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Original Article

Determination of thiol-disulphide homeostasis in premenstrual syndrome during adolescence

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ABSTRACT

Background. Premenstrual syndrome (PMS) characterized by cyclic symptoms during the luteal phase of the menstrual cycle, presents an uncertain etiology in adolescents involving hormonal fluctuations and serotonin-related neurotransmitters with a limited existing literature on the impact of oxidative stress. This study aimed to explore the potential association between PMS and oxidative stress in adolescents.

Methods. In a cross-sectional study conducted at a university hospital, involving 45 adolescent girls aged 12 to 18, participants were categorized based on the presence or absence of PMS using the cut-off point of 110 on the PMS Scale developed by Gençdoğan. Oxidative stress was assessed through dynamic thiol-disulfide homeostasis. The shift from the balance towards disulfide form is associated with oxidative stress, whereas towards thiol it shows a greater antioxidant capacity.

Results. Thirty out of the forty-five participants were found to have PMS with a mean age of 15.5 years. The PMS group demonstrated a significant increase in antioxidant markers, specifically elevated native (631.6±57.55 vs 598.2±41.08, p=0.048) and total thiol levels (675.15±3.4 vs 639.3±44.9, p=0.031). Despite a significant increase in thiol, thiol to disulfide ratio was not found to be significant (p=0.849).

Conclusion. Contradictory to other studies in adults, we have demonstrated an increase in the antioxidant markers in adolescents with PMS. Elevated antioxidant status in adolescents with PMS may be an adaptive response to acute cyclic inflammation in the adolescent period, which might decrease with the progression of age. Further research is needed to investigate the complex interaction between oxidative stress and PMS across different age groups.

Key words: premenstrual syndrome, thiol-disulphide homeostasis, adolescence, oxidative stress.

Premenstrual syndrome (PMS) is a complex and prevalent condition characterized by cyclic physical, behavioral, and psychological symptoms during the luteal phase of the menstrual cycle, typically resolving with menstruation onset. PMS induces various discomforts in the days preceding menstruation, including breast tenderness, headaches, joint pain, mood changes (irritability or mood swings), and behavioral alterations in sleep patterns or appetite.[1]

PMS etiology remains unknown; however, some studies suggest that the hormonal fluctuations or sensitivity to these changes during the menstrual cycle play a significant role in the manifestation of PMS symptoms. Symptoms have also been shown to be associated with serotonin and other related neurotransmitters. Studies have also demonstrated associations between PMS and lifestyle factors, including eating and drinking habits.[2] Additionally, there

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is growing evidence indicating the involvement of inflammatory processes in PMS.[3]

Inflammation is a vital immune response, but when it becomes dysregulated, it can contribute to the development and aggravation of various health issues. It is suggested that progesterone and estrogens exhibit anti-inflammatory effects, however, excessive doses may have a pro-oxidative impact and lead to oxidative stress. Although individuals with PMS have hormone levels similar to those of healthy women, they may be more sensitive to hormonal changes during the luteal phase. Additionally, estrogen conversion products, such as catechol estrogens, can generate oxygen radicals. Inflammatory molecules in PMS may trigger the release of pain-associated prostaglandins and influence neurotransmitters, potentially worsening premenstrual symptoms and contributing to mood disturbances.[4]

Thiols are organic compounds containing a sulfhydryl group (-SH) that can be oxidized to form disulfides. A disulfide is a chemical bond formed between two sulfur atoms of the sulfhydryl groups from two thiol molecules. This thiol-disulfide exchange is crucial in protein structure and function, acting as a switch to regulate protein activity and maintaining cellular redox homeostasis. The comparative analysis of thiol and disulfide levels is a biochemical indicator that can be utilized to assess the cellular oxidative stress status and overall antioxidant defense mechanisms. The abundance of thiols in cells may indicate the active nature of these defense mechanisms and their ability to provide protection against potential oxidative damage. If thiol levels are higher than disulfide levels, it generally indicates that cells are in a reduced state rather than experiencing oxidative stress. In other words, the redox balance in cells leans towards a more reduced (reductive) state, suggesting a high antioxidant capacity and a lower risk of oxidative damage. On the contrary, if disulfide levels are higher than thiols, this situation indicates that cells are exposed to oxidative stress and are in a more oxidized state. Oxidative stress can lead to damage in cells and tissues and may play a role in the development of chronic diseases such as PMS.[4,5]

The association between PMS and different parameters related to inflammation, oxidative stress, and antioxidative stress has previously been studied. Limited evidence indicates that women experiencing PMS may exhibit higher levels of inflammatory parameters, lower antioxidant status, and higher oxidation levels compared to those without PMS although results are inconsistent. These findings may suggest a potential link between oxidative stress, inflammation, and the development of PMS.[3,6] However, the majority of these studies have predominantly focused on adult women, there was high heterogeneity in the study designs, and the diagnosis of PMS was not always made with a validated method.[3]

Focusing on the adolescent age group can provide a unique framework for investigating the relationship between PMS and oxidation in this period of life.[7,8] Therefore, this study aimed to explore the potential association between PMS and oxidative stress in adolescent girls.

