Total Oxidant/Antioxidant and Paraoxonase-1 Levels in Premenstrual Dysphoric Disorder: A Follow-Up Study

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ABSTRACT:
Total oxidant/antioxidant and paraoxonase-1 levels in premenstrual dysphoric disorder: a follow-up study

Objective: Premenstrual dysphoric disorder (PMDD) is a severe form of premenstrual syndrome (PMS) that was categorized as a mood disorder in the most recent version of the Diagnostic and Statistical Manual for Mental Disorders. In addition to a history of PMS, a PMDD diagnosis requires prospective symptom assessment for 2 consecutive months. Although the effects of some oxidants-antioxidants were previously studied in PMS, their possible effects in PMDD remain unknown. Paraoxonase-1 is a new high-density lipoprotein-associated enzyme with many antioxidative effects. We hypothesized that assessing serum total oxidant-antioxidant and paraoxonase-1 (PON-1) levels could clarify the role of oxidant/antioxidant system in PMDD.

Methods: All participants (n=50) were assessed by an experienced psychiatrist for PMDD by using the Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV), Premenstrual Assessment Form and Daily Record of Severity of Problems-Short Form (DRSP) or possible psychiatric disorders including depression, anxiety, and sleep disorders. Serum estrogen, progesterone, total oxidant-antioxidant, and PON-1 levels were measured in the serum of 20 participants with PMDD and 30 asymptomatic controls during the follicular and luteal phases of two consecutive menstrual cycles. Sleep quality, depression, and anxiety symptoms were assessed with the Pittsburg Sleep Quality Index (PSQI), Hamilton Depression Rating Scale (HDRS), and Hamilton Anxiety Rating Scale (HARS), respectively.

Results: There were no significant intergroup differences in estrogen, progesterone, oxidant-antioxidant, or PON-1 levels or PSQI scores. However, the mean HDRS and HARS scores were statistically significantly higher for patients with PMDD than for controls. Levels of estrogen, progesterone, and total oxidant-antioxidant were not correlated with HDRS, HARS, or PSQI scores.

Conclusions: Considering the lack of differences in hormonal and biochemical levels between the two groups, it may be more efficient and discriminative to longitudinally assess biochemical and cellular stress-related parameters in subjects with PMDD.

Keywords: estrogen, progesterone, oxidant, antioxidant, paraoxonase-1, premenstrual dysphoric disorder

INTRODUCTION
It is estimated that at least 75% of women experience minor premenstrual symptoms¹, and 20-32% and 3-8% would fulfill the diagnostic criteria for premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD), respectively²,³. PMDD, which was first defined as a
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severe form of PMS in the fourth text revision of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV-TR), was listed as a depressive disorder in the DSM-5, and a diagnosis requires the prospective assessment of two consecutive menstrual periods4-6.

Premenstrual dysphoric disorder (PMDD) is a cyclic pattern of symptoms that begins during the late luteal phase of the menstrual cycle and subsides shortly after the onset of menstruation; it is diagnosed by the existence of at least one affective symptom (depressed mood, anxiety, affective lability, marked anger, or irritability) and five or more total symptoms including affective symptoms4-5. Numerous biological factors have been proposed to influence PMDD pathogenesis, including progesterone deficiency, serotonergic dysfunction, and disturbed endorphin modulation by gonadal steroids and their products7-11. Environmental and nutritional factors including lack of exercise and poor dietary habits have also been blamed for PMDD12. Interestingly, a growing body of evidence suggests that subjects with PMDD have normal ovulatory conditions and do not show evidence of hormonal imbalances13-15.

Oxidative stress is a situation that reflects an imbalance between oxidant production and antioxidant defenses16,17. Measuring the total antioxidant capacity (TAC) requires the simultaneous quantification of several antioxidant markers and is one indicator of oxidative status5. Oxidative stress may lead to premature cell death and is associated with many ischemic and inflammatory diseases, degenerative disorders, and psychiatric disorders such as depression, bipolar disorder, schizophrenia, obsessive compulsive disorder, sleep disorders, and anxiety disorders18-23. In addition, almost all steps of inflammation and cellular immune activation have been linked to depression pathogenesis24-28. Besides depressive symptoms, PMDD may involve increased inflammatory processes because it involves the influx of a large amount of inflammatory cells29. The inflammatory processes that may trigger tissue desquamation at menstruation remain to be clarified, but some studies have shown alterations in inflammation markers in relation to menstrual30 and premenstrual symptom severity31. A recent study described increased lipid hydroperoxide (LHP), which is commonly used to assess oxidative levels, and decreased TAC in subjects with PMS in their luteal phases5. Another group reported that subjects with PMS had total oxidant status (TOS) levels non-significantly higher that and TAC levels similar with those of controls32. In another study, elevated levels of nitric oxide (NO), a well-known antioxidant, were reported in PMS patients at the beginning of the menstrual cycle. Therefore, it was suggested that the increasing NO levels at the follicular phase of the menstrual cycle were involved in the amelioration of PMS symptoms that after menstruation onset33. Paraoxonase-1 (PON-1) is a high-density lipoprotein-associated enzyme with many antioxidative effects. Decreased PON-1 plasma activity was found to be a trait marker for major depression34. However, PON-1 levels have not yet been examined in PMDD.

