Objective: To explore whether oxidative stress has any role in premenstrual syndrome (PMS).

Methods: Female volunteers suffering from PMS, in the age group of 20–24 years were compared to their asymptomatic normomennorhoeic counterparts in follicular phase and late luteal phase for ferric reducing antioxidant power of plasma (FRAP), plasma protein thiols (PPT) and protein carbonyls (PPC) levels.

Results: There was no significant change in FRAP and PPC levels in controls and PMS groups but PPT decreased significantly in luteal phase of PMS (P < 0.05) when compared to follicular phase.

Conclusions: Estrogen and progesterone, might be responsible for a healthy antioxidant profile in PMS. However, a marked decrease in PPT in luteal phase of PMS group may be due to pro-oxidant nature of estrogen-active in this phase of PMS leading to consumption of the sacrificial antioxidant–protein thiol.

1. Introduction

A number of diseases including many mood affective disorders like depression and anxiety and neurological disorders like schizophrenia have exhibited oxidative stress[1-5]. Free radical toxicity can be a contributory factor or a consequence of the disease itself. Such oxidative stress, brought about by reactive oxygen species (ROS), reactive nitrogen species and reactive sulphur species, leads to the injury of the cell/tissue and finally its death. The damaging effects of pro-oxidants vary considerably with the organisms studied, age, physiological state and diet[5]. Pro-oxidants are highly toxic to all types of biomolecules including DNA, proteins, lipids and carbohydrates. In our body such pro-oxidants are scavenged by various antioxidants. Only when the pro-oxidants and antioxidants homeostasis is disturbed, there is oxidative stress.

Premenstrual syndrome (PMS) which affects 30%–80% of the reproductive female age group has many features similar to that of depression and anxiety which however is limited to the late luteal phase of the menstrual cycle. The spectrum of this disorder ranges from mild (mere notice of symptoms) to a severe state of impairment of mood and behaviour (premenstrual dysphoric disorder, PMDD) upsetting the personal, professional and social life. The severe form PMDD, as categorized by DSM-IV, is seen in 3%–8% of the total female population[6].

The most common spectrum of symptoms observed in PMS are acne formation ~70%, irritability ~69%, depression ~63%, swelling/bloating ~ 63%. Further, PMS is just not one syndrome. It can be classified into different syndrome classes[7]. Most of the women fall under general discomfort syndrome and water retention syndrome. Depressive features fall under agitated–anxious or hostile type of atypical or non–endogenous depression[7]. Anxiety disorder features are present to a lesser extent than the depressive features[8,9]. There have been links between PMS and chronic schizophrenia as well as PMS and bulimia[10]. Past history of psychiatric illness and/or family history of depression[10–12], suicidal attempts[10,13] and a future risk of major depressive disorder have been correlated with PMS. However, the intensity of physical and emotional premenstrual complaints do not correlate[10]. It is also possible that premenstrual changes do not have the magnitude to qualify as a disorder as in some women they
are actually positive changes[10]. Many etiologies have been put forward from hormonal factors to nutritional disturbances to explain the underlying pathology which however seems to be multifactorial.

Since, PMS manifests various debilitating symptoms, in the present study we have tried to explore the role of oxidative stress in this condition. A preliminary study in PMS with a smaller sample size (n=6) had led us to infer that there is no indication of oxidative stress in such cases[14]. Therefore, in the present study we explored with a larger sample size and also with more sensitive markers of oxidative stress such as protein oxidation (protein thiols and carbonyls) and total antioxidant power of plasma.

Proteins being the most abundant biomolecules in the plasma, cysteine and methionine amino acid residues of protein undergo dynamic and reversible oxidation in the earliest stage of oxidant attack protecting the oxidation at other sites forming disulfide linkage with other proteins or other low molecular weight thiols or glutathione resulting in decreased concentration of the total reduced thiols. Estimating the reduced thiols indicates the antioxidant power of proteins, a drop in their levels being the earliest indication of oxidative stress[15-18]. The persistence of the oxidants in the body manifests as irreversible oxidized protein products, amongst which the most prominent are the protein carbonyls indicating the extent of the damage to proteins. On the other hand, the tests which measure the combined antioxidant effect of the nonenzymatic defenses in biological fluids may be useful in providing an index of the antioxidant system e.g. FRAP which measures the total antioxidant capacity of the plasma, serum and other biological fluids directly[19-21]. Sixty percent of FRAP is contributed by uric acid followed by ascorbic acid (15%), protein (10%), alpha–tocopherol (5%), bilirubin (5%) and others. There is no apparent interaction between antioxidants in the FRAP assay. FRAP however does not assay the sulfhydryl (SH) containing antioxidants[20].

Thus, the ferric reducing antioxidant power of plasma (FRAP), plasma protein thiol (PPT) and plasma protein carbonyl (PPC) levels have been chosen as the study parameters in the present study.