Materials and Methods

Study participants

The participants included in this cross-sectional study were healthy females with regular menstrual cycles, aged between 12-18 years, who presented to the adolescent medicine clinic at Hacettepe University, İhsan Doğramacı Children's Hospital between January 2022 and October 2022 for a well-child visit. Regular menstrual cycles were defined as lasting between 21 and 45 days, a range chosen based on established criteria in the literature for defining menstrual regularity among adolescent females.[9] As PMS is triggered by ovulation, we only included participants with regular menstrual cycles to ensure that they were ovulating. Adolescents with a history of physical or mental illnesses and/or using any medication were excluded from the study.

Written informed consent was acquired from the parents and written assent was obtained from all adolescents. Study approval (GO 21/67) was obtained from the Hacettepe University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee.

Heights of the adolescents were measured by a Harpenden stadiometer, and body weights were measured by a digital scale. Body mass index (BMI, kg/m^2) was then calculated by dividing the body weight (kg) with the square of the height (m). Age of menarche was noted.

Evaluation of PMS

The diagnosis of PMS was determined using the Premenstrual Syndrome Scale developed by Gençdoğan in 2006.[10] It is a 44-item, five-point (none, very little, sometimes, often, always) Likert-type questionnaire with nine subgroups. A score of "one point" was assigned to the "none" option, and a score of "five points" was assigned to the "always" option. The minimum and maximum total scores that could be obtained from each subscale were as follows: 7-35 for depressive mood, 7-35 for anxiety, 6-30 for fatigue, 5-25 for irritability, 7-35 for depressive thoughts, 3-15 for pain, 3-15 for appetite changes, 3-15 for sleep changes, and 3-15 for bloating. The lowest possible score that could be obtained from the entire scale is 44, and the highest score is 220. An increase in scores indicates the intensity of PMS. The presence of PMS is assessed based on whether the total and subscale scores exceed 50% of the maximum possible score. The reliability of the scale, assessed by Cronbach's Alpha, is 0.75. Participants who scored 110 or higher on the scale were assigned to the PMS group, while those scoring lower than 110 were assigned to the control group.

Assessment of oxidative stress parameters

In this study, blood samples were collected for the determination of thiol and disulfide values and prepared for laboratory analyses. We utilized thiol and disulfide levels as biomarkers to estimate oxidative stress in this study. To assess thiol-disulfide hemostasis parameters, a blood sample was collected from subjects into plain tubes following an eight-hour fasting period. Following rapid centrifugation of blood samples at a speed of 1500 rpm for a duration of 10 minutes, the plasma and serum components were separated. The serum samples were subsequently stored at a temperature of -80 °C. Blood samples were collectively sent to the Medical Biochemistry Laboratory of Ankara Bilkent City Hospital's for the analysis of oxidative stress parameters. The whole research cohort was examined collectively during a single session. The study investigated the balance of serum thiol-disulfide levels using a completely automated analytical technique established by Erel and Neselioglu.[11] The dynamic disulfide level was calculated by dividing the difference between total thiol and native thiol into two. Following the process of dynamic disulfide, we measured the levels of native and total thiol. We then computed the ratios of disulfide to native thiol, disulfide to total thiol, and native thiol to total thiol. Measurements were analyzed using the spectrophotometric method developed by Erel. According to the Erel method[11], the levels of plasma disulfide were measured to be 17.29±5.32 μmol/L, native thiol levels were 397±62 μmol/L, and the ratio of disulfide to native thiol percentage was 4.32±1.49 in healthy subjects. Blood was not drawn at a particular phase of the menstrual cycle.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 21. The distribution of data was assessed using the Kolmogorov-Smirnov test. For the comparison of independent variables showing a normal distribution, independent t- test was used, while the Mann-Whitney U test was employed for data that did not exhibit a normal distribution. Qualitative data were evaluated using the chi-square test and Fisher's exact test. Pearson or Spearman correlation tests were conducted as applicable. A p value <0.05 was accepted as statistically significant.

