IFN-g stimulation, the HLA-G expression of MDA-MB-231 cells was significantly decreased compared with the US groups (p=0.0032), but not in the IFNg-50 group. These decreases were more significant in the IFNg-10/MSC and IFNg-50/MSC co-culture groups (p<0.0001 and p<0.0001, respectively) (Figure 2 and Figure 3).

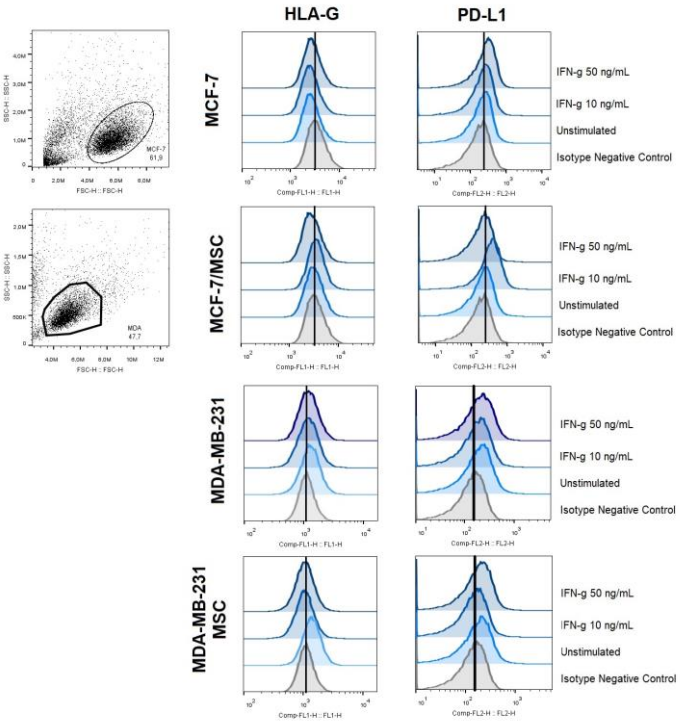

Figure 2: The histogram charts of HLA-G and PD-L1 flow cytometry analyses of MCF-7 and MDA-MB-231 cells with and without MSCs in different IFN-g stimulations.

We found no significant change in the PD-L1 MFI values of the US MCF-7 and US MCF-7/MSC groups. However, there was a significant increase in the IFN-10 group compared to IFN-10/MSC group (p < 0.0001). Interestingly, this increase was reversed for the IFNg-50 and IFNg-50/MSC groups, and the PD-L1 expression of MCF-7 cells was significantly decreased in the presence of MSCs (p < 0.0001) (Figure-2 and Figure-3). We detected that the PD-L1 expression of MDA-MB-231 cells was consistently reduced in the presence of MSCs. However, the difference was only significant between the IFNg-10 and IFNg-10/MSC groups (p < 0.0001).

We detected that the IDO expression of MCF-7 and MDA-MB-231 tumor cells were not affected by IFN-g stimulation. However, in the presence of MSCs, the IDO expression of MDA-MB-231 cells was significantly increased in response to 50 ng/ml IFN-g stimulation in contrast to the remaining groups (p < 0.0001 for all groups).

Discussion

In this study, we stimulated breast tumor cell lines, MDA-MB-231 and MCF-7, with increased doses of IFN-g to mimic the inflammatory environment. We found that the HLA-G and PD-L1 expression of MDA-MB-231 and MCF-7 cells differed and was altered by the presence of MSCs. In addition, we observed that the IDO expression of breast tumor cells did not change after IFN-g stimulation; however; it significantly increased in the presence of MSCs.

There are numerous studies in the current literature about the relationship between HLA-G and tumor progression. Most have been performed using immunohistochemical methods

and have reported a strong correlation between increased HLA-G expression and poor tumor prognosis [15,23]. In BC, HLA-G is also associated with poor prognosis, and some researchers determined that HLA-G might be increased in a subset of tumor cells called cancer stem cells [24,25]. Based on the literature data, we investigated the HLA-G expression status of IFN-g-stimulated invasive breast tumor cells; i.e., MDA-MB-231 and non-invasive MCF-7 cells and explored how MSCs affected this status. There are a very low number of reports on the HLA-G expression of these cell lines. Elliott et al. showed that MDA-MB-231 and MCF-7 cells were be expressed by the mRNA of HLA-G in contrast to healthy breast tissue [26]. Our experiments confirmed that MDA-MB-231 cells had HLA-G expression, and this basal expression significantly decreased through IFN-g stimulation. In addition, we observed that this inhibition was stronger when IFN-g and MSCs were combined (Figure 2 and 3). According to our data, the HLA-G expression of MDA-MB-231 cells was significantly suppressed by IFN-g stimulation, which was potentiated by the presence of MSCs. The other breast tumor cell line MCF-7 is considered as non-invasive and has been shown to also express HLA-G [24,27]. In our experiments, we found that the HLA-G expression of MCF-7 cells did not change after IFN-g stimulation in a dose-dependent manner. However, we observed a significant increase in the HLA-G expression of the US-MCF and IFNg-10/MSC groups caused by MSCs. Interestingly, the MSC-induced HLA-G increase was not present in the IFN-g-50 group. According to our findings, MSCs seem to be an important factor for HLA-G expression in the basal and low-density inflammation of MFC-7 cells. However, this positive effect may be lost in high-intensity inflammation (Figure-2 and 3). In this study, we were not able to perform a cytotoxicity assay, but the possibility of occurrence of cell death due to high IFN-g stimulation applied should be considered.

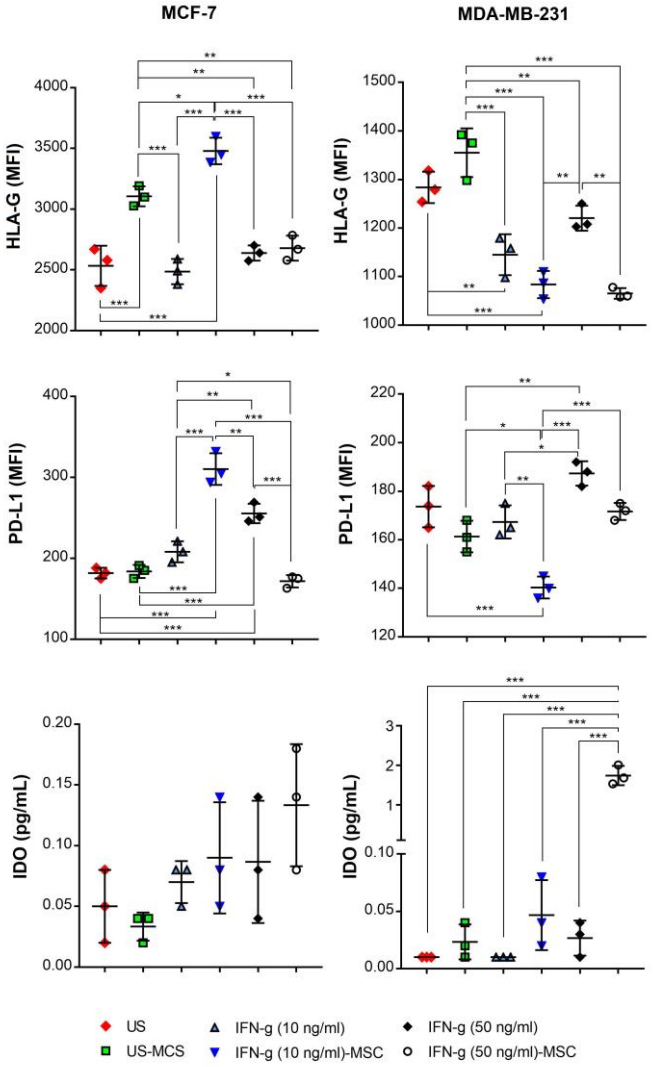

Figure 3: The comparison charts of the HLA-G, PD-L1 and IDO expressions of MDA-MB-231 and MCF-7 cells with and without MSCs in different IFN-g stimulations. The data are presented as mean ± standard deviation (SD) (* statistically significant; $p < 0.05$).

Another important immune evasive molecule of tumor cells is PD-L1, which is expressed in many tumor types, including breast tumor cells. There are many studies on the expression of PD-L1 in breast tumor cells, particularly in MDA-MB-231 and MCF-7 cells [28,29]. It was shown that IFN-g with pleiotropic effects increased the levels of immunosuppressive molecules, IDO, PD-L1 and CTLA-4, thereby limiting T cell functions [30,31]. In our study, we observed that both MDA-MB-231 and MCF-7 cells expressed PD-L1, and this expression significantly increased after 50 ng/ml IFN-g stimulation, but not after 10 ng/ml IFN-g. We consider that this finding may be due to the pleiotropic effect of IFN-g. The PD-L1 expression of MDA-MB-231 cells may have been significantly reduced in the presence of MSCs, and this decrease may not have been related to IFN-g stimulation. As a result, the interaction between MSCs and MDA-MB-231 cells in response to IFN-g stimulation, may result in more efficient suppression of both HLA-G and PD-L1 expression. Similar to HLA-G expression, the PD-L1 expression of MCF-7 cells significantly increased in the IFNg-10/MSC co-culture group and significantly decreased in the IFNg-50/MSC co-culture group. This finding suggests that IFN-g levels may be a critical factor in the interaction between MCF-7 and MSCs (Figure 2).

In many studies, tumor cells have also been shown to express IDO. However, the main sources of IDO in tumor tissue are tumor microenvironment cells, such as tumor-associated macrophages, cancer-associated fibroblasts, myeloid-derived suppressor cells, and MSCs [32,33]. It has been reported that the expression of IDO in breast tumor tissues are correlated with advanced tumor stage and poor prognosis [34]. In addition, the IDO mRNA expression has been detected in breast tumor cells lines, MDA-MB-231 and MCF-7 [35]. In this study, we investigate the contribution of MSCs to the IDO expression of tumor cells and found that the basal IDO expression of both MDA-MB-231 and MCF-7 cells was not affected by increased levels of IFN-g stimulation. However, we also determined that the IDO levels of the IFNg-50/MSC group were significantly higher in MDA-MB-231 cells than the remaining groups. The IDO expression of MCF-7 cells was unchanged in the presence of MSCs. This finding indicates that when the MSCs interacting with MDA-MB-231 cells, MSCs may be the main source of IDO expression.

In conclusion, the responses of two different breast tumor cells for the molecules investigated greatly differed. We found that MSCs interacting with MCF-7 cells significantly increased the HLA-G and PDL-1 expression in the presence of low IFN-g stimulation, but high IFN-g stimulation reversed this effect. The interaction of MDA-MB-231 cells with MSCs resulted in a significant reduction in the HLA-G and PD-L1 expression. In MDA-MB-231 cells, MSCs play a protective role because they reduce the expression of HLA-G and PD-L1 that are involved in the suppression of cytotoxic cells and exhaustion of T cells. However, MSCs may be an important source of increased IDO levels, and therefore may suppress the anti-tumor immune response. The data of our study were obtained only from the interactions of tumor cells and MSCs. In order to confirm our data and provide a better understanding of the contribution of MSCs to tumor immune evasion, further studies including immune cells are needed.

Acknowledgements: This project was supported by the Scientific Research Projects Coordination Unit of Manisa Celal Bayar University (Project number 2017-224).

We would like to thank Dr. Ayşe Nalbantsoy for her help in flow cytometry analyses.

References

1. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–7.
2. Sangiorgi B, Panepucci RA. Modulation of Immunoregulatory Properties of Mesenchymal Stromal Cells by Toll-Like Receptors: Potential Applications on GVHD. Stem Cells Int. 2016;2016:e9434250.
3. Chen P-M, Yen M-L, Liu K-J, Sytwu H-K, Yen B-L. Immunomodulatory properties of human adult and fetal multipotent mesenchymal stem cells. J Biomed Sci. 2011;18:49.
4. Gao F, Chiu SM, Motan D a. L, Zhang Z, Chen L, Ji H-L, et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis. 2016;7:e2062.
5. Özdemir RBÖ, Özdemir AT, Sarıboyacı AE, Uysal O, Tuğlu Mİ, Kırmaz C. The investigation of immunomodulatory effects of adipose tissue mesenchymal stem cell educated macrophages on the CD4 T cells. Immunobiology. 2019;224:585–94.
6. Castro-Manrreza ME, Montesinos JJ. Immunoregulation by Mesenchymal Stem Cells: Biological Aspects and Clinical Applications. J Immunol Res. 2015; 2015:e394917.
7. Erbey F, Atay D, Akcay A, Ovali E, Ozturk G. Mesenchymal Stem Cell Treatment for Steroid Refractory Graft-versus-Host Disease in Children: A Pilot and First Study from Turkey. Stem Cells Int. 2016;2016:1641402.
8. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 2007;449:557–63.
9. Ridge SM, Sullivan FJ, Glynn SA. Mesenchymal stem cells: key players in cancer progression. Mol Cancer. 2017;16:31.
10. Wang Y, Liu J, Jiang Q, Deng J, Xu F, Chen X, et al. Human adipose-derived mesenchymal stem cell-secreted CXCL1 and CXCL8 facilitate breast tumor growth by promoting angiogenesis. Stem Cells. 2017;35:2060-70.
11. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome Res. 2014;24:743–50.
12. Dahlberg CIM, Sarhan D, Chrobok M, Duru AD, Alici E. Natural Killer Cell-Based Therapies Targeting Cancer: Possible Strategies to Gain and Sustain Anti-Tumor Activity. NK Cell Biol. 2015;6:605.
13. Eskicioğlu F, Özdemir AT, Özdemir RB, Turan GA, Akan Z, Hasdemir SP. The association of HLA-G and immune markers in recurrent miscarriages. J Matern Fetal Neonatal Med. 2016;29:3056-60.
14. Özgül Özdemir RB, Özdemir AT, Oltulu F, Kurt K, Yiğittürk G, Kırmaz C. A comparison of cancer stem cell markers and nonclassical major histocompatibility complex antigens in colorectal tumor and noncancerous tissues. Ann Diagn Pathol. 2016;25:60–3.
15. Amiot L, Ferrone S, Grosse-Wilde H, Seliger B. Biology of HLA-G in cancer: a candidate molecule for therapeutic intervention? Cell Mol Life Sci. 2011;68:417–31.
16. Goldman-Wohl DS, Ariel I, Greenfield C, Hanoch J, Yagel S. HLA-G expression in extravillous trophoblasts is an intrinsic property of cell differentiation: a lesson learned from ectopic pregnancies. Mol Hum Reprod. 2000;6:535–40.
17. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, et al. PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Front Pharmacol. 2017;8:561.
18. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways. Am J Clin Oncol. 2016;39:98–106.
19. Francisco LM, Sage PT, Sharpe AH. The PD-1 Pathway in Tolerance and Autoimmunity. Immunol Rev. 2010;236:219–42.
20. Soliman H, Mediavilla-Varela M, Antonia S. Indoleamine 2,3-dioxygenase: is it an immune suppressor? Cancer J Sudbury Mass. 2010;16:354–9.
21. Routy J-P, Routy B, Graziani GM, Mehraj V. The Kynurenine Pathway Is a Double-Edged Sword in Immune-Privileged Sites and in Cancer: Implications for Immunotherapy. Int J Tryptophan Res. 2016;9:67–77.
22. Takikawa O, Tagawa Y, Iwakura Y, Yoshida R, Truscott RJW. Interferon-Gamma-Dependent/Independent Expression of Indoleamine 2,3-Dioxygenase. Adv Exp Med Biol. 1999;467:553-7.
23. Bukur J, Jasinski S, Seliger B. The role of classical and non-classical HLA class I antigens in human tumors. Semin Cancer Biol. 2012;22:350–8.
24. He X, Dong D, Yie S, Yang H, Cao M, Ye S, et al. HLA-G expression in human breast cancer: implications for diagnosis and prognosis, and effect on allocytotoxic lymphocyte response after hormone treatment in vitro. Ann Surg Oncol. 2010;17:1459–69.
25. Ozgul Ozdemir RB, Ozdemir AT, Oltulu F, Kurt K, Yigitturk G, Kirmaz C. The Expressions of Cancer Stem Cell Markers and Nonclassical HLA antigens in Breast Tumors. Istanb Med J. 2017;18:128–34.
26. Elliott RL, Jiang XP, Phillips JT, Barnett BG, Head JF. Human leukocyte antigen G expression in breast cancer: role in immunosuppression. Cancer Biother Radiopharm. 2011;26:153–7.
27. Pangault C, Amiot L, Caulet-Maugendre S, Brasseur F, Burtin F, Guilloux V, Drenou B, Fauchet R, Onno M. HLA-G protein expression is not induced during malignant transformation. Tissue Antigens. 2002;53:335–46.
28. Mazel M, Jacot W, Pantel K, Bartkowiak K, Topart D, Cayrefourcq L, et al. Frequent expression of PD-L1 on circulating breast cancer cells. Mol Oncol. 2015;9:1773–82.
29. Rom-Jurek E-M, Kirchhammer N, Ugocsai P, Ortmann O, Wege AK, Brockhoff G. Regulation of Programmed Death Ligand 1 (PD-L1) Expression in Breast Cancer Cell Lines In Vitro and in Immunodeficient and Humanized Tumor Mice. Int J Mol Sci. 2018;13:19.
30. Castro F, Cardoso AP, Gonçalves RM, Serre K, Oliveira MJ. Interferon-Gamma at the Crossroads of Tumor Immune Surveillance or Evasion. Front Immunol. 2018;4;9.
31. Osum KC, Burrack AL, Martinov T, Sahli NL, Mitchell JS, Tucker CG, et al. Interferon-gamma drives programmed death-ligand 1 expression on islet β cells to limit T cell function during autoimmune diabetes. Sci Rep. 2018;8:1–12.
32. Holmgaard RB, Zamarin D, Munn DH, Wolchok JD, Allison JP. Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4. J Exp Med. 2013;210:1389–402.
33. Munn DH, Mellor AL. IDO in the Tumor Microenvironment: Inflammation, Counter-Regulation, and Tolerance. Trends Immunol. 2016;37:193–207.
34. Larrain MTI, Rabassa ME, Lacunza E, Barbera A, Cretón A, Segal-Eiras A, et al. IDO is highly expressed in breast cancer and breast cancer-derived circulating microvesicles and associated to aggressive types of tumors by in silico analysis. Tumor Biol. 2014;35:6511–9.
35. Wei L, Zhu S, Li M, Li F, Wei F, Liu J, Ren X. High Indoleamine 2,3-Dioxygenase Is Correlated With Microvessel Density and Worse Prognosis in Breast Cancer. Front Immunol. 2018;9:724.

