Evaluation of Oxidative Stress and Antioxidant Status during Normal Menstrual Cycle

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Abstract: Background & Objectives: Oxidative stress has been investigated to explain various physiological as well as pathological bases of many medical conditions. But very few data concerning the oxidative stress during normal menstrual cycle of eumenorrheic women are available. Thus, the purpose of study was to examine the physiological role of oxidative stress during normal menstrual cycle. **Methods:** 120 young healthy female subjects of reproductive age group (17-27 yrs), having regular menstrual cycle, were examined. Serum malondialdehyde (MDA), an oxidative stress biomarker and serum ascorbic acid (vitamin-C), an antioxidant vitamin were assessed in the follicular phase (on 7th day) and in the luteal phase (on 21st day) of normal menstrual cycle. **Results:** In the present study, significant higher ($p<0.0001$) levels of MDA and lower but non-significant ($p>0.05$) levels of ascorbic acid were observed in the luteal phase when compared to the follicular phase. Non-significant negative correlations were also observed between MDA and ascorbic acid in both the phases of normal menstrual cycle. Significant increase in serum MDA level coincided with the increased progesterone and estrogen levels during the luteal phase. High levels of estrogen may be the initiator of lipid peroxidation process which eventually ends up with cellular injury during the luteal phase. **Interpretation & Conclusion:** Oxidative stress has an important role to play in physiological phenomenon of the menstruation.

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Key Words: Antioxidants, ascorbic acid, malondialdehyde (MDA), menstrual cycle, oxidative stress.

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Introduction: Menstrual cycle is the result of complex interacting processes involving interaction of the hypothalamus, pituitary, ovaries, uterus, prostaglandins and neuroendocrine factors[1].

The normal menstrual cycle is a twenty eight day period which is divided into two phases i.e. follicular phase and luteal phase. The follicular phase is characterised by a low level of estrogen and progesterone which is followed by rise in estradiol, lutenizing hormone, and follicle stimulating hormone at the time of ovulation, while the luteal phase is associated with increased levels of progesterone and estrogen. These phases are associated with various changes in blood parameters along with variations in the sex hormones.

Several powerful reactive oxygen species or free radicals or oxidants are produced during the course of metabolism in blood cells and most other cells of the body. These oxidants are very reactive molecules that can react with proteins, nucleic acids, lipids and other molecules which changes physicochemical status of the cell to alter their structure and cause tissue damage. Lipid peroxidation is a well established mechanism of cellular injury in human and is used as an indicator of oxidative stress in cells and tissues. Malondialdehyde (MDA) is one of the important byproduct of lipid peroxidation process which is widely used as an indicator of lipid peroxidation[2].

These free radicals are the target for the enzymatic and non-enzymatic scavenging systems[3], which includes the antioxidants such as superoxide dismutase, glutathione peroxidase, vitamin-A, vitamin-C, vitamin-E etc. that scavenge the free radicals and protect the tissues from oxidative stress.

Oxidative stress has been implicated in various physiological as well as pathological bases of many medical conditions including reproductive system. Evidences have shown the dual effects of oxygen radicals in the physiological reproductive processes such as oocyte maturation, ovulation, menstruation, luteolysis, luteal maintenance in pregnancy, implantation and blastocyst development [4,5,6,] as well as in the pathological conditions like spontaneous abortions and infertility in females[7].

The imbalance between free radicals and antioxidants resulting from either an overproduction of free radicals or a deficit in

antioxidant protection leads to oxidative stress[8]. Although, reports regarding variations of oxidative stress across the normal menstrual cycle in eumenorrheic women have been published, especially in reference to MDA (a marker of oxidative stress and an important byproduct of lipid peroxidation), but these are sparse and have conflicting trend.

Thus, the purpose of this study was to examine the role of oxidative stress during the different phases of normal menstrual cycle by measuring the MDA which is served as an oxidative stress biomarker and ascorbic acid (vitamin-C), served as an antioxidant.

Material and Methods: The present study was carried out on 120 normal healthy and regular menstruating female subjects aged between 17 and 27 years (mean age 20.53 ± 2.9 years) selected from the S.M.S. Medical College & attached hospitals, Jaipur. Study was carried out in the Upgraded Department of Physiology, S.M.S. Medical College, Jaipur. Permission was also obtained from institutional ethical committee for carrying out the research work. After seeking an informed written consent and recording the detailed menstrual history (i.e. age at menarche, date of last menstruation, cycle length and days of bleeding), the subjects were then subjected to sample collection. Serum malondialdehyde (MDA) and serum ascorbic acid (vitamin-C) levels were assayed during their monthly menstrual cycle. All subjects were neither obese nor on any medication of any known pathologies.

5 ml of fasting blood samples were drawn from antecubital vein from each subject during the follicular phase (on 7th day of the cycle) and luteal phase (on 21st day of the cycle) after taking full antiseptic precautions.

