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Correlation between Levels of Serum Antioxidants and Numerous Hormones In Primary Infertility of Women

Sundus Majeed Hamza[*1], Saheb Jumaa Abd-alrahman[2], Saleh Muhammad Raheem[3]

*[1]Biology Department, Garmian University, Iraq
[2,3]Biology Department, Tikrit University, Iraq

ABSTRACT

The aim of this study was to evaluate serum antioxidants and reproductive hormones levels in primary infertile women, and to reveal the correlation between them. A total of 120 infertile women with different etiologies and 45 fertile women as a control were investigated. For all women, the levels of LH, FSH, TSH, prolactin, E2, progesterone, anti-mullerian hormone, inhibin B and leptin were estimated. As well as the levels of serum SOD activity, TAC and GSH concentration were estimated. The current results were revealed that the levels of LH and FSH, TSH, progesterone and leptin were non- significantly increased, while there was a significant increase in prolactin and E2 levels and a significant decrease in anti-mullerian hormone, inhibin B, TAC, SOD and GSH levels in infertile group as compared with fertile group. There was a positive correlation between SOD and AMH (r= 0.231) and inhibin B (r= 0.364) and negative correlation with E2 (r= -0.269). TAC exhibited a positive correlation with inhibin B (r= 0.368) and negative correlation with FSH (r= -0.244). GSH had a positive correlation with AMH (r= 0.274) and inhibin B (r= 0.223). In conclusion, serum antioxidants levels were positively effect on anti-mullerian hormone and inhibin B levels in serum of women with primary infertility and serum inhibin B is a good diagnostic marker for antioxidants imbalance in women with primary infertility.

Keywords: Super oxide dismutase, Total Antioxidant Capacity, Inhibin B, Anti-Mullerian Hormone and Infertility

I. 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 [1]. Globally estimation indicates that nearly 72 million couples expertise fertility problems. Infertility has been classified into primary and secondary infertility [2].

Oxidative Stress is a disruption in the balance between antioxidants and pro-oxidant molecules. Oxidative Stress increases when the generation of ROS and other radicals like RNS overrides the scavenging ability of antioxidants, either in result of the excessive production of ROS or inadequate amounts of antioxidants [3]. Antioxidants are a family of vitamins, minerals and other nutrients that help to protect the body from the oxidative damage. They work as a defense system. Glutathione is present inside and outside the cells in human body and its effects on oocyte and embryo quality. Follicular fluid GSH is found in high concentrations of different sized follicles (small, medium and large); and may act as an antioxidant to protect oocytes from free radicals during oocyte growth and maturation [4]. Peritoneal fluid from patients with endometriosis has been shown to exhibit inadequate antioxidant defenses, including low total antioxidant capacity (TAC) and significantly reduced levels of individual antioxidant enzymes such as superoxide dismutase (SOD). It was found that infertile women with endometriosis have lower concentrations of SOD than fertile controls and total thiol level is used as a predictive factor of TAC [5].

Inhibin-B is produced by granulosa cells of preantral and early antral follicles, and its levels are usually high during the follicular phase of the menstrual cycle and

low during the luteal phase [6]. Inhibin-B is positively in combination with 17β-estradiol and with the number of oocytes, and it correlates with the pregnancy rate in patients receiving treatment with in-vitro fertilization [7]. There is a significant decrease in inhibin B levels and number of oocytes retrieved in patients with endometriosis than in the healthy group. It peaked in the mid-follicular phase or on the day of hCG administration [8]. anti-mullerian hormone (AMH) is produced by granulosa cells from pre-antral and antral follicles and its levels relatively remain stable during the menstrual cycle. AMH main role is to inhibit follicular development from primordial to primary follicular stages [9]. AMH seems also to be a better predictive of menopause than other markers such as inhibin B [10].
The aim of the current study was to evaluate the correlation between SOD, TAC and GSH with many sex hormones, and to verify the effect of age on studied antioxidants and hormones levels in women serum with primary infertility.

II. METHODS AND MATERIAL

A. Subjects

We investigated 120 infertile women and 45 fertile women as control, 18-45 year of age, 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 smoking, 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 the body mass index (BMI) was recorded.

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.

