Role of Ischemia and Oxidative Stress in Primary Dysmenorrhea Pathogenesis

Primer Dismenore Patogenezinde İskemi ve Oksidatif Stresin Rolü

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Abstract

Objectives: Primary dysmenorrhea is a pelvic pain without pathologic reasons during the menstrual period, induced by prostaglandin synthesis. Last studies have shown the relation of primary dysmenorrhea with ischemia/hypoxia. Ischemia-Modified Albumin (IMA) is a marker used for detecting the early period of ischemia. In this study we planned to investigate role of ischemia and oxidative stress in etiopathogenesis of primary dysmenorrhea according to the severity of its symptomatology.

Method: Volunteer 47 female medical faculty students with primary dysmenorrhea were included in this study. The investigation conforms to the principles outlined in the Declaration of Helsinki and the Ethical Committee approved the study. Each student passed through the full physical and gynecological examination. Visual Analog scale (VAS) was used to measure pain intensity (no pain score of 0; worst imaginable pain score of 10). VAS grading from 1-4 was accepted as mild; 5-7 as moderate; and 8-10 as severe pain. Blood samples were collected from all participants on the third day of menstrual period. After separation of serum, they were kept at -80°C until analyzed. Serum IMA levels were measured by albumin cobalt binding (CAB) test and recorded as absorbance units (AUs). The results were corrected by using serum albumin values expressed as corrected IMA (C-IMA). MDA levels were measured by using thiobarbituric acid reactive substance (TBARS) and the results were expressed as m̄mol/L.

Results: C-IMA values were: 0.867±0.23 in mild; 1.279±0.31 in moderate and 1.222±0.20 AU/g albumin in severe pain group. There were significant difference between the averages of groups with One-way ANOVA (p<0.024). By using Tukey test the C-IMA values in group with mild pain found significantly lower than the C-IMA values of the group with moderate pain (p=0.021). MDA results were: 9.01±6.4 in the moderate and 15.20±6.86 m̄mol/L severe pain group. The difference between groups with One-way ANOVA was statistically significant (p<0.016). Group comparisons with Tukey test showed significant difference between the group with mild dysmenorrhea and the group with severe pain (p<0.016).

Conclusion: C-IMA and MDA levels increased in patients with primary dysmenorrhea. Their levels were related with the severity of the pain, suggesting roles of ischemia and oxidative stress in primary dysmenorrhea. Probably pain-generating mechanisms also produce oxidative stress and ischemia. Molecular mechanisms which induce oxidative stress together with ischemia and pain should be investigated in further studies.

Keywords: Primary dysmenorrhea, ischemia-modified albumin, malondialdehyde
Introduction
Primary dysmenorrhea (PD) is lower abdominal and pelvic pain during the menstrual period which is not related with any pathologic reasons. The definite pathogenesis of PD remains unclear, but studies have shown that prostaglandins and vasoactive mediators are increased in the endometrium and menstrual specimens. Recent studies have also shown that some hormonal or vascular endothelial functional changes, such as increased vasopressin and serum ADMA levels, cause vasoconstriction, uterine contractions, and eventually uterine ischemia related to the menstrual pain in PD. PD also affects other systems and may lead to an increase in the risk of cardiac arrhythmia or mental status change during the menstrual period. Ischemic and hypoxic conditions and oxidative stress constitutes the major part of PD pathogenesis. Major amount of prostaglandins are secreted during the first 48 hours of menstruation, which concur with the greatest intensity of the symptoms. It is well known that if the free radical concentrations are elevated and/or antioxidant potential is lowered, oxidative stress is formed. It has also been reported that dysmenorrhea led to an increase in lipid peroxidation, an index of oxidative stress. Malondialdehyde (MDA) is a biomarker of oxidative damage to lipids caused by oxidants. MDA can be produced as a decomposition product of oxidized lipids. While oxidation of polyunsaturated fatty acids is the major source of MDA in vivo, other minor sources exists such as byproducts of free radical generation by ionizing radiation and of the biosynthesis of prostaglandins. Excessive amount of prostanoids are secreted from the endometrium during menstruation. The uterus is induced to contract, with increased basal tone and increased active pressure. Uterine hypercontractility lead to reduced uterine blood flow by the way hypoxia too. Ischemia modified albumin is a biomarker for acute ischemia. IMA constitutes via the modification in albumin where reactive oxygen species are formed due to ischemia. When exposed to ischemic conditions, the N-terminus of albumin is damaged, which makes it unable to bind metals and capable of being measured by an albumin cobalt-binding test. Under the normal conditions IMA is the 1-2 % of total albumin while ischemic conditions this amount become 6-8 %. Very low or high concentration of albumin and the presence of lactic acidosis effects IMA measurement. To eliminate the effect of albumin to IMA we used corrected IMA index. 1g/dL change at albumin causes, 2.6 % amount change at level of IMA. Because its levels in the blood increase within minutes of the onset of ischemia, remains elevated for 6 to 12 hours and return to normal within 24 hours, IMA has been implicated in the detection of acute ischemia prior to necrosis.

