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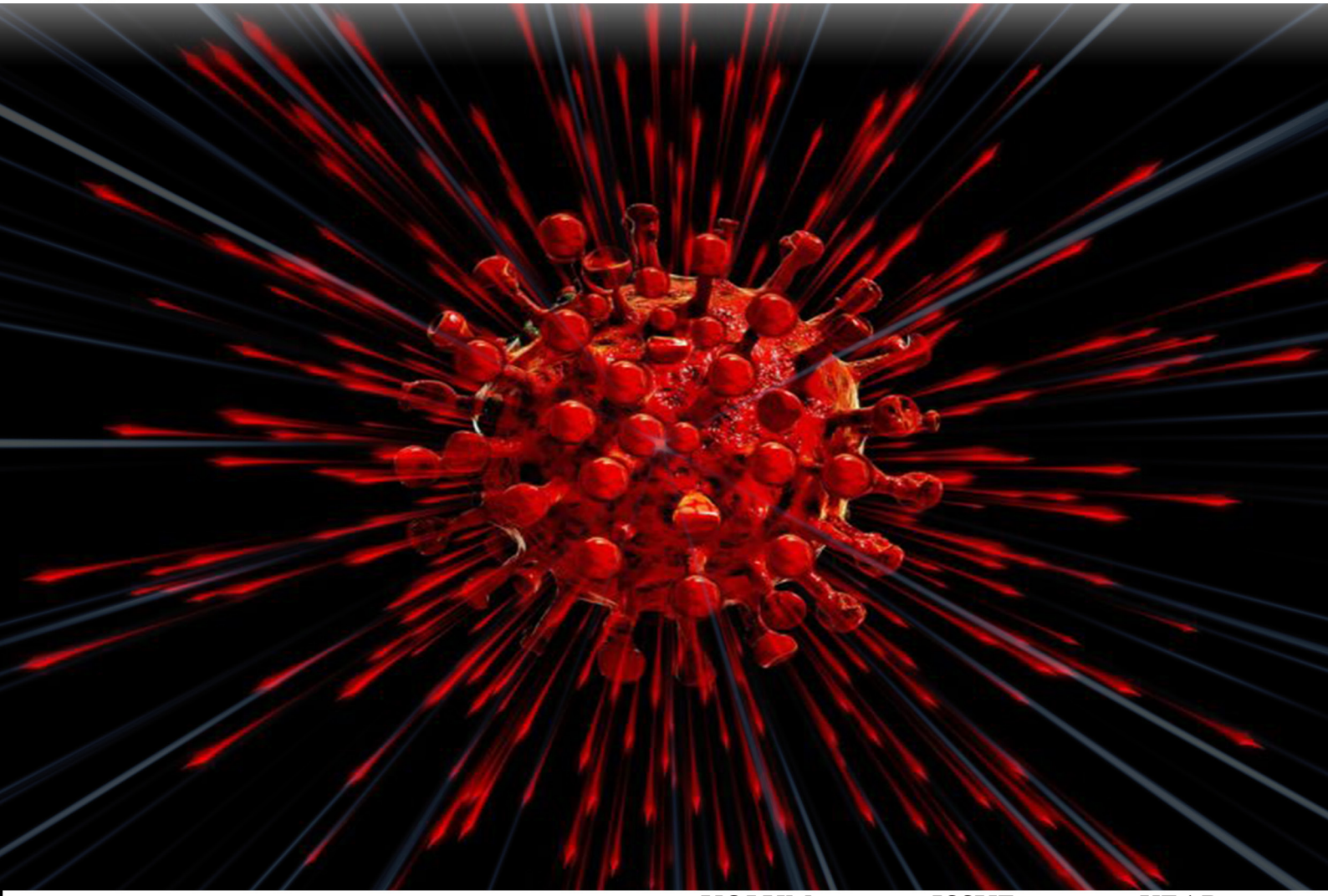

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Fatih Şamil ULUDAĞ
(fsuludag@gmail.com)

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MediHealth Academy Publishing
Emniyet Mah., Yukarı Sk., 6/1, Yenimahalle, Ankara, Türkiye
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Dear esteemed readers of ACMJ,

Here we are again with a new issue. We are proud to publish the fourth issue of our journal for 2021 with 13 articles. In this issue, there are 12 original articles and 1 case report that we think will attract your attention. All of our articles are in English. In this way, we aim to both get more citations and get the ISI (Web of Science) index, which will open the way to enter indices such as SCI and SCI-E. We know that our journal, which increases its scientific quality with each new issue, is also eagerly awaited by you, our valuable readers. To further increase the scientific quality of our journal, we work hard, including those on the editorial board. We were very pleased with the contribution of our colleagues, who were psychologically burn out during the COVID-19 pandemic.

Wish you a pleasant fall of 2021.

Sincerely yours

Assoc. Prof. Muhammed Kızılgül, MD
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Anatolian Curr Med J 2021; 3(4); 264-268

A comparison of cleavage-stage embryo transfer and blastocyst transfers in patients with low anti-Mullerian hormone levels

Nur Dokuzeylül Güngör[1], Tuğba Gürbüz[2], Arzu Yurci[3]

[1]Bahçeşehir University, Göztepe Medikal Park Hospital Complex, Gynecology and IVF Clinic, İstanbul, Turkey
[2]Medistate Hospital, Gynecology and Obstetric Clinic, İstanbul, Turkey
[3]Memorial Hospital, IVF Clinic, Kayseri, Turkey

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ABSTRACT

Aim: Decreased ovarian reserve is a common problem that many women struggle with and seek help by assisted reproductive techniques (ART). Embryo transfer (ET) is an important step in ART that can be performed on cleavage-stage (day 2 or 3) or blastocyst stage (day 5 or 6). This study aims to investigate the effect of ET day on pregnancy results in patients with low levels of anti-Mullerian hormone (AMH).

Material and Method: This retrospective study enrolled 239 women with low AMH levels referred to the Bahçeşehir University, Göztepe Medikal Park Hospital In-vitro Fertilization (IVF) Clinic from May 2015 to April 2020. The patients were divided into two groups: blastocyst transfer group and cleavage-stage transfer group. Among the participants, blastocyst transfer was performed in 30 women and cleavage-stage ET on 209 women,respectively.

Results: Among 239 participants, 30 (12.6%) were in the blastocyst group, and 209 (87.4%) were in the cleavage-stage group. There was no significant difference in the total number of retrieved oocytes between the two groups (p=0.1). The number of mature oocytes (MII) was significantly different between the two groups (p=0.006). Examining pregnancy results between the two groups showed no significant difference between positive and negative pregnancy tests (p=0.4). Even though a higher live birth rate for the blastocyst transfer group was observed with 15(50%), no significant difference was found between the number of live births and miscarriages in the two groups (p=0.1).

Conclusion: The transfer day does not affect the pregnancy results in women with low AMH levels.

Keywords: Anti-Mullerian hormone, assisted reproductive technique, blastocyte, cleavage, embryo transfer

INTRODUCTION

Infertility is a problem that one in six couples struggles with and may seek help with assisted reproductive technique (ART). About 30-40% of the causes of infertility in women are related to ovarian disorders, and the rest are related to uterine disorders, immune factors, and systemic diseases (1). The cause of infertility has always been discussed in ART treatments, and patients with various causes such as endometriosis, male factor, tubular causes, and polycystic ovary syndrome (PCOS) are treated, and the effect of hormonal disorders has always been discussed (2,3).

Anti-Mullerian hormone (AMH) is a bi-chain glycoprotein from the family of cell growth and differentiation factors. AMH levels are associated with the number of primary follicles and have a higher specificity than the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol on the third day of the menstrual cycle (2). Unlike FSH, which must be measured on the second or third day of menstruation, AMH can be measured on any day of the cycle. AMH level reflects ovarian function, and it decreases with age. Also, the AMH level is low in women who do not respond well to ovarian stimulation drugs in in-vitro fertilization (IVF) programs (2-4).

Embryo Transfer (ET) from the laboratory to the uterus is an important step in ART. Traditionally, ET is done on day 2-3 or cleavage-stage, but with the advancement of IVF and embryo culture in recent decades, blastocyst transfer was preferred (5). It is said that blastocyst transfer may be physiologically more appropriate because it is closer to the time of natural implantation (6,7). In the recent decade, blastocyst transfer in ART cycles has

Corresponding Author: Tuğba Gürbüz, drtgurbuz@hotmail.com

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increased significantly (8). Simultaneously with this increase in the use of blastocysts transfer, other advances have been made in the fields of assisted reproduction, such as sequential culture media, time-lapse systems, and vitrification programs to replace freezing techniques that enable pre-implantation genetic screening. These advances have led to the re-discussion of the cleavage-stage transfer as an effective method instead of the blastocyst transfer method (13-15). Therefore, this study aimed to investigate the effectiveness of transfer day on pregnancy results in patients with low AMH levels.

MATERIAL AND METHOD

Ethical approved this study by Research and Project Development Ethics Committee of Beykoz University (Date: 21.12.2020, Decision No: 2020/04). All procedures conducted in our study conformed to the national or institutional research committee's ethical standards and the 1964 Helsinki Declaration and its later amendments.

This retrospective study included 239 infertile women referred to the Bahçeşehir University Göztepe Medical Park Hospital IVF Clinic from May 2015 to April 2020. The patients were divided into two groups: blastocyst transfer group and cleavage-stage transfer group. Among the participants, blastocyst transfer was performed in 30 women and cleavage-stage ET on 209, respectively. Inclusion criteria were: age ≤40 years and AMH level<1ng/ml. Exclusion criteria were: patient reluctance to participate in the study, systemic diseases, women over 40, and couples with severe male infertility.

Statistical Analysis

The results of the Klomogrov test show that not all quantitative variables have a normal distribution. Mann-Whitney test is used to examine the relationship between quantitative variables in the two groups. The Chi-square test and Fisher's exact test are used to examine qualitative variables. For all tests, a significance level of 0.05 will be considered. Data were analyzed using SPSS for Windows, Version 23.0 (Armonk, NY: IBM Corp).

RESULTS

Among 239 participants, 30 (12.6%) were in the blastocyst group, and 209 (87.4%) were in the cleavage group. The participants' age was a minimum of 20 and a maximum of 40, with a mean of 33.6 (±4.9). Participants' body mass index (BMI) was a minimum of 19 and a maximum of 29, with a mean of 24.2 (±2.10). The number of ART attempts averaged 1.6 (±1.60) with a minimum of 0 and a maximum of 9 times. The mean AMH level in participants was 0.47 (±0.18) with a minimum of 0.1 and a maximum of 0.9. Among the participants, the minimum total number of oocytes was 1, and the maximum was 13, with a mean of 3.4 (±1.8). The mean number of mature oocytes (MII) was 2.7 (±1.5), with a minimum of 1 and a maximum of 11. Out of 239 participants, 219 (91.6%) did not smoke, and 20 (8.4%) smoked. **Table 1** shows these characteristics. All patients were primary inferility.

Table 1. Descriptive statistics of variables

Variable	N	Minimum	Maximum	Mean	Standard deviation (SD)
Age	239	20	40	33.6	4.9
BMI	239	19	29	24.2	2.10
Number of attempts	239	0	9	1.6	1.60
AMH	239	0.1	0.9	0.47	0.18
Total oocyte	239	1	13	3.4	1.8
Number of mature oocytes (MII)	239	1	11	2.7	1.5
Smoke		**Number**			**Frequency**
No		219			91.6
Yes		20			8.4
Group					
Blastocyte transfer		30			12.6
Cleavage transfer		209			87.4

Out of 239 participants, one embryo was transferred to 188 (78.3%) participants, and two embryos were transferred to 51 (21.3%) participants. Examination of participants' pregnancy results showed that 108 (45.2%) participants had positive pregnancy results, and 131 (54.8%) participants had negative pregnancy results. Of the 108 participants who tested positive for pregnancy, 83 (76.8%) had a live birth, and 25 (23.1%) had a miscarriage. These results are shown in **Table 2**.

Table 2. Descriptive statistics of pregnancy results

Pregnancy result	Number	Frequency (%)
Live birth	83	34.7
Missed	25	10.5
Negative	131	54.8
Result		
Positive	108	45.2
Negative	131	54.8
Number of embryos transferred		
1	188	78.7
2	51	21.3

Mann-Whitney test was used to evaluate the quantitative variables between the two groups of blastocyst transfer and cleavage-stage transfer. There was no significant difference between the age (p=0.2) and BMI (p=0.4) of the two groups. There was also no significant difference between AMH (p=0.3) and the number of attempts of the two groups (p=0.2). There was no significant difference between total oocytes between the two groups (p=0.1). The MII variable was significantly different between the two groups (p=0.006). The results of this test are given in **Table 3**.

Table 3. The comparison of variables between groups

Variable	Group		p value
	Blastocyte transfer mean (Sd)	Cleavage transfer mean (Sd)	
Age	32.9 (3.9)	33.7 (5.04)	0.2
BMI	24 (2.3)	24.3 (2.1)	0.4
Number of attempts	1.3 (1.7)	1.7 (1.6)	0.2
AMH	0.5 (0.2)	0.4 (0.2)	0.3
Total oocyte	4.03 (2.2)	3.4 (1.8)	0.1
Number of mature Oocytes (MII)	3.5 (1.8)	2.6 (1.4)	0.006

Chi-square and Fisher's exact tests were used to evaluate the qualitative variables between the two groups of blastocyst transfer and cleavage transfer. Examining pregnancy results between the two groups showed no significant difference between positive and negative pregnancy tests (p=0.4). Even though a higher live birth rate for the blastocyte transfer group was observed with 15 (50%), no significant difference was found between the number of live births and miscarriages in the two groups (p=0.1). There was a significant difference in the number of transferred embryos between the two groups (p<0.001) (**Table 4**).

Table 4. The comparison of pregnancy results between groups

Variable	Group		p value
	Blastocyte transfer frequency (%)	Cleavage transfer frequency (%)	
Pregnancy result			0.1
Live birth	15 (50)	68 (32.5)	
Missed	1 (3.3)	24 (11.5)	
Negative	14 (46.7)	117 (56)	
Result			0.4
Positive	16 (53.3)	92 (44)	
Negative	14 (46.7)	117 (56)	
Number of embryos transferred			0.000
1.00	11 (36.7)	177 (84.7)	
2.00	19 (63.3)	32 (15.3)	

DISCUSSION

In the present study, pregnancy outcomes were compared in two groups: the cleavage-stage transfer vs. the blastocyst transfer in patients with low AMH levels. Currently, age, antral follicle count (AFC), and AMH levels are generally acknowledged as the best predictors for ovarian reserve (1). In the last decade, despite the results of contradictory clinical trials, there has been an increasing trend towards blastocyst transfer (2). Numerous studies have shown that the blastocyst transfer method can produce viable, genetically more normal embryos (1-6). Our results showed that no significant difference was found between the two groups despite of a higher live birth rate in the blastocyst transfer group. Miscarriage rates were also not significantly different between the two groups. This result is consistent with the results of previous studies (7-9). Jones et al. (10) showed that continuing to culture embryos until the fifth day could provide more competent embryos. However, they concluded that blastocyst transfer could not completely prevent chromosomal abnormalities. Probability of success with IVF cycle largely depends on a woman's ovarian reserve and her ability to produce a large number of high-quality mature oocytes in a cycle after COH. Average serum AMH is 4 ng/ml in healthy young women with normal ovarian reserve (11). However, a recent consensus reported in La Marca et al. (12) considered poor response at AMH under 1 ng/ml and high response when AMH is over 3 ng/ml. Nikmard et al. (13) considered normal AMH range at 1.3–2.6 ng/ml obtaining good ovarian response and clinical outcomes after ART.Our data showed a pregnancy rate of 53.3% for the blastocyst transfer group and 44% for the cleavage group. Despite the higher pregnancy rate in the blastocyst transfer group, this difference is not significant. The results of miscarriage also did not show a significant difference between the two groups. These results are consistent with the results of some studies (14-16). Coskun et al. (17) did not find a difference in pregnancy rate and implantation rates between the blastocyte and cleavage stage transfer. Also, Levron et al. (18) found similar results in their comparing the ET day. Lundqvist et al. (19) showed that increasing embryo culture days did not affect the pregnancy rate and the take-home baby rate.On the other hand, these results are inconsistent with some studies as in Frattarelli (20) and Van der Auwera et al. (21) which showed that the rate of pregnancy and implantation in the blastocyst transfer method is higher than the cleavage transfer method. Wilson et al. (22) and Levron et al. (18) also showed that the blastocyst transfer can increase pregnancy rates in patients over 35. However, they reported higher miscarriage rates for the blastocyst transfer group in contrast to our study.Schwarzler et al. (23) also found higher take-home baby rates in the blastocyst transfer group than in the cleavage-stage transfer group.The American Society for Reproductive Medicine (ASRM), in its guidelines for blastocyte transfer in ART (24), showed no significant association between increased birth rate per cycle by blastocyst transfer compared to cleavage-stage transfer. In our study, no significant differences were observed between blastocyst transfer and cleavage-stage ET group among patients with low AMH levels. All our cases were fresh ET. However, different conditions such as frozen or fresh transfer of the embryo (25), the number of transferred blastocytes (26), or diseases and underlying conditions (27) can affect the results. Therefore, further studies should be performed to further investigate the effect of various factors such as AMH on selecting the best transfer day.In a study conducted at an ART database in Canada, 3206 blastocyst transfers were compared with

9506 cleavage-stage ET. In contrast to our study the live birth rate was significantly higher following blastocyst transfer than following cleavage-stage transfer (26). Some studies have shown that blastocyst transfer can increase the rate of multiple pregnancies in addition to the pregnancy rate (28-30). Also, Karacan et al. (31) have shown that the rate of multiple pregnancy in blastocyst transfer is significantly higher in blastocyst transfer than in cleavage-stage transfer. In contrast to these studies, we have no multiple pregnancy because of limited number of transferable embryos. In the present study, all participants had AMH level <1 ng/ml. Very recently, qualitative embryo characteristics as blastulation and aneuploidy rate in addition to clinical IVF outcomes were analyzed by Morin et al. (32) in a large retrospective study including 3457 patients. The authors reported that women younger than 38 years old with evidence of diminished ovarian reserve (DOR) did not display an oocyte qualitative decline. Indeed, fertilized oocytes retrieved from young patients with DOR formed blastocysts of high quality, euploid and were able to produce live births as those of women of the same age with high AMH values (32). As far as we know, no studies have been performed on the role of AMH in the selection of embryo transfer day. Our results showed no significant difference between the two methods in patients with low levels of AMH. One of the limitations of this study was the lack of a control group for the AMH variable. In future studies, control groups with normal AMH levels should be considered to examine the effects of AMH more accurately in each transfer method. Data from this study were also obtained from a single clinic. In subsequent studies, data from several clinics can be combined to obtain more comprehensive conclusions. Indeed, endometrial receptivity in patients with low AMH is not affected as it thought, but the only difference is the number of obtained embryos with good quality to select the best ones for ET.

CONCLUSION

In this study, both blastocyte and cleavage-stage transfer in subjects with low AMH levels were compared. No significant difference was observed between the live birth and miscarriage rate between the two groups. Further studies should be performed considering the control group for AMH and larger populations to confirm the findings of this study.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical approved this study by Research and Project Development Ethics Committee of Beykoz University (Date: 21.12.2020, Decision No: 2020/04).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Lehmann P, Velez MP, Saumet J, et al. Anti-Mullerian hormone (AMH): a reliable biomarker of oocyte quality in IVF. J Assist Reprod Genet 2014; 31: 493–8.
2. Papanikolaou EG, Fatemi H, Venetis C, et al. Monozygotic twinning is not increased after single blastocyst transfer compared with single cleavage-stage embryo transfer. Fertil Steril 2010; 93: 592-7.
3. Källén B, Finnström O, Lindam A, Nilsson E, Nygren K-G, Olausson PO. Blastocyst versus cleavage stage transfer in in vitro fertilization: differences in neonatal outcome? Fertil Steril 2010; 94: 1680-3.
4. Kokkali G, Traeger-Synodinos J, Vrettou C, et al. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of β-thalassaemia: a pilot study. Human Reprod 2007; 22: 1443-9.
5. Mangalraj AM, Muthukumar K, Aleyamma T, Kamath MS, George K. Blastocyst stage transfer vs cleavage stage embryo transfer. J Hum Reprod Sci 2009; 2: 23-6.
6. Milki AA, Jun SH, Hinckley MD, Behr B, Giudice LC, Westphal LM. Incidence of monozygotic twinning with blastocyst transfer compared to cleavage-stage transfer. Fertil Steril 2003; 79: 503-6.
7. Barrenetxea G, de Larruzea AL, Ganzabal T, Jiménez R, Carbonero K, Mandiola M. Blastocyst culture after repeated failure of cleavage-stage embryo transfers: a comparison of day 5 and day 6 transfers. Fertil Steril 2005; 83: 49-53.
8. Chang HJ, Lee JR, Jee BC, Suh CS, Kim SH. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. Fertil Steril 2009; 91: 2381-90.
9. Desai N, Ploskonka S, Goodman LR, Austin C, Goldberg J, Falcone T. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. Reprod Biol Endocrinol 2014; 12: 1-10.
10. Jones GM, Trounson AO, Gardner DK, Kausche A, Lolatgis N, Wood C. Evolution of a culture protocol for successful blastocyst development and pregnancy. Hum Reprod 1998; 13: 169-77.
11. Muttukrishna S, Suharjono H, McGarrigle H, et al. Inhibin B and anti-mullerian hormone: markers of ovarian response in IVF/ICSI patients?. BJOG 2004; 111: 1248–53.
12. La Marca A, Ferraretti AP, Palermo R, et al. The use of ovarian reserve markers in IVF clinical practice: a national consensus. Gynecol Endocrinol 2016; 32: 1–5.
13. Nikmard F, Aflatoonian B, Hosseini E, et al. A comparative study on the results of agonist and antagonist protocols based on serum AMH levels in patients undergoing intracytoplasmic sperm injection. Int J Reprod Biomed 2016; 14: 769-76.

14. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev 2016; 6: CD002118.
15. Ishihara O, Kuwahara A, Saitoh H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: an analysis of single embryo transfer cycles in Japan. Fertil Steril 2011; 95: 1966-9.
16. Martins W, Nastri C, Rienzi L, Van Der Poel S, Gracia C, Racowsky C. Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: a systematic review and meta-analysis. Hum Reprod 2016; 31: 2561-9.
17. Coskun S, Hollanders J, Al-Hassan S, Al-Sufyan H, Al-Mayman H, Jaroudi K. Day 5 versus day 3 embryo transfer: a controlled randomized trial. Hum Reprod 2000; 15: 1947-52.
18. Levron J, Shulman A, Bider D, Seidman D, Levin T, Dor J. A prospective randomized study comparing day 3 with blastocyst-stage embryo transfer. Fertil Steril 2002; 77: 1300-1.
19. Lundqvist M, Rova K, Simberg N, Lundkvist Ö. Embryo transfer after 2 or 5 days of IVF culture: a retrospective comparison. Acta obstetricia et gynecologica Scand 2002; 81: 126-32.
20. Frattarelli JL, Leondires MP, McKeeby JL, Miller BT, Segars JH. Blastocyst transfer decreases multiple pregnancy rates in in vitro fertilization cycles: a randomized controlled trial. Fertil Steril 2003; 79: 228-30.
21. Van der Auwera I, Debrock S, Spiessens C, et al. A prospective randomized study: day 2 versus day 5 embryo transfer. Hum Reprod 2002; 17: 1507-12.
22. Wilson M, Hartke K, Kiehl M, Rodgers J, Brabec C, Lyles R. Integration of blastocyst transfer for all patients. Fertil Steril 2002; 77: 693-6.
23. Schwärzler P, Zech H, Auer M, et al. Pregnancy outcome after blastocyst transfer as compared to early cleavage stage embryo transfer. Hum Reprod 2004; 19: 2097-102.
24. Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion. Fertil Steril 2013; 99: 667-72.
25. Zeng M, Su S, Li L. Comparison of pregnancy outcomes after vitrification at the cleavage and blastocyst stage: a meta-analysis. J Assist Reprod and Genet 2018; 35: 127-34.
26. Zhu Q, Zhu J, Wang Y, et al. Live birth rate and neonatal outcome following cleavage-stage embryo transfer versus blastocyst transfer using the freeze-all strategy. Reprod Biomed Online 2019; 38: 892-900.
27. Vajta G, Parmegiani L. Cleavage stage vs. blastocyst transfer: a more considerate analysis suggested. Hum Reprod 2020; 35: 2399-400.
28. Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. Mol Hum Reprod 2014; 20: 117-26.
29. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril 2014; 101: 128-33.
30. Maheshwari A, Kalampokas T, Davidson J, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril 2013; 100: 1615-21.
31. Karacan M, Ulug M, Arvas A, et al. Comparison of the transfer of equal numbers of blastocysts versus cleavage-stage embryos after repeated failure of in vitro fertilization cycles. J Assist Reprod Genet 2014; 31: 269-74.
32. Morin SJ, Patounakis G, Juneau CR, Neal SA, Scott RT, Seli E. Diminished ovarian reserve and poor response to stimulation in patient <38 years old: A quantitative but not qualitative reduction in performance Hum Reprod 2018; 33: 1489-98.

Original Article

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Social media and breastfeeding: an Instagram study

Selda Bülbül[1], Cansu Çelik[1], Sevim Gonca Kocagözoğlu[2], Aliye Gülbahçe[1]

[1]Kırıkkale University, Faculty of Medicine, Department of Social Pediatrics, Kırıkkale, Turkey
[2]Kahramankazan Hamdi Eriş State Hospital, Clinic of Pediatrics, Ankara, Turkey

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ABSTRACT

Background: Human milk has many proven benefits but breastfeeding rates are far below the desired optimal level. Social media plays an important role in accessing information and advice on health-related issues. There are several studies investigating how the social media has an impact on the community about health-related issues and decisions. We aimed to reveal how much Instagram has touched on breastfeeding and related contents about infant feeding.

Material and Method: This is a descriptive cross-sectional study. We surveyed on Turkish Instagram accounts which only share about children health and diseases between June 2018 and January 2019. We searched the hashtags about child health and infant feeding and we enrolled the accounts that have more than 1000 followers. The obtained data were analyzed by SPSS 16.0 for Windows. The significancy level was accepted as $p<0.05$.

Results: We enrolled 75 Turkish Instagram accounts. The mean number of posts was 743.77 (22-11349) and the mean number of posts of human milk and formula/bottle feeding were 9.80 and 2.07 ($p=0.006$). Human milk-related posts received a mean of 1368.3 likes, while those associated with formula/bottle feeding received 437.3 ($p=0.007$).

Conclusion: As a result, it is seen that posts on human milk and breastfeeding were significantly more than those on formula and bottle feeding in social media. The expanding world of the internet today, led us to think that social media can be used as an important tool to increase breastfeeding rates. This should be obviously taken into consideration in future plans of policy makers.

Keywords: Bottle feeding, breastfeeding, formula, human milk, social media

INTRODUCTION

Human milk has many proven benefits for mothers, infants and society with reduced infant mortality rates, protection against infections, and prevention of chronic diseases both in mothers and children. Although these benefits are well known, breastfeeding rates are far below the desired optimal level (1). Facing attitudes that avoid breastfeeding in the community, inadequate support from the partner and close environment, restrictive working conditions, aggressive advertisement campaigns of formula companies can be listed as some of the effecting factors (2).

People who were born between 1980s and early 1990s are known as "Generation Y" or the Internet generation as tending to use the internet for everything. Also, they use social media and online forums, as reliable sources of information (3).

New mothers need support at any sites where she feels insufficient. Social media plays an important role, particularly during the stressful postpartum transition period, in communicating with other people who have similar problems, dealing with stress of being a parent, and accessing information and advice on nutrition, healthcare and nurturing their children (3-6).

Google and Facebook are the most popular networking platforms for providing health related answers and to reduce feelings of social isolation especially in young mothers (3,7). Instagram is another famous social media platform that has more than 700 million active users worldwide and allows sharing photos and videos. "Blogger moms" have become an increasingly widespread phenomenon some of who have thousands of followers (8).

Corresponding Author: Sevim Gonca Kocagözoğlu, goncadeprem@yahoo.com

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There are studies investigating how the social media has an impact on the community about health-related issues and decisions. These studies showed positive and negative results on the impact of social media on public health (9,10).

In social media, where false information can spread very quickly, it becomes crucial that the information and advices given by accounts that share about children's health and diseases are medically correct and it has great importance that these accounts focus on essential health issues and topics perceived wrongly by the community (11). The good thing is that, especially when seeking information about breastfeeding issues, women are tending to prefer accessing mostly academic websites or websites that were moderated by health professionals (3).

In this study, we aimed to reveal how much Instagram has touched on human milk, breastfeeding and related contents about infant feeding such as formulas and bottles. Moreover we aimed to see how the followers react to these posts.

MATERIAL AND METHOD

This descriptive cross-sectional study was conducted between the dates June 2018 and January 2019. This study was conducted by examining social media accounts. Due to the fact that the data is available to the public, neither ethics committee approval nor informed consent were required. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Content analysis was done for user info of Turkish Instagram accounts that share posts on child health issues, infant feeding and childhood diseases. We used the hashtags in Turkish such as; #cocuksagligi (#childhealth), #pediatri (#pediatrics), #annesütü (#humanmilk), #emzirme (#breastfeeding), #cocukdoktoru (#pediatrician), #cocukbeslenmesi (#childfeeding), #mama (#formula), #biberon (#bottle) and scanned similar account suggestions. We didn't obtain an informed consent because the account owners had accepted to share their data in Instagram. We reviewed related posts and searched submissions about human milk, breastfeeding, bottle and formula and we planned to enroll accounts with more than 1000 followers. We examined the account owners' characteristics as gender, occupation, marital status and having children by using descriptive statistical methods. Among the submissions, the posts about human milk with breastfeeding and bottle feeding with formula were divided into two separate categories. The number of received likes and the rates of participation in the accounts were used for statistical analyses. The obtained data were analyzed by SPSS 16.0 for Windows. For statistical analyses, descriptive statistical methods were used and the results were given as numbers, percentages, and mean values. For comparison of means we conducted one-sample t-test. The significancy level was accepted as $p<0.05$.

RESULTS

During the study period, 56,189 posts were evaluated and 75 Turkish Instagram accounts with more than 1000 followers who had posts on child health and infant feeding were included in the study. The number of posts related to hashtags about child health and infant feeding between the study period was given in **Table 1**. Of the accounts' owners 14.7% (n=11) were male and 82.7% (n=62) were female; 85.3% (n=64) were married; %80 (n=60) had at least one child. Among the whole group 54.7% (n=41) were working in the health sector, 9% (n=7) were housewives and 36.3% (n=27) were from other professions (**Table 2**).

Table 1. Number of posts related to hashtags about child health and infant feeding between June 2018-January 2019

Name of hashtag	Number of shared posts
#annesütü (#humanmilk)	127,000
#emzirme (#breastfeeding)	180,000
#çocuksağlığı (#childhealth)	127,000
#pediatri (#pediatrics)	123,000
#çocukdoktoru (#pediatrician)	74,600
#çocukbeslenmesi (#childfeeding)	70,700
# biberon (#bottlefeeding)	55,200
#mama (#formula)	62,000

Table 2. Demographic characteristics of the account owners

	N	%*
Gender		
Female	62	82.7
Male	11	14.7
Corporate	2	2.6
Marital status		
Married	64	85.2
Single	2	2.7
Unknown	9	12
Parental status		
Parent	60	80
Not a parent	13	17.3
Corporate account	2	2.7
Occupation		
Healthcare worker	41	54.7
Others	27	36.3
Not working/housewife	7	9
TOTAL	**100**	**100**

*column percentage is given

The mean number of posts on the analyzed pages were 743.77 (min 22- max 11,349). The mean number of posts related to human milk/breastfeeding and baby bottle were 9.80 (0-89) and 2.07 (0-9), respectively (p=0.006). The ratio of human milk related and formula related posts to all posts were as 2.57 and 0.75 respectively. Human milk related posts received a mean of 1368 (min 0- max 25,370) likes, while those associated with formula/bottle feeding received 437.3 (0-30660) likes with a statistically significant difference (p=0.007) (**Table 3**).

When only female page owners were taken into account, the mean number of posts was 783.18 (min 22- max 11,349), and the mean number of posts related to human milk and bottle feeding were 10.68 (min 0- max 89) and 2.35 (min 0- max 9) respectively. The ratio of posts related to human milk to all posts was 2.52, while those associated with formula to all posts was 0.87. The mean of like numbers of human milk and of formula feeding/bottle feeding posts were 14,662 (min 13- max 25,370), and 448 (min 0- max 3066) respectively (**Table 3**).

DISCUSSION

Social media is an available communication tool for health-related questions, exchanging ideas with other people who have the same problems or gaining information on specific health problems (12). Social media networks are excellent tools for spreading messages to public, while the cost to deliver information to a large number of people in a very short time and reach young adults is quite low (13,14). With increasing number of followers day by day, which reached up to a maximum of 979,000 of the Turkish Instagram pages 75 were analysed in this study. This huge number of Instagram users only in Turkey can support the above idea.

As compatible with our results (82.7% of the account owners in the study were women), recent social media utilization data indicate that the majority of adults who use social media are women and over 80% are in the 18–40 years age group (11,12). It is not possible to obtain the age information from the social media accounts, so we used the data given by the statistics page of Instagram on January 2018. These data showed that 31% of the page owners were in 18-24 years age group and 30% were in 24-35 years age group (15). According to 2017 Turkey Statistical Institute data it was determined that the average age of first-time being a mother was 28.7 years (16). When these data were evaluated together, it can be said that social media could be accepted as the modern-day information and communication platform for young people and women in reproductive age.

It has been shown that, the most searched topics on child health issues by the parents were; sleep problems, mental health issues, car safety and childhood vaccines (11). Infant feeding seems to be a less researched topic by parents on social media. Griauzde et al. (7) also showed that mothers were not using social media or other internet sources for gaining information on infant feeding. Our results also reveal that posts on infant feeding (breast feeding/bottle feeding) are relatively low [mean number of posts on the analysed pages was (743.77), mean number of posts related to human milk/breastfeeding was 9.80 and bottle feeding/formula was 2.07]. For this reason, Turkish Ministry of Health should encourage healthcare professionals to use social media more actively and share correct baby feeding recommendations.

Due to the interactive and unifying structure of social media, breastfeeding could be a health activity that can be easily campaigned via these networks. Common problems, encountered difficulties, fears about breastfeeding can be discussed and resolved and breastfeeding can be strongly encouraged on these platforms by the comments, sharing of stories and information posted under these issues (17-19). In previous studies, it has been shown that campaigns conducted via the Internet significantly increased the level of knowledge, positive beliefs, and desire of breastfeeding compared to information provided by "offline" healthcare professionals (20,21). In our study breast milk related posts received more mean of likes than formula/bottle feeding with a statistically significant difference. Considering as a preliminary study, our study results might give a positive impression that social media can have a positive effect on breastfeeding rates. Taking into account the ages of those who frequently use social media, strategic campaigns for adolescent girls and boys would be an innovative approach to promote and support breastfeeding in the next generation (22).

In recent years, use of internet has increased among the young pregnant women and mothers for getting information on baby care (23). However, this increase also poses a risk for the spread of false information. Even though the fact that social media is not open to control in terms of information accuracy, it is obvious

Table 3. Distribution of characteristics of breastfeeding and formula feeding related posts by gender and working in a health related sector

	Female mean (min-max)	Male mean (min-max)	Healthcare W mean (min-max)	Total mean (min-max)
Number of human milk posts	10.68 (0-89)	5.62 (0-28)	13.76 (0-89)	9.8 (0-89)
Number of baby bottle posts[1]	2.35 (0-9)	0.69 (0-2)	1.49 (0-8)	2.07 (0-9)
Number of formula posts[1]	1.92 (0-8)	0.46 (0-2)	1.54 (0.7)	1.67 (0-8)
Likes of human milk posts[2]	14,662 (13-25,370)	9375 (0-8793)	630 (13-5259)	1368.3 (0-25,370)
Likes of Formula posts[2]	4.48 (0-3066)	375.39 (0-1762)	352 (27-2075)	437.33 (0-3066)
Human milk posts/Total posts	2.52	2.82	3.36	2.57
Formula posts/total posts	0.87	0.19	0.67	0.75

[1]: p= 0.006; [2]: p=0.007

that healthcare professionals provide social and medical support to these mothers with accurate information on their platforms (24). Our study group (determined accounts) was also mostly composed of healthcare professionals (54.7%). Therefore, it seems that, though, healthcare professionals are already using these platforms and this number is increasing day by day, it is still not enough.

There are relatively few studies on the visibility and communication of nursing mothers and healthcare professionals on social media and how this communication can be effectively used to support breastfeeding. In this sense, our study is important as it is one of the first studies in terms of the visibility of breastfeeding and breastmilk on social media. Moreover, it can provide valuable preliminary information to establish a systematic network of "online" breastfeeding support and information.

Limitation

This study has some limitations. The ages of the account holders and their purpose of the social media usage couldn't be reached. We could only define the occupations and genders of them. The undefined characteristics of the account holders could be an important part of the information. We believe that, valuable information can be gained by organizing a study focusing on the detailed demographic characteristics and expectations of people using social media to share information on child health and infant feeding.

CONCLUSION

In conclusion, it seems that posts on breast milk and breastfeeding were significantly more than those about formula in social media. However, it is still not at the desirable levels. At present, social media could be accepted an important and easily available platform for physicians to serve accurate and scientific information to large number of people.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was conducted by examining social media accounts. Due to the fact that the data is available to the public, neither ethics committee approval nor informed consent were required.

Referee Evaluation Process: Externally peer-reviewed.

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

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REFERENCES

1. Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet 2016; 387: 475-90.
2. Sriraman K, Kellams A. Breastfeeding: What are the barriers? why women struggle to achieve their goals. J Women's Health 2016; 25: 714-22.
3. Alianmoghaddam N, Phibbs S, Benn C. "I did a lot of Googling": A qualitative study of exclusive breastfeeding support through social media. Women Birth 2019; 32: 147-56.
4. Umberson D, Pudrovska T, Reczek C. Parenthood, childlessness, and well-being: a life course perspective. J Marriage Fam 2010; 72: 612-29.
5. Bartholomew MK, Schoppe-Sullivan SJ, Glassman M, Kamp Dush CM, Sullivan JM. New parents' Facebook use at the transition to parenthood. Fam Relat 2012; 61: 455-69.
6. Doty JL, Dworkin J. Online social support for parents: a critical review. Marriage Fam Rev 2014; 50: 174-98.
7. Griauzde DH, Kieffer EC, Domoff SE, et al. The influence of social media on child feeding practices and beliefs among Hispanic mothers: A mixed methods study. Eat Behav 2020; 36: 101361.
8. Number of monthly active Instagram users from January 2013 to September 2017 (in millions). Statista.com (Access March 2019)
9. Asiodu IV, Waters CM, Dailey DE, Lee KA, Lyndon A. Breastfeeding and social media among first-time African American mothers. J Obstet Gynecol Neonatal nurs 2015; 44: 268-78.
10. Tomfohrde O, Reinke J. Breastfeeding mothers' use of technology while breastfeeding. Comput Hum Behav 2016; 64: 556-61.
11. Bryan MA, Evans Y, Morishita C, Midamba N, Moreno M. Parental perceptions of the internet and social media as a source of pediatric health information. Acad Pediatr 2020; 20: 31-8.
12. Shieh C, Khan I, Umoren R. Engagement design in studies on pregnancy and infant health using social media: Systematic review. Prev Med Rep 2020; 19: 101113.
13. Strecher V. Internet methods for delivering behavioral and health-related interventions (eHealth). Ann Rev Clin Psychol 2007; 3: 53-7.
14. Vance K, Howe W, Dellavalle R P. Social internet sites as a soure of public health information. Dermatol Clin 2009; 27: 133-6.
15. Distribution of Instagram users worldwide as of January 2018, by age group website: https: //www.statista.com/statistics/325587/instagram-global-age-group/ (Access december 2018)
16. Türkiye Nüfus ve Vatandaşlık İşleri Müdürlüğü, TÜİK, Doğum istatistikleri, 2018 (Turkey Directorate of Population and Citizenship Affairs, TSI, Birth Statistics, Access : December 2018)
17. Dornan B A, Oermann MH. Evaluation of breastfeeding web sites for patient education. Am J Matern Child Nurs 2006; 31: 18-23.
18. Riordan J. Teaching breastfeeding on the web. J Hum Lact 2000; 16: 231-4.
19. Thomas J, Shaikh U. Electronic communication with patients for breastfeeding support. J Hum Lact 2007; 23: 275-9.
20. O'Connor ME, Brown EW, Lewin LO. An Internet-based educational program improves breastfeeding knowledge of maternal-child healthcare providers. Breastfeed Med 2011; 6: 421-7.

