Investigation of oxidative balance in patients with dysmenorrhea by multiple serum markers

Dismenoresi olan hastalarda çeşitli serum belirleyicileri ile oksidatif dengenin araştırılması

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Abstract

Objective: To investigate the level of oxidative stress in patients with dysmenorrhea by multiple serum markers including malondialdehyde (MDA), nitrotyrosine (3-NT), deoxyguanosine (8-OHdG) and superoxide dismutase (SOD).

Material and Methods: Fifty-eight women, aged between 20 and 34, who had had regular menses for at least six previous cycles, were involved. The women were divided into two groups. The study group consisted of 33 patients with primary dysmenorrhea, and the control group consisted of 25 healthy women.

Results: Demographic characteristics of patients were similar between the two groups. The serum MDA levels were 1.32±0.46 and 0.91±0.26 nmol/mL for the dysmenorrhea and control groups, respectively ($p<0.001$). The differences in plasma levels of 3-NT, SOD and serum 8-OhdG were similar in both groups ($p>0.05$). Also, no correlation was found between the severity of dysmenorrhea and the levels of oxidative markers.

Conclusion: Oxidative stress is slightly aggravated in patients with dysmenorrhea. (J Turkish-German Gynecol Assoc 2012; 13: 233-6)

Key words: Primary dysmenorrhea, malondialdehyde, nitrotyrosine, deoxyguanosine, superoxide dismutase

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Özet

Amaç: Dismenoresi olan hastalarda malondialdehit (MDA), nitrotirozin (3-NT), deoksigunaozin (8-OhdG) ve süperoksit dismutaz (SOD) gibi belirleyiciler ile oksidatif stres düzeyinin araştırılması.

Gereç ve Yöntemler: Yaşları 20 ile 34 arasında değişen, en az 6 aydır düzenli adet gören 58 kadın çalışmaya dâhil edildi. İki gruba ayrıldılar. Primer dismenoreli 33 hasta çalışma grubunu, sağlıklı 25 hasta ise kontrol grubunu oluşturdu.

Bulgular: Hastaların demografik karakteristik özellikleri her iki grupta da birbirine benzerdi. MDA düzeyleri, dismenore ve kontrol grupları için sırasıyla; 1.32±0.46 ve 0.91±0.26 nmol/mL idi ($p<0.001$). 3-NT, SOD plazma ve 8-OhdG serum düzeyleri her iki grupta da biribirine benzerdi ($p>0.05$). Dismenorenin şiddeti ile oksidatif belirleyicilerin düzeyleri arasında herhangi bir bağlantı bulunamadı.

Sonuçlar: Oksidant/Antioksidant dengesi, dismenoreli hastalarda hafifçe reaktif oksijen radikalleri lehine değişikliğe uğramıştır.

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Anahtar kelimeler: Primer dismenore, malondialdehit, nitrotirozin, deoksiguanozin, süperoksit dismutaz

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Introduction

Primary dysmenorrhoea is defined as pelvic pain around the time of menstruation in the absence of an identifiable pathologic lesion (1). Dysmenorrhoea occurs due to myometrial contraction induced by prostaglandins originating from the secretory endometrium. The secretory endometrium contains sunstantial stores of arachidonic acid, which is converted to prostaglandin F2α (PGF2α), prostaglandin E2 (PGE2), and leukotrienes during menses. Other symptoms associated with dysmenorrhoea including headache, nausea and vomiting, backache and diarrhea are related to the elevation of circulatory prostaglandins (PGF2α, PGE2) and their metabolites. The posterior pituitary peptides, vasopressin and oxytocin, have also been implicated in the aetiology.

In a study, both vasopressin and PGF2 alpha are higher and markedly fluctuating vasopressin levels were found in the women with dysmenorrhea (2, 3). Furthermore the differences in oxytocin, vasopressin, FSH and 17beta-E2 concentrations found in women with dysmenorrhea plasma suggest an involvement of these hormones in mechanisms of primary dysmenorrhea (4).

