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RESEARCH ARTICLE

Serum Monoamine Oxidase (A and B) Activities and Oxidative Stress Status in Iraqi Infertile Women Undergoing *In Vitro* Fertilization

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Abstract

Serum monoamine oxidase (MAO-A and B) activities were measured in 98 infertile women undergoing *in vitro* fertilization (Intracytoplasmic Sperm Injection ICSI) attending Kamal AL-Samaree Hospital for Infertility and *In Vitro* Fertilization at Baghdad after treatment with standard long protocol. Methods: Participants in the study were 98 infertile women subdivide according to infertility cause into PCOS, Unknown cause, and Male factor cause. The activities of MAOs, some antioxidant, and oxidant parameters (catalase, thiol levels, and lipid peroxidation product (MDA) were measured. Luteinizing hormone (LH), Follicle stimulating hormone (FSH), LH/FSH ratio, TSH and prolactin were measured. Results: The activities show significant variations among the three infertility groups. Serum MAOs activities (MAO-A, MAO-B) in sera of PCOS ($P<0.01$), as well, MDA, and total thiol levels were higher significantly when compared with other groups .While, the activity of catalase was lower significantly ($p<0.001$). Conclusion: Our study reveals the existence of an imbalance between serum oxidant and antioxidant status in infertile women with PCOS undergoing IVF.

Keywords: *Monoamine Oxidase Activity, Ovary Polycystic Syndrome, Unknown Infertility, Oxidative Stress.*

Introduction

Infertility is defined as the inability to have children naturally without using contraceptive [1]. It is considered a public health problem in addition to it is costly & stressful in detection and treatment. Although the oxidative stress (OS) was found associated with decreased female fertility [2], but still not proved if OS is one of the important causes of female infertility [3]. The OS occurs when the production of reactive oxygen species (ROS) and other free radical species are higher than the antioxidants amount, which ceases the balance in the ratio oxidative/antioxidants [4].

It is important to mention that having a limited amount of ROS is necessary for the development of cell functions where afterwards every molecule returns to its normal state [4]. But over production of ROS making an environment unsuitable for normal women physiological reactions and that affect the physiological female reproductive system may lead to diseases such as endometriosis, PCOS, and another types of infertility in addition to problem over pregnancy, such spontaneous abortion, continual pregnancy loss , preeclampsia, and intrauterine growth restriction [6,7].Monoamine oxidases (MAOs) (EC 1.4.3.4) are family of enzymes, includes (MAO-A, MAO-B) enzymes that catalyze the oxidative deamination of biogenic & xenobiotic amines, as well as small-molecule monoamines and polyamines [8]. In the intracellular metabolite of monoamines, including neurotransmitters like dopamine (DA), serotonin; aldehydes and hydrogen peroxide (H_2O_2) are formed.

Although, the main reason for raised generation of ROS in PCOS could be associated with the weakness of the mitochondrial electron transport chain (ETC) [9], it was confirmed that MAOs produce more H_2O_2 than electron transport chain [10]. To the better of our knowledge, this is often the primary study to research the level of

MAO enzymes activities in the serum of infertility female. The aim of this study is to explore the oxidative stress status in serum from infertile female undergoing IVF/ICSI.

Materials and Methods

Subjects and Sampling

This study was performed within the period from Nov 2017 to April 2018 on ninety-eight women undergoing ICSI who were attending Kamal AL-Samaree Hospital for infertility and *In Vitro* Fertilization at Baghdad. A written approval from each participant and from the local ethical committee was taken.

The patients were classified according to type of infertility into 3 groups: Thirty three female with PCOS (mean ±SD of age 26.85±4.91year), thirty three female with Male factor infertility (mean ±SD of age 29.15± 6.38 year), and thirty two female with Unknown infertility cause (mean ±SD of age 31.31±5.88year). Mean ±SD level of Body Mass Index for each group was 30.20±3.67 kg/m^2 for PCOS group, 26.19± 3.82 kg/m^2 for Male factor group, and 26.30± 3.50 kg/m^2 for Unknown infertility.

Diagnosis of PCOS was based on the criteria of the ESRHE/ASRM Rotterdam consensus meeting in 2003[11]. A standard long protocol was utilized in all patients for ovarian stimulation of ICSI. Gonadotrophic releasing hormone (GnRH- a) (0.1 mg/24 hours) was administered from day twenty-one of the cycle. From day three of the following cycle, female was treated with human menopausal gonadotropin (hMG). The first dose of the administered gonadotropin was set at 150 IU / day and raised at last by steps of 75 IU each 3-4 days while controlling follicular growth under ultrasound.

Ovulation was stimulated by human chorionic gonadotropin (10000 IU of hCG). When the three leading follicles had reached a diameter of eighteen millimeter, the gametocyte retrieval was performed by transvaginal aspiration thirty six hours after hCG administration. A venous blood sample was withdrawn from women undergoing ICSI before entering the operation, the blood was placed in gel-containing tubes. After clotting, the specimens were centrifuged at 3000 rpm for 10 min to collect serum, which was divided into aliquots in Eppendroff tube, and stored at -20 °C.

Methods

Lipid Peroxidation

The lipid peroxidation was assessed in serum by estimating the concentration of TBARS [12].

Thiol Group Levels

Protein thiol group concentrations in serum were measured using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid; DTNB) [13].

Catalase Activity

The activity of catalase in serum was measured using a simple and accurate colorimetric method [14].