2. Materials and methods

Female volunteers of age 20–24 years from Manipal University were enrolled in this study. They had regular menstrual cycles with no other illness and were not on any medications. The study was conducted in department of Biochemistry, Kasturba Medical College, Manipal, from 7th June 2005 to 6th July 2006. A total of 154 volunteers participated in this study. None of the subjects had any history of polycystic ovarian disease, smoking, alcohol consumption, drug abuse, insulin resistance and use of contraceptive pills. 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/m²). The subjects were divided into two major groups on the basis of COPE that is Calendar of Premenstrual Experiences[22]. The groups were: normal/controls (who do not experience the premenstrual symptoms, COPE score=0) with median age=24 years, n=80; PMS (premenstrual syndrome; luteal score – follicular score ≥ 30% of COPE score, follicular phase score<40) with median age=23 years, n=74.

Under aseptic conditions, 1.5 mL of venous blood was collected in EDTA vacutainers. The blood samples were collected from each volunteer at two time points namely, follicular phase sample (seven day period following menstruation) and luteal phase sample (seven day period before menstruation). The plasma was separated immediately after collection of sample by centrifuging at 4 000 rpm for two minutes. The separated plasma was transferred and stored in eppendorff tubes in the freezer at −20 °C. The parameters were assayed immediately or within next two days of collection. The plasma samples were allowed to thaw to room temperature before assay.

This study was carried out after clearance through the institutional ethical committee. The samples were obtained from the volunteers after their written consent.

FRAP was measured according to the spectrophotometric method of Benzie et al[21]. PPT was estimated by treating the plasma with dithionitrobenzoic acid (DTNB)[15,16] and the absorbance was measured at 412 nm. PPC was analyzed by treating the plasma with 2,4-dinitrophenylhydrazine (DNPH)[23] and the absorbance peak was measured at 355 nm. The assay was based on the fact that several ROS can attack amino acids residues in proteins (particularly histidine, arginine, lysine, proline) to produce carbonyl functions that can react with DNPH to generate chromophobic dinitrophenylhydrazine which is measured spectrophotometrically.

Statistical analysis was carried out using the SPSS package (version 11.0).

3. Results

There was no significant difference in the FRAP, PPT and PPC levels between the control subjects and the PMS group at both the time points, follicular as well as the luteal phases (Table 1). Further, an intra group comparison of the levels in the follicular and the luteal phases in the controls also showed no significant changes. However, a comparison of the PPT levels between the follicular and luteal phases of the PMS group indicated a significant decrease in PPT in the luteal phase (P<0.05) (Table 1), the FRAP and PPC levels remaining unchanged.

4. Discussion

Under aerobic conditions, cells are always threatened with the insult from ROS which however is overcome by the highly
powerful antioxidant systems of the cell or body without any untoward effect, one such antioxidant being estrogen. In the present study therefore, the PMS group which is definitely associated with numerous stress symptoms\cite{8,10,12,13} and vulnerable to the ROS attack shows a significant change in PPT levels (P<0.05) between the follicular phase and luteal phase when compared to their asymptomatic counterparts. However, FRAP and PPC levels remain unaffected when an intra- as well as inter-group comparison of the two phases between controls and PMS subjects was made. Earlier work carried out in our laboratory\cite{14} and views expressed elsewhere\cite{24-28} strongly support the observation that estrogens as well as progestins may exert protective and adaptive response as potent antioxidants to the symptoms of PMS combating any oxidative stress. Further reports on plasma TBARS and erythrocyte antioxidant enzyme activities showed that in healthy eumennorrheic women, the levels were not altered in either of the two phases of the menstrual cycle except for the enzyme glutathione peroxidase\cite{29,30}. A significant decrease in the PPT level in the luteal phase of the PMS group in this study, may be due to the overactivity of estrogen\cite{31-34} in this phase which is responsible for the PMS symptoms on one hand and is also a potent antioxidant, on the other hand\cite{24-30} thus, leading to an increase in the total turnover of PPT to combat any oxidative damage thereon, resulting in a marked decrease in its level in the plasma during this phase. Excessive activity of estrogens in the luteal phase of PMS group may stimulate its prooxidant nature also, which in turn significantly lowers the PPT levels, a sacrificial antioxidant\cite{16}. Estrogens are converted to catecholestrogens which in turn produce oxygen radicals which bring about various types of damage\cite{35}. However, the hormonal status of all the subjects in this study was well within normal limits in both the phases. Moreover, other workers\cite{36} have also suggested that neuroendocrine basis for the biobehavioral symptoms of PMS may involve oxytocin in conjunction with the female reproductive hormones and endogenous opioid peptides especially endorphins\cite{6,37-39} promoting a better response to stress. Furthermore, estrogen influences the action of neurotransmitters like oxytocin and enkephalin probably affecting their antioxidant activity\cite{10,40}. Hence, it can be hypothesized that hormones like estrogen, progesterone, oxytocin and neuropeptides could have attributed to the healthy antioxidant profile in PMS despite the transient decrease in the PPT levels in luteal phase of the PMS group.

Conflict of interest statement

We declare that we have no conflict of interest.

References

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