21. Pate B. A systematic review of the effectiveness of breastfeeding intervention delivery methods. J Obstet Gynecol Neonatal Nurs 2009; 38: 642-3.
22. Harding K, Aryeetey R, Carroll G, Lasisi O, Pérez-Escamilla R, Young M. Breastfeed4Ghana: Design and evaluation of an innovative social media campaign. Matern Child Nutr 2020; 16: e12909.
23. Shaikh U, Scott B J. Extent, accuracy and credibility of breastfeeding information on the internet. J Hum Lact 2005; 21: 175-83.
24. Bautista JR, Zhang Y, Gwizdka J. Healthcare professionals' acts of correcting health misinformation on social media. J Med Inform 2021; 148: 104375.

Original Article

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Are the blood urea nitrogen/creatinine ratio and the mean platelet volume able to act as predictors of mortality and morbidity in patients with upper gastrointestinal bleeding?

Burçak Kantekin[1], Mücahit Avcil[2], Mücahit Kapçı[3], Adnan Bilge[4], Sema Avcı[5]

[1]İstanbul Acıbadem Maslak Hospital, Department of Emergency Medicine, İstanbul, Turkey
[2]Uşak Training and Research Hospital, Department of Emergency Medicine, Uşak, Turkey
[3]İstanbul Başakşehir Çam ve Sakura City Hospital, Department of Emergency Medicine, İstanbul, Turkey
[4]Manisa Celal Bayar University Medical Faculty, Department of Emergency Medicine, Manisa, Turkey
[5]Uşak Training and Research Hospital, Department of Emergency Medicine, Uşak, Turkey

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ABSTRACT

Background: Upper gastrointestinal (GI) bleeding originates above the Treitz ligament and blood urea nitrogen (BUN)/Creatinine (Cr) ratio ≥30 was shown to indicate the presence of upper GI bleeding and it might be an independent risk factor for mortality. The mean platelet volume (MPV) was reported as an indicator of platelet function and activation and was shown to be useful in the diagnosis and follow-up of vascular-origin disorders. In the present study, we aimed to investigate whether BUN/Cr ratio and MPV were predictors of mortality and morbidity in patients with upper GI bleeding.

Material and Method: This retrospective study enrolled a total of 141 patients admitted to the Emergency Department with symptoms of upper GI bleeding and had undergone endoscopy between January 2013-January 2015. Demographics, hemoglobin, hematocrit, MPV, BUN/Cr, the hospitalization unit, the duration of stay, outcome, and the endoscopy reports of the patients were recorded. Statistical analysis was performed using SPSS 17.0.

Results: It was found that the MPV level did not predict mortality and morbidity in our patients with upper GI bleeding. The likelihood of active bleeding was higher in patients with a BUN/Cr ratio of above 30 when compared to those with a ratio of below 30.

Conclusion: In patients presenting to the emergency department with symptoms of GI bleeding but without signs of renal failure, calculation of admission BUN/Cr ratio may be beneficial for making treatment and follow-up plans.

Keywords: Upper gastrointestinal bleeding, blood urea nitrogen/creatinine ratio, mean platelet volume, morbidity, mortality

INTRODUCTION

Upper gastrointestinal (GI) bleeding originates from proximal sites of the Treitz ligament, and mostly occurs due to gastric or duodenal ulcers. GI bleeding is a significant cause of mortality and morbidity that should be ruled out in patients presenting with hematemesis, hypotension, blood in stool, and darkening of stool color. Despite all advances in diagnosis and management, 8-10% of patients presenting to emergency departments (EDs) with upper GI bleeding are currently lost (1).

The annual incidence of acute massive upper GI bleeding in the population is 40-150 per 100,000 (2). Its incidence is lower in the young population, and it has started to be seen even more commonly in elderly due to several factors such as the increased prevalence of comorbidities, and more frequent use of non-steroidal anti-inflammatory agents (3).

The findings on physical examination include orthostatic symptoms, postural changes, pallor, palpitations, fatigue, chest pain, dyspnea, tachypnea, and abdominal pain. The digital anorectal examination should be performed both to investigate the presence of anorectal disease, and to confirm stool color described by the patient. The findings on physical examination, decreased hemoglobin level, abnormal coagulation parameters, and occult blood in stool do not necessarily indicate the presence or the severity of GI bleeding. It is essential to diagnose upper GI bleeding by esophagogastroduodenoscopy (4,5).

Corresponding Author: Sema Avcı, dr.semaavci@outlook.com

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In upper GI bleeding, the level of blood urea nitrogen (BUN) rises due to volume contraction and absorption of blood proteins, resulting in an increased BUN/Creatinine (Cr) ratio (6). The BUN/Cr ratio ≥30 was shown to indicate the presence of GI bleeding, and has been suggested in various reports that it might have been an independent risk factor for mortality (7).

The mean platelet volume (MPV) is an easy-to-measure parameter, indicating the platelet function and activation. The platelets are indispensable for hemostasis, and their dysfunction leads to bleeding and coagulation disorders (8). It has been suggested that MPV might be useful in the diagnosis and follow-up of many disorders, mainly those with vascular origin (9).

In this study, we aimed to investigate whether the BUN/Cr ratio calculated at admission of the patient and the MPV level were predictors of mortality and morbidity in patients who had presented to the ED with symptoms of upper GI bleeding and had undergone endoscopic examination.

MATERIAL AND METHOD

Approval of the Ethics Committee

Our study was approved by the Adnan Menderes University Faculty of Medicine Non-invasive Clinical Research Ethics Committee (Date: 12.02.2015, Decision No: 2015/546-9). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Properties of the Patient Group

This retrospective study enrolled the patients aged 18 years and over, who had presented to the ED with symptoms of GI bleeding and had undergone an endoscopic examination between January 1st, 2013 and January 1st, 2015. The data on their medical charts were recorded and reviewed. Patients admitted to ED with chronic renal failure, known high urea levels or ongoing hemodialysis program and under age of 18 were excluded from the study.

Data Collection

A data sheet was prepared to record patient information. This data sheet included information about the patient characteristics including name, gender, age, and medical chart number, the hemoglobin (Hgb), hematocrit (Htc), MPV, BUN, and Cr values at the time of admission, the unit that the patient had been hospitalized (intensive care unit (ICU)/regular ward), the duration of stay in the hospital, the patient outcome (discharged/deceased), and the endoscopy reports. After recording all relevant data on the data sheet, the BUN/Cr ratio at the time of admission to the ED was calculated and recorded.

Statistical Analysis

All results were reported as mean± standard deviation. All study data were analyzed with the Statistical Package for Social Sciences (SPSS) 17.0 software package. The T-test, the Chi-square and the Mann-Whitney U test methods were used in the analysis and comparison of the data and the groups. $p<0.05$ was considered statistically significant.

RESULTS

Of 141 patients, 49 (35%) were female, and 92 (65%) were male. The male-to-female ratio was 1.87. The mean patient age of all patients was 67 years. Approximately 50% of our patients were aged between 60-80 years and particularly 75-80 years (25%).

The mean ages of the patients admitted to the ICU and the regular ward were 68 years and 65 years, respectively. The mean Hgb value of all patients at the time of admission was 9.5±2.5 g/dL. The mean Hgb level was 8.7±2.4 g/dL among patients admitted to ICU, and 10.5±2.33 g/dL among others. The mean Htc levels of the entire study population, the patients in ICU, and those in the ward were 29.5±7.1%, 26.9%, and 32.6%, respectively. The mean BUN/Cr ratios of the entire study population, the patients hospitalized in ICU and the regular ward were 34.1±15.0, 36.4, and 31.0, respectively. The analysis of MPV levels revealed an overall mean MPV level of 9.8±1.1 fL. The mean MPV level was 9.8 fL in both the patients admitted to ICU and the regular ward. **Table 1** shows the values and the comparison of age, gender, Hgb, Htc, and MPV, the BUN/Cr ratio, the rates of active bleeding and mortality according to the units in which the patients were hospitalized. Statistically significant differences were found to be present between the patients hospitalized in ICU and the regular ward regarding Hgb and Htc values, the BUN/Cr ratio, and the mortality rate. The patient group in ICU had significantly lower levels of Hgb and Htc, and significantly higher BUN/Cr ratio and mortality rate compared to the patient group hospitalized in the regular ward ($p<0.05$).

Table 1. The distribution of the study population according to the unit that the patients were hospitalized

		ICU group	Regular ward group	P
Sex	Male	48	44	
	Female	29	20	
Mean age (years)		68	65	0.104
Hemoglobin (g/dL)		8.7	10.5	0.000
Hematocrit (%)		26.9	32.6	0.000
MPV (fL)		9.8	9.8	0.805
BUN/Creatinine		36.4	31.0	0.027
Active bleeding		11%	3%	0.111
Mortality rate		8%	0.00	0.031

ICU: Intensive care unit; MPV: Mean platelet volume; BUN: Blood urea nitrogen

The analysis of the study group based on patient age under vs. over 60 years showed that there were 42 patients (35 males, 7 females) under the age of 60 years, and 99 patients (57 males, 42 females) aged over 60 years. The mean Hgb levels were 9.1 g/dL and 10.3 g/dL in the patients aged over and under 60 years, respectively. The mean Htc level was 31.5% in patients aged under 60 years and 28.7% in those aged over 60 years. The age-based analysis of the MPV levels revealed that the mean MPV level was 9.8 fL in both the patients under and over the age of 60 years. The active bleeding rate was 4.0% in the patients aged over 60 years whereas it was 17.1% in those aged under 60 years. **Table 2** depicts the age-based comparison of sex, ICU admission rate, Hgb, Htc, MPV, BUN/Cr ratio, active bleeding, and mortality rates. There were statistically significant differences between the patients aged over and under 60 years regarding Hgb and Htc values and the active bleeding rates ($p<0.05$). The patient group aged over 60 years had significantly lower Hgb and Htc values than those aged under 60 years; however, the active bleeding rate was significantly higher in the patient group aged under 60 years when compared to the patient group with age over 60 years.

Table 2. The distribution and comparison of the study population according to age being under and over 60 years

		Age >60	Age <60	P
Sex	Male	57	35	
	Female	42	7	
ICU admission rate		58%	47%	0.255
Hemoglobin (g/dL)		9.1	10.3	0.009
Hematocrit (%)		28.7	31.5	0.034
MPV (fL)		9.8	9.8	0.992
BUN/Creatinine		33.8	34.7	0.561
Active bleeding		4.0%	17.1%	0.015
Mortality rate		5%	2%	0.670

ICU: Intensive care unit; MPV: Mean platelet volume; BUN: Blood urea nitrogen

A total of 61 (43%) patients (44 males, 17 females) had a BUN/Cr ratio below 30. BUN/Cr ratio was above 30 in a total of 79(56%) patients (47 males, 32 females).

The analysis of the laboratory results of patients with a BUN/Cr ratio below 30 revealed that Hgb, Htc, and MPV levels were 10.0 gr/dL, 31.4%, and 9.7 fL, respectively; the corresponding figures for those with a ratio greater than 30 were 9.0 gr/dL, 27.8%, and 9.9 fL, respectively. The mean age of patients with a BUN/Cr ratio lower than 30 was 73 years whereas the mean age was 67 years in those with a BUN/Cr ratio greater than 30. The rate of active bleeding in endoscopy was 18% in the patients with a BUN/Cr ratio below 30 whereas it was 82% among those with a ratio above 30. The rate of ICU admission was 32% in patients with a BUN/Cr ratio below 30 and 67% for those with a ratio over 30. **Table 3** shows BUN/Cr ratio-based comparison of sex, ICU admission rate, age, Hgb, Htc and MPV values, together with the active bleeding and mortality rates. There were significant differences between the patient groups having a BUN/Cr ratio over and under 30 regarding ICU admission rate, and Hgb and Htc values. The ICU admission rate was significantly higher and Hgb and Htc levels were significantly lower in the patient group with a BUN/Cr ratio over 30 when compared to those with a ratio under 30 ($p<0.05$).

Table 3. The distribution and comparison of the study population according to the BUN/Creatinine ratio

		BUN/Creatinine >30	BUN/ Creatinine <30	P
Sex	Male	47	44	
	Female	32	17	
ICU admission rate		67%	32%	0.003
Hemoglobin (g/dL)		9.0	10.0	0.012
Hematocrit (%)		27.8	31.4	0.003
MPV (fL)		9.9	9.7	0.429
Mean Age (years)		67	73	0.377
Active bleeding		82%	18%	0.113
Mortality rate		67%	33%	0.699

ICU: Intensive care unit; MPV: Mean platelet volume; BUN: Blood urea nitrogen

DISCUSSION

Upper GI bleeding constitutes a significant portion of the disorders in patients admitted to EDs. Ten to thirty percent of cases with upper GI bleeding results in rebleeding and two to fifteen percent result in death (10). While the etiology of upper GI bleeding is considerably variable, peptic ulcer, erosive gastritis, and esophageal varices have been reported as the most common causes, constituting 90% of all incidents with GI bleeding (11).

The epidemiology of upper GI bleeding is characterized by a lesser rate of peptic ulcer bleeding among the young population and an increased incidence of peptic ulcer bleeding in the elderly population due to increased use of aspirin and non-steroidal anti-inflammatory drugs. Factors increasing mortality and morbidity in GI bleeding include being over 50 years of age, comorbidities such as cardiovascular, hepatic, and renal disorders, encephalopathy, malignancy, major surgery, physiological stress due to sepsis or trauma, a low Htc level, and prolonged prothrombin time. Performing a risk classification especially in the triage setting would be useful for making correct medical decisions, treatment planning, prediction of disease course, and use of available resources, even though it is difficult to prove that patients benefit from such a classification regarding mortality.

The gender distribution in our study was consistent with the reports published in the medical literature. Okutur et al. (12), in their study conducted on 230 patients with upper GI bleeding, reported that 71% of patients were male and 29% were female. In their study involving 336 patients, Sezgin et al. (13) reported that 71% of patients were male and 29% were female, with the mean age of 57.7 years (13).

The age distribution found in our study was also compliant with the literature. Seventy percent of all acute upper GI bleedings occur in patients older than 60 years. Advanced age is an independent risk factor for mortality in upper GI bleeding. The review of the literature revealed that affected patients were predominantly over 50 years of age. Uyanıkoğlu et al. (14) reported that 19% of patients had an age between 51 and 60 years, and Yenigün et al. (15) reported that 26% of their patients were aged between 70 and 79 years.

Hemoglobin level is essential for follow-up, prognosis, and treatment planning in patients with upper GI bleeding. The mean Hgb level in our study was consistent with previously conducted studies. In patients with upper GI bleeding, Di Fiore et al. (15) reported a mean admission Hgb level of 9.5 g/dL, Chassaignon et al. (16) 9.8 g/dL, Yenigün et al. (17) 8.8 g/dL, Göksu et al. (18) 9.9 g/dL, and Kaltar et al. (19) 9.30 g/dL.

The mean Htc value determined in our study was also in accordance with the reports published in the literature. Okutur et al. (12) found an admission Htc level of 28.5±8.1%. They reported the values of 26.97±6.85% in patients admitted to ICU, and 32.61±6.33% for others.

The analysis of the MPV levels of our patients with upper GI bleeding showed that there was no significant correlation between age, hospitalization in ICU, and MPV level. Our results were in accordance with the reports in the literature. Altun et al. (20) showed that the MPV level during admission to ICU was not predictive of mortality. Yavuzcan et al. (21), on the other hand, showed that MPV level, which is a peripheral marker of the systemic inflammatory response, was not changed when the hematological parameters of patients with abnormal vaginal bleeding who were diagnosed either with endometrium cancer or benign endometrial pathology were compared.

GI bleeding involving the upper tract increases serum BUN level due to the absorption and digestion of Hgb. A BUN/Cr ratio of ≥30 was reported by Witting et al. (22) to indicate that the bleeding was more likely of upper GI origin. Other reports have also shown that patients with a BUN/Cr ratio above 30 had higher rates of active bleeding, ICU admission, and mortality when compared to the patients with a ratio below 30 (7). Our study produced similar results as in the literature.

Limitations

The limitations of the study are the retrospective design of the study, the advanced mean age of the patients, and the inclusion criteria being only those with renal dysfunction and patients under 18 years of age.

CONCLUSION

Our results suggest that the MPV level is not a significant predictor of mortality and morbidity in patients presenting to the ED with signs of upper GI bleeding and, the MPV level during admission is not useful for prediction of mortality and morbidity in patients with upper GI bleeding in whom an endoscopic examination is performed. However, in patients presenting with symptoms of GI bleeding, the BUN/Cr ratio is a significant predictor of the location of bleeding in the upper or lower tract, the likelihood of active bleeding, and mortality and morbidity rates. In addition to indicating the upper gastrointestinal tract as the origin of a hemorrhagic incident, a BUN/Cr ratio greater than 30 also increases the likelihood of active bleeding. The mortality rate is higher in patients with a BUN/Cr ratio above 30 when compared to those with a ratio below 30.

In clinical practice, the BUN/Cr ratio may give clues about the location of bleeding, the presence of active bleeding, and mortality. This study suggests that the BUN/Cr ratio may prove beneficial for treatment planning and follow-up.

ETHICAL DECLARATIONS

Ethics Committee Approval: Our study was approved by the Adnan Menderes University Faculty of Medicine Non-invasive Clinical Research Ethics Committee (Date: 12.02.2015, Decision No: 2015/546-9).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Feldman M, Friedman LS, Sleisenger MH. Gastrointestinal bleeding. Fordtran's Gastrointestinal and Liver Disease; 2002. page: 211-3.

2. Longstreth GF. Epidemiology of hospitalization for acute upper gastrointestinal hemorrhage: a population-based study. Am J Gastroenterol 1995; 90: 206-10.
3. Tırnaksız B. Gastrointestinal kanamanın cerrahi yönetimi. Yoğun Bakım Derg 2005; 5: 174-81.
4. Osman D, Djibre M, Da Silva D, Goulenok C. Management by the intensivist of gastrointestinal bleeding in adults and children. Ann Intensive Care 2012; 2: 46.
5. Colle I, Wilmer A, Le Moine O, et al. Upper gastrointestinal tract bleeding management: Belgian guidelines for adults and children. Acta Gastroenterol Belg 2011; 74: 45-66.
6. Uzunismail H. Akut üst gastrointestinal kanama. İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Sürekli Tıp Eğitimi Etkinlikleri Acil Hekimlik Sempozyumu Oct, 16-17th, 1997; İstanbul. page: 233-45.
7. Wu KH, Shih HA, Hung MS, Hsiao CT, Chen YC. The association between blood urea nitrogen to creatinine ratio and mortality in patients with upper gastrointestinal bleeding. Arab J Gastroenterol 2018; 19: 143-7.
8. Akpolat T, Utaş C. Günlük nefroloji ve böbrek yetmezliğinde ilaç kullanımı. 2006.
9. O'Malley T, Langhorne P, Elton RA, Stewart C. Platelet size in stroke patients. Stroke 1995; 26: 995-9.
10. Wang CH, Chen YW, Young YR, Yang CJ, Chen IC. A prospective comparison of 3 scoring systems in upper gastrointestinal bleeding. Am J Emerg Med 2013; 31: 775-8.
11. Krupp MA, Chatton MJ. Current Medical Diagnosis and Treatment. 1984.
12. Okutur SK, Alkım C, Bes C, Gürbüz D, Kınık Ö. Akut üst gastrointestinal kanamaları. Akademik Gastroenteroloji Derg 2007; 6: 30-6.
13. Sezgin O, Altıntaş E, Tombak A. Effect of seasonal variations on acute upper gastrointestinal bleeding and its etiology. Turk J Gastroenterol 18: 172-6.
14. Uyanıkoğlu A, Danalıoğlu A, Davutoğlu C, Alimoğlu O, Baş G. Akut üst gastrointestinal sistem kanaması: endoskopi sonuçlarının retrospektif değerlendirilmesi. İstanbul Üniversitesi Tıp Fakültesi Derg 2008; 71: 120-3.
15. Yenigün EC, Pirpir A, Aytan P, Ulusal G, Yıldırım İS. Üst gastrointestinal sistem kanamalı hastaların özelliklerinin değerlendirilmesi. Akademik Gastroenteroloji Derg 2006; 5: 116-22.
16. Di Fiore F, Lecleire S, Merle V, et al. Changes in characteristics and outcome of acute upper gastrointestinal haemorrhage: a comparison of epidemiology and practices between 1996 and 2000 in a multicentre French study. Eur J Gastroenterol Hepatol 2005; 17: 641-7.
17. Chassaignon C, Letoumelin P, Pateron D, Group HD. Upper gastrointestinal haemorrhage in Emergency Departments in France: causes and management. Eur J Emerg Med 2003; 10: 290-5.
18. Göksu E, Erken Ö, Erçetin Y. Akdeniz Üniversitesi Hastanesi Acil Servisine üst gastrointestinal sistem kanaması ile başvuran hastalarda mortaliteyi belirleyen faktörler ve demografik özellikleri. Türkiye Acil Tıp Derg 2004; 4: 121-6.
19. Katlar T. Üst gastrointestinal sistem kanamalı hastalarda steroid olmayan antiinflamatuar ilaç kullanımı ve bu hastalarda hemostaz parametrelerine etkilerinin incelenmesi. 2007.
20. Altun Y, Demircan F, Mengeloğlu FZ, Namuslu M. Yoğun bakım hastalarında ortalama trombosit hacminin mortalite ile ilişkisi. J Clin Exp Invest 2011; 2: 408-11.
21. Yavuzcan A, Çağlar M, Erdem H, et al. Anormal uterin kanamalı hastalarda endometrium kanseri ve benign endometrial patoloji tanısı alanların hematolojik parametrelerinin karşılaştırılması. Van Tıp Derg 2014; 21: 148-53.
22. Witting MD, Magder L, Heins AE, Mattu A, Granja CA, Baumgarten M. ED predictors of upper gastrointestinal tract bleeding in patients without hematemesis. Am J Emerg Med 2006; 24: 280-5.

Original Article

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Baseline demographic, clinical and laboratory risk factors for predicting admission to intensive care unit in patients diagnosed with COVID-19 in the emergency department

Bülent Güngörer

University of Health Sciences, Ankara City Hospital, Department of Emergency Medicine, Ankara, Turkey

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ABSTRACT

Aim: The COVID-19 pandemic has caused very significant morbidity and mortality throughout the world. Predicting the need for intensive care in these patients is important in terms of proper planning of health services and developing cost-effective management strategies. In this study, we sough to investigate the predictability of whether patients with COVID-19 would need intensive care by looking at some clinical, hematological and biochemical parameters.

Material and Method: All of the patients who applied to the adult emergency department of our hospital with the diagnosis of COVID-19 and were hospitalized were included in the study. The age, physical examination findings, comorbidities, and first laboratory parameters of the patients admitted to our hospital between March 2020 and June 2020 were retrospectively analyzed. A multivarible logistic regression model was constructed to determine the significant predictors of admission to ICU.

Results: A total of 1,005 patients were included in the study. Logistic regression analyses revealed that age (OR: 1.094, p<0.001), chronic renal failure (OR: 4.735, p=0.036), cancer diagnosis (OR: 3.957, p=0.021), higher levels of lactate dehydrogenase (OR: 1.006, p<0.001), and ferritin (OR: 1.001, p=0.001), and lower levels of lymphocyte count (OR: 0.879, p=0.021) were the independent risk factors in predicting the intensive care unit admission of the patients

Conclusion: Age, chronic renal failure, cancer, higher levels of LDH and ferritin, and lower levels of lymphocyte are found to be independent risk factors in predicting intensive care admission for patients admitted to the emergency department with the diagnosis of COVID-19.

Keywords: COVID-19, emergency department, intensive care unit

INTRODUCTION

The coronavirus 2019 disease (COVID 19) caused by a new strain of coronavirus, SARS-CoV 2, has caused a pandemic. It led to millions of people being hospitalized and being hospitalised in intensive care unit (1,2). For a long time, health systems caused serious problems in many countries due to lack of space and intensive care beds, and people were unable to access health services. Emergency services became the most important actor of this pandemic and had to decide which patients will have a poor prognosis and which patients will be treated as outpatients, and doing this in a very short time has become an important approach for the management of patients (3). In some studies, clinical clues such as the age of the patients, underlying diseases, some hematological parameters and radiological findings were investigated whether the patients should be hospitalized and whether they needed intensive care (4,5). During the pandemic in Turkey, especially large hospitals were accepted as pandemic hospitals and took an active role in the management of the pandemic. In this regard, *** has become a very important center in Turkey with the number of patients admitted, the number of beds and the number of intensive care units. We planned this retrospective study with the thought that with the decrease in the number of applications today, there are clues about which patients will have a poor prognosis and which patients may need intensive care, and it will be of great help to the clinician. With this research, we investigated the predictability of whether patients with COVID-19 would need intensive care by looking at some clinical, hematological and biochemical parameters.

Corresponding Author: Bülent Güngörer, gungorerbulent@gmail.com

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MATERIAL AND METHOD

The study was carried out with the permission of Ankara City Hospital No. 2 Clinical Researchs Ethics Committee (Date: 14.07.2021, Decision No: E2-21-739). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

All of the patients who applied to the adult emergency department with the diagnosis of COVID-19 and were hospitalized were included in the study. The ages, examination findings, comorbidities, and first laboratory parameters of the patients admitted between March 2020 and June 2020 were retrospectively analyzed. All hospitalized patients older than 18 years with positive polymerase chain reaction (PCR) tests were included. Patients whose laboratory findings were missing and clinical information could not be reached were excluded from the study. The patients were separated into two groups as those admitted to the normal service and those requiring intensive care, and compared in terms of these parameters. The patients' ages, pregnancy status, presence of hypertension, diabetes, coronary artery disease, cronic obstructive pulmonary disease, chronic kidney failure, cancer, immunodeficiency, pneumonia, and whether the patient was a healthcare worker were recorded. Hematological data of the patients, hemoglobin levels, white blood cell counts, lymphocyte count, platelet count were obtained from the hospital data system. Among the biochemical parameters, creatinine level, sodium, potassium, lactate dehydrogenase, erythrocyte sedimentation rate, c reactive protein, interleukin 6 level, procalcitonin level, ferritin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and troponin levels were recorded on patient follow-up forms.

Admission to intensive care unit was done according to current guidelines (6). Severe illness, severe respiratory tract infection (severe pneumonia; respiratory rate ≥30/min and/or severe respiratory distress (dyspnea, use of extra respiratory muscles) and/or oxygen saturation in room air ≤90%), hypotension (systolic blood pressure < 90 mmHg and more than 40 mmHg decrease from normal systolic blood pressure and mean arterial pressure < 65 mmHg, tachycardia > 100/min, troponin elevation, presence of skin disorders such as capillary return disorder and cutis marmaratus, acute respiratory distress dyndrome, sepsis, septic shock, myocarditis, arrhythmia and cardiogenic shock, metabolic acidosis, coagulation disorder and multiple organ failure.

Statistical Analysis

All statistical analysis entered to Stata computer based programme (version16.0 MP; StataCorp). The distribution of continuous variables was determined using Kolmogorov-Smirnov. Of the continuous data, those with normal distribution were presented as mean±standard deviation (SD), and data without normal distribution were presented as median (range). Categorical data were defined as the number of cases. Statistical analysis differences in variables those with normal distribution were compared.

Student's t-test was used for two different groups (ICU and non-ICU patients) with normal distribution, Mann-Whitney U test was used for data not normally distributed. Categorical variables were compared using Pearson's Chi square test. A univariate logistic regression model was constructed for each variable to show significant predictors of ICU admissions, and then those with $p<0.1$ values were tested in the multivariate logistic regression model. Odds ratios (ORs) and their 95% confidence intervals (CI) for ICU admission are presented. $p<0.05$ was considered significant in all statistical analyses.

RESULTS

A total of 1,005 patients were included in the study. While 847 patients were admitted to the normal inpatient service, 158 of them were hospitalised to the intensive care unit. The mean age of the patients admitted to the intensive care unit was higher than the patients admitted to the normal service (42.9±15.7 vs 67.3±14.5, $p<0.001$). There was no difference between the groups in terms of gender and pregnancy. Hypertension, diabetes, chronic obstructive pulmonary disease, chronic kidney failure, cancer, pneumonia and being a healthcare worker differed significantly between the groups. These data are summarized in **Table 1**.

When the laboratory data were compared between the two groups, it was seen that all other laboratory data were statistically different between the two groups, except for platelet, sodium and potassium levels. All these parameters are also summarized in **Table 2**.

Table 1. Baseline clinical and demographical findings of the study population

	Non-ICU (n=847)	ICU (n=158)	p-value
Age, mean (SD)	42.9 (15.7)	67.3 (14.5)	<0.001
Male, n (%)	438 (51.7%)	88 (55.7%)	0.36
Pregnancy, n (%)	16 (9.8%)	0 (0.0%)	0.083
Hypertension, n (%)	79 (9.3%)	48 (30.4%)	<0.001
Diabetes mellitus, n (%)	56 (6.6%)	19 (12.0%)	0.017
Chronic obstructive pulmonary disease, n (%)	16 (1.9%)	18 (11.4%)	<0.001
Coronary artery disease, n (%)	11 (1.3%)	4 (2.5%)	0.24
Chronic renal failure, n (%)	5 (0.6%)	7 (4.4%)	<0.001
Cancer, n (%)	9 (1.1%)	13 (8.2%)	<0.001
Immun deficiency, n (%)	2 (0.2%)	1 (0.6%)	0.40
Pneumonia, n (%)	359 (42.4%)	88 (55.7%)	0.002
Health-care workers, n (%)	44 (5.2%)	1 (0.6%)	0.011

Table 2. Initial laboratory findings of the study population

	Non-ICU (n=847)	ICU (n=158)	p-value
Hemoglobin, mean (SD)	13.8 (1.7)	12.7 (2.1)	<0.001
White blood cell, mean (SD)	5.6 (2.5)	7.5 (6.3)	<0.001
Neutrophil, mean (SD)	3.6 (2.1)	5.7 (4.9)	<0.001
Lymphocyte, median (IQR)	1.4 (1.0, 1.9)	0.9 (0.5, 1.2)	<0.001
Platelet, mean (SD)	226.8 (80.4)	217.9 (102.3)	0.23
Creatinine, mean (SD)	0.8 (0.5)	1.1 (0.7)	<0.001
Sodium, mean (SD)	139.1 (3.2)	138.6 (7.3)	0.15
Potassium, mean (SD)	4.1 (0.4)	4.0 (0.5)	0.19
Lactate dehydrogenase, median (IQR)	214.0 (181.8, 270.5)	331.3 (234.5, 424.0)	<0.001
C-reactive protein, median (IQR)	5.0 (1.1, 25.6)	86.9 (25.9, 165.0)	<0.001
Erythrocyte sedimentation rate, mean (SD)	24.9 (23.5)	52.5 (40.4)	<0.001
Interleukin-6, median (IQR)	8.1 (1.1, 37.9)	41.7 (12.9, 90.4)	<0.001
Procalcitonin, mean (SD)	0.1 (0.3)	0.4 (0.9)	<0.001
Ferritin, median (IQR)	112.9 (26.7, 293.4)	380.2 (187.0, 721.2)	<0.001
Alanine aminotransferase, mean (SD)	32.5 (29.5)	47.5 (74.5)	<0.001
Aspartate aminotransferase, mean (SD)	26.3 (20.9)	52.0 (61.8)	<0.001
Alkaline phosphatase, mean (SD)	73.3 (32.8)	91.5 (77.5)	<0.001
Troponin, median (IQR)	3.4 (0.4, 6.3)	13.1 (5.4, 20.7)	0.011

IQR: Interquartile Range, SD: Standard deviation

When we look at the results of the logistic regression analysis, it was seen that the clinical demographic parameters, age, chronic renal failure and cancer diagnosis, high lactate dehydrogenase and ferritin levels, and low lymphocyte count were the only independent risk factors in predicting the intensive care unit admission of the patients (**Table 3**). The bars showing the relationship between age and gender distribution and the intensive care unit are presented in **Figure 1**, and the trends of LDH, lymphocyte count and ferritin levels are presented in **Figure 2A, 2B, 2C**.

Table 3. Predictors of ICU patients according to multivariable logistic regression analysis

	Adjusted odds ratio (95% confidence interval)	p value
Age	1.094 (1.077-1.112)	<0.001
Chronic renal failure	4.735 (1.117-20.067)	0.036
Cancer	3.957 (1.225-12.776)	0.021
Lactate dehydrogenase	1.006 (1.003-1.008)	<0.001
Lymphocyte	0.879 (0.789-0.980)	0.021
Ferritin	1.000 (1.000-1.001)	0.001

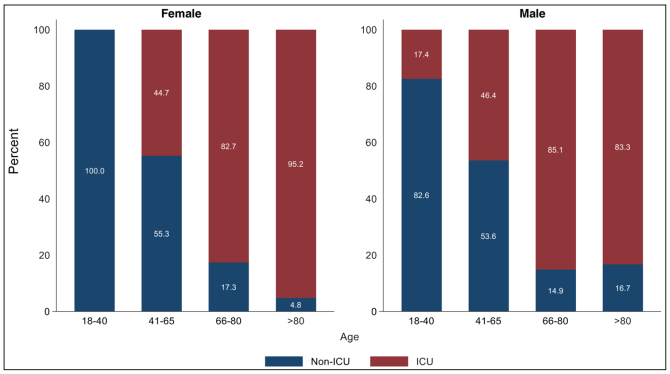

Figure 1. Non-intensive care unit (ICU) vs ICU patients' distributions according to sex and age subgroups

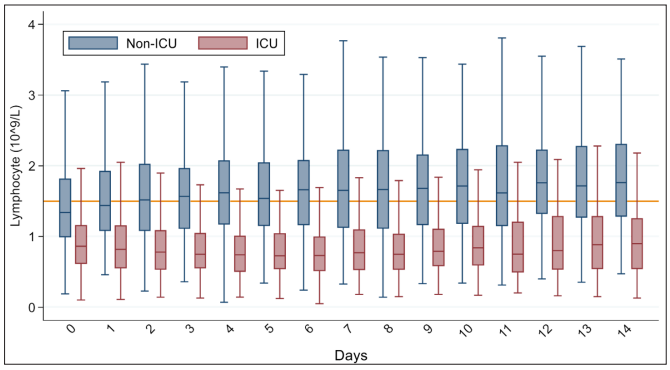

Figure 2B. Dynamic trends of Lymphocyte count according to Non-intensive care unit (ICU) and ICU patients up to 14-days

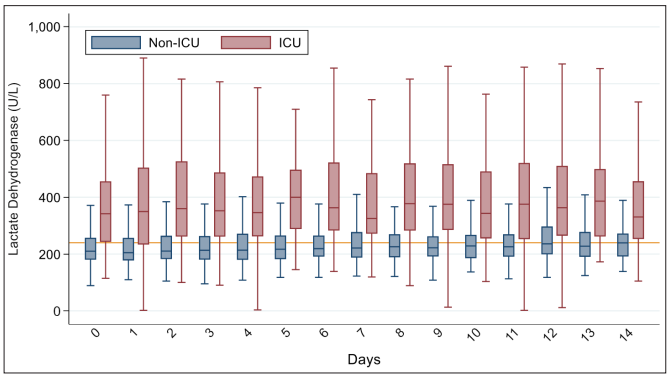

Figure 2A. Dynamic trends of Lactate Dehydrogenase according to Non-intensive care unit (ICU)and ICU patients up to 14-days

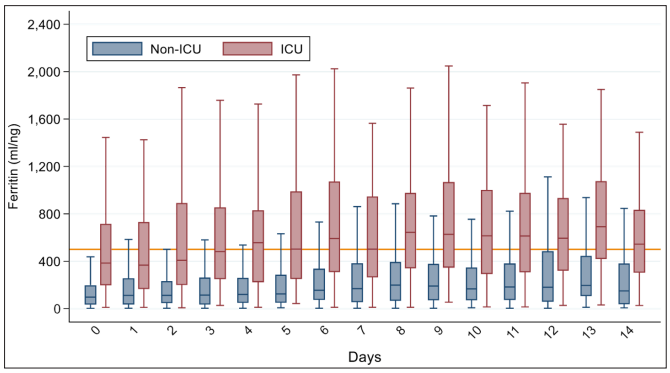

Figure 2C. Dynamic trends of Ferritin according to Non-intensive care unit (ICU) and ICU patients up to 14-days

DISCUSSION

According to the results of this study, age, additional morbidities such as chronic kidney failure and cancer, and laboratory data, high lactate dehydrogenase and ferritin and low lymphocyte count alone are independent risks in predicting whether patients who are admitted to the emergency department with the diagnosis of COVID-19 and hospitalized will need intensive care or not. factor was found. The fact that it included a fairly sufficient number of patients and that different parameters were found to be independent risk factors in predicting intensive care admission are the peculiarities of our study.

In the literature, there are different studies on the parameters taken during the intensive care unit admission of COVID-19 patients. In some studies, diabetes and HbA1c are predictive, and some studies have shown that pneumonia and chronic lung problems are important in ICU admission (7,8). Age is also seen in the studied and published data among these parameters. Many parameters can be used to decide on the hospitalization of patients in adverse situations such as in cases where there are too many patient admissions, the number of beds is insufficient, and the limited usage conditions. Some of these can be added as troponin, lung radiology and clinical findings (8,9). Although clinical findings are at the forefront in intensive care admission, it may be useful to look at additional parameters and make decisions based on independent risk factors in places with limited number of beds. For this reason, the results of our study can support healthcare professionals serving the emergency department in their decision-making and can be used during practical applications in predicting intensive care admission.

In the study of Luo et al. (10), age, neutrophil and platelet count were found to be significantly correlated with CRP and severe disease and poor prognosis. Similarly, in the study of Marin et al. (11), increased age was shown to be associated with lymphopenia and chronic renal failure suffocation, mortality and poor prognosis. In the same study, an increase in LDH, which indicates cellular injury, is associated with mortality and poor prognosis. Covino et al. (12) similar to other studies in the literature, it was shown that advanced age is closely related to intensive care unit admission and mortality. Wu et al. (13) found a close relationship between ferritin level and the development of ARDS in patients with COVID-19 pneumonia. Similarly, Şan et al. (14) found that the complete blood count parameters can help to identify and classify COVID-19 patients into non-severe to severe groups.

In the study conducted by Carlino et al. (5), a significant relationship was found between patients with a history of cancer and admission to intensive care unit. In the study of Hu et al. (15), no significant difference was found between comorbidities (anemia, cancer, hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, cerebral infarction) and serious disease. We attributed this situation to the small number of patients included, unlike our study. Lee et al. (16) in his study, a significant correlation was found between patients with a history of lymphopenia, cancer, and chronic renal failure and mortality. Similarly, Bhargava et al. (17) found a significant relationship between chronic renal failure and severe disease in their study.

This study may have some limitations, one of them being a retrospective study, but the sufficient number of cases is an important factor in closing this gap. The high number of patients in our hospital, the high number of emergency service applications and the large bed capacity can be counted as the strengths of our study.

