RESEARCH ARTICLE / Araştırma Makalesi

Role of Ischemia and Oxidative Stress in Primary Dysmenorrhea Pathogenesis

Primer Dismenore Patogenezinde İskemi ve Oksidatif Stresin Rolü

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Özet

Amaç: Primer dismenore, menstrual dönemde patolojik nedenlere bağlı olmayan ve prostaglandin sentezi tarafından uyarılabilen pelvik ağrıdır. Son çalışmalarda, primer dismenore ile iskemi/hipoksi arasındaki ilişki gösterilmiştir. İskemi Modifiye Albumin (IMA) iskeminin erken döneminin saptanmasında kullanılan yeni bir belirteçtir. Bu çalışmada, iskemi ve oksidatif stresin primer dismenore etiyopatogenezindeki rolünü ve ağrı şiddeti ile ilişkisini araştırmayı planladık..

Yöntem: Bu çalışmaya primer dismenoreli 47 gönüllü tıp fakültesi öğrencisi dahil edildi. Çalışma, Helsinki Deklarasyonu'nda belirtilen esaslara uygun yapıldı ve Etik Komite tarafından onaylandı. Her öğrencinin tam bir fizik ve jinekolojik muayenesi yapıldı. Ağrı şiddetini ölçmek için Görsel Analog Skala (VAS) kullanıldı (ağrı yok- 0, hayal edilebilen en şiddetli ağrı-10). Görsel Analog Skala ile, 1-4 arası skorlar hafif; 5-7 arası orta; ve 8-10 arası şiddetli ağrı olarak kabul edildi. Kan örnekleri, menstrüel döneminin üçüncü günü toplandı. Serum ayrıldıktan sonra analiz edilinceye kadar -80 °C'de saklandı. Serum IMA düzeyleri albumin kobalt bağlama (CAB) testi ile ölçüldü ve absorbans üniteleri olarak kaydedildi(AUs). Bu sonuçlar kullanılarak ölçülen serum albümin değerlerine göre düzeltilmiş-IMA (C-IMA) değerleri hesaplandı. C-İMA sonuçları AU/g albumin olarak verildi. Malondialdehit (MDA) düzeyleri tiyobarbitürik asit reaktif madde (TBARS) kullanılarak ölçüldü ve sonuçlar µmol/L olarak verildi.

Bulgular: C-IMA değerleri: hafif ağrılı grupta 0.867 ± 0.23; orta ağrılı grupta 1.279 ± 0.31 ve şiddetli ağrılu grupta 1.222 ± 0.20 AU/g albumin bulundu. Oneway ANOVA ile karşılaştırıldığında, grupların ortalamaları arasında anlamlı fark vardı (p <0.024). Tukey testi kullanılarak hafif ağrılı gruptaki C-IMA değerlerinin orta ağrılı olan grubun C-IMA değerlerinden anlamlı derecede düşük olduğu görüldü (p = 0,021). MDA sonuçları: hafif ağrılı grupta 9.01 ± 0.64; orta ağrılı grupta15.20 ± 6.86 ve şiddetli ağrılı grupta 11.78 ± 1.97 µmol/L bulundu. Oneway-ANOVA ile gruplar arasında istatistiksel anlamlı fark vardı (p <0.016). Tukey testi ile grup karşılaştırmaları, hafif dismenoreli grup ile şiddetli ağrılı grup arasında anlamlı fark olduğunu gösterdi (p <0.016).

Sonuç: Primer dismenore hastalarında C-IMA ve MDA düzeyleri artmaktadır. Ayrıca, bunların seviyeleri, ağrının şiddeti ile de ilişkiliydi. Bu bulgular, primer dismenorede iskemi ve oksidatif stresin rol oynadığını düşündürmektedir. Muhtemelen ağrı oluşturan mekanizmalar, aynı zamanda oksidatif stres ve iskemiye de neden olmaktadır. İskemi ve ağrı ile birlikte oksidatif stres oluşturan bu mekanizmalar daha ileri araştırmalar ile aydınlatılmalıdır. **(Sakarya Tıp Dergisi 2017, 7(4):205-210)**

Anahtar Kelimeler: Primer dismenore, iskemi-modifiye albumin, malondialdehit

Abstract

Objective: Primary dysmenorrhea is 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. **(Sakarya Med J 2017, 7(3):136-144)**

