Alterations in Some Infertility Related Biomarkers in the blood of Women with primary Infertility

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Abstract

The aims of this study focus on the role of oxidative stress and immunoactivity in female infertility and revealed the correlation between them. A total of 120 infertile women with different etiologies and 45 fertile women as a control were investigated, their age ranged between (16-45) years old. For all women the BMI was calculated, levels of interleukin- 1β (IL- 1β) and interleukin-8 (IL-8) were estimated by ELISA method, Malondialdehyde (MDA) and catalase activity levels were determined spectrophotometrically, and sodium, magnesium and zinc were estimated by inductively coupled plasma optical emission spectroscopy (ICP-OES). The obtained data was revealed non- significant difference ($p<0.05$) in BMI between infertile and fertile groups. Also there was non- significant elevation in the mean ±SD of serum IL-1β (3.05±0.73) and IL-8 (35.00±5.95) between infertile group and fertile one.

A significant increase in MDA level (4.23± 1.79) and a significant decreased in catalase activity (4.33± 0.15) was found between infertile and fertile groups. According to the results, infertile group exhibited a significant decreased in the levels of Na (2.87± 0.35), Mg (20.59± 3.18) and Zn (0.46± 0.19) as compared with the fertile group.

Age was positively correlated with BMI (r= 0.451) and IL- 1β (r=0.258). Positive correlation was also found between Na and Mg (r=0.350), Na and Zn (r=0.248), and Mg with Zn (r=0.319). On another hand, there was a negative correlation between MDA with Mg (r= -0.219) and Zn (r= -0.255). In conclusion, the decreased in serum antioxidants levels was influenced female infertility and there was no correlation between interleukins and oxidative stress in women with primary infertility.

KEYWORDS: Interleukin-1β, Catalase, Malondialdehyde, Zinc, Infertility.

1. Introduction

Infertility is known as a failure to achieve a clinical pregnancy after one year or more of regular unprotected sexual intercourse, so infertility is not a disease with a particular etiology (Aflatoonian *et al.*, 2011). Or infertility is the inability to carry pregnancy over one year despite adequate, regular, unprotected sexual intercourse. Woman, man, or both disorders can be cause infertility (Jenkins and Carrell, 2012). Global estimates indicate that nearly 72 million couples expertise fertility problems (Khan *et al.*, 2015). Female infertility is classified into primary and secondary infertility (Adamson *et al.*, 2011). Infertility may produce by oxidative stress (Sharma *et al.*, 2013) and increasing in the secretion of pro-inflammatory cytokines such as interleukin-1β (IL -1β) and interleukin-8 (IL -8) (Ahn *et al.*, 2015).

Catalase enzyme has a major role in the first defense line of enzymatic antioxidant and very important enzyme in reproductive reactions. Catalase enzyme catalyzes hydrogen peroxide (H_2O_2) to form water and molecular oxygen, it possesses largest turnover number of all enzymes; each molecule of catalase enzyme can convert millions molecules of hydrogen peroxide each second (Goodsell, 2004 and Fang, 2013), despite catalase enzyme is distributed over the cell, it concentrates in peroxisomes and mitochondria (Franks *et al.*, 2008).

Malondialdehyde (MDA) is an endogenous product of enzymatic and free radicals -induced lipid peroxidation (Niedernhofer *et al.*, 2008). Therefore, MDA levels measurement can be widely used as an important indicator of oxidative stress and lipid peroxidation (Jetawattana, 2005). It was observed that erythrocyte MDA levels were significantly increased but erythrocyte reduced glutathione level and catalase activity were significantly decreased in patients with polycystic ovary syndrome when compared to control group. Serum homocysteine levels were significantly higher in polycystic ovary syndrome patients than in the controls (Mohan and Priya, 2009). Catalase activity level were significantly decreased in females with primary infertility when compared with those of the fertile group, but MDA levels were significantly increase in females with primary infertility when compared with those of the fertile group (Hussain *et al.*, 2013).

The aim of the current study was to evaluate the correlation among the studied biomarkers, and to verify the effect of age on body mass index (BMI), IL- 1β, IL- 8, catalase, MDA, Na, Mg and Zn levels in the sera of women with primary infertility.

2. Materials and Methods

2.1 Subjects

We investigated 120 infertile women and 45 fertile women as control, with age ranging between 18-45 years old, who attended the Sheray Naqeeb hospital in Kalar city. The exclusion criteria include none of the women had previous ovarian surgery, no contraceptive methods, no receiving sex steroids or any drug known to affect ovarian function for at least 2 months, and all of them with primary infertility, also BMI was recorded, it was calculated as the ratio of body weight in Kg/ heigh in meter square. They were subdivided into three groups according to the age, as following: **Group 1:** It has been contained 55 women aged between 16-25 years, 40 of them infertile and 15 were fertile. **Group 2:** It has been contained 55 women aged between 26-35 years, 40 of them infertile and 15 were fertile. **Group 3:** It has been contained 55 women aged between 36-45 years, 40 of them infertile and 15 were fertile.