CONCLUSION

This study revealed that age, chronic renal failure, and cancer are the only independent risk factors in predicting intensive care admission for patients admitted to the emergency department with the diagnosis of COVID-19, and that high LDH, ferritin levels, and low lymphocyte count are laboratory independent risk factors.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No. 2 Clinical Research Ethics Committee (Date: 14.07.2021, Decision No: E2-21-739).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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REFERENCES

1. Sahin D, Tanacan A, Erol SA, et al. A pandemic center's experience of managing pregnant women with COVID-19 infection in Turkey: A prospective cohort study. Int J Gynecol Obstetr 2020; 151: 74-82.
2. Immovilli P, Morelli N, Antonucci E, Radaelli G, Barbera M, Guidetti D. COVID-19 mortality and ICU admission: the Italian experience. Critical Care 2020; 24: 1.

3. Walton H, Navaratnam AV, Ormond M, Gandhi V, Mann C. Emergency medicine response to the COVID-19 pandemic in England: a phenomenological study. Emerg Med J 2020; 37: 768-72.
4. Assandri R, Buscarini E, Canetta C, Scartabellati A, Viganò G, Montanelli A. Laboratory biomarkers predicting COVID-19 severity in the emergency room. Arch Med Res 2020; 51: 598-9.
5. Carlino MV, Valenti N, Cesaro F, et al. Predictors of intensive care unit admission in patients with Coronavirus disease 2019 (COVID-19). Monaldi Arch Chest Dis 2020; 9.
6. https://COVID19.saglik.gov.tr/Eklenti/40781/0/COVID-9rehberiagirpnomoniardssepsisveseptik sokyontemipdf.pdf
7. Prattichizzo F, de Candia P, Nicolucci A, Ceriello A. Elevated HbA1c levels in pre-COVID-19 infection increases the risk of mortality: a sistematic review and meta-analysis. Diabetes Metab Res Rev 2021: e3476.
8. Zhou Y, Yang Z, Guo Y, et al. A new predictor of disease severity in patients with COVID-19 in Wuhan, China. MedRxiv 2020.03.24.20042119.
9. Nie SF, Yu M, Xie T, et al. Cardiac troponin I is an independent predictor for mortality in hospitalized patients with COVID-19. Circulation 2020; 142: 608-10.
10. Luo X, Zhou W, Yan X, et al. Prognostic value of C-reactive protein in patients with Coronavirus 2019. Clin Infect Dis 2020; 71: 2174-9.
11. Gallo Marin B, Aghagoli G, Lavine K, et al. Predictors of COVID-19 severity: a literature review. Rev Med Virol 2021; 31: 1-10.
12. Covino M, Sandroni C, Santoro M, et al. Predicting intensive care unit admission and death for COVID-19 patients in the emergency department using early warning scores. Resuscitation 2020; 156: 84-91.
13. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020; 180: 934-43.
14. Şan İ, Gemcioğlu E, Davutoğlu M, et al. Which hematological markers have predictive value as early indicators of severe COVID-19 cases in the emergency department? Turk J Med Sci 2021; 10.3906/sag-2008-6.
15. Hu H, Du H, Li J, et al. Early prediction and identification for severe patients during the pandemic of COVID-19: A severe COVID-19 risk model constructed by multivariate logistic regression analysis. J Glob Health 2020; 10: 020510.
16. Lee J, Park SS, Kim TY, Lee DG, Kim DW. Lymphopenia as a biological predictor of outcomes in COVID-19 patients: a nationwide cohort study. Cancers 2021; 26; 13: 471.
17. Bhargava A, Fukushima EA, Levine M, et al. Predictors for severe COVID-19 infection. Clin Infect Dis 2020; 71: 1962-8.

Original Article

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Blood gas analysis syringes containing spray-dosed droplet liquid heparin may decrease sample rejection ratios

Kamil Taha Uçar[1], Nilhan Nurlu[2]

[1]Bilecik Public Health Laboratory, Medical Biochemistry, Bilecik, Turkey
[2]İstanbul Gaziosmanpaşa Training and Research Hospital, Medical Biochemistry, İstanbul, Turkey

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ABSTRACT

Aim: The objective of this study is to evaluate blood gas analysis (BGA) sample rejection ratios (SRRs) in our laboratory and investigate the effect of various BGA syringes on SRR.

Material and Method: 3 groups were formed based on the type and use period of BGA syringes. Syringes containing spray-dosed droplet liquid Lithium Heparin were used in Group 1 (November 2018–May 2019), syringes containing lyophilized dried Lithium Heparin were used in Group 2 (July 2019–January 2020), and another syringes containing spray-dosed droplet liquid Lithium Heparin were used in Group 3 (March 2020–September 2020), and the groups were determined based on such use. SRRs of these groups were calculated, causes for sample rejection were identified, and department-based investigations were conducted. Comparisons between groups were performed according to the indicated variables.

Results: Mean SRRs of the groups by percentage (%) were calculated as 6.1±1.5, 10.0±0.9, and 3.8±0.9, respectively, and showed a statistically significant difference ($p<0.001$). Based on the post-hoc Scheffé's test, a lower SRR was calculated in Group 3 ($P<0.05$). The most frequent causes for sample rejection by percentage were found as clotted sample (73.4±10.7), insufficient sample (14.7±9.1), and inappropriate (nonconforming) sample (5.5±2.0). No statistically significant difference was observed based on the causes for sample rejection among the groups. Based on the frequency of SRRs by percentage, the departments were determined as the Emergency Department (ED) (44.3±11.6), Intensive Care Unit (ICU) (22.2±6.5), and Pediatric & Neonatal Emergency Department (PNED) (16.8±6.6). For department-based results among the groups, SRRs for ED and PNED were found to be higher in terms of statistical significance in Group 2 whereas a lower SRR for ICU was determined in Group 3 ($P<0.05$).

Conclusion: It was observed that BGA syringes containing spray-dosed droplet liquid Lithium Heparin decreased SRRs. Therefore, SRR follow-up may help clinics and laboratories evaluate sample quality as well as developing solutions.

Keywords: Blood gas analysis, sample rejection ratio, preanalytical phase, blood collection device, lithium heparin

INTRODUCTION

Blood gas analysis (BGA) is of vital importance for emergency departments (EDs) and intensive care units (ICUs) as it provides highly accurate results for oxygenation, acid-base equilibrium, and electrolyte levels of patients under life-threatening emergency conditions within a short period (1,2). Fast and accurate results as provided by BGA may allow for faster diagnosis and treatment opportunities (3). Based on the evaluation of the Total Testing Process (TTP) of BGA, it was reported that the most problematic part was the preanalytical (prior to analysis) phase (1,4). In addition to this, it can be concluded that preanalytical phase for BGA is more vulnerable in general and poses a higher threat to patient safety as it often involves sampling and use of analyzers by non-laboratory staff (5,6).

It was reported that sample rejection ratios (SRRs) of BGA particularly in case of use under emergency conditions and for samples received from EDs were higher than those of outpatient/inpatient departments (7,8). Additionally, BGA collection is technically challenging for the phlebotomy staff and may also be painful for the patient from whom the blood sample is collected (9). Therefore, the requirement for resampling in addition to causing a delay in the diagnosis and treatment opportunities in case of BGA sample rejection can be troublesome (10,11). Moreover, other problems such as obtaining inaccurate results or equipment failure may also arise due to lack to identify inappropriate BGA samples (e.g., micro clots, air bubbles, etc.) (12,13). Considering the fact that such equipment is also frequently used by non-

Corresponding Author: Kamil Taha Uçar, drktahaucar@hotmail.com

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laboratory staff, e.g., during the quality assessment of samples and equipment maintenance, etc., this process may turn into a difficult challenge for staff who works in an intensive environment under emergency conditions (6). Any failure to provide accurate BGA results in a timely manner may pose a potential threat to patient safety (11). For all such reasons, it is crucial for BGA to use quality indicators (QI), calculate SRRs, and perform routine evaluations (14).

Literature review results in a considerable number of clinical evaluations in relation to BGA. These studies evaluated comparative results among BGA syringes of various manufacturers (15-18), made comparisons between BGA results and clinical biochemical analyzers results (19,20), investigated the effects of preanalytical conditions on clinical results (21) as well as making comparisons among various BGA analyzers (22,23). In addition, the effect of hemolysis on BGA was investigated in particular (24–28). Moreover, quality indicators (QI) and SRRs in relation to BGA were also evaluated in a smaller number of studies (10,14,29,30). It was reported that there was a direct relationship between the sample quality and blood collection devices used, and BGA samples were among the types of samples determined as the most vulnerable to such effect (31). The authors were unable to access any publication in which SRR causes for BGA were investigated in detail, including sample quality assessment in connection with blood collection devices, and discussion of recommendations for solutions.

Our objective in this study is to investigate the TTP for BGA in our laboratory based on SRRs as well as conducting a department-based investigation of SRRs, and a comparison of blood gas analysis (BGA) syringes of various types (i.e., dried, or sprayed liquid lithium heparin) used in the routine procedure in different periods.

MATERIAL AND METHOD

This observational ambispective study was conducted including BGA samples accepted to the laboratory during the period between November 2018–September 2020 in line with the Declaration of Helsinki (1964) following the approval of the Ethics Committee of Gaziosmanpasa Training and Research Hospital (Date: 31.03.2021, Decision No: 247/2021).

Study Design and Data Collection Process

Due to a significant increase in BGA SRRs subject to monthly follow-up in our laboratory, it was determined to perform a root cause analysis. For that purpose, the root causes for such an increase in SRRs were investigated and it was found that BGA syringes were replaced. In order to perform a statistical evaluation for such replacement, two separate groups were determined for the previous and the current BGA syringes taking the time of replacement of BGA syringes as the baseline, and SRRs were calculated accordingly. It was determined that BGA syringes used prior to replacement were contained 50 I.U. spray-dosed droplet liquid Lithium Heparin Calcium Balanced/ml of blood whereas the new BGA syringes used were contained 72 I.U. lyophilized dried Lithium Heparin Calcium Balanced/ml of blood.

Since the most significant cause of the increase in SRRs was determined as the replacement of BGA syringes based on the root cause analysis, BGA syringes were replaced by new syringes. For new ones, syringes that contained 72 I.U. spray-dosed droplet liquid Lithium Heparin Calcium Balanced/ml of blood were selected. During the replacement of BGA syringes, the relevant nurses, and authorized staff responsible for blood collection as well as EDs and ICUs were contacted and necessary information on the subject matter was provided accordingly.

Two BGA analyzers are available in our hospital and they are in our emergency laboratory. The selected analyzers consists of two systems, namely, Siemens RAPIDLab 1200 (Siemens Healthcare, Camberley, UK) and Siemens RAPIDPoint 405 (Siemens Healthcare, Camberley, UK). BGA collection and transfer of samples are provided by non-laboratory staff (i.e., physicians, physician residents, and nurses for blood collection, and assigned department staff for transfer of samples) whereas processing of samples as well as the evaluation and reporting of the results were performed by laboratory technicians and specialists. During this period, approximately 5000 BGA samples/month on average were processed in our laboratory. Most of these samples were received from the ED and the ICU. The samples received by the laboratory mainly contain arterial BGA samples; however, venous BGA samples are preferred in patients at our outpatient clinics. In this study, no differentiation was made for the types of BGA samples collected.

Sample Rejection Causes Evaluation

As in most of the laboratories in Turkey, our laboratory also follows the recommendations published by the Ministry of Health of the Republic of Turkey for the purpose of SRR evaluation (32). Out of such criteria, the most frequently encountered 10 criteria for sample rejection as applicable to BGA evaluation were listed below:

1. Clotted sample;
2. Insufficient sample;
3. Inappropriate sample (e.g., air bubbles, micro clots, etc.);
4. Blank (empty) sample;
5. Prolonged storage time (>30 min. at room temperature);

6. Wrong barcode;
7. Wrong sample container;
8. Rejection resulting from equipment failure;
9. Rejection resulting from laboratory technician failure;
10. Samples delivered under nonconforming transport conditions (e.g., samples coming into any direct contact with ice, samples delivered using pneumatic systems, etc.).

Since BGA syringes were replaced in June 2019, an SRR evaluation vs. Group 2 was conducted as a reference period to ensure that the periods during which the BGA syringes have remained in use were equal. June 2019 was considered as a familiarization period and the monthly SRRs during the period between July 2019 and January 2020 were included in the calculation. Since the second syringes remained in use for 7 months, the evaluation period for Group 1 was determined as the period between November 2018 and May 2019. Finally, February 2020 was considered as a familiarization period since the use of BGA syringes containing spray-dosed droplet liquid lithium heparin was reintroduced in February 2020, and the period between March 2020 and September 2020 was determined as the evaluation period for Group 3.

SRRs of all these groups were obtained from the Laboratory Information System (ALIS, Ventura, Turkey). SRRs, causes for sample rejection, and SRRs based on the departments were calculated. Following the monthly calculation of SRRs, the mean SRRs of the groups were compared against each other. In addition, the causes of sample rejection were also compared among these 3 groups. Finally, department based SRRs were calculated and compared among 3 groups.

Statistical Analysis

For comparative analysis of the groups, compliance to normal distribution was determined by the Shapiro-Wilk test. The results were presented in the form of mean±standard deviation (SD) since the groups comply with a normal distribution and comparisons among the groups were conducted by the one-way analysis of variance (ANOVA). A post-hoc Scheffé's test was conducted to determine differences among the groups since the variances of the groups were equal among such groups. The level of statistical significance was determined as $p<0.05$ (two-way). Microsoft Office 365 (Microsoft Excel Software, Microsoft Corporation, USA) and MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium) software were used to create tables and charts as well as conducting statistical analyses.

RESULTS

Mean SRRs of 3 groups by percentage (%) were calculated as 6.1±1.5, 10.0±0.9, and 3.8±0.9, respectively. The one-way analysis of variance (ANOVA) resulted in a statistically significant difference ($p<0.001$). Based on the post-hoc Scheffé's test, it was found that each group showed statistically significant differences from one another, and a lower SRR was calculated in Group 3 as compared to other groups (vs. Group 1 $p=0.005$, vs Group 2 $p<0.001$). The most frequent causes for sample rejection by percentage for each group were found as clotted sample (73.4±10.7), insufficient sample (14.7±9.1), and inappropriate sample (5.5±2.0), respectively. No statistically significant difference was observed among the causes for sample rejection among the groups with the replacement of BGA syringes. Based on the frequency of SRRs among total SRRs by percentage, the departments were determined as the ED (44.3±11.6), ICU (22.2±6.5), and Paediatric & Neonatal Emergency Department (PNED) (16.8±6.6), respectively. For department-specific results among the groups, SRRs for ED and PNED were found to be higher in terms of statistical significance in Group 2 (for ED; vs. Group 1 $p<0.001$, vs Group 3 $p<0.001$, and for PNED; vs. Group 1 $p=0.006$, vs Group 3 $p<0.001$) whereas a lower SRR for ICU samples was determined in Group 3 (vs. Group 1 $p=0.005$, vs Group 2 $p<0.001$).

Mean SRRs and causes of sample rejection for each group are presented in **Table 1**, and mean SRRs based on the department-specific are presented in **Table 2**. Frequencies of sample rejection causes and departments within total SRR based on the frequency of SRR during the evaluation period are found in **Figure 1**, and **Figure 2**, respectively. Total SRRs and mean SRR differences among the groups based on causes for sample rejection and departments are illustrated in **Figures 3**, **4**, and **5**, respectively.

Table 1. Frequency of sample rejection causes (%) and total sample rejection ratios (%) by groups

Groups	Clotted samples	Insufficient samples	Inappropiate samples	Other causes	Total SRR*(%)
1	71.7±14.5	18.8±12.1	4.4±1.9	4.4±2.8	6.1±1.5
2	79.4±5.5	9.88±2.4	5.7±2.3	4.4±2.4	10.0±0.9
3	69.2±8.8	15.4±8.8	6.4±1.3	7.9±3.1	3.8±0.9

Data (%) are presented as mean and SD. Group 1: Between November 2018 and May 2019, used spray-dosed droplet liquid Lithium Heparin BGA syringes, Group 2: Between June 2019 and January 2020, used lyophilized dried Lithium Heparin BGA syringes, and Group 3: Between March 2020 and September 2020, used spray-dosed droplet liquid Lithium Heparin BGA syringes. SRR: Sample rejection ratio *Total SRRs in 3 groups were found to be statistically significantly different from each other ($p<0.05$).

Table 2. Sample rejection ratios of departments (%) by groups

Groups	Emergency department (ED)	Pediatric and neonatal emergency department (PNED)	Intensive care unit (ICU)
1	6.0±2.0	6.3±1.9	5.2±1.8
2	13.7±2.6*	9.5±1.5*	5.4±0.4
3	3.9±1.2	4.6±1.5	2.6±1.0**

Since the number of samples from other departments was insufficient for data comparison, three departments with the most frequent sample rejection ratios were evaluated. Data (%) are presented as mean and SD. Group 1: Between November 2018 and May 2019, used spray-dosed droplet liquid Lithium Heparin BGA syringes, Group 2: Between June 2019 and January 2020, used lyophilized dried Lithium Heparin BGA syringes, and Group 3: Between March 2020 and September 2020, used spray-dosed droplet liquid Lithium Heparin BGA syringes *It is statistically significantly higher than the other groups ($p<0.05$) **It is statistically significantly lower than the other groups ($p<0.05$)

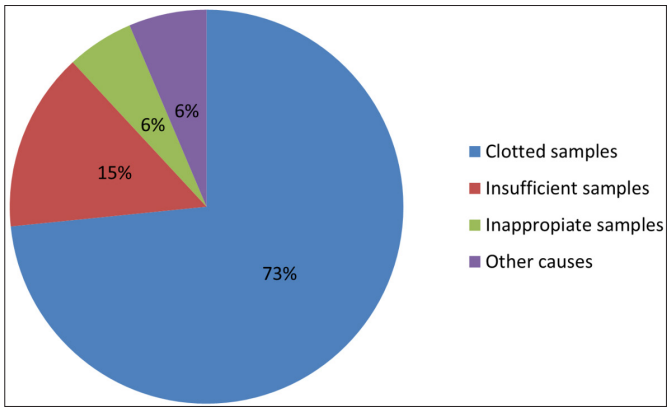

Figure 1. The frequencies (%) of sample rejection causes

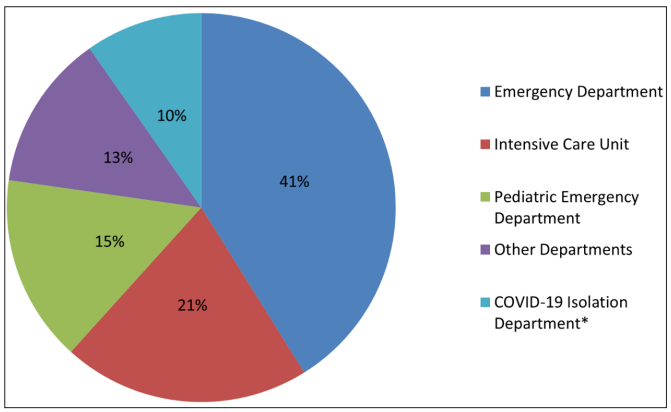

Figure 2. The frequencies (%) of services sample rejection ratios in total sample rejection ratios.
* The COVID-19 Isolation Department worked only in the 3rd group period.

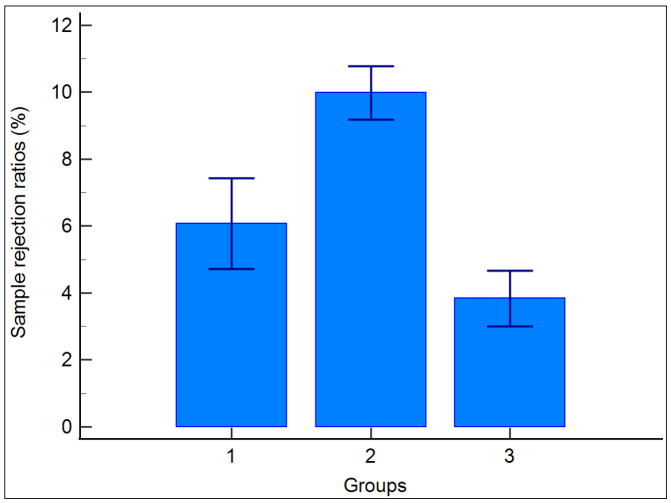

Figure 3. Mean and 95% CI of total sample rejection ratios (%) for groups are presented. Group 1: Between November 2018 and May 2019, used spray-dosed droplet liquid Lithium Heparin BGA syringes, Group 2: Between June 2019 and January 2020, used lyophilized dried Lithium Heparin BGA syringes, and Group 3: Between March 2020 and September 2020, used spray-dosed droplet liquid Lithium Heparin BGA syringes.

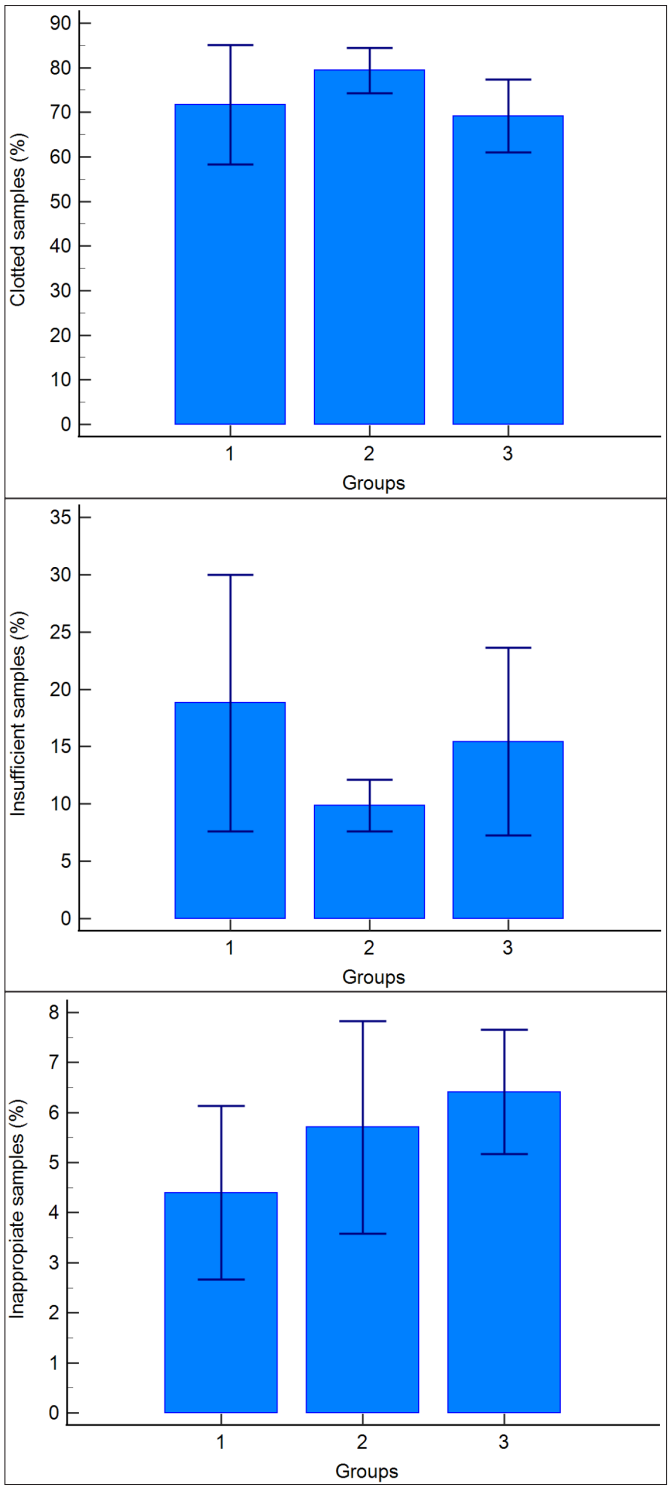

Figure 4. The mean and 95% CI of the sample rejection ratios (%) according to the causes in the groups are presented. Group 1: Between November 2018 and May 2019, used spray-dosed droplet liquid Lithium Heparin BGA syringes, Group 2: Between June 2019 and January 2020, used lyophilized dried Lithium Heparin BGA syringes, and Group 3: Between March 2020 and September 2020, used spray-dosed droplet liquid Lithium Heparin BGA syringes.

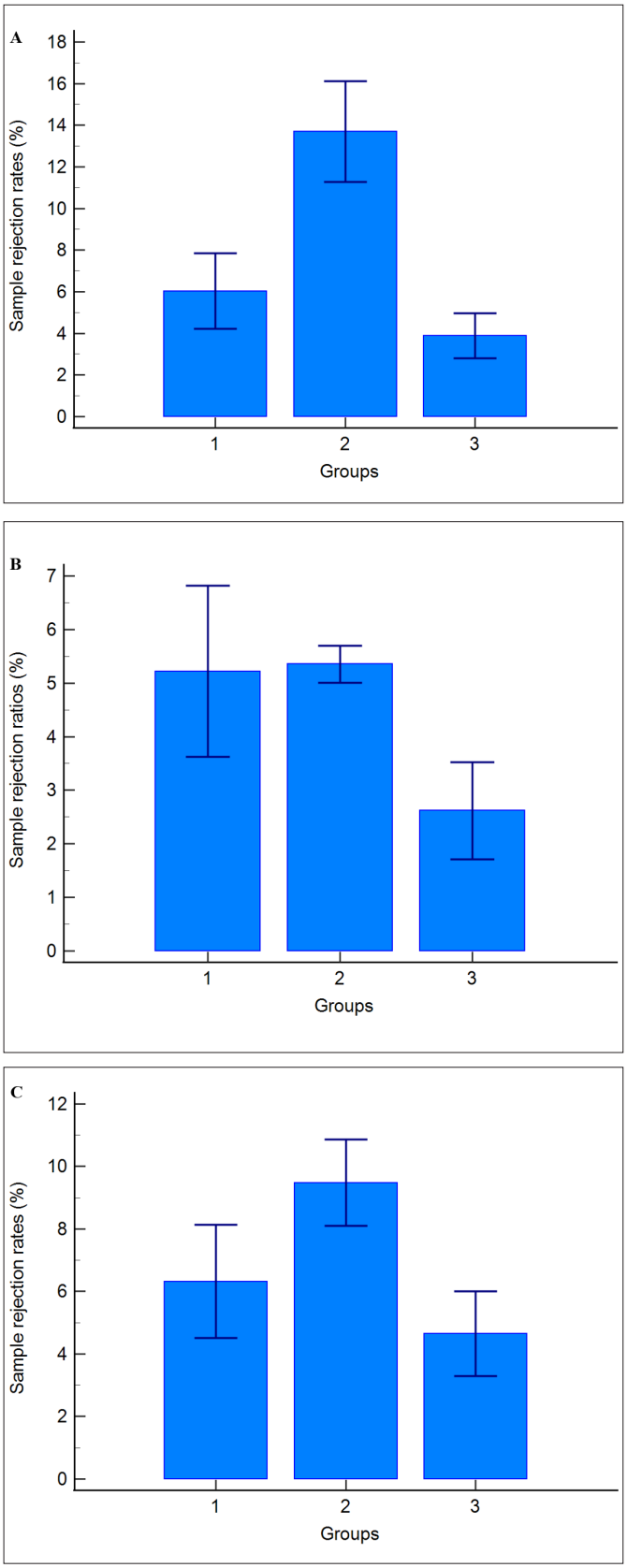

Figure 5. The mean and 95% CI of the sample rejection ratios (%) according to the services in the groups are presented. A: Emergency department, B: Intensive care unit, C: Paediatric and Neonatal Emergency department. Group 1: Between November 2018 and May 2019, used spray-dosed droplet liquid Lithium Heparin BGA syringes, Group 2: Between June 2019 and January 2020, used lyophilized dried Lithium Heparin BGA syringes, and Group 3: Between March 2020 and September 2020, used spray-dosed droplet liquid Lithium Heparin BGA syringes.

DISCUSSION

In our study, a decrease in total BGA SRRs with the use of BGA syringes containing spray-dosed droplet liquid Lithium Heparin was indicated. Such a decrease is considered to be associated with the ability of liquid heparin to mix with whole blood much better compared to lyophilized dried heparin. It is assumed that anticoagulant activity in whole blood samples is crucial for sample quality and such activity may be enhanced in line with the ability to mix well with whole blood. On the other hand, no significant difference was found in SRRs in terms of the type of BGA syringes and the causes for sample rejection. As a result of the evaluation of department specific SRRs, a decrease in SRRs for BGA samples received from ED, PNED, and ICU after the replacement of BGA syringes was observed.

Kume et al. (33) found that BGA SRR as 17% and reported that the most frequent cause for BGA sample rejection consisted of clotted samples. Similarly, during a 1-year SRR follow-up, Dikmen et al. (8) found BGA SRR as 9.2% and reported that the most frequent two causes of sample rejection were clotted samples and insufficient samples, respectively. In our study, it was indicated that an SRR higher than 10% was found only in Group 2 and this was in fact associated with the specifications of the BGA syringe. Cantero et al. (14) did not provide any total SRR in their study; however, they found that the sample rejection ratio of the samples rejected due to clotted BGA samples or equipment failure to total accepted samples was 15.8% for the point-of-care testing (POCT) analyzer in the Neonatal unit and 3.3% in the Central lab. In addition, they also reported that the ratios for insufficient samples for the two groups were 2.9% and 0.9%, respectively. Similarly, Oliver et al. (10) did not provide any specific total SRR; however, they reported that BGA SRR specific to each department would be expected to be less than 10% . Accordingly, they reported that only BGA SRRs of the samples received from the Delivery room exceeded 10% during certain months and the most frequent causes for sample rejection were often associated with the preanalytical phase. Moreno et al. (30) calculated SRRs for BGA samples associated with the preanalytical phase as 2.3-4.2%. In the same study, they indicated that POCT SRRs were higher (Central lab: 2.6%, POCT: 4.2%) for the first year, during which SRRs in the Central lab and the POCT in the Nephrology department were evaluated, whereas there was no statistically significant difference between two groups for the second year (Central lab: 2.8%, POCT: 2.3%). On the other hand, O'Kane et al. (29) stated that the total SRR for BGA samples was 0.52% and reported that 77.5% of this SRR was associated with equipment failures. In our study, the most frequent causes for sample rejection were found as

clotted, inappropriate, and insufficient samples resulting from preanalytical processes, similar to the findings of the studies except for the study conducted by O'Kane et al. (29) It was assumed that the difference between the findings of O'Kane et al. (29) and our findings was due to the differences between the two studies in terms of the QIs and the method for SRR follow-up.

In fact, there are studies on the follow-up of BGA/POCT processes (4,5,12,34). Nevertheless, a generally accepted quality specification approach is not available since the QIs evaluated in TTP could not be efficiently aligned with BGA/POCT processes (14). For these reasons, it is considered that laboratories may not effectively carry out the follow-up of such processes. Future studies particularly focusing on BGA/POCT processes are considered to have the potential to contribute to this area and the safety of patients, physicians, and laboratories in general can be improved as a result. BGA and POCT processes are not exclusively controlled by laboratories (1). For this reason, follow-up, and evaluation of the preanalytical phase, which is the most vulnerable stage of TTP, can be more challenging for BGA and POCT (5). In this context, the organization of activities, symposiums, and work groups, involving other physicians, phlebotomists, and nurses, in addition to the laboratory staff as well as publishing guidelines can be beneficial for the determination of problems and finding possible solutions (6,13).

For the laboratory quality management procedures, all aspects are required to be addressed with an integrated approach (35). Both root cause analysis for sample rejection and clinical evaluations makes a great contribution to finding solutions to the problems (36). At this point, it should not be overlooked that blood collection systems are one of the key variables of this process (37). Blood collection systems and sets should be ergonomic and easy to use with all types of samples and for BGA/POCT samples in particular (1). In this way, it is possible to reduce the number of inappropriate samples causing sample rejection (38). In this context, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)-Working Group for Preanalytical Phase (WG-PRE), in an opinion paper, suggested that laboratories should also perform technical evaluations for blood collection systems (39). Especially in smaller laboratories, such technical evaluations may not be possible under routine conditions. The authors suggest that technical evaluations may be carried out using SRR follow-up. A close follow-up for SRRs could significantly contribute to laboratories in the evaluation of sample quality and blood collection systems.

This study had certain restrictions. Firstly, taking into consideration the turnaround time change in the BGA process within the relevant period in addition to calculation and comparison of SRRs as well as technical service and maintenance requirements for BGA analyzer may allow for more accurate results. In addition, since the evaluation of hemolysis in BGA samples is challenging in routine procedure, it was excluded from this study. However, since hemolysis is one of the most frequent causes for inappropriate samples and spurious hemolysis is common especially in BGA samples, it is considered useful to evaluate BGA samples with hemolysis and take necessary steps applicable in routine procedure.

CONCLUSION

Our study demonstrated that SRRs decreased following the use of syringes containing spray-dosed droplet lithium heparin instead of those containing lyophilized dried lithium heparin. In addition to clinical evaluations, technical evaluations of blood collection systems used may empower laboratories in quality management. Routine SRR follow-up is considered as one of the key tools to help laboratories determine the causes and find solutions for inappropriate/nonconforming samples.

ETHICAL DEVCLARATIONS

Ethics Committee Approval: To conduct the study, Ethics Committee of Gaziosmanpasa Training and Research Hospital (Date: 31.03.2021, Decision No: 247/2021).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Lippi G, Baird GS, Banfi G, et al. Improving quality in the preanalytical phase through innovation, on behalf of the European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). Clin Chem Lab Med 2017; 55: 489–500.
2. Lippi G, Panteghini M, Bernardini S, et al. Laboratory testing in the emergency department: An Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) and Academy of Emergency Medicine and Care (AcEMC) consensus report. Clin Chem Lab Med 2018; 56: 1655–9.
3. Davis MD, Walsh BK, Sittig SE, Restrepo RD. AARC clinical practice guideline: blood gas analysis and hemoximetry: 2013. Respir Care 2013; 58: 1694–703.

4. Baird G. Preanalytical considerations in blood gas analysis. Biochem Medica 2013; 23: 19–27.
5. Shaw JLV. Practical challenges related to point of care testing. Pract Lab Med 2016; 4: 22–9.
6. Quig K, Wheatley EG, O'Hara M. Perspectives on blood-based point-of-care diagnostics. Open Access Emerg Med 2019; 11: 291–6.
7. Aykal G, Keşapli M, Aydin Ö, et al. Pre-test and post-test applications to shape the education of phlebotomists in a quality management program: An experience in a training hospital. J Med Biochem 2016; 35: 347–53.
8. Gunnur Dikmen Z, Pinar A, Akbiyik F. Specimen rejection in laboratory medicine: Necessary for patient safety? Biochem Medica 2015; 25: 377–85.
9. CLSI. Procedures for the collection of diagnostic blood specimens by Venipuncture; approved standard—sixth edition. CLSI document GP41-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
10. Oliver P, Fernandez-Calle P, Mora R, et al. Real-world use of key performance indicators for point-of-care testing network accredited by ISO 22870. Pract Lab Med 2020; 22.
11. Lippi G, Betsou F, Cadamuro J, et al. Preanalytical challenges-time for solutions. Clin Chem Lab Med 2019; 57: 974–81.
12. Dukić L, Kopčinović LM, Dorotić A, Baršić I. Blood gas testing and related measurements: National recommendations on behalf of the Croatian society of medical biochemistry and laboratory medicine. Biochem Medica 2016; 26: 318–36.
13. Peck Palmer OM, Wheeler SE, Plebani M, Patterson PD, Korpi-Steiner NL, Martin C. Recognition of the prehospital preanalytical phase: collaborative efforts between laboratory medicine and emergency medicine to ensure quality testing. Clin Chem 2020; 66: 998–1005.
14. Cantero M, Redondo M, Martín E, Callejón G, Hortas ML. Use of quality indicators to compare point-of-care testing errors in a neonatal unit and errors in a STAT central laboratory. Clin Chem Lab Med 2015; 53: 239–47.
15. Tang NY, Leanse JH, Tesic V, van Wijk XMR. A comparison of venous blood gas analysis with paired specimens collected in syringes and evacuated blood collection tubes. Clin Chim Acta 2020; 510: 671–4.
16. Uyanik M, Sertoglu E, Kayadibi H, et al. Comparison of blood gas, electrolyte and metabolite results measured with two different blood gas analyzers and a core laboratory analyzer. Scand J Clin Lab Invest 2015; 75: 97–105.
17. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Different manufacturers of syringes: A new source of variability in blood gas, acid-base balance and related laboratory test? Clin Biochem 2012; 45: 683–7.
18. Gruber MA, Felbermeir S, Lindner R, Kieninger M. Preanalytics: The (in-)stability of volatile POCT parameters and the homogeneity of blood in syringes at the market. Clin Chim Acta 2016; 457: 18–23.
19. Triplett KE, Wibrow BA, Norman R, et al. Can the blood gas analyser results be believed? A prospective multicentre study comparing haemoglobin, sodium and potassium measurements by blood gas analysers and laboratory auto-analysers. Anaesth Intensive Care 2019; 47: 120–7.
20. Oyaert M, Van Maerken T, Bridts S, Van Loon S, Laverge H, Stove V. Analytical and pre-analytical performance characteristics of a novel cartridge-type blood gas analyzer for point-of-care and laboratory testing. Clin Biochem 2018; 53: 116–26.
21. Küme T, Şişman AR, Solak A, Tuğlu B, Çinkooğlu B, Çoker C. The effects of different syringe volume, needle size and sample volume on blood gas analysis in syringes washed with heparin. Biochem Medica 2012; 22: 189–201.
22. Mohammed-Ali Z, Bagherpoor S, et al. Performance evaluation of all analytes on the epoc® Blood Analysis System for use in hospital surgical and intensive care units. Pract Lab Med 2020; 22: e00190.
23. Chothia MY, Kassum P, Zemlin A. A method comparison study of a point-of-care blood gas analyser with a laboratory auto-analyser for the determination of potassium concentrations during hyperkalaemia in patients with kidney disease. Biochem Medica 2020; 30: 1–7.
24. Salvagno GL, Lippi G, Gelati M, Guidi GC. Hemolysis, lipaemia and icterus in specimens for arterial blood gas analysis. Clin Biochem 2012; 45: 372–3.
25. Liu D, Li YX, Huang Y. Analysis of hemolysis, icterus and lipemia in arterial blood gas specimens. Clin Chem Lab Med 2017; 55: e69–71.
26. Lippi G, Ippolito L, Fontana R. Prevalence of hemolytic specimens referred for arterial blood gas analysis. Clin Chem Lab Med 2011; 49: 931–2.
27. Duhalde H, Skogö J, Karlsson M. Point-of-care hemolysis detection in blood gas specimens directly at the emergency department. Scand J Clin Lab Invest 2019; 79: 283–7.
28. Lippi G, Fontana R, Avanzini P, Sandei F, Ippolito L. Influence of spurious hemolysis on blood gas analysis. Clin Chem Lab Med 2013; 51: 1651–4.
29. O'Kane MJ, McManus P, McGowan N, Lynch PLM. Quality error rates in point-of-care testing. Clin Chem 2011; 57: 1267–71.
30. Moreno ALQ, Sáez PO, Calle PF, et al. Clinical, operative, and economic outcomes of the point-of-care blood gases in the nephrology department of a third-level hospital. Arch Pathol Lab Med 2020; 144: 1209–16.
31. Hedberg P, Majava A, Kiviluoma K, Ohtonen P. Potential preanalytical errors in whole-blood analysis: Effect of syringe sample volume on blood gas, electrolyte and lactate values. Scand J Clin Lab Invest 2009; 69: 585–91.
32. Orhan B, Sonmez D, Cubukcu HC, et al. The use of preanalytical quality indicators: a Turkish preliminary survey study. Clin Chem Lab Med 2021; 59: 837–43.
33. Küme T, Ali R, Özkaya A, Çoker C. Preanalytical errors of specimens sent from the emergency department to the laboratory. J Turkish Clin Biochem 2009; 7: 49–55.
34. Dukić L, Šimundić AM. Institutional practices and policies in acid-base testing: A self reported Croatian survey study on behalf of the Croatian society of medical biochemistry and laboratory medicine Working Group for acid-base balance. Biochem Medica 2014; 24: 281–92.
35. Lippi G, Becan-McBride K, Behúlová D, et al. Preanalytical quality improvement: In quality we trust. Clin Chem Lab Med 2013; 51: 229–41.
36. West J, Atherton J, Costelloe SJ, Pourmahram G, Stretton A, Cornes M. Preanalytical errors in medical laboratories: a review of the available methodologies of data collection and analysis. Ann Clin Biochem 2017; 54: 14–9.
37. Lima-Oliveira G, Volanski W, Lippi G, Picheth G, Guidi GC. Pre-analytical phase management: a review of the procedures from patient preparation to laboratory analysis. Scand J Clin Lab Invest 2017; 77: 153–63.
38. Lippi G, von Meyer A, Cadamuro J, Simundic AM. Blood sample quality. Diagnosis (Berlin, Ger) 2019; 6: 25–31.
39. Lippi G, Cornes MP, Grankvist K, Nybo M, Simundic AM. EFLM WG-Preanalytical phase opinion paper: Local validation of blood collection tubes in clinical laboratories. Clin Chem Lab Med 2016; 54: 755–60.