The MDA levels were measured by thiobarbituric acid assay method[9]. The principle of the method was based on the spectrophotometric measurement of the colour developed during the reaction of thiobarbituric acid with MDA. The concentration of thiobarbituric acid reactive substances was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex. Serum ascorbic acid was estimated spectrophotometrically, by using 2, 4-dinitrophenyl hydrazine[10]. The underlying principle was based on the oxidation of ascorbic acid to dehydroascorbic acid followed by coupling with 2, 4- dinitrophenyl hydrazine under controlled conditions, in the presence of Thiourea as a mild reducing agent which gives red coloured osazones.

The data thus obtained was subjected to statistical analysis using the PRIMER OF BIOSTATISTICS software. The comparison of variables was done using the Student paired 't' test and correlation between variables was estimated by using Karl Pearson's correlation coefficient. The significance level was considered at $p<0.05$.

Results: The mean serum MDA and serum ascorbic acid levels during the follicular and luteal phases of menstrual cycle are presented as in Table 1. In the present study, the mean serum MDA (nmol/ml) level was greater during the luteal phase (4.294±1.60 nmol/ml) as compared to follicular phase (2.119±0.83 nmol/ml). The difference between MDA levels in the luteal phase was highly significant ($p<0.0001$) when compared with the follicular phase.

Lower values of serum ascorbic acid were found in luteal phase when compared to follicular phase. The mean values observed were 0.9399±0.40 mg/dl & 0.8963±0.37 mg/dl in follicular phase and luteal phase, respectively. But, the changes were not significant statistically ($p>0.05$).

Table 1: Mean±SD Levels of Serum Malondialdehyde (MDA) and Serum Ascorbic Acid during Follicular and Luteal Phases of Menstrual Cycle.

Parameters	Follicular phase (Mean ± SD)	Luteal phase (Mean ± SD)	p value
Serum MDA (nmol/ml)	2.119± 0.83	4.294± 1.60	<0.0001*
Serum Ascorbic acid (mg/dl)	0.9399± 0.40	0.8963± 0.37	>0.05**

Data expressed as mean and SD, *Highly significant, **Non-significant.

In this study, a negative correlation was also observed between serum ascorbic acid (mg/dl) and serum MDA (nmol/ml) levels in both follicular and luteal phases but the correlation was found to be non-significant in both follicular phase (r value=-0.026, p>0.05) as well as in luteal phase (r value=-0.010, p>0.05).

Discussion: Present study has revealed the role of oxidative stress in the physiology of menstruation. We observed significant higher levels of MDA while non-significant lower levels of ascorbic acid in the luteal phase when compared with the follicular phase of the menstrual cycle.

Previous studies also have correlated increased serum MDA levels during the luteal phase with the physiological phenomenon of menstruation in the healthy normal menstruating females which support our study results[6,11]. While, no significant differences in MDA levels throughout the normal menstrual cycle also have been reported[12,13,14].

Unlike our study results, a progressive significant rise in plasma ascorbic acid levels has been reported from menstrual to ovulation[15]. Similarly, "a sharp increase in the fasting level of plasma Vitamin C" also has been showed in some women during the middle of the menstrual cycle[16]. Whereas, some other researchers found no evidences of unusual variability of plasma ascorbic acid values throughout the menstrual cycle, which supports our study[17].

The significant rise of MDA and depletion of ascorbic acid levels during the luteal phase of menstrual cycle coincides with the increased levels of estrogen and progesterone, which is a characteristic feature of this phase[18]. Increased levels of estrogen and progesterone cause the proliferation of uterus while the initiation of sloughing of the endometrium is mainly due to the rise in the estrogen levels[18,19,20].

Thus, the high estrogen level from developing follicles may be the initiator of lipid peroxidation process[21], which eventually causes the cellular injury followed by release of cytokines especially tumour necrosis factor-alpha, which generates reactive oxygen species from the tissues which in turn causes lipid peroxidation[22]. The generated free radicals may play an important role in spasm of the highly vascularised vessels leading to vascular necrosis and menstrual flow, when hormonal support for the endometrium is diminished.

High levels of oxidative stress have been coincided with high levels of female sex hormone such as estradiol in previous studies also[6,21,23]. While, in other studies a significant negative correlation between these parameters in regularly menstruating females also have been established[24]. Whereas, some evidences show no significant correlation between ovarian hormones and oxidative stress during the follicular and luteal phases of menstrual cycle[25]. Present study perhaps therefore suggests that the high MDA level in the luteal phase may play an important role in the initiation of menstruation which is a well-established cellular injury based phenomenon.

Conclusion: In conclusion, these results suggest that increase of serum MDA levels may play an important role in the physiological phenomenon of menstruation.