Arch Clin Exp Med 2019;4(3):132-137. e-ISSN: 2564-6567

DOI:10.25000/acem.605006 Araştırma makalesi / Research article

Radiologically guided percutaneous nephrostomy: A 6-year single-center experience

Görüntüleme kılavuzluğunda perkütan nefrostomi: 6 yıllık tek merkez deneyimi

Mehmet Şeker [1], Türkmen Turan Çiftçi [2], Devrim Akıncı [2], Okan Akhan [2]

Abstract

Aim: To retrospectively analyze the indications, underlying pathologies, technical success rate, complications and benefit of percutaneous nephrostomies in a single centre.

Materials and Methods: Data of 578 patients who underwent radiologically guided percutaneous nephrostomy between January 1999 and December 2004 were retrospectively reviewed. The mean age of the patients was 42.5 years (range, 6 days–90 years). The indications were urinary obstruction without urinary infection (77.9%), urinary obstruction with urinary infection (13.1%), urinary diversion (6.9%) and diagnostic testing (2.1%).

Results: The technical success rate was 99.4%. There was no procedure related mortality. Major hemorrhage or sepsis were not observed in children. Major hemorrhage occurred in 1.55% and sepsis occured in 2.65% of adult patients. Catheter dislodgement was the commonest complication with an overall rate of 11.4%. In 7.2% patients, percutaneous nephrostomy was successful in managing patients without further intervention. 36.5% of patients had surgery and 14.7% had ureteral stenting as definitive treatment.

Conclusion: Radiologically guided percutaneous nephrostomy, can be used effectively, and safely in a wide variety of indications with high technical success and low complications rates.

Keywords: Percutaneous nephrostomy, urinary system obstruction, urinary leakage, urinary fistula, interventional radiology.

Öz

Amaç: Perkütan nefrostomilerin, endikasyonlarını, altta yatan patolojileri, teknik başarı oranını, komplikasyonları ve genel faydalarını retrospektif olarak incelemek.

Materyal ve Metod: Ocak 1999 ile Aralık 2004 tarihleri arasında görüntüleme kılavuzluğunda perkütan nefrostomi yapılan 578 hastanın verileri retrospektif olarak incelenmiştir. Hastaların ortalama yaşı 42,5 yıl idi (6 gün–90 yıl). İşlem endikasyonları üriner enfeksiyon olmadan obstrüksiyon varlığı (% 77,9), üriner enfeksiyon ile beraber obstrüksiyon varlığı (%13,1), üriner diversiyon (%6,9) ve böbrek fonksiyonları değerlendirme (%2,1) idi.

Bulgular: Teknik başarı oranı % 99,4 idi. İşlemle ilişkili mortalite izlenmedi. Çocuklarda major hemoraji ya da sepsis görülmemişti. Erişkin hastalarda major kanama oranı % 1,55, sepsis oranı ise %2,65 idi. Kateter dislokasyonu en sık ortaya çıkan komplikasyondu ve oranı toplamda %11,4 idi. Hastaların %7,2'sinde başka girişim yapılmadan perkütan nefrostomi ile başarılı tedavi sağlandı. Hastaların %36,5'inde cerrahi ile ve %14,7'sinde ise üreteral stent yerleştirilerek kesin tedavi sağlandı.

Sonuç: Radyoloji kılavuzluğunda yapılan perkütan nefrostomi, yüksek teknik başarı ve düşük komplikasyon oranları ile çok çeşitli endikasyonlarda etkili ve güvenli bir şekilde kullanılabilir.

Anahtar kelimeler: Perkütan nefrostomi, üriner sistem obstrüksiyonu, üriner sistem kaçağı, üriner fistül, girişimsel radyoloji.

[1] **Medipol University, Faculty of Medicine, Department of Radiology, Bağcılar, Istanbul, Turkey.**

[2] **Hacettepe University, Faculty of Medicine, Department of Radiology, Çankaya, Ankara, Turkey.**

MŞ: 0000-0002-6745-0159
TTÇ: 0000-0002-1284-859X
DA: 0000-0002-8189-4688
OA: 0000-0002-6864-0229

Ethics Committee Approval: The study was approved by the local ethical authority (28.04.2005; LUT 05/31).
Etik Kurul Onayı: Çalışma lokal etik komite tarafından onaylanmıştır (28.04.2005; LUT 05/31).

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Geliş Tarihi / Received: 22.08.2019
Kabul Tarihi / Accepted: 06.11.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:

Mehmet Şeker
Adress/Adres: Medipol Mega Hospital, No: 1, Bağcılar, 34214, Istanbul/Turkey.

E-posta: hikmet.irfan@hotmail.com
Tel/Phone: +90 533 453 38 29
Fax: +90 0212 460 70 70

Introduction

Interventional radiology plays a fundamental role in terms of diagnostic and therapeutic support in the field of nephrology and urology. With the accumulation of experience within years, interventional radiologic procedures have been found to be better tolerated than the surgical procedures and they have fully or partially replaced the standard treatment with open abdominal surgery. Percutaneous nephrostomy (PCN) is one of these interventional procedures [1-4].

PCN has conventionally been performed under fluoroscopic guidance and anatomic landmarks or a radiopaque target (eg, stone, opacified collecting system with contrast) are used to localize the collecting system and to select the appropriate entry site. But along with the technical developments in imaging modalities, more procedures were performed with the use of cross-sectional techniques. The use of cross-sectional imaging modalities, alone or in combination with fluoroscopy, to guide the procedure have increased the technical success and reduced the associated complications [2, 3].

Early in its development, PCN was used almost always for temporary or permanent drainage in cases of an obstructed kidney and for evaluation of renal function. Increased success rates and reduced associated complications have significantly broadened the indications. These indications include relieving urinary obstructions without or with infection, urinary diversion for a leak or fistula, and accessing the collecting system for diagnostic and therapeutic procedures [1-4]. Although the advancement of modern endourological techniques has led to a decline in the indications for PCN, it still plays an important role in the treatment of multiple urologic conditions [2-4].

PCN is one of the commonest procedures performed in urologic and interventional radiology units and several reports were published for assessing the indications and outcome of PCN. However, since there is no recently published article on the indications and outcomes of PCN, we conducted this retrospective study to evaluate our experience of PCN. In this retrospective study we aim to analyze the indications, underlying pathologies, technical success rate, complications and overall benefit of PCN.

Material and methods

For this study approval of Ethics Committee of Hacettepe University was obtained (28/April/2005; decision no. LUT 05/31). Informed consent was obtained from all individual participants included in the study. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written consent could not be taken due to the retrospective design of the study.

Data collection and study population

We retrospectively reviewed the hospital charts and radiology records of all patients whom PCN procedure were performed in non-vascular interventional radiology unit of Hacettepe University between January 1999 and December 2004.

Hemorrhage and sepsis rates were calculated based on the number of patients treated and other complication and technical success rates were reported based on the number of procedures performed according to the Percutaneous Nephrostomy Guideline of Society of Interventional Radiology (SIR) [2, 3].

Preparation before PCN and technique

International normalized ratio (INR) and platelet count of all cases were evaluated and abnormal parameters (INR higher than 1.5 or platelet count less than 80000/mm3) were corrected before procedure. A single dose of prophylactic broad spectrum antibiotic was administered one hour before the procedure to all patients unless the patient was already receiving antibiotics.

The procedures were carried out by an interventional radiologist or by a radiology resident supervised by an interventional radiologist.

Procedures of children were carried out under sedation or general anesthesia with supervision of a specialist an anesthesiologist. In all adult patients, sedation were used.

All procedures, except two, were performed with Seldinger technique under combined use of ultrasonography (US) and fluoroscopy. In the remaining 2 patients, only US was used for guidance.

The patients were placed on the fluoroscopy table in prone position. After sterilely cleansed and draped, the skin and fascia were incised with a 11G blade and an 18-21G Chiba needle was inserted through the incision under US guidance and aimed at the direction previously determined. The sonographic view as well as urine confirmed that the needle was at the desired site. A small volume of dilute contrast was injected to opacify the collecting system to verify needle positions. Following succesfull access, needle was removed and a 0.035 or 0.018 inch guidewire (Amplatz Superstiff or 0.018 inch Nitinol guide wire; Boston Scientific, Watertown, MA) was advanced through the needle under fluoroscopic guidance. Nephrostomy tract was dilated with dilators and a PCN catheter (6-12 Fr) was advanced over guidewire. All PCN catheters used in our series had self-retaining mechanisms (650 with and 153 without locking strings) and fixed to the skin. Catheters were placed to a gravity drainage bag. The technique of PCN is shown step by step in figure 1.

The patients were followed in their referring clinics after the procedure.

Statistical analysis

Descriptive statistics, including means and percentages, were used to summarize the data.

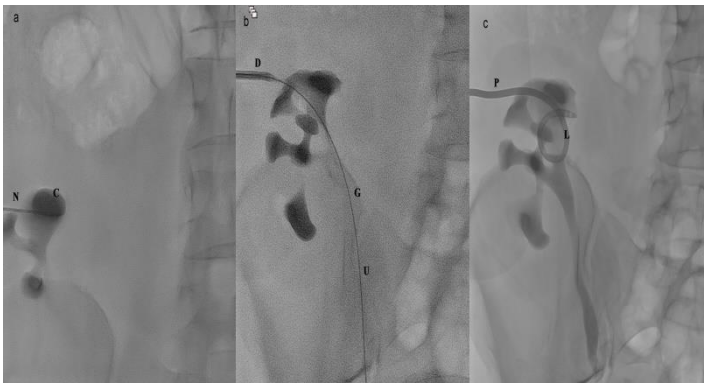

Figure 1. Radiographs show the basic technique of percutaneous nephrostomy. (a) With the patient in the prone position, a 21G Chiba needle (N) is passed into the upper pole calyx (C). After urine sample is collected, a small volume of diluted contrast material is injected to opacify the collecting system to verify needle positions. (b) Following succesfull access, needle is removed and a guidewire (G) is advanced through the needle into the ureter (U) under fluoroscopic guidance. Nephrostomy tract is than sequentially dilated with dilators (D). (c) A PCN catheter (P) with self-retaining mechanism is advanced over the wire, and the loop (L) is formed.

Results

Study population

A total of 803 PN procedures were performed in 578 patients (353 male and 225 female). Mean age of these patients was 42.5 years. 160 PCN procedures were performed in 126 patients, aged between 6 days to 15 years (mean age, 4.93 years). The remaining 452 patients were aged between 16 to 90 years (mean age, 52.9 years) and a total of 643 PCN procedures were performed in these patients.

The most common indication was urinary obstruction without urinary infection (77.9%). The other indications were urinary obstruction with urinary infection (13.1%), urinary diversion (6.9%) and diagnostic testing (2.1%). The most common underlying pathologies considered as PCN indication in children and adults were congenital abnormalities (80.1%) and malignities (53.6%), respectively. The indications of PCN procedures and underlying pathologies are shown in tables 1-3.

Table 1: Indications of PCN procedures and underlying pathologies

Indication	Malignity	Urinary stone disease	Congenital pathology+	Other pathology*	RPF	Total
Obstruction without infection	216 (48)	107 (23.8)	94 (20.9)	24 (5.3)	9 (2)	450 (77.9)
Obstruction with infection	19 (25)	29 (38.1)	18 (23.7)	10 (13.2)	0	76 (13.1)
Urinary diversion	7 (17.5)	3 (7.5)	1 (2.5)	29 (72.5)	0	40 (6.9)
Diagnosting testing	3 (25)	4 (33.3)	3 (25)	2 (16.7)	0	12 (2.1)
Total	245 (42.4)	143 (24.7)	116 (20.1)	65 (11.2)	9 (1.6)	578 (100)

n (%): numbers of patients and percentage, PCN: percutaneous nephrostomy, RPF; retroperitoneal fibrosis, +: includes ureteropelvic and ureterovesical junction obstruction, vesicoureteral reflux, posterior uretral valve, ureterocele, *: includes urinary, gynecologic and other surgery, trauma, radioterapy.

Table 2. Indications of PCN procedures and underlying pathologies in children.