Original Article

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Levels of methylated arginines and L-arginine in patients with polycystic ovary syndrome: a promising approach in clinical evaluation

Özgür Öztan[1], Gökçen Erdoğan[2], Coşkun Şimşir[3], Vugar Ali Türksoy[4], Gülsüm Abuşoğlu[5], Lütfiye Tutkun[6], Serdar Deniz[7], Mehmet Erdem Alagüney[8]

[1]HLC Medical Center, Department of Medical Management, Ankara, Turkey
[2]Yakındoğu University, Faculty of Medicine, Department of Obstetrics and Gynecologia, Lefkoşa, TRNC
[3]Yüksek İhtisas University, Faculty of Medicine, Department of Obstetrics and Gynecologia, Ankara, Turkey
[4]Yozgat Bozok University, Department of Public Health, Yozgat, Turkey
[5]Selcuk University, Vocational School of Health, Department of Medical Laboratory Techniques, Konya, Turkey
[6]Gazi University, Health Science Faculty, Department of Nutrition and Dietetics, Ankara, Turkey
[7]Malatya Provincial Health Directorate, Malatya, Turkey
[8]Health Sciences University, Konya City Hospital, Occupational Medicine Department, Konya, Turkey

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ABSTRACT

Aim: Polycystic ovary syndrome (PCOS) is a prevalent disease in women. PCOS is related with metabolic syndrome and associated with cardiovascular disorders. Methylated arginine is involved in endothelial dysfunction, inflammation and atherosclerosis pathophysiology. Our aim is to determine the association between PCOS and methylated arginine metabolites in order to investigate the role of vascular factors in the etiopathogenesis of PCOS.

Material and Method: This is a case-control study. The study group is consisted of 45 PCOS cases and 45 controls. The case group is patients who applied to Department of Gynecology and diagnosed with PCOS. Control group is consisted of healthy volunteers who applied to the outpatient clinics for other reasons. The study took place in Department of Gynecology in XX hospital. Data collection was held between 2018 October to 2019 June. Methylated arginine derivatives such as ADMA, SDMA, L-NMMA (L-NG-monomethyl Arginine Acetate) and also arginine and citrulline were determined.

Results: ADMA, SDMA, L-NMMA, arginine, citrulline, Arginine/ADMA, SDMA/ADMA and total methylarginine parameters showed statistically significant differences between groups. Strong positive relation was determined between scoring of Ferriman-Gallway (FGS) and luteinizing hormone (LH), glucose, insulin, ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels.

Conclusions: High levels of methylated arginine/NO pathway metabolites in PCOS patients may be related with cardiovascular outcomes of PCOS.

Keywords: PCOS, ADMA, SDMA, methylated arginine

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogenous disorder characterized by increased androgen levels, menstrual disorders, and cysts in the ovaries and affects 7% of adult women (1). Health complications in PCOS include menstrual dysfunction, infertility, hirsutism, acne, obesity, and metabolic syndrome (2-4). It is one of the most common endocrine disorders in women of reproductive age (5). The effects of lifestyle on clinical features reveals that the etiopathogenesis of the disease is affected not only by genetics but also by environmental factors. Intrauterine exposure to Bisphenol A and phthalates is the most commonly accused environmental factors in the development of the disease (6,7). Despite the accusation of genetic and environmental factors in etiology, the factors causing the disease have not been determined exactly.

Major criteria of PCOS are hyperandrogenism and / or hyperandrogenemia, oligo-ovulation and the exclusion of the other known disorders and they were defined at

Corresponding Author: Erdem Alagüney, alaguney@gmail.com

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the NIH-National Institute of Child Health and Human Development Conference in 1990. A consensus was reached in Rotterdam in 2003 that at least two of the following three criteria: clinical and/or biochemical hyperandrogenism, oligo/anovulation and polycystic ovaries, excluding other endocrinopathies, would be sufficient for the diagnosis of PCOS (8,9).

Symptoms are in chronic nature in PCOS. It usually starts during adolescence and progresses gradually over time. Some conditions can cause changes in the characteristics of these symptoms. For example, weight gain, anovulation and hirsutism can lead to aggravation in symptoms (10,11). The levels of "follicular-stimulating hormone" (FSH) do not change in the hormonal profile but it is typical for "luteinizing hormone" (LH) and "gonadotropin-releasing hormone" (GnRH) levels to increase (12,13).

Insulin resistance is the most important cause of reproductive disorders and metabolic problems (14). This causes an increased risk of impaired glucose tolerance, type 2 diabetes mellitus and cardiovascular disorders (15,16). It has been shown in studies that women with PCOS have increased cardiovascular mortality, especially in the postmenopausal period (17,18).

As an endogenous inhibitor of nitric oxide synthetase (NOS), asymmetric dimethylarginine (ADMA) is an important amino acid that play role in a wide range of human diseases especially cardiovascular diseases (19). It has been demonstrated by many studies that ADMA and SDMA (Symmetric dimethylarginine) are involved in endothelial dysfunction, inflammation and atherosclerosis pathophysiology (20-22). On the other hand, arginine / homoarginine pathway is also known to play a role in endothelial dysfunction and atherosclerosis (23,24).

In our study, we aimed to determine the relation between PCOS and methylated arginine/NO pathway metabolites in order to investigate the role of vascular factors in the etiopathogenesis of PCOS. These metabolic markers can be used to show the severity of the endothelial dysfunction when there is no apparent clinical outcome. This may help taking preventing measures before the worse clinical outcomes become overt. With the results of this study, we will be able to understand the biochemical pathways in development of PCOS.

MATERIAL AND METHOD

The study was carried out with the permission of Liv Hospital Ankara Ethics Committee (Date: 2018, Decision No: 2018-003-005). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Participants and setting

The study group is consisted of 45 PCOS cases and 45 controls. The study took place in Department of Gynecology in Liv Hospital Ankara hospital. Data collection was held between 2018 October to 2019 June. The case group is patients who applied to Liv Hospital Ankara Hospital, Department of Gynecology and diagnosed with PCOS. Control group is consisted of healthy volunteers who applied to the outpatient clinics for other reasons.

Collection of Biological Samples and Biochemical Measurements

Eight milliliters of venous blood samples were collected into tubes (BD Vacutainer, USA) by venipuncture from each participant and analyzed according to stability procedures. After the centrifugation at 3500 x g for 10 min at 40C, sera were collected and stored at −800C until transfer the samples for biochemical measurements. These samples were transferred to Yozgat Bozok University Medical Biochemistry Laboratory under cold chain conditions. Methylated arginine derivatives such as ADMA, SDMA, L-NMMA (L-NG-monomethyl Arginine Acetate) and also arginine and citrulline (Sigma, Karlsruhe, Germany) of serum were determined via high performance liquid chromatography (Shimadzu LC-20AD system (Tokyo, Japan) tandem mass spectrometry [Applied Biosystems MDS SCIEX (Foster City, CA, USA) API 3200]. This electrospray ionization (ESI) technique was operated in positive mode with a high-resolution chromatography column [Phenomenex (Torrance, CA, USA) Luna C18] (25,26). According to this procedure, 100 µL of internal standard [(deuterated7-ADMA (Cambridge Isotopes, Tewksbury, MA, USA)] dissolved in methanol (Merck, Darmstadt, Germany) were added to 200 µL of sample and proteins were separated after centrifugation via 6000 g for 10 min. The clear supernatant was taken and evaporated under a nitrogen gas flow at 600C. Two hundred microliters of fresh butanol (Merck, Darmstadt, Germany) solution including 5% (vv−1) acetyl chloride (Merck, Darmstadt, Germany) were used for derivatization of samples. The mixture was incubated at 600C for 20 min. The mixture was dried under nitrogen flow at 600C. The bulks were dissolved in 100 µL of water (Merck, Darmstadt, Germany)–methanol (Merck, Darmstadt, Germany) (90:10, vv−1) containing 0.1% (vv−1) formic acid (Sigma, Karlsruhe, Germany) and 40 µL were used for injection to chromatographic column. Mobile phase A and B consist of high-performance liquid chromatography grade water containing 0.1% (vv−1) formic acid and methanol containing 0.1% (vv−1), respectively with a total binary flow of 0.8 mL. Chromatographic separation was performed on Phenomenex Luna C18 column (Torrance, CA, USA) (250 x4.6 mm, 5 µm, 100 Å) in 5 min analysis

time. Mass spectrometric parameters were such as: ion source gas 1: 60; ion source gas 2: 60; entrance potential: 7.5; collision cell exit potential: 4; ion spray voltage: 5500 V; declustering potential: 40; collision gas: 5; collision energy: 24; temperature: 5500C. Methylated arginine derivatives' analyses were assessed by an optimization procedure with an infusion of a 50 μM solution of each molecule. According to this method, either intra-day coefficient variation (CV) or inter-day CV values for methylated arginine molecules were both under 20%. The observed bias for all added concentrations was <±17% and recoveries were between 80 and 92% (80% for L-NMMA).

Statistical Analysis

Statistical analyses were performed using the SPSS version 20.0 statistical software package. The conformity of continuous variables to normal distribution was tested with Kolmogorov-Smirnov test. The descriptive statistics of continuous variables were expressed as mean ± standard deviation. The presence of a statistically significant difference between the groups in terms of continuous variables was examined with Student's t test for parametric parameters. The difference between the groups (case-control) for non-normally distributed parameters was tested with the Mann-Whitney U test. The presence of a correlation between the groups was analyzed with Spearman correlation test. $p<0.05$ and $p<0.01$ values were considered significant.

RESULTS

A total of 90 participants (45 controls and 45 PCOS) were included in the study. Clinical parameters of control and PCOS groups are shown in **Table 1**. Age and Body Mass Index (BMI) levels of the both groups were found similar ($p>0.05$). LDL, Total cholesterol, Luteinizing hormone (LH), glucose, insulin levels and Ferriman-Gallway Scores (FGS) were significantly different between groups ($p<0.01$). However, HDL level was determined statistically lower in the PCOS group than control. On the other hand, gravidity and parity of control and PCOS groups were found statistically significantly different (gravidity: 3 and 1; parity: 2 and 1; respectively, $p<0.01$). Triglyceride (TG), Follicle-stimulating hormone (FSH), Estradiol (E2), Waist-hip ratio (WHR) and Total testosterone (T. Testosterone) did not show statistically significant difference between control and PCOS groups ($p>0.05$).

All methylated arginine parameters such as ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA, total methylarginine, arginine/ADMA ratio and SDMA/ADMA ratio were found statistically significant different between control and PCOS groups ($p<0.01$). The

Table 1. Clinical parameters of control and PCOS groups

	Group	Mean	Median	STD	Minimum	Maximum	p
Age (year)	0	29.86	29.50	5.56	20.00	39.00	0.094
	1	27.68	26.00	6.17	18.00	39.00	
TG (mg/dL)	0	95.41	89.00	44.27	39.00	297.00	0.686
	1	96.50	82.50	48.38	45.00	298.00	
LDL (mg/dL)	0	80.01	79.00	25.04	34.00	132.00	0.003
	1	63.59	59.50	24.74	27.00	136.00	
HDL (mg/dL)	0	65.75	65.50	9.23	46.00	84.00	0.043
	1	62.00	60.00	7.84	51.00	84.00	
T. Cholesterol (mg/dL)	0	160.27	162.50	33.56	90.00	225.00	0.009
	1	142.57	138.00	28.57	98.00	231.00	
LH (mIU/mL)	0	6.08	4.22	4.24	0.65	17.61	<0.001
	1	9.71	9.13	5.51	2.07	29.35	
FSH (mIU/mL)	0	6.74	6.45	3.08	2.25	21.18	0.238
	1	7.04	6.59	2.14	2.32	14.50	
Estradiol, E2 (mIU/mL)	0	33.55	29.50	21.34	7.00	111.00	0.226
	1	36.62	36.00	18.58	5.00	75.00	
BMI (kg/m2)	0	22.03	21.61	3.13	16.18	29.14	0.157
	1	23.66	22.62	4.59	15.06	35.46	
WHR	0	0.79	0.78	0.07	0.66	0.95	0.887
	1	0.79	0.78	0.07	0.66	0.95	
Glucose (mg/dL)	0	84.00	82.00	7.47	71.00	98.00	<0.001
	1	91.07	91.00	2.54	82.00	99.00	
Insulin (μIU/mL)	0	8.45	6.90	5.86	2.94	38.38	0.003
	1	10.98	9.92	5.68	3.06	33.84	
FGS (%)	0	4.30	5.00	2.22	1.00	10.00	<0.001
	1	11.82	11.50	4.55	1.00	22.00	
T. Testosterone (ng/dl)	0	1.65	1.46	0.79	0.60	3.97	0.263
	1	1.43	1.36	0.57	0.60	3.39	

0: control group (n=45), 1: PCOS group (n=45); PCOS: polycystic ovary syndrome; STD: Standard Deviation; TG: triglyceride; LDL: LDL cholesterol; HDL: HDL cholesterol; T. Cholesterol: Total cholesterol; LH: luteinizing hormone; FSH: follicle-stimulating hormone; E2: Estradiol; BMI: Body Mass Index; WHR: Waist-hip ratio; FGS: Ferriman-Gallway Score; T. Testosterone: Total testosterone

Table 2. The methylated arginine parameters of control and PCOS groups

	Group	Mean	Median	STD	Minimum	Maximum	p
ADMA (µmol/L)	0	0.33	0.37	0.12	0.07	0.50	<0.001
	1	0.52	0.51	0.09	0.41	0.70	
SDMA (µmol/L)	0	0.35	0.38	0.12	0.07	0.52	<0.001
	1	0.48	0.45	0.13	0.31	0.92	
Arginine (µmol/L)	0	171.47	175.00	78.71	55.60	437.00	0.006
	1	205.89	200.50	50.21	91.30	312.00	
Citrulline (µmol/L)	0	23.14	20.75	7.30	11.30	53.80	<0.001
	1	38.86	35.80	14.28	16.50	87.30	
Homoarginine (µmol/L)	0	2.53	2.48	0.81	0.79	4.23	<0.001
	1	4.01	3.75	1.26	1.82	8.78	
L-NMMA (µmol/L)	0	0.04	0.04	0.01	0.01	0.06	<0.001
	1	0.05	0.05	0.01	0.01	0.09	
Total methylarginine (µmol/L)	0	0.73	0.80	0.28	0.14	1.56	<0.001
	1	1.09	1.01	0.24	0.83	1.94	
Arginine/ADMA ratio	0	589.73	519.94	351.10	224.18	2385.09	<0.001
	1	406.39	410.75	112.56	196.77	759.12	
SDMA/ADMA ratio	0	1.09	1.03	0.31	0.83	2.76	<0.001
	1	0.95	0.89	0.29	0.53	2.25	

0: control group (n=45), 1: PCOS group (n=45); PCOS: polycystic ovary syndrome; STD: Standard Deviation.

methylated arginine parameters of control and PCOS groups are shown in **Table 2.**

The correlation between clinical parameters is shown in **Table 3**. Positive correlations were found between LH and FSH (r=0.433; p<0.01), estradiol (r=0.260; p<0.05), ADMA (r=0.250; p<0.05), homoarginine (r=0.238; p<0.05) and total methylarginine (r=0.318; p<0.01). A strong positive relationship was determined between FGS and LH, glucose, insulin, ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels, respectively (r=0.358, r=0.402, r=0.330, r=0.538, r=0.545, r=0.466, r=0.356, r=0.462; p<0.01). **Figure 1** presents relationship between FGS and methylated arginines such as ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels. Strong negative correlations were determined between FGS and Arginine/ADMA, SDMA/ADMA ratios, respectively (r=-0.339, r=-0.355; p<0.01). On the other hand, negative relationship was found with FGS and LDL cholesterol levels (r=-0.258; p<0.05). The relationship between FGS and insulin, glucose, LH, Estradiol (E2), LDL cholesterol levels are shown in **Figure 2**.

DISCUSSION

All methylated arginine parameters such as ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA, total methylarginine, arginine/ADMA ratio and SDMA/ADMA ratio were found statistically significant different between control and PCOS groups (**Table 2**). With the results of this study, we will be able to understand the biochemical pathways in development of PCOS. Krishna et al. (27) reported that women with PCOS showed reduced plasma NOx (nitrate plus nitrite), high ADMA synthesis and reduced arginine bioavailability. Similarly, in our study, serum ADMA levels are high in the PCOS group. ADMA, which is the most analyzed member of methylarginines, is frequently analyzed in the detection of metabolic changes and cardiovascular risk resulting from endothelial dysfunction in PCOS. One of the advantages in our study is that all methylarginine in the ADMA pathway were analyzed. The high level of total methylarginine (ADMA+SDMA+L-NMMA) in the PCOS group supports this thesis. Reduced arginine bioavailability reduces nitric oxide formation. In our study, although the serum ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA and total methylarginine levels were higher in the PCOS group compared to the control group, this difference was statistically significant. One explanation for this could be a compensator increasing of the levels of arginine, the precursor molecule, to eliminate endothelial dysfunction and to increase nitric oxide synthesis again. Elci et al. (28) found that serum ADMA levels were significantly higher in the PCOS group, both obese and non-obese, compared to the control group. In our PCOS group, obesity or non-obesity classification has not been made, however, similar to this study, serum ADMA levels were high. In spite of that BMI levels in our study of the both groups were found similar.

Obesity and weight gain are factors that can speed up the process of developing insulin resistance and the formation of vascular damage. In our study, serum glucose and insulin levels were found to be high in the PCOS group with high ADMA levels. In a study conducted by Burchall et al. (29), in support of our data, it was found that in patients with insulin resistance and high serum glucose values in the PCOS group, serum ADMA value

Table 3. The correlation between all clinical parameters

	TG	LDL	HDL	T. Cholesterol	LH	FSH	Estradiol, E2	BMI	WHR	Glucose	Insulin	FGS	T.Testosteron	ADMA	SDMA	Arginine	Citrulline	Homoarginine	L-NMMA	Total methylarginine	Arginine/ ADMA ratio	SDMA/ADMA ratio
Age	.069	.012	.001	-.008	.022	.015	-.081	-.191	-.174	-.135	-.106	-.192	-.045	-.103	-.059	.047	-.128	-.185	-.131	-.098	.048	.083
TG	1	.309**	-.085	.466**	.130	-.125	-.007	.069	.059	.015	-.010	.025	.107	-.037	-.074	.124	.038	-.022	-.016	-.082	.038	-.080
LDL		1	.287**	.786**	-.164	-.047	-.101	.067	.063	-.117	-.122	-.258*	.101	-.315**	-.212*	-.121	-.250*	-.243*	-.249*	-.233*	.139	.127
HDL			1	.428**	-.257*	-.113	-.009	-.138	-.036	-.123	.183	-.097	.152	-.254*	-.298**	-.109	-.137	-.228*	-.227*	-.321**	.116	.069
T. Cholesterol				1	-.172	-.085	-.120	.176	.168	-.130	-.037	-.182	.159	-.315**	-.220*	-.084	-.144	-.214*	-.160	-.315**	.196	.126
LH					1	.433**	.260*	-.001	-.075	.138	.020	.358**	-.181	.250*	.125	.066	.118	.238*	.105	.318**	-.114	-.045
FSH						1	.195	-.053	-.056	-.043	.073	.105	-.094	.081	.127	.128	-.080	.187	.152	.245*	.048	.049
Estradiol, E2							1	.045	-.158	.035	.108	.212*	-.021	.161	.083	-.065	.140	.052	.187	.164	-.166	-.122
BMI								1	.341**	.062	-.042	.088	-.008	.064	.051	-.015	.192	-.055	.206	.027	.026	-.050
WHR									1	.025	-.054	-.011	.017	.073	.203	.143	-.058	.060	.150	.018	.065	.068
Glucose										1	.519**	.402**	-.065	.277**	.156	.004	.236*	.148	.149	.209	-.198	-.146
Insulin											1	.330**	.016	.223*	.083	.088	.194	.068	.137	.165	-.096	-.126
FGS												1	-.117	.538**	.250*	.094	.545**	.466**	.356**	.462**	-.339**	-.355**
T.Testosteron													1	-.148	.029	.112	.015	-.043	.073	-.186	.154	.186
ADMA														1	.606**	.322**	.457**	.586**	.505**	.869**	-.599**	-.592**
SDMA															1	.520**	.328**	.419**	.521**	.759**	-.197	.156
Arginine																1	.184	.238*	.362**	.440**	.413**	.041
Citrulline																	1	.346**	.306**	.437**	-.210*	-.247*
Homoarginine																		1	.444**	.558**	-.369**	-.298**
L-NMMA																			1 0	.528**	-.224*	-.171
Total methylarginine																				1	-.412**	-.320**
Arginine/ ADMA ratio																					1	.577**

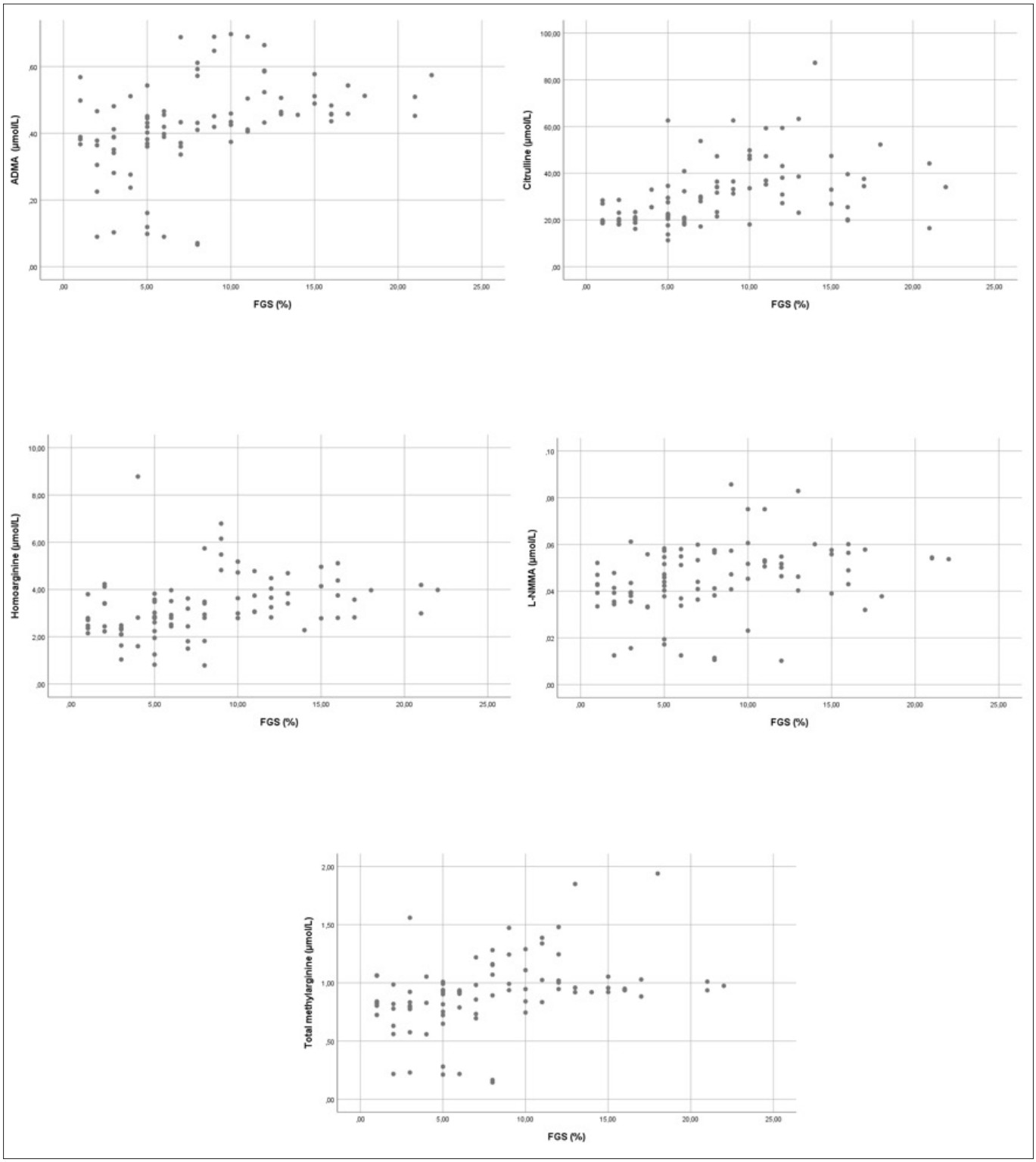

Figure 1. The relation between FGS with ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels

was higher than the control group, regardless of age and BMI. None the less age and BMI levels in our study of the both groups were obtained similar.

Taslipinar et al. (30) reported high serum ADMA levels as an endothelial dysfunction indicator in the PCOS patient group. When all parameters are compared in terms of distinguishing PCOS disease from the control group, the use of classical laboratory parameters and the clinical scoring of Ferriman-Gallway (FGS) are less predictive to detect disease compared to serum methylarginine levels which is a new biomarker. The relation between FGS with ADMA, citrulline, homoarginine, L-NMMA and total methylarginine levels are shown in **Figure 1**. The measurements of ADMA, SDMA, arginine, citrulline, homoarginine, L-NMMA parameters were found to be more effective in predicting the disease. The reason for this may be that derivatives of methylarginines other

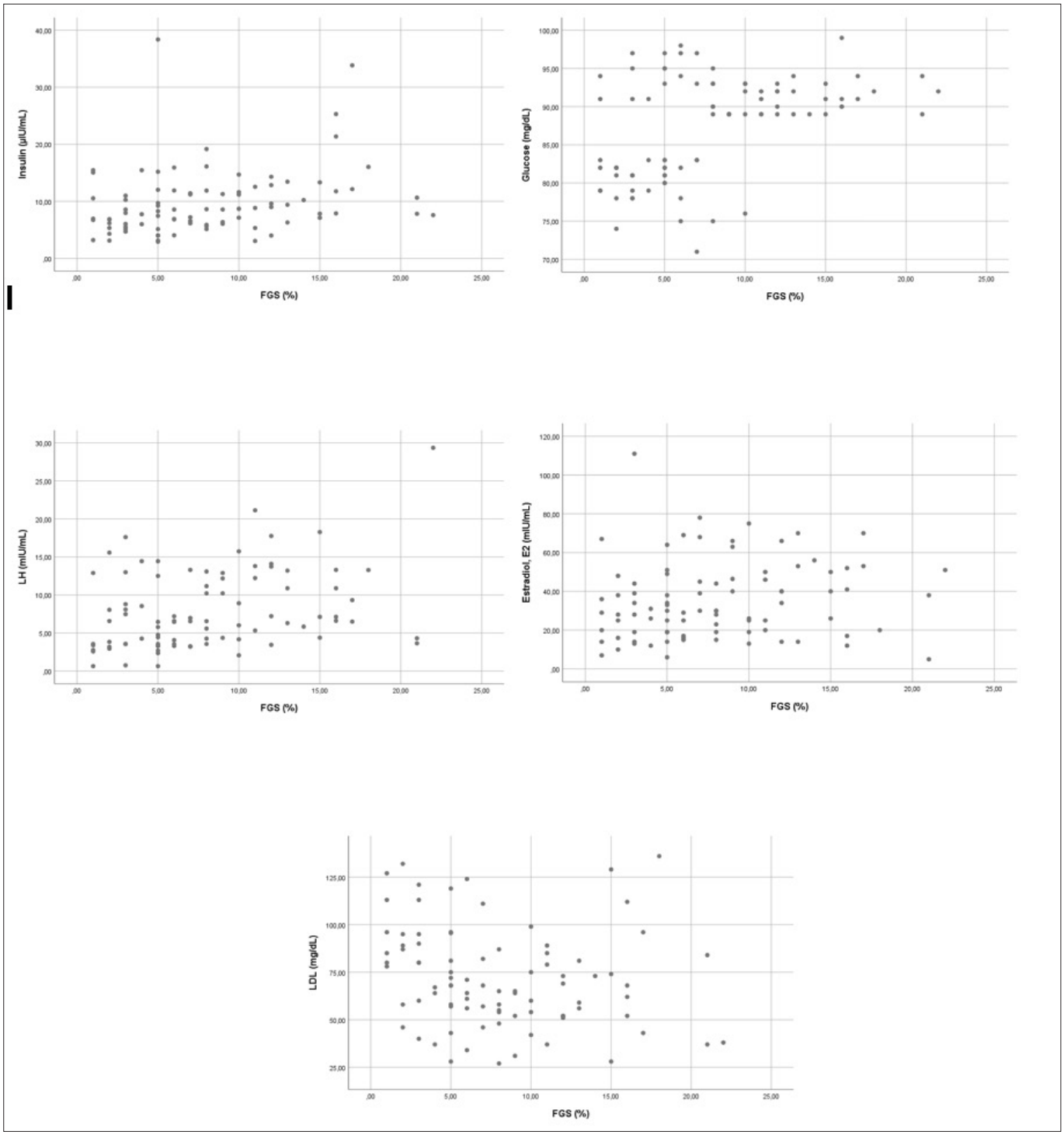

Figure 2. The relation between FGS and insulin, glucose, LH, Estradiol (E2), LDL cholesterol levels

than ADMA are known to be effective in other metabolic variations. Arginine levels even increases with high protein content diet. Also, plasma SDMA levels can increase due to impaired kidney function.

It is known that estrogens increase the expression of endothelial-dependent and inducible nitric oxide synthase enzymes in vascular cells. Karakurt et al. (31) reported that high serum ADMA levels decreased with estrogen therapy in the PCOS group. These hormonal changes in PCOS can lead to increased vascular damage and permeability, especially as a result of disruption of nitric oxide synthesis and increased levels of methylarginine. In our study, serum methylarginine levels were found to be higher in the PCOS group compared to the control group. This may be important to prevent occurrence of vascular damage. Moran LJ et al. (32) reported that weight loss did not alter serum ADMA levels statistically when compared in PCOS and healthy groups.

In this study, serum homoarginine parameter, which has not previously been studied in the PCOS group, was analyzed.

Previous literature shows that low circulating homoarginine as well as high levels of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) have been associated with impaired cardiovascular (CV) outcome and mortality in patients at risk and in the general population (33). Another study shows that high L-homoarginine (hArg) levels are directly associated with several risk factors for cardiometabolic diseases (34). ADMA, SDMA, and homoarginine are non-proteinogenic amino acids structurally related to L-arginine. hArg has been shown to serve as an alternative substrate for NOS and to inhibit arginase. Thus, it is considered to increase NO formation. In addition, low circulating concentrations of homoarginine have been proposed as a cardiovascular risk factor (35). In our study, serum homoarginine levels were found to be high in the PCOS group. This may mean suppression of nitric oxide synthesis. To our knowledge, no other study investigated serum homoarginine levels in the PCOS group.

CONCLUSIONS

Radiological imaging and physical examination are important in diagnosis of PCOS; however, these metabolic markers are found to be significantly higher in the study population, therefore, may have shed a light understanding the pathogenesis of PCOS. These markers can be used to show the severity of the endothelial dysfunction when there is no apparent clinical outcome. This may help taking preventing measures before the worse clinical outcomes become overt. Our study is unique as there are no studies investigating all ADMA parameters in patients with PCOS.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Liv Hospital Ankara Ethics Committee (Date: 2018, Decision No: 2018-003-005).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet 2007; 370: 685-97.
2. Archer JS, Chang RJ. Hirsutism and acne in polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004; 18: 737-54.
3. Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH. Screening women with polycystic ovary syndrome for metabolic syndrome. Obstet Gynecol 2005; 106: 131-7.
4. van der Spuy ZM, Dyer SJ. The pathogenesis of infertility and early pregnancy loss in polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004; 18: 755-71.
5. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod 2016; 31: 2841-55.
6. Schonfelder G, Friedrich K, Paul M, Chahoud I. Developmental effects of prenatal exposure to bisphenol a on the uterus of rat offspring. Neoplasia 2004; 6: 584-94.
7. Vagi SJ, Azziz-Baumgartner E, Sjodin A, et al. Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol A in polycystic ovary syndrome: a case-control study. BMC Endocr Disord 2014; 14: 86.
8. Cheung AP, Cog F. Polycystic ovary syndrome: a contemporary view. J Obstet Gynaecol Can 2010; 32: 423-5.
9. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81: 19-25.
10. Ribeiro VB, Kogure GS, Lopes IP, et al. Effects of continuous and intermittent aerobic physical training on hormonal and metabolic profile, and body composition in women with polycystic ovary syndrome: A randomized controlled trial. Clin Endocrinol (Oxf) 2020; 93: 173-86.
11. Balen AH, Morley LC, Misso M, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update 2016; 22: 687-708.
12. Blank SK, McCartney CR, Marshall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update 2006; 12: 351-61.
13. Willis DS, Watson H, Mason HD, Galea R, Brincat M, Franks S. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. J Clin Endocrinol Metab 1998; 83: 3984-91.
14. Hansen SL, Svendsen PF, Jeppesen JF, et al. Molecular mechanisms in skeletal muscle underlying insulin resistance in women who are lean with polycystic ovary syndrome. J Clin Endocrinol Metab 2019; 104: 1841-54.
15. Ganie MA, Dhingra A, Nisar S, et al. Oral glucose tolerance test significantly impacts the prevalence of abnormal glucose tolerance among Indian women with polycystic ovary syndrome: lessons from a large database of two tertiary care centers on the Indian subcontinent. Fertil Steril 2016; 105: 194-201.
16. Forslund M, Landin-Wilhelmsen K, Trimpou P, Schmidt J, Brannstrom M, Dahlgren E. Type 2 diabetes mellitus in women with polycystic ovary syndrome during a 24-year period: importance of obesity and abdominal fat distribution. Hum Reprod Open 2020; 2020: hoz042.
17. Merz CN, Shaw LJ, Azziz R, et al. Cardiovascular Disease and 10-Year Mortality in Postmenopausal Women with Clinical Features of Polycystic Ovary Syndrome. J Womens Health (Larchmt) 2016; 25: 875-81.
18. Zhao L, Zhu Z, Lou H, et al. Polycystic ovary syndrome (PCOS) and the risk of coronary heart disease (CHD): a meta-analysis. Oncotarget 2016; 7: 33715-21.

19. Walch K, Kolbus A, Hefler-Frischmuth K. Polymorphisms of the endothelial nitric oxide synthase gene in premenopausal women with polycystic ovary syndrome. Maturitas 2008; 61: 256-9.
20. Sibal L, Agarwal SC, Home PD, Boger RH. The role of asymmetric dimethylarginine (ADMA) in endothelial dysfunction and cardiovascular disease. Curr Cardiol Rev 2010; 6: 82-90.
21. Mookerjee RP, Dalton RN, Davies NA, et al. Inflammation is an important determinant of levels of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) in acute liver failure. Liver Transpl 2007; 13: 400-5.
22. Notsu Y, Yano S, Shibata H, Nagai A, Nabika T. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. Atherosclerosis 2015; 239: 61-6.
23. Haghikia A, Yanchev GR, Kayacelebi AA, et al. The role of L-arginine/L-homoarginine/nitric oxide pathway for aortic distensibility and intima-media thickness in stroke patients. Amino Acids 2017; 49: 1111-21.
24. Surdacki A, Martens-Lobenhoffer J, Wloch A, et al. Elevated plasma asymmetric dimethyl-L-arginine levels are linked to endothelial progenitor cell depletion and carotid atherosclerosis in rheumatoid arthritis. Arthritis Rheum 2007; 56: 809-19.
25. Di Gangi IM, Pirillo P, Carraro S, et al. Online trapping and enrichment ultra performance liquid chromatography-tandem mass spectrometry method for sensitive measurement of "arginine-asymmetric dimethylarginine cycle" biomarkers in human exhaled breath condensate. Anal Chim Acta 2012; 754: 67-74.
26. Perticone F, Sciacqua A, Maio R, et al. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. J Am Coll Cardiol 2005; 46: 518-23.
27. Krishna MB, Joseph A, Thomas PL, Dsilva B, Pillai SM, Laloraya M. Impaired arginine metabolism coupled to a defective redox conduit contributes to low plasma nitric oxide in polycystic ovary syndrome. Cell Physiol Biochem 2017; 43: 1880-92.
28. Elci E, Kaya C, Cim N, Yildizhan R, Elci GG. Evaluation of cardiac risk marker levels in obese and non-obese patients with polycystic ovaries. Gynecol Endocrinol 2017; 33: 43-7.
29. Burchall GF, Piva TJ, Linden MD, Gibson-Helm ME, Ranasinha S, Teede HJ. Comprehensive assessment of the hemostatic system in polycystic ovarian syndrome. Semin Thromb Hemost 2016; 42: 55-62.
30. Yavuz Taslipinar M, Kilic N, Bayraktar N, et al. Endothelial dysfunction and insulin resistance in young women with polycystic ovarian syndrome. Turk J Med Sci 2014; 44: 787-91.
31. Karakurt F, Carlioglu A, Kaygusuz I, Gumus, II, Uz B, Akdeniz D. Effect of ethinyl estradiol-cyproterone acetate treatment on asymmetric dimethyl-arginine levels in women with polycystic ovary syndrome. Arch Gynecol Obstet 2014; 289: 135-40.
32. Moran LJ, Noakes M, Wittert GA, Clifton PM, Norman RJ. Weight loss and vascular inflammatory markers in overweight women with and without polycystic ovary syndrome. Reprod Biomed Online 2012; 25: 500-3.
33. Atzler D, Schwedhelm E, Nauck M, Ittermann T, Boger RH, Friedrich N. Serum reference intervals of homoarginine, ADMA, and SDMA in the study of health in Pomerania. Clin Chem Lab Med 2014; 52: 1835-42.
34. Seppala I, Oksala N, Jula A, et al. The biomarker and causal roles of homoarginine in the development of cardiometabolic diseases: an observational and Mendelian randomization analysis. Sci Rep 2017; 7: 1130.
35. Loso J, Lund N, Avanesov M, et al. Serum biomarkers of endothelial dysfunction in fabry associated cardiomyopathy. Front Cardiovasc Med 2018; 5: 108.

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Association between serum ferritin level and thyroid hormones in hypothyroid pediatric patients

Ayşegül Alpcan[1], Yaşar Kandur[1], Serkan Tursun[1], Meryem Albayrak[2], Ayça Törel Ergür[3]

[1]Kırıkkale University, School of Medicine, Department of Pediatrics, Kırıkkale, Turkey
[2]Kırıkkale University, School of Medicine, Department of Pediatric Hematology and Oncology, Kırıkkale, Turkey
[3]Ufuk University, Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey

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ABSTRACT

Aim: The present study aimed to investigate the iron store status in patients with hypothyroidism due Hashimoto thyroiditis and subclinical hypothyroidism.