References:

1. Joseph L. Mayo. A healthy menstrual cycle. Clinical Nutrition Insights 1997; 5(9): 1-8.
2. Raharjo S, Sofos JN and Schmitt GR. Solid-phase acid extraction improves thiobarbituric acid methods to determine lipid oxidation. J Food Sci 1993; 58: 921-932.
3. Grunert RR and Phillips PH. A modification of nitroprusside method of analysis for glutathione. Arch Biochem 1951; 30(2): 217-225.
4. Sawada M and Carlson J. Superoxide radical production in plasma membrane samples from regressing corpora lutea. Can J Physiol Pharmacol 1989; 67(5): 465-471.
5. Harvey AJ, Kind KL and Thompson JG. REDOX regulation of early embryo development. Reproduction 2002; 123: 479-486.
6. Akande AA and Akinyinka AO. Serum malondialdehyde levels during menstrual cycle. Afr J Biotechnol 2005; 4(11): 1297-1299.
7. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative

stress on female reproduction: a review. Reprod Biol Endocrinol 2012; 10: 49.
8. Terada LS. Specificity in reactive oxidant signalling: Think globally, act locally. J Cell Biol 2006; 174: 615-623.
9. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978; 90(1): 37-43.
10. Natelson S. Determination of ascorbic acid by using 2, 4 – dinitrophenyl hydrazine. Techniques of Clinical Chemistry. 3rd ed. Springfield, USA: Charles C. Thomas. 1971: pp. 165-166.
11. Karowicz-Bilinska A, Plodzidym M, Krol J, Lewinska A, Bartosz G. Changes of markers of oxidative stress during menstrual cycle. Redox Rep 2008; 13(5): 237-240.
12. Serviddio G, Loverro G, Vicino M, et al. Modulation of endometrial redox balance during the menstrual cycle: relation with sex hormones. J Clin Endocrinol Metab 2002; 87(6): 2843-2848.
13. Elhadd TA, Neary R, Abdu TA, et al. Influence of the hormonal changes during the normal menstrual cycle in healthy young women on soluble adhesion molecules, plasma homocysteine, free radical markers and lipoprotein fractions. Int Angiol 2003; 22(3): 222-228.
14. Browne RW, Bloom MS, Schisterman EF, et al. Analytical and biological variation of biomarkers of oxidative stress during the menstrual cycle. Biomarkers 2008; 11: 160-183.
15. Michos C, Kiortsis DN, Evangelou A, Karkabounas S. Antioxidant protection during the menstrual cycle : The effects of estradiol on ascorbic – dehydroascorbic acid plasma levels and total antioxidant plasma status in eumenorrheic women during the menstrual cycle. Acta Obstet Gynecol Scand 2006; 85(8): 960-965.
16. Mickelson O, Dippel AL and Todd RL. Plasma Vitamin C levels in women during the menstrual cycle. J Clin Endocrinol Metab 1943; 3(11): 600-602.
17. Hauck HM. Plasma levels and urinary excretion of ascorbic acid in women during the menstrual cycle. J Nutr 1947; 33(5): 511-514.
18. Ganong. Gonads: Development and function of the reproductive system. In: Review of Medical Physiology. William F. 13th ed. USA. Prentice-Hall Int. Inc. 1987; pp. 364-369.
19. Llewellyn-Jones D. Everywomen - A gynaecological guide for life. 4thed. The Chancer press. Great Britain: 1986: pp. 48.
20. Whitley RJ, Wayne M and Nelson BW. Endocrinology. In: Teitz Textbook of Clinical Chemistry. Carl Burtis and Edward Ashwood (editors). Philadephia: Saundera Company. 1992: pp. 1879.
21. Schisterman EF, Gaskins AJ, Mumford SL, et al. Influence of Endogenous Reproductive Hormones on F2-Isoprostane Levels in Premenopausal Women. Am J Epidemiol 2010; 172(4): 430-439.
22. Romero FJ, Bosch-Morell F, Romero MJ, Jareno EJ, Romero B, Roma MN. Lipid peroxidation products and antioxidants in human diseases. Environ Health Perspect 1998; 106: 1229-1234.
23. Sowers M, McConnell D, Jannausch ML, et al. Oestrogen metabolites in relation to isoprostanes as a measure of oxidative stress. Clin Endocrinol (Oxf) 2008; 68(5): 806-813.
24. Bednarek–Tupikowska G, Bohdanowicz–Pawlak A, Bidzinska B, Milewicz A, Antonowicz–Juchniewicz J, Andrzejak R. Serum lipid peroxide and superoxide dismutase activity in premenopausal and postmenopausal women. Gynecol Endocrinol 2001; 15(4): 298-303.
25. Lutoslawska G, Tkaczyk J, panczenco-kresowska B, Hubner-Wozniak E, Skierska E, Gajewski AK. Plasma TBARS, blood GSH concentrations, and erythrocyte antioxidant enzyme activities in regularly menstruating women with ovulatory and anovulatory menstrual cycles. Clin Chim Acta 2003; 331(1-2): 159-163.

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