2.2. Blood Collection

All women had blood collecting on the second day of menstrual cycle, venous blood samples were collected from them in gel separated tubes. The sera were separated by centrifuge (Hittch, Germany) at 3000 rpm for 10 minutes; then transferred by micropipette (eppendorf , Germany) to eppendorf tubes and stored at (-30) ºC in deep freezer (LABKITS, China).

2.3 Biochemical Assays

A. Estimation of IL- 1β and IL-8

The kits provided by (KOMA BIOTECH, Korea), they are a quantitative kits, and use Sandwich-ELISA assay. The optical density (OD) is measured using microplate reader (Dynex, USA) at 450 nm.

B. Estimation of MDA Level

The analysis of MDA by 2-thiobarbituric acid assay has been employed in this study for the assessment of lipid peroxidation. Under acidic and boiling conditions, one molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid to yield a chromophore, the absorbance read spectrophotometrically at 532 nm (Wallace and Kelsey, 2010).

C. Estimation of Catalase Enzyme Activity

Catalase enzyme activity was measured following the method of L. goth (Goth, 1991). The rate of consumption of H_2O_2 is relative to the concentration of catalase enzyme in the serum. The activity was determined spectrophotometrically by measuring the decrease in H_2O_2 absorbance at 240 nm**.**

D. Estimation of Na, Mg and Zn Levels

The inductively coupled plasma optical emission spectroscopy (ICP-OES) (Spectro Arcos,Germany) used for elemental analysis. Serum sample was gone under deproteinization process; the upper layer supernatant was transferred to a test tube for measurement of Na, Mg and Zn.

2.4 Statistical Analysis

Statistical analysis of the data was performed using significant F test and ANOVA. The multiple comparisons between the means were analyzed by Duncan Post Hoc test and a value of P <0.05 was statistically considered significant**.** Data were expressed as Mean ±SD.

3. Results

A. Infertility Effects on Biomarkers Levels

In the current study, the obtained data was verified that BMI has non-significant difference (p<0.05) between infertile and fertile groups (29.10±4.64 and 27.30±4.57), shown in (Figure 1), there was non- significant elevation in the level of serum IL-1β between infertile (3.05±20.73) and fertile group (2.03± 0.53), as well as IL-8 level was not significantly increased in infertile group (35.00±5.95) as compared with fertile group (24.65± 3.27), shown in (Figure 2).

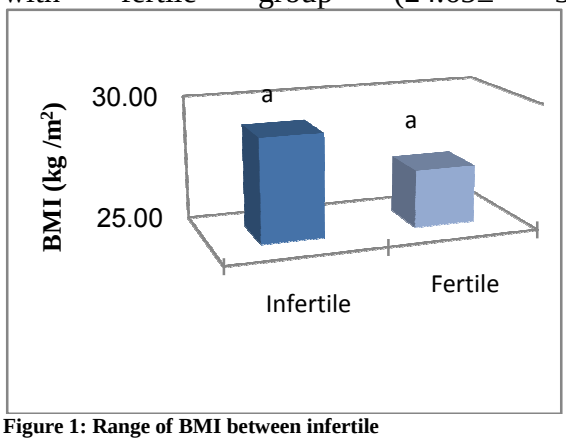

Figure 1: Range of BMI between infertile and fertile groups

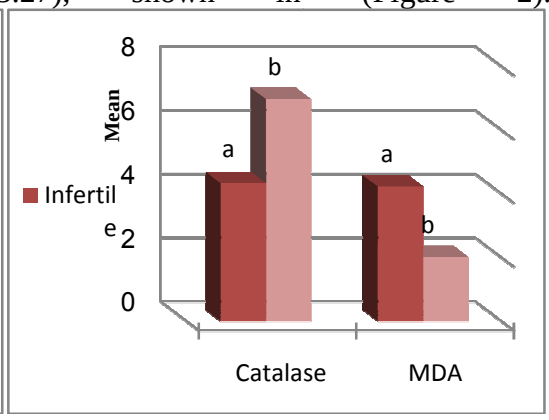

Figure 2: Levels of MDA and catalase activity between infertile and fertile groups

The data demonstrated a significant increase in MDA level in infertile group (4.23± 1.79) as compared with fertile group (2.02± 0.51), and a significant decreased in catalase activity between infertile (4.33± 0.15) and fertile (6.98± 2.11) group, see

(Figure 3). According to (Figure 4) the results demonstrated that in infertile group the levels of Na (2.87± 0.35), Mg (20.59± 3.18) and Zn (0.46± 0.19) were decreased significantly as compared with the fertile group.