Material and Method: We retrospectively reviewed the medical records of patients who were followed up at our university hospital with the diagnosis of hypothyroidism due to Hashimoto thyroiditis and subclinical hypothyroidism between 2010-2021.

Results: Nineteen patients with Hashimoto thyroiditis, 17 with subclinical hypothyroidism, and 20 controls were enrolled in this study. There was no significant difference in mean age among three groups (138.3±46.8 months, 116.0±68.1 months, 151.8±50.3 months in groups of Hashimoto thyroiditis, subclinical hypothyroidism and controls respectively p=0.169) . There was a significant female preponderance in HT group (68.5%, p=0.001). There was no significant difference for mean ferritin levels among groups (p=0.440). There was no correlation between TSH and ferritin level (r=-0.132, p=0.410) but we found a positive correlation between ferritin and free T4 level (r=0.340, p=0.030).

Conclusion: We found a positive relationship between ferritin and thyroxine hormone that made us think that iron supplementation may be effective in the treatment of hypothyroidism. But this hypothesis warrants further prospective and experimental studies.

Keywords: Children, ferritin, hypothyroidism

INTRODUCTION

Iron deficiency may impair thyroid functions. Previous studies have suggested that iron deficiency may impair thyroid hormone metabolism. These studies showed that in iron-deficient-anemic subjects, serum T3 and T4 levels were significantly decreased (1,2). Although some studies showed a correlation between ferritin level and hypothyroidism, (3,4) yet there is no agreement on this subject and the study groups were not composed of patients with thyroid disorders. The present study aimed to investigate the iron store status in patients with hypothyroidism, Hashimoto thyroiditis (HT) and subclinical hypothyroidism (SH).

MATERIAL AND METHOD

We retrospectively reviewed the medical records of patients who were followed up at our university hospital with the diagnosis of hypothyroidism, HT and SH between 2010-2021. The control group was selected from healthy children aged 1 month to 17 years who were examined at the healthy child division and whose serum ferritin levels and thyroid function tests were measured. The demographic characteristics, medical history, and laboratory findings of the patients were collected from the patients' medical records. Patients with hyperthyrodism, and congenital hypothyroidism were excluded. Patients with ultrasonographic findings and the presence of antibodies against thyroid peroxidase (TPO antibodies) confirmed the diagnosis of HT (5). SH is defined as isolated hyperthyrotropinemia with high serum TSH levels, and normal free T4 concentrations (6). The ethics committee approval of the study was obtained from the Kırıkkale University Non-interventional Researchs Ethics Committee (Date: 30.06.2021, Decision No: 2021.05.01). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Corresponding Author: Ayşegül Alpcan, ozcalk@yahoo.com

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Statistical Analysis

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 20.0 software (SPSS Inc.,USA). We presented categorical variables in the form of frequency and percentage and continuous variables in the form of mean and standard deviation (SD). The continuous variables were compared with One Way ANOVA variance analysis test among three groups. Binary comparisons were done with post hoc Tukey and Duncan tests. Chi-square test and Fisher's exact test were used to compare categorical variables among three groups where applicable. The possible correlation between thyroid hormones and ferritin level was analysed with Spearman's rank correlation test. A two-sided p value of less than 0.05 was considered statistically significant.

RESULTS

Nineteen patients with HT, 17 with SH, and 20 controls were enrolled in this study. The demographic chracteristics and laboratory parameters of all study population were presented on **Table**. There was no significant difference in mean age among three groups (138.3±46.8 months, 116.0±68.1 months, 151.8±50.3 months in groups of HT, SH and controls respectively p=0.169) . There was a significant female preponderance in HT group (68.5%, p=0.001). The TSH levels in HT and SH were significantly greater and the free T4 levels were lower than the control group as expected (**Table**). There was no significant difference for mean ferritin levels among groups (p=0.440). Nor hemoglobin neither mean corpuscular volume levels showed significant difference among three groups. Also there was no significant difference among groups for parameters of mean vitamin B12 (p=0.555) and folic acid levels (p=0.879).

There was no correlation between TSH and ferritin level (r=-0.132, p=0.410) but we found a positive correlation between ferritin and free T4 level (r=0.340, p=0.030). Also there was a positive correlation between hemoglobin and ferritin level as expected (r=0.538, p=<0.001).

DISCUSSION

In our study, we observed female preponderance in HT patients compared to SH and controls and we observed a positive correlation between ferritin and free T4 level. The iron load can be evaluated with plasma ferritin level that is a simple, accessible and inexpensive method (7). Although Shamshirsaz et al. (8) found no correlation between ferritin level and hypothyroidism, Chirico et al. (9) found such a correlation. But, ferritin level did not show a significant difference between controls and patients with thyroid disorder in Chirico's study similar to our results.

Sakata et al. (10) found that ferritin levels were higher in patients with SH than in controls. But in our study, the ferritin level was slightly higher in the SH group but it was insignificant. In our study population we found lower levels of ferritin in the HT group in comparison to controls but it was insignificant. We thought that this result may arise from the high percentage of female patients in this group whose ferritin levels were under the effect of menorrhagia.

Hess et al. (11) have shown that thyroid peroxidase activity is significantly decreased in iron deficiency anemia. On the contrary Tienboon et al. (12) found statistical difference in thyroid hormones of the iron-deficient anemic children. And they found a positive and significant correlation between the serum ferritin level and the free T4 levels similar to our results. This finding may be the result of deficiency in iron-dependent enzymes such as thyroid peroxidase that have had influence on thyroid metabolism.

We observed that the free T4 level was lower in HT group than in the control group but it was an insignificant difference at thyroid-stimulating hormone level. Moreover Takamatsu et al. (13) showed that serum ferritin levels increased in all hypothyroid patients with HT when euthyroidism was achieved with L-T4 therapy. Nevertheless we thought that ferritin level had an effect on free T4 level.

Table. Comparison of demographic characteristics and laboratory parameters among three groups.

Parameters	Hashimoto thyroiditis (N=19)	Subclinical hypothyroidsm (N=17)	Control (N=20)	P value
Age (month)	138.3±46.8	116.0±68.1	151.8±50.3	0.169
Female gender n (%)	15 (68.5)	7 (42)	10 (50)	0.001
TSH (uIU / mL)	17.2±6.8	7.3±3.1	2.7±0.9	0.043
Free T4 (ng/dL)	1.06±0.24	1.57±0.71	1.31±0.17	0.003
Ferritin (ng/mL)	30.8±22.1	44.0±29.1	42.2±11.7	0.440
Hg (g/dL)	13.2±1.6	13.4±1.7	14.0±1.7	0.332
MCV (fL)	78.2±7.7	79.4±4.6	83.1±7.0	0.08
Vitamin B12 (pg/mL)	567	325±165	373±139	0.555
Folic acid (ng/mL)	8.4±4.7	9.2±4.1	9.1±2.7	0.879

The values are presented as mean±SD and n(%). Hg: Hemoglobin, TSH:Thyroid stimulating hormone, T4: levothyroxine, MCV: mean corpuscular volume.

We found no significant difference between ST and HT groups for mean ferritin level. Ferritin is also an acute-phase reactant that is elevated in many conditions sush as infection (viral and bacterial), autoimmunity, stem cell transplant, malignancy and renal disease (14). However in some auto-immune inflammatuary diseases such as the rheumatoid arthritis (RA), serum ferritin may not be increased (15). We suggested that like RA, HT is a disease where ferritin increment is not observed as an acute phase reactant reaction. There may be different pathogenic pathways to explain this condition in HT.

The insignificant difference of vitamin B12 and folate levels among our groups may also showed us that thyroid hormones had no effect on gastrointestinal absorption. Studies of intestinal absorptive functions in 11 hyperthyroid and 14 hypothyroid patients showed that steatorrhoea was common in hyperthyroid patients and rare in hypothyroid patients (16). Since our study group composed of hypothyroid patients we found no difference for vitamin B12 and folate levels as expected.

The present study has the following limitations. Firstly, because of its retrospective nature and reliance on medical records, some of the investigated data were not available for all patients such serum iron levels. Secondly the small sample study population limits the power of study. And our single-center study design does not project the whole population. So that our findings should be confirmed by further multicenter prospective studies with a larger sample size.

CONCLUSION

We found a positive relationship between ferritin and thyroxine hormone that made us think that iron supplementation may be effective in the treatment of hypothyroidism. The underlying mechanisms of ferritin role in the modulation of thyroid functions and its specific feature except acute phase reactant for HT, warrant further prospective and experimental studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The ethics committee approval of the study was obtained from the Kırıkkale University Non-interventional Researchs Ethics Committee (Date: 30.6.2021, Decision No: 2021.05.01).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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REFERENCES

1. Martinez-Torres C, Cubeddu L, Dillman E, et al. Effect of exposure to low temperature on normal and iron-deficient subjects. Am J Physiol 1984; 246: R380-3.
2. Beard JL, Borel MJ, Deer J. Impaired thermoregulation and thyroid function in iron deficiency anemia. Am J Clin Nutr 1990; 52: 813-9.
3. Eshragi P, Tamaddoni A, Zarifi K, et al. Thyroid function in major thalassemia patients: Is it related to height and chelation therapy? Caspian J Intern Med 2011; 2: 189.
4. İpek İÖ, Kaçmaz E, Bozaykut A, Sezer RG, Seren L, Paketçi C. The effect of iron deficiency anemia on plasma thyroid hormone levels in childhood. Turk Arch Pediatr. 2011;46:122–5.
5. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev. 2014;13(4-5):391-7
6. Gallizzi R, Crisafulli C, Aversa T, et al. Subclinical hypothyroidism in children: is it always subclinical? Ital J Pediatr 2018; 44: 25.
7. Tiwari R, Saharia GK, Mangaraj M. Evaluation of serum ferritin and anti-thyroid peroxidase antibody status in newly diagnosed subclinical cases of hypothyroidism. Endocr Metab Immune Disord Drug Targets 2020.
8. Shamshirsaz AA, Bekheirnia MR, Kamgar M, et al. Metabolic and endocrinologic complications in beta-thalassemia major: a multicenter study in Tehran. BMC Endocr Disord 2003; 3: 4.
9. Chirico V, Antonio L, Vincenzo S, et al. Thyroid dysfunction in thalassaemic patients: ferritin as a prognostic marker and combined iron chelators as an ideal therapy. Eur J Endocrinol 2013; 169: 785–93.
10. Sakata S,Nagai K, Maekawa H, et al. Serum ferritin concentration in subacute thyroiditis. Metabolism 1991; 40: 683-8.
11. Hess SY, Zimmermann MB, Arnold M, Langhans W, Hurrell RF. Iron deficiency anemia reduces thyroid peroxidase activity in rats. J Nutr 2002; 132: 1951-5
12. Tienboon P, Unachak K. Iron deficiency anaemia and thyroid function. Asia Pac J Clin Nutr 2003; 12: 198-202.
13. Takamatsu J, Majima M, Miki K, Kuma K, Mozai T. Serum ferritin as a marker of thyroid hormone action on peripheral tissues. J Clin Endocrinol Metab. 1985; 614: 672-6.
14. Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM. Ferritin for the clinician. Blood Rev. 2009; 23: 95–104.
15. Kumon Y, Suehiro T, Nishiya K, Hashimoto K, Nakatani K, Sipe JD. Ferritin correlates with C-reactive protein and acute phase serum amyloid A in synovial fluid, but not in serum. Amyloid 1999; 6: 130-5.
16. Misra GC, Bose SL, Samal AK. Malabsorption in thyroid dysfunctions. J Indian Med Assoc 1991; 89: 195-7.

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The validity and agreement of PI-RADS v2 in the diagnosis of prostate cancer

Şehnaz Tezcan, Ülkü Bekar, Müge Gürbüz Onbaşıoğlu, Giray Ergin

Koru Hospital, Ankara, Turkey

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ABSTRACT

Aim: The purpose of this study was to evaluate accuracy of multi-parametric MRI (mpMRI) in detection of clinically significant (CS) prostate cancer (PC) and determine agreement of Prostate Imaging Reporting and Data Systems version2 (PI-RADS v2) among three readers.

Material and Method: The study included 65 (32 malignancy, 33 benign) patients with clinically suspected PC who were underwent mpMRI between January 2017 and January 2020 followed by biopsy or prostatectomy. The images were evaluated by three readers who were blinded to patient data. The inter-observer agreement was analyzed with Cohen's weighted kappa statistics.

Results: 74 lesions were detected in 46 patients among 65 patients. When a PI-RADS assessment category ≥3 (K value, 0.406-0.632) was considered positive for CS PC for readers, higher sensitivity, lower specificity and lower agreement was found than PI-RADS ≥4 (K value, 0.545-0.667). The sensitivity and specificity of index lesion detection ranged from 71.8%-90.6%, 60.6%-72.7%, respectively. We found moderate to substantial agreement for index lesion detection. The agreement of PZ lesions was higher than TZ lesions. The agreement in DWI scores was higher than the agreement in T2 scores between readers.

Conclusion: By using PI-RADS v2, high sensitivity but moderate specificity was found in detection of index lesion. The agreement in PI-RADS category assignment was moderate among readers. The agreement and sensitivity in threshold of PI-RADS 4 was higher than PI-RADS 3. TZ lesions showed more variability among radiologists than PZ lesions by using PI-RADS v2.

Keywords: The prostateimaging reporting and data systems version 2, prostate cancer, inter-observer agreement

INTRODUCTION

The use blood prostatic specific antigen (PSA) is the main screening method to detect prostate cancer (PC). However, low specificity and false positive results of PSA may result in unnecessary biopsy procedures (1). Hence, in recent years, multi-parametric magnetic resonance imaging (mpMRI) has become a widely used modality for diagnosis of clinically significant (CS) PC prior to biopsy (2,3). European Society of Urogenital Radiology (ESUR) has developed the Prostate Imaging Reporting and Data Systems version 1 (PI-RADS v1) to provide a global standardization of diagnosis of PC in 2012 (4). In PI-RADS v1, lesions were scored 1 to 5 in each individual pulse sequence. However, this categorization caused variability in assessing PC among radiologists due to lack of strength in determination of final overall score. Subsequently, PI-RADS version 2 (v2) was published in 2015 to improve inter-observer agreement (IOA) of prior PI-RADS system (5). In PI-RADS v2, the dominant sequence was determined for each zone which was diffusion-weighted images (DWI) in peripheral zone (PZ) and T2-weighted images (T2WI) in transition zone (TZ) (5,6). If a lesion score cannot be defined with dominant sequence, contrast enhancement in PZ and diffusion restriction in TZ is used to specify PI-RADS score (5).

Previous studies revealed that PI-RADS v2 had high sensitivity rates (%70-90) but low to moderate reproducibility to detect CS PC (2,3,5-9). In previous reports, IOA of PI-RADS v2 has been studied with preselected lesion which was determined by study coordinator (3,7-9). In the studies analyzing preselected lesions, the diagnostic performance of mpMRI to determine malignancy cannot be evaluated properly due to bias. In the literature, the reproducibility among readers

Corresponding Author: Şehnaz Tezcan, sehnazce@yahoo.com

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in lesion detection and characterization as would be done in routine clinical practice was analyzed in limited reports (2,6,10). This study technique provides to analyze the ability of radiologists to differentiate malignancy from benign lesions with minimizing the bias. The aim of this study was to evaluate diagnostic performance of mpMRI for detection, localization and characterization of lesions among three readers in a routine clinical practice and determine the agreement of PI-RADS v2.

MATERIAL AND METHOD

The study was carried out with the permission of Health Science University, Dr. Abdurrahman Yurtarslan Oncology Health Application and Research Center Clinical Researchs Ethics Committee (Date: 10.03.2021, Decision No: 2021-03/1071). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

This retrospective study requirement for informed consent was waived. All patients underwent 1.5 T MRI. 146 patients with clinically suspected PC based on blood PSA or clinical examination with no prior biopsy or with prior negative biopsy who were underwent mpMRI between January 2017 and January 2020 were enrolled in this study. Of these patients, 41 patients who had not histopathologic evaluation in our hospital were excluded from this study. 105 patients that were followed by radical prostatectomy (RP), trans-vesical prostatectomy or systematic 12-core trans-rectal ultrasound (TRUS)-guided biopsy, were included in this study. 34 of the 105 patients included in this study were positive for PC by histopathologic analysis. Of these patients, two patients with PC were excluded from this study because of poor image quality of contrast enhanced images. The remaining 71 patients were negative for PC. Of these patients, 33 patients were randomly selected to reach a ratio of approximately 1: 1 cancer to non-cancer control group. 33 patients were chosen from the box included patients with negative for PC (N=71) by study coordinator due to simple random sampling method. The total study population was 65 patients (32 patients with PC, 33 control subjects). A flowchart of patients who participated in the study is shown in **Figure 1**. Characteristics of patients are demonstrated in **Table 1**.

Table 1. Patient characteristics

Characteristics	Cancer group	Control group	P value
No.	32	33	
Age (y)[a]	66.3±8.2	64.6±6	0.346
PSA value[a]	13.5±15.3	8.1±6.3	0.06
PSA density[a]	0.33±0.43	0.11±0.08	0.008
Prostate volume[a]	50.4±29.9	92.7±73.5	0.004
GS/ISUP (no.)			
6/1	8 (RP:6, TRUS: 2)[a]	-	
7/2	9 (RP:8, TRUS: 1)[b]	-	
7/3	7 (RP:5, TRUS: 2)[b]	-	
8/4	4 (RP:1, TRUS: 3)[b]	-	
9/5	4 (RP:4 TRUS: 0)[b]	-	

[a]Values are number or mean with standard deviation, [b]the method of obtaining histopathologic specimens. Dash (-) represents not applicable. GS, Gleason score; ISUP, The International Society of Urological Pathology classification; RP, radical prostatectomy; TRUS, trans-rectal ultrasound (TRUS)-guided biopsy

MRI Protocol

All MRI examinations were performed using 1.5 T MRI (GE Optima 360, USA®) with 8 channel body/torso array coil. All patients were examined in supine position. A routine protocol was performed including T2WI, DWI with ADC map, T2 fat-sat, T1WI and dynamic contrast-enhanced (DCE) images. **Table 2** indicates the MRI acquisition parameters and sequences in this study. The DCE images were obtained after administration of 0.1 mmol/kg of gadoteric acid. DWI was performed using b values of 50, 1000 s/mm².

Study Design

All MRI examinations were read by 3 radiologists (10, 5 and 8 years, respectively) in prostate MRI. The images were analyzed by readers who were blinded to each patient's data and clinical findings. All readers had experience with PI-RADS v2 prior to this study. The lesion localization was not given to readers. Readers were asked to detect lesion and determine the characteristics of lesion such as localization, measurement, shape, margin, capsule invasion, extra-prostatic extension (EPE) and contrast enhancement. Readers recorded the number of lesions and determine index lesion using PI-RADS v2. And also readers assigned T2 score, DWI score and a final overall score using PI-RADS v2 of each patient. Once readers detected a lesion, they marked it and recorded the number of slice and sequence. The recorded reports for each lesion were compared among readers to determine the agreement in detection.

Table 2. Multi-parametric MRI acquisition protocol

	Axial T2WI	Sagittal T2WI	Coronal T2WI	Axial DWI	Axial T2 fat-sat	Axial T1WI	Axial DCE
Sequence	FSE	FSE	FSE	EPI	FSE	FSE	3D FSPGR
TR (ms)	5594	4300	6200	5400	5357	486	5
TE (ms)	90	102	100	80	78	35	1.7
FOV (mm²)	20×20	24×24	22×22	20×20	20×20	20×20	30×30
Flip angle (˚)	160	160	160	-	160	160	45
Matrix	320×224	320×224	320×244	140×70	320×224	288×244	192×128
B Values	-	-	-	50, 1000	-	-	-
Slice thickness (mm)	3	3	3	3	3	3	3

TR: repetition time, TE: echo-time, FOV: field of view, FSE: fast spin echo, EPI: echo planar imaging; FSPGR, fast spoiled gradient-echo; DCE, dynamic contrast enhanced

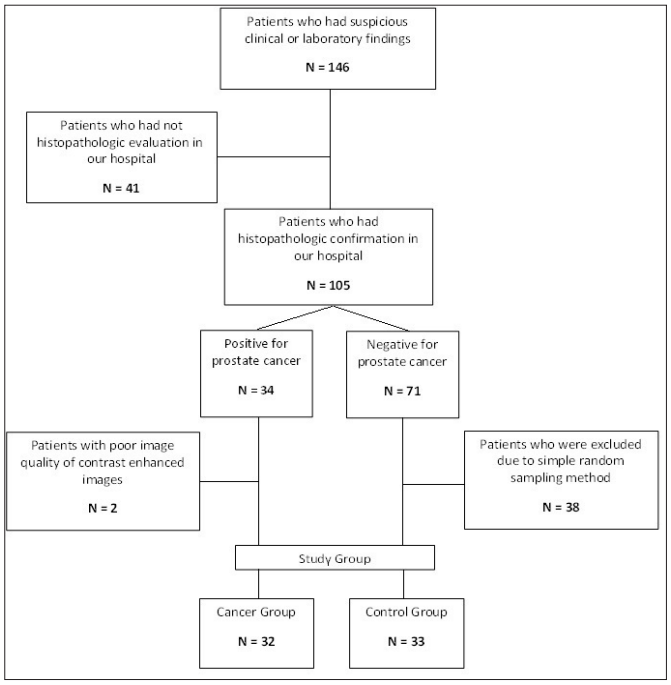

Figure 1. Flowchart of inclusion and exclusion criteria of study sample

Histopathologic Analysis

64 of 65 patients underwent a systemic 12 core TRUS-guided biopsy by 13-years experienced urologist. In cancer group (N=32), 25 of them had been performed with RP following TRUS-guided biopsy in our hospital. In remaining 7 patients with CS PC, RP had not been performed in our hospital, therefore only histopathologic results of TRUS-guided biopsy included in this study for those patients. In one participant (1/65), trans-vesical prostatectomy had been performed. The histopathologic results of TRUS-guided biopsy (39 patients), RP (25 patients) and trans-vesical prostatectomy (1 patient) were analyzed in this study. Gleason score (GS) ≥7, and/or volume ≥0.5 cc, and/or EPE were defined as CS PC. Index lesion was defined as the highest grade lesion on pathological specimens. The International Society of Urological Pathology (ISUP) classification was used to categorize the lesions.

Statistical Analysis

The Kolmogorov-Smirnov test was used to analyze the normal distribution of data. Descriptive statistics were applied to compare demographics, PSA levels and prostate volumes. The reader sensitivity, specificity and accuracy of lesions were analyzed at both patient and lesion level. The final overall PI-RADS category at patient level was defined as the PI-RADS v2 category of index lesion, was classified into two groups which were PI-RADS thresholds ≥3 and ≥4. The IOA was assessed to PI-RADS category, T2 score and DWI score, DCE positivity, lesion detection, index lesion detection and assignment of capsule invasion and EPE at patient level. The assignment and agreement of zone location of lesions were analyzed at lesion level. IOA between each pair of readers for lesions was evaluated by using Cohen's weighted kappa statistics, considering categories according to Landis and Koch recommendations (Kappa (K) value; <0 Poor; 0.00-0.20 Slight; 0.21-0.40 Fair; 0.41-0.60 Moderate; 0.61-0.80 Substantial; 0.81-1.00 Almost Perfect) with the 95% confidence intervals (CIs). Analyses were performed using SPSS (version 22). Statistical significance was accepted at a p-value of less than 0.05.

RESULTS

In 19 patients, no lesion was reported by all readers among 65 patients. In 16 of 19 patients, histopathologic workup showed no CS PC in specimens. In remaining 3 patients, CS PC was detected in PZ (ISUP 1 in two patients, ISUP 2 in 1 patient). Among 65 patients, 74 lesions were detected in 46 patients by at least one reader. Of these 74 lesions, 55 (74.3%) lesions in PZ, 19 (25.6%) lesions in TZ were labeled. Histopathologic analysis revealed 46 (39 in PZ, 7 in TZ) CS PC among these 74 lesions. In PZ lesions, ISUP 1 in 11 (28%), ISUP 2 in 10 (25.6%), ISUP 3 in 8 (20.5%), ISUP 4 in 5 (12.8%), ISUP 5 in 5 (12.8%) lesions were reported. In TZ lesions, ISUP 1 in 1 (14.2%), ISUP 2 in 2 (28.5%), ISUP 3 in 1 (14.2%), ISUP 5 in 3 (42.8%) lesions were reported. The sensitivity and specificity for all readers at patient-level was presented in **Table 3**. When a PI-RADS category ≥3 was considered positive for CS PC for all readers, the sensitivity was found to be higher than PI-RADS ≥4 for all lesions (p<0.000). The specificity decreased in PI-RADS ≥3 at versus PI-RADS ≥4 (p<0.000).

Reader agreement was defined as the agreement of PI-RADS category assessment at least two readers of three readers. While the sensitivity of reader agreement (at least 2/3 readers) was higher in PI-RADS ≥3 than PI-RADS ≥4 (84.3% vs 75%), the specificity was higher in PI-RADS ≥4 (72.7% vs 81.8%, **Table 3**). When a PI-RADS category ≥3 was considered positive for CS PC, all false positive cases were in category of ISUP 1. When a PI-RADS category ≥4 was considered positive for CS PC, the majority of false positive cases were in category of ISUP 1 (6/8). The sensitivity of reader agreement (at least 2/3 readers) was found to be similar to sensitivity of the average sensitivity of 3 readers in PI-RADS ≥3 (84.3% vs 82.2%, **Table 3**). The specificity of reader agreement (at least 2/3 readers) was found to be higher than the average specificity of 3 readers in PI-RADS ≥3 (72.7% vs 64.6%, **Table 3**). The reader agreement (at least 2/3 readers) had higher accuracy than did each readers in PI-RADS ≥3 (p<0.000, **Table 3**). While the average sensitivity of 3 readers (69.7%) was lower than that of the reader agreement (75%), the accuracy was higher in reader agreement (at least 2/3 readers) than accuracy of each readers in PI-RADS ≥4 (**Table 3**).

Table 3. Validity analysis of All lesions at patient-level

Category Assignment	Reader 1 (%)	Reader 2 (%)	Reader 3 (%)	Reader agreement* (%)
≥PI-RADS 3				
Sensitivity	90.6 (74.9-98)	84.3 (67.2-94.7)	71.8 (53.2-86.2)	84.3 (67.2-94.7)
Specificity	60.6 (42.1-77)	60.6 (42.1-77)	72.7 (54.4-86.7)	72.7 (54.4-86.7)
Accuracy	75.3 (63.1-85.2)	72.3 (59.8-82.6)	72.3 (59.8-82.6)	78.4 (66.5-87.6)
≥PI-RADS 4				
Sensitivity	68.7 (49.9-83.8)	78.1 (60-90.7)	62.5 (43.6-78.9)	75 (56.6-88.5)
Specificity	87.8 (71.8-96.6)	69.7 (51.2-84.4)	81.8 (64.5-93)	81.8 (64.5-93)
Accuracy	78.4 (66.5-87.6)	73.8 (61.4-83.9)	72.3 (59.8-82.6)	78.4 (66.5-87.6)

Values in parenthesis are 95% confidence intervals, *Reader agreement necessitated at least 2 of 3 readers assigned a lesion

Table 4 shows the sensitivity, specificity and accuracy for all readers at patient-level in the detection of index lesion.

The sensitivity and specificity of positive enhancement were 54.5% and 68.7% (reader 1), 42.4% and 87.5% (reader 2) and 45.4% and 62.5% (reader 3), respectively.

Table 5 demonstrates the sensitivity and specificity of all lesions, PZ lesions and TZ lesions for all readers at the lesion-level. The sensitivity of all lesions was higher than specificity in the majority of readers (**Table 5**). The average sensitivity of PZ lesions was higher than TZ lesions (PZ vs TZ; 71.3% vs 48.1%, respectively), but the difference wasn't significant (p=0.361). The TZ lesions had higher average specificity than did PZ lesions, however, it wasn't significant (p=0.415). The sensitivity and specificity of index lesions were higher than all remaining lesions (**Table 4, 5**).

Table 6 shows agreement for all readers in assessing lesions with PI-RADS v2. While K value of the IOA was higher in PI-RADS ≥4 than PI-RADS ≥3, the agreement between the readers was moderate to substantial in both thresholds of PI-RADS ≥3 and PI-RADS ≥4 for all lesions. In the lesion based analysis, the agreement of PZ lesions which was in moderate agreement category was higher than the agreement of TZ lesions which was in poor to fair agreement category. And also, the agreement of TZ lesions between readers wasn't significant (p >0.05). We found moderate to substantial agreement for index lesion detection and slight to moderate agreement for all lesions. The slight to fair agreement was found in T2 scores. The agreement in DWI scores which was in substantial to almost perfect agreement categories, was higher than the agreement in T2 scores between readers. In the threshold of DWI score ≥4, the agreement was higher than the threshold of DWI score ≥3. The agreement in DCE positivity was found to be fair to moderate among readers. While the agreement in capsule invasion and EPE were substantial between reader 1 and 2, the agreement between the other readers was fair to moderate (**Table 6**). Representative images were featured in **Figure 2** and **Figure 3**.

Table 4. Validity analysis of ındex lesions at patient-level

	Reader 1 (%)	Reader 2 (%)	Reader 3 (%)
Sensitivity	90.6 (74.9-98)	84.3 (67.2-94.7)	71.8 (53.2-86.2)
Specificity	60.6 (42.1-77)	60.6 (42.1-77)	72.7 (54.4-86.7)
Accuracy	75.3 (63.1-85.2)	72.3 (59.8-82.6)	72.3 (59.8-82.6)

Values in parenthesis are 95% confidence intervals

Table 5. Validity analysis of all lesions at lesion-level

	Reader 1 (%)	Reader 2 (%)	Reader 3 (%)
All lesions			
Sensitivity	83.6 (70.3-92.6)	71.4 (56.7-83.4)	51 (36.3-65.5)
Specificity	65.9 (50-79.5)	65.9 (50-79.5)	79.5 (64.7-90.2)
PZ lesions			
Sensitivity	80.9 (65.8-91.4)	76.1 (60.5-87.9)	57.1 (40.9-72.2)
Specificity	88.6 (75.4-96.2)	65.9 (50-79.5)	86.3 (72.6-94.8)
TZ lesions			
Sensitivity	87.5 (42.1-99.6)	42.8 (9.9-81.5)	14.2 (0.36-57.8)
Specificity	75.5 (60.4-87.1)	100 (92.1-100)	93.3 (81.7-98.6)

Values in parenthesis are 95% confidence intervals; PZ, peripheral zone; TZ, transitional zone

Table 6. Inter-reader Agreement in Prostate Imaging Reporting and Data Systems version 2

	Reader 1-2 K value (p value)	Reader 1-3 K value (p value)	Reader 2-3 K value (p value)
PI-RADS Category			
≥PI-RADS 3	0.406 (0.001)	0.449 (<0.000)	0.632 (<0.000)
≥PI-RADS 4	0.545 (<0.000)	0.615 (<0.000)	0.667 (<0.000)
T2 score			
≥T2 score 3	0.400 (0.001)	0.299 (0.016)	0.212 (0.078)
≥T2 score 4	0.277 (0.014)	0.149 (0.208)	0.256 (0.008)
DWI score			
≥DWI score 3	0.746 (<0.000)	0.810 (<0.000)	0.935 (<0.000)
≥DWI score 4	0.954 (<0.000)	0.915 (<0.000)	0.970 (<0.000)
DCE positivity	0.344 (0.003)	0.464 (<0.000)	0.368 (0.002)
Lesion Localization			
All lesions	0.170 (0.09)	0.144 (0.12)	0.494 (<0.000)
PZ lesion	0.493 (<0.000)	0.450 (<0.000)	0.570 (<0.000)
TZ lesions	0.113 (0.196)	-0.033 (0.733)	0.235 (0.08)
Index lesion detection	0.406 (0.001)	0.449 (<0.000)	0.632 (<0.000)
Capsule invasion	0.789 (<0.000)	0.380 (0.006)	0.412 (0.008)
EPE	0.728 (<0.000)	0.380 (0.006)	0.343 (0.033)

* K value; Kappa value of Cohen's weighted kappa statistics, CI; confidence interval; DWI, diffusion-weighted imaging; DCE, dynamic contrast enhancement, PZ, peripheral zone; TZ, transitional zone; EPE, extra-prostatic extension

Figure 2. 70-year-old man with prostate cancer (group 3). A. Diffusion-weighted image (DWI). B. Apparent diffusion coefficient (ADC). C. Axial-T2 image. The lesion in left posterolateral segment of apex is hyperintense on DWI, hypointense on ADC and T2. PI-RADS 4 category was assigned by all readers for this patient.

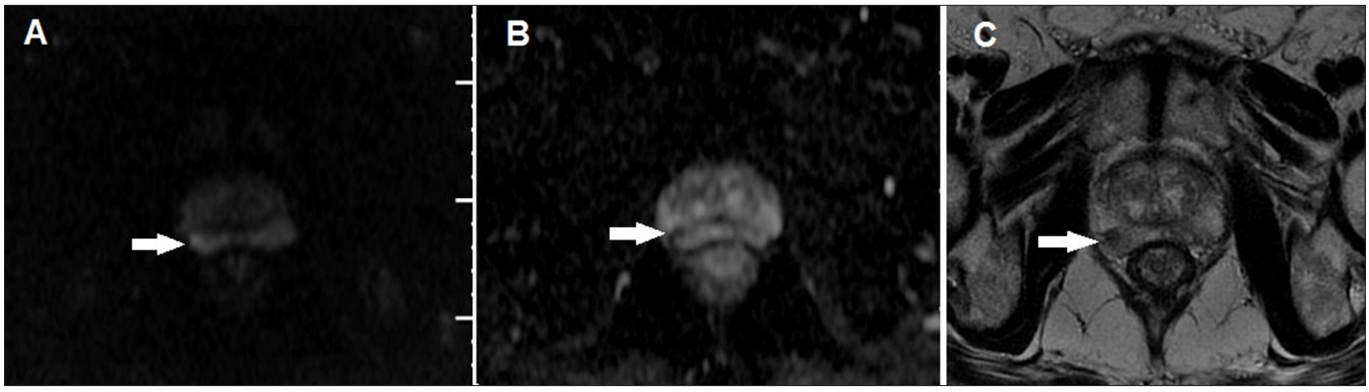

Figure 3. 70-year-old man with prostate cancer (group 1). A. Diffusion-weighted image (DWI). B. Apparent diffusion coefficient (ADC). C. Axial-T2 image. The lesion in the posterolateral segment of right midgland is hyperintense on DWI, hypointense on ADC and T2. PI-RADS 3 category was assigned by two readers in this patient. One reader assigned PI-RADS4 category.

DISCUSSION

In this study, we evaluated the agreement among three readers using PI-RADS v2 and analyzed the validity of mpMRI to detect the CS PC. The mpMRI provides additional information for the decision of biopsy besides the clinical findings. The readers evaluated the images without knowing the data of patients to minimize the bias. The determination of optimal PI-RADS category threshold for the decision of biopsy is still a confusing issue. In our study, when PI-RADS 4 was used as threshold value for biopsy indication, we observed that an increase in specificity and decrease in sensitivity than threshold of PI-RADS 3. The preference of PI-RADS 4 category for biopsy indication provided the diagnosis of benign patients more accurately and reduced unnecessary biopsy procedures. However, using PI-RADS 4 as a threshold also resulted in missing some malignant patients. When PI-RADS 3 was used as threshold value for biopsy, the unnecessary biopsy ratios increased, but the risk of misdiagnosis of malignancy has been reduced. The diagnostic accuracy of thresholds of PI-RADS 4 and PI-RADS 3 were similar to each other in this study. Previous studies also reported higher sensitivity and lower specificity for ≥PI-RADS 3 than for ≥PI-RADS 4 similar to our study (2,3,11). And also, the agreement of ≥PI-RADS 4 was higher than ≥PI-RADS 3 in this study similar to previous studies (2,3,7,11). Rosenkrantz et al. (11) found a moderate agreement among 6 readers (k values of 0.509-0.593 for ≥PI-RADS4 and 0.386-0.534 for ≥PI-RADS3) and Purysko et al. (3) reported substantial and almost perfect agreement among 2 readers (k values of 0.91 for ≥PI-RADS4 and 0.63 for ≥PI-RADS3) from different medical centers, evaluating the preselected lesions. Although, readers from different centers may improve the results due to eliminating similar approaches of readers from single center, the study design with evaluating the preselected lesions may lead to bias in evaluating reproducibility and accuracy of the results. The agreement of the both PI-RADS 3 and 4 thresholds were moderate to substantial among 3 readers in our study. We believe that the definition of PIRADS 3 is inadequate for differentiation of malignancy from benign lesions. In the description of PI-RADS 3, the subjective term of mildly or moderately signal intensity may result in variability among readers due to lack of clear definition of assessment in PI-RADS 3. In TZ, some benign nodules may show hyper-intensity on DWI and hypo-intensity on ADC, thus the morphologic features of TZ lesions on T2 predominates for the assessment of PI-RADS category. When heterogeneous signal intensity with obscured margins is detected in TZ on T2, PI-RADS category assigned as 3 due to PI-RADS v2. However, in

PI-RADS category assessment of TZ lesions, the greater amount of benign prostatic nodules may lead to difficulty in evaluating the margin and morphology of lesions on T2. The majority of the previous studies reported poor agreement in TZ than PZ among readers due to the uncertainty definition which may lead to variability in PI-RADs v2 (2,3,7,8,11). In current study, slight agreement was found in TZ lesion that was lower than PZ lesion among readers.

DCE provides information to discriminate the PI-RADS 4 from PI-RADS 3 due to the principle of early enhancement of malignancy as compared to adjacent normal prostatic tissue. However, direct visual assessment of enhancement without any qualitative evaluation such as kinetic curves or semi-quantitative methods may lower the agreement among readers. Kim et al. (12) found a limited efficiency of time-intensity curves to predict the malignancy. Rosenkrantz et al. (13) reported higher sensitivity of semi-quantitative model than qualitative model with no association of both models with GS. In the literature, the impact of DCE on PI-RADS v2 is still controversial (2,7,11-13). In this study we found slight to moderate agreement and moderate sensitivity and specificity in positivity of early enhancement of lesions similar to previous studies (2,7,11). Although, previous studies reported various validity and reproducibility results of DCE, the impact of the usefulness of mpMRI as compared to bi-parametric MRI was emphasized in the literature (14,15). Nevertheless, further studies which analyze the qualitative evaluation or quantitative and semi-quantitative methods in predicting the strength of DCE besides the direct visual evaluation on PI-RADS categorization with larger patient group are required to decide whether contrast agent is needed for the diagnosis of CS PC in MRI.

Double reading of MRI may be used in training to increase the experience of radiologists and also may improve the diagnostic performance of work-up. It is widely used in screening mammography to increase the accuracy (16). In mpMRI, double reading is not a common method in interpretation of MRI which has a moderate reproducibility among readers, in particular for low-experienced or nonspecialized radiologists (2,3,7,8,17). In this study, the sensitivity, specificity and accuracy were mildly higher in double reading than the average of 3 readers in both threshold of PI-RADS 3 and 4. In double reading, the majority of false positive cases had low grade PC in the current study. Previous studies also reported lower accuracy results in low grade tumors than high grade tumors in mpMRI (18). Although we found a mild increase in the validity of double reading than readers individually, it cannot be an advice for using the double reading as a method in interpretation of mpMRI due to our results.

