JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH

How to cite this article:
TULADHAR ET, KAMATH A, RAO A. PLASMA PROTEIN OXIDATION AND TOTAL ANTIOXIDANT POWER IN PREMENSTRUAL DYSPHORIC DISORDER AND MENSTRUATING YOUNG ADULT FEMALES.Journal of Clinical and Diagnostic Research [serial online] 2010 December [cited: 2010 December10]; 4:3409-3413.

Available from
http://www.jcdr.in/article_fulltext.asp?issn=0973-709x&year=2010&volume=4&issue=6&page=3409-3413&issn=0973-709x&id=822

ORIGINAL ARTICLE

Plasma Protein Oxidation And Total Antioxidant Power In Premenstrual Dysphoric Disorder And Menstruating Young Adult Females

EANS TARA TULADHAR*, KAMATH A**, ANJALI RAO***

ABSTRACT

Purpose: Oxidative damage has been associated with many human diseases encompassing mood affective disorders. Premenstrual dysphoric disorder (PMDD) has features which are similar to these, along with physical symptoms of stress. The purpose of the present study was, to assess whether oxidative stress has any role in PMDD. **Method:** Female subjects suffering from PMDD , in the age group of 20-24 years, were compared to their eumennorhic counterparts and also with those with premenstrual syndrome (PMS), in the follicular phase and in the late luteal phase for the ferric reducing antioxidant power of plasma (FRAP), plasma protein thiol (PPT) and protein carbonyl (PPC) levels. **Results**: There were no significant changes in the FRAP and PPC levels in the controls, in the PMS and the PMDD groups, but PPT levels decreased significantly in the luteal phase of the PMS (P=0.015) and the PMDD groups (P= 0.018) when compared to those in the follicular phase. Besides, PPT levels exhibited a significant increase (P=0.015) in the follicular phase of the PMDD subjects. **Conclusion**: A marked decrease in PPT levels in the luteal phase of the PMS and the PMDD groups may be due to the pro-oxidant nature of oestrogen - active in this phase of PMS, leading to the consumption of the sacrificial antioxidant – protein thiol. Further, to compensate this loss, a large reserve of PPT gets accumulated in the follicular phase of the PMDD group, thus indicating a dynamic turnover of this antioxidant between the two phases.

Key words: Ferric reducing antioxidant power of plasma; premenstrual dysphoric disorder; protein thiol; protein carbonyl

Key message: PMS/PMDD involve oxidative stress.
The body tries to adapt to this stress by utilizing endogenous antioxidants.
Plasma protein thiols appear to be more sensitive antioxidants.

*MD, Department of Biochemistry; **PhD, Department of Biochemistry; ***M Phil, Department of Community Medicine, Kasturba Medical College, Manipal, Karnataka, India
Address for correspondence:
Dr. Anjali Rao, Professor of Biochemistry, Kasturba Medical College, Manipal- 576 104, Karnataka, India, Phone: 918202922326, Fax: 91820 2570062, Email: dranjalirao@hotmail.com

Introduction

Premenstrual dysphoric disorder (PMDD) is a severe form of premenstrual syndrome (PMS) that affects 3-8 % of menstruating women. The symptoms of the disorder as categorized by DSM-IV, include marked depression, anxiety, moodiness, irritability, tension, etc. The quality of life of such women is considerably reduced. The pathogenesis of PMDD is not known and the only cure would be menopause [1] or the administration of selective serotonin receptor inhibitors (SSRIs), which is not without the danger of its side effects. Oxidative stress has been associated with many human diseases, including mood

affective disorders. Premenstrual dysphoric disorder (PMDD) has features which are similar to these, along with physical symptoms of stress**.** It is now well documented that oxidative stress may have a role in PMS.[2-4] However, no such reports are available in its debilitating form, which is called PMDD. Hence, in the present study, the antioxidant status in PMDD, PMS and normal females was studied by using the ferric reducing antioxidant power of plasma (FRAP), plasma protein thiol (PPT) and plasma protein carbonyl (PPC) levels.

Materials and Methods

Study Design: Cross sectional study

Study population: Females aged 20-24 years from a University setting

Sample size: By anticipating the prevalence of PMDD among females of 20-24 years as 5%, for a level of significance of 5% and an absolute precision of 3%, 203 women were screened. There was a drop out of 20.1% women in the study. As a result, only 162 females participated in the study.

Sampling: Convenient sampling

The study duration was from June 2005 to July 2006. Inclusion criteria: Females having regular menstrual cycles with no other illness and those who were not on any medications.

Exclusion criteria: No history of polycystic ovarian disease, smoking, alcohol consumption, drug abuse, insulin resistance and the use of contraceptive pills.