These results revealed that infertility has a positive relation with the levels of BMI, IL-1β, IL-8, and MDA, and a negative relation with the catalase activity and the levels of Na, Mg and Zn levels.

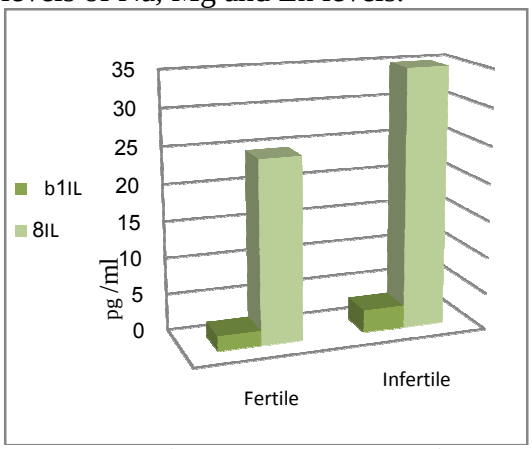

Figure 3: Levels of IL- 1β and IL- 8 between infertile and fertile groups

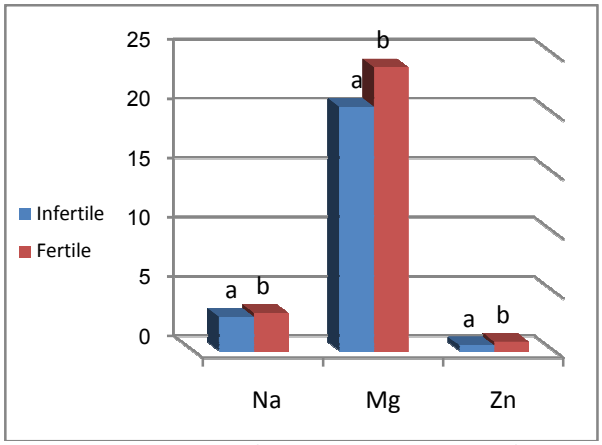

Figure 4: Levels of Na, Mg and Zn between infertile and fertile groups

B. Age Effects on Biomarkers Levels

According to the data that were shown in (Figure 5) the level of BMI in age group 3 (31.631±4.652) exhibited a positive significant ($p<0.05$) difference with both age groups 1and 2. The (Figure 6) was shown that the level of IL-1β was significantly increased in age group 3 (4. 15± 1.15) as compared with age groups 1(2.30± 0.74). Whereas, the level of IL-8 was increased in significant in age group 2 (47.31± 6.03) when compared with age groups 1 and 3 (28.83± 3.43 and 28.88± 5.69) respectively.

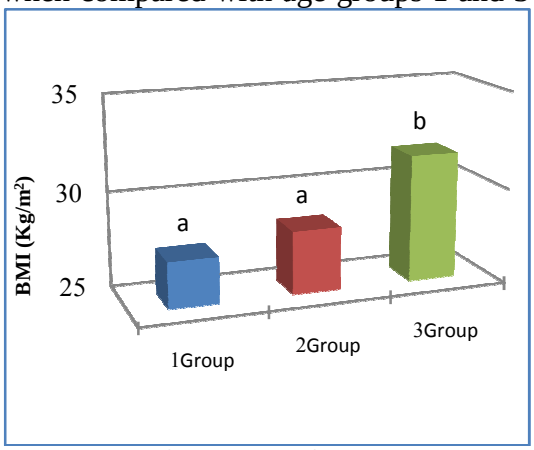

Figure 5: Range of BMI among infertile groups in relation to age

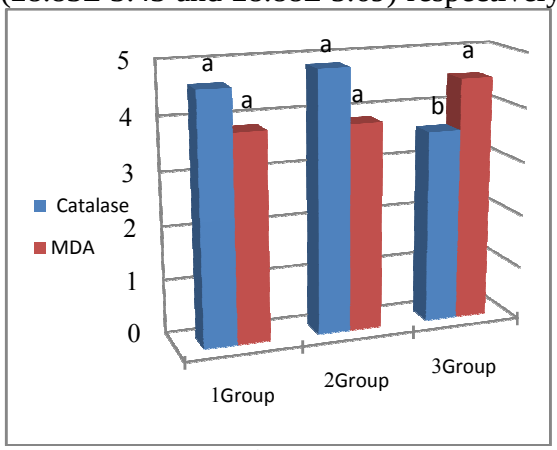

Figure 6: Levels of MDA and catalase activity among infertile groups in relation to age

As illustrated in (Figure 7) the MDA level were not significantly increased among the age groups of infertile women, whereas the evidence signified that was a significant reduction of catalase activity in age group 3 (3.57 ±0.79) comparing with age group 2.