B. Blood Collection

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

C. Biochemical Assays

1) Estimation of Reproductive Hormones

LH, FSH, prolactin, TSH, Progesterone and E_2 assays were achieved by electrochemiluminescence immunoassay (Roche-Hitachi Cobas e 411) using quantitative kits (Cobas, Japan).

2) Estimation of AMH

AMH was estimated by using Human AMH (Anti-Mullerian Hormone) ELISA Kit (MyBioSource, USA). This kit is quantitative; the detection depends on the reaction between biotinylated detection antibody and Avidin-HRP conjugate which appears as a blue colored solution. The optical density (OD) is measured using microplate reader (Dynex, USA) at 450 nm. The concentration of AMH in the samples is calculated by comparing the OD of the samples to the standard curve prepared using professional software.

3) Estimation of Inhibin B Hormone

Inhibin B hormone was estimated by using Human INHB (Inhibin B) ELISA Kit (MyBioSource, USA). This kit is quantitative; In the presence of inhibin B in the samples, the combination of biotinylated detection antibody specific for inhibin B and Avidin-Horseradish peroxidase (HRP) conjugate will appear as a blue color. The OD is measured using microplate reader at 450 nm.

4) Estimation of Leptin Hormone

Leptin hormone was estimated by using Leptin ELISA Kit (LDN, Germany). This enzyme immunoassay test based on conjugation of a monoclonal antibody specific for leptin in the microwell plate and another monoclonal antibody specific for a different epitope of leptin with biotin, forming a blue colored compound according to the amount of leptin present. The absorbance was read using microplate reader at 450 nm.

5) Estimation of SOD Activity

SOD activity is estimated by using SOD Assay Kit-WST (Dojindo, Japan). SOD Activity was measured by inhibiting the reduction of nitroblue tetrazolium to produces a water-soluble formazan dye with the superoxide anion using microplate reader (StateFax, USA) at 450 nm.

6) Estimation of TAC

TAC concentration was estimated by using TAC Fast Track (LDN, Germany). This assay is based on the reaction of peroxides with peroxidase and the reaction of tetramethylbenzidine to form a blue colored compound, it spectrophotometrically measured at 450 nm.

7) Estimation of Reduced Glutathione

Reduced glutathione (GSH) in the serum was estimated according to the method of Moron et al. [11], the reaction of GSH with 5,5-dithio-bis (2 -nitrobenzoic acid) [DTNB] gives an yellow colored compound that absorbs spectrophotometrically at wavelength of 412 nm.

D. Statistical Analysis

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

III. RESULTS AND DISCUSSION

Although of the physiological role of ROS during ovulation and regulation of ovarian mesenchyme growth [12], increased ROS causes oxidative stress which is involved in numerous pathological conditions of female infertility [13].

The results that were shown in Fig. (1) revealed that the levels of LH, FSH and TSH hormones were increased but not significantly, while there was a significant increase ($p<0.05$) in prolactin level in infertile group when compared with fertile group, This mean that infertility has positive effects on the levels of pituitary hormones, our results in agreement with that of [14]. Fig. (3) demonstrated that the levels of E2 and leptin were increased, while the levels of AMH and inhibin B hormones were significantly decreased. These hormonal imbalances of (LH, FSH and prolactin) have an impact on ovulation and menstruation because FSH and LH are needed for follicular maturation at the beginning of the monthly cycle, hence LH stimulates theca cells to produce estrogens and FSH stimulates recruitment of secondary ovarian follicles and the secretion of estradiol from granulosa cells [15]. Increasing LH may occurs in result of physiological changes in the endometrium [16], also hypothalamic-pituitary axis disorders increases the pulsatic releasing of LH [17]. Increasing FSH levels in age group 3 is due to decrease the antral follicle count which not produces enough inhibin B to reduce FSH levels. Regarding the decreased of FSH and LH hormones levels when prolactin increased in the age group 2, shown in Fig. (2), may due to the high level of prolactin that can work at both central and ovarian lines [18]. If high levels of prolactin present, the ovulation may be inhibited due to the inhibition of GnRH secretion and this interference with hypothalamic-pituitary-gonadal axis through a positive feedback effect on dopamine secretion. Increase dopamine reduces GnRH secretion by suppressing arcuate nucleus function in the pituitary gland [19].

It was also observed that ROS participates in the loss of sensitivity of granulosa cells to LH and FSH hormones, in the loss of steroidogenic function [20] and damage to DNA of ovarian epithelium or cell apoptosis.

