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Correlation between some pro-inflammatory markers and serum trace elements during menstrual cycle of women

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A B S T R A C T

Many physiological alterations occurs during menstrual cycle, this cycle is controlled and regulated through several hormonal mechanisms that associated with immune system. A total of 40 women aged 16–50 years were included in this study to investigate the changes occurring in the levels of some cytokines and trace minerals (interlukine-6, C-reactive protein, Zinc, and Copper). Pro-inflammatory cytokine IL-6 and C- reactive protein reveals significantly elevation in their levels in sera during menstruation. Trace minerals level in sera of women with menstruation showed significant changes ($P<0.05$) among all age groups. Changes in pro-inflammatory cytokine concentration, acute phase protein that occurs during menstruation led to changes in serum zinc and copper concentrations this may be attributed to fluctuations of estrogen and progesterone hormone levels during menstrual cycle.

Introduction

Menstrual cycle define as a chain of physiological change which occurs in fertile females of human and animals within limited time of life (Strassman, 1996). Menstrual cycle can be classified into three stages: follicular phase, ovulatory phase and luteal phase. At these phase, endometrium undergoes several histological and physiological changes. The period of menstrual cycle is different from woman to woman, but the normal time range in most fertile women is approximately 28 day. At the age of 50, the most women stop menstrual flow and entering the menopause (Hitchcock, 2008). The menstrual cycle is controlled and regulated through several hormonal mechanisms. Hypothalamus-pituitary axis on ovaries is involved in the regulation of menstrual cycle (Jones *et al.*, 1997). The immune system exerts essential role in the physiological regulation and defense mechanism of menstruation (Chisty *et al.*, 1996; Islam, 2004). Endometrium represents important site for evolutionary defense mechanisms, fertilization and maintenance of pregnancy. Infection of uterus with pathogenic microbes accounts to prevent fertilization. Leukocytes and their

enzymes act to form barrier against any infectious microbe. The branch of immune system with other cytokines also involved in the defense mechanisms (Hancock and Diamond, 2000; Cole *et al.*, 2002).

Materials and Methods

A total of forty women aged 16–50 years old were recruited from college of science for women and of Babylon teaching hospital for gyniatrics and obstetric.

Blood samples drown from women with regular menstrual cycle, at both follicular and luteal phase. Women of this study have free from chronic disease and no use of contraceptive drugs. Determination of interleukin-6 (IL-6) involved three steps to determine IL-6 according to kit instructions supplied by Ray Bio Company, Italy. Determination of C-reactive protein (CRP), two methods were used to determine CRP, the first was involved qualitative method and performed by latex material (Plasmatic laboratory Company). The second method included semi qualitative determination of CRP and tube agglutination test. Measurement of serum copper and zinc was done according to instruments that supplied by LTA, Italy. In this method, serum copper reacts with chromogen dye resulted in blue complex color formation; its intensity is proportional directly with zinc concentration (kit supplied by LTA, Italy). All data were analyzed using completely randomized design and $P<0.05$ was used as lowest significant deference to compare among different groups.

Result and Discussion

Enzyme linked immunosorbent assay (ELISA) showed an increase in serum IL6 concentrations of women during menstrual cycle. There is a significant enhancement ($P<0.05$) in the levels of interleukin-6 in the age group of 46–50 years old recorded a high level (40.00pg/ml) where as the age group (20–16) years old recorded lower level (12.91pg/ml) (Table 1).

C-reactive proteins shows increase in their value in all women at menstrual cycle. These values were appeared significantly higher in all age group with noticeable variation in their concentrations among all age groups (Table 2).

The age group (46–50) years recorded a higher level in a comparison with age group (16–20) years. Mineral levels in serum of women at menstrual cycle show variation in their values. Serum zinc level revealed high (12.35 g/dl) at age group 26–30, whereas age group 36–40 revealed higher level of copper 23.28 g/dl in sera of women at menstrual cycle (Table 3).

