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Evaluating Activity of Antioxidant Enzymes during Human Menstrual Cycle

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Abstract

Introduction: There is a complex interaction between the hypothalamus, pituitary, and ovary in the human menstrual cycle. There is increasing evidence about the role of oxidative stress on female reproductive tract.

Methods: The purpose of this study was to determine the activities of antioxidant enzymes; superoxide dismutase, glutathione peroxidase, catalase and total antioxidant capacity during the menses, follicular and luteal phases of the menstrual cycle in twenty women. In addition, the relationship between the activity of antioxidant enzyme and estradiol, progesterone, LH, FSH, and testosterone were evaluated.

Results: There was no significant difference between the activity of superoxide dismutase, glutathione peroxidase, catalase and total antioxidant capacity during the menses, follicular and luteal phases of the menstrual cycle. We found a significant correlation between LH and FSH with the activity of superoxide dismutase and glutathione peroxidase during the luteal phase ($p<0.05$).

Conclusion: Our results showed that there were not significant differences in activity of antioxidant enzymes during the menstrual cycle.

Keywords: Oxidative stress; Antioxidants; Glutathione peroxidase; Superoxide dismutase; Catalase; Menstrual cycle

Introduction

The production of the highly reactive free radicals (reactive oxygen species) is an innate trait of normal cellular metabolism(1). Reactive oxygen species (ROS) are the main trigger of tissue damage and can cause dysfunction in the regulation of enzyme activity, gene expression and transcription(2, 3). In these destructive process antioxidant defense systems -including antioxidant molecules (non-enzymatic antioxidant), such as vitamin A, vitamin E, ascorbic acid, urate, cysteamine, transferrin, taurine hypotaurine, albumin and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutaredoxin(4-7)- work together synergistically to create the right balance of oxidant - antioxidant. When this balance tends to increase the oxygen reactive species, oxidative stress occurs(8). There is a question, whether reactive oxygen species can be beneficial or not? In a response to this question can be said, ROS is a double-edged sword, which has been localized in the female reproductive tract(9). As we know it increased during the die down of the lute-

al phase, which inhibits the secretion of progesterone (10, 11). Moreover, protection mechanisms against damage by free radicals in the reproductive system are matched as well and reproductive system highly developed to use the advantage of these free radicals(1) including oocyte maturation, ovulation and luteal growth in the female reproductive system(12-14). Nevertheless, detriments are still strongly there and as a result of excessive production of free radicals or impairment in the antioxidant system oxidative stress occurs(1, 15). Studies indicate oxidative stress not only affects female fertility(example;; spontaneous abortions(16), infertility(1) endometriosis and follicular growth (17, 18)) but also has an unclear role in the period after (menopause)(1). It also plays a role in fertility decline with increasing age. Even there is growing evidence about its effects on the involvement in the pathophysiology of preeclampsia, Hydatidiform moles and birth defects(1).

Despite all these destructive processes the reproductive system tries to protect from itself. During this protection, some changes take place that need be proved. it has been shown that the rate of oxidative stress may be altered by fluctuations in the concentration of endogenous hormones during the reproductive cycle (19) and changes in the concentration of endogenous hormones are effective in a number of antioxidants(20-22). These changes in oxidative stress plasma concentration during the menstrual cycle still remain unclear.

Therefore In this study, we evaluate the activity of SOD, glutathione peroxidase, glutathione reductase, catalase and total antioxidant capacity during the menses, follicular and luteal phases of the menstrual cycle. And also, the relationship between the activity of antioxidant enzyme and estradiol, progesterone, LH, FSH, and testosterone were evaluated.

Patients and methods:

This is a longitudinal study carried out in Hamedan, Iran from 26-30 days intervals from October 2007 to October 2008. In this study, 20 medical sciences students with regular menses aged between 18-30 years old selected randomly using cluster sampling methods among those studying in Hamedan University of Medical Sciences. A written consent form was obtained from all participants and the study was approved by the ethical committee of the Hamedan University of Medical Sciences.

7cc fasting blood from each person from an antecubital vein in different menstrual phase was taken in three episodes, the first episode of blood sampling was performed on the third day of menstruation, second episode three days after termination of bleeding and third episode three days after ovulation (according to rising in body basal temperature). The blood was transferred to a heparinized tube and centrifuged for 10 minutes after separation plasma from RBCs, plasma freeze in -20C, and then RBCs washed with physiologic serum four times.