Ischemic and hypoxic conditions and oxidative stress constitutes the major part of PD pathogenesis. IMA and MDA are good markers of ischemia and oxidative stress respectively. That’s why, the objective of our study was to show the role of oxidative stress and hypoxic/ischemic conditions in the pathogenesis of PD, by using IMA and MDA. In this study we also investigated the relationship of these parameters with the severity of dysmenorrhea.

Materials and Methods
Forty-seven female medical students with primary dysmenorrhea were included in this study. Their main complaint was dysmenorrhea which was mostly located in lower abdominal and pelvic area. All the participants underwent a standardized clinical assessment, which included detailed medical history, and the systemic physical and full gynecological examination. Participants having any gynecological disease, rheumatic, renal, cardiovascular, endocrine and metabolic disorders, inflammatory bowel disease, fibromyalgia, known malignancy, and taking any oral contraceptive drugs were excluded. Informed consent was obtained from all participants prior to the study. The investigation conforms to the principles outlined in the Declaration of Helsinki and it was approved by local ethics committee (71522473/050.01.04/205).

Pain intensity was measured by using the Visual Analog Scale (VAS) which is derived from a 10-cm scale, with end points of 0 for “no pain” and 10 for “the imaginable most severe pain”. The use of the scale was clearly explained to all participants. Patients were asked to make a mark on the line representing their pain intensity. Grading from 1-4 was accepted as mild; 5-7 was accepted as moderate; and 8-10 was accepted as severe pain.

All reagents, unless otherwise noted, were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemicals (St. Louis,
Following an overnight fasting, venous blood samples were collected on the 3rd day of menses. After clotting and centrifugation at 400xg for 10 min, serum samples were separated in Eppendorf tubes and frozen immediately at -80 °C until analysis. Routine biochemical parameters were measured by automated colorimetric methods with commercially available kits (ARCHITECT c16000 auto analyzer -Abbott Laboratories, Abbott Park, Illinois, USA).

The levels of lipid peroxide in blood plasma were measured using a thiobarbituric acid reactive substance (TBARS) assay, which monitors MDA production, based on the method of Beuge and Aust. The amount of MDA was calculated using an extinction coefficient of 1.56 x 105 M⁻¹cm⁻¹. The concentrations of MDA were expressed as micromoles/L in serum samples.

To detect ischemia modified albumin we use the albumin cobalt binding (CAB) test reported by Bar-Or et al., spectrophotometrically. Briefly, a 200 μl serum sample was added into 50 μl of 0.1% cobalt chloride solution, and mixed. After 10 min incubation at room temperature for cobalt albumin binding, 50 μl Dithiothreitol (0.15 %solution) was added, after 2 min incubation, the reaction was stopped by adding 1.0 ml of 0.85% NaCl. The absorbance of the samples and blanks were taken at 470 nm by using Shimadzu spectrophotometer. IMA values were expressed as absorbance units (AUs).