In this study, we aimed to determine whether there are measurable differences in total oxidant statuses and antioxidant capacity levels in subjects with PMDD. We investigated sleep quality; symptoms of depression and anxiety; and the levels of serum ovarian hormones (estrogen and progesterone), total oxidant-antioxidant, and PON-1 levels in four consecutive blood samples during the follicular and luteal phases of two consecutive menstrual cycles.

MATERIAL AND METHODS

Study Design and Setting

This was a prospective cohort study conducted at our university hospital after the study protocol was approved by the local ethics committee and all participants provided written informed consents. Between September and November 2012, 550 participants were randomly selected from the population of female medical students at the university. After a clinical evaluation procedure, 71 participants were selected for follow-up during
two consecutive menstrual cycles. At the end of their second menstrual cycle, their records were analyzed, and 20 participants were diagnosed with PMDD. Although 37 participants had no premenstrual symptoms, 2 of them did not attend the study sessions, and 5 of the asymptomatic participants were not interested in completing the following assessments and did not provide a reason. Ultimately, 30 participants who had no premenstrual symptoms were enrolled as the control group. The exclusion and inclusion criteria, assessment steps, and participant recruitment are summarized in Figure 1.

**Procedure and Participants**

During the follow-up period, an experienced psychiatrist assessed all participants (n=50) for PMDD based on the Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV) and Daily Record of Severity of Problems-Short Form (DRSP) or possible psychiatric disorders including depression, anxiety, and sleep disorders. The participants underwent a medical examination and blood sampling. Serum TOS, TAC, estrogen, progesterone, and PON-1 levels were assessed during the luteal (days 21-25) and follicular (days 6-8) phases of 2 consecutive menstrual cycles in all 50 subjects. Ovulation was determined by evaluating plasma progesterone levels on day 21 (the subject was considered to be ovulating when plasma progesterone levels were \(\geq 10.0 \text{ ng/mL}\)). Sleep quality and depressive and anxiety-related symptoms were assessed at the first and second luteal phases using the instruments listed below.
Instruments

**Sociodemographic data form:** Each participant completed a data form that asked about dysmenorrhea, nutritional habits, smoking habits, exercise levels, and caffeine consumption.

**Daily Record of Severity of Problems (DRSP):** This tool was developed by Endicott et al. to aid the diagnosis and evaluation of DSM-IV PMS. To enhance the assessment of specific DSM-IV PMDD criteria, the 11 physical and psychological symptoms from criterion A were described using 11 separate items in the short form. An additional three items described specific types of functional impairment caused by the symptoms. The participants were asked to fill out the DRSP every day during two consecutive menstrual cycles. A six-point severity scale was used to score each item “to indicate the degree to which the problems were experienced”. Participants who scored at least 4 in depressive symptoms and irritability items, ≥4 in 5 of the 11 items, and ≥4 in functionality items (items 12-14) for at least 2 days during their luteal phase (days 21-25) for 2 consecutive cycles were diagnosed as having PMDD.

**The Premenstrual Assessment Form (PAF):** This form was developed to categorize reported premenstrual changes. The development of the PAF and its categorical and dimensional scoring systems are described in detail elsewhere. The PAF comprises 95 items, each of which are rated using a 6-point severity scale that focuses on the degree of change from the typical premenstrual state to that during the full menses flow.

**Pittsburg Sleep Quality Index (PSQI):** The PSQI includes 19 items that measure different aspects of sleep quality and sleep disturbances during a 1-month period. A cut-off score of 5 was found to correctly identify 88.5% of patients with sleep disturbances. The sum of scores yields a global score that ranges from 0 to 21, with higher scores indicating poorer sleep quality.

**The Hamilton Depression Rating Scale (HDRS):** The HDRS comprises 17 items selected to measure the severity and variability of depression symptoms. Higher scores indicate more severe depression.

**The Hamilton Anxiety Rating Scale (HARS):** The HARS includes 14 items designed to measure the severity and variability of anxiety symptoms. Higher scores indicate more severe anxiety.