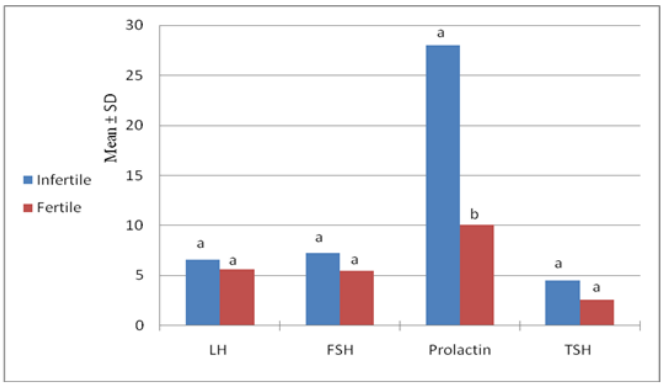

Figure 1. Pituitary gland hormones levels in infertile and fertile group

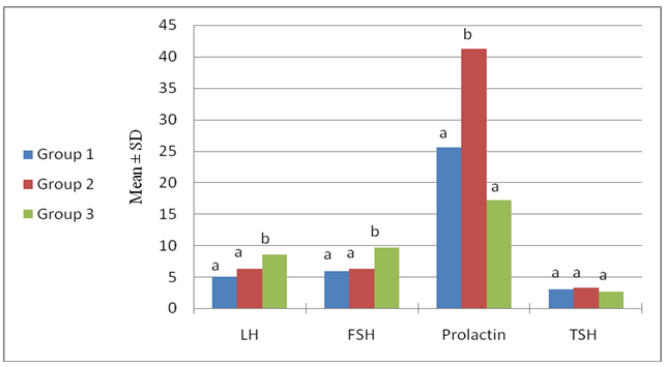

Figure 2. Pituitary gland hormones levels among infertile groups in relation to age

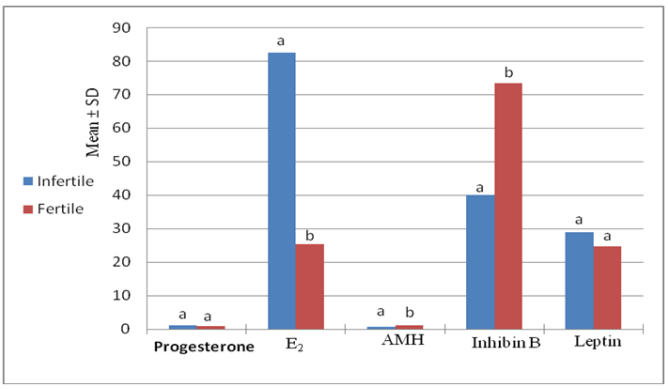

Figure 3. Reproductive hormones levels in infertile and fertile group

In Fig. (4) our results found no significant decline in the serum AMH level in age group 3, this result was compatible with Serdar Aydın et al. (2015) study whose revealed that serum concentrations of AMH decrease with advanced age due to concomitant decline in the number of primordial follicles, intern decrease the number of granulosa cells [21]. Although in Fig. (4), decreased inhibin B corresponding with age makes it to be a marker of ovarian activity rather than ovarian reserve [10].