Indication	Malignity	Urinary stone disease	Congenital abnormality					Other pathology*	Total
			UP	UV	VUR	Other+	Total		
Obstruction without infection	3 (3.2)	11 (11.6)	41 (43.2)	25 (26.3)	8 (8.4)	5 (5.2)	79 (83.1)	2 (2.1)	95 (75.5)
Obstruction with infection	(0)	2 (9.1)	4 (18.2)	9 (40.9)	3 (13.6)	2 (9.1)	18 (81.8)	2 (9.1)	22 (17.5)
Urinary diversion	(0)	(0)	1 (25)	0	0	0	1 (25)	3 (75)	4 (3.1)
Diagnosting testing	(0)	1 (20)	3 (60)	(0)	(0)	(0)	3 (60)	1 (20)	5 (3.9)
Total	3 (2.4)	14 (11.1)	49 (38.9)	34 (26.9)	11 (8.7)	7 (5.6)	101 (80.1)	8 (6.4)	126 (100)

n (%); numbers of patients and percentage, PCN; percutaneous nephrostomy, UP; ureteropelvic junction obstruction, UV; ureterovesical junction obstruction, VUR, vesicoureteral reflux. +includes posterior uretral valve and ureterocele; *; includes surgery and trauma.

Technical success

The procedure was successfully completed in 798 out of 803 procedures (99.4%). In 80 % of patients with technical failure, no or minimal dilatation were observed in the collecting system.

Complications

There was no procedure related death. In a 10-day-old neonate, methemoglobinemia due to local anesthesia and a subsequent cardiac arrest was observed. The patient survived after succesful resuscitation. Mortality rate within 30 days of PCN was 6.4%. Most of these deaths (91.9%) were as a result of underlying malignancy.

Table 3. Indications of PCN procedures and underlying pathologies in adults.

Indication	Malignity	Urinary stone disease	Congenital pathology	Other pathology*	RPF	Total
Obstruction without infection	213 (60)	96 (27)	15 (4.3)	22 (6.2)	9 (2.5)	355 (78.5)
Obstruction with infection	19 (35.2)	27 (50)	0	8 (14.8)	0)	54 (11.9)
Urinary diversion	7 (19.5)	3 (8.3)	0	26 (72.2)	(0)	36(8)
Diagnosting testing	3 (42.9)	3 (42.9)	0	1 (14.2)	(0)	7 (1.6)
Total	242 (53.6)	129 (28.5)	15 (3.3)	57 (12.6)	9(2)	452 (100)

PCN; percutaneous nephrostomy, RPF; retroperitoneal fibrosis, numbers in parentheses are raw data, *; includes urinary, gynecologic and other surgery, trauma, radiotherapy.

Complications such as neighboring organ injury, hydro or pneumothorax were not observed in any patient. Major hemorrhage was not observed in children. Major hemorrhage was occurred in 1.55% of adult patients (in 7 of 452 patients). In these patients, stabilization was achieved without any treatment except blood transfusion.

The overall rate of minor hemorrhage was 6.1%. Perirenal hematoma developed in 1.7% of all procedures and all of them resolved spontaneously without any treatment. There was no sepsis in children. In adult patient group, sepsis rate was 2.65%. Of these patients the most common indication was urinary obstruction with urinary infection (91.7%).

The catheter dislodgement rate was 10.6% in children and 11.7% in adults. 52.9% of these children and 41.3% of these adult patients had bilateral PCN catheters. The rate of catheter dislodgement was 10.3% for those with locking strings and 16.4% for those without locking strings.

The major and minor complications are summarized in tables 4 and 5, respectively.

Table 4. Major complications of PCN.

Complication	Children	Adult	Total
Failed PCN+	0	5/643 (0.78)	5/803 (0.6)
Methemoglobinemi due tolocal anesthesia*	1/126 (0.79)	0	1/578 (0.17)
Hemorrhage requiring blood transfusion*	0	7/452 (1.55)	7/578 (1.2)
Sepsis*	0	12/452 (2.65)	12/578 (2.1)
Urinoma requiring percutaneous intervention+	1/160 (0.63)	1/643 (0.16)	2/803 (0.25)
Perirenal abscess+	0	4/643 (0.62)	4/803 (0.49)
PCN catheter dislodgement+	17/160 (10.6)	75/643 (11.7)	92/803 (11.4)

n (%); numbers of patients and percentage, numbers are raw data, PCN; percutaneous nephrostomy, +; represents the number of number of PCN procedures and *; represents the number of patients.

Table 5. Minor complications of PCN.

Complication	Children	Adult	Total
Hemorrhage not requiring blood transfusion*	4/126 (3.2)	31/452 (6.9)	35/578 (6.1)
Urinoma not requiring percutaneous intervention+	1/160 (0.63)	0	1/803 (0.13)
Urine extravasation not requiring percutaneous intervention+	2/160 (1.25)	8/643(1.24)	10/803 (1.25)

n (%); numbers of patients and percentage, numbers are raw data, PCN; percutaneous nephrostomy, *; Represents the number of patients, +; represents the number of PCN procedures.

Outcomes of the procedure

In 95.1% of patients who underwent PCN due to azotemia, creatinine levels decreased after the procedure and in 42.5% of these patients, the creatinine levels decreased even to normal levels.

Among adult patients who underwent PCN for urinary diversion, successfull treatment was achieved with PCN without further intervention in 19.4% and by with ureteral stenting in 11.1% of patients. 50% children (2 of the 4 children) who underwent PCN for urinary diversion were succesfully treated with percutaneous catheterization alone. Leaks or fistulas were caused by benign causes in 85% of the patients treated with percutaneous treatment and conservative approach. In two patients, there was no urinary leak or fistula on antegrade pyelography. PCN was used for permanent urinary diversion in 13.9% of the patients.

PCN was performed in 12 patients for renal function evaluation. After catheterization, renal function was considered to be sufficient in 25% of these patients.

PCN catheters were placed in transplanted kidneys in 10 patients. Of these patients all except one were adults. The most common indication for PCN procedures performed in this group was urinary obstruction without urinary infection (60%). Of these 6 patients, 83.3% had obstruction in the UVJ and 16.7% had obstruction in the UPJ. 33.3% of these patients were treated succesfully with surgery and 66.7% with PCN, percutaneous ureteral stent placement and-or balloon dilatation without the need for surgery. In the remaining 4 patients the indications were urinary leakage from UVJ and urinary obstruction with infection (30% and 10%, respectively). In one of the 3 patients treated for urinary leakage, no leakage was found after antegrade pyelography. The other 2 patients underwent surgical treatment after catheterization.

The mean duration of PCN drainage was 34.9 days (1 to 480 days) in adults and 28.4 days (2 to 365 days) in children. The mean duration of PCN drainage was longest in patients who were treated for urinary leakage or fistula (46.4 days) and the mean duration of PCN drainage was longer in the procedures performed due to malignant underlying pathology (45.2 days for malignancies, 22 days for urinary tract stones and 21.8 days for retroperitoneal fibrosis). In 14.4% of adult patients, catheters were used for permanent drainage and the most common underlying pathology in these patients was malignancy (83.7%). In 1.6% of children, the catheters were used for long-term drainage and these patients had UPJ obstruction.

In 7.2% patients, PCNs were successful in managing patients without further intervention. 36.5% of patients had surgery and 14.7% had ureteral stenting as definitive treatment. 8.3% of patients underwent nephrectomy. In 4.6% of patients, the catheters were withdrawn as the kidney was nonfunctional. In 11.6% patients, catheters were used for long term drainage and discharged from the hospital with their catheters. 10.5% of patients died. The remaining patients were out of follow-up.

Discussion

The technical success rates of PCN under imaging guidance were reported to reach 98-100% in many reports [1-5]. In our series, technical success rate was 99.4%. Although high success rates have been also reported under sole fluoroscopy guidance, the success rates of combined guidance is higher. The use of US guidance for puncture, reduces the number of puncture attempts. But in some cases it may be compulsory to perform the next steps under fluoroscopy guidance due to the difficulty of monitoring the guidewire and catheter with US [5-7]. In addition the use of US during puncture decreases the risk of complications such as adjacent organ injury and hydro or pneumothorax [7]. In our study, adjacent organ injury and hydro or pneumothorax were not observed.

Dilatation of renal collecting system is an another factor which is thought to be effective on technical success [4, 5]. In recent studies it was reported that in almost all procedures where technical failure was observed, there was no collecting system dilatation and the use of US during puncture increases the success even in the existence of non or minimally dilated collecting systems [2, 5, 7]. In our study, there was no or minimal dilatation in 80% of the cases with technical failure.

The relief of urinary obstruction without or with infection represents the most common indication for PCN, representing 85% to 90% of patients in several large series [1-5]. In our study, this ratio was 91 % in all patients.

The two most common causes of renal obstruction without infection in adults are malignancies and urinary tract stones. In large series, these rates vary between 38.2% to 61% and 26% to 40.6%, respectively [1, 8, 9]. In our study, the rates were similar with the literature and were calculated as 60% and 27%, respectively.

In our series, the most common underlying pathology in adult patients who underwent PCN for obstruction with infection was urinary tract stones (50%). This result is also consistent with the literature [3].

Congenital abnormalities are the most common underlying pathology in children. In the literature [10, 11], common underlying pathologies in patients undergoing PCN for the relief of obstruction with or without infection are UPJ obstruction (49.5%), UVJ obstruction (21%) and urinary tract stones (13.6%) and these are similar with our study (38.5%, 29.1% and 11.1%, respectively).

PCN is often the simplest method for the initial management of obstructive renal failure and meets the need for safe, effective and urgent urinary drainage until the cause of obstruction is eliminated in these cases [1]. Complete recovery of the glomerular filtration rate can be expected with one week of complete obstruction but after 12 weeks, very little recovery may be seen. In addition, relief of obstruction may also contribute to the improvement of renal function [4]. In our series, creatinine levels were improved in almost all patients (95.1%) who underwent PCN due to azotemia. Beyond this, the creatinine levels decreased to normal levels in 42.5% of the these. However there is a dilemma in the therapeutic approach in the presence of malignant obstruction. But PCN or other percutaneous or endourological methods may at least protect the patient from the adverse effects of uremia and save time for selecting and performing the appropriate treatment [12, 13].

On the other hand an infected, obstructed kidney requires emergent drainage. Evacuation of pus and the release of intrarenal pressure with PCN, reduce the risk of aggravation of infection and improve renal perfusion and function [1-4]. Although PCN and retrograde stenting have been shown to have equivalent patient outcomes in some reports, PCN has recently

become the first-line therapy especially in seriously ill patients based on its reduced manipulation of the obstructed, infected ureter [4].

The most common nonobstructive indication for PCN in the literature is urinary diversion [14, 15]. The most common causes of urinary leakage or fistulas, are malignancies, surgical procedures, radiotherapy and trauma [16, 17]. In our study, the most common cause of urinary leakage-fistula was gynecological and urological surgeries (44.4%) in adults and trauma (50%) in children.

Conservative treatment or surgical reconstruction is performed in the presence of urinary fistula or leakage. PCN with or without antegrade ureteric stenting are used effectively both as a primary treatment and as an adjunctive method to surgery. They are also used as a palliative treatment for patients who are not suitable for surgery and may increase the quality of life of these patients [17, 18]. In our series, 32.5% of the patients whom PCN were performed for urinary diversion, were succesfully treated without the need of surgery.

It is thought that the renal function and how much of its function can recover may be evaluated by PCN. Other than diagnostic benefit, functional recovery in kidneys with poor function after PCN has been reported [4]. In our series, after an avarage of 9.8 days follow up, it was decided that 25% of the patients had sufficient renal function and the preprocedurel treatment decision (nephrectomy) was changed.

In our series the indications for PCN in transplanted kidneys were, obstruction without infection, urinary diversion for urinary leakage and obstruction with infection (60%, 30% and 10%, respectively). In the literature urinary leakage are most commonly seen from the ureteroneocystostomy site and obstructions are often observed in the distal ureter proximal to the ureteroneocystostomy [1, 19]. Similar results were observed in our study.

Traditionally surgical treatment is the preferred method in the presence of urinary leakage in transplantated kidneys. However, PCN saves time for patient preparation if surgery is indicated [19]. In addition, PCN can provide diagnostic benefits because in some cases antegrade pyelography is required for definitive diagnosis. In our series, in one of 3 patients who underwent PCN for urinary leakage, no leakage was detected after antegrade pyelography and the catheter was withdrawn. The other two patients underwent surgical treatment after catheterization.

PCN can also contribute to diagnosis and treatment in the presence of obstruction in transplanted kidneys. In the studies performed in transplant kidneys with obstruction, functional improvement was observed in almost all patients by percutaneous intervention in the early postoperative period. However, percutaneous intervention success was low in obstruction diagnosed late in postoperative period [20]. In our series, 33.3% of the patients who underwent PCN for this indication were treated with surgery and 66.7% of the patients were treated successfully with PCN, percutaneous ureteral stent placement and-or balloon dilatation without the need for surgery.

The mortality rates associated with PCN are as low as 0.046-0.7% and PCN can be performed safely even in patients with poor general condition [1]. In our series, there was no procedure related mortality.

Minor hemorrhages which are often due to small vessel or venous bleeding are common after PCN [1]. The rate of minor hemorrhage was 6.1% in our series. The rates of major hemorrhage which can take the form of hematuria or retroperitoneal hemorrhage, vary between 1-4% in different series [1-3]. This rate was 1.55% in adults in our series. There was no major hemorrhage in children.

Procedure-related major hemorrhages are usually caused by lacerations in major branches of renal artery and vein. The use of US guidance during puncture reduces the risk of vessel injury by providing an appropriate selection of the entry site. In addition, US guidance allows these hemorrhages to be noticed during the procedure most of the time [4]. Persistent gross hematuria more than 3 to 5 days after PCN or significant drop in hemoglobin levels may indicate arterial injury, and angiographic evaluation may be required in rare cases. Fortunately, these vascular injuries can be managed with endovacular approach without the need for surgery [1, 4]. In our series, in one patient angiography was required. But no pathology was found in angiography and hemorrhage was controlled by catheter upsizing.

Patel et al [21] reported the rate of major hemorrhage as relatively high (7%) in their series compared with others. In this study, PCN procedures were performed in kidneys that had no dilatation in their collecting systems and the authors claimed that the risk of major hemorrhage was higher in the procedures performed in such cases. However, in our series as some other studies, in most of the procedures in which hemorrhage occurred, there were advanced or moderate dilatation in the collecting system and no relationship was found between hemorrhage and collecting system dilatation [8].

There are different reports that coagulation disorders being a risk factor for PCN-related hemorrhage. However, many authors suggest to correct coagulation parameters before the procedure [8, 13]. In our series, 71.5% of patients (5 of 7 patients) with major hemorrhage had coagulopathy. With these findings, we also think that coagulopathies should be corrected before the procedure.

Another important complication of PCN is sepsis and in different series it was reported to be 1-9% [1-3, 8]. In our series sepsis was not observed in children and 2.65% of adult patients developed sepsis despite all were given antibiotic prophylaxis. However in some reports, the prevalence of septic complications were reported to be 25% or higher [22, 23]. This discrepancy may be due a number of factors including differences in the definition of sepsis which are accepted as complications, the differences in the use of prophylactic antibiotics and differences in underlying pathologies.

The use of prophylactic antibiotics is not universally accepted in PCN. Some feel that prophylactic antibiotics is not indicated for routine PCN [21, 24] whereas others give prophylactic antibiotics to all patients undergoing PCN [25]. Some authors defined a group at high risk for development of sepsis including but not limited to patients with positive urine culture or urinalysis, urinary tract stones (especially struvite calculi), a urinary ostomy and those who are immunosuppressed. Their findings showed that administration of antibiotics decreased the frequency of sepsis in the high-risk group and had a beneficial effect in the low-risk group as well [8, 23, 24].

