Arch Gynecol Obstet (2007) 275:361–365
DOI 10.1007/s00404-006-0269-1

ORIGINAL ARTICLE

Malon dialdehyde, nitrite and adrenomedullin levels in patients with premenstrual syndrome

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Received: 13 August 2006 / Accepted: 28 September 2006 / Published online: 25 October 2006

Abstract
Objective To assess the levels of malon dialdehyde (MDA); a lipid peroxide product, total nitrite; a stabile product of nitric oxide (NO), and adrenomedulin (AM), to determine whether their levels are altered in premenstrual syndrome (PMS) and to search for their possible pathophysiological role in this peculiar syndrome.
Study design Twenty-one patients aged between 28 and 37 years, who had regular menses for at least six previous cycles, and were in general good health condition, were taken into the study. Blood samples were obtained from each patient at the 3rd and 21st day of their menstrual cycles. AM, nitrite, MDA and estradiol levels have been assessed in these samples for each patient.
Results No statistically significant difference in terms of age, parity and body mass index was detected among groups ($P > 0.05$). Nitric oxide levels were higher on the 3rd day, compared to 21st day in the study group, and this difference was statistically significant ($P < 0.05$). In the study group, 21st day AM levels were significantly higher when compared to the control group ($P < 0.05$).
Conclusion Even though various stress symptoms are present in PMS, there is no change in the levels of MDA, an oxidative distress indicator but AM and NO may have a pathophysiological role on this enigmatic disease.

Keywords Premenstrual syndrome · Nitric oxide · Adrenomedullin · Malon dialdehyde

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Introduction

Premenstrual syndrome (PMS) is one of the most common complaints of women of reproductive age characterized by breast tenderness, weight gain, headache, diarrhea and mood changes that are observed after mid-luteal phase of menstrual cycle. Difficulty in concentration, irritability, and anxiety are only a few symptoms of emotional and mental disturbances of these women. More than 90% of women had one or more of those signs and symptoms that affect their daily activities and social relations [1].

Since first described by Frank in 1931 [2], many theories have been forwarded concerning its etiology. However, till date, there has not been one single

explanation, which is widely accepted. Currently, there is little evidence to show that absolute concentrations (deficiency or excess) of sex hormones cause PMS, or that, women with PMS have different hormonal patterns compared to those without PMS. It has also been suggested that the disease might result from an abnormal central nervous response to normal gonadal steroids or their metabolites [3]. Such central effects may occur on the brain by modulating neurotransmitter receptor responses such as γ-aminobutyric acid A (GABA-A) receptor complex. It is also speculated that oxidative damage may have a role; however there is no such well-designed prospective study to test this hypothesis.

Oxygen-free radicals promote the formation of a number of hormones or cytokines that are highly deleterious to cellular macromolecules. An end product of ROS-induced peroxidation is malon dialdehyde (MDA), by itself potentially damaging to polypeptides and known to induce cross-links in protein [7]. Since it is an indicator of oxidative distress, measuring MDA gives a clue about the oxidative process in the body.

Adrenomedullin (AM) and nitric oxide (NO) are two potent vasodilators. AM has biological actions on various organs, most of which appear to be involved in the regulation of cardiovascular function and body fluid electrolyte homeostasis [4]. This peptide may also be important both as a paracrin and auotocrine factor, and may serve as a classical circulating hormone. Although the main source of plasma AM is considered to be the vascular endothelial cells and vascular smooth muscle cells, AM gene is expressed in most organs including adrenal gland, cardiovascular system, central nervous system, kidney, respiratory tract, gastro-intestinal tract, skin, and blood cells [5]. NO, an oxygen-radical, acts as a major messenger molecule that regulates vasodilatation and neurotransmission in the nervous system. It is produced by certain cell types to facilitate intracellular communication [6]. NO is a very labile molecule and is oxidized to nitrite and then nitrate, in a few seconds, where it is produced. Therefore, the indirect measurement of NO, via the oxidized metabolites, is an established technique for in vivo studies and plasma nitrite and nitrate (total nitrite) concentrations were accepted as an index of NO.