Results

A total of 53 patients were considered eligible for this study. However, three patients were excluded after being diagnosed with other illnesses during the study, three others declined to provide blood samples, and two patients were excluded due to incomplete PMS scale responses. Our research examined a cohort of 45 adolescent girls with a mean age of 15.4±1.4 years. While 30 adolescents (66.6%) reported a PMS score over 110 with a mean score of 150.6±27.5, 15 (33.4%) reported a PMS score lower than 110 with a mean score of 87.2±21.8 (P<0.001). The clinical parameters of the PMS (+) and PMS (-) groups are presented in Table I, and a comparative analysis of the anthropometric and clinical data between the two groups did not yield any statistically significant differences.

The comparison of native thiol, total thiol and disulfide values, along with the comparison of these values are presented in Table II. The results indicated a significant elevation in native (p=0.048) and total thiol (p=0.031) levels in the PMS group. We did not find a significant difference in disulfide levels between the two groups.

The Pearson correlation analysis was utilized because the variables demonstrated a normal distribution. The analysis revealed no strong linear correlations, whether positive or negative, between PMS scores and thiol, disulfide, or thiol-disulfide ratios. The associations observed between PMS scores and these biochemical parameters were generally weak. Although low positive correlations were identified between PMS scores and native thiol, total thiol, and various disulfide ratios, none of these reached statistical significance (p > 0.05). Notably, the correlation between disulfide and PMS score had a relatively higher coefficient (r = 0.262) compared to other variables, but this too did not achieve statistical significance (p = 0.093). Overall, the findings do not indicate a strong relationship between PMS scores and the biochemical measurements evaluated. The correlation analysis results are presented in Table III.

Table I. The clinical parameters of the adolescents with and without PMS.

Clinical variables	PMS(+) (n=30)	PMS(-) (n=15)	P value
Age, mean±SD (years)	15.5±1.3	15.3±1.5	0.544
Body weight, mean±SD (kg)	61.7±11.3	67.7±20.7.5	0.232
Height, mean±SD (cm)	160.2±4.6	162.6±6.3	0.168
Body mass index, mean±SD (kg/m^2)	24.1±4.6	25.3±6.5	0.476
Menarchial age, mean±SD (years)	11.8±1.3	11.5±1.2	0.594
PMS score, mean±SD	150.6±27.5	87.2±21.8	<0.001

PMS: premenstrual syndrome

Table II. Comparison of oxidative stress in adolescents with and without PMS.

Laboratory variables	PMS (+) (n=30)	PMS (-) (n=15)	P value
Native thiol (μmol/L)	631.6±57.55	598.2±41.08	**0.048**
Total thiol (μmol/L)	675.1±53.4	639.3±44.9	**0.031**
Disulfide (μmol/L)	21.7±5.5	20.5±5.7	0.503
Disulfide / native thiol	3.5±1.0	3.4±0.9	0.839
Disulfide / total thiol	3.3±0.9	3.2±0.8	0.847
Native thiol / total thiol	93.5±1.7	93.6±1.6	0.849

PMS: premenstrual syndrome

Table III. Thiol-disulfide levels and PMS correlation.

Variables	Correlation Coefficient (r)	P value
PMS score vs. native thiol	0.155	0.326
PMS score vs. total thiol	0.213	0.176
PMS score vs. disulfide	0.262	0.093
PMS score vs. (disulfide / native thiol) * 100	0.199	0.207
PMS score vs. (disulfide / total thiol) * 100	0.197	0.210
PMS score vs. (native thiol / total thiol) * 100	-0.197	0.210

PMS: premenstrual syndrome. Pearson correlation analysis was used to evaluate the relationships between PMS scores and biochemical parameters.

Discussion

In our study, we demonstrated that the PMS group displayed a statistically significant increase in both native and total thiol levels. The difference was not statistically significant for disulfide levels. These results suggest that adolescents with PMS have higher antioxidant status compared to the control group.

Oxidation and inflammation are closely interrelated biological processes. Oxidative stress is a condition where there is an imbalance between oxidants such as free radicals and reactive oxygen species and the body's antioxidant defense system. These reactive species can cause oxidation of lipids, proteins, and DNA, leading to cellular damage and inflammation. Numerous studies have been conducted regarding the relationship between various gynecological issues and oxidative stress.[12] Studies focusing on the relationship between PMS and oxidative stress, utilized a broad range of different biomarkers directly indicating inflammation or oxidative stress.[3]