In PI-RADS v2, the index lesion is defined as a lesion which has the highest PI-RADS assessment category. Our results showed high sensitivity, moderate specificity and moderate to substantial agreement in detecting the index lesions similar to previous studies (2,3,6-8). We found higher sensitivity, specificity and reproducibility in index lesions than all remaining lesions for all readers. Reporting the index lesion provides convenience to determine the overall PI-RADS category and to guide subsequent biopsy for cognitive biopsy technique. Although, biopsy is performed to multiple cores to improve the diagnostic performance, predicting the index lesion with mpMRI prior to procedure may be helpful to focus on the highest suspicious core for multiple tissue sampling.

Our study had some limitations. First, the study design was retrospective and sample size was small. Second, this study evaluated agreement among three readers without considering the experience levels of readers which may have an influence on the agreement. Third, as previously noted, all examinations were performed at a single center with using same protocol by readers from single center which could induce similar approaches. Nevertheless, we established the study method including the evaluation as it should be in routine clinical practice without analyzing preselected lesions to minimize this bias. Even though, studies evaluating the preselected lesions may ensure that all readers analyzing same lesion, in current study the lesions were marked by readers to avoid misinterpretation. In the current study, we included patients who had been performed TRUS-guided biopsy besides patients subjected to radical prostatectomy which would induce a bias toward high-risk PC. However, TRUS-guided biopsy may lower the accuracy of results due to the lack of evaluation of all segments of prostate on specimens which was the fourth limitation of this report.

CONCLUSION

By using PI-RADS v2, radiologist can detect the malignant lesions in particular for index lesions with high sensitivity rates that enable to make decision on biopsy beside blood PSA levels and physical examination. And also, the determination of index lesion prior to biopsy using mpMRI forms an advantage to focus on the most suspicious region while tissue sampling. Specificity was found to be moderate that may result in unnecessary biopsy procedures in some cases. The moderate agreement in PI-RADS category assignment has confirmed the results of previously reported studies. PZ lesions showed lesser variability among radiologists than TZ lesions by using PI-RADS v2. The agreement in threshold of PI-RADS 4 was higher than PI-RADS 3 that can be resulted due to the inadequate definition of PI-RADS3 in PI-RADS v2.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Health Science University, Dr. Abdurrahman Yurtarslan Oncology Health Application and Researchs Center Clinical Research Ethics Committee (Date: 10.03.2021, Decision No: 2021-03/1071).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Kohestani K, Wallström J, Dehlfors N, et al. Performance and inter-observer variability of prostate MRI (PI-RADS version 2) outside high-volume centres. Scand J Urol 2019; 53: 304-11.
2. Greer MD, Shih JH, Lay N, et al. Interreader variability of prostate imaging reporting and data system version 2 in detecting and assessing prostate cancer lesions at prostate MRI. AJR Am J Roentgenol 2019; 1-8.
3. Purysko AS, Bittencourt LK, Bullen JA, Mostardeiro TR, Herts BR, Klein EA. Accuracy and interobserver agreement for prostate imaging reporting and data system, version 2, for the characterization of lesions identified on multiparametric MRI of the prostate. AJR Am J Roentgenol 2017; 209: 339-49.
4. Barentsz JO, Richenberg J, Clements R, et al. European Society of Urogenital Radiology. ESUR prostate MR guidelines 2012. Eur Radiol 2012; 22: 746-57.
5. American College of Radiology. Prostate imaging reporting and data system version 2. www.acr.org/Quality-Safety/Resources/PIRADS.Published 2015.
6. Greer MD, Brown AM, Shih JH, et al. Accuracy and agreement of PIRADSv2 for prostate cancer mpMRI: a multireader study. Comparative Study J Magn Reson Imaging 2017; 45: 579-85.
7. Mussi TC, Yamauchi FI, Tridente CF, et al. Interobserver agreement of PI-RADS v. 2 lexicon among radiologists with different levels of experience. J Magn Reson Imaging 2020; 51: 593-602.
8. Mussi TC, Yamauchi FI, Tridente CF, et al. Interobserver agreement and positivity of PI-RADS version 2 among radiologists with different levels of experience. Acad Radiol 2019; 26: 1017-22.
9. Seibert MK, Lehmann T, Aschenbach R, et al. Assessment of PI-RADS v2 for the detection of prostate cancer. Eur J Radiol 2016; 85: 726-31.
10. Popita C, Popita A, Andrei A, et al. Interobserver agreement in prostate cancer detection using multiparametric MRI. J BUON 2018; 23: 1061-9.
11. Rosenkrantz AB, Ginocchio LA, Cornfeld D, et al. Interobserver reproducibility of the PI-RADS version 2 lexicon: a multicenter study of six experienced prostate radiologists. Radiology 2016; 280: 793-804.
12. Kim SH, Choi MS, Kim MJ, et al. Role of semi-quantitative dynamic contrast-enhanced MR imaging in characterization and grading of prostate cancer. Eur J Radiol 2017; 94: 154-9.
13. Rosenkrantz AB, Sabach A, Babb JS, et al. Prostate cancer: comparison of dynamic contrast-enhanced MRI techniques for localization of peripheral zone tumor. AJR Am J Roentgenol 2013; 201: W471-8.
14. Franco FB, Fennessy FM. Arguments against using an abbreviated or biparametric prostate MRI protocol. Abdom Radiol (NY) 2020; 45: 3982-9.
15. Xu L, Zhang G, Shi B, et al. Comparison of biparametric and multiparametric MRI in the diagnosis of prostate cancer. Cancer Imaging 2019; 19: 90.
16. Trimboli RM, Verardi N, Cartia F, Carbonaro LA, Sardanelli F. Breast cancer detection using double reading of unenhanced MRI including T1-weighted, T2-weighted STIR, and diffusion-weighted imaging: a proof of concept study. AJR Am J Roentgenol 2014; 203: 674-81.
17. Puech P, Randazzo M, Ouzzane A, et al. How are we going to train a generation of radiologists (and urologists) to read prostate MRI? Curr Opin Urol 2015; 25: 522-35.
18. Holtz JN, Silverman RK, Tay KJ, et al. New prostate cancer prognostic grade group (PGG): can multiparametric MRI (mpMRI) accurately separate patients with low-, intermediate-, and high-grade cancer? Abdom Radiol (NY) 2018; 43: 702-12.

Original Article

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Bilayer repair technique with mesh in petit lumbar hernia

Ramazan Topcu[1], İsmail Sezikli[1], Hülya Topcu[2]

[1]Hitit University, Faculty of Medicine, Department of General Surgery, Çorum, Turkey
[2]Hitit University, Faculty of Medicine, Department of Anesthesia and Reanimation, Çorum, Turkey

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ABSTRACT

Aim: Lumbar hernias are rare; Petit's lumbar hernia (PLH), which is among them, is a rarer hernia type. Surgical repair is a standard treatment after the diagnosis of lumbar hernia. Controversy continues regarding the surgical technique of repairing Petit's lumbar hernia. With this study, we aim to share short term results we obtained from the application of bilayer repair technique with mesh (BRTM), which is a new method in petit lumbar hernia treatment.

Material and Method: Five patients who were diagnosed with lumbar hernia and treated between 2017 and 2020 were examined retrospectively.

Results: Most patients consisted of women (n=4). The mean age was 58.2 years. As for the etiological reason of the disease, 3 patients were trauma-related and 2 patients were previous operations-related. The petit lumbar hernia defect diameter was 6.42. In all patients, BRTM was used. The mean length of hospitalization was 3.6 days. The mean follow-up duration was 13.8 months. There was no recurrence in any of the patients after follow-up.

Conclusion: PLH requires a difficult management from diagnosis to treatment. Thanks to such a strong BRTM, not merely tissue integrity can be ensured, but also anatomical structures will become more organized, and intra-abdominal pressure will be evenly distributed to the preperitoneal area, creating a suspensory mechanism, also, since recurrence will be minimized, we predict that the surgery operation can be performed safely through BRTM in petit lumbar hernia. To support this technique, long-term follow-up and multi-centered studies are necessary.

Keywords: Petit lumbar hernia, bilayer repair technique, recurrence

INTRODUCTION

Even in the times of advanced medicine, it is possible that the rarity of a condition complicates diagnosis and treatment; such a difficulty is found in a lumbar hernia. Lumbar hernia was first proposed by Barbette in the late 17th century, and Garageot published his first case in 1731. Lumbar hernia is a protrusion of intraperitoneal or extraperitoneal contents through a defect in the posterolateral abdominal wall (1). The most common site for lumbar hernia formation is in the upper lumbar triangle (Grynfeltt) and less commonly in the lower lumbar triangle (Petit) (2).

Grynfeltt is an inverted triangle bordered by the 12th rib superiorly, laterally (anterior obliquely) and quadratus lumborum medial (posterior). The floor is the transverse fascia and the aponeurosis of the transversus abdominis muscle. The roof is the external oblique and latissimus dorsi muscles.

Petit is a smaller right triangle located between the posterior border of the external oblique muscle lateral (anterior), the lateral border of the latissimus dorsi muscle medially (backward), and inferior iliac crest. It consists of the lumbodorsal fascia (3,4).

Twenty percent of lumbar hernias are congenital; 80 percent are acquired (5), which may be either primary (spontaneous) or secondary to trauma or surgery such as nephrectomy, adrenalectomy, liver resection, and abdominal aortic aneurysm repair Posttraumatic and post-incision lumbar hernias are larger and more diffuse than primary lumbar hernias, may not observe the triangular borders outlined above and are therefore often referred to as lateral hernias (5).

Clinically, the most common presentation of a lumbar hernia is a palpable posterolateral mass that increases in

Corresponding Author: Ramazan Topcu, topcur58@gmail.com

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size with coughing and strenuous activity (6). The mass is usually shrinkable and disappears when the patient assumes the decubitus position (7). The risk of intestinal confinement is low (< 10 percent) because the neck hernia is wide at the mouth and is located within the abdominal wall of the hernia (5,8). Lumbar hernias may also present as vague back pain, bowel obstruction, urinary obstruction, pelvic mass, or rarely retroperitoneal or gluteal abscess.

Petit lumbar hernia (PLH) comprises a difficult identification process for surgeons, from diagnosis to treatment in terms of both its rarity and complex anatomical localization (9).

Since lumbar region hernia has risk of strangulation and/or incarceration, it should be treated surgically (10). It is also a matter of debate what surgical repair technique will end up with. Many techniques such as primary repair, musculoaponeurotic reconstruction, prosthetic mesh repair and laparoscopic repair have been defined (10). Whereas some sources discuss that approximation of these hernia boundaries only is sufficient, other sources suggest that the use of mesh necessarily should be used. Surgical treatment of lumbar hernia may be the best site for tension-free mesh placement in the preperitoneal region (11).

Lumbar hernias are rare, yet PLH, which is one of them, is a rarer hernia. Discussion on surgical technique of PLH repair still continues. BRTM steps forth more than other techniques in terms of placing sublay mesh, which constitutes the main rationale of the laparoscopic repair technique, increasing the protection at the lower edge of the iliac crest, and strengthening the muscle groups depicting the petit triangle with onlay mesh.

With this study, we aim to share short term results we obtained from the application of BRTM, which is a new method in petit lumbar hernia treatment using both sublay mesh and onlay mesh.

MATERIAL AND METHOD

The study was approved by the Hitit University Non-interventional Researchs Ethics Committee (Date: 30.04.2021, Application No :2021-80, Decision No: 2021-66). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total 5 patients who were operated upon the diagnosis of PLH between 2017 and 2021 were investigated retrospectively from the hospital's database and clinical archive. The patients were examined in terms of age, gender, etiology of the disease, side, surgical technique, presence of postoperative complications, radiological imaging, length of hospital stay, follow-up time and recurrence.

Bilayer Repair Technique

The patient was positioned laterally. A transverse incision was made about 6-8 cm above the iliac crest and next to the paraspinal muscular system. A hernia sac emerges in the subcutaneous dissection, lower part of the latissimus dorsi muscle, top edge of the iliac crest and medial of the external oblique muscle (**Figure 1**).

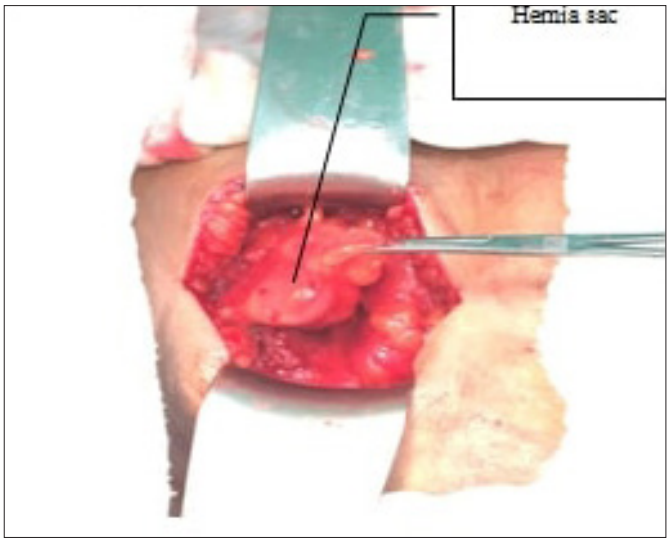

Figure 1. Hernia sac after dissection of subcutaneous tissue

To reduce the hernia defect, the edges of the petit triangle were approximated to the musculoaponeurotic tissues with 2/0 polyglactin. It was placed as "onlay" to close the lower boundary on the iliac crest, and fixation sutures were made on the musculoaponeurotic tissue with 2/0 polypropylene after the musculoaponeurotic reconstruction. The subcutaneous hemovac drain was fixed. Subcutaneous tissue was covered with absorbable 3/0 polyglactin sutures. The skin was closed with 3/0 polyprolen seam and compressed dressing was applied (**Figure 3**).

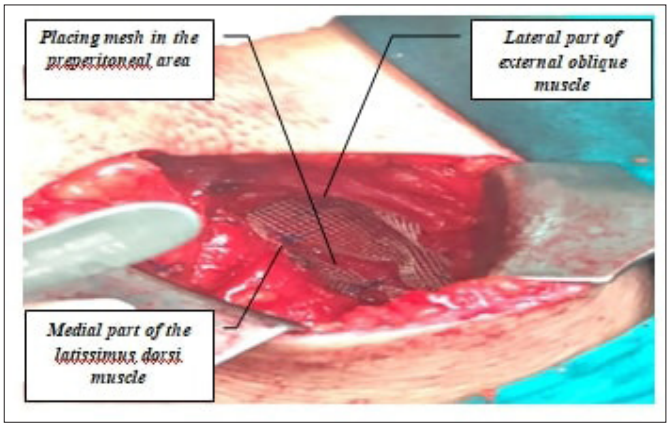

Figure 2. Placement of sublay mesh to preperitoneal area and its detection

Figure 3. Placing onlay mesh on musculoaponeurotic tissue

RESULTS

Five patients who were included in the study were operated and followed up by the same surgeon. Most patients consisted of women (n=4). The mean age was 58.2 years. As for the etiological reason of the disease, 3 patients were trauma-related and 2 patients were previous operations-related. The most common reason for hospital admission was left side pain and swelling. For diagnosis, all patients were asked to have computerized tomography (CT) (**Figure 4**).

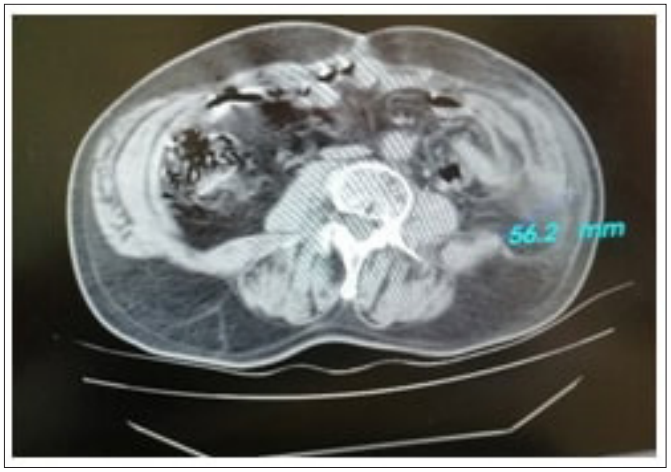

Figure 4. Left lumbar petit hernia in CT

The petit lumbar hernia defect diameter was 6.42. In all patients, the bilayer hernia repair technique with mesh was used. The mean length of hospitalization was 3.6 days. The mean follow-up duration was 13.8 months. There was no recurrence in any of the patients after follow-up. Patients' data are given in **Table 1**. The drains were removed the third day post-operation. The patients were discharged the third or fourth day in general. The patients have been followed up so far and no complication has been found regarding the wound site. The patients do their daily activities without any limitation in their life.

DISCUSSION

PLH is defined as herniation of retroperitoneal fat through aponeurosis of the internal abdominal oblique muscle between erector spinae muscles in the lower lumbar triangle. The neck of this hernia is usually large and so, it has lower risk of strangulation compared to other hernias (12). The PLH was defined by Petit in 1783. The edges of the lower lumbar (Petit) triangle consist of the iliac crest, the sides of the latissimus dorsi muscle, and the external abdominal oblique muscles. In the lower lumbar triangle base, the internal abdominal oblique muscle is seen.

These hernias should not be treated conservatively due to two reasons. The first reason is that about 25% of these hernias are tended to incarceration and 10% are tended to strangulation that emerges with acute abdominal features and requires emergency surgery (13). The second one is that these hernias tend to increase in size over time. Surgical repair of a large hernia is difficult. Thus, surgical repair is a preferred treatment without any delay (14).

Petit lumbar hernia requires a difficult management from diagnosis to treatment. Many options such as primary repair, musculoaponeurotic reconstruction, prosthetic mesh repair and laparoscopic repair have been offered as surgical techniques. Surgical treatment is open or laparoscopy and has an equal rate of success (15). Recurrences are known in laparoscopy and open surgical repair. Patients with a risk of recurrence are those who have common lumbar hernias, hernias larger

Table 1. Patients' data

Case	Age	Sex	Etiology	Application complaint	Defect diameter (cm)	Operation	Recurrence	Length of hospital stay (day)	Postoperative complication	Follow-up duration (month)
1	57	M	Trauma	Left side pain, swelling	5.6	BRTM	No	4	No	20
2	58	F	Trauma	Right side pain, swelling	6	BRTM	No	4	No	18
3	76	F	Previous operation	Left side pain	8	BRTM	No	3	No	17
4	50	F	Previous operation	Right side pain	6.4	BRTM	No	4	No	7
5	50	F	Trauma	Right side pain	6.1	BRTM	No	3	No	7

BRTM: Bilayer repair technique with mesh

than 16 cm and hernias associated with muscular atrophy (16). Therefore, preference on surgical repair depends on the patient factor and expertise of the surgeon.

Considering the triangle region where the petit lumbar hernia is formed, it becomes a three dimensional space resulting from the destruction caused by the hernia content (11). The repair with sublay mesh alone, which constitutes the main rationale of the laparoscopic repair technique, protects the muscles in this area and the spaces between the muscles. The onlay mesh, making the second layer of the bilayer repair technique, offers a more solid and reliable repair option by increasing the protection in the lower edge of the iliac crest and strengthening the muscle groups that picture the petit triangle. There are two studies on bilayer repair with mesh (BRWM), one of which is case report. The other is the comparison of onlay mesh repair and BRWM in the lumber incisional hernias and is offered as a new technique (17). They stated that this technique could be used on patients safely and they could do recurrence and daily activities without pain in their follow-up. BRWM was applied in all cases of this study and no complication or recurrence was seen in their 13.8 months of follow-up. The patients can easily do their daily activities without any limitation. The current studies support this study.

Before choosing a technique, a full diagnosis must be made so that we know the size, location and content of the defect. Therefore, CT should be performed for the correct planning of the surgery. The operation to be performed on the patient should be decided by considering the benefits or harms of each technique. The anterior approach is highly traumatic and requires extensive dissection to identify the damaged planes and locate the defect, but has the advantage of enabling us to perform a complete parietal reconstruction (18-20). The laparoscopic approach has the advantage of being minimally invasive. It also prevents large dissections, provides the exact location of the lesion and offers excellent visualization, thus avoiding possible lesions to adjacent structures (eg ureter, colon, nerves). However, the laparoscopic approach does not allow for parietal reconstruction or repair under controlled tension (21-30). Therefore, we believe that the double mesh technique should be performed as the repair must be done under a certain degree of tension in order to guarantee an adequate aesthetic and functional result.

It is divided into two types which are congenital (20%) and acquired (80%) according to etiology. Congenital herniated disc is common in babies and results from the musculoskeletal defects. Acquired herniated disc can be seen in two types: primary idiopathical (55%) and secondary (25%) PLH developed after trauma, iliac crest graft, infection, etc. (31) In this study, PLHs occurred in 3 patients after a traffic accident and in 2 patients due to previous operations.

They are generally asymptomatic and, either a palpable mass or not, they may cause back pain in the sciatic nerve distribution area. According to Light, back pain is the most possible diagnosis in young women and sporters (32). Also, abscess, hematoma, lipoma, kidney tumors or twitching should be considered in the diagnosis. For screenings, CT is known to be a routine diagnosis technique to examine patients with herniated disc (8,33). Most patients went to the hospital with non-specific complaints, and only one of them went to the emergency service with serious flank pain. This patient had strangulation and was operated under emergency conditions. Although the palpable mass was considered to be lipoma, hematoma or a deep abscess at first, the screening showed that it was a strangulated intestinal structure. There are two points to be considered here. In emergency applications, the presence of trauma becomes important in the differential diagnosis of strangulated lumbar hernia, hematoma or deep abscess, particularly in the palpable mass in the flank area. It should be kept in mind that the palpable structure in the non-traumatic flank area can spring from lumbar hernia and so, an evaluation should be made using CT in the differential diagnosis (13).

In addition, one patient in this study went to the plastic surgery outpatient clinic with the complaint of right flank pain and swelling, and the superficial sonography was compatible with lipoma, and when it was noticed that there was a hernia during excision under local anesthesia, the operation was abandoned and directed to the polyclinic. The mass was palpated in the sitting position. A diagnosis of PLH was made on CT. The gold standard for diagnosing PLH is a CT with 98% sensitivity. The benefits of CT are; distinguishing the fascial and muscle layers, detecting a defect in one of these layers, evaluating the nature of the possible herniated content and making the differential diagnosis of soft tissue lesions such as lipoma, hematoma (33).

The diagnosis and treatment of lumbar hernias are complicated by its low incidence, resulting in scarce experience and lack of consensus in the literature. Heterogeneity in the population, based on the subdivision in etiology, increases this problem. Publication bias, due to the design of the included studies, might result in an overestimation of the complaints and complications of the patient at presentation, like incarceration. On the other hand, it can cause an underestimation of the recurrence rates. More high-quality research is needed, in which, unfortunately, is the incidence of a lumbar hernia a severely limiting factor. Centralising care for this rare entity would increase specialisation and produce the volumes needed for adequate research. Furthermore, cooperation between specialised abdominal wall centres would increase the awareness of this type of dorsal lumbar abdominal wall hernia.

CONCLUSION

Thanks to such a strong bilayer repair technique with mesh, not merely tissue integrity can be ensured, but also anatomical structures will become more organized, and intra-abdominal pressure will be evenly distributed to the preperitoneal area, creating a suspensory mechanism, also, since recurrence will be minimized, we predict that the surgery operation can be performed safely through bilayer repair technique with mesh in petit lumbar hernia. To support this technique, long-term follow-up and multi-centered studies are necessary.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Hitit University Non-interventional Researchs Ethics Committee (Date: 30.04.2021, Application No :2021-80, Decision No: 2021-66).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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REFERENCES

1. Burt BM, Afifi HY, Wantz GE, Barie PS. Traumatic lumbar hernia: report of cases and comprehensive review of the literature. J Trauma Inj Infect Crit Care 2004; 57: 1361–70.
2. Wakhlu A, Wakhlu AK. Congenital lumbar hernia. Pediatr Surg Int 2000; 16: 146-8.
3. Cavallaro G, Sadighi A, Paparelli C, et al. Anatomical and surgical considerations on lumbar hernias. Am Surg 2009; 75: 1238.
4. Beffa LR, Margiotta AL, Carbonell AM. Flank and lumbar hernia repair. Surg Clin North Am 2018; 98: 593.
5. Teo KA, Burns E, Garcea G, et al. Incarcerated small bowel within a spontaneous lumbar hernia. Hernia 2010; 14: 539.
6. Liang TJ, Tsai CY. Images in clinical medicine. Grynfeltt hernia. N Engl J Med 2013; 369: 14.
7. Orcutt TW. Hernia of the superior lumbar triangle. Ann Surg 1971; 173: 294.
8. Moreno-Egea A, Baena EG, Calle MC, et al. Controversies in the current management of lumbar hernias. Arch Surg 2007; 142: 82.
9. Cesar D, Valadão M, Murrahe RJ. Grynfelt hernia: case report and literature review. Hernia 2012; 16: 107-1.
10. Bathla L, Davies E, Fitzgibbons RJ Jr, et al. Timing of traumatic lumbar hernia repair: is delayed repair safe? Report of two cases and review of the literature. Hernia 2011; 15: 205-9.
11. Bigolin AV, Rodrigues AP, Trevisan CG, et al. Petit lumbar hernia-a double-layer technique for tension-free repair. Int Surg 2014; 99: 556-9.
12. Petit JL. Traite des maladies chirurgicales, et des operations qui leur convenient. Vol. 2. T.F. Didot; Paris: 1774. pp. 256–8.
13. Ahmed ST, Ranjan R, Saha SB, et al. Lumbar hernia: a diagnostic dilemma. BMJ Case Rep 2014; 2014: bcr2013202085.
14. Esposito C, Settimi A, de Marco M, et al. Congenital lumbar hernia: two case reports and a review of the literature. J Paediatr Surg Spec 2009; 3: 40–2
15. Mingolla GP, Amelio G. Lumbar hernia misdiagnosed as a subcutaneous lipoma: a case report. J Med Case Rep 2009;103: 9322.
16. Moreno-Egea A, Alcaraz AC, Cuervo MC. Surgical options in lumbar hernia: laparoscopic versus open repair. a long-term prospective study. Surg Innov 2013; 20: 331–44.
17. Parikh APK, Parikh P, Vadodariya D, Dave JP, Bhatt JG. Triple mesh technique in repair of recurrent lumbar incisional hernia. Int J Res Med Sci 2019; 7: 4803-6.
18. Lichtenstein IL. Repair of large diffuse lumbar hernias by an extraperitoneal binder technique. Am J Surg 1986; 151: 501-4.
19. Sutherland RS, Gerow RR. Hernia after dorsal incision into lumbar region: a case report and review of pathogenesis and treatment. J Urol 1995 ;153: 382-4.
20. Bolkier M, Moskovitz B, Ginesin Y, et al. An operation for incisional lumbar hernia. Eur Urol 1991; 2052-3.
21. Burick AJ, Parascandola S. Laparoscopic repair of a traumatic lumbar hernia: a case report. J Laparoendosc Surg 1996; 6: 259-2.
22. Woodward AM, Flint L, Ferrara J . Laparoscopic retroperitoneal repair of recurrent postoperative lumbar hernia. J Laparoendosc Adv Surg Tech A 1999; 9: 181-6.
23. Arca MJ, Heniford BT, Pokorny R, et al. Laparoscopic repair of lumbar hernias. J Am Coll Surg 1998; 187: 147-2.
24. Moreno-Egea A, Torralba JA, Morales G, et al. Open vs laparoscopic repair of secondary lumbar hernias: a prospective nonrandomized study. Surg Endosc 2005; 19: 184-7.
25. Bickel A, Haj M, Eitan A. Laparoscopic management of lumbar hernia. Surg Endosc 1997; 11: 1129-0.
26. Heniford BT, Iannitti DA, Gadner M. Laparoscopic inferior and superior lumbar hernia repair. Arch Surg 1997; 132: 1141-4.
27. Shekarriz B, Graziottin TM, Gholami S, et al. Transperitoneal preperitoneal laparoscopic lumbar incisional herniorrhaphy. J Urol 2001; 166: 1267-9.
28. Meinke AK. Totally extraperitoneal laparoendoscopic repair of lumbar hernia. Surg Endosc 2003; 17: 734-7.
29. Postema RR, Bonjer HJ. Endoscopic extraperitoneal repair of a Grynfeltt hernia. Surg Endosc 2002; 167-9.
30. Habib E. Retroperitoneoscopic tension-free repair of lumbar hernia. Hernia 2003; 7: 150-2.
31. Lillie GR, Deppert E. Inferior lumbar triangle hernia as a rarely reported cause of low back pain: a report of 4 cases. J Chiropr Med 2010; 9: 73–5.
32. Light HG. Hernia of the inferior lumbar space. A cause of back pain. Arch Surg 1983; 118: 1077–80.
33. Suarez S, Hernandez JD. Laparoscopic repair of a lumbar hernia: report of a case and extensive review of the literature. Surg Endosc 2013; 27: 3421–9.

Original Article

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Prediction of hospitalization and blood transfusion requirement in patients with macroscopic hematuria

Hasan Basri Çetinkaya, Harun Güneş

Balıkesir University, School of Medicine, Department of Emergency Medicine, Balıkesir, Turkey

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ABSTRACT

Aim: It was aimed to find out simple parameters that can be used in the emergency triage and can discriminate patients requiring hospitalization or blood transfusion (BT) among those presenting with macroscopic hematuria (MH).

Material and Method: Medical records of the patients presenting to the emergency department (ED) with MH were investigated retrospectively. The patients' vital signs including pulse rate (PR), systolic blood pressure (SBP) and diastolic blood pressure, and laboratory test results including hemoglobin, platelet count and international normalized ratio were recorded. Shock index (SI) was calculated using SBP and PR of each patient.

Results: Seventy seven patients were recruited into the study. PR and SI were found to significantly differ between the patients discharged from the ED and those who needed hospitalization. Hospitalized patients were seen to have higher PR values and SI compared to discharged ones. PR and SI were higher in patients requiring BT compared to those who did not require transfusion.

Conclusion: Use of PR and SI in the emergency triage may be helpful while discriminating patients who need hospitalization or BT among those presenting with MH.

Keywords: Macroscopic hematuria, hospitalization, blood transfusion, shock index, pulse rate

INTRODUCTION

Macroscopic hematuria (MH) is one of the leading symptoms which cause considerable anxiety although it is not one of the commonest symptoms encountered in the emergency department (ED). The most significant final diagnosis is urologic cancer which may occur in renal, ureteric, bladder or prostatic level. The other possible diagnoses include benign prostatic hyperplasia, urolithiasis, urinary tract infection and renal problems. Less commonly urinary tract injury may also lead to MH (1-5). MH patients generally admit to the hospital through the ED. Recognition of those patients and determining a suitable diagnostic and therapeutic management strategy are significant (6). Most of the MH patients can be discharged from the ED and seen again for more detailed evaluation at the outpatient clinic within 2 weeks (6-8). On the other hand, some of the patients may need admission for hospitalized follow-up or rarely for surgical intervention. There are no guidelines for management of MH patients in ED so those patients are often inappropriately discharged or referred to another medical facility (9).

We suggest that a simple and catchy method to recognize MH patients would help physicians. Blood pressure and pulse rate (PR) are routinely measured in EDs to determine the severity of the patient's clinical condition. Shock index (SI) can be calculated easily in the early period during the ED triage. Literature includes previous studies reporting that SI can be used to predict prognosis in patients with hypovolemia, sepsis, myocardial infarction and pneumonia (10-12). SI is a simple parameter calculated with the formula PR divided by systolic blood pressure (SBP), and it provides more reliable information about the hemodynamic status than PR or SBP does individually (13). Normal range of SI is 0.5 to 0.7 (14).

The current study aimed to find out simple parameters which help the physician determine the patients with more critical condition in the early period by helping discriminate those requiring hospitalization or blood transfusion (BT) among all admissions related to MH.

Corresponding Author: Hasan Basri Çetinkaya, hbcetinkaya@gmail.com

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MATERIAL AND METHOD

The study was approved by the Balıkesir University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 19.08.2020, Decision No: 2020/140). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The present study was performed by retrospectively investigating the hospital records of MH patients above 18 years of age who admitted to ED of a university hospital. Patients below 18 years of age and those who refused the treatment and left the ED before his or her observation period completed were excluded from the study. A patient was recruited into the study if he or she was diagnosed with MH by the emergency physician and the diagnosis was confirmed by the consulting urologist. Gender, age, comorbidities, blood-thinning medication (anticoagulant or antiplatelet) use and vital signs (PR, SBP and diastolic blood pressure (DBP)) of the patients were taken from their charts stored in the hospital's archive. Results of laboratory tests including hemoglobin (Hmg), platelet count (Plt) and international normalized ratio (INR) were recorded. The patients were grouped in terms of BT requirement in the ED and their outcome (hospitalized or discharged from the ED).

Statistical Analysis

Shapiro-Wilk test was used to test the normality of variables. Continuous variables were presented as mean±standard deviation for normally distributed variables and with median (25^{th} percentile-75^{th} percentile) values for non-normal variables. Categorical variables were expressed by number and percentages. Receiver operating characteristics (ROC) curve analysis was performed to evaluate and compare the performances of diagnostic markers. Youden J index was used to obtain optimal cut-off value, and related sensitivity, specificity, positive predictive and negative predictive values were given. Significance level was taken as α=0.05. Statistical analyses were performed with IBM SPSS Statistics version 22.0 (IBM Corp., USA) and MedCalc version 12.3.0.0.

RESULTS

The study included 77 patients, with a median age of 73 (66-83). Among 77 patients, 67 (87%) were male. The demographic and laboratory data of the patients were given in **Table 1**.

Of the 77 patients, 27 (35.06%) were discharged from the hospital and 50 (64.94%) patients were hospitalized. While 26 (33.77%) of the patients required BT, 51 (66.23%) did not require. We performed ROC curve analyses to evaluate the diagnostic performances of SI, PR, SBP and DBP in discriminating discharged patients from those who were hospitalized. Considerably good diagnostic performances were obtained for SI (AUC=0.647, p=0.028) and PR (AUC=0.689, p=0.004). But these two parameters were seen to not differ significantly in terms of their diagnostic performances while predicting the patients who needed hospitalization (p=0.380). Also ROC curve analyses were performed to evaluate the diagnostic performances of these variables in discriminating patients who required BT from those who didn't require. SI (AUC=0.646, p=0.039) and PR (AUC=0.662, p=0.016) also had relatively good performances in discriminating patients who needed BT from those who didn't. They were also seen to not differ significantly in terms of their diagnostic performances while predicting the patients who needed BT (p=0.728). Optimal cut-off values were obtained according to Youden J index; corresponding sensitivity and specificity values are given (**Table 2**, **Figure 1, 2**).

Table 1. Patient characteristics and laboratory test results

Variables	Descriptive statistics
Gender (male)[a]	67 (87)
Age (years)[b]	73 (66-83)
SBP[b]	133 (120-150)
DBP[c]	76±13
PR[b]	87 (78.5-100)
Comorbidity status[b]	49 (63)
Blood-thinning medication use[b]	29 (37.7)
Hb[c]	11.06±3.01
PLT[b]	260000 (206500-337500)
INR[b]	1.10 (1.04-1.23)

Data given as [a]: n (%), [b]: median (25^{th} percentile-75^{th} percentile) or [c]: mean±standard deviation, SBP: systolic blood pressure, DBP: diastolic blood pressure, PR: Pulse rate, Hb: Hemoglobin, PLT: Platelet count, INR: International normalized ratio

Table 2. ROC curve analyses for SI, PR, SBP and DBP in discriminating patients who were discharged and hospitalized, and who required BT and who didn't

		AUC	p value	cut-off value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Hospitalization requirement	SBP	0.505	0.944	≤117	28.00 (16.20-42.50)	88.89 (70.80-97.60)	82.40 (59.50-93.70)	40.00 (34.90-45.30)
	DBP	0.567	0.333	≤70	48.00 (33.70-62.60)	66.67 (46.00-83.50)	72.70 (59.30-83.00)	40.90 (32.20-50.20)
	Pulse rate	0.689	0.004	>80	76.00 (61.80-86.90)	55.56 (35.30-74.50)	76.00 (66.90-83.20)	55.60 (40.70-69.40)
	SI	0.647	0.028	>0.68	62.00 (47.20-75.30)	74.07 (53.70-88.90)	81.60 (69.30-89.70)	51.30 (40.90-61.50)
Blood transfusion requirement	SBP	0.567	0.325	≤132	61.54 (40.60-79.80)	56.86 (42.20-70.70)	42.10 (31.90-53.00)	74.40 (62.80-83.30)
	DBP	0.620	0.078	≤66	38.46 (20.20-59.40)	82.35 (69.10-91.60)	52.60 (34.00-70.50)	72.40 (65.40-78.50)
	Pulse rate	0.662	0.016	>91	61.54 (40.60-79.80)	72.55 (58.30-84.10)	53.30 (40.00-66.20)	78.70 (68.90-86.10)
	SI	0.646	0.039	>0.8	42.31 (23.40-63.10)	90.20 (78.60-96.70)	68.70 (46.10-85.00)	75.40 (68.60-81.20)

AUC: Area under the curve, CI: Confidence interval, SBP: systolic blood pressure, DBP: diastolic blood pressure, SI: shock index

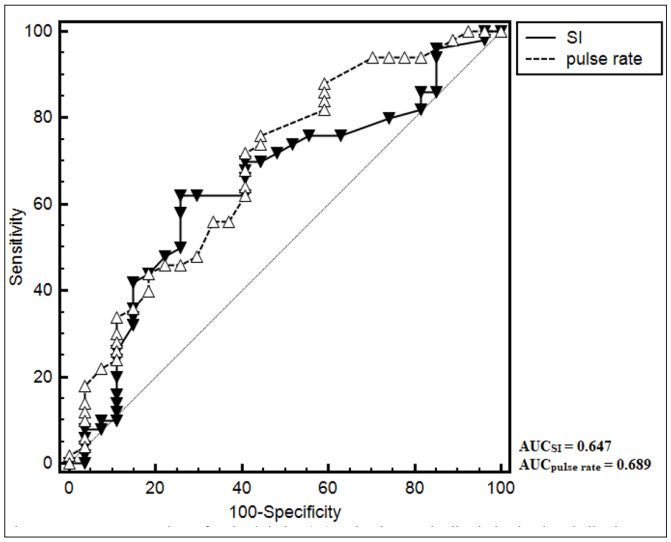

Figure 1. ROC curve analyses for shock index (SI) and pulse rate in discriminating hospitalized patients from those who were discharged

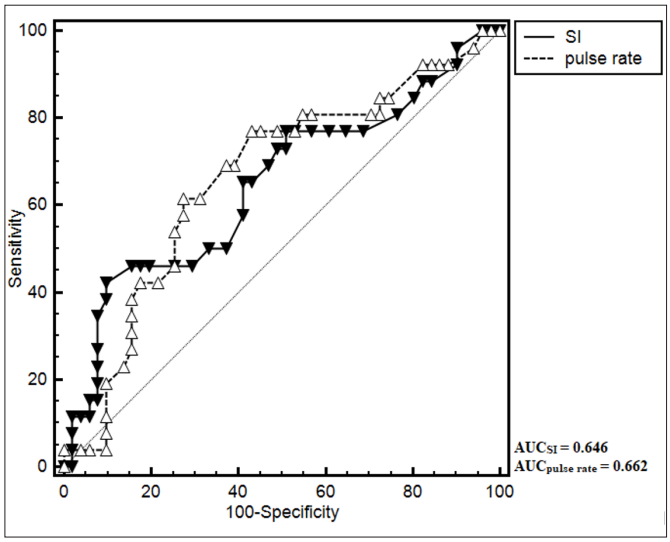

Figure 2. ROC curve analyses for shock index (SI) and pulse rate in discriminating patients who required BT and those who didn't

DISCUSSION

Urinary bladder-originated persistent hematuria refractory to therapeutic interventions may be life-threatening, and is still a hard-to-treat problem. When bladder irrigation with triple lumen urinary catheter and fulguration of bleeding lesions fails to stop bleeding, and the rate of BT cannot catch up that of bleeding, a life-threatening condition may ensue. Any patient with signs of cardiovascular compromise related to blood lose should be resuscitated with volume replacement, correction of coagulopathy and therapies directed to restore hemostasis in the ED (15). Patients without any cardiovascular compromise may be managed on an out-patient setting if they do not have any other feature necessitating in-patient follow-up such as sepsis, acute renal failure, thrombus retention, inability to take sufficient fluids orally, serious comorbidities and social concerns (9).