The levels of Na, Mg and Zn were not significantly changed in all age groups of infertile women, shown in (Figure 8). These results revealed that age had only significant effect on BMI, IL-1β, IL-8 levels and catalase activity.

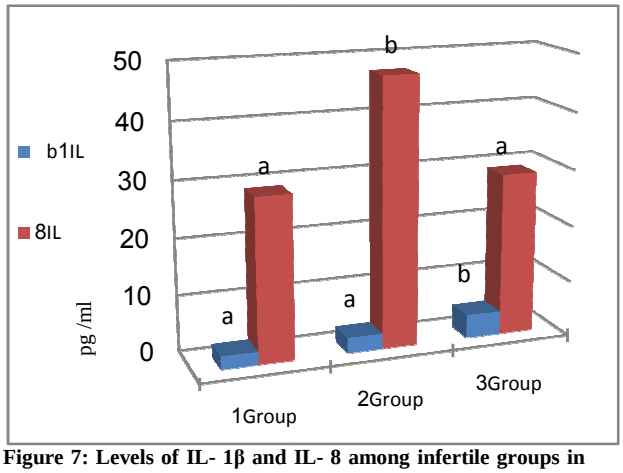

Figure 7: Levels of IL- 1β and IL- 8 among infertile groups in infertile groups in relation to age

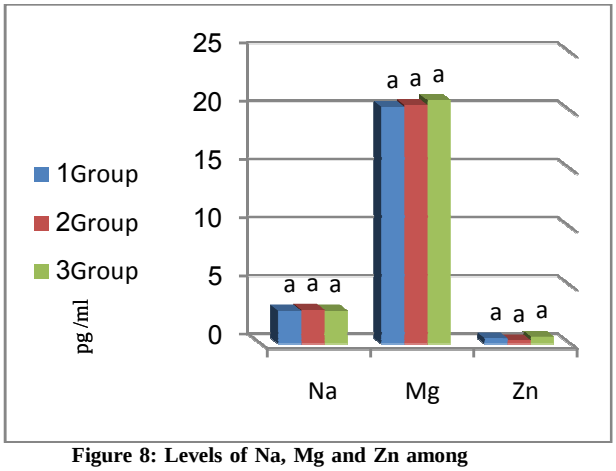

Figure 8: Levels of Na, Mg and Zn among relation to age

C. Correlations among the Studied Biomarkers

In this study, Pearson correlation was used to investigate the relationships between the biomarkers. Age showed a positive correlation with BMI (r= 0.451) and IL- 1β (r=0.258). Positive correlation was found between Na and Mg (r=0.350), Na and Zn (r=0.248), and Mg with Zn (r=0.319). On another hand, there was a negative correlation between MDA with Mg (r= -0.219) and Zn (r= -0.255).

4. Discussion

Decreasing in antioxidants activities and increasing ROS levels is a good marker of oxidative stress which is effect on female fertility (Sharma *et al.*, 2013). Our result agreed with Ogbuji study in (2010) has been compared obese women (have BMI more than 30 kg/m^2) with normal-weight patients; obese women patients had a higher prevalence of infertility and higher rate of early miscarriage and congenital anomalies. Since ROS and antioxidants are closely in balance to get a healthy body; we examine the levels of catalase and MDA.

MDA represents the end product of polyunsaturated fatty acid oxygenation, is commonly used as the lipid peroxidation level marker and the presence of oxidative stress (Grotto *et al.*, 2010). In our study MDA increasing may be explained by the increased ROS production as a result of higher lipid peroxidation, also according to previous studies there is a direct relation between increased MDA and decreased TAC (Farzadi *et al.*, 2013).

The present results found that decreased catalase activity may participate in the infertility because previous researches proved that antioxidants prevented apoptosis effectively, and the depletion of reduced glutathione causes apoptosis in many organs such as the corpus luteum and thus preventing pregnancy (Farzadi *et al.*, 2013). ROS raises follicles apoptosis, while reduced glutathione and FSH are necessary for follicles growing. FSH stimulation increases the levels of estrogen and exciting the generation of catalase in the dominant follicle, and preventing apoptosis (Behrman *et al.*, 2001). Women with endometriosis often have high macrophage levels which may produce excessive ROS in the peritoneal medium, this increased ROS levels negatively alter fertilization ability and embryonic development (ALL-Ahmed *et al.*, 2015).