Considering the vasoregulatory and neurotransmitting effects of these peptides, we aimed to determine if there is any relation of these substances to PMS. For this reason, we measured the levels of MDA, a lipid peroxide product, total nitrite, a stabile product of NO, and AM, to determine whether their levels are altered in PMS and to search for their possible pathophysiological role in this peculiar syndrome.

Materials and methods

Twenty-one patients, admitted between June 2003 and December 2003 were enrolled in the study. Patients were separated into two groups. Group I (study group) was consisting of 11 patients with PMS, and group II (control group) was consisting of 10 normal women. All patients were aged between 28 and 37 years, had regular menses for at least six previous cycles, and were in good health condition. Patients, who were pregnant, under treatment for PMS or with a history of psychiatric disorders or those taking oral contraceptives, were excluded. Each patient included in the study underwent psychiatric consultation and complete physical examination. PMS was diagnosed according to American College of Obstetrics and Gynecology criteria [8]. In detail, PMS was diagnosed by the evaluation of the symptom charts kept by the subjects for three consecutive months, and if the subjects had at least five of the following negative symptoms: abdominal bloating, unprovoked anger or irritability, mood swings, crying spells, headache, weight gain, fatigue, lack of energy, less libido, change in drinking and eating patterns, pain and tension in breasts, and edema in extremities, and if the symptom/s show a cyclic occurrence, and if the symptom/s are sufficiently severe to interfere with physical, psychological and/or social functioning of the subject, and if the symptom/s appear with consistent and predictable relationship to the menses, and if the symptom/s appear during the last 2 weeks of the menstruation, and if all the other possible reasons including seizure disorders, thyroid and other endocrine disorders, cancer, systemic lupus erythematosus, anemias, endometriosis, and various infections can be excluded by psychological and clinical measures.

Ovulation was determined in each patient by measuring serum progesterone concentrations on the 21st day of menstrual cycle. Blood samples were obtained from each patient at the 3rd and 21st days of their menstrual cycles. AM, nitrite, MDA and estradiol levels have been assessed in these samples for each patient.

Measurement of plasma total nitrite level

We deproteinised 300 μl of plasma by adding 600 μl of 75 mmol/l $ZnSO_4$ solution, stirring, and centrifuging at 10,000*g* for at least 1 min at room temperature, after which 600 μl of 55 mmol/l NaOH was added. Again the solution was stirred and centrifuged at 1,000*g* for 3 min and the supernatant was recovered. Total nitrite was quantitated by means of the Griess reaction after incubation of plasma samples with *Escherichia coli* reductase

to convert NO_3^- to NO_2^- [9]. Griess reagent (1 ml, 1% sulfanilamide, 0.1% naphthylene diamine hydrochloride, and 2.5% phosphoric acid) (Sigma Chemical Co., St. Louis, MO, USA) was then added to 1 ml of plasma specimens. Absorbance was read at 545 nm after a 30-min incubation. Standard curves were prepared with known concentrations (1–100 μmol/L) of sodium nitrite. The coefficients of variation were 4.20–5.62% for intra- and inter-assay precision, respectively.

Measurement of plasma adrenomedullin levels

Plasma samples were applied to supelcosil C18 columns (Cecil 100HPLC) after extraction and purification. Loaded material was eluted 60% acetonitrile in 0.1% trifluoroacetic acid [10]. Rat adrenomedullin (1–50) (Phoenix Pharmaceuticals, Inc.) was used as the standard in the determination of plasma AM levels. The coefficients of variation were 2.40–3.90% for intra- and inter-assay precision, respectively.

Measurement of MDA

Thiobarbituric acid (TBA) reacts with lipoperoxidation aldehydes, such as MDA, as the most common method to assess lipid peroxidation in biological samples. The procedure was modified from Buege and Aust [11]. Briefly, 0.5 ml of plasma was added to a reaction mixture (1.0 ml) formed by equal parts of 15% trichloroacetic acid, 0.25 N HCl, and 0.375% TBA, plus 2.5 mM BHT and 0.1 ml of 8.1% SDS, followed by 30 min heating at 95°C; pH value of the analytical reaction mixture was about 0.9. BHT was used to prevent lipid peroxidation during heating. After cooling either incubation, the chromogen was extracted with *n*-butanol and read spectrophotometrically at 532 against a reaction mixture "blank" lacking plasma but subjected to the entire procedure and extracted with n-butanol. To correct for the background absorption, absorbance values at 572 nm were subtracted from those at 532 nm, the latter representing the absorption maximum of the 2:1 TBA:MDA, adduct [12]. A molar extinction coefficient of 154,000 was used.