Among inflammatory markers, interleukins, tumor necrosis factor α, interferon γ, high-sensitivity C-reactive protein, granulocyte-macrophage colony-stimulating factor, and anti-heat shock protein 27 have been evaluated. While some studies have reported statistically significant results regarding increased inflamation, there are also other studies that have not found a significant association.[13,14] Oxidative stress markers, including total oxidative stress indicators, lipid peroxidation levels, protein oxidation products, and other related parameters, have been studied in relation to PMS. In the research conducted by Incebiyik et al., total oxidative stress was assessed using total oxidant status and the oxidative stress index. However, no statistically significant differences were observed between the PMS group and the control group.[15] Non-enzymatic antioxidant parameters and total antioxidant capacity (TAC) have been investigated between PMS and control groups. In one study, a statistically significantly lower TAC was found in women with PMS compared to the control group, but there are also studies that did not find such a difference.[16,17] These findings are inconsistent and do not directly indicate a higher inflammatory and oxidant status in PMS cases, suggesting that the relationship between PMS and oxidative stress related inflammation is not firmly established.[3]

Thiols and disulfides are used as antioxidant markers in many various studies, and to the best of our knowledge, there is just one study that evaluated these markers in PMS, and it included adult women.[16] In this study, oxidant status was evaluated using lipid hydroperoxide (LHP), malondialdehyde (MDA), and protein carbonyl (PC), while antioxidant status was evaluated using total thiol and TAC. Biomarkers were examined on the follicular (3rd day) and luteal (21st day) phases of the menstrual cycle, and no statistically significant differences were found between the study and control groups during the follicular phase. During the luteal phase, although no significant differences were observed between the groups in terms of MDA, PC, and total thiol levels, it was noted that LHP

levels increased in the study group compared to the control group, and TAC levels decreased.[16] In contrast to this study, we have demonstrated a significant elevation of antioxidant levels in the PMS group.

Our findings lack alignment with the limited information in the literature showing either an increase or no change in oxidant levels and a decrease or no change in antioxidant status, which can be explained in several ways. Population differences should be considered in the obtained results. Adolescence, characterized by distinct and unique features from adulthood, accompanies significant biological, psychological, and social changes, indicating that the etiological causes of PMS might differ during the adolescent period.[18-20] While prolonged and severe stress conditions often lead to a decrease in thiol levels, in certain cases, cells may elevate thiol levels to cope with this stress, possibly by activating defense mechanisms or regulating signals.[3,18] During adolescence, the rise in antioxidant levels might suggest the presence of acute onset inflammation during the premenstrual phase, resulting in a reactive and activated response of the antioxidant system rather than chronic, prolonged stress observed in adults. Similarly, the oxidation and antioxidation processes change with aging. As individuals age, the antioxidant defense mechanisms in the body generally weaken, and coping with damage caused by free radicals may become less effective. Additionally, as metabolism slows down with age, the repair processes of cells can change, making the body more vulnerable to oxidative damage. Enzymes functioning as antioxidants may decrease in production or become less effective.[19,21] All these factors can explain the age-related oxidative stress parameter differences between adolescents and adults with PMS.

Our study has several limitations. The small sample size and blood samples obtained irrespective of the menstrual cycle phase are the major limitations. Considering the complex hormonal fluctuations in the menstrual cycle, it can be assumed that oxidation levels may vary during different menstrual phases, particularly the premenstrual period. Another limitation is the lack of oxidant status measurements. Future studies examining both oxidant and antioxidant biomarkers at follicular and luteal phases of the cycle might provide additional data. Another limitation is the lack of nutritional habits, exercise levels, and healthy lifestyle preferences of adolescents, which may have both affected the PMS symptomatology and oxidative stress status.[22,23] Lifestyle factors such as a balanced and healthy diet, regular exercise, limiting tobacco and alcohol consumption, and stress management can support the body's capacity to cope with oxidative stress and help reduce oxidative damage.[24] Factors such as family support, education, and cultural influences may also play important roles in shaping adolescents' experiences with PMS.

In conclusion, there is uncertainty regarding the role of increased oxidative stress in PMS, and data specific to the adolescent period is very limited. Our study demonstrating elevated antioxidant status in adolescents with PMS might occur in response to acute cyclic inflammation during the premenstrual phase and act as an adaptive mechanism in reaction to increased oxidative stress. As age progresses the antioxidant capacity might decrease resulting in some studies demonstrating heightened oxidative stress and decreased antioxidant status in adults with PMS. Future research with more robust methodologies, including longitudinal studies with larger and more diverse age populations, is needed to better understand the complex interaction between oxidative stress and PMS.

Ethical approval

The study was approved by Hacettepe University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (date: 19.01.2021, number: 2021/02-47).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SA, DA; data collection: DA, LJ, ÖE; analysis and interpretation of results: MPK, LJ, ÖE; draft manuscript preparation: LJ. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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