The other factor for this discrepancy may be the underlying pathologies. In the study of Cohran et al [23] in which there was a higher rate of sepsis, 75% of patients who developed sepsis had urinary tract stones. In this study, it was also found that, the probability of a patient with a struvite stone developing signs of sepsis was significantly increased compared with patients with other types of stones. They recommended to use appropriate antibiotics, on the basis of urine test results before PCN and continue for 48-72 hours after the procedure in high-risk group and for 24-48 hours in low-risk group [23, 24].

Urinoma related to PCN procedure is a very rare complication and was reported to be 0.2-0.5% [5]. Small urinomas usually resolve spontaneously but if urinoma is large or infected, percutaneous drainage is required. In our series urinoma

requiring percutaneous intervention encountered in one child and one adult patient after the procedure.

Amongst the complications, catheter dislodgement is the commonest problem. We had an overall catheter dislodgement complication rate of 11.4% which was comparable with other studies [1, 9, 26]. Catheter dislodgements are more common with those without self-retaining mechanisms but dislodgements can be seen in any type of catheter. In our series all PCN catheters had self-retaining mechanisms but the rate of catheter dislodgement rate for those without locking strings was higher than those with locking strings (16.4% and 10.3%, respectively). Fixation of the catheter at the skin is also used in order to keep the catheter in place. However, none of these can completely prevent catheter dislodgements. The common opinion is that the most important way to prevent catheter dislodgements is to inform patients and their relatives about catheter care [27].

This was a retrospective study and has limitations. Because the patients were sent back to the referring clinics, a number of complications, especially minor ones, might not have been reported to us.

In conclusion, our technical success and complications rates were within the accepted target ranges proposed by the Society of Interventional Radiology Standards of Practice Committee. Combined US and fluoroscopy guided PCN, can be used effectively, and safely in a wide variety of indications with high technical success and low complications rates.

References

1. Dagli M, Ramchandani P. Percutaneous nephrostomy: technical aspects and indications. Semin Interv Radiol. 2011;28:424–37.
2. Pabon-Ramos WM, Dariushnia SR, Walker TG, d'Othée BJ, Ganguli S, Midia M, et al. Quality improvement guidelines for percutaneous nephrostomy. J Vasc Interv Radiol. 2016;27:410–4.
3. Ramchandani P, Cardella JF, Grassi CJ, Roberts A.C, Sacks D, Schwartzberg M.S, et al. Quality improvement guidelines for percutaneous nephrostomy. J Vasc Interv Radiol. 2003;14:277–81.
4. Young M, Leslie SW. Percutaneous Nephrostomy. [Updated 2019 Jun 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493205/
5. Efesoy O, Saylam B, Bozlu M, Çayan S, Akbay E. The results of ultrasound-guided percutaneous nephrostomy tube placement for obstructive uropathy: A single-centre 10-year experience. Turk J Urol 2018; 44: 329-34.
6. Lodh B, Gupta S, Singh AK, Sinam RS. Ultrasound guided direct percutaneous nephrostomy (pcn) tube placement: stepwise report of a new technique with its safety and efficacy evaluation. J Clin Diagn Res 2014;8:84-7.
7. Montvilas P, Solvig J, Johansen TE. Single-centre review of radiologically guided percutaneous nephrostomy using "mixed" technique: success and complication rates. Eur J Radiol. 2011;80:553–8.
8. Farrell TA, Hicks ME. A review of radiologically guided percutaneous nephrostomies in 303 patients. J Vasc Interv Radiol. 1997;8:769–74.
9. Radecka E, Magnusson A. Complications associated with percutaneous nephrostomies. A retrospective study. Acta Radiol. 2004;45:184–8.
10. Gupta DK, Chandrasekharam VV, Srinivas M, Bajpai M. Percutaneous nephrostomy in children with ureteropelvic junction obstruction and poor renal function. Urology. 2001;57:547–50.
11. Hogan M, Coley BD, Jayanthi VR, Shiels WE. Percutaneous Nephrostomy in Children and Adolescents: Outpatient Management. Radiology 2001;218:207–10.
12. Chitale SV, Scott-Barrett S, Ho ET, Burgess NA. The management of ureteric obstruction secondary to malignant pelvic disease. Clin Radiol. 2002;57:1118–21.
13. Meira MDS, Barbosa PNVP, Bitencourt AGV, Almeida MFA, Tyng CJ, Costa MAF et al. Retrospective analysis of computed tomography-guided percutaneous nephrostomies in cancer patients. Radiol Bras. 2019;52:148–54.
14. Millward SF. Percutaneous nephrostomy: a practical approach. J Vasc Interv Radiol 2000;11:955-64.
15. Patel U, Hussain FF. Percutaneous nephrostomy of nondilated renal collecting systems with fluoroscopic guidance: technique and results. Radiology. 2004;233:226-33.
16. Gayer G, Zissin R, Apter S, Garniek A, Ramon J, Kots E, et al. Urinomas caused by ureteral injuries: CT appearance. Abdom Imaging. 2002;27:88–92.
17. Avritscher R, Madoff DC, Ramirez PT, Wallace MJ, Ahrar K, Morello FA, et al. Fistulas of the lower urinary tract: percutaneous approaches for the management of a difficult clinical entity. Radiographics, 2004;24:S217–S36.
18. Titton RL, Gervais DA, Hahn PF, Harisinghani MG, Arellano RS, Mueller PR. Urine leaks and urinomas: diagnosis and imaging-guided intervention. Radiographics 2003;23:1133–47.
19. Hunter DW, Castaneda-Zuniga WR, Coleman CC, Herrera M, Amplatz K. Percutaneous techniques in the management of urological complications in renal transplant patients. Radiology. 1983;148:407-12.
20. Gregory MC, Micklos J, Miller FJ, et al. Percutaneous dilatation and stenting of ureteral stenosis in renal transplantation. Clin Trans. 1988;2:107-9c.
21. Patel U, Hussain FF. Percutaneous nephrostomy of nondilated renal collecting systems with fluoroscopic guidance: technique and results. Radiology. 2004;233:226-33.
22. Ferral H, Stackhouse DJ, Bjarnason H, Hunter DW, Castaneda-Zúñiga WR, et al. Complications of percutaneous nephrostomy tube placement. Semin Intervent Radiol 1994;11:198–206.
23. Cochran ST, Barbaric ZL, Lee JJ, Kashfian P. Nephrostomy tube placement: an outpatient procedure? Radiology 1991;179:843–7.
24. Moon E, Tam MD, Kikano RN, Karuppasamy K. Prophylactic antibiotic guidelines in modern interventional radiology practice. Semin Intervent Radiol. 2010;27:327–37.
25. Smith TP, Hunter DW, Letourneau JG, Cragg AH, Darcy MD, Castaneda-Zuniga WR, et al. Urine leaks after renal transplantation: Value of percutaneaous pyelography and drainage for diagnosis and treatment. AJR Am J Roentgenol. 1988;151:511–3.
26. Sim LS, Tan BS, Yip SK, Ng CK, Lo RH, Yeong KY, et al. Single centre review of radiologically-guided percutaneous nephrostomies: a report of 273 procedures. Ann Acad Med Singapore 2002;31:76-80.
27. Wah TM, Weston MJ, Irving HC. Percutaneous nephrostomy insertion: outcome data from a prospective multi-operator study at a UK training centre. Clin Radiol. 2004;59:255–61.

Arch Clin Exp Med 2019;4(3):142-147. e-ISSN: 2564-6567

DOI:10.25000/acem.623975 Araştırma makalesi / Research article

Effect of palmitate-induced steatosis on paraoxonase-1 and paraoxonase-3 enzymes in human-derived liver (HepG2) cells

İnsan kaynaklı karaciğer (HepG2) hücrelerinde palmitat ile oluşturulan yağlanmanın paraoksonaz-1 ve paraoksonaz-3 enzimlerine etkisi

Gülben Sayılan Özgün [1], Eray Özgün [1], Kıymet Tabakçıoğlu [2], Selma Süer Gökmen [1], Sevgi Eskiocak [1]

Abstract

Aim: Palmitate is one of the most abundant fatty acid in both liver of healthy individuals and in patients with non-alcoholic fatty liver disease. Palmitate-induced steatosis in HepG2 cells is an in vitro non-alcoholic fatty liver disease model to investigate acute harmful effects of fat overaccumulation in the liver. Non-alcoholic fatty liver disease is strongly associated with atherosclerosis. Paraoxonase-1 and paraoxonase-3 are anti-atherosclerotic enzymes which are bound to high density lipoprotein in circulation and they are primarily synthesized by liver. There is no study that investigated the effect of palmitate-induced steatosis on paraoxonase-1 and paraoxonase-3 enzymes. The aim of present study was to investigate the effect of palmitate-induced steatosis on paraoxonase-1 and paraoxonase-3 enzymes in HepG2 cells.

Methods: To induce steatosis, cells were incubated with 0.4, 0.7 and 1 mM palmitate for 24 hours. Cell viability was evaluated by 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. Cells were stained with oil red O and triglyceride levels were measured. Paraoxonase-1 and paraoxonase-3 protein levels were measured by western blotting, their mRNA expression were measured by quantitative PCR and arylesterase activity was measured spectrophotometrically.

Results: All palmitate concentrations caused a significant increase on paraoxonase-1 mRNA levels. Palmitate concentrations did not cause a significant change on paraoxonase-1 and paraoxonase-3 protein levels, paraoxonase-3 mRNA levels and arylesterase activities.

Conclusion: Our study showed that palmitate-induced steatosis up-regulates paraoxonase-1 mRNA, has no effect on paraoxonase-1 and paraoxonase-3 protein levels, paraoxonase-3 mRNA and arylesterase activity in HepG2 cells.

Keywords: Palmitate, paraoxonase-1, paraoxonase-3, arylesterase, HepG2, non-alcoholic fatty liver disease.

Öz

Amaç: Palmitat, hem sağlıklı bireylerin hem de non-alkolik karaciğer yağlanması hastalarının karaciğerinde en fazla bulunan yağ asitlerinden biridir. HepG2 hücrelerinde palmitat ile oluşturulan yağlanma, karaciğerdeki yağ birikiminin akut zararlı etkilerinin araştırılmasında kullanılan in vitro non-alkolik yağlı karaciğer hastalığı modelidir. Non-alkolik yağlı karaciğer hastalığı ateroskleroz ile yakından ilişkilidir. Paraoksonaz-1 ve paraoksonaz-3 dolaşımda yüksek dansiteli lipoproteine bağlı anti-aterosklerotik enzimlerdir ve esas olarak karaciğerde sentezlenirler. Palmitat ile oluşturulan yağlanmanın paraoksonaz-1 ve paraoksonaz-3 enzimleri üzerine etkisini araştıran bir çalışma bulunmamaktadır. Bu çalışmanın amacı HepG2 hücrelerinde palmitat ile oluşturulan yağlanmanın paraoksonaz-1 ve paraoksonaz-3 enzimlerine etkisini araştırmaktır.

Yöntemler: Yağlanma oluşturmak için hücreler 0.4, 0.7 ve 1 mM palmitat ile 24 saat inkübe edildi. Hücre canlılığı 3-(4,5-Dimetil-2-tiazolil)-2,5-difenil-2H-tetrazolium bromür testi ile değerlendirildi. Hücreler oil red O ile boyandı ve trigliserit düzeyleri ölçüldü. Paraoksonaz-1 ve paraoksonaz-3 protein düzeyleri western blot ile, mRNA'ları ise kantitatif PCR ile ve arilesteraz aktivitesi spektrofotometrik olarak ölçüldü.

Bulgular: Tüm palmitat konsantrasyonları paraoksonaz-1 mRNA düzeylerinde anlamlı bir artışa yol açtı. Palmitat konsantrasyonları paraoksonaz-1 ve paraoksonaz-3 protein düzeylerinde, paraoksonaz-3 mRNA düzeylerinde ve arilesteraz aktivitesinde anlamlı bir değişime yol açmadı.

Sonuç: Çalışmamız, HepG2 hücrelerinde palmitat ile oluşturulan yağlanmanın paraoksonaz-1 mRNA düzeyini arttırdığını, paraoksonaz-1 ve paraoksonaz-3 protein düzeylerine, paraoksonaz-3 mRNA düzeylerine ve arilesteraz aktivitesine etkisi olmadığını gösterdi.

Anahtar kelimeler: Palmitat, paraoksonaz-1, paraoksonaz-3, arilesteraz, HepG2, non-alkolik yağlı karaciğer hastalığı.

[1]Trakya University, School of Medicine, Department of Medical Biochemistry, Edirne, Turkey.
[2]Trakya University, School of Medicine, Department of Medical Biology, Edirne, Turkey.

GSÖ: 0000-0001-6990-3484
EÖ: 0000-0002-6744-1519
KT: 0000-0002-7345-0825
SSG: 0000-0001-5701-4962
SE: 0000-0002-0813-2345

Ethics Committee Approval: HepG2 cells were commercially available and due to the fact that this study was no human or animal study, no ethical approval has been taken.
Etik Kurul Onayı: HepG2 hücreleri ticari olarak temin edildi ve bu çalışma insan veya hayvan çalışması olmadığı için etik kurul onayı alınmamıştır.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Geliş Tarihi / Received: 24.10.2019
Kabul Tarihi / Accepted: 13.11.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:

Gülben Sayılan Özgün

Adres/Address: Department of Medical Biochemistry, Trakya University School of Medicine 22030 Edirne, Turkey.

e-posta: gulben_syln@hotmail.com

Tel/Phone: +90 546 4191946
Fax: +90 284 2357652

Introduction

Non-alcoholic fatty liver disease (NAFLD) can be defined as the presence of hepatic steatosis without significant alcohol consumption and it's the major cause of liver disease with high global prevalence and incidence [1, 2]. NAFLD is associated with metabolic syndrome and there is an increased triglyceride accumulation in hepatocytes of patients with NAFLD [3]. Although NAFLD is a primary liver-related disease, it also affects extra-hepatic organs and regulatory pathways. NAFLD increases risk of diabetes mellitus, cardiovascular and kidney diseases [4]. NAFLD also plays an independent role in the atherogenic dyslipidemia and is strongly associated with atherosclerosis [3, 5].

Palmitate is a 16-carbon saturated fatty acid. It is one of the most abundant fatty acid in both liver of healthy individuals and in patients with NAFLD [6]. HepG2 cells are commercially available human-derived hepatoma cells and retain many biological characteristics of hepatocytes. These cells are useful tools in the understanding of hepatic protein biosynthesis [7]. Palmitate-induced steatosis in HepG2 cells is an experimental NAFLD model to investigate acute harmful effects of fat overaccumulation in liver [8].

Paraoxonase (PON)1 and PON3 are the members of PON enzyme family and they are primarily synthesized by liver [9]. In circulation, PON1 and PON3 are bound to high density lipoprotein (HDL) which is the anti-atherosclerotic lipoprotein. HDL mediates reverse cholesterol transport and have antioxidant and anti-inflammatory properties [10]. PON1 and PON3, antioxidant enzymes, prevent oxidation of HDL and low density lipoprotein and therefore prevent atherosclerosis [11]. Although most known PON enzyme activities are paraoxonase and arylesterase, PON1 has mainly paraoxonase and arylesterase activities but all PON enzymes are primarily lactonases [9].

Although there are some reports that investigated PON1 and PON3 mRNA expression [12, 13], PON1 protein levels [14] and activity [15] in patients with NAFLD or experimental NAFLD models, the effect of NAFLD on PON1 and PON3 enzymes have not been fully understood.