Most of the release of prostaglandins during menstruation occurs within the first 48 hrs, which coincides with the greatest intensity of the symptoms (5). It is well known that the presence of elevated concentrations of free radicals and/

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or lowered antioxidant potential leads to oxidative stress (OS). Recently, oxidative stress has been implicated in more than 100 diseases (6, 7). Dysmenorrhea also has been reported to lead to an increase in lipid peroxidation, an index of oxidative stress (8). Previous studies about OS and dysmenorrhea investigated only malondialdehyde (MDA) levels as a marker of oxidative status (9, 10). However, measuring MDA levels is limited in detecting oxidative stress in some cases, and might not be sufficient to show real oxidative stress in dysmenorrhoea (11, 12). Reactive oxygen species (ROS) produced either endogenously or exogenously can attack lipid, protein and nucleic acid simultaneously in living cells (13). In order to understand the extent of oxidative stress in dysmenorrheal women, in addition to MDA we examined nitrotyrosine (3-NT), which is a marker of the oxidation chain of protein and deoxyguanosine (8 OHdG) level, which is also a sensitive marker of oxidative DNA damage in cells (14, 15). To our knowledge, there are no other studies in the literature investigating the protein and DNA oxidation pathway in women with dysmenorrhea. The development of oxidative stress in dysmenorrhea may be related to its severity. Furthermore, redox levels may modulate the severity and the dynamics of dysmenorrhea. To the best of our knowledge there are no data revealing the relation of redox levels and severity of dysmenorrhea. In the present study, we evaluated oxidative stress and relation between redox levels and severity of dysmenorrhea.

Material and Methods

Fifty-eight eligible women were enrolled in this study, between June 2007 and July 2008 in the Department of Obstetrics and Gynecology, Fatih University. The study was approved by the Institutional Review Board of Fatih University and written informed consents were obtained from each of the women before the start of the study. The inclusion criteria for women were as follows: Women with a history of primary dysmenorrhoea, nulliparous, aged between 21 and 32, body mass index (BMI) < 23kg/m^2, who use an acceptable method of barrier contraception, but who do not use an intrauterine contraceptive device or an oral contraceptive. Patients with pelvic pathology or a history of alcohol and tobacco use were excluded. Care was taken to make sure that each participant had not taken any analgesic within 24 hrs prior to the study and to be in their most painful phase of menstrual cycle.

The following criteria are used to define dysmenorrhoea:

- Onset of pain within 6-12 hrs after menstruation.
- Lower abdominal or pelvic pain associated with onset of menses and lasting 8-72 hrs.
- Lower back pain during menses.
- Medial or anterior thigh pain.
- Menstrual pain with associated features such as headache, diarrhea, nausea and vomiting (1).

In addition, the severity of dysmenorrhea in all patients was scored on a 5-point scale ranging from 0 to 4 (i.e. 0=no pain, 1=mild pain requiring no medication, 2=moderate pain responding to mild pain relievers, 3=severe pain necessitating potent pain relievers, and 4=incapacitating pain unresponsive to potent pain relievers (16).

Patients were allocated into two groups. The study group consisted of 33 patients (Group 1) with primary dysmenorrhea, and the control group (Group 2) consisted of 25 healthy women with matching demographics. At the first screening visit, women had a complete history taken and pelvic examination to rule out uterine irregularity, cul de sac tenderness, or nodularity, which may suggest endometriosis, pelvic inflammatory disease, or a pelvic mass. Pelvic ultrasonography was also performed to evaluate the presence of leiomyomata or ovarian cysts consistent with endometriosis. All patients had a pregnancy test, a Pap smear, and whole blood cell counts and blood biochemical parameters including glucose, liver and kidney functions. Whole blood samples (~5 mL) were drawn from a peripheral vein in the morning hrs (8:00-10:00) after an overnight fast. Serum samples for clotting were kept in flat tubes with gel at room temperature for 30 minutes, then they were centrifuged at 2700 g for 10 minutes. After centrifuging the blood samples at 1500 g at +4°C for 20min, plasma samples were kept at -80°C until use.in K3 EDTA tube.

Measurement of plasma malondialdehyde levels

MDA level of the plasma was measured by the TBA method (17). The resulting pink stained TBA was determined in a spectrophotometer at 532 nm. The calibration curve was performed using 1,1,3,3-tetramethoxypropane subjected to the same treatment as that of the samples. Intra- and inter-assay coefficients for TBA assay were 4.5% n=8 and 4.7% n=10 respectively. Results were expressed as nanomoles per milliliter (nmol/mL).