**Protocol**

Four consecutive fasting blood samples were obtained from each participant at two different menstrual periods between 8:00 and 9:00 a.m. by venipuncture and were collected in anticoagulant-free vacuum tubes. The samples were allowed to clot and then centrifuged at 3500 × g for 5 min. Serum aliquots were stored at -80°C and thawed immediately prior to the measurement of biochemical parameters.

**Estrogen and progesterone assessment**

Estrogen and progesterone serum concentrations were determined using commercially available radioimmunoassay kits (RadimSpA®, Pomezia, Italy). Automated TAC and TOS assays were performed using plasma samples from healthy volunteers and patients using commercially available kits (Rel Assay Diagnostics®, Gaziantep, Turkey).

A fully automated assay for PON-1 activity in plasma samples used commercially available kits (Rel Assay Diagnostics®) containing two different sequential reagents.

**Data Analysis**

Descriptive statistics were used to assess sociodemographic data and analyze participant scale scores. The Kolmogorov-Smirnov test was used to test the hypothesis that the distribution was normal. Independent sample t or Mann Whitney U tests were performed to compare the serum total oxidant-antioxidant, estrogen, and
progesterone levels between the control and PMDD groups. Spearman correlation analyses were used to examine relationships between biochemical, hormonal, and other parameters. Analyses for total oxidant-antioxidant, progesterone, and estrogen levels were performed for both menstrual cycles in both the luteal and follicular phases. Two-way repeated-measures analyses of variance (ANOVs) were used to assess the luteal and follicular differences between dependent variables (biochemical parameters) in the factors (groups and menstrual phases).

**RESULTS**

The mean ages and body mass indexes (BMIs) of the PMDD and control groups were 21.8±1.4 and 21.6±1.8 years and 21.7±2.1 and 21.2±2.0 kg/m², respectively. There were no statistically significant differences between groups in terms of age, BMI,

| Table 1: TAC, TOS, and PON-1 levels in patients with PMDD (premenstrual dysphoric disorder) and controls (Student’s t test). |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Biochemical assessment**  | **PMDD** Mean±SD            | **Control** Mean±SD         | **P**                       |
| TOS (Total Oxidant Status, Eq/L) | 36.9±12                     | 35.4±7.2                    | 0.63                       |
| TAC (Total Antioxidant Capacity, µmol H₂O₂/L) | 326.5±85.0                  | 332.2±81.5                  | 0.83                       |
| PON-1 (Paraoxonase-1 levels, U/L) | 2723.3±315.9               | 2808.1±367.7                | 0.44                       |

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<tr>
<th>Table 2: TAC, TOS, PON-1, estrogen, and progesterone levels in patients with PMDD (premenstrual dysphoric disorder) and controls.</th>
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<td><strong>Follicular phase</strong></td>
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<td>TAC (Total antioxidant capacity) (mmol/TroloxEq/L)</td>
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<td><strong>First cycle</strong></td>
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<td><strong>Mean of both cycles</strong></td>
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<td>ΔTotal antioxidant* (median, percentiles 25%-75%)</td>
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<td>TOS (Total oxidant status) (µmol H₂O₂/L)</td>
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<td><strong>First cycle</strong></td>
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<td>ΔTotal oxidant* (median, percentiles 25%-75%)</td>
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<td>PON-1 (Paraoxonase-1 levels) (U/L)</td>
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<td>ΔPON-1* (median, percentiles 25%-75%)</td>
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<td>Estrogen levels (pg/mL)</td>
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<td><strong>First cycle</strong></td>
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<td>ΔEstrogen* (median, percentiles 25%-75%)</td>
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<td>ΔProgesterone* (median, percentiles 25%-75%)</td>
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*Mann Whitney U test, others t test. For Δ values median (percentiles 25%-75%), for others mean±SD.
There were no statistically significant differences in the mean estrogen and progesterone levels in the PMDD and control groups (83.4±28.4 pg/mL vs. 100.6±66.6 pg/mL and 5.0±3.9 vs. 5.8±5.4 pg/mL, respectively). Their nutritional habits, exercise levels, and caffeine consumptions were also similar. Some of the participants diagnosed with PMDD were also diagnosed with minor-moderate depression (4/20), anxiety disorder (2/20), and primary insomnia (1/20). None of the controls were diagnosed with any of these conditions.

Total antioxidant and PON-1 levels did not differ between the PMDD and control groups in the luteal or follicular phases of the two menstrual cycles (Table 1). Total antioxidant levels in both the phases were similar between the PMDD and control group, but total oxidant levels were statistically significantly lower in the PMDD group compared to controls in the second follicular phase (Table 2).