All participants were healthy and those with using any kind of OCP and steroids in the recent year and NSAID in recent 2weeks, history of smoking, any chronic diseases, history pregnancy and lactation in the recent year and BMI≤18, BMI≥25 were excluded. For data analysis repeated measurement ANOVA was used for menstrual, follicular and luteal phase, Pearson test for the relation between sexual hormones and antioxidants and statistics were calculated using SPSS (version 16).

Results:

In this study, 20 women with regular cycles participated. Three times sampling in menstrual, follicular and luteal phase was performed. In luteal phase there is positive relation between SOD and FSH, LH(r=0.42, p<0.05 r=0.54, p<0.05), glutathione peroxidase and LH (r=0.44, p<0.05).The results are presented in tables (1-5).

Activity	Menstrual phase	Follicular phase	Luteal phase	P value
Superoxide dismutase(Iu/gHb)	527 ± 1850	888 ± 1957	509 ± 1847	<0.05
Glutathione peroxidase(Iu/gHb)	14 ± 4/35	12.7 ± 37.4	13.3 ± 39.1	<0.05
Glutathione reductase(Iu/gHb)	1.8 ± 6.2	1.6 ±6.5	1.7 ± 7.1	<0.05
catalase(k/g Hb)	50.8 ± 296.8	63.3 ± 303.5	85.5 ± 306.2	<0.05

Table 1.The amount of super oxide dismutase, glutathione peroxidase, glutathione reductase and catalase

activity in RBCs in menstrual, follicular and luteal phase

Concentration	Menstrual phase	Follicular phase	Luteal phase	P value
Estradiol(pg/mL)	10.9 ± 25.0	17.9 ±39.1	45.4 ± 85.2	0.05<
Progestrone(ng/mL)	0.2 ± 0.45	1.1± 0.76	7.3 ±8.3	0.05<
LH(mIU/mL)	4.5±2.5	7.5 ± 8.1	8.6 ± 10.3	0.05<
FSH(mIU/mL)	5.1 ± 6.5	1.9 ± 6.1	2.1 ± 3.5	0.05<
Testosterone(ng/mL)	0.23 ±0.58	0.24 ± 0.60	0.22 ± 0.61	0.05<

Table 2.Estradiol, progesterone, LH, FSH and testosterone concentration in menstrual, follicular and lute-

Activity	FSH (mIU/mL)	LH (mIU/mL)	progestrone (ng/mL)	Estradiol (pg/mL)
Superoxide dismutase(U/g Hb)	0.13	0.27	0.14-	0.10-
Glutation peroxidase(U/g Hb)	0.16	0.22	0.90-	0.15
Glutation reductase(U/g Hb)	0.22-	0.020-	0.36	0.18
Catalase(k/g Hb)	0.19-	0.12	0.02	0.35
Total antioxidant capacity(mmol/L)	0.06-	0.08	0.036-	- 0.2

Table 3.The relation between estrogen, progesterone, LH, FSH activity with superoxide dismutase, glutation peroxidase, glutation reductase, catalase in RBCs and total antioxidant capacity in menstrual phase

Activity	Testosteron(ng/mL)
Superoxide dismutase(U/g Hb)	0.1-
Glutation peroxidase(U/g Hb)	0.18-
Glutation reductase(U/g Hb)	0.18
Catalase(k/g Hb)	0.25
Total antioxidant capacity(mmol/L)	0.25-

Table 4.The relation between testosterone with superoxide dismutase, glutation peroxidase, glutation reductase, catalase in RBCs and total plasma antioxidant capacity in menstrual phase (n=20)

	Estradiol (pg/mL)	Progesterone (ng/mL)	LH (mIU/mL)	FSH (mIU/mL)
Superoxide dismutase(Iu/gHb)	-0.13	-0.26	0.42*	*0.54
Glutathione peroxidase(Iu/gHb)	0.08	360.	0.44*	0.35
Glutathione reductase(Iu/gHb)	-0.16	-0.37	0.12	0.15
Catalase(k/g Hb)	0.12	0.12	-0.25	-0.20
Total antioxidant capacity(mmol/L)	0.13	-0.03	0.11	0.22

Table 5. The relation between estradiol, progesterone, LH and FSH with superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase in RBCs and total antioxidant capacity in luteal phase (n=20)

Discussion:

It is important to further elucidation the role of oxidative stress in unexplained infertility and recurrent early pregnancy losses and thus design strategies to overcome its adverse effects.