The nutritional difference was insignificant, as they belonged to the same socioeconomic strata. The body mass index (BMI) for all the subjects was well within the normal range (22-24 kg/m^2). A Calendar of Premenstrual Experiences (COPE) questionnaire was used to identify the females who experienced PMS and PMDD. [5]

The screen positive PMDD females were considered as cases. The rest of the group comprised of PMS cases and the normals. The questionnaire screening identified eight females who experienced PMDD. Thus, 24 each of the normal and PMS females were randomly selected from the rest of the group to form the group of Controls and the PMS subjects.

Blood sample collection:

Under aseptic conditions, 1.5 ml of venous blood was collected in EDTA containing vacutainers. The blood samples were collected from each volunteer at two time points, namely, the follicular phase sample (seven day period following menstruation) and the luteal phase sample (seven day period before menstruation). The plasma was separated immediately after the collection of the sample by centrifuging at 4000 rpm for two minutes. The separated plasma was transferred and stored (if needed) at -20ºC. The parameters were assayed immediately or within the next two days of the collection of the samples. The plasma samples were allowed to thaw to room temperature before the assay was carried out.

This study was carried out after clearance from the institutional ethical committee. The samples were obtained from the volunteers after their written consent was taken. FRAP was measured according to the spectrophotometric method of Benzie et al. [6] At low pH, reduction of a ferric tripyridyltriazine (Fe^{III}-TPTZ) complex to the ferrous form, which has an intense blue colour, can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is directly related to the combined or "total" reducing power of the electron donating antioxidants which are present in the reaction mixture. PPT was measured in plasma by a spectrophotometric method by using di thionitrobenzene (DTNB)-Ellman's method. [7],[8] Ellman's reagent or 5,5'-dithiobis (2-nitrobenzoate, DTNB) is a symmetrical aryl disulfide which readily undergoes the thiol-disulfide interchange reaction in the presence of a free thiol. The TNB dianion has a relatively intense absorbance at 412 nm as compared to both the disulfides. The protein thiol concentration in plasma was determined by using the molar extinction coefficient of the TNB complex in the assay mixture at 412 nm which was obtained after using known standard concentrations and their absorbance values.

PPC was analyzed by treating the plasma with 2,4- dinitrophenylhydrazine (DNPH) [9] and the absorbance peak was measured at 355 nm. The assay was based on the fact that several reactive oxygen species (ROS) can attack amino acid residues in proteins (particularly

histidine, arginine, lysine and proline) to produce carbonyl functions that can react with DNPH to generate chromophobic dinitrophenylhydrazone, which can be measured spectrophotometrically.

Statistical analysis was carried out by using the SPSS package (version 11.0). Continuous data was summarized by using median and interquartile ranges, as the data was skewed. Comparison of the median between the three groups was done by using the Kruskal Wallis test, followed by a pair wise comparison of the groups by using the Mann Whitney test with Bonferroni correction for the type I error.

Results:

There was no significant difference in the FRAP and PPC levels between the control subjects and the PMS and PMDD groups at both the time points ie. the follicular as well as the luteal phases [Table 1].

[Table 1]: FRAP and plasma protein oxidative markers in controls, PMS and PMDD, Median (IQR)*

Parameters	Controls (n=24)	PMS (n=24)	PMDD (n=8)
Follicular phase			
FRAP (μ mols)	810 (740-1115)	920 (780-1135)	820 (805-1050)
Protein thiols (μ mols)	399 (370-410)	429 (390-466)	471[b] (409-488)
Protein carbonyls (μ mols/mg protein)	0.78 (0.67-0.99)	0.81 (0.74-1.05)	0.70 (0.65-0.76)
Luteal phase			
FRAP (μ mols)	870 (707-1005)	900 (800-1130)	850 (705-1047)
Protein thiols (μ mols)	403 (370-463)	410[a] (356-468)	354[c] (306-429)
Protein carbonyls (μ mols/mg protein)	0.76 (0.62-0.88)	0.96 (0.79-1.19)	0.82 (0.69-0.95)

*Values are expressed as median (Interquartile Range), nonparametric Mann Whitney test.

[a]P=0.015 when two phases of PMS were compared, Wilcoxon's Signed Rank test

[b] P=0.015 between the groups, Kruskal Wallis test (Anova)

[c] P=0.018 when two phases of PMDD were compared, Wilcoxon's Signed Rank test

Further, an intra group comparison of these levels in the follicular and the luteal phases in the controls and PMS subjects also showed no significant changes. However, a comparison of the PPT levels between the follicular and luteal phases of the PMS and the PMDD groups indicated a significant decrease (P=0.015 and P= 0.018, respectively) in PPT in the luteal phase [Table 1], the FRAP and PPC levels remaining unchanged. Moreover, an inter group comparison exhibited a markedly significant (P=0.015) PPT reserve in the follicular phase of the PMDD group [Table 1].