There are no studies that investigated the effect of palmitate-induced steatosis on PON1 and PON3 enzymes. Also, we could not encounter any studies which investigate the effect of NAFLD on PON3 protein levels. In the present study, we aimed to investigate the effect of palmitate-induced steatosis on PON1 and PON3 enzymes in HepG2 cells. For this purpose, HepG2 cells were incubated with 0.4, 0.7 and 1 mM palmitate for 24 hours to induce steatosis. PON1 and PON3 protein levels were measured by western blotting, their mRNA expression were measured by quantitative PCR and arylesterase activity was measured spectrophotometrically. With present study, effect of palmitate on PON1 and PON3 enzymes are investigated for the first time and we found that palmitate-induced steatosis up-regulates PON1 mRNA, has no effect on PON1 and PON3 protein levels, paraoxonase-3 mRNA and arylesterase activity in HepG2 cells.

Material and methods

Chemicals

Human HepG2 cells were purchased from ATCC (Middlesex, UK). Sodium palmitate, 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), oil red O and phenylacetate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Minimum Essential Medium, fetal bovine serum (FBS), antibiotic-antimycotic, trypsin-EDTA, RNA isolation kit, High-Capacity cDNA reverse transcription kit, TaqMan probes for PON1, PON3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and horseradish peroxidase (HRP) chemiluminescent substrate were purchased from Thermo Fisher (Waltham, MA USA). PON1, PON3, alpha tubulin primary antibodies and goat anti-mouse IgG H&L HRP secondary antibody were purchased from Abcam (Cambridge, UK). Fatty acid free bovine serum albumin, radioimmunoprecipitation assay (RIPA) lysis buffer system was purchased from Santa Cruz (Heidelberg, Germany). Polyvinylidene fluoride (PVDF) membrane was purchased from Bio-Rad (Hercules, CA, USA). Other chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) or Merck (Darmstadt, Germany). All reagents were of analytical grade.

Cell culture and experimental design

HepG2 cells were cultured in minimum essential medium with glutamine containing 10% FBS, 1% sodium pyruvate and 1% antibiotic-antimycotic (100 units/mL penicillin and 100 µg/mL streptomycin and 25 µg/mL of Gibco Amphotericin B) in a humidified environment at 37 °C and 5% CO2 atmosphere.

Sodium palmitate was dissolved in sterile 0.9 % NaCl at 70 °C and complexed with 0.7 mM fatty acid free albumin which dissolved in medium without phenol red at 37 °C. For all experimental groups, total albumin concentration in medium was 0.7 mM which was similar to human serum albumin concentrations [16]. Cells were cultured with medium containing 0 (control), 0.4, 0.7 and 1 mM palmitate for 24 hours. Palmitate concentrations were choosen according to previous studies in literature [8, 16].

Cell viability assays

Effect of palmitate on cell viability was evaluated by MTT assay [17]. 104 cells were seeded into the 96 well plates. Cells were cultured with medium containing different palmitate (0, 0.4, 0.7 and 1 mM) concentrations for 24 hours. At the end of treatment, mediums were removed and 10 µl of MTT (5 mg/mL) solved in phosphate-buffered saline (PBS) and 100 µl of medium without phenol red were added to each well. Cells were then incubated for 4 hours in a humidified environment at 37 °C and 5% CO_2 atmosphere. MTT-containing medium was then removed and formazan crystals were then dissolved by adding 200 µL dimethyl sulfoxide and 25 µL Sorensen buffer (0.1 M glycine, 0.1 M sodium chloride equilibrated to pH 10.5 with 0.1 M NaOH). Optical density of plates were measured using a microplate reader at 570/630 nm [18]. Optical density of each sample was then compared with the mean optical density value of control group optical density.

Oil red O staining

Cells were seeded into 6-well plates. After the cells reached 80-90% confluence, cells were cultured with medium containing different palmitate (0, 0.4, 0.7 and 1 mM) concentrations for 24 hours. After 24 hours, cells were washed with PBS and fixed with 10% paraformaldehyde for 1 hour. Then cells were washed with 60% isopropanol and incubated with oil red O in 60% isopropanol for 30 minutes. 350 mg oil red O dissolved in 100% isopropanol as 100 mL of stock solution. 60% isopropanol containing oil red O solution was prepared freshly and filtered before using. After incubation with oil red O, cells were washed with distilled water and photos were taken with inverted microscope [19].

Intracellular triglyceride assay

Cells were seeded into 25 cm^2 flask. After the cells reached 80-90% confluence, cells were cultured with medium containing different palmitate (0, 0.4, 0.7 and 1 mM) concentrations for 24 hours. After 24 hours, cells were washed twice with PBS and scrapped with RIPA lysis buffer system. Intracellular triglyceride levels were analyzed by AU 5800 clinical chemistry analyzer (Beckman Coulter lnc, Brea, CA, USA) using its original enzymatic kit (Beckman Coulter lnc, Brea, CA, USA). Protein concentrations were measured according to Lowry et al. [20] by using bovine serum albumin as standard. Triglyceride levels were calculated relative to total protein levels and expressed as fold change relative to control by dividing to mean triglyceride value of control group.

PON1 and PON3 mRNA expression

Cells were seeded into 6 well plates. After the cells reached 80-90% confluence, cells were cultured with medium containing different palmitate (0, 0.4, 0.7 and 1 mM) concentrations for 24 hours. RNA was isolated from cells by using commercial RNA isolation kit and cDNA was generated from 1 μg of total RNA by using commercial high-capacity cDNA reverse transcription kit. These reaction products were subject to quantitative PCR by using TaqMan gene expression assays for PON1 and PON3. GAPDH was used as housekeeping gene. Results were calculated using the $2^{-\Delta\Delta CT}$ method [21].

Western blot analysis of PON1 and PON3 proteins

Cells were seeded into 75 cm^2 flask. After the cells reached 80-90% confluence, cells were cultured with medium containing different palmitate (0, 0.4, 0.7 and 1 mM) concentrations for 24 hours. After 24 hours, cells were washed twice with PBS and scrapped with RIPA lysis buffer system. Samples were homogenized and then centrifuged at 4 °C for 10 minutes at 15,000 × g [22]. Supernatants were used for protein determination and western blotting. Protein concentrations were measured according to Lowry et al. [20] by using bovine serum albumin as standard.

20 μg of total protein was separated by 5-8% sodium dodecyl sulfate-polyacyrlamide gel electrophoresis and transferred to PVDF membrane by using semi-dry blotting system. Membranes were blocked with 5% skim milk powder for 1 hour at room temperature. After blocking, membranes were incubated with monoclonal primary antibodies (PON1:1/1000 dilution and PON3:1/1000 dilution) overnight at 4°C and then with secondary antibody (HRP goat anti-mouse: 1/10000 dilution) at room temperature for one hour. PON1 and PON3 protein bands were visualised by using electrochemiluminescence detection system with HRP chemiluminescent substrate and were quantified by Image J [23]. Results were calculated relative to alpha-tubulin as loading control and expressed as fold change relative to control for each blot.

Arylesterase activity

Cells were seeded into 75 cm^2 flask. After the cells reached 80-90% confluence, cells were cultured with medium containing different palmitate (0, 0.4, 0.7 and 1 mM) concentrations for 24 hours. After 24 hours, cells were washed twice with PBS and scrapped with 50 mM Tris-HCl buffer (pH:8) containing 1mM CaCl2, 1% protease inhibitor cocktail and 0.1% Triton-X100. Arylesterase activity was determined by the measuring initial rate of substrate hydrolysis at 270 nm in the assay mixture containing 1 mM $CaCl_2$, 2 mM phenylacetate in 50 mM Tris-HCl buffer (pH 8.0) [24, 25]. Measurements were performed at 25 °C and the blank sample containing incubation mixture without cell lysate was run simultaneously for the correction of spontaneous substrate breakdown. Results were calculated relative to total protein levels and expressed as fold change relative to control.

Statistical analysis

Results were given as means ± standard deviation (SD). The one-way analysis of variance test was used for comparison of biochemical parameters among the groups, and then, Tukey and Tamhane post-hoc tests were used for multiple comparisons when the significant difference obtained. SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA) statistical software was used for statistical analysis. P value < 0.05 was considered as statistical significant.

Results

The mean percentages of cell viabilities of palmitate-incubated cells were 85% for 0.4 mM palmitate, 76% for 0.7 mM palmitate and 63% for 1 mM palmitate. All palmitate concentrations (0.4, 0.7, 1 mM) caused a significant decrease on cell viabilities as compared with control (P<0.05 for all). Increasing palmitate concentrations (0.4, 0.7, 1 mM) caused a significant decrease on cell viabilities (P<0.05 for all) (Figure 1).

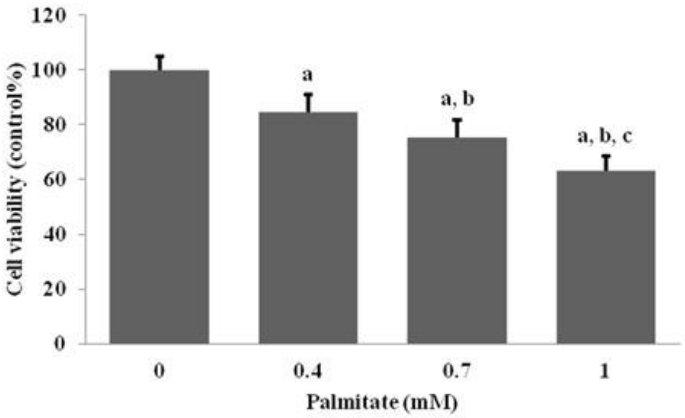

Figure 1: Effect of palmitate on cell viabilities of HepG2 cells. Results are expressed as mean ± SD of twenty four results for all groups, a: p<0.05, compared with control, b: p<0.05, compared with 0.4 mM palmitate-incubated cells, c: p<0.05, compared with 0.7 mM palmitate-incubated cells.

Palmitate-incubated HepG2 cells were stained with oil red O to show intracellular lipid content. Increasing palmitate concentrations (0.4, 0.7, 1 mM) caused an increase on intracellular lipid content of HepG2 cells which were visually observed microscopically by oil red O staining (Figure 2a).

Intracellular triglyceride levels of palmitate-incubated cells were 1.12-fold, 1.43-fold and 1.77-fold for 0.4, 0.7 and 1 mM palmitate, respectively. All palmitate concentrations (0.4, 0.7, 1 mM) caused a significant increase on intracellular triglyceride levels as compared with control (P<0.05 for all). Also increasing palmitate concentrations (0.4, 0.7, 1 mM) caused a significant increase on intracellular triglyceride levels (P<0.05 for all) (Figure 2b).

PON1 mRNA expression of palmitate-incubated cells were 2.09-fold, 1.82-fold and 1.79-fold for 0.4, 0.7 and 1 mM palmitate, respectively. PON1 mRNA expression in 0.4, 0.7 and 1 mM palmitate incubated cells were significantly increased as compared to those in control cells (P<0.05 for all) (Figure 3a).

PON1 protein levels of palmitate-incubated cells were 1.11-fold, 1.07-fold and 0.97-fold for 0.4, 0.7 and 1 mM palmitate, respectively. There was no significant difference

between PON1 protein levels of groups (p>0.05 for all) (Figure 3b).

PON3 mRNA expression of palmitate incubated cells were 0.88-fold, 0.95-fold and 0.98-fold for 0.4, 0.7 and 1 mM palmitate, respectively. There was no significant difference between PON3 mRNA expression of groups (p>0.05 for all) (Figure 3a).

PON3 protein levels of palmitate-incubated cells were 1.21-fold, 1.26-fold and 1.20-fold for 0.4, 0.7 and 1 mM palmitate, respectively. There was no significant difference between PON3 protein levels of groups (p>0.05 for all) (Figure 3b).

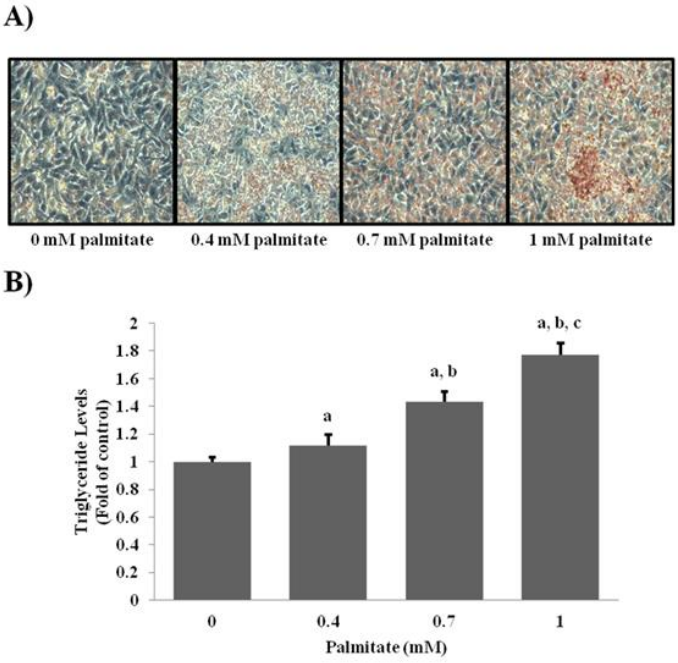

Figure 2: Effect of palmitate on steatosis in HepG2 cells. a) Microscopic images of oil red O stained (x400 magnification) and b) intracellular triglyceride levels of HepG2 cells. Data are expressed as the mean ± SD of six results from three independent experiments for all groups, a: p<0.05, compared with control, b: p<0.05, compared with 0.4 mM palmitate-incubated cells, c: p<0.05, compared with 0.7 mM palmitate-incubated cells.

Arylesterase activities of palmitate incubated cells were 1.04-fold, 1.03-fold and 1.04-fold for 0.4, 0.7 and 1 mM palmitate, respectively. There was no significant difference between arylesterase activities of groups (p>0.05 for all) (Figure 4).

Discussion

Palmitate-induced steatosis in HepG2 cells is an in vitro NAFLD model to investigate acute harmful effects of fat overaccumulation in the liver [8]. In our study, increasing palmitate concentrations significantly increased steatosis and decreased cell viability in HepG2 cells. Our results confirmed steatosis and cytotoxicity caused by palmitate and were compatible with the literature [26, 27].

For the first time, the effect of palmitate-induced steatosis on PON1 and PON3 enzymes in HepG2 cells and the effect of NAFLD on PON3 protein levels were investigated with the present study. We showed that palmitate at different concentrations (0.4, 0.7 and 1 mM) increases PON1 mRNA expression, but it does not change PON1 protein level and arylesterase activity in HepG2 cells.