Patients’ serum albumin concentrations were measured by bromocresol green staining method according to manufacturer instruction (Biolabo, Les Hautes Rives, 02160, Maizy, France). After albumin concentrations were found IMA results (AUs) were corrected by using serum albumin values-expressed as corrected IMA (C-IMA), and compared groups. C-IMA values were found as 0.867±0.23 AU/g albumin (n=15) in the group with mild pain (VAS Score 1-4); 1.279±0.31 AU/g albumin (n=22), in the group with moderate pain (VAS Score 5-7) and 1.222±0.20 AU/g albumin (n=10) in the group with severe pain (VAS Score 8-10). There were significant differences between the averages of groups with One way ANOVA (p<0.024). By using Tukey Test the C-IMA values in group with mild pain found significantly lower than the C-IMA values of the group with moderate pain (p = 0.021) (Fig. 1). MDA results were: 9.01±0.64 in the mild; 11.78±1.97 in the moderate and 15.20± 6.86 μmol/L severe pain group (Fig. 2). The difference between groups with One way ANOVA was statistically significant (p<0.016). Further analysis with Tukey showed significant difference between the groups with mild and the severe dysmenorrhea (p<0.016) (Fig. 2). MDA values increased parallel to VAS scores, but only the difference between the mild and severe groups reached statistical significance.

Discussion
This study has showed the role of ischemic conditions and oxidative stress in PD by using serum IMA and MDA levels. We graded...
dysmenorrhea pain as mild, moderate, severe and evaluated the association of serum IMA and MDA levels. In comparisons among pain groups, serum IMA and MDA levels significantly differed between the moderate and mild pain intensity groups and severe and mild pain intensity groups respectively. However, there was no significant difference between moderate to severe pain groups in serum IMA and MDA levels. Thus, we also found that more severe pain was associated with higher serum IMA and MDA levels. This study has showed role of hypoxic/ischemic conditions and oxidative stress in pathogenesis of PD by using IMA, for the first time in the literature. The association of IMA with severity of PD symptomatology was also shown for the first time.

Figure 1. IMA concentrations of PD patients according to severity of their symptomatology.

IMA results expressed as corrected IMA in AUs which were adjusted according to their serum albumin concentrations. In the group with mild pain VAS Scores were 1-4 (n=15); in the group of moderate pain VAS Score were 5-7 (n=22); and in the group with severe pain VAS Scores were 8-10 (n=10). The differences between groups with Oneway ANOVA were significant (p<0.024). C-IMA values in group with mild pain were significantly lower compared to the group with moderate pain (p = 0.021).

Oxidative stress due to production of reactive oxygen species (ROS) or decreased antioxidant protection have been implicated in the pathogenesis of many disorders in the human body. Reactive oxygen species attack macromolecules such as protein lipid and DNA. There are several studies that reported the role of reactive oxygen species and lipid peroxidation in ethiopathogenesis of PD. In their study, Dikensoy et al. showed that serum malondialdehyde as a marker of lipid peroxidation were significantly higher in subjects with dysmenorrhea compared to control. In their study, MDA, nitric oxide (NO) and adrenomedullin serum levels were increased in subjects with primary dysmenorrhea. Similarly, we showed increased serum levels of MDA in the subjects with primary dysmenorrhea compared to the controls in the present study. Moreover, our study revealed an association between lipid peroxidation and severity of dysmenorrhea.

Figure 2: MDA levels of PD patients according to severity of their symptomatology.

MDA results were expressed as μmole/Liter. The difference between groups with Oneway-ANOVA was statistically significant (p<0.016). MDA levels of severe pain group were significantly higher than the levels of mild pain group (p<0.016).

Ischemia Modified Albumin has been found to be very useful marker for the detection of acute myocardial ischemia but now it is being used to investigate the relation of many diseases to hypoxia/ischemia such as preeclampsia, appendicitis, hepatitis B-related chronic liver diseases, intestinal ischemia necrotizing enterocolitis. Similarly, we used IMA to demonstrate hypoxia/ischemia in the etiopathogenesis of PD in our study. The secretory endometrium contains plenty amount of arachidonic acid, which is used for production of various prostaglandins and leukotrienes during menses. These prostaglandins and their metabolites are responsible for the symptomatology of PD such as pain, headache, nausea and vomiting, and backache. By stimulating prolonged myocardial constructions they also may play a role in formation of hypoxia/ischemia in myometrium. In our study, we showed that presence of ischemia in primary dysmenorrhea. According to
our findings, C-IMA and MDA levels were related with the severity of the pain, suggesting roles of ischemia and oxidative stress in primary dysmenorrhea. Probably pain-generating mechanisms also produce oxidative stress and ischemia. Although underlying mechanisms are not fully known yet prostaglandins are one of the possible candidates as a common denominator. Interestingly, Akdemir et al. showed that serum ADMA levels were higher in patients with moderate and severe dysmenorrhea compared with patients with mild dysmenorrhea according to their VAS scores.6 They also have shown a significant positive correlation between serum ADMA and AMH levels in primary dysmenorrhea. These findings are consistent with our results, as ADMA is a marker of endothelial dysfunction.30