Measurement of plasma level of 3- Nitrotyrosine

Plasma Nitrotyrosine levels were measured using an ELISA kit according to the manufacturer's protocol (Hycult Biotechnology Elisa Kit, Holland). Measurable concentration range of Hycult Biotechnology Elisa Kit 2-1.500 nM. Intra-assay coefficients of variation were 6.1%.

Measurement of serum level of 8-OH Deoxyguanosine

Serum values of 8-OH Deoxyguanosine were determined with test kits by the enzyme linked immunabsorbant assay (ELISA) method (Assay Designs DNA Damage ELISA Kit 8- hydroxy-2'-deoxyguanosine, USA) The sensitivity of Assay Designs's DNA Damage ELISA kit were determined to 0.59 ng/mL. Inter and intra-assay coefficients of variation were 4.1% and 5.2% respectively.

Measurement of plasma level of Superoxide dismutase (SOD)

SOD activity was determined according to the method of Sun et al. (18). The principle of the method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthinexanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate after 1.0 mL ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount of enzyme causing 50% inhibition of the NBT reduction rate. SOD activity was expressed as units per milliliter (U/mL).

Statistical Analysis

Data analysis was performed by using Statistical Package for Social Sciences (SPSS) version 11.5 software (SPSS Inc.,

Chicago, IL, United States). Shapiro-Wilk test was used to test the normality of distribution for continuous variables. Data were expressed as mean±standard deviation or median (minimum-maximum), where applicable. Nominal data were shown as the number of case and (%). While the mean age, height, weight, 8-OhdG and MDA were compared by Student's t test, the Mann Whitney U test was applied for the evaluation of menarche age, nitrotyrosine and SOD levels. A p value less than 0.05 was considered statistically significant.

Results

The demographic data for both groups are summarized in Table 1. In both groups, there were no significant differences in terms of body mass index, age, age at menarche. The mean plasma levels of SOD (14.98±2.08, 14.92±1.49 U/mL; P=0.48) were similar in both groups (P>0.05). The mean serum levels of corrected [8-OhdG] (25.93±3.89, 27.85±3.24 ng/mL, respectively; P=0.05) and nitrotyrosine (97.94±41.16, 85.04±18.8 nM/mL, respectively; P=0.48) were similar between the study and control groups (P>0.05). However, mean plasma levels of MDA were significantly higher in the study group compared to the control groups (1.32±0.46; 0.91±0.26, P<0.001, respectively) (Table 2). The dysmenorrhea group was divided into Grade II (moderate) group and Grade III (severe) group according to MDS classification. Biochemical findings were compared between the groups of Grade II and Grade III. MDA level in Grade II group was 1.12±0.38 nmol/mL whereas in Grade III group was 1.42±0.47 nmol/mL (p>0.05). 8-OHdG level in Grade II dysmenorrheal group was 25.19±2.99 ng/mL and was 26.30±4.28 ng/mL in Grade III. Nitrotyrosine was demonstrated as (81.4 (51.6- 240.8 nM/mL), SOD was (14.5 (12.9-16.8 U/mL) in Grade II and Nitrotyrosine was 82.4 (49.6–166.7 nM/mL), SOD was 14.7 (10.6-23.6 nM/mL) in Grade III, respectively. There were no significant differences between these findings either (Table 3).

Discussion

Plasma levels of MDA were higher in the subjects with primary dysmenorrhea compared to those in the control group. Currently, it is reported that reactive oxygen species (ROS) have been implicated in the pathogenesis of a variety of injury models. It is possible that dysmenorrhea is one of these conditions (8). Several studies investigated the role of free radicals in dysmenorrhea. However, no researches were able to clarify the balance of ROS and antioxidant systems in dysmenorrhea. Previously, Yeh et al. (10) showed that plasma MDA and interleukin-6 levels were significantly higher in subjects with dysmenorrhea compared to those in healthy subjects. Similarly, plasma levels of MDA in the subjects with primary dysmenorrhea were found to be higher compared to those in the control group in the present study. Dikensoy et al. (9) also reported that the plasma levels of MDA increased in subjects with primary dysmenorrhea. In their study, in addition to plasma MDA levels, serum nitric oxide (NO) and adrenomedullin (AM) levels also increased. However, no acceptable (limited number of study investigated antioxidant markers) antioxidant markers were studied in any of the above studies. In study of Dikensoy et al. (9) they used only AM, which is not a specific antioxidant; it has multipotent properties, including vasodilator function. Furthermore, they reported that AM levels were increased by compensatory mechanisms in patients with dysmenorrhea. However, oxidative stress occurs when an increase in the amount of reactive oxygen species or depletion in the levels of antioxidants occurs. As SOD levels, which is a specific antioxidant marker, were unchanged between two groups, our study