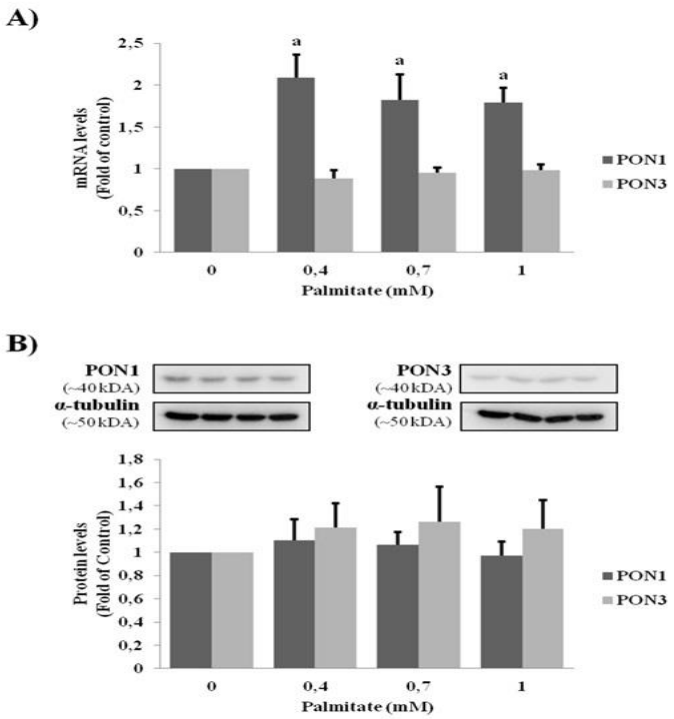

Figure 3: Effect of palmitate on PON1 and PON3. a) mRNA and b) protein levels in HepG2 cells. Data are expressed as the mean ± SD of five results from five independent experiments for protein levels and nine results for mRNA levels from three independent experiments for all groups, a: p<0.05, compared with control.

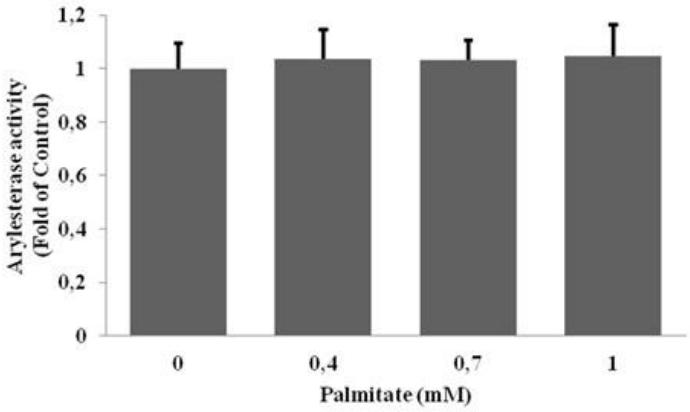

Figure 4: Effect of palmitate on arylesterase activity in HepG2 cells. Data are expressed as the mean ± SD of twelve results from three independent experiments for all groups.

Our result indicating that PON1 mRNA expression increases in palmitate-induced steatosis supports Desai et al. [14] who reported that liver PON1 mRNA expression increases in paediatric non-alcoholic steatohepatitis. It is known that PON1 prevents oxidative stress and fights with inflammation [11]. It was reported that oxidative stress [28, 29] and inflammation [30, 31] are increased in palmitate-induced steatosis in HepG2 cells. An increase in PON1 mRNA expression in palmitate-induced steatosis in HepG2 cells may be associated with cell defense against to oxidative stress and/or inflammation.

PON enzymes have different activities for different substrates. PON1 has mainly arylesterase activity whereas PON3 has very low arylesterase activity [9]. In the present study, although palmitate-induced steatosis increased PON1 mRNA expression in HepG2 cells, it did not change PON1 protein levels and arylesterase activities. Our arylesterase activity results are compatible with Kudchodkar et al. [32] who reported dietary tripalmitin treatment does not alter PON1 activity in rats and also

compatible with Bosham et al. [33] who reported palmitic acid composition of HDL is not associated with the PON1 activity. Whereas Wang et al. [15] reported that oleic acid-induced NAFLD decreases PON activity in L02 cells. On the other hand, Gomez-Lechon et al. [8] reported that, oleic acid-induced steatosis represents chronic cell model of NAFLD whereas palmitate-induced steatosis represents acute harmful effects of fat overaccumulation. This may be responsible for the difference between PON activities of these two studies. Desai et al. [14] also reported that liver PON1 protein levels are increased in paediatric non-alcoholic steatohepatitis. Whereas we found that palmitate-induced steatosis does not change PON1 protein levels. Unchanged PON1 protein levels in palmitate-induced steatosis could have resulted from the incubation of cells with palmitate for a limited time. It is also known that palmitate induces endoplasmic reticulum stress and inhibits protein synthesis [34]. This may also be responsible from unchanged PON1 proteins and arylesterase activities in our study.

PON3 is not as well studied protein such as PON1. In the present study, for the first time, we showed that PON3 mRNA expression and protein levels are not changed in palmitate-induced steatosis. PON3 mRNA results of our study were compatible with the previous NAFLD animal studies [12,13]. Our finding indicating that PON3 mRNA and protein levels do not change in palmitate-induced steatosis in HepG2 cells points out that PON3 enzyme is not affected by increased oxidative stress and inflammation [28-31]. The finding of Reddy et al. [35] who reported that, different from PON1, PON3 is not regulated by oxidized lipids supports this idea.

Although PON1 and PON3 are the members of the same enzyme family and they have many similarities, we found that lipoic acid up-regulates PON3 but down-regulates PON1 mRNA expression, and caffeine increases PON1 protein levels but does not change PON3 protein levels in HepG2 cells in our previous studies [24, 36]. Taking into consideration the findings we have mentioned above, we can say that PON1 and PON3 are regulated by different mechanisms.

Limitation of present study is not to able to show any PON3 enzyme activity because PON3 lactonase activities are below or very close to detection limits in cell lysates with assay methods using dihydrocoumarin and decanolactone [37]. Also this is an in vitro study and it needs to be supported by future animal and human studies.

In conclusion, we showed that palmitate-induced steatosis up-regulates PON1 mRNA expression but it does not change PON1 and PON3 protein levels, PON3 mRNA expression and arylesterase activities in HepG2 cells.

References

1. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005-23.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer, M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73-84.
3. DeFilippis AP, Blaha MJ, Martin SS, Reed RM, Jones SR, Nasir K, et al. Nonalcoholic fatty liver disease and serum lipoproteins: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 2013;227:429-36.
4. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol. 2015; 62: 47-64.
5. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. J Hepatol. 2008;49:600-07.
6. Araya J, Rodrigo R, Videla LA, Thielemann L, Orellana M, Pettinelli, P et al. Increase in long-chain polyunsaturated fatty acid n - 6/n - 3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. Clin Sci (Lond). 2004;106:635-43.
7. Bouma ME, Rogier E, Verthier N, Labarre C, Feldmann G. Further cellular investigation of the human hepatoblastoma-derived cell line HepG2: morphology and immunocytochemical studies of hepatic-secreted proteins. In Vitro Cell Dev Biol. 1989;25:267-75.
8. Gómez-Lechón MJ, Donato MT, Martínez-Romero A, Jiménez N, Castell JV, O'Connor JE. A human hepatocellular in vitro model to investigate steatosis. Chem Biol Interact. 2007;165:106-16.
9. She ZG, Chen HZ, Yan Y, Li H, Liu DP. The human paraoxonase gene cluster as a target in the treatment of atherosclerosis. Antioxid Redox Signal. 2012;16:597-632.
10. Podrez EA. Anti-oxidant properties of high-density lipoprotein and atherosclerosis. Clin Exp Pharmacol Physiol. 2010;37:719-25.
11. Précourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E, et al. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis. 2011;214:20-36.
12. Hussein O, Zidan J, Abu Jabal K, Shams I, Szvalb S, Grozovski M, et al. Paraoxonase activity and expression is modulated by therapeutics in experimental rat nonalcoholic Fatty liver disease. Int J Hepatol. 2012;2012:265305.
13. Pereira RR, de Abreu IC, Guerra, JF, Lage NN, Lopes JM, Silva M, et al. Açai (Euterpe oleracea Mart.) Upregulates Paraoxonase 1 Gene Expression and Activity with Concomitant Reduction of Hepatic Steatosis in High-Fat Diet-Fed Rats. Oxid Med Cell Longev. 2016;2016:8379105.
14. Desai S, Bake SS, Liu W, Moya DA, Browne RW, Mastrandrea L, et al. Paraoxonase 1 and oxidative stress in paediatric non-alcoholic steatohepatitis. Liver Int. 2014;34:110-17.
15. Wang B, Yang RN, Zhu YR, Xing JC, Lou XW, He YJ, et al. Involvement of xanthine oxidase and paraoxonase 1 in the process of oxidative stress in nonalcoholic fatty liver disease. Mol Med Rep. 2017;15:387-95.
16. Yang X, Chan C. Repression of PKR mediates palmitate-induced apoptosis in HepG2 cells through regulation of Bcl-2. Cell Res. 2009;19:469-86.
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55-63.
18. Ahmadian S, Barar J, Saei AA, Fakhree, MA Omidi Y. Cellular toxicity of nanogenomedicine in MCF-7 cell line: MTT assay. J Vis Exp. 2009;26:1191.
19. Jang E, Shin MH, Kim KS, Kim Y, Na YC, Woo HJ, et al. Anti-lipoapoptotic effect of Artemisia capillaris extract on free fatty acids-induced HepG2 cells. BMC Complement Altern Med. 2014;14:253.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.
21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8.
22. Beltowski J, Jamroz-Wiśniewska A, Borkowska E, Wójcicka G. Differential effect of antioxidant treatment on plasma and tissue paraoxonase activity in hyperleptinemic rats. Pharmacol Res. 2005;51:523-32.
23. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9:671-75.
24. Ozgun E, Sayilan Ozgun G, Tabakcioglu K, Suer Gokmen S, Sut N, Eskiocak S. Effect of lipoic acid on paraoxonase-1 and paraoxonase-3 protein levels, mRNA expression and arylesterase activity in liver hepatoma cells. Gen Physiol Biophys. 2017;36:465-70.
25. Gan KN, Smolen A, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos. 1991;19:100-6.
26. Wang GL, Fu YC, Xu WC, Feng YQ, Fang SR, Zhou XH. Resveratrol inhibits the expression of SREBP1 in cell model of steatosis via Sirt1-FOXO1 signaling pathway. Biochem Biophys Res Commun. 2009;380:644-49.
27. Gorgani-Firuzjaee S, Adeli K, Meshkani R. Inhibition of SH2-domain-containing inositol 5-phosphatase (SHIP2) ameliorates palmitate induced-apoptosis through regulating Akt/FOXO1 pathway and ROS production in HepG2 cells. Biochem Biophys Res Commun. 2015;464:441-46.
28. Liu JF, Ma Y, Wang Y, Du ZY, Shen JK, Peng HL. Reduction of lipid accumulation in HepG2 cells by luteolin is associated with activation of AMPK and mitigation of oxidative stress. Phytother Res. 2011;25:588-96.
29. Ma S, Yang D, Li D, Tan Y, Tang B, Yang Y. Inhibition of uncoupling protein 2 with genipin exacerbates palmitate-induced hepatic steatosis. Lipids Health Dis. 2012;11:154.

30. Choi YJ, Choi SE, Ha ES, Kang Y, Han SJ, Kim DJ, et al. Involvement of visfatin in palmitate-induced upregulation of inflammatory cytokines in hepatocytes. Metabolism. 2011;60:1781-89.
31. Joshi-Barve S, Barve SS, Amancherla K, Gobejishvili L, Hill D, Cave M, et al. Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. Hepatology. 2007;46:823-30.
32. Kudchodkar BJ, Lacko AG, Dory L, Fungwe TV. Dietary fat modulates serum paraoxonase 1 activity in rats. J Nutr. 2000;130:2427-33.
33. Boshtam M, Razavi AE, Pourfarzam M, Ani M, Naderi GA, Basati G, et al. Serum paraoxonase 1 activity is associated with fatty acid composition of high density lipoprotein. Dis Markers. 2013;35:273-80.
34. Perry BD, Rahnert JA, Xie Y, Zheng B, Woodworth-Hobbs ME, Price SR. Palmitate induced ER stress and inhibition of protein synthesis in cultured myotubes does not require Toll-like receptor 4. PLoS One. 2018;13:e0191313.
35. Reddy ST, Wadleigh DJ, Grijalva V, Ng C, Hama S, Gangopadhyay A. Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. Arterioscler Thromb Vasc Biol. 2001;21:542-47.
36. Sayılan Özgün G, Özgün E, Tabakçıoğlu K, Süer Gökmen S, Eskiocak S, Çakır E. Caffeine Increases Apolipoprotein A-1 and Paraoxonase-1 but not Paraoxonase-3 Protein Levels in Human-Derived Liver (HepG2) Cells. Balkan Med J. 2017;34:534-39.
37. Draganov DI, Stetson PL, Watson CE, Billecke SS, La Du BN. Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. J Biol Chem. 2000;275:33435-42.

Arch Clin Exp Med 2019;4(3):148-151. e-ISSN: 2564-6567

DOI: 10.25000/acem.589711 Olgu Sunumu / Case Report

Successful management of lower rectal carcinoma recurrence on perineal pseudo-continent colostomy: A case report and review of literature

Perineal psödo-kontinan kolostomi üzerinde alt rektal karsinom rekürrensinin başarılı tedavisi: Bir olgu sunumu ve literatürün gözden geçirilmesi

Malek Bouhani [1], Olfa Jaidane [1], Mohamed Amine Bouida [1], Radhi Bennaceur [2], Riadh Chargui [1], Khaled Rahal [1]

Abstract

Recurrence of lower rectal carcinoma on perineal pseudo-continent colostomy is rarely reported in the literature. It presents a real challenge for the physician. The aim of this case report was to document an exceptional recurrence and how to manage it.

A 56-year-old man presented with stage II adenocarcinoma of the lower rectum. He received concomitant chemoradiation followed by abdominoperineal resection with perineal pseudo-continent colostomy. Three years later, he developed a local recurrence of his prior adenocarcinoma, on the perineal pseudo-continent colostomy. He underwent wide excision, followed by reconstruction with a rectus abdominis myocutaneous flap with an inferior pedicle and an oblique skin paddle. He underwent adjuvant chemotherapy. The patient is free of disease with three years follow up.

Extended resection should be considered as an initial treatment for locally recurrent rectal cancer.

Keywords: rectal cancer, recurrence, colostomy, reconstructive surgical procedures.

Öz

Perineal psödo-kontinan kolostomi üzerinde alt rektal karsinomun rekürrensi literatürde nadiren bildirilmektedir ve hekimler için gerçek bir zor durum oluşturmaktadır. Bu çalışmada, rektum kanserinde nadir bir rekürrens ve yönetimi sunulmuştur.

56 yaşında bir erkek hasta alt rektum yerleşimli evre II adenokarsinom ile başvurdu. Eş zamanlı kemoradyoterapi ve ardından perineal psödo-kontinan kolostomi ile abdominoperineal rezeksiyon yapıldı. Üç yıl sonra, perineal psödo-kontinan kolostomi üzerinde önceki adenokarsinomunun lokal rekürrensi gelişti. Hastaya geniş eksizyon uygulandı, ardından rektus abdominis miyokutan flebi ile inferior pedikül ve oblik cilt adasıyla rekonstrüksiyon yapıldı. Hasta adjuvan kemoterapi aldı. Hasta 3 yıllık takip süresinde hastalıksız olarak izlenmektedir.

Kapsayıcı bir rezeksiyon, lokal rekürren rektum kanseri için ilk tedavi olarak düşünülmelidir.

Anahtar Sözcükler: rektal kanser, rekürrens, kolostomi, rekonstrüktif cerrahi işlemler.

[1] **Salah Azaiz Institute, Department of Oncologic Surgery, Tunis, Tunisia.**
[2] **Salah Azaiz Institute, Department of Plastic Surgery, Tunis, Tunisia.**

MB: 0000-0001-5913-911X
OJ: 0000-0002-8157-0544
MAB: 0000-0002-2491-307X
RB: 0000-0002-7841-5105
RC: 0000-0002-4518-4048
KR: 0000-0002-6093-4257

Informed Consent: The written consent was received from the patient who was presented in this study.
Hasta Onamı: Çalışmada sunulan hastadan yazılı onam alınmıştır.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

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

Finansal Destek: Yazarlar bu olgu için finansal destek almadıklarını beyan etmişlerdir.