In summary of our study, hypoxia/ischemia is one component of the etiopathogenesis of primary dysmenorrhea. The increased IMA and MDA levels were related with the severity of the pain, suggesting roles of ischemia and oxidative stress in primary dysmenorrhea. However this study has some limitations and could be supported with further research with respect to the following points primarily. The absence of a control group, absence of follow-up data, single blood sampling, and small sample size are limitations of this study. Design of our does not permit to draw any conclusion on a causal relationship between the underlying interactions in primary dysmenorrhea. Our sample size was relatively small, which may have led to the inability to catch the difference between three groups of primary dysmenorrhea according to their VAS scores. To clarify underlying mechanisms, it is necessary to confirm the findings in a further study with a larger sample size.

Ethical approval: The study protocol was previously reviewed and approved by the ethics committee of the University of the Sakarya, Faculty of Medicine. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (71522473/050.01.04/205).

Conflict of Interest: The authors report no conflicts of interest.

Contributors:
FBSC, NA, BA and HC researched literature and conceived the study. HH, IK, LS, FBSC, BA, NA, ZK and HC were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. FBSC, BA, NA and HC wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.
1. Oladosu FA, Tu FF, Hellman KM. Nonsteroidal antiinflammatory drug resistance in dysmenorrhea: epidemiology, causes, and treatment. Am J Obstet Gynecol. 2017;216(5):502-509. PMID: 28885592

2. Dawood MY. Primary dysmenorrhea: advances in pathogenesis and management. Obstet Gynecol. 2006;108(2):428-41. PMID: 16808317

3. Iacovides S, Avdon I, Baker FC. What we know about primary dysmenorrhea today: a critical review. Hum Reprod Update. 2015;21(6):762-78. PMID: 26346058

4. Akerlund M. Involvement of oxytocin and vasopressin in the pathophysiology of preterm labor and primary dysmenorrhea. Prog Brain Res. 2002;139:359-65. PMID: 12434699

5. Valtonen P, Punnkonen K, Saarela H, Heiskanen N, Raitakari OT, Jousilahti P. Oxidatively modifed albumin: could it be a new oxidative stress biomarker for markers of oxidative balance in patients with dysmenorrhea by multiple serum markers. J Turk Ger Gynecol Assoc. 2012;13(4):233-6. PMID: 24592048

6. Piwowar A. Oxidatively modifed forms of albumin in patients with risk factors of metabolic syndrome. J Endocrinol Invest. 2014;37(9):819-27. PMID: 24957167

7. Turhan N, Çelik H, Duvan CI, Onaran Y, Aydın M, Armutcu F. Investigating Enterocolitis: A Systematic Review. Pediatr Gastroenterol Nutr. 2017;31(1):69-74. PMID: 28395790

8. Ye S, Zhou X, Lin J, Chen P. Asymmetric Dimethylarginine induced apoptosis and dysfunction of endothelial progenitor cells: Role of endoplasmic reticulum stress pathway. Biomed Res Int. 2017;2017:6395601. PMID: 28589144

9. Dikensoy E, Balat O, Pençe S, Balat A, Cekmen M, Yurekli MJ. Malondialdehyde, nitric oxide and adrenomedullin levels in patients with primary dysmenorrhea. Arch Gynecol Obstet. 2018;398(3):1049-53. PMID: 19012707

10. Aksoy AN, Lafoglu E, Ozkaya AL, Yilmaz EP. Serum heme oxygenase-1 levels in patients with primary dysmenorrhea. Arch Gynecol Obstet. 2017;295(4):929-934. PMID: 28236018

11. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, Knight KA. The correlation of serum asymmetric dimethylarginine and anti-Müllerian hormone in primary dysmenorrhea. Kaohsiung J Med Sci. 2016;32(8):414-9. PMID: 27523455

12. Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. Rev Endocr Metab Disord. 2007;8(2):129-6. PMID: 17335519