The changes (Δ) in total antioxidant, estrogen, and progesterone levels were similar between the PMDD and control groups in both menstrual cycle phases (Table 2). However, the ΔPON-1 in the PMDD group was statistically significantly higher in the luteal phase compared to the control group. We expected an increase in TOS in the PMDD group, but TOS was statistically significantly lower in the PMDD group in the second follicular phase compared to controls (Table 2). We also evaluated the total antioxidant/total oxidant ratio. No statistically significant difference was found in the luteal, follicular, mean, or Δtotal antioxidant/total oxidant ratio between the PMDD patients and controls in either cycle.

Two-way ANOVAs revealed no statistically significant differences between the groups in terms of TAC, TOS, or PON-1 levels (Figures 2-4). The respective f and p values were 0.601 and 0.616 for TAC, 1.164 and 0.327 for TOS, and 0.238 and 0.849 for PON-1.

The mean HDRS scores were higher for patients with PMDD than for controls in (8.2±4.2 vs 5.3±5.2, p=0.047) and HARS scores were higher for patients with PMDD than for controls (15.0±6.4 vs 11.0±8.7, p=0.046) respectively.
As PMDD is known for symptoms that occur during the luteal phase, we examined the correlation between luteal phase blood markers and clinical symptoms (Only statistically significant results on correlation analysis were given for PMDD group). The total antioxidant capacity was statistically significantly, negatively and strongly correlated with PON-1 levels (r=-0.729, p<0.001). The mean luteal HDRS score was positively and near to statistically significantly correlated with the HARS score (r=0.461, p=0.06). The mean luteal HARS score was negatively and near to statistically significantly correlated with mean PON-1 in the luteal phase (r=-0.474, p=0.06). The HDRS and PSQI scores did not show any statistically significant correlation with oxidant, antioxidant, PON-1, estrogen, or progesterone levels. The mean luteal progesterone level was negatively, moderately and statistically significantly correlated with mean luteal antioxidant level (r=-0.628, p=0.005). The total PAF score that evaluates the premenstrual symptom severity was positively, mildly and statistically significantly correlated with total oxidant levels in second follicular phase (r=0.559, p=0.03) and was positively, mildly and statistically significantly correlated with estrogen levels in both luteal phases (for both r=0.505, p=0.33).

**DISCUSSION**

In this study, although total antioxidant and PON-1 levels did not differ between the PMDD and control groups in the luteal or follicular phases of the two menstrual cycles, total oxidant levels were statistically significantly lower in the PMDD group compared to controls in the second follicular phase. Additionally, ΔPON-1 in the PMDD group was statistically significantly higher in the luteal phase compared to the control group. Also we found opposite patterns for serum TAC, TOS, and PON-1 in PMDD and controls.

**Role of oxidative stress and PON-1 in PMDD**

Previous studies have reported decreased antioxidant levels and increased oxidative and nitrosative stress pathway activities in subjects with depression\(^44-46\). Further, antidepressants have been shown to reduce oxidative and nitrosative stress pathway activations in both clinical depression and animal models, strongly indicating that inflammatory processes are involved in depression\(^46\).

Recently, the hypothesis about that PMDD may involve increased inflammatory processes\(^29\) has been expanded to include a role of progesterone withdrawal in the release of inflammatory products such as nuclear factor-κB and tumor necrosis factor\(^47\). However, to date conflicting results have been obtained with regard to the involvement of oxidative stress and PMS. In a preliminary study, subjects with PMS showed no evidence of oxidative damage\(^18\). In two studies that used malondialdehyde (MDA) as a marker of oxidative stress, no difference was observed between PMS patients and controls\(^5,49\). Conversely, a study by Duvan et al.\(^5\) reported a significant difference in LPH levels between controls and patients with
PMS, and they found that TAC was significantly decreased in the PMS group. Importantly, the authors did not evaluate well-known confounding factors such as depression, anxiety, and sleep quality. In this study, we did not find any statistically significant differences in mean serum total oxidant, antioxidant, or PON-1 levels between the PMDD and control groups of two menstrual cycles.

We did measure a statistically significant difference in ΔPON-1 between the PMDD and control groups. This protein is produced primarily in the liver but is also expressed in cells throughout the body, including endothelial cells and macrophages. A polymorphism on PON-1 (Q192R) was previously associated with depression and bipolar disorder. A previous study described lower levels of PON-1 in patients with depression compared with controls and found altered PON-1 levels in patients following treatment with citalopram. This suggests that PON-1 may play a role in PMDD pathophysiology.