Table 1. Demographic data of the subjects

	Group I (n=25)	Group II (n=33)	p value
Age (years)	25.0±2.9	24.2±3.1	0.340[a]
BMI (kg/m^2)	22.4±3.5	21.9±2.7	0.547[a]
Age at menarche (year)	12.7±1.0	12.3±0.7	0.096[b]
Pain (n%)			-
Moderate	-	11(33.3%)	
Severe	-	22 (66.7%)	

BMI: Body mass index, Values expressed as mean±SD, [a]Student's t test, [b]Mann Whitney U test

Table 2. Malondialdehyde, Corrected [8-OhdG], Nitrotyrosine and SOD levels of Study and Control groups

	Group I (n=25)	Group II (n=33)	p value
Corrected [8-OhdG] ng/mL	27.8±3.25	25.9±3.89	0.051[a]
Nitrotyrosine nM/mL	81.4 (53.6-117.1)	81.4 (49.6-240.8)	0.489[b]
SOD U/mL	14.7 (14.0-21.8)	14.7 (10.6-23.6)	0.956[b]
MDA nmol/mL	0.9±0.26	1.3±0.46	<0.001[a]

SOD: Superoxide Dismutase, MDA: Malondialdehyde, Values expressed as mean±SD, [a]Student's t test, [b]Mann Whitney U test

Table 3. Biochemical findings of Grade II and Grade III in patients with dysmenorrhea

	Moderate (n=11)	Severe (n=22)	p
Corrected [8-OhdG] ng/mL	25.2±2.99	26.3±4.29	0.447[a]
Nitrotyrosine nM/mL	81.4 (51.6-240.8)	82.4 (49.6-166.7)	0.778[b]
SOD U/mL	14.5 (12.9-16.8)	14.7 (10.6-23.6)	0.440[b]
MDA nmol/mL	1.1±0.38	1.4±0.47	0.077[a]

SOD: Superoxide Dismutase, MDA: Malondialdehyde, Values expressed as mean±SD, [a]Student's t test, [b]Mann Whitney U test

supported the opinion that increased oxidative stress in dysmenorrhea depended only on excess of free oxygen radicals. Additionally, in the present study, we did not detect any assocation between the severity of dysmenorrhea and these markers. Dysmenorrhea is caused by frequent and prolonged PG-induced uterine contractions that decrease blood flow to the myometrium, resulting in ischemia. Substantial evidence suggests that hypoxia-ischemia activates phospholipase A2, a lipolytic enzyme that hydrolyses the acylglycerolipids and generates free fatty acids, especially arachidonic acid. Hence, arachidonic acid accumulates during the hypoxic-ischemic period. Upon perfusion, when oxygen is available, arachidonic acid is metabolized mainly by three different groups of enzymes-cyclooxygenase, lipooxygenase, and cytochrome P450-resulting in eicosanoid formation and the generation of activated oxygen species (19). Studies on oxidative stress and dysmenorrhea measured only MDA levels as an oxidative stress marker. However, it is well known that oxidative stress may disturb the destruction of all major classes of biological macromolecules, including nucleic acids, proteins, carbohydrates, and lipids (20). Hence, to make any conclusion about the level of oxidative stress in patients with dysmenorrhea, all of these oxidative stress indicators should be measured. In the present study, in addition to the MDA level, we also investigated plasma levels of 3-NT, which are markers of oxidative damage of protein and 8 OhdG, which also shows the oxidation of DNA. To our knowledge, there are no other studies in the literature investigating the detail of oxidation including protein and DNA oxidation in dysmenorrhea. In addition, no study has investigated antioxidant status by real antioxidant markers in patients with dysmenorrhea.

In conclusion, according to our results, as we only detected lipid peroxidation while protein and DNA oxidation is normal, we can suggest that increased oxidative stress may ocur in dysmenorrhea, but this oxidative stress is not really prevalent in dysmenorrhea. Before any other suggestions, further clinical researches with larger numbers of patients are required to clarify the relation of oxidant/antioxidant balance in dysmenorrhea.

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

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