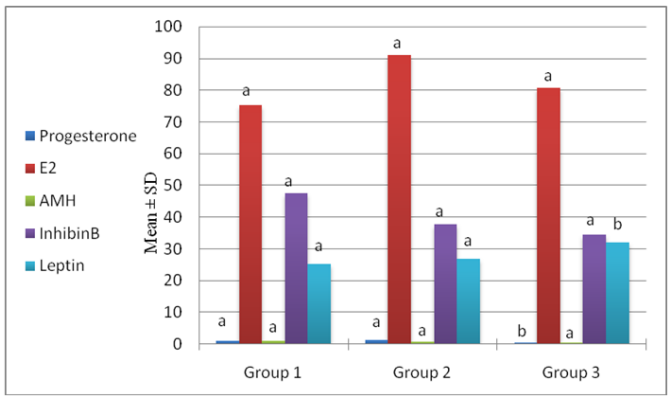

Figure 4. Reproductive hormones levels among infertile groups in relation to age

In the current study, the results were obtained demonstrated a significant decreased in each of TAC, SOD, catalase activity and GSH levels and a significant increase in MDA levels in infertile group as compared with fertile group, shown in Fig. (5). Several studies have been accorded with our results in that women with infertility have insufficient antioxidant defense, with lower total TAC and significantly reduced SOD levels [22]. Kuscu and Var in (2009) explained in their study increased in MDA levels and unregulated SOD activity in patients with PCOS compared to controls [23]. This increasing in lipid peroxidation may result in the consumption of antioxidants and depletion of glutathione and SOD and other antioxidants [24], also oxidative stress effects on graffian follicles which directly damage the defense role of antioxidants and disrupting the ova and it may be damage the ovum DNA, leads to disorder and defect in fertilization and congenital malformations in the embryo [25].

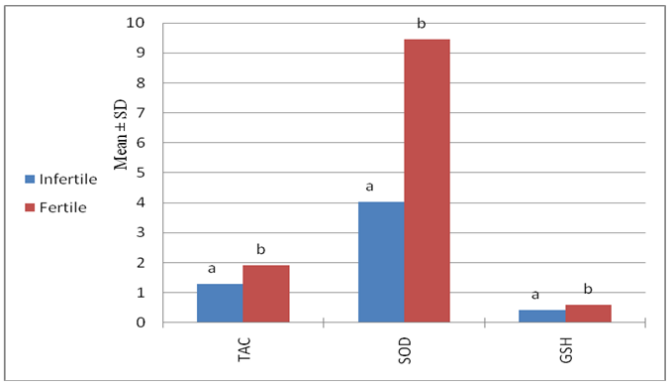

Figure 5. Antioxidants levels in infertile and fertile group

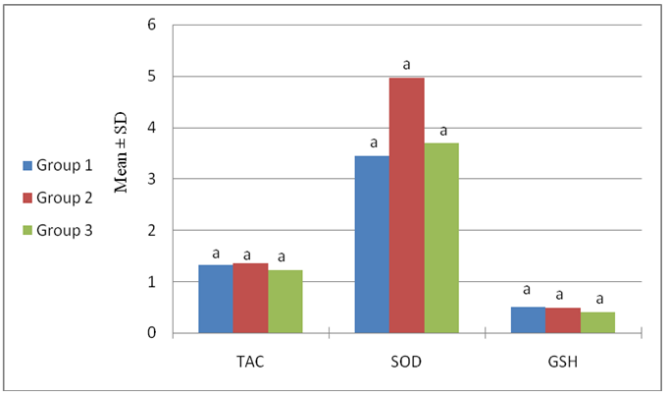

Figure 6. Antioxidants levels among infertile groups in relation to age

A correlation between biomarkers can provide more accurate information about the infertility causes, in the present study there was a positive correlation between SOD and AMH (r= 0.231) and inhibin B (r= 0.364) and negative correlation with E2 (r= -0.269). While TAC exhibited a positive correlation with inhibin B (r= 0.368) and negative correlation with FSH (r= -0.244). GSH had a positive correlation with AMH (r= 0.274) and inhibin B (r= 0.223). These positive correlations for SOD with AMH and inhibin B were corresponded with Asada et al. in (2008) who found that there was a positive correlation between Cu, Zn-SOD levels and the number of preovulatory follicles and collected oocytes [26]. In another study follicular fluid TAC appeared positive correlation with pregnancy rates in IVF and it reflects the antioxidant activity of granulosa cells [27], also decreased TAC declined fertilization potential. GSH positively correlated with AMH and inhibin B, in agreement with another study that showed that GSH activity was associated with failed fertilization in women [28].

In our study, the moderate elevation in LH levels at different age groups was positively corresponded with MDA level elevation at the different age groups; as previously describe oxidative stress was in correlation with the concentration of LH [24]. It was possible that this elevated LH levels in infertile women denoted a response to increased ROS induced oxidative DNA damage. These positive correlations between TAC and SOD with ovarian hormones suggested that these hormones may have a role in the ovarian antioxidant–oxidant balance.

IV. CONCLUSION

It can be concluded that there was a positive and inverse relationship between two different aetiologies of female infertility which are hormones abnormality and oxidative stress. The results of our study indicate that oxidative stress has an impact on the production of FSH from anterior pituitary and steroid hormones from the granulosa cells of the ovary, in particular on AMH, inhibin B and E2 levels, which are an important predictor of ovarian response.

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