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 mens 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 unites (AUs). The results were corrected by using serum albumin values-expressed as corrected IMA(C-IMA) (AU/g albumin). Malondialdehyde (MDA) levels were measured by using thiobarbituric acid reactive substance (TBARS) and the results were expressed as µmol/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 Oneway 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±0.64 in the mild; 11.78±1.97 in the moderate and 15.20± 6.86 µmol/L severe pain group. The difference between groups with Oneway-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. **(Sakarya Med J 2017, 7(4):205-210)**

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.[1] The definite pathogenesis of PD remains unclear, but studies have shown that prostaglandins and vasoactive mediators are increased in the endometrium and menstrual specimens.[2,3] 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.[2,4-6] 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.3 Ischemic and hypoxic conditions and oxidative stress constitutes the major part of PD pathogenesis.[2,7] Major amount of prostaglandins are secreted during the first 48 hours of menstruation, which concur with the greatest intensity of the symptoms.[8] 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.[7,9,10] 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.[11] Excessive amount of prostanoids are secreted from the endometrium during menstruation.[12] The uterus is induced to contract, with increased basal tone and increased active pressure. Uterine hyper-contractility lead to reduced uterine blood flow by the way hypoxia too.[2] Ischemia modified albumin is a biomarker for acute ischemia.[13,14] IMA constitutes via the modification in albumin where reactive oxygen species are formed due to ischemia.[15] 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.[15,16] 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.[17,18] 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. 18-20 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.[15]

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,

MO, USA).

Following an overnight fasting, venous blood samples were collected on the 3rd day of menses. After clotting and centrifugation 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.[21] The amount of MDA was calculated using an extinction coefficient (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., spectrophotometricly.22 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) to avoid effect of the variability on concentration of albumin within patients in the group. C-IMA values were calculated according to the equation as below C-IMA = Patient serum albumin (g/dL)*patient IMA (AU)/median albumin concentration of group (g/dL)[17].

Statistical analyses

Data were analyzed via statistical software SPSS, version 10.0 [SPSS Inc, Chicago, IL, USA]. The distribution characteristics of continuous data were determined using histogram examination and the one-sample Kolmogorove Smirnov test. Normally distributed data were presented as mean± standard deviation (SD) and compared with one-way analysis of variance (ANOVA). Further analyses were performed using Tukey's honestly significant difference test as needed. Statistical significance was defined as $p \leq 0.05$.

Results

There were 47 women with primary dysmenorrhea in the study. Mean and standard deviation of age was 20±2.1. BMI of participants was 22.4 ±1.9 (Mean±SD). They were categorized according to their VAS scores, serum IMA and MDA levels were evaluated between groups. To avoid effect of the variability on concentration of albumin within patients in the group, IMA results expressed as AUs were adjusted according to their serum albumin concentrations -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 difference between the averages of groups with Oneway 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 Oneway-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.

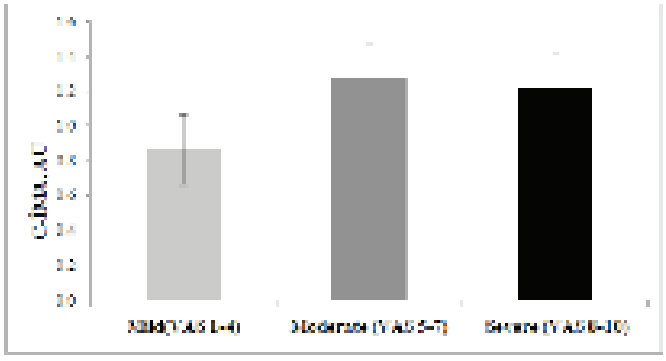

Figure 1. IMA concentrations of PD patients according to severity of their symptomatology.

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.[7,9,23] Previously, Dikensoy et al. showed that serum malondialdehyde as a marker of lipid peroxidation were significantly higher in subjects with dysmenorrhea compared to control.[9] 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.

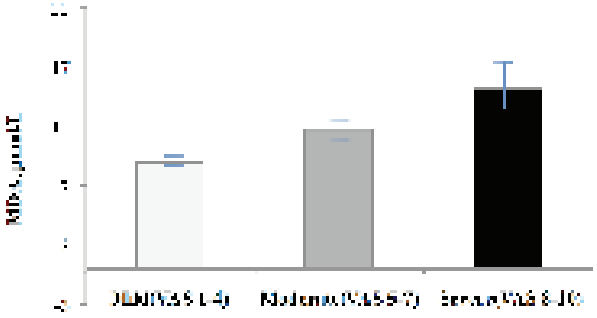

Figure 2: MDA levels of PD patients according to severity of their symptomatology.

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.[22,24-28] 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.2[9] 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 ethiopathogenesis 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.

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