Geliş Tarihi / Received: 10.07.2019
Kabul Tarihi / Accepted: 21.08.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:

Bouhani Malek
Adres/Address: Department of oncologic surgery, Salah Azaiz Institute, Boulevard 9th April 1009, Tunis, Tunisia.
e-mail: bouhani_malek@hotmail.fr
Tel/Phone: 0021623604048

Introduction

The management of ultra-low rectal cancer less than 2 cm from the dentate line is still challenging and one of the reasons is the lack of definition and standardization of surgery in low rectal cancer. Sphincter preservation is a major concern in cancer treatment. There is only two procedures: an intersphincteric resection with a coloanal anastomosis and abdominoperineal resection (APR) with a definitive iliac colostomy or perineal pseudo-continent colostomy (PPCC) [1]. Local recurrence of lower rectal cancer is difficult to treat, may cause severe and disabling symptoms, and usually has a fatal outcome.

We present a case of local recurrence of rectal adenocarcinoma on PPCC. This case presented a challenge for the oncologic surgeon, reconstructive surgeon, and oncologist. We reported how we did manage this rare entity and its oncologic outcomes.

Case report

We present the case of a 56-year-old male patient with adenocarcinoma of the lower rectum. The tumor was situated 1 cm from the anal ring and has been classified as T2N0M0 of the TNM classification.

He underwent concomitant radiochemotherapy. He received a capecitabine-based chemotherapy regimen at 1250 mg/m², 2 doses per day, and 2 weeks per 3. The radiotherapy regimen consisted of pelvic irradiation (45 Gy total dose) in 25 fractions over 5 weeks daily, in a dose of 1.8 Gy per session. The patient underwent APR with total mesorectum excision and a PPCC.

The histological exam showed a moderately differentiated adenocarcinoma with the presence of vascular embolus. The tumor measured 5 cm in its greatest axis. It was right anterolateral, with a circumferential margin greater than 1 mm. The lymph node dissection brought back 12 lymph nodes that were all negative. The tumor was classified as ypT2N0M0.

The patient did not undergo adjuvant chemotherapy. He has been regularly followed up.

Three years later, he presented a prolapsed mass situated on the perineal pseudo continent colostomy. Physical exam showed a large budding, ulcer mass of perineum, measured 20 cm in diameter (Figure 1). The mass was mobile. A biopsy was performed. The pathology of the punch biopsies revealed a recurrence of his prior adenocarcinoma on his CCPP. The CT body scan did not show any metastatic lesion. And tumor markers were negative (Carcinoembryonic antigen and CA 19-9).

We decide to perform a wide excision of the local recurrence with definitive left iliac colostomy and reconstruction with a rectus abdominis myocutaneous (RAM) flap with an inferior pedicle and an oblique skin paddle (Taylor flap).

The patient is placed in a gynecological position. The tumor was non-occlusive, large, ulcer, and budding mass. It measured 20 cm in its greatest diameter and goes up to 10 cm in height of the CCPP. We performed a median laparotomy from the xiphoid to the pubis, passing to the right of the umbilicus. We proceeded to disconnect the CCPP, passing 2 cm from the tumor in the circumferential margin and 5 cm in height (Figure 2). The distal colonic stump was left pending. Reconstruction passing through the large muscle right of the abdomen, and we ensured that the right flap chosen was well vascularized (Figure 3). Once the flap was raised, it was switched to intra-abdominal in 180 degrees and down to the perineum. We assured its vascular safety in pelvic position (Figure 4). We performed the colostomy, we carried out systematic prosthetic parietal reconstruction and then we closed the abdominal wall at the same time. Then we performed modeling and suturing of the flap.

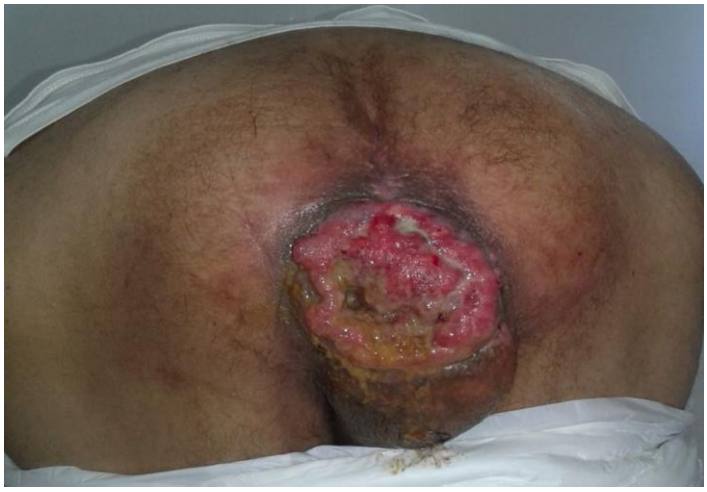

Figure 1: A large budding, ulcer mass on the perineal pseudo continent colostomy.

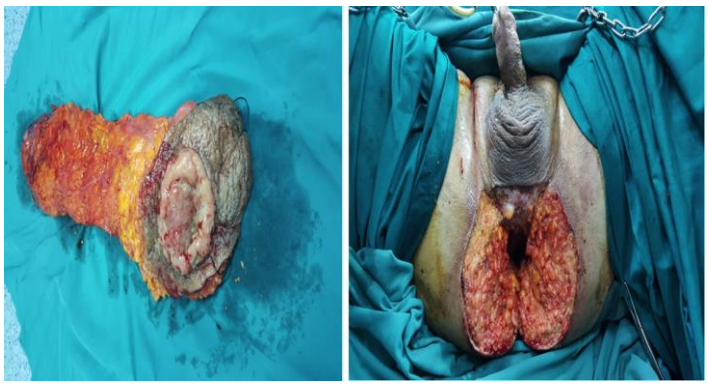

Figure 2: The perineal pseudo continent colostomy is disconnected.

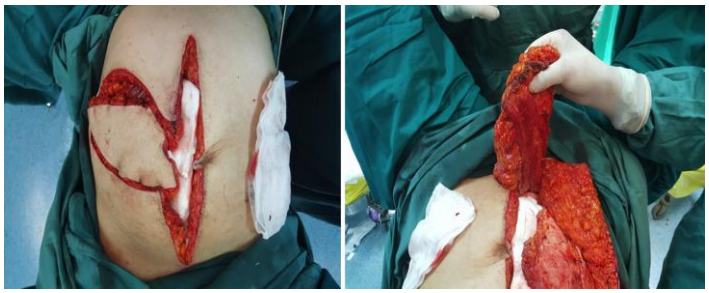

Figure 3: Reconstruction passing through the large muscle right of the abdomen.

The postoperative management is straightforward and the patient was discharged from the hospital on the tenth day. The definitive healing was achieved in 30 days.

The definitive histological exam showed a moderately differentiated adenocarcinoma with a vascular embolus. We found 2 lymph nodes that were negative. The circumferential margin was 20 mm, and the distal margin was free at 40 mm. The patient underwent adjuvant chemotherapy. The regimen chemotherapy was six cycles of Folfox.

The patient is free of disease with a 3-year-follow up.

The written consent was taken from the patient.

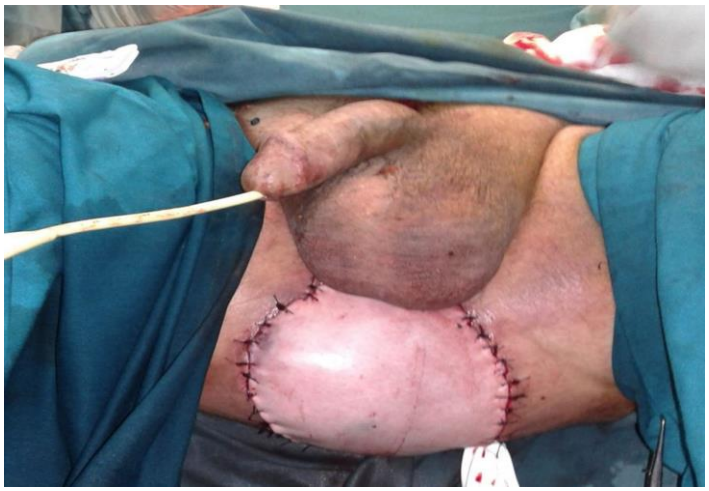

Figure 4: The flap was switched to intra-abdominal in 180 degrees and down to the perineum. We assured its vascular safety in pelvic position.

Discussion

Regardless of whether or not preoperative chemoradiotherapy or radiotherapy are performed, local recurrence of curatively resected rectal cancer has been reported to vary from 3.7% to 13.0%. [2-4].

The sites of local recurrences are so various [2, 5]. Before the progress of total mesorectal excision, the most common local types of recurrence expected to be central (perianastomotic and anterior). Lateral and posterior types (presacral), nevertheless, have become more common as multimodal treatments have come into use [2]. In our case despite combined treatment and the total mesorectal excision, the recurrence was central.

Surgeons try to maximize the preservation of the sphincter, so APR is infrequently used for the treatment of lower rectal cancer and canal anal cancer. However, after this sort of surgery, restoration and digestive continuity through definitive left iliac colostomy continued to date the most common reconstruction process [6].

PCPC was firstly described by Schmidt [1], and then it was used previously by many surgeons [6, 7]. Actually, this technique is not used currently for the treatment of rectal cancer.

Lasser et al. [6] defined the selection criteria for this technique: young patients, healthy, able to perform perineal irrigations and, above all, motivated by the choice of this technique. Obesity is an adverse factor in the implementation of this technique. A prolonged sitting position does not constitute a contraindication to PCPC, nor is the use of adjuvant chemotherapy. However, postoperative radiotherapy is a contraindication to the use of this reconstruction, as is the presence of a large fixed tumor [6].

Moreover, Kuzu et al. [8] mentioned that social, physical, sexual, and psychological aspects of life, in addition to religious worship, are severely impaired by sphincter sacrificing surgery in the Islamic population. As an alternative Skouda et al. [9] demonstrated that PCPC provides a high degree of patient satisfaction without compromising oncological results. It is a good option in selected patients, especially in Muslim countries.

In our study, we proposed the PCPC technique to our patients believing that it would be more adapted to their economic situation, social and religious specificities of our population. By allowing body image preservation, PCPC makes easy the social reinsertion and avoids the alteration of the quality of life due to permanent iliac colostomy, especially in Muslim patients. These reasons could explain the high rate of satisfaction among our patients.

The main oncological advantage of PPCC technique is to allow early diagnosis of pelvic recurrences by rectal examination or by echo-endoscopy [7, 9], like in our case, the local recurrence was identified by clinical rectal inspection and confirmed by histological findings before adequate treatment was initiated.

Treatments of loco-regional recurrences of rectal cancer present a real challenge for physicians.

Bosman et al. [10] reported that reirradiation (with concomitant chemotherapy) was associated with high morbidity. Many studies have clearly proven the survival benefits of surgical resection of locally recurrent rectal cancer [2, 11, 12].

Surgical resection of locally recurrent rectal cancer prolongs survival after diagnosis of recurrence, regardless of R0 resection [11, 13]. So, such resection should be considered as an initial treatment for locally recurrent rectal cancer. Furthermore, the wide surgical resection is conflated with an aesthetic challenge to handle the perineal defect. In our case, excision of the local recurrence was coupled with a large perineal defect that required Taylor's flap.

Whereas perineal scarring is a major issue in terms of the quality of life for patients after an abdominal-perineal amputation. The extensive resections and irradiation make this extremely random and long healing [14]. The complications such as infections and abscess, disunity or chronic wounds are quite frequent under these conditions (up to 65% depending on the series).

One of the alternatives to Taylor's flap is direct closure on omentoplasty. Lefèvre et al. [15], concluded that Taylor's flap was a technique which reduced perineal complications and the time of healing in patients with abdominal-perineal amputation for anal cancer without increasing morbidity in the abdominal wall. Other roofing flaps can also be used as the gracilis flap [16], lower gluteal [17] or deep inferior epigastric perforators (DIEP). The gracilis flap [16] has many disadvantages compared to the one of Taylor. Small in size, it does not allow to cover the great loss of substance. Its reliability, much lower with necroses present in 10 to 25% of cases depending on the series makes it a second-line flap in case failure or impossibility of making a flap of large right. As for the lower gluteal flap, the ransom scarring, low mobility and chronic pain [17] also make it a second choice flap. As for DIEP, its main advantage is the absence of secondary venting [18]. But the complexity and the cumbersomeness of realization for patients often fragile in the background also a second choice flap. In addition, the reliability of a microanatomist free flap in multi-operated and irradiated patients is much less than that of a much safer Taylor flap [18].

Taylor's flap allows for immediate reconstruction in large cases [15]. The minimal complications of the receiving site (disunity, abscesses, partial necroses and etc.) and the donor site (vents and etc), encourages us to carry out an immediate reconstruction.

The choice of immediate or secondary reconstruction can also be discussed. In our case, the patient received an immediate reconstruction. Early filling of the loss of substance reduces the risk of complications. The immediate reconstruction is good because it makes the length of hospitalization and cares much shorter.

As for the risks of suffering from the flap, it will agree to ensure the absence of intrinsic compression (bladder, uterus and etc.) at the rotation point [19].

In conclusion, surgical resection of locally recurrent rectal cancer prolongs survival after diagnosis of recurrence, regardless of R0 resection. So, such resection should be

considered as an initial treatment for locally recurrent rectal cancer. However, this resection is often associated with a large defect that leads to a problem of coverage.

Locally recurrent rectal cancer yields wide perineal defects and Taylor flap would be a good alternative for the reconstruction.

Thus, the benefit for the patient is both functional and psychological: The quality of surgical resection as R0 affords to the patient long survival term, and Taylor's flap ensures a good quality of life.