To the best of the authors' knowledge, there are no studies in the English literature evaluating the predictive power of possible simple parameters while determining hospitalization or BT requirement in patients presenting to ED due to MH. The current study seems to be the first one evaluating this topic. All of the patients recruited into the current study were consulted with the Urology Department and admitted to hospital in the case of need for in-patient follow-up. It was seen in a study by Goonewardena et al. (16) that 130 of 156 macroscopic hematuria patients were male and only 26 patients were female; the current study also showed that a significantly higher number of the patients presenting to the ED with MH were male. A study by Rockall et al. (17) showed that incidence of hematuria increases after the age of 40 which is also a threshold for significantly increased risk of urologic malignancy of which prostatic cancer is the most important one. And, the mean age of the patients was found 73 years in the present study. We suggest that higher number of the patients having comorbidities in the current study may be related to higher number of older ones among all recruited patients. Rate of blood-thinning medication use was found to be low in the present study. Culclasure et al. (18) suggested that information regarding anti-coagulant medication use related to cardiac or cerebral vascular disease must be gathered but their results indicated that anti-coagulants do not cause hematuria because incidence of hematuria was found to be lower in the group using anti-coagulant medication compared to the control group.

The results of the largest retrospective study evaluating predictive power of SI in determining hospitalization requirement among all ED admissions indicated that SI may be a fast and accurate marker while predicting need for hospital admission (19). SI was also shown to predict the need for intensive care unit admission in patients with gastrointestinal bleeding (20). In the present study, most of the patients with MH were admitted to the hospital, and SI (AUC=0.647, p=0.028) and PR (AUC=0.686, p=0.004) were seen to have significantly good diagnostic performances. While predicting need for hospitalization, cut-off values for SI and PR were calculated as >0.68 and >80 beats/min, respectively. Saffouri et al. (21) found that SI is useful when predicting need for major transfusion, which means more than 4 units of erythrocyte suspension, in patients with upper gastrointestinal hemorrhage. El-Menyar et al. (22) reported that SI can be used to predict massive BT or laparotomy requirement and mortality in trauma patients. The optimal cut-off value of SI when predicting need for massive BT was 0.81. Terceros-Almanza et al. (23) stated that SI is a good predictor of massive hemorrhage in trauma patients and, it can be added to the initial diagnostic work-up of patients with

severe trauma. It was seen in the current study that some of the patients with MH needed BT, and SI (AUC=0.646, p=0.039) and PR (AUC=0.662, p=0.016) had significant predictive powers when determining those with the need for BT. Cut-off values for PR and SI related to BT requirement were >91 beats/min and >0.8, respectively.

The present study has mainly two significant limitations; the first one is relatively small number of the patients recruited into the study and the second one is design of the study as a single-center one.

CONCLUSION

Although some of the patients with MH can be managed on an out-patient setting, the others need in-hospital follow-up and BT, and the results of this study suggests that use of PR and SI in the emergency triage may be helpful while discriminating patients who need hospitalization or BT. However, none of them had perfect diagnostic test criteria results so the physicians should not solely rely on these parameters

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Balıkesir University Faculty of Medicine Clinical Research Ethics Committee (Date: 19.08.2020, Decision No: 2020/140).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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REFERENCES

1. Amling CL. Diagnosis and management of superficial bladder cancer. Curr Probl Cancer 2001; 25: 219–78.
2. Smet CE, De Hurtado JM, Pareja RR, et al. Analysis of 895 consultations for hematuria in the emergency department in an urology unit. J Urol (Paris) 1996; 102: 168–71.
3. Mommsen S, Aagaard J, Sell A. Presenting symptoms, treatment delay and survival in bladder cancer. Scand J UrolNephrol 1983; 17: 163–7.
4. Goonewardena SA, AbeygunasekeraAM. Haematuria as the presenting symptom: experience from a tertiary referral centre in Sri Lanka. Ceylon Med J 1998; 43: 156–8.
5. Rodgers M, Nixon J, Hempel S, et al. Diagnostic tests and algorithms used in the investigation of haematuria: systematic reviews and economic evaluation. Health Technol Assess 2006; 18: 259.
6. Paul AB, Collie DA, Wild SR, Chisholm GD. An integrated haematuria clinic. Br J ClinPract 1993; 47: 128–30.
7. Wallace DM, Bryan RT, Dunn JA, et al. Delay and survival in bladder cancer. BJU Int 2002; 89: 868–78.
8. Thiruchelvam N, Mostafid H. Do patients with frank haematuria referred under the two-week rule have a higher incidence of bladder cancer? Ann R CollSurg Engl 2005; 87: 345–7.
9. Hicks D, Li CY. Management of macroscopic haematuria in the emergency department. Emerg Med J 2007; 24: 385-90.
10. Sankaran P, Kamath AV, Tariq SM, et al. Are shock index and adjusted shock index useful in predicting mortality and length of stay in community-acquired pneumonia? Eur J Intern Med 2011; 22: 282-5.
11. Semerci E, Durukan P, Yıldırım S, Baykan N, Yakar Ş, İpekten F. The effect of shock index and hematocrit levels on mortality in patients with gastrointestinal bleeding. Turk J Acad Gastroenterol 2018; 17: 85-9.
12. Mutschler M, Nienaber U, Münzberg M, et al. The shock index revisited - a fast guide to transfusion requirement? A retrospective analysis on 21,853 patients derived from the Trauma Register DGU®. Crit Care 2013; 17: R172.
13. Rassameehiran S, Teerakanok J, Suchartlikitwong S, Nugent K. Utility of the shock index for risk stratification in patients with acute upper gastrointestinal bleeding. South Med J 2017; 110: 738-43.
14. Otero R, Nguyen B. Şoktaki hastaya yaklaşım. In: Yıldıray Ç., editor. Acil Tıp KapsamlıBirÇalışmaKlavuzu. 7th edition. İstanbul: Nobel Tıp Kitabevi; 2013. p. 165-172
15. Choong SK, Walkden M, Kirby R. The management of intractable haematuria.BJU Int 2000; 86: 951–9.
16. Goonewardena SA, Abeygunasekera AM. Haematuria as the presenting symptom: experience from a tertiary referral centre in Sri Lanka. Ceylon Med J 1998; 43: 156-8.
17. Rockall AG, Newman-Sanders AP, al-Kutoubi MA, et al. Haematuria. Postgrad Med J 1997; 73: 129-36
18. Culclasure TF, Bray VJ, Hasbargen JA. The significance of hematuria in the anticoagulated patient. Arch Intern Med 1994; 154: 649-52.
19. Al Jalbout N, Balhara KS, Hamade B, Hsieh YH, Kelen GD, Bayram JD. Shock index as a predictor of hospital admission and inpatient mortality in a US national database of emergency departments. Emerg Med J 2019; 36: 293-7.
20. Kocaoğlu S, Çetinkaya HB. Use of age shock index in determining severity of illness in patients presenting to the emergency department with gastrointestinal bleeding. Am J Emerg Med 2021; 47: 274-8.
21. Saffouri E, Blackwell C, Laursen SB, et al. The shock index is not accurate at predicting outcomes in patients with upper gastrointestinal bleeding. Aliment PharmacolTher 2020; 51: 253–60.
22. El-Menyar A, Goyal P, Tilley E, Latifi R. The clinical utility of shock index to predict the need for blood transfusion and outcomes in trauma. J Surg Res 2018; 227: 52-9.
23. Terceros-Almanza LJ, García-Fuentes C, Bermejo-Aznárez S, et al. Prediction of massive bleeding. shock index and modified shock index. Med Intensiva 2017; 41: 532-8.

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Combined effects of the implementation of magnesium and ascorbic acid on myocardial ischemia-reperfusion in open heart surgery

Yıldırım Gültekin[1], Abdulkadir Güzel[2], Atalay Karakaya[3], Yavuz Beşoğul[4]

[1]Kırıkkale University, School of Medicine, Department of Cardiovascular Surgery, Kırıkkale, Turkey
[2]Beylikdüzü State Hospital, Department of Cardiovascular Surgery, İstanbul, Turkey
[3]İstanbul Medipol University, Bağcılar Mega Hospitals Complex, Department of Cardiovascular Surgery, İstanbul, Turkey
[4]Eskişehir Osmangazi University, School of Medicine, Department of Cardiovascular Surgery, Eskişehir, Turkey

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ABSTRACT

Aim: This study aimed to investigate the combined effects of magnesium (Mg) and high dose ascorbic acid on cardiac ischemia-reperfusion (IR) injury.

Material and Method: This study was performed on 45 patients that were scheduled for coronary artery bypass graft (CABG) operations. The patients were divided into three equal groups. Group C received 50 mg/kg ascorbic acid; Group CM received the same dose of ascorbic acid plus 30 mg/kg Mg; Group K received neither ascorbic acid nor Mg. At various times during the operation, the blood levels of malondialdehyde (MDA), serum creatine kinase MB (CK-MB), and lactate dehydrogenase (LDH) levels were analyzed.

Results: There were statistically significant decreases in arrhythmia requiring intervention in Group CM compared to Group K (P=0.026). MDA levels increased in all groups but MDA 2 and MDA 3 levels were found to be statistically significantly lower in Group C and Group CM than in Group K (P=0.009, P=0.012, P=0.009 and P=0.006 respectively). However, inhibition of lipid peroxidation in both Group C and Group CM was not parallel to cardiac enzymes and hemodynamic measurements. There was no significant statistical difference in the cardiac enzyme levels between Group K, Group C, and Group CM (p>0.05).

Conclusion: To reduce the IR, Mg with a high dose of ascorbic acid may be efficacious in patients undergoing cardiac surgery. A larger population group is needed to prove the results of this study.

Keywords: Open heart surgery, ascorbic acid, magnesium, ischemia-reperfusion

INTRODUCTION

Coronary artery bypass graft (CABG) surgery is one of the preferred treatments for ischemic heart disease. Despite advances in anesthesia techniques, myocardial preservation methods, and surgical techniques, myocardial IR injury is still one of the most significant causes of mortality and morbidity in cardiac surgery (1). Cardiopulmonary bypass (CPB) and cardioplegic arrest have some disadvantages, such as, systemic inflammatory response, myocardial damage, neurological damage, and renal and pulmonary insufficiency. Despite these disadvantages, the most important advantage of CPB is the ability to reach the target vessels in the immobile, hollow heart and to perform complete revascularization by working in a bloodless environment (2).

Normalization of myocardial blood flow after CPB, which is ischemic due to coronary artery disease, may result in IR injury. The degree of IR damage depends on the duration of the ischemic period and the degree of oxidative stress that occurs during reperfusion. The physiopathological mechanisms underlying IR injury are completely unknown (3). However, it has been suggested that excessive production of free oxygen radicals (FOR) and intracellular calcium (Ca) overload or redistribution may be the cause (4). IR damage presents clinically as microvascular dysfunction including myocardial stunning, reperfusion arrhythmia, myocyte death, endothelial dysfunction, and inadequate flow (5).

Corresponding Author: Yıldırım Gültekin, dr.ygultekin@hotmail.com

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Different pharmacological treatments have been tried to reduce myocardial IR damage and these can be classified into several categories. However, it is true that the choice of treatment, the method of administration, and the timing, i.e., before ischemia, before reperfusion, immediately after, or hours after reperfusion, is a critical issue (6).

Antioxidants are substances that delay or prevent the oxidation of substrates. Since FOR are constantly formed in normal metabolism, all aerobic cells develop protective mechanisms against their harmful effects. Antioxidants either play a preventive role by removing the FOR precursors from the environment and deactivating the catalysts, or they act by breaking the oxidation reaction chain that has started (7). Ascorbic acid (vitamin C) with its hydrophilic structure is an important antioxidant in both plasma and cytosol (8).

Calcium is an essential cation for normal cell metabolic functions. Calcium and FOR mechanisms are not separate, but two aspects of the same mechanism. While FOR cause membrane dysfunction and Ca redistribution, Ca accumulation may increase the damage initiated by FOR and even facilitate FOR production (9). It has been shown that Ca channel blockers are beneficial with various mechanisms of action in myocardial IR injury. Magnesium (Mg) is a physiological Ca antagonist and cell membrane stabilizer. Together with Ca and phospholipids, Mg plays an important role in the regulation of cell permeability. It replaces Ca competitively in its binding sites and simultaneously inhibits Ca's entry into the cell, while speeding up its exit from the cell (10).

In this study, we aimed to compare the effectiveness of the use of a high dose of ascorbic acid in reducing myocardial IR damage with the effectiveness obtained by adding Mg to ascorbic acid.

MATERIAL AND METHOD

This prospective study was carried out on 45 patients with coronary artery disease who were scheduled to undergo CABG operations at the Department of Cardiovascular Surgery, Faculty of Medicine Osmangazi University. This study was conducted in accordance with the 1964 Declaration of Helsinki and subsequent changes or comparable ethical standards. All the patients participating in the study signed the informed consent form, and the study was carried out with the permission of Scientific Researches Ethics Committee of Osmangazi University Faculty of Medicine (Date: 30.06.2009, Decision No: 60).

Patients whose physical characteristics conformed to class I, II, or III according to the New York Heart Association (NYHA) classification and who were between the ages of 44 and 75 (mean: 63.86±8.59) with an ejection fraction (EF) of 40% or above, were included in the study. Patients who had myocardial infarction in the last six weeks, required emergency surgery and had previous cardiac surgery were excluded from the study. Patients who were known to be using Ca channel blockers or medication known to have antioxidant properties prior to the operation, were not included in the study. These antioxidant drugs are trimetazidine, statins, angiotensin-converting enzyme (ACE) inhibitors, N-acetyl cysteine (NAC), xanthine oxidase inhibitors, vitamins (α-tocopherol (Vit E), β-carotene (Pro-vit A), pyridoxal-5-phosphate), folic acid, nitric oxide donors (L-arginine), endothelin-1 receptor antagonists (bosentan), glucose-insulin-potassium (GIK) solutions, platelet inhibitors and adenosine. The patients were divided into three equal groups of 15 patients. The patients were randomly placed in groups and were not selected in terms of distribution of coronary artery lesions, ventricular function, age, gender, body surface area (BSA), NYHA class, or EF. While one of the groups was followed as the control group (Group K), Group C received 50 mg/kg intravenous (IV) ascorbic acid (Vitabiol C 500 mg ampoule, IE Ulagay, İstanbul, Turkey) after induction. Group CM was given 50 mg/kg IV ascorbic acid and 30 mg/kg IV Mg (Magnesium Sulphate Biofarma 15% Ampoule, Biofarma, Mamak, Ankara) after induction. Preoperative routine tests were performed for the surgery, and none of the patients had undergone CABG surgery before. The same team performed all operations and the operation time, CPB time, cross clamp (CC) time and the number of bypass grafts applied were recorded.

Surgical Technique

Standard premedication and anesthetic methods were used for all patients. Diazepam 10 mg tb was given the night before the operation. When the patients were taken into the operating theatre, electrocardiography (ECG) monitoring was initiated and monitored (Datex-Ohmeda S/5, Datex-Ohmeda Inc., Madison, WI, USA) throughout the operation. At the same time, ECG segment analysis was performed for ischemia monitoring. Anesthesia induction was completed with 8 cg/kg fentanyl, 0.15 mg/kg midazolam, and 0.1 mg/kg pancuronium bromide, after 1 mg/kg IV lidocaine was administered to reduce the hemodynamic response to laryngoscopy and endotracheal intubation at the beginning of induction. Maintenance of anesthesia was accomplished by infusion of 5–10 cg/kg/hour fentanyl and inhalation of 0.2–1.5% isoflurane plus 50% air-oxygen mixture. The neuromuscular blockade was maintained with the addition of 0.05 mg/kg pancuronium bromide to the pump reservoir at the beginning of the CPB, and the termination of the isoflurane inhalation. A median sternotomy was performed on all patients, and heparinization was applied so that the activated coagulation time (ACT) was above 400 seconds before CPB started.

During the operation, arterial cannulation was performed from the ascending aorta, and venous cannulation was performed from the right atrial appendage with a two-stage cannula. All cardioplegia doses were administered antegrade through the cannula placed in the aortic root. CPB was performed on all patients by nonpulsatile perfusion (Terumo® Advanced Perfusion System 1, Terumo Medical Corporation, Ann Arbor, Michigan, USA), with a membrane oxygenator (Capiox SX 18R®, Terumo Medical Corporation, Somerset, NJ, USA). At the onset of CPB, mild systemic hypothermia was applied to all patients until the temperature reached 28°C. Intermittent hyperkalemic cold blood cardioplegia was used for myocardial protection. Before the CC was lifted, 10 ml/kg hot-shot application was applied to provide controlled reperfusion. The presence of arrhythmia was noted at the beginning of the re-contractions of the heart after the removal of the CC. During the operation, no medication known to have an antioxidant effect was administered to the patients. Heparin neutralization was achieved with a 1.3/1 protamine sulfate infusion after CPB was terminated.

To evaluate the hemodynamics of the patients, mean arterial pressure and heart rate were recorded before incision, before CPB, five minutes after protamine infusion and at the postoperative second hour. In addition, patients who needed perioperative inotropes were recorded.

Arterial blood samples taken for an MDA level were taken at the following times: before induction (1), just before the cross clamp was lifted (2), and five minutes after the end of the protamine infusion (3). Plasma MDA determination was performed by applying the Esterbauer method which is based on a spectrophotometric measurement at a wavelength of 532 nm. The color intensity of the complex formed by thiobarbituric acid, one of the degradation products of lipid peroxidation, was measured and results were reported in nmol/g (11).

Venous blood samples were taken preoperatively and two hours after the operation for CK-MB and LDH levels.

Statistical Analysis

All statistics were performed using SPSS (Statistical Package for Social Science) version 15.0 for Windows (IBM Corporation, New York, USA). Data was summarized as mean±standard deviation (mean±std). The compliance of the data to normal distribution was tested with Shapiro-Wilk. ANOVA (analysis of variance) was used to determine the difference in group averages. The Tukey test, one of the POSTHOC tests, was used to determine the means between the different groups. Only the Kruskal–Wallis test was used to compare the NYHA class between the groups, and Fischer's exact test was used to compare the presence of arrhythmia. The $p<0.05$ was considered statistically significant.

RESULTS

There was no statistically significant difference between the three groups in terms of demographic and preoperative data (P>0.05). The data are shown in **Table 1**.

Table 1. Demographic data of patients

	Group K (n=15)	Group C (n=15)	Group CM (n=15)	P value
Age (years)	64.3±8.8	66.7±7.4	60.6±9.5	>0.05
Gender (M / F)	8/3	8/2	8/2	>0.05
BSA(m^2)	1.77±0.16	1.85±0.19	1.84±0.14	>0.05
NYHA(I/II/III)	0/8/2	2/7/1	1/8/1	>0.05
EF (%)	46.5±10.7	49.5±11.5	47.0±4.21	>0.05

BSA: Body surface area; NYHA: New York Heart Association; EF: ejection fraction

There was no statistically significant difference between the three groups in terms of intraoperative data (P>0.05). The data are shown in **Table 2**.

Table 2. Intraoperative parameters of patients

	Group K (n=15) (mean±std)	Group C (n=15) (mean±std)	Group CM (n=15) (mean±std)	P value
Operation time (min)	358.0±55.9	351.0±55.6	315.0±57.2	>0.05
CPB time (min)	127.2±39.4	132.9±32.1	125.6±33.1	>0.05
CC time (min)	70.9±26.9	85.0±28.2	81.4±23.1	>0.05
Bypass grafts count	3.7±0.4	3.8±0.4	3.5±0.8	>0.05

CPB: Cardiopulmonary bypass; CC: cross clamp; std: standard deviation; min: minute.

Preoperative CK-MB (CK-MB1) and postoperative CK-MB (CK-MB 2) levels, preoperative LDH (LDH1) and postoperative LDH (LDH2) levels of the groups are shown in **Table 3**. In all three groups, the levels obtained in the second hour postoperatively for CK-MB and LDH increased significantly compared to preoperative level (P<0.001). There was no significant difference between the groups in preoperative and postoperative CK-MB and LDH levels (P>0.05). However, although not statistically significant, both enzyme levels were found to be lower in Group CM than in Group K and Group C (**Figures 1** and **2**).

Table 3. CK-MB and LDH levels of the groups

	Group K (n=15) (mean±std	Group C (n=15) (mean±std)	Group CM (n=15) (mean±std)	P value
CK-MB 1 (m/l)	16.90±4.05	17.80±6.32	17.30±4.59	>0.05
CK-MB 2 (m/l)	64.00±8.30*	73.10±19.89*	69.32±15.43*	>0.05
LDH 1 (m/l)	418.2±124.3	486.2±114.1	497.8±182.7	>0.05
LDH 2 (m /l)	992.1±225.1*	1013.0±306.9*	1037.2±256.6*	>0.05

std: standard deviation. * P<0.001 compared with the preoperative value.

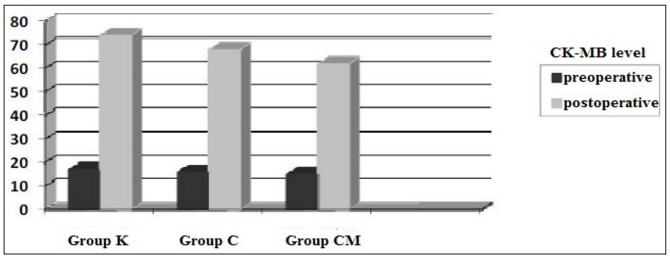

Figure 1. Preoperative and postoperative CK-MB levels in the groups. (When compared with the preoperative values of the postoperative values within the group, P<0.001).

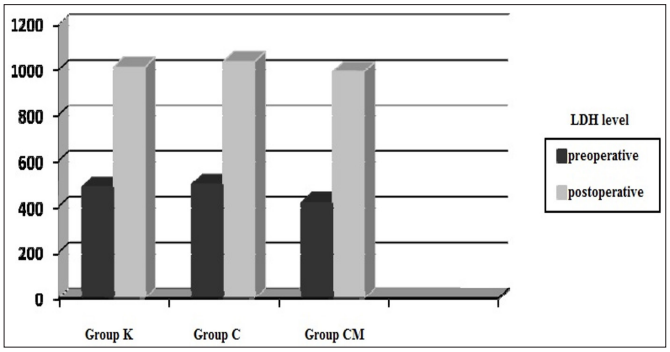

Figure 2. Preoperative and postoperative LDH levels in the groups. (When compared with the preoperative values of the postoperative values within the group. (P<0.001)).

Preoperative and perioperative MDA level are shown in **Table 4** and **Figure 3**. In Group K, MDA 2 and MDA 3 value were statistically significantly higher than the MDA 1 value (P=0.011 and P=0.007). When Group C and Group CM's MDA value were compared within the group, no statistically significant difference was found (p>0.05). MDA 2 and MDA 3 value were found to be statistically significantly lower in Group C and Group CM than in Group K (P=0.009, P=0.012, P=0.009 and P=0.006 respectively)

Table 4. MDA levels of the groups

	Group K (n=15) (mean±std	Group C (n=15) (mean±std)	Group CM (n=15) (mean±std)
MDA 1 (nmol/g)	2.00±0.34	2.16±0.55	2.21±0.96¶
MDA 2 (nmol/g)	3.04±0.88*	2.54±0.74‡	2.55±0.62‡§
MDA 3 (nmol/g)	3.64±0.81*†	2.87±0.61‡	2.62±0.68‡§

MDA: Malondialdehit; std: standard deviation. * P<0.05 compared with MDA 1 value; † P<0.05 compared with MDA 2 value; ‡ P<0.05 compared with Group K; § P>0.05 compared with Group C value; ¶ P>0.05 compared with Group C and Group K.

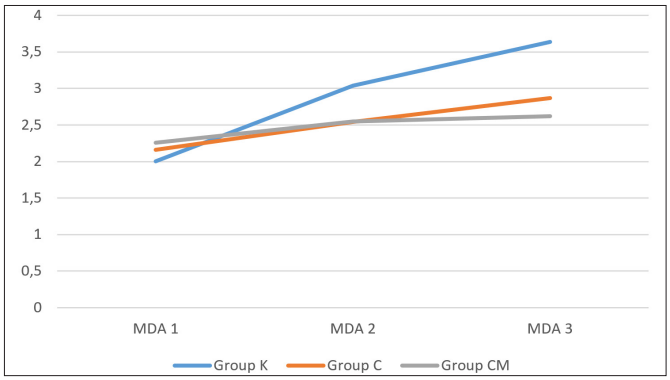

Figure 3. Comparison of MDA (malondialdehit) values in the groups (MDA 1:before induction ; MDA 2: just before cross clamp removal; MDA 3: after protamine infusion).

When the CC was removed, arrhythmia requiring intervention was observed in six patients in Group K, three patients in Group C, and one patient in Group CM. A statistically significant difference was determined only between the K Group and CM Group (P=0.026). The data are shown in **Figure 4**.

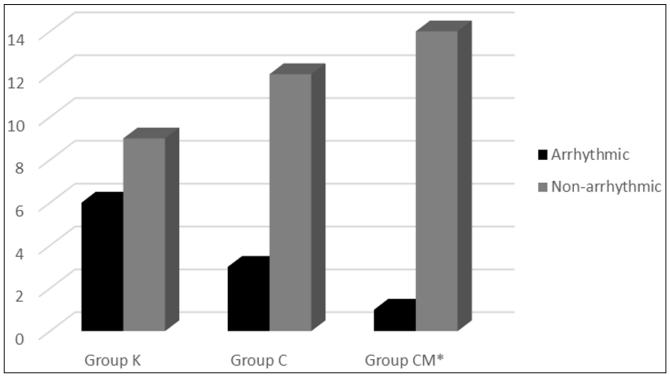

Figure 4. Arrhythmia requiring intervention when the cross clamp is lifted in all three groups (*P=0.026 compared to Group K).

Table 5. Hemodynamic measurements obtained in three groups

	Group K (n=15) (mean±std	Group C (n=15) (mean±std)	Group CM (n=15) (mean±std)
Mean arterial pressure 1 (mmHg)	81.20±19.75	68.30±10.07	72.10±8.86
Mean arterial pressure 2 (mmHg)	73.90±9.31	74.40±11.42	74.30±12.48
Mean arterial pressure 3 (mmHg)	66.40±5.13*	65.80±9.25	68.30±6.73
Mean arterial pressure 4 (mmHg)	78.00±8.68	70.10±6.98	73.70±9.65
Heart rate 1 (beats/min)	70.9±13.8	66.9±16.0	72.6±11.8
Heart rate 2 (beats/min)	81.9±16.6	77.5±15.2	86.2±13.2*
Heart rate 3 (beats/min)	88.1±19.2*	93.0±19.1*†	86.8±10.8*
Heart rate 4 (beats/min)	93.1+18.7*	99.5±16.4*†	100.4±16.4*†‡

std: standard deviation; min: minute. * P<0.05 compared to 1st value; † P<0.05 compared to 2nd value; ‡ P<0.05 compared to 3rd value.

The mean arterial pressure and the heart rate results obtained in the three groups, before incision (1), before CPB (2), five minutes after protamine infusion (3) and at the postoperative second hour (4) are shown in **Table 5**. There was no statistically significant difference between the groups (P>0.05).

The need for inotropes was detected in five patients in Group K, three patients in Group C, and four patients in Group CM. There was no statistically significant difference between the groups (P>0.05).

DISCUSSION

This study demonstrated the efficacy of the combined application of ascorbic acid and Mg in the IR injury caused by CPB in CABG surgery. The main findings of the study were as follows: high-dose ascorbic acid and Mg were shown to prevent lipid peroxidation and arrhythmias requiring intervention.

Despite the advances in pharmacological intervention, surgical methods and CPB system in open heart surgery, ischemia due to CPB and reperfusion injury that develops after the removal of the CC and coronary flow is damaging to the heart. Systemic and local hypothermia, antegrade and retrograde hypothermic crystalloid or blood cardioplegia were used for intraoperative myocardial protection (12). Experimental studies aiming to protect organs against IR damage have been carried out using different surgical, pharmacological, and genetic methods. However, few of the studies that seem successful in the experimental setting have been applied clinically. The reason for this is that IR damage is too complex to be prevented by blocking a single step or mediator (13). The antioxidant system has an important role in protecting damage that could be caused by FOR. There are mechanisms in the antioxidant system for binding transition metal ions, preventing the formation of FOR, collecting or suppressing the formed radicals, breaking radical chain reactions, repairing, or removing damaged target molecules from the environment (14).

Antioxidant and free radical scavenger treatments were created to determine the overproduction of FOR and the role of antioxidant enzymes in the pathogenesis of myocardial damage due to IR (15). For this, pharmacological agents such as calcium channel blockers, antioxidant agents, melatonin, immunosuppressive agents, adenosine, glutamate-aspartate, α-tocopherol, methylprednisolone, ACE inhibitors, trimetazidine, and endothelin-1 receptor antagonists were used (16). Braunwald and Kloner (17) reported that the total antioxidant capacity decreased and oxidant damage occurred during the IR periods during CPB. In addition, it has been shown that the total antioxidant capacity was suppressed, and lipid peroxidation increased for 72 hours after the operation in patients with CPB. During CBP, blood levels of vitamin A, vitamin E and vitamin C were tested. There was a slight decrease in the vitamin A and vitamin E level, and a significant decrease in the vitamin C level during and after the operation (18). The decrease in the ascorbic acid level has been attributed to either a direct reaction with FOR, or its role in the regeneration of other antioxidants. In addition, in studies conducted with ascorbic acid, its inhibitory effect on lipid peroxidation and its protective role of the myocardium from IR damage has been demonstrated (19). In the study conducted by Dingchao et al. (20), it was shown that high-dose ascorbic acid administered intravenously at a dose of 250 mg/kg had a myocardial protective effect.

It is known that reperfusion causes ventricular fibrillation, ventricular tachycardia, idioventricular rhythm, and ventricular premature beats (21). It has been argued that the increase of FOR, which occurs in the early stage of reperfusion, affects the membrane lipids and thus the ion pumps, making the myocardium susceptible to arrhythmia (22). In the study of Seitelberger et al. (23), conducted on 120 patients, it was determined that Ca channel blockers decrease the number of ventricular premature complexes. In another study, it has been shown that ascorbic acid prevents AF (24), and in our study, when the CC was removed, arrhythmia requiring intervention was observed in six patients in Group K, three patients in Group C, and one patient in Group CM. Although the difference between Group K and Group CM is statistically significant, the detection of arrhythmia in a smaller number of patients compared to Group K with high-dose ascorbic acid administration and a further decrease in this number with the addition of Mg to ascorbic acid supports the additive antiarrhythmic effect advocated by Hearse and Tosaki (25).

One of the widely accepted theories about IR injury is the increase in FOR and intracellular free Ca (26). Uncontrolled increase in intracellular Ca levels causes ischemic contracture and structural changes. With the application of hypocalcemic reperfusion, and the addition of Ca channel blockers to the cardioplegia solution and the IV, intracellular Ca increase was prevented (27). Mg is a physiological Ca antagonist and reduces myocardial damage during IR by blocking the FOR products generated by reperfusion (28). In a study, Mg infused intraoperatively from the aortic root showed positive effects on ischemia, IR damage, and arrhythmia during cardiac surgery (29). Prophylactic Mg has been reported to reduce postoperative arrhythmia in patients under CPB (30). If arrhythmia occurs despite Mg, it appears that the rate of recovery of these arrhythmias is then higher with other antiarrhythmic drugs (31).

During and after CPB, it has been shown that Mg levels decrease postoperatively in up to 70% of the patients when it is not replaced, and then gradually increase until the 4th postoperative day (32). The mechanism of the relationship between cellular Mg and serum Mg has not been fully determined. However, it has been demonstrated that measuring intracellular Mg concentration by radiographic microanalysis shows a Mg deficit in cardiac patients (33). Although the intracellular Mg level was not examined as a criterion of our study, we believe that these results are reliable as we have monitored the ionized fraction, which is physiologically

the most important part. Studies have claimed that the perioperative Mg level can be reliably assessed by monitoring the ionized fraction in the serum in patients undergoing cardiac surgery (34).

FOR, which occur during myocardial IR are very reactive and although they have a lifespan that is measured in microseconds, their presence can be demonstrated in tissue using spin resonance spectroscopy (35). Oxidation of polyunsaturated fatty acids with FOR in biological systems is called lipid peroxidation, and the presence of FOR reaction in studies has been proven by the demonstration of lipid peroxidation markers (36). The most frequent method used for the determination of lipid peroxidation is the measurement of the MDA level. In our study, MDA levels obtained before and after the administration of protamine increased in all three groups compared to the preoperative MDA level, but it was found that this increase was less in Groups C and CM. MDA 2 and MDA 3 value were found to be statistically significantly lower in Group C and Group CM than in Group K. Although the increase in MDA was less in group CM compared to Group C, there was no statistically significant difference between them ($p>0.05$). These results show us that high dose ascorbic acid and an ascorbic acid–magnesium combination protects the myocardium against FOR.

Many studies have shown an increase in cardiac enzyme levels after CPB (37). In studies where antioxidant agents and Mg were used to prevent myocardial IR damage, cardiac enzyme levels were measured to show myocardial damage and conflicting results were obtained (38). Although there are studies showing that high dose ascorbic acid and Mg reduce CK-MB release from the myocardium, the clinical significance of these results could not be determined (39). In our study, CK-MB and LDH levels obtained at the second hour postoperatively showed a statistically significant increase in all three groups compared to the preoperative values ($P<0.001$), but there was no additional finding suggesting myocardial damage during this period. There was no significant change in mean arterial pressure. Although not statistically significant, postoperative enzyme levels were lower in the CM group than in the other two groups. Therefore, we believe that CK-MB and LDH levels are not fully specific for myocardial damage, and that the value of cardiac enzymes has not been fully investigated in similar studies.

It has been argued that FOR occurs during myocardial IR at the same time as cellular Ca loading occurs, and endogenous defense mechanisms are insufficient. In our study, this opinion was supported by the increase in the level of MDA in Group K, which is a product of lipid peroxidation known to occur in the presence of FOR. When ascorbic acid and Mg were used to prevent the damage caused by FOR, it was shown that lipid peroxidation and arrhythmias requiring intervention were prevented. However, there was no statistically significant difference between the groups in terms of cardiac enzyme levels and hemodynamic measurements. This result is parallel to the findings in the study by Demirağ et al. (40), where diltiazem was added to the ascorbic acid. Despite this result, we believe that advanced myocardial protection methods are required in high-risk patients with preoperative ventricular dysfunction, and the protective effect of Mg and high dose ascorbic acid will be reflected in clinical findings in patients with high risk. To reach a definitive conclusion on this subject, we believe that it is necessary to conduct studies involving a greater sample size and higher risk patients.

Limitation of the Study

The first limitation of this study is that it was conducted in a single center. The second limitation of this study is that the amount of intracellular magnesium could not be shown. The third limitation is that most patients were in the low to intermediate risk group according to the NYHA.

CONCLUSION

In conclusion, the combined use of high-dose ascorbic acid and Mg in CABG operations of low to medium risk patients, did not have a greater impact in preventing myocardial IR damage during CPB in terms of cardiac enzyme levels and hemodynamic measurements than cold blood cardioplegia. However, prevention of lipid peroxidation and arrhythmias that require intervention suggests that Mg and high-dose ascorbic acid may be beneficial in a high-risk patient group.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Scientific Researches Ethics Committee of Osmangazi University Faculty of Medicine (Date: 30.06.2009, Decision No: 60).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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REFERENCES

1. Trachiotis GD, Weintraub WS, Johnston TS, et al. Coranary artery bypass grafting in patients with advanced left ventriculer dysfunction. Ann Thorac Surg 1998; 66: 1632-9.
2. Allman KC, Shaw LJ, Hachamovitch R, Udelson RJ. Myocardial viability testing and impact of revascularization on prognosis in patients with coronary arter disease and left ventrikular dysfonction: A meta analysis. J Am Coll Card 2002; 39: 1151-8.
3. Ozcan V, Beşogul Y, Tunerir B, et al. The effect of glutamate and aspartate on myocardial protection at cardiopulmonary bypass. Anadolu Kardiyol Derg 2004; 4: 114-9.
4. Piper MH, Meuter K, Schafer C. Cellular mechanism of ischemia-reperfusion injury. Ann Thorac Surg 2003; 75: 644-8.
5. Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Ames MM. High dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. N Engl J Med 1985; 312: 137-41.
6. Moukarbel GV, Ayoub MC, Abchee AB. Pharmacological therapy for myocardial reperfusion injury. Curr Opin Pharmacol 2004; 4: 47-53.
7. Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. Cardivasc Res 2000; 47: 446-56.
8. Meerson FZ, Belkina LM, Sazontova TG, Saltykova VA, Arkhipenko Y. The role of lipid peroxidation in pathogenesis of arrhythmias and prevention of cardiac fibrillation with antioxidants. Basic Res Cardiol 1987; 82: 123-37.
9. Inserte J, Garcia-Dorado D, Agullo L, Paniagua A, Soler-Soler J. Urodilatin limits acute reperfusion injury in the isolated rat heart. Cardiovasc Res 2000; 45: 351-9.
10. Mak IT, Komarov AM, Wagner TL, Stafford RE, Dickens BF, Weglicki WB. Enhanced nitric oxide production during magnezyum defeciency and its role in madiating red cell glutation loss. Am J Physiol 1996; 271: 385-90.
11. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hidroxynonenal. Methods Enzymol 1990; 186: 407-21.
12. Josephson RA, Silverman HS, Lakatta EG, Stern MD, Zweier JL. Study of the mechanisms of hydrogen peroxide and hydroxyl free radical–induced cellular injury and calcium overload in cardiac myocytes. J Biol Chem 1991; 266: 2354-61.
13. Britton J, Pavord I, Richards K, Wisniewski A, Knox A, Lewis S. Diatery magnesium lung function, wheesing and airway hiperactivity in a random adulth population sample. Lancet 1994; 334: 356-62.
14. Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc Res 2004; 61: 461-70.
15. Gao WD, Liu Y, Mellgren R, Marban E. Intrinsic myofilament alterations underlying the decreased contractility of stunned myocardium: a consequence of Ca2-dependent proteolysis? Circ Res 1996; 78: 455-65.
16. Wallerta M, Zieglera M, Wanga X, et al. α-Tocopherol preserves cardiac function by reducing oxidative stress and inflammation in ischemia/reperfusion injury. Redox Biology 2019; 26: 101292-303.
17. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? J Clin invest 1985; 76: 1713-9.
18. Bulkley BH, Hutchins GM. Myocardial consequences of coronary artery bypass graft surgery: the paradox of necrosis in areas of revascularization. Circulation 1977; 56: 906-13.
19. Hsu CC, Wang JJ. L-ascorbic acid and alpha-tocopherol attenuates liver ischemia-reperfusion induced of cardiac function impairment. Transplantation Proceedings 2012; 44: 933-6.
20. Dingchao H, Zhiduan Q, Liye H, Xiaodong F. The protective effects of high dose askorbic acid on myocardium against reperfusion injury during and after cardiopulmonary bypass. Torac Cardiovasc Surgeon 1994; 42: 276-8.
21. Chung MK. Cardiac surgery: postoperative arrhythmias. Crit Care Med 2000; 28: 136-44.
22. Hearse DJ, Tosaki A. Free radicals and calcium: Simultaneous interacting triggers as determinants of vulnerability to reperfusion induced arrhythias in the rat heart. J Mol Cell Cardiol 1988; 20: 213-23.
23. Seitelberger R, Hannes W, Gleichauf M, Keilich M, Christoph M, Fasol R. Effects of diltiazem on perioperative ischemia, arrhythmias and myocardial function in patients undergoing elective coronary bypass grafting. J Thorac Cardiovasc Surg 1994; 107: 811-21.
24. Baker WL, Coleman CI. Meta-analysis of ascorbic acid for prevention of postoperative atrial fibrillation after cardiac surgery. Am J Health-System Pharm 2016; 73: 2056-66.
25. Hearse DJ, Tosaki A. Reperfusion-induced arrhythmias and free radicals: studies in the rat heart with DMPO. J Cardiovasc Pharmacol 1987; 9: 641-50.
26. Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 1999; 79: 609-34.
27. Gao WD, Liu Y, Mellgren R, Marban E. Intrinsic myofilament alterations underlying the decreased contractility of stunned myocardium: a consequence of Ca2-dependentproteolysis? CircRes. 1996; 78: 455-65.
28. Woods KL, Barnett DB. Magnesium in acute myokardial infarction. BMJ. 1995; 310: 1669-70.
29. Beşogul Y, Tunerir B, Ozdemir C, Aslan R. Magnesium-flush infusion into the aortic root just before reperfusion reduces the requirement for internal defibrillation and early post-perfusion arrhythmias. J Int Med Res 2003; 31: 202-9.
30. Baradari1 AG, Zeydi AE, Ghafari R, Aarabi M, Jafari M. A double-blind randomized clinical trial comparing different doses of magnesium in cardioplegic solution for prevention of atrial fibrillation after coronary artery bypass graft surgery. Cardiovascular Therapeutics 2016; 34: 276-82.
31. Kobara M, Tatsumi T, Kambayashi D, et al. Effects of ACE inhibition on myocardial apoptosis in an ischemia–reperfusion rat heart model. J Cardiovasc Pharmacol 2003; 41: 880-9.
32. Beyesdorf F, Allen BS. Myocardial protection with integrated blood cardioplegia. In: Franco KL, Verrior ED, eds. Advanced therapy in cardiac surgery, 2nd ed. Hamilton London: B C Decker inc; 1999: 16: 201-13.
33. Horn B. Magnesium and the cardiovascular system. Magnesium 1987; 6: 109-11.
34. Armstrong SC. Protein kinase activation and myocardial ischemia/reperfusion injury. Cardiovasc Res 2004; 61: 427-36.
35. Schafer C, Ladilov Y, Inserte J, et al. Role of the reverse mode of the Na+/Ca2+ exchanger in reoxygenationinduced cardiomyocyte injury. Cardiovasc Res 2001; 51: 241-50.
36. Inserte J, Garcia-Dorado D, Ruiz-Meana M, et al. Effect of inhibition of Na+/Ca2+ exchanger at the time of myocardial reperfusion on hypercontracture and cell death. Cardiovasc Res 2002; 55: 739–48.