The significant reduction in catalase activity in age group 3 comparing with age group 2 in our study, was accorded with the study of (Appasamy *et al.*, 2008) that found there was no significant relationship between age of the woman and plasma

TAC and the study of (Hussain *et al.*, 2013) that was observed a negative impact of age on catalase values in infertile women which categorized into three age groups. Likewise, catalase activity exhibited significant decreases in three age related groups of patients as compared to those of the control groups. The reason may due to increased iron levels in body at menopause which lead to increased iron stores in the body and thus may induce oxidative imbalance. Menopause also causes decrease in estrogen and lack of its protective effects of endometrium against ROS damage (Kayar *et al.*, 2015).

Based on these documented results, we can prove that oxidative stress and antioxidants play a potent role in regulation of female reproduction and that oxidative stress was increased with aging as antioxidant defense system has been declined. Therefore, oxidative equilibrium may persist throughout infertility.

Additional research found that sodium bicarbonate improved cervical mucus viscoelasticity and sperm penetration, when compared with basic sodium chloride (Everhardt *et al.*, 1990). In women, gently saturating the vagina and cervix with organic sodium bicarbonate may positively impact vaginal ecology, and increasing vaginal mucus conductivity thereby promoting conception (Dittman, 2008).

The significant decreased magnesium concentration in the current study may participate in infertility because magnesium deficiency associates with variable complications of female reproductive system such as the production and function of sex hormone, the risk of miscarriage and pre-term birth. When magnesium levels decrease, estrogen cannot bind to its receptor due to the importance of magnesium in estrogen receptor binding process. Magnesium also plays a principal role in modulating the process of FSH binding to its receptors on ovarian membrane, ant its deficiency is also result in reduced legibility of fallopian tube in consequence of increasing smooth muscle cell contraction in the tube wall (De Ridder and Gossen, 1988).

As the present study has given interest in the role of zinc in female infertility, many studies have paid particular attention to zinc role in human reproduction. Our results revealed that serum zinc concentration was significantly reduced in infertile group in comparison with the fertile group, this showed that zinc deficiency can play a negative significant role in female infertility and this was related to other previous studies demonstrated the same like (Rajeswari and Swaminathan, 2015) study, this low concentration may involve in infertility because it is part of superoxide dismutase composition and of those proteins that repair damaged DNA, or it cause hypogonadism and decreases in the development of secondary sexual characteristics (Zhao *et al.*, 2016). The current result was corresponded with (Kalu et al., 2007) study on infertile women, which found that serum level of IL-1β was not significantly different in the patients and controls.

Indeed, IL-1β levels are reported to be decreased in endometrium of women with recurrent miscarriage, suggesting that a fail in activating the pathways downstream of IL-1β may contribute to pregnancy failure (Rossi *et al.*, 2005). Also it was seen that any increasing of follicular fluid IL-1β level was associated with fertilization. Previous studies suggested that ability to generate a healthy fetus and successful in vivo fertilization occur in the oocytes in follicles with lower concentration of IL-1β in follicular fluid (Revelli *et al.*, 2009), the reason may due to IL-1β role in suppressing estradiol and progesterone hormones release from granulosa and luteal cells (Mahdi, 2011). The level of IL-8 we found within in our study is

lower than that recorded in some previous studies, whereas correspond to other studies. IL-8 is cytokine produced by T lymphocytes and macrophages, it also produced by numerous ovarian cells like granulosa cell, theca cell and stromal cells (Malizia *et al.*, 2010).

the level of IL-1β was significantly increased in age group 3 as compared with age groups 1 and 2, this finding is agree with (Malizia *et al.*, 2010) study who reported that follicular fluid IL-1β concentration is in positive correlation with infertile women age. Anti- IL-8 antibody treatment has been used to inhibit cell proliferatioin because IL-8 work as stimulator of follicular cell proliferation and preparer in formation of corpus luteum therefore its concentration increased, it also work as attractant for neutrophil from blood and attachment of them to endothelial cells of the ovaries. The most important function of IL-8 related to ovulation.

Regarding to correlation between age and BMI, the weight is increased with age due to high levels of leptin hormone which works in the neuroendocrine sensation to starvation. in normal weight women, most obese persons have high serum leptin concentrations as a result of adipocytes expansion that induced by diet, on another hand low leptin concentrations was seen weakened individuals such as patients with type 1 diabetes, this low concentration possibly due to increased circulating levels of free fatty acids from lipolysis (Chou and Mantzoros, 2014).

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