13. Apple FS, Wu AH, Mair J, Ravkilde J, Panteghini M, Tate J, Pagani F, Chris-tenson RH, Mockel M, Danne O, Jaffe AS; Committee on Standardization of Markers of Cardiac Damage of the IFCC. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. Clin Chem. 2005;51(5):810-24. PMID: 15774573

14. Dominguez-Rodriguez A, Abreu-Gonzalez P. Current role of ischemia-modifed albumin: could it be a new oxidative stress biomarker for colorectal carcinoma? Gut Liver. 2013;7(6):675-80. PMID: 24312708

15. Bar-Or D, Curtis G, Rao N, Bampou N, Lau E. Characterization of the Co2+- and Ni2+- binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. Eur J Biochem. 2001;268(7):42-7. PMID: 1112100

16. Yeh ML, Chen HH, Su EC, Liu C-F. A study of serum malondialdehyde and interleukin-6 levels in young women with dysmenorrhea in Taiwan. Life Sci 2004; 75: 669–673. PMID: 15172716

17. Denks JP, Schellens DH, Acosta S. Serological markers for human intestinal ischemia: A systematic review. Best Pract Res Clin Gastroenterol. 2017;31(1):69-74. PMID: 28395790

18. Terrin G, Stronti L, Cucchiara S, De Curtis MJ. Serum Markers of Necro-tizing Enterocolitis. A Systematic Review. Pediatr Gastroenterol Nutr. 2017 Apr 5. PMID: 28379923

19. Koke H, Egawa H, Ohtsuka T, Yamaguchi M, Ikennoue T, Mori N. Correlation between dysmenorrheic severity and prostaglandin production in women with endometriosis. Prostaglandins Leukot Essent Fatty Acids 2002; 66: 425–432. PMID: 11745733

20. Ye S, Zhou X, Lin J, Chen P. Asymmetric Dimethylarginine induced apop-tosis and dysfunction of endothelial progenitor cells: Role of endoplasmic reticulum stress pathway. Biomed Res Int. 2017;2017:6395601. PMID: 28589144

21. Iacovides S, Avdon I, Baker FC. What we know about primary dysmenorrhea today: a critical review. Hum Reprod Update. 2015;21(6):762-78. PMID: 26346058

22. Lippi G, Montagnana M, Salagino GL, Guidi GC. Standardization of isch-e mia-modified albumin testing: adjustment for serum albumin. Clin Chem Lab Med. 2017;55(1):261-62. PMID: 27331959

23. Sharanu E, Georgiadou P, Voudris V. Ischemia modified albumin changes: review and clinical implications. Clin Chem Lab Med 2011;49:177-84. PMID: 21083441

24. Dominguez-Rodriguez A, Abreu-Gonzalez P. Current role of ischemia-modified albumin in routine clinical practice. Biomarkers. 2010;15(8):655-62. PMID: 20874662

25. Lee E, Eom JE, Jeon KH, Kim TH, Kim E, Jhon GJ, Kwon Y. Evaluation of albumin structural modifications through cobalt-albumin binding (CAB) assay. J Pharm Biomed Anal. 2014;91:17-23. PMID: 2443427

26. Evrard-Cloche E, Grebryk E, Manczynski D, Szymańska-Chabowska A, Piwowar A. Oxidatively modified forms of albumin in patients with risk factors of metabolic syndrome. J Endocrinol Invest. 2014;37(9):819-27. PMID: 24957167

27. Lippi G, Montagnana M, Salagino GL, Guidi GC. Standardization of ische-mia-modified albumin testing: adjustment for serum albumin. Clin Chem Lab Med. 2017;55(1):261-62. PMID: 27331959

28. Khoei S, Hafezieh M, Vahidnia A. Clinical relevance of bio-markers of oxidative stress. Antioxid Redox Signal. 2015;20(14):1144-70. PMID: 26415143.

29. Lee E, Eom JE, Jeon KH, Kim TH, Kim E, Jhon GJ, Kwon Y. Evaluation of albumin structural modifications through cobalt-albumin binding (CAB) assay. J Pharm Biomed Anal. 2014;91:17-23. PMID: 2443427

30. Ye S, Zhou X, Lin J, Chen P. Asymmetric Dimethylarginine induced apoptosis and dysfunction of endothelial progenitor cells: Role of endoplasmic reticulum stress pathway. Biomed Res Int. 2017;2017:6395601. PMID: 28589144