It seems that women with regular menstrual cycles are able to somehow adjust the activity of body's antioxidant defense system during the menstrual cycle no to dispose to increasing reactive oxygen species (ROS) and oxidative stress (23). Physiologic periodical changes in women with regular menstrual cycles have no effects on enzymatic and non-enzymatic antioxidant system. We can assume that in physiological changes such as menstruation and ovulation or luteal phase antioxidant system can regulate these changes. This study shows that antioxidant activity including SOD, glutathione reductase, catalase and total antioxidant capacity in menstrual, follicular and luteal phase has no significant difference and in menstrual phase there is no significant difference between steroid hormones and antioxidant enzymatic activity. Also in the study of Brown and et al in 2008 with more than 20 indicators of oxidative stress including antioxidant enzymes, lipid peroxidation markers, and antioxidant vitamins during different days of the menstrual of 9 women studied no significant differences in antioxidant activity and concentrations of these factors were found which is consistent with the results of our study(24). Although the study of Michos et al reported that total plasma antioxidant capacity during the ovulation phase significantly increased, the difference observed between these two studies is likely due to the differences in the intake of anti-oxidants in the compounds between different individuals under investigation in these two studies that need more investigation in terms of diet and the intake of antioxidants. The results showed between sex hormone levels and activity of antioxidant enzymes during bleeding phase does not exist any significant association. In other words, antioxidant enzyme activities during this stage are not influenced by sex hormones. During the follicular phase, a significant inverse association between plasma progesterone concentrations and glutathione peroxidase activity observed, and during the luteal phase statistically significant positive correlation be-

tween the concentration of LH, FSH and glutathione peroxidase with superoxide dismutase (SOD) activity (red blood cells) perceived. At this stage, a significant correlation between the concentration of LH and glutathione peroxidase activity (red blood cells), was obtained. It is possible that FSH and LH indirectly have an impact on enzymatic activity.

Michos et al evaluate total antioxidant capacity, FSH, LH, estradiol and progesterone in 13 women in the menstrual cycle. In their study, total antioxidant capacity had significant increasing during ovulation and there is a positive relation between total antioxidant capacity and estradiol in all phase of the menstrual cycle (25). It is possible that the difference between these two studies related to the difference between antioxidant consumption in cases of two studies.

However, in this study, a direct connection between estradiol concentrations and the antioxidant system did not obtain but the observed association between LH and FSH with superoxide dismutase and glutathione peroxidase also emphasized the fact that hormonal changes during the menstrual cycle are effective on antioxidant system and can protect against oxidative stress.

Massafra et al evaluated the effects of estrogen and androgen on antioxidant enzymatic activity in RBCs of 12 women they found that in late follicular phase and early luteal phase there was increasing in glutathione peroxidase activity. Also, there was a significant relationship between estradiol and glutathione peroxidase in the menstrual cycle (26).

The relation between glutathione peroxidase and estradiol in 14 women on the menstrual cycle was studied by Ha et al (27). The difference between results of studies may be due to the difference of selenium consumption in the diet of cases. This element can effect on glutathione peroxidase activity. Wira et al reported the effects of menstrual cycle on immunologic parameters. In spite of other mucosal systems, the female genital tract has mucosal immunologic system due to contact with surface antigens of sperms (28).

Conclusion:

The results of this study showed that antioxidant activity and total antioxidant capacity had no changes in the different phase of the regular menstrual cycle, but glutathione peroxidase, glutathione reductase, and catalase activity were increased in menstrual, follicular and luteal phase. This may be a mechanism of the human body against free oxygen radicals. On the other hand, we can say that physiologic system of women with regular menstrual cycle can protect the human body against oxidative stress and some part of this action is due to LH and FSH hormonal action. This question still remained, whether this antioxidant system exists in women with the irregular menstrual cycle or not? Or whether the antioxidant system can affect menstrual cycle or not? More studies are needed to respond to the questions.

Authors' Contributions:

MFS designed the study and drafted the manuscript. NM and FPM helped in manuscript drafting and data acquisition. APD and ZY helped in manuscript drafting and data analysis. All authors have approved the final version of manuscript.

Conflict of Interest Disclosures:

There are no conflicts of interest in terms of the present manuscript.

Ethical approval/Consideration:

This study was registered at ethics committee of Hamedan University of Medical Sciences, Hamedan, Iran. A written informed consent was taken from patients for participating in this study. All the personal information remained anonymous.

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