Statistical analysis

Data were analyzed by student's *t* test for normally distributed data and Mann–Whitney *U* test for skewed data. Paired samples *t* test was used for intra group analysis. $P < 0.05$ was considered statistically significant.

Results

The mean age of patients was 30.24 ± 2.18 years. These women were otherwise healthy. Age, parity, and body mass index of study and control group were 34.2 ± 3.5 and 32.9 ± 3.1, 2.9 ± 0.8 and 2.6 ± 0.6, 23.8 ± 4.7 and 24.1 ± 5.1, respectively. There was no difference between groups in terms of age, parity, and body mass index. All patients were multiparous. Eleven patients were in group 1 (study group) and 10 patients were in group 2 (control group).

Symptom profiles of the patients with PMS were shown in Table 1. All values of MDA, NO, AM, and estradiol levels were shown in Table 2. There was no statistical difference between 3rd and 21st day MDA and AM levels in the study group. However, NO levels were higher in 3rd day, compared to 21st day in the study group, and this difference was statistically significant ($P < 0.05$).

Table 1 Sypmtom profiles of the subjects with premenstrual syndrome (PMS)

Patients no.	Symptoms											
	Abdominal bloating	Unprovoked anger or irritability	Mood swings	Crying spells	Headache	Weight Gain	Fatigue	Lack of energy	Less libido	Food cravings	Pain and tension in breasts	Edema in extremities
1	+		+		+	+	+	+		+	+	
2	+	+	+	+		+	+	+			+	
3	+	+	+	+		+	+	+		+		
4			+		+	+			+		+	
5	+	+	+		+	+	+	+			+	
6	+	+	+		+	+	+	+			+	
7	+	+	+		+	+	+	+	+		+	
8			+	+	+	+					+	
9	+	+	+		+	+	+	+		+		+
10	+		+		+	+	+	+			+	
11	+	+		+	+	+	+	+			+	

Table 2 Malon dialdehyde (MDA), Nitric oxide (NO), Adrenomedullin (AM) levels of study and control groups on day 3 and day 21

	Study group $n = 11$		Control group $n = 10$	
	D_3^a	D_{21}^a	D_3^a	D_{21}^a
MDA	1.9 ± 0.1	1.4 ± 0.1	0 ± 0.2	1.6 ± 0.1
NO	49 ± 4.1	42 ± 2.4	56 ± 3.8	43 ± 2.0
AM	35 ± 0.8	37 ± 2.2	34 ± 2.1	26 ± 1.4
Estradiol	31 ± 4.5		33 ± 6.5	

[a] Mean ± SD

D_3 = Third day of menstrual cycle

D_{21} = 21st day of menstrual cycle

There was no statistical difference between MDA, NO, and AM levels in day 3 and day 21 in the control group ($P > 0.05$) (Fig. 1).

Comparison between the study and control groups revealed no statistical difference between two groups, in terms of day-3 and day-21 MDA and NO levels and day-3 estradiol levels. In the study group, 21st day AM levels were significantly higher when compared to the control group ($P < 0.05$) (Fig. 1).

Comment

Premenstrual syndrome affects about 3% women of reproductive age and has the characteristics of various stress symptoms [13]. While the etiology has not been completely understood yet, the importance of stress in this particular syndrome has been reported. Kalia et al. [14], in their preliminary study, had reported that despite the clearly manifested stress symptoms, there was no evidence of oxidative damage in PMS patients. Similarly, in our study, we did not demonstrate any increase in MDA levels, both within and between groups, as an indication of an oxidative process. Although this finding suggests that there may be no oxidative disturbance in PMS, protective role of female hormones, estrogen, and progesterone, may be responsible for this situation since these hormones were shown to prevent oxidative stress [15]. Moreover, etiological studies have proven the increase in these hormones during premenstrual phase [16]. On the other side, measuring MDA levels is limited in detecting oxidative stress in some cases [11,12], and may not be sufficient enough to show oxidative stress in PMS.