We noted that PON-1 was higher during the follicular phase in subjects with PMDD and in the luteal phase in controls (Figure 3). Indeed, we found opposite patterns for serum total oxidant, antioxidant, and PON-1 levels in PMDD and controls. Although these findings were not statistically significant, they might reflect different onsets of inflammatory reactions that cause women to be symptomatic or asymptomatic during their late luteal phases.

**Role of estrogen and progesterone in PMDD pathophysiology**

As expected, progesterone and estrogen levels were found higher during the luteal phase, but we found no statistically significant difference between PMDD patients and controls. We found that estrogen levels in both luteal phases were found as positively, mildly and statistically significantly correlated with PAF scores that suggests the severity of the premenstrual syndrome. Etiological studies have described increases in these hormones during the premenstrual cycle, but another group reported no differences in estrogen and progesterone levels in subjects with PMS. In a study that examined to reduce progesterone sensitivity by altering receptor levels and in another study, an increased proportion of subjects with PMS were found to have an estrogen receptor polymorphism. We speculated that increases in estrogen and progesterone levels in PMDD might have been a compensatory mechanism to ameliorate oxidant mechanisms. The protective functions of these female hormones could underlie these unexpected results since estrogen and progesterone have been shown to prevent oxidative stress. Another group demonstrated how estrogen might enhance an antioxidant mechanism via anti-inflammatory effects. Collectively, the evidence in the literature suggests that estrogen and progesterone may have indirect roles in PMDD pathophysiology via their receptors rather than a direct effect due to their serum levels.

**Correlation of sleep, depression, and anxiety with PON-1 and oxidative stress levels**

Sleep, depression, and anxiety scores were evaluated in subjects with PMDD, as were the relationships of these variables with total oxidant-antioxidant and PON-1 levels. A limited number of clinical studies have evaluated how sleep effectiveness impacts oxidative stress. It has been proposed that cerebral free radicals accumulate during wakefulness and are removed during sleep. Interestingly, women with severe PMDD reported subjectively but significantly poorer sleep quality during the late luteal phase; however, polysomnograms indicated no evidence of
disturbed sleep specific to symptoms. We used the PSQI and did not find a difference in sleep quality between PMDD patients and controls in either menstrual phase.

A lifetime comorbidity history of anxiety or other mood disorders has been reported in more than 50% of women presenting with PMS. Anxiety and oxidant and antioxidant levels were explored in patients with sleep bruxism; however, the effect of anxiety itself on oxidant and antioxidant levels has not been explored. Impaired immune function and increased cytokine release, probably due to augmented cortisol secretion that could lead to oxidative stress as indicated by lowered plasma TAC has been reported in women with anxiety. Whether an imbalanced oxidant/antioxidant status underlies gamma-aminobutyric acid (GABA)ergic activity in PMS patients or is secondary to excessive opioid activity in PMS remains unknown. In our research, HARS and HDRS scores were higher in PMDD patients than controls, but this was not due to increased oxidative stress in the blood, and further investigations on other possible mechanisms underlying vulnerability to depression and anxiety in PMDD patients are warranted. Interestingly, the mean luteal HARS score was negatively near to statistically significantly correlated with the mean luteal PON-1 level in the same cycle. We think that this data deserves further analysis as PON-1 levels may be important in the pathophysiology of anxiety or vice versa. Similarly, PAF scores were positively correlated with second-cycle follicular phase oxidant levels, suggesting that some PMDD symptoms may also manifest in the follicular phase.

To our knowledge, this is the first study evaluating estrogen, progesterone, total oxidant-antioxidant, and PON-1 levels in patients with PMDD and examining possible correlations with depression, anxiety, and sleep quality.

Limitations

We only assessed a small number of subjects including young females, which may explain the lack of significant findings. Despite this shortcoming, we controlled for possible confounding factors that would impact oxidant and antioxidant levels, such as age, BMI, dietary habits. Moreover, subjects were excluded if they reported any of the following: smoking, long-term medication usage, hypertension, hyperlipidemia, or diabetes.

CONCLUSION

In conclusion, the serum levels of estrogen, progesterone, oxidant-antioxidants, and PON-1 were not found to be altered in the PMDD patients. Hence, these findings of the present study did not prove our initial hypothesis that oxidative stress and neuroendocrine factors might influence PMDD symptoms. Therefore, evidence to support the hypothesis proposed by earlier studies that suggest associations between PMDD and oxidative stress and related cellular mechanisms remains elusive. Additional longitudinal studies with larger sample sizes that will examine possible role of inflammatory processes in the pathophysiology of PMDD are needed to clarify PMDD pathogenesis.

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