Serum levels of C- reactive protein and minerals fluctuated with the concentration of interleukin 6, results pointed out that zinc and copper concentration for women with menstruation were fluctuated and correlated with the changes of IL-6 concentration (Figure 1).

Results showed a significant changes in IL-6 levels ($P<0.05$) of all age. The age group 46–50 years old showed a higher level of IL-6 than other age groups.

Previous studies showed that estrogen hormone has dominant receptor located at different cells of the body such as T-Cell, B-cell, bone marrow, osteoblasts and osteoclast (Becker, 2006). These studies indicated that deficiency in the production of sex hormone (estrogen) induces B-cell to release different inflammatory cytokines especially IL-6, IL-17 and tumor necrosis factor α (INFα). These cytokines in turn

interact to differentiate and stimulate bone resorptive cell (osteoclasts), but IL-7 acts to inhibit such cells (Weizmann and Pacific, 2005). This phenomenon led the researchers to consider the osteoporosis occurring with age in women as specific autoimmune diseases resulted from estrogen deficiency and this consistent with data obtained from present study. For instance, with age several diseases developed such as diabetes mellitus type-2, thyroid disorders, cardiovascular disease and bone diseases. The prevalence of these diseases had been found associated with a high amount of cytokines production especially TNFα and IL-6. It has been found that the adipose tissues which to become accumulate with age in turn tend to release high levels of IL-6 in women have obesity (Mohammed-Ali *et al.,* 1997; Bastard, 2000). Other studies showed that the production of cytokines have been increased directly with body mass index (Kovacs and Olson, 1996). Bantalena *et al.,* (1994) confirmed a relationship between thyroid disease and levels of cytokines. However, through experimental studies showed that the levels of tri iodothyronine (T3) in euthyroidism decrease because negative feedback mechanisms of IL-6 on hypothalamus –pituitary-thyroid axis. Study of Medonnell and Norris (2002) indicated that estrogen deficiency resulted in elevation of IL-6 in blood circulation. The present study included determination of C-reactive protein (CRP). As we know that CRP used as indicator for inflammatory processes within the body. Previous studies found that estrogen hormone acts to elevate the amount of such proteins and progesterone work in opposite line (Wander, 2008). Other studies confirmed that factor (Leptin) and menstrual cycle during follicular phase (Mannucci *et al.,* 1999; Raid *et al.,* 1998).

Also, it has been found increased CRP to peak level in mid cycle and 31% in luteal phase (Termaa *et al.,* 1998). As for other studies showed there is a negative effect of estrogen on CRP while progesterone exerts positive effects (Vegato *et al.,* 2010; Prestwood *et al.,* 2004). The present study showed variations in the levels of serum zinc (Zn^{+2}) in different age groups within menstrual cycle. It had been found a high level of Zn in age group 26–30 years old during luteal phase, and low level of Zn in age group 41–45 years old during follicular phase. Zinc and other minerals are as cofactor for several enzymatic systems. Liver, bones, red blood cells have profound amount of zinc pools. Previous studies indicated that zinc becomes exhaustive with age and its reservoirs in RBCs, bones and liver are decreased (Buchinger *et al.,* 1988; Dursun *et al.,* 1995; Simck *et al.,* 1997). Other studies showed that deficiency of zinc which acts as cofactor with anti-oxidant enzymatic system such as Cu, Zn-SOD enzyme, in turn increase oxidative stress (Iwas *et al.,* 1993). Other researchers showed that with age there is increased zinc excretion with urine (Dolev *et al.,* 1988). Study of Chen *et al.,* (2000) suggested that there is a correlation among thyroid hormones-leptin and some trace minerals in the body. The present study suggests that mineral of the body because of hormonal imbalance.