References

1. Schmidt E. The continent colostomy. World J Surg. 1982;6:805–9.
2. Yu TK, Bhosale PR, Crane CH, Iyer RB, Skibber JM, Rodriguez-Bigas MA, et al. Patterns of locoregional recurrence after surgery and radiotherapy or chemoradiation for rectal cancer. Int J Radiat Oncol Biol Phys. 2008;71:1175–80.
3. Yun JA, Huh JW, Kim HC, Park YA, Cho YB, Yun SH, et al. Local recurrence after curative resection for rectal carcinoma: The role of surgical resection. Medicine (Baltimore). 2016;95:e3942.
4. Sauer R, Becker H, Hohenberger W, Rödel C, Wittekind C, Fietkau R, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med. 2004;351:1731–40.
5. Kusters M, Marijnen CA, van de Velde CJ, Rutten HJ, Lahaye MJ, Kim JH, et al. Patterns of local recurrence in rectal cancer; a study of the Dutch TME trial. Eur J Surg Oncol. 2010;36:470–6.
6. Lasser P, Dubé P, Guillot JM, Elias D. Pseudocontinent perineal colostomy following abdominoperineal resection: technique and findings in 49 patients. Eur J Surg Oncol. 2001;27:49-53.
7. Souadka A, Majbar MA. Perineal colostomy may be the solution of phantom rectum syndrome following abdominoperineal resection for rectal cancer. J Wound Ostomy Continence Nurs. 2014;41:15–6.
8. Kuzu MA, Topcu O, Ucar K, Ulukent S, Unal E, Erverdi N, et al. Effect of sphincter-sacrificing surgery for rectal carcinoma on quality of life in Muslim patients. Dis Colon Rectum. 2002;45:1359–66.
9. Souadka A, Majbar MA, El Harroudi T, Benkabbou A, Souadka A. Perineal pseudocontinent colostomy is safe and efficient technique for perineal reconstruction after abdominoperineal resection for rectal adenocarcinoma. BMC Surg. 2015;5:40.
10. Bosman SJ, Holman FA, Nieuwenhuijzen GA, Martijn H, Creemers GJ, Rutten HJ. Feasibility of reirradiation in the treatment of locally recurrent rectal cancer. Br J Surg. 2014;101:1280–9.
11. Dresen RC, Gosens MJ, Martijn H, Nieuwenhuijzen GA, Creemers GJ, Daniels-Gooszen AW, et al. Radical resection after IORT containing multimodality treatment is the most important determinant for outcome in patients treated for locally recurrent rectal cancer. Ann Surg Oncol. 2008;15:1937–47.
12. Heriot AG, Byrne CM, Lee P, Dobbs B, Tilney H, Solomon MJ, et al. Extended radical resection: the choice for locally recurrent rectal cancer. Dis Colon Rectum. 2008;51:284–91.
13. Rahbari NN, Ulrich AB, Bruckner T, Münter M, Nickles A, Contin P et al. Surgery for locally recurrent rectal cancer in the era of total mesorectal excision: is there still a chance for cure? Ann Surg 2011;253:522–33.
14. Wiig JN, Poulsen JP, Larsen S, Braendengen M, Waehre H, Giercksky KE. Total pelvic exenteration with preoperative irradiation for advanced primary and recurrent rectal cancer. Eur J Surg. 2002;168:42–8.
15. Lefèvre JH, Corte H, Tiret E, Boccara D, Chaouat M, Touboul E, et al. Abdomino-perineal resection for squamous cell anal carcinoma: survival and risk factors for recurrence. Ann Surg Oncol. 2012;19:4186–92.
16. Ouar N, Mazouz-Dorval S, Revol M, Sorin T. Perineal reconstruction: the use of a gracilis muscle flap for urethral fistula coverage, our point of view. Ann Chir Plast Esthet. 2017;62:163–6.
17. Ferron G, Martel P, Querleu D. Vaginal reconstruction after pelvic exenteration: when and which techniques? Bull Cancer. 2003;90:435–40.
18. Leclère FM, Mordon S, Ramboaniaina S, Schoofs M. Breast reconstruction with a free DIEP flap complicated by spontaneous rupture of internal mammary artery. Ann Chir Plast Esthet. 2010;55:593–6.
19. Lahmidani S, Zakri B, Elotmany A. Pelvic reconstruction with a vertical rectus abdominis flap (VRAM): flap ischemia caused by a bladder globe. Ann Chir Plast Esthet. 2010;55:251–2.

Arch Clin Exp Med 2019;4(3):152-154. e-ISSN: 2564-6567

DOI: 10.25000/acem.641952 Olgu Sunumu / Case Report

Anorectal malignant melanoma: A case report and treatment options

Anorektal malign melanom: Bir olgu sunumu ve tedavi seçenekleri

Hacı Hasan Abuoğlu [1], Mehmet Gençtürk [1], Mehmet Kamil Yıldız [1], Onur İlhan [1], Mehmet Gülmez [1], Kübra Kaytaz [1], Selvinaz Özkara [2]

Abstract

Anorectal malignant melanoma (AMM) is a rare malignant disease with a poor prognosis. This disease is often confused with hemorrhoids. The most common site of malignant melanoma following skin and eye involvement is the anorectal region. This is the most commonly involved site in the gastrointestinal tract. We report the case of a 67-year-old patient with lower gastrointestinal hemorrhage for 4 months and hemorrhoid treatment for 2 months. The imaging revealed no distant metastasis but histopathologically, lymph node metastasis and invasion of surrounding tissues. Laparoscopic abdominoperineal resection (APR) was performed.

Key words: Anorectal diseases, malignant melanoma, surgery.

Öz

Anorektal melanom (AMM) ender görülen, kötü prognozlu malign bir hastalıktır. Bu hastalık sıklıkla hemoroid ile karışmaktadır. Malign melanomanın deri ve göz tutulumundan sonra en sık görüldüğü bölge anorektal bölgedir. Bu bölge gastrointestinal sistemde en sık olarak tutulan bölgedir. Biz 67 yaşında, 4 aydır makattan kanama şikayetleri olan, 2 aydır da hemoroid tedavisi uygulanan hastayı sunduk. Yapılan görüntülemelerde hastanın uzak metastazının olmadığı ancak histopatolojik olarak lenf nodu metastazının ve çevre dokulara invazyonun olduğu izlendi. Hastaya laparoskopik abdominoperineal rezeksiyon (APR) yapıldı.

Anahtar Kelimeler: Anorektal hastalıklar, malign melanom, cerrahi.

[1] University of Health Sciences, Haydarpaşa Training and Research Hospital, Department of General Suregry, Istanbul, Turkey.

[2] University of Health Sciences, Haydarpaşa Training and Research Hospital, Department of Pathology, Istanbul, Turkey.

HHA: 0000-0002-2285-0685
MG: 0000-0002-6172-0736
MKY: 0000-0003-2565-1208
Oİ: 0000-0001-5369-5510
MG: 0000-0002-3782-6039
KK: 0000-0002-1898-3119
SÖ: 0000-0002-5931-3760

Informed Consent: The written consent was received from the patient who was presented in this study.
Hasta Onamı: Çalışmada sunulan hastadan yazılı onam alınmıştır.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

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

Finansal Destek: Yazarlar bu olgu için finansal destek almadıklarını beyan etmişlerdir.

Geliş Tarihi / Received: 07.11.2019
Kabul Tarihi / Accepted: 15.11.2019
Yayın Tarihi / Published: 01.12.2019

Sorumlu yazar / Corresponding author:

Mehmet Gençtürk

Adres/Address: Kurtköy Mah,. Ankara Cd., 390/3, 34912 Pendik/ İstanbul
e-mail:drgencturk@hotmail.com
Tel/Phone: +90 5079427492

Introduction

Malignant melanoma is a rare disease, but it has usually a poor diagnosis because it is diagnosed late [1]. It can be mistaken for hemorrhoids. The incidence of malignant melanoma is 0.1-4.6% of all rectal malignancies [3]. The patient's complaints included rectal bleeding, palpable mass, breech pain, tenesmus and pruritis [5]. Its treatment is surgical, with extensive local excision or abdominoperineal resection [6]. In our 67-year-old woman, we performed abdominoperineal resection due to locally advancing of the tumor.

Case report

A 67-year-old female patient was treated with diagnosis of hemorrhoids in another hospital due to lower gastrointestinal hemorrhage, 4 months ago. The patient received medical treatment for 2 months. Physical examination revealed an irregular mass on the anorectal junction. Colonoscopy was planned. The colonoscopy showed a mass located at 3.cm from the anal canal and surrounding the anal canal (Figure 1). Histopathological examination revealed mucosal malignant melanoma (Figure 2). No other features were found in the other system examinations of our patient.

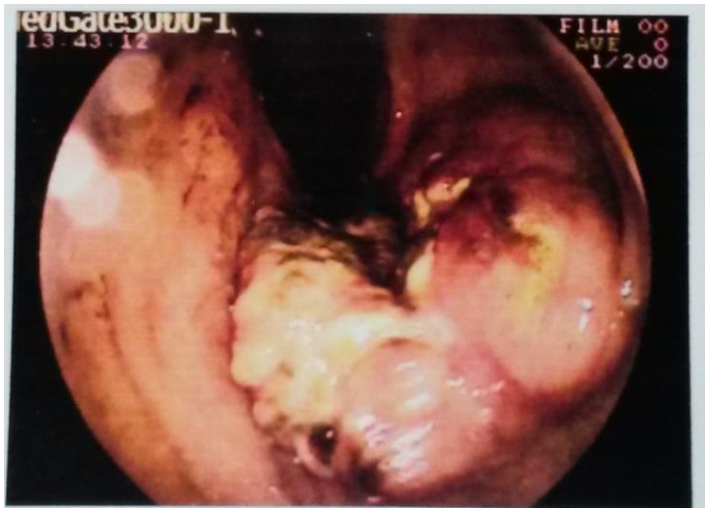

Figure 1: Colonoscopic image of anorectal malignant melanoma.

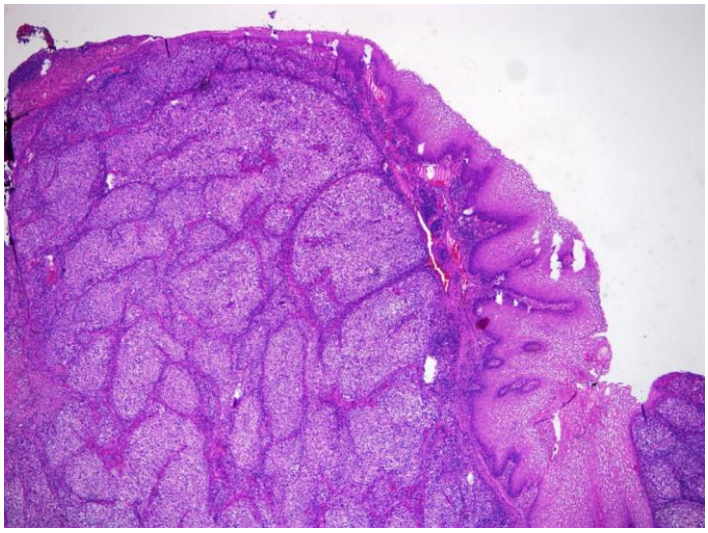

Figure 2: Histopathological imaging of anorectal malignant melanoma.

Abdominal computed tomography showed a mass lesion of 2.5 cm in diameter at the thickest site, and lymph nodes in the pararectal region, the largest of which was 3 * 1.5 cm in size. Increased attenuation suggesting infiltration was observed in perirectal fat plans (Figure 3). In addition, multiple diverticula were observed in the sigmoid colon and the descending colon (diverticulosis coli).

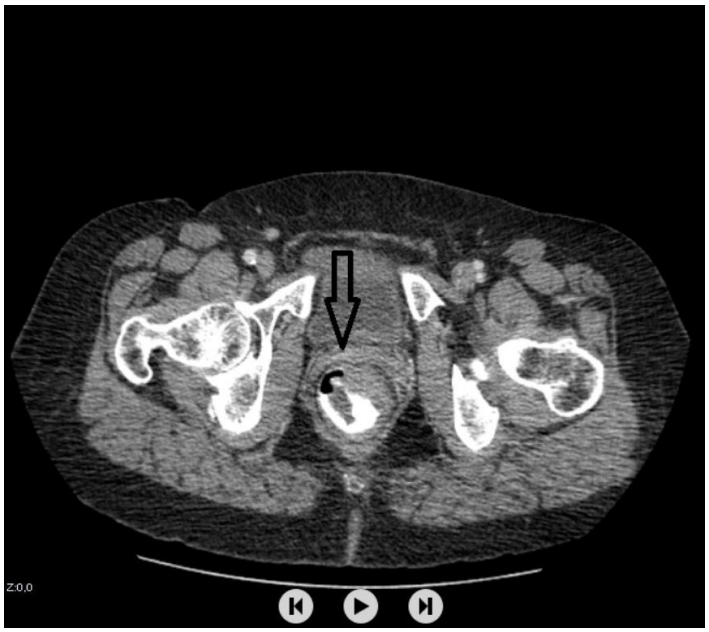

Figure 3: Computed tomography image of anorectal malignant melanoma.

Following preoperative evaluations, laparoscopic abdominoperinal resection was planned. Invasion of the mass into the posterior wall of the vagina was observed during surgery. The patient was consulted with gynecologist peroperatively. Total excision of the mass was completed including the vagina in the posterior wall. Histopathological examination revealed that the tumor was 5.5 X 4 X 1.2 cm in size, passed through the muscularis propria and showed direct invasion to the adipose tissue and surrounding structures. The patient had uneventful postoperative period and was discharged on the 7th day.

Informed consent was obtained from the patient.

Discussion

Anorectal malignant melanoma is an extremely rare disease of neuroectodermal origin, which is aggressive and has a poor prognosis, and is usually fatal with late diagnosis [1]. Malignant melanoma of the anus and rectum was first reported by Moore in 1857 [2]. Rectal malignant melanomas constitute 0.2-3% of all malignant melanomas and 0.1-4.6% of rectal malignancies [3]. The most common symptom is rectal hemorrhage, so it is confused with hemorrhoidal disease and causes delays in diagnosis. Our patient also had medical treatment for a long time with the diagnosis of hemorrhoids. The most common symptoms of AMM include palpable mass, pain in the breech, changes in bowel habits, pruritis, tenesmus, and anorectal region [1, 5]. In our patient, bleeding and severe pain in the breech area were prominent. The contribution of chemotherapy and radiotherapy is limited. Surgical treatment is still controversial even today [9]. In previous studies, radical resections such as abdominoperineal resection were accepted for treatment, whereas in recent studies there was no significant difference in survival and prognosis with wide local excision (WLE) technique [4]. Thibault et al. [5] recommended WLE

with a negative surgical margin of at least 1 cm in treatment and recommended APR for palliative or non-WLE-only tumors for obstructing tumors. There is no consensus on the surgical margin in WLE and it should be noted that even a 1 cm negative surgical margin can lead to anal incontinence. Therefore, if negative surgical margin can be achieved without causing anal incontinence, WLE should be performed [6]. However, some studies have suggested that aggressive treatment with APR has a better survival rate, possibly associated with lymphadenectomy, that can control lymphatic spread (especially mesenteric lymph nodes) and that wider negative surgical margins and lower local recurrence rates can be achieved. [4, 8]. Yap et al. [1] reviewed 17 series published and reported that there was no statistically superiority between two techniques in all stages of the disease in their 5-year results. To sum up, treatment of anorectal malignant melanoma should be patient-specific [7].

In conclusion, in light of all these information, we preferred APR because of pathological appearance LAPs in the patient's pararectal region, infiltration in the perirectal fatty area and vaginal invasion. The closeness of the mass to the anal canal played a major role in this decision.

References

1. Yap LB, Neary P. A comparison of wide local excision with abdominoperineal resection in anorectal melanoma. Melanoma Res. 2004;14:147-50.
2. Meguerditchian AN, Meterissian SH, Dunn KB. Anorectal melanoma: diagnosis and treatment. Dis Colon Rectum. 2011;54:638-4.
3. Damodaran O, Morgan A, Mendelsohn G. Primary malignant melanoma in the anorectum: an uncommon cancer. N Z Med J. 2008;121:66-8.
4. Yeh JJ, Shia J, Hwu WJ, et al.The role of abdominoperineal resection as surgical therapy for anorectal melanoma. Ann Surg. 2006;244:1012-17.
5. Thibault C, Sagar P, Nivatvongs S, Ilstrup DM, Wolff BG. Anorectal melanoma - an incurable disease. Dis Colon Rectum. 1997;40:661-8.
6. Malik A, Hull T, Floruta C. What is the best surgical treatment for anorectal melanoma? Int J Colorectal Dis. 2004;19:121-23.
7. Fazio VW, Church JM, Delaney CP. Current therapy in colon and rectal curgery. Second Edition. Philadelphia: Elsevier Mosby 2005;79-81.
8. Pessaux P, Pocard M, Elias D, et al.Surgical management of primary anorectal melanoma. Br J Surg. 2004;91:1183-7.