Discussion

Stress is one of the most common symptoms which are experienced during PMS and more so, in PMDD. [1],[10] Now, there are also reports on the involvement of oxidative stress in the pathophysiology of both PMS and PMDD.[2] Thus, in the present study, a significant depletion of PPT, a sensitive antioxidant in the luteal phase, both in the PMS and the PMDD groups over that in the follicular phase as compared to the controls, indicated that this sacrificial antioxidant was being consumed to probably counteract the oxidative challenge which was thrust at that period of time. Further, the increased levels of PPT in the follicular phase of the PMDD subjects only, could be used to neutralize the free radical toxicity which was generated during the luteal phase of these subjects. This fact could be explained on the basis of previous observations [11] on the prooxidant and antioxidant effects of oestrogens. The excessive activity of oestrogen in the luteal phase of the PMS/PMDD groups may stimulate its prooxidant nature. Oestrogens can get converted to catecholoestrogens which act as oxidants and in turn, significantly lower the PPT levels.[12] However, in the present study, the hormonal status of all the subjects was found to be well within the normal limits. Furthermore, the antioxidant activity of oxytocin, enkephalin and endorphin was influenced by oestrogens. [13-15] Moreover, dietary influence on the antioxidants and the hormone levels may also play a role in PMDD.[16] Thus, such a confluence of hormone action involving oestrogens [17] also may lead to oxidative stress in PMDD, as observed in this study.

References

[1] Steiner M, Born L . Diagnosis and treatment of premenstrual dysphoric disorder : an update. Int Clin Psychopharmacol 2000 ; 15 : S5-S17.

[2] Duvan CI, Nilgun AC, Turhan O, Karasu C, Kafali H. Oxidant/antioxidant status in premenstrual syndrome. Arch Gynecol Obstet DOI 2010;10.1007/s00404-009-1347-y.

[3] Kalia G, Sudheendran S, Rao A. Antioxidant status and lipid peroxidation in premenstrual syndrome: a preliminary study. Clin Chim Acta 2001; 309 : 97- 99.

[4] Tuladhar ET, Rao A. Plasma protein oxidation and total antioxidant power in premenstrual syndrome. Asia Pacific J Trop Med 2010; 3 : 237-240.

[5] Ling FW. Recognizing and treating premenstrual dysphoric disorder in the obstetrics, gynecologic and primary care practices. J Clin Psychiatry 2000; 61(suppl): 9-16.

[6] Benzie IFF, Strain JJ. Ferric reducing /antioxidant power of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 1999; 299: 15-27.

[7] Hu ML. Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol 1994; 233:380-385.

[8] Motichnik PA, Frei B, Ames BN. Measurement of antioxidants in human blood plasma. Methods Enzymol 1994; 234:269-279.

[9] Reznnick AZ, Packer L. Oxidative Damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 1994;233:357-363.

[10] Kaur G, Gonsalves L, Thacker HL. Premenstrual dysphoric disorder: a review for the treating practitioner. Cleve Clin J Med 2004; 71: 303-318. Review.

[11] Natan L, Chaudhari G. Antioxidant and prooxidant action of estrogens: potential physiological and clinical implications. Semi Reprod Endocrinol 1998; 16: 309-1314.

[12] Beral V, Banks E, Reeves G. Evidence from randomized trials on the long term effects of hormone replacement therapy. Lancet 2002; 360 : 942-944.

[13] . Reed SC, Levin FR, Evans SM. Changes in mood, cognitive performance and appetite in the late luteal and follicular phases of the menstrual cycle in women with and without PMDD (premenstrual dysphoric disorder). Horm Behav 2008; 54 : 185-193.

[14] Straneva PA, Maixner W, Light KC, Pedersen CA, Costello NL, Girdler SS. Menstrual cycle, beta-endorphins, and pain sensitivity in premenstrual dysphoric disorder. Health Psychol 2002; 21 : 358-367.

[15] Moosmann B, Behl C. Secretory peptides hormones are biochemical antioxidants: structure activity relationship. Mol Pharmacol 2002; 61:260-268.

[16] Bloomer RJ, Fisher-Wellman KH. Lower postprandial oxidative stress in women compared with men. Gender Medicine 2010; 7 :340-349.

[17] Karowicz-Bilinska A, Plodzidym M, Krol J, Lewinska A, Bartosz G Changes of markers of oxidative stress during menstrual