37. Kroner A, Seitelberger R, Schirnhofer J, et al. Diltiazem during reperfusion preserves high energy phosphates by protection of mitochondrial integrity. Eur J Cardiothorac Surg 2002; 21: 224-31.

38. Ballmer PE, Reinhart WH, Jordan P, Bühler E, Moser UK, Gey KF. Depletion of plazma vitamin C but not vitamin E in response to cardiac operations. J Thorac Cardiovasc Surg 1994; 108: 311-20.

39. Dingchao H, Zhiduan Q, Liye H, Xiaodong F. The protective effects of high dose askorbic acid on myocardium against reperfusion injury during and after cardiopulmonary bypass. Torac Cardiovasc Surgeon 1994; 42: 276-8.

40. Demirag K, Askar FZ, Uyar M, et al. The protective effects of high dose ascorbic acid and diltiazem on myocardial ischaemia-reperfusion injury. Middle East J Anaesthesiol 2001; 16: 67-79.

Original Article

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The relationship between 68Ga-PSMA uptake and Gleason Score and PSA levels in patients with prostate cancer

Şadiye Altun Tuzcu[1], Ali uyar[1], Fatih Güzel[1], İhsan Kaplan[2], Yunus Güzel[2], Mansur Dağgülli[3], Bekir Taşdemir[1]

[1]Dicle University, Faculty of Medicine, Department of Nuclear Medicine, Diyarbakır, Turkey
[2]Diyarbakır Gazi Yaşargil Research and Education Hospital, Department of Nuclear Medicine, Diyarbakır, Turkey
[3]Dicle University, Faculty of Medicine, Department of Urology, Diyarbakır, Turkey

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ABSTRACT

Aim: PSMA expression has been observed in increased levels in patients with high Gleason scores. Current information on Ga-68 PSMA PET/CT shows that primary staging with PET/CT is important in patients with high-risk PC. Ga-68 PSMA PET/CT may also have a place in patients with intermediate risk PC, but only a few data are available at present. In this study, we aimed to elucidate the relationship between PSMA expression value in the prostate gland, total PSA levels and GS in patients diagnosed with prostate cancer.

Material and Method: A total of 98 patients who were pathologically diagnosed with prostate cancer that did not receive any treatment and underwent Ga-68 PSMA PET/CT imaging for staging were included in the study. Findings detected in Ga-68 PSMA PET/CT imaging were categorized as prostate, lymph node, bone, and visceral organ. The focal increased PSMA expression values observed in the prostate gland were recorded as SUVmax. The patients were divided into two separate groups according to their GS score (GS>7 and GS≤7). Correlations between prostate PSMA SUVmax, GS score and total PSA scores were investigated.

Results: PSMA SUVmax levels of the group with a Gleason score of >7 were found to be significantly higher than the group with a Gleason score of ≤7 (p=0.03). The rates of lymph node metastasis, bone metastasis and visceral organ metastases were found to be significantly higher in the group with a Gleason score >7 compared to the group with a Gleason score ≤7. A positive correlation was observed between PSMA SUVmax and Total PSA (r=0.260, p=0.01). A positive correlation was observed between PSMA SUVmax and Gleason score (r=0.260, p=0.01). A positive correlation was observed between total PSA and Gleason score (r=0.320, p=0.001).

Conclusion: In conclusion, PSMA SUVmax and Total PSA levels were higher in the group with high Gleason score. There is a positive correlation between PSMA SUVmax and total PSA. Clinicians should be careful in this regard, as the possibility of metastasis will be high in groups with high Gleason scores.

Keywords: Prostate specific membrane antigen, prostate cancer, Ga-68 PSMA PET/CT

INTRODUCTION

Prostate cancer (PC) is the second most common type of cancer seen in men all over the world and ranks fourth among all cancers (1). While the 5-year survival rate is approximately 100% in patients with localized PC, this rate drops to 31% in patients with distant metastases (2). Despite all the advances in early diagnosis and treatment, including prostate-specific antigen (PSA) screening as well as imaging methods and genetic analysis, prostate cancer is the second leading cause of cancer-related death in men after lung cancer. A significant proportion of prostate cancer patients are metastatic at the time of diagnosis.

PSMA has been an ideal molecular target for both the diagnosis and treatment (theranostic) of prostate cancer in nuclear medicine. The intense expression of PSMA in prostate cancer cells is up to 1000 times compared to normal prostate cells, which is not released freely into the circulation. This constitutes an important molecular target in the diagnosis and treatment of metastatic castration resistant prostate cancers (mCRPC) (3).

In general, increased PSMA expression is observed in poorly differentiated, metastatic and hormone-

Corresponding Author: Şadiye Altun Tuzcu, sadiyetuzcu@yahoo.com.tr

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refractory PC. PSMA expression has been observed in increased levels in patients with high Gleason scores. Involvement is directly proportional to aggressiveness, metastasis and hormone therapy resistance and is an important indicator for prognosis (4). Current information on Ga-68 PSMA PET/CT shows that primary staging with PET/CT is important in patients with high-risk PC.

It was previously stated that Ga-68 PSMA PET/CT is a more appropriate technique for detecting the focus or foci of recurrence, especially at low PSA levels (0.2-2.0 ng/mL) after primary treatment, compared to conventional imaging techniques. The sensitivity of Ga-68 PSMA PET/CT increases in relation to GS and PSA kinetics (PSA doubling time) (5).

The most important advantage of Ga-68 PSMA is that it has been shown to be superior to F-18 choline and other currently FDA-approved agents (C-11 choline, F-18 Flucyclovin) in PET imaging at low PSA values in detecting PC recurrence (mean sensitivity 76%- 86, specificity 86-100%) (6). Although there is no FDA approval for the recommendation of Ga-68 PSMA PET/CT imaging for PC, in the current version of the National Comprehensive Cancer Network (NCCN), it can be performed in clinical trials and controlled studies (6). According to the EAU guideline, it is recommended after radical prostatectomy (RP), and in biochemical recurrence (BR) in radiotherapy (RT).

Finally, in terms of "optimal imaging in advanced PC" in ASCO guideline, there are recommendations under the definition of new generation imaging (NGI) as PSMA PET imaging in addition to conventional imaging under the conditions specified in advanced PC (7).

Study Hypothesis

In this study, we aimed to elucidate the relationship between PSMA expression value in the prostate gland, total PSA levels and GS in patients diagnosed with prostate cancer.

MATERIAL AND METHOD

The study was carried out with the permission of Dicle University Faculty of Medicine Non-interventional Clinical Research Ethics Committee (Date: 2021, Decision No: 359). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 98 patients who were pathologically diagnosed with prostate cancer and applied to Dicle University Faculty of Medicine, Department of Nuclear Medicine between April 2019 and May 2021 has been retrospectively analyzed. Informed consent has been obtained from all participants.

Individuals that did not receive any treatment and underwent Ga-68 PSMA PET/CT imaging for staging were included in the study. Findings detected in Ga-68 PSMA PET/CT imaging were categorized as prostate, lymph node, bone, and visceral organ. The focal increased PSMA expression values observed in the prostate gland (with or without corresponding lesions on CT) were recorded as SUVmax. The maximum standardized uptake value (maximum standardized uptake value-SUVmax) values of all lesions that were considered positive were measured and recorded. In addition, lymph nodes, bones and visceral organs were evaluated whether there was metastasis according to PSMA involvement. Imaging with any pathological finding was considered positive, and imaging without any pathological finding was considered negative. Except for the areas of physiological involvement, the involvement above the ground activity was considered significant.

Areas with involvement were anatomically localized with nondiagnostic CT images. The pathology reports of the patients were evaluated, and the Gleason score (GS) values were recorded. Prostate Specific Antigen (PSA) data measured in the last 1 month according to the imaging date of the patients were obtained.

In addition, the patients were divided into two separate groups according to their GS score (GS>7 and GS≤7). We investigated whether there were differences in total PSA, Prostate PSMA SUVmax values between these two groups. In addition, correlations between prostate PSMA SUVmax, GS score and total PSA scores were investigated.

Ga-68 PSMA PET/CT images of the patients were evaluated by two Nuclear Medicine specialists.

Statistical Analysis

IBM SPSS 21.0 for Windows statistical package program was used for the statistical evaluation of the data. Measurable variables were presented as mean±standard deviation, and categorical variables as numbers and percentages (%). The chi-square ($\chi 2$) test was used for comparing categorical variables. Independent-t test was used for comparing measurable variables. In addition, Spearman correlation test was utilized for the correlation between Gleason score and PSMA SUVmax and total PSA. Pearson correlation test was performed to examine the correlation between PSMA SUVmax and total PSA. A P value ≤0.05 indicated statistically significant difference.

RESULTS

The mean ages were not different between the group with a Gleason score of ≤ 7 and the group with a Gleason score of >7.

Total PSA level was found to be significantly higher in the group with Gleason score >7 than in the group with Gleason level ≤7 (p=0.001).

PSMA SUVmax levels of the group with a Gleason score of >7 were found to be significantly higher than the group with a Gleason score of ≤7 (p=0.03) (**Table 1**).

Table 1. Gleason Scores according to age, total PSA and PSMA SUVmax

	Gleason score ≤7 (n=50)	Gleason score >7 (n=48)	P value
Age (years)	65.40±7.1	65.3±7.6	>0.05
Total PSA	28.5±33.6	52.6±39.7	0.001
PSMA SUVmax	11.8±7.6	15.2±7.89	0.03

The rates of lymph node metastasis, bone metastasis and visceral organ metastases were found to be significantly higher in the group with a Gleason score >7 compared to the group with a Gleason score ≤7 (**Table 2**).

Table 2. Gleason Scores according to lymph node metastasis, bone metastasis and visceral organ metastasis

	Gleason score ≤7 (n=50)	Gleason score >7 (n=48)	P value
Lymph node metastasis (yes/no)	16 (32%)/ 34 (68%)	32 (66.7%)/ 16 (33.3%)	0.001
Bone metastasis (yes/no)	12 (24%)/ 38 (76%)	27 (56.3%)/ 21 (43.8%)	0.002
Visceral organ metastasis (yes/no)	1 (2%)/ 49 (98%)	8 (16.7%)/ 40 (93.7%)	0.01

A positive correlation was observed between PSMA SUVmax and Total PSA (r=0.260, p=0.01) (**Figure 1**).

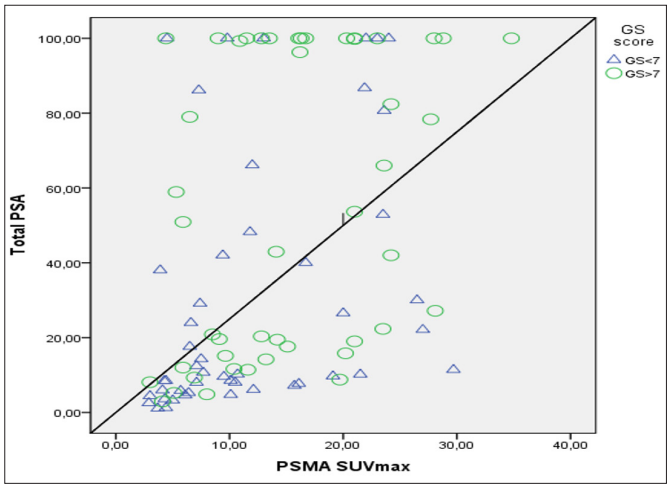

Figure 1. Scatter graph showing the correlation between Total PSA and PSMA SUV max

A positive correlation was observed between PSMA SUVmax and Gleason score (r=0.260, p=0.01). A positive correlation was observed between total PSA and Gleason score (r=0.320, p=0.001)(**Table 3**)(**Figure 2**).

Tablo 3. Correlation between Gleason score PSMA SUVmax and Total PSA

	Gleason score	Total PSA
PSMA SUVmax	R=0.260. p=0.01	R=0.524. p=0.001
Total PSA	R= 0.320 p=0.001	

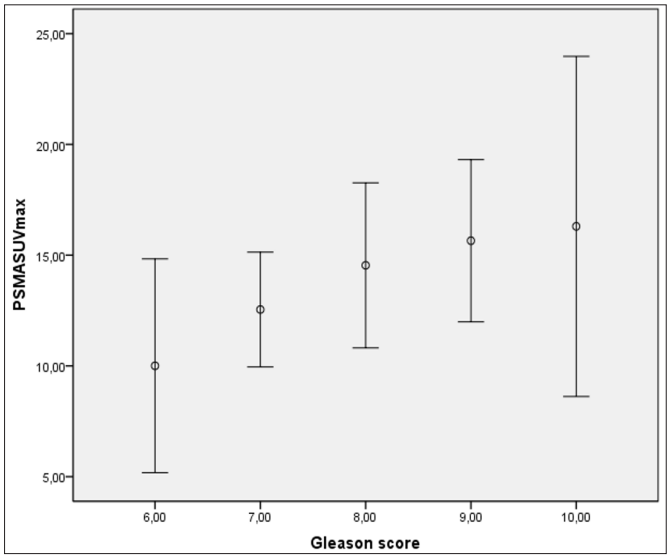

Figure 2. Correlation between PSMA SUVmax and Gleason score

DISCUSSION

Ga-68 PSMA PET/CT is being used more and more frequently in the staging of prostate cancer, determining the treatment plan, and detecting the recurrent lesion and its localization even at low PSA levels in patients with biochemical recurrence. Studies evaluating the relationship between the findings in Ga-68 PSMA PET/CT, pathology and laboratory parameters are increasing day by day. These studies also derive a significant relationship was between PSMA involvement and GS (8,9).

The rates of lymph node metastasis, bone metastasis and visceral organ metastases were found to be significantly higher in the group with a Gleason score >7 compared to the group with a Gleason score ≤7. In a study, it was reported that distal metastases such as lymph nodes, bones and liver were observed more frequently in patients with a Gleason score above 7. These findings are consistent with the results of our study (10). In a retrospective series, the sensitivity and specificity of Ga-68 PSMA was reported as 98-100% and 88-100%, respectively, for the detection of skeletal metastases at the initial staging of PC, while it was reported as 86-89% and 60-96% in bone scintigraphy (11) . Similarly, Thomas et al (12). showed that Ga-68 PSMA was superior to bone scintigraphy in detecting skeletal metastases in PC, and Ga-68 PSMA detected approximately twice as many lesions. In a recent study, Ga-68 PSMA PET/CT had significantly higher sensitivity and accuracy than bone scintigraphy (96.2% vs. 73.1% and 99.1% vs. 84%). 1) has been reported to

show. Therefore, current guidelines recommend the use of PSMA PET/CT to enable early detection of recurrences and salvage treatment options (4,13).

In this study we have found that the total PSA level was significantly higher in the group with Gleason score >7 than in the group with Gleason level ≤7 (p=0.001). PSMA SUVmax levels of the group with a Gleason score of >7 were found to be significantly higher than the group with a Gleason score of ≤7 (p=0.03). In previous literature, Ga-68 PSMA PET/CT imaging is recommended for patients diagnosed with prostate cancer and those in the medium-high risk group (14,15). In this study, according to the D'Amico risk classification, 33 staging patients were in the high risk group and 7 were in the intermediate risk group (TRUS-Bx was performed 3 times in one patient and adenocarcinoma was diagnosed in the last biopsy, Gleason score was not given in the report). Uprimny et al. (16), in their study with 90 patients diagnosed with prostate cancer, found that SUVmax values measured from the prostates of patients with GS 7 and below (GG3 and below) and patients with a GS above 7 (GG3 and above) differed significantly. In the study of Uprimny et al. (16), a significant difference was found between patients with a PSA value of 10 ng/ml and below and patients with a PSA value above 10 ng/ml in terms of prostate SUVmax values.

We have also found relation of PSA and other variables. In this study a positive correlation was observed between PSMA SUVmax and Total PSA (r=0.260, p=0.01). A positive correlation was observed between PSMA SUVmax and Gleason score (r=0.260, p=0.01). A positive correlation was observed between total PSA and Gleason score (r=0.320, p=0.001). In a study by Klingenberg et al. (17) a significant positive correlation was found between prostate PSMA SUVmax values and International Society of Urological Pathology (ISUP) grade. In the same study, a positive correlation between PSA levels and prostate PSMA SUVmax was shown, which is consistent with the findings of our study. PSMA SUVmax and Total PSA levels were higher in the group with high Gleason score. There is a positive correlation between PSMA SUV max and total PSA. Eiber et al. (18) retrospectively evaluated 248 patients with prostate cancer diagnosed with biochemical recurrence after radical prostatectomy. In this study, pathological findings were detected in Ga-68 PSMA PET/CT imaging in 89.5% of the patients. They found that PSA values differed significantly between patients with negative imaging and patients with positive imaging. In this study, a significant difference was found between PSA values. Eiber et al. (18) also found that the Ga-68 PSMA PET/CT positivity rates of patients with GS 7 and below and patients with 8 and above differed significantly (18).

In a study by Chun et al. (19), the results of transrectal US-guided prostate biopsy (TRUS-Bx) and radical prostatectomy results of 2982 prostate cancer patients were compared and GS upgrade has been detected in 29% of the patients and downgrade in 14% of the patients. It was thought that this might be one of the reasons why there was no correlation between SUVmax values and GS/GG values. In fact, this finding suggests that the prognosis and treatment of the patient may change due to the lower reporting of GS/GG in patients diagnosed with TRUS-Bx, and SUVmax value can be used as a more valuable prognostic factor than GS/GG (19). Ceci et al. (20) retrospectively analyzed 70 patients with prostate cancer who had undergone radical prostatectomy or received radical RT for primary malignancy and had biochemical recurrence or persistent PSA elevation and therefore had Ga-68 PSMA PET/CT imaging. They accepted the imaging as positive Ga-68 PSMA PET/CT in which they detected any pathological finding. Accordingly, 74% of the patients are positive for Ga-68 PSMA PET/CT (20).

Obek et al. (20) found the sensitivity, specificity, and accuracy in the subgroup with ≥15 lymph nodes removed with histopathological correlation (n=37) were 67%, %, respectively. 88 and 81%, morphological imaging (MR, CT) found 20%, 99% and 72% in primary lymph node staging of Ga-68 PSMA PET/CT in 51 newly diagnosed high-risk PC patients. The authors stated that Ga-68 PSMA PET/CT is superior to morphological imaging in detecting lymph node metastasis, while surgical dissection remains the gold standard for lymph node staging. Therefore, Ga-68 PSMA PET/CT can replace conventional abdominal-pelvic CT in staging lymph node metastasis in PC (21).

In the ASCO guideline, although the role of new generation imaging including Ga-68 PSMA PET/CT in non-metastatic CRPC is not clear due to limited data, it is superior to conventional techniques in demonstrating metastatic, non-metastatic disease and will contribute to patient management with local treatment strategies (22,23). In addition, the role of Ga-68 PSMA PET/CT is not yet known when there is PSA progression in metastatic CRPC and if conventional imaging is negative, and although it is not routinely recommended when there is radiological progression, it is stated that it may have a role in evaluating the extent of progression and monitoring the findings (7,22). In prospective and retrospective studies, it has been reported that Ga-68 PSMA PET/CT, with its high positive predictive value and recurrence detection rate at low PSA levels, causes up to 50% change in patient management in general (24,25).

Limitations of the Study

The main limitation of the study could be attributed to its retrospective nature. The other limitation was elaborated as single-center patient enrollment but this might be rationalized by the retrospective data collection.

The strength of this study lies beneath the fact that staging patients who have not received any treatment were included in the analysis. The other upside could be emphasized as it is an emerging era.

CONCLUSION

In conclusion, PSMA SUVmax and Total PSA levels were higher in the group with high Gleason score. There is a positive correlation between PSMA SUV max and total PSA. Clinicians should be careful in this regard, as the possibility of metastasis will be high in groups with high Gleason scores. According to the results of this study one can say that Ga-68 PSMA PET/CT imaging for staging suggests that it should be put into routine use in medium-high risk patients during staging purposes. Regarding the association between Ga-68 PSMA PET/CT findings and SUVmax values, histopathological findings and laboratory findings, SUVmax values are also can be used as a prognostic factor.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dicle University Faculty of Medicine Non-interventional Clinical Research Ethics Committee (Date: 2021, Decision No: 359).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients

Referee Evaluation Process: Externally peer-reviewed.

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

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: 359-86.
2. 2.Siegel RL, Sahar L, Portier KM, et al. Cancer death rates in US congressional districts. CA Cancer J Clin 2015; 65: 339-44.
3. Thomas L, Balmus C, Ahmadzadehfar H, Essler M, Strunk H, Bundschuh RA. Assessment of bone metastases in patients with prostate cancer-a comparison between (99m)Tc-bone-scintigraphy and [(68)Ga]Ga-PSMA PET/CT. Pharmaceuticals (Basel) 2017; 31: 10.
4. Lengana T, Lawal IO, Boshomane TG, et al. (68)Ga-PSMA PET/CT replacing bone scan in the initial staging of skeletal metastasis in prostate cancer: a fait accompli? Clin Genitourin Cancer 2018; 16: 392-401.
5. Verburg FA, Pfister D, Heidenreich A, et al. Extent of disease in recurrent prostate cancer determined by [(68)Ga]PSMA-HBED-CC PET/CT in relationto PSA levels, PSA doubling time and Gleason score. Eur J Nucl Med Mol Imaging 2016; 43: 397- 403.
6. National Comprehensive Cancer Network. NCCN Clinical Guidelines in Oncology. Prostate Cancer. Version 2.2020. 2020; https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf
7. Fendler WP, Eiber M, Beheshti M, et al. 68Ga-PSMA PET/CT: Joint EANM and SNMMI procedure guideline for prostate cancer imaging: version 1.0. Eur J Nucl Med Mol Imaging 2017; 44: 1014-24.
8. Kirby M, Hirst C, Crawford ED. Characterising the castration-resistant prostate cancer population: a systematic review. Int J Clin Pract 2011; 65: 1180-92.
9. Siegel RL, Sahar L, Portier KM, et al. Cancer death rates in US congressional districts. CA Cancer J Clin 2015; 65: 339-44.
10. Topuz ÖV, Aksu A, Erinç SR, Tamam MÖ.Correlations of 68 Ga-PSMA PET/CT in the initial staging of prostate cancer patients. Hell J Nucl Med 2021; 24: 60-65.
11. Pyka T, Okamoto S, Dahlbender M, et al. Comparison of bone scintigraphy and (68)Ga-PSMA PET for skeletal staging in prostate cancer. Eur J Nucl Med Mol Imaging 2016; 43: 2114-21.
12. Sridhar SS, Freedland SJ, Gleave ME, et al. Castration-resistant prostate cancer: from new pathophysiology to new treatment. Eur Urol 2014; 65: 289-99.
13. Cornford P, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG guidelines on prostate cancer. Part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. Eur Urol 2017; 10
14. Bailey J, Piert M. Performance of 68Ga-PSMA PET/CT for prostate cancer management at initial staging and time of biochemical recurrence. Curr Urol Rep 2017; 18: 84.
15. Lenzo NP, Meyrick D, Turner JH. Review of Gallium-68 PSMA PET/CT imaging in the management of prostate cancer. Diagnostics (Basel, Switzerland) 2018; 8: 16.
16. Uprimny C, Kroiss AS, Decristoforo C, et al. 68Ga-PSMA-11 PET/CT in primary staging of prostate cancer: PSA and Gleason score predict the intensity of tracer accumulation in the primary tumour. Eur J Nucl Med Mol Imaging 2017; 44: 941–9.
17. Klingenberg S, Jochumsen MR, Ulhøi BP, et al. (68)Ga-PSMA PET/CT for primary lymph node and distant metastasis NM staging of high-risk prostate cancer. J Nucl Med 2021; 62: 214-20.
18. Eiber M, Maurer T, Souvatzoglou M, et al. Evaluation of hybrid 68Ga-PSMA ligand PET/CT in 248 patients with biochemical recurrence after radical prostatectomy. J Nucl Med 2015; 56: 668–74.
19. Chun FK-H, Steuber T, Erbersdobler A, et al. Development and internal validation of a nomogram predicting the probability of prostate cancer Gleason sum upgrading between biopsy and radical prostatectomy pathology. Eur Urol. 2006; 49: 820–6.
20. Ceci F, Uprimny C, Nilica B, et al. (68)Ga-PSMA PET/CT for restaging recurrent prostate cancer: which factors are associated with PET/CT detection rate? Eur J Nucl Med Mol Imag 2015; 42: 1284–94.

21. Öbek C, Doğanca T, Demirci E, et al. Members of Urooncology Association, Turkey. the accuracy of 68Ga-PSMA PET/CT in primary lymph node staging in high-risk prostate cancer. Eur J Nucl Med Mol Imaging 2017; 44: 1806-12.

22. Mottet N, Bellmunt J, Briers E. et al. Members of the EAU-ESTRO-ESUR-SIOG Prostate Cancer Guidelines Panel. EAU-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer. Edn. presented at the EAU Annual Congress Amsterdam 2020. 978-94-92671-07-3. Arnhem: EAU Guidelines Office; 2020.

23. Calais J, Fendler WP, Eiber M, et al. Impact of 68Ga-PSMA-11PET/CT on the management of prostate cancer patients with biochemical recurrence. J Nucl Med 2018; 59: 434-41.

24. Sonni I, Eiber M, Fendler WP, et al. Impact of 68Ga-PSMA-11 PET/CT on staging and management of prostate cancer patients in various clinical settings: a prospective single center study. J Nucl Med 2020; 61: 1153-60.

25. Uprimny C, Kroiss AS, Decristoforol C, et al. 68Ga-PSMA-11 PET/CT in primary staging of prostate cancer: PSA and Gleason score predict the intensity of tracer accumulation in the primary tumour, Eur J Nucl Med Mol Imaging 2017; 44: 941–9.

Case Report

ANATOLIAN CURRENT MEDICAL

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Seven cases of imported malaria with recurrence

Fatma Kesmez Can, Handan Alay, Mehmet Parlak

Atatürk University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Erzurum, Turkey

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ABSTRACT

Malaria is an infectious disease caused by Plasmodium species parasites and transmitted to humans by the Anopheles mosquito. The malaria parasites responsible for infection in humans are *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium falciparum*, *Plasmodium malaria*, and Plasmodium knowlesi. Although malaria cases originating from Turkey no longer occur, cases originating from abroad are still seen. This report evaluates the epidemiological and clinical characteristics and histories of seven imported malaria cases. All seven cases in this report had a history of travel to African countries together with fever (>39°C), weakness, headache, tremor, sweating, cough, general body pain, and severe shortness of breath. Appropriate treatment was arranged once the necessary examinations had been performed. All patients but one responded to treatment. The non-responsive patient re-presented after 15 days with similar complaints. Primaquine therapy at 15 mg tablet 2×1 was administered, and that patient was discharged after 14 days. The most effective means of bringing malaria under control involves rapid diagnosis and effective treatment. We wish to emphasize that imported cases may be seen in non-endemic regions due to international travel. The travel history of patients in sporadic regions should be investigated, and malaria should be considered in the presence of fever in order to ensure early diagnosis and treatment.

Keywords: Malaria, fever, *Plasmodium falciparum*, infectious disease

INTRODUCTION

Malaria remains a common infectious disease in much of the world. The agent is protozoan parasites from the family *Plasmodidae*. The disease is transmitted to humans by the Anopheles mosquito (1).

The World Health Organization (WHO) World Malaria Report for 2017 stated that 237 million cases were seen in 2010, decreasing to 216 million in 2016. In addition, 445,000 individuals died from the disease (2). Symptoms include periodic fever, shivering, anemia and splenomegaly. Diagnosis is based on the presence of Plasmodia at thick drop and thin smear tests. Where the disease was contracted, and its resistance, are important factors in terms of treatment (1).

Although the numbers of malaria cases are decreasing as a result of measures being adopted worldwide, sporadic cases can still be seen in non-endemic regions for reasons such as migration and seasonal labor. We describe seven cases presenting with histories of travel and fever in which malaria needed to be considered at differential diagnosis.

CASE REPORT

Seven male patients, aged 22-56 years, were admitted to our outpatient clinic between June and September. These had complaints of fever (>39°C), weakness, headache, chills, chills, sweating, coughing, generalized body pain, and severe shortness of breath. All patients had a history of stays of 1-6 months in African countries. No drugs had been used for prophylaxis before travel, although they had employed unknown medicines for these complaints while in Africa. The seven cases' laboratory tests were examined (**Table**). Hepatosplenomegaly was present in all patients. Thick drop and thin smear methods were applied to blood samples. Giemsa staining was applied, and a signet ring-shaped plasmodium trophozoite was observed (**Figure**). In the light of the region from which the patients had arrived, *Plasmodium falciparum* (*P. falciparum*) was suspected as the cause of malaria. This was regarded as resistant to chloroquine, and treatment with Artemether (20 mg)-lumefantrine (120 mg) 2×4 tablets was initiated (three days). Tetracycline was also given (4×500 mg/day for seven days). One

Corresponding Author: Fatma Kesmez Can, fatma.can@atauni.edu.tr

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Table. Laboratory findings of cases before treatment

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
WBC K/mm³	10340	3400	6400	5200	4900	4400	5100
Hemoglobin g/dL	11.3	11.2	10.2	10.4	11.3	10.4	11.5
PLT /L	148000	113000	26000	76000	134000	39000	120000
AST U/L	43	37	55	56	35	43	30
ALT U/L	53	45	61	33	37	56	52
LDH U/L	336	303	208	649	276	439	253
Total bilirubin µmol/L	1.0	1.2	1.1	5.4	0.9	0.7	0.8
Creatinine µmol/L	0.8	0.9	1.0	1.2	0.7	0.8	0.5

WBC: White blood cell count, ALT: Alanin aminotransferaz, AST: Aspartat aminotransferaz

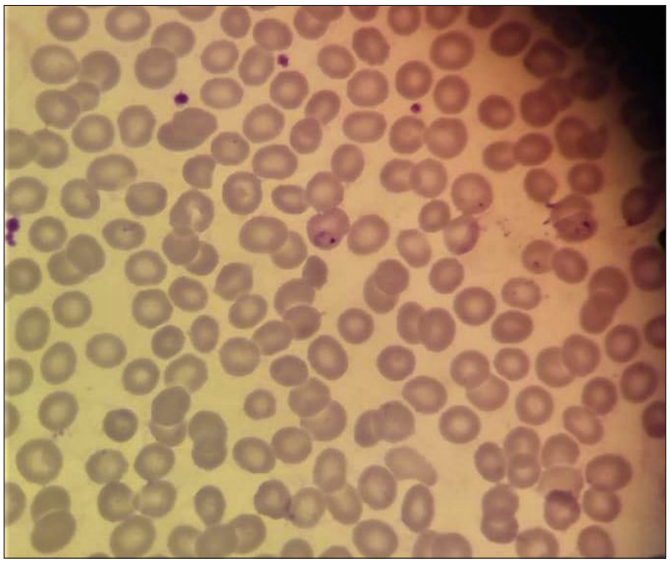

Figure. Signet Ring Shape *Plasmodium trophozoite*
* It was stained with Giemsa paint and a signet ring shape plasmodium trophozoite was seen.

patient (Case 4) again reported chills, high fever and nausea, vomiting, and dizziness 15 days after discharge. Trophozoites were observed at smear preparation examination, and mixed type malaria was suspected. Primaquine therapy was initiated at 15 mg tablet 2×1 (14 days). Patients with clinical and laboratory improvement were discharged, and no relapse was observed at six-month follow-up.

DISCUSSION

Thanks to its effective malaria control program, Turkey was included in the elimination phase by the WHO, reducing its case numbers from 11,381 in 2000 to 90 in 2010 (2). While a major decrease has occurred in local malaria cases, 251 cases of malaria imported from abroad were reported in 2013, 249 in 2014, 221 in 2015, and 209 in 2016 (2). Imported cases are seen in Turkey due to increased travel, international workforce mobility, and migration, and malaria remains an important health problem (1,3). For climatic reasons, malaria in our region assumes the form of sporadic cases. The majority of cases are imported from abroad, and the most important problems are experienced in diagnosis and treatment. Malaria is most commonly seen between June and October (3). In Turkey, the disease exhibits travel-related seasonal variation. Travelers to endemic regions must be started on chemoprophylaxis, and this must be maintained for four weeks after their return. None of our cases had employed chemoprophylaxis before traveling (4).

The characteristic feature of malaria is that it emerges in the form of rising body temperature with chills and episodes. Febrile episodes occur regularly once every 48 h in *Plasmodium vivax* (*P. vivax*) and *Plasmodium ovale* (*P. ovale*) infections, once every 72 h in *Plasmodium malaria* (*P. malaria*), and irregularly once every 36-48 h in *P. falciparum* (1). Fever exhibited an intermittent irregular pattern in all our cases. Shivering, fever, sweating, headache, fatigue, listlessness, and joint and skeletal pain were also present in all seven patients. Accompanying shortness of breath and cough were also present in four patients. Anemia, thrombocytopenia, splenomegaly, hepatomegaly, jaundice, petechiae, conjunctival bleeding, and herpes labialis can also be seen in cases of malaria (5). Hepatosplenomegaly, anemia, mild hepatic enzyme elevation, and thrombocytopenia were present in our cases. Bilirubin elevation and icterus were also observed in Case 4. No cutaneous lesions were detected, but vesicular eruptions, which are uncommon in malaria, were observed in the febrile period in one patient.

Histories of travel to African countries have been determined in cases of imported malaria previously reported from Turkey, and as in the present cases, no history of prophylaxis use prior to travel was present in those cases. Patients had similarly previously presented with fever, shivering, nausea, vomiting, and headache. Quinine-sulfate and tetracycline therapy was administered in the light of the possibility of resistance in cases thought to be caused by *P. falciparum* based on the regions of travel involved and Giemsa staining. Primaquine, effective against the hypnozoite form seen in *P. vivax* and *P. ovale*, was also given in cases thought to be mixed type, as in our case. The importance of considering malaria in individuals traveling to endemic regions was also emphasized (3,8).

Microscopic examination of peripheral blood specimens is still the gold standard for the diagnosis of malaria. Diagnosis is based on the presence of the parasite in thick drop and thin smear preparates from peripheral blood (6). Diagnosis was based on observation of trophozoites in all our cases. All seven patients had used unidentified medications due to similar symptoms while in the African countries where they were working. All presented to our clinic with recurrence of infection.

Manifestations of reinfection despite appropriate treatment or recrudescence (unsuccessful treatment) can be seen in *P. falciparum malaria*. Failure of treatment may be associated with various factors, such as drug resistance, weak medication passage into the body or dosages being below the requisite levels, vomiting, and inappropriate drug pharmacokinetics (4). Artemisinin performs an important function in the rapid reduction of the parasite burden, immediate improvement of symptoms, and prevention of resistance to other drugs (5). Artemisinin combination therapy is recommended by the WHO. Artemisinin/lumefantrine is well tolerated and highly effective. This was used in all our patients, and responses to treatment were achieved in all cases (4,7).

Lumefantrine, artemisinin and tetracycline were used in the treatment of our patients. Since the patients had been treated in various different countries, tetracycline was added to the artemisinin regimen. Smears were repeated due to recurrence of fever and vomiting after 15 days in only one case. Trophozoites were observed at examination, and mixed type malaria was suspected.

Primaquine therapy, which is effective in the hypnozoite form seen in *P. vivax* and *P. ovale*, was started in the form of 15 mg tablet 2×1. No recurrence or relapse was observed in our patients at six-month follow-up.

CONCLUSION

Malaria is one of the diagnoses requiring primary consideration in case of fever in individuals traveling from countries where the disease is endemic. The most effective means of bringing malaria under control is rapid diagnosis and effective treatment. Individuals due to travel to endemic regions must be given chemoprophylaxis, and presenting cases must be treated in the light of the drug-resistance status of the region in question. Finally, we wish to emphasize that imported cases may be seen in non-endemic regions due to international travel

ETHICAL DECLARATIONS

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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REFERENCES

1. Topçu AW, Soyletir G, Doğanay ME, editors. Infectious diseases and microbiology. İstanbul: Nobel Medical Bookstores; 2017. p. 887-905.
2. World Health Organization. World malaria report 2017, WHO Press, Geneva, Switzerland.
3. Parlak E, Ertürk A, Çayır Y, Parlak M. Four malaria-import patterns: sporadic region. Turkiye Parazitol Derg 2013; 37: 161-7.
4. World Health Organisation (WHO). Guidelines for the treatment of malaria. Second edition.2015.Availableat:https://www.who.int/malaria/publications/atoz/mpac_report_september_2014.pdf (Accessed on 3 July 2021).
5. Ulçay A, Karaahmetoğlu G, Turhan V, et al. The management of therapeutic failure in a *falciparum malaria* patient under oral arthemether lumefantrine therapy. Turkiye Parazitol Derg 2014; 38: 61-7.
6. Valecha N, Srivastava P, Mohanty SS, et al. Therapeutic efficacy of artemether-lumefantrine in uncomplicated *falciparum malaria* in India. Malar J 2009; 8: 107.
7. Askling HH, Bruneel F, Burchard G, et al. Management of imported malaria in Europe. Malar J 2012; 11: 328.
8. Bayındır Y, Aycan OM, Atambay M, et al. The first appearance in Malatya of falciparum malaria originating in Uganda: Two cases, Turkiye Parazitol Derg 2005; 29: 157-9.

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