Data obtained from present study showed a significant differences in copper concentration ($P<0.05$) among age groups. The age group (46–50 years old) recorded higher levels in a comparison with that copper (16–20). Previous studies confirmed that copper deficiency is a predisposing factor for prevalence of autoimmune diseases especially in women, since, women need to copper more than men. The copper element is used as cofactor for enzymes responsible for the conversion of progesterone to estrogen hormone, as for

males need zinc to convert progesterone to testosterone (Klevay and Moor, 1990; Lu *et al.,* 1990). Other experimental studies showed that treatment of patients affected with Wilson's disease by using d-Penicillamine causes to chelate copper out of the body and resulted in developing autoimmune disease such as lupus. Ecological studies confirmed that some heavy minerals such as Cadmium, aluminum, and mercury become accumulated within the body at advanced age lead to chelate copper (Bjork *et al.,* 2000). Uany *et al.,* (1998) showed that copper essential role in activation of cellular immunity and vital role of white blood cells functions.

Trace mineral also have been found to perform multiple physiological and immune -biochemical function within the body. Copper and zinc have vast functions represented through DNA –replication, cell division and hormone biosynthesis, Trace mineral act as a cofactor to several enzymatic system engaged with vital functions of the cells (Harris, 1997; Cousins, 2006). In conclusion, sex hormones play essential role in regulating biochemical and immune system during menstrual cycle.

Table.1 Levels of IL6 for different age group women at menstrual cycle

Age groups (years)	**IL-6 levels (pg/ ml)**
21–25	12.91± 100.1
26–30	23.1± 100.1
31–35	37.87±223.54
36–40	23.11± 71.29
41–45	31.22± 16.6
46–50	40.0± 300.0
LSD at P< 0.05 =50.124	

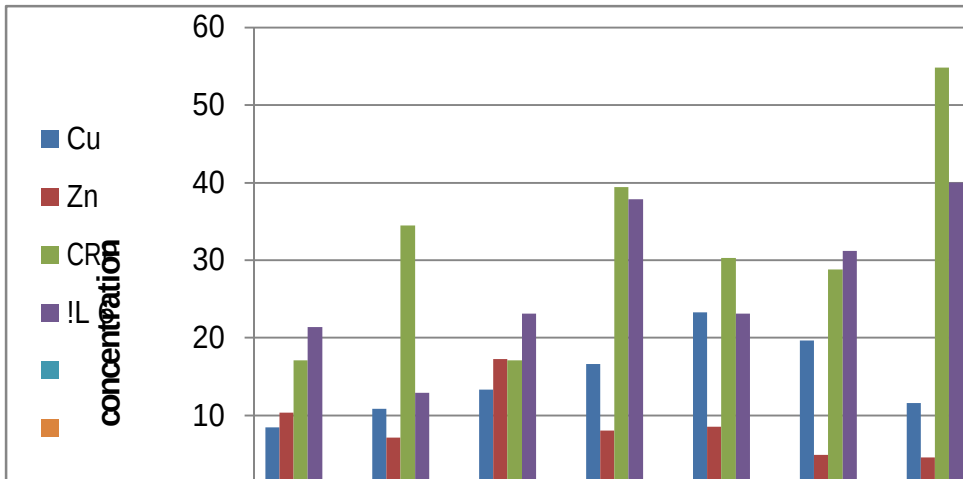
Table.2 Levels of C- reactive proteins for different age group women at menstrual cycle

Age groups (years)	**C-reactive proteins levels**
	Women with M.C
16–20	17.142±6.33
21–25	34.50± 11.4
26–30	17.14± 1.71
31–35	39.42±15.86
36–40	30.85± 13.5
41–45	28.8± 15.96
46–50	54.85± 11.37
LSD at P< 0.05 = 12.96	

Table.3 The levels of serum zinc and copper g/dl at different age groups of menstrual cycle

Age group year	Mean ± stander error	
	Serum zinc	Serum copper
16–20	10.45±32.59	8.45±115.47
21–25	7.13±28.05	10.83±125.95
26–30	12.35±40.35	13.30±128.31
31–35	8.03±23.98	16.65±134.30
36–40	8.59±32.92	23.28±150.22
41–45	4.92±15.56	19.64±125.46
45–50	4.63±17.52	11.61±169.52

Figure.1 Relationship of IL6 with minerals and CRP concentrations



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