Adrenomedullin, which was first isolated from human pheochromocytoma [4], has recently been shown to have multipotent properties [17]. Besides its vasodilator function, the protective role against oxidative stress and organ damage has been shown [18]. When we analyzed our results, there was an increase in ADM levels in PMS patients during premenstrual period as compared to healthy women. The increment in AM levels may be the result of a compensatory mechanism, and thus, may be an explanation for its

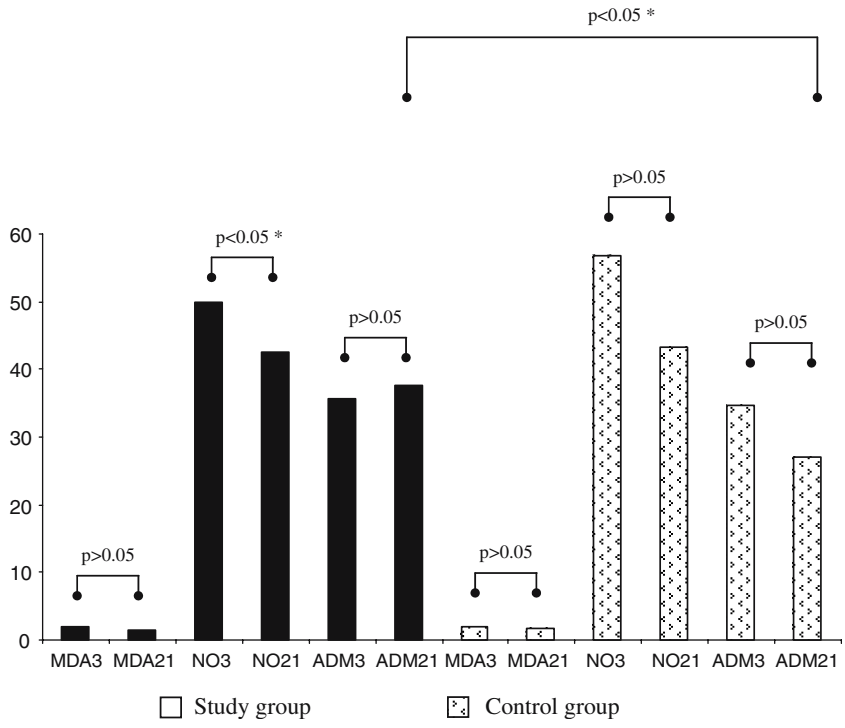

Fig. 1 Malon dialdehyde, Nitric oxide, Adrenomedullin values of the study and control groups on 3rd and 21st days of menstrual cycle

inability to detect the oxidative stress marker MDA. Also, this increment in AM may be a response to vascular reaction rather than oxidative distress.

Additionally, we have shown an increase in NO levels in the beginning of menstrual cycle in PMS patients. Although the mechanism by which AM carries out its function have not been fully clarified, it is thought that stimulation of the NO synthesis is one of the mechanisms [19]. Hattori et al. [20] had clearly demonstrated that AM enhances nitric oxide synthatese (iNOS) expression and results in an increment in NO levels. Hence, the increased NO levels in PMS patients may be a reflection of increased AM levels during the premenstrual phase. There is a great interest in the role of NO in neuropsychiatric disorders. NO has been implicated in a great number of normal and pathological functions [21]. Therefore, another speculation may be that the increased level of NO at the beginning of menstrual cycle is responsible for the relief of the symptoms after beginning of menstruation.

Besides the effect of AM on NO levels, recent studies have shown the effect of AM on the granulose cell of ovary, to stimulate progesterone production [22]. Since it is already known that the ovarian steroid hormones and their metabolites have potent effect on brain function by binding to GABA receptors, which have strong relation with PMS, increased ADM level may lead to PMS symptoms indirectly over progesterone, which is blamed in etiology of PMS.

In conclusion, according to the findings in our study, even though various stress symptoms are present in PMS, there is no change in the levels of MDA, an oxidative distress indicator but AM and NO may have a pathophysiological role on this enigmatic disease. Further studies may strengthen their links to the etiology of PMS.

Acknowledgment The authors thank Assoc. Prof. Dr. Ayse Balat, MD for editing the manuscript

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