Activities of MAO-A, and MAO-B

The MAO-A activity was measured in the serum samples after using deprenyl-HCl as selective inhibitor of MAO-B activity [15]. Briefly, the serum (0.1 ml) was mixed with 0.1 ml deprenyl-HCl (0.001mM), and incubated for 30 minutes at 37 °C. The solution included both potassium phosphate buffer (1.65ml; 100 mM; pH 7.2) and tyramine (50µl; 100 µM) were added, and then was incubated for 60 minutes.

The addition of 0.2 ml of 2M HCl was to stop the reaction. The absorbance was determined using UV- spectrophotometer at 275nm. The same method was used to measure MAO-A and MAO-B activities after inhibiting SSAO enzyme by semicarbazide (10 mM). One unit of Monoamine oxidase activity represents the degradation of one µmol of the substrate per min under well-defined conditions.

Total Protein Levels

Modified Biuret method [16] was used to determine total protein in serum using bovine serum albumin as standard.

The Calculation of Enzymes' Specific Activity

The specific activity of the enzymes was expressed as follow:

Specific activity (U/mg) =total activity (U/L) /Protein concentration (mg/L).

Measurement of LH, FSH, TSH and Prolactin Levels

Serum LH, FSH, TSH and prolactin levels were quantitatively determined using

sandwich chemiluminescence immune assay method.

Determination of Lipid Profile and Atherogenic Index

Total cholesterol, triglyceride and HDL measurements were performed by using commercial kits.

LDL-Cholesterol, VLDL-Cholesterol, atherogenic index, atherogenic ratio-1, and atherogenic ratio-2 were calculated.

Statistical Analysis

Statistical Packages for Social Science – Version 20 (SPSS-20) was applied for statistical analysis of data. It was showed in simple measures, mean & standard deviation (±SD). To compare between the three infertility groups (PCOS, Unknown infertility, and Male infertility); One-Way Analysis of Variance (ANOVA) was applied followed by post hoc test. The value of P was considered statistically significant or highly significant when it was lower than 0.05, 0.01 or 0.001 respectively.

Results

The mean level of hormones mentioned in the Table [1] showed that TSH, LH, and LH/FSH mean levels of PCOS group were higher significantly ($p<0.001$) when compared with other groups, while mean level of prolactin between groups showed non- significant difference ($P>0.05$). The FSH mean level of PCOS group were lower significantly ($p<0.001$) when compared with Male infertility and Unknown infertility groups. The LH, FSH, LH/FSH, prolactin and TSH mean levels showed non- significant difference between Unknown infertility group and Male infertility group.

Table1: The mean (±SD) levels of LH, FSH, LH/FSH, Prolactin and TSH hormones in serum of all groups

Hormones	Group Male infertility (n=33)	Group Unknown infertility (n=32)	Group PCOS (n=33)
LH µlU/ml	4.10± 1.60	4.08± 1.54	6.95± 2.26***a,***b
FSH µlU/ml	6.84± 1.92	7.22± 2.77	4.69± 1.45***a,***b
LH/FSH	0.60± 0.14	0.58± 0.17	1.51± 0.36***a,***b
Prolactin ng/ml	15.26± 4.07	17.09± 6.33	17.40± 3.71
TSH ng/ml	1.75± 0.73	1.83± 0.58	2.36± 0.75**a,**b

***P< 0.001, **: P< 0.01, *: P<0.05. One-way analysis of variance (ANOVA) was used to compare the parameters, Luteinizing hormone (LH), Follicle stimulating hormone (FSH, a: significant of PCOS & Unknown infertility in comparison to Male factor infertility. b: significant of PCOS with Unknown infertility.

Results in Table [2] of the lipid profile in the PCOS group showed that all the lipid profile parameters (TC,TG ,VLDL-C and LDL-C) were higher when compared with other groups except for HDL-C which had lower mean levels in PCOS group than other groups. The mean levels of TC and LDL-C of Unknown infertility group were higher significantly (P< 0.01) than Male infertility group. The AIP, atherogenic ratio-1 and atherogenic ratio-2 mean level of PCOS group were higher significantly ($p<0.001$) when compared with Male infertility and Unknown infertility groups. The AIP, atherogenic ratio-1 and atherogenic ratio-2 mean level of Unknown infertility group showed non-significance difference ($P>0.05$) when compared with Male infertility group.

Table 2: Mean (±SD) levels of serum lipid profile, AIP, atherogenic ratio-1 and atherogenic ratio-2 of all groups

Parameter	Group Male infertility (n=33)	Group Unknown infertility (n=32)	Group PCOS (n=33)
TC (mg/dl)	143.17± 17.80	162.70± 19.96**a	174± 22.97***a
TG (mg/dl)	118.36± 20.16	119.12± 24.23	137.15± 20.20**a**b
HDL-C (mg/dl)	45.13± 8.05	45.03± 8.15	34.89± 6.95***a,***b
LDL-C (mg/dl)	78.71± 16.32	93.84± 16.12**a	110.79± 16.27***a,***b
VLDL-C (mg/dl)	23.67± 4.03	23.82± 4.84	27.43± 4.04**a,**b
AIP	0.41±0.1	0.42± 0.09	0.6±0.12***a,***b

atherogenic ratio-1	3.24± 0.57	3.69± 0.59	5.16± 1.19***a,***b
atherogenic ratio-2	1.79±0.46	2.15± 0.54	3.30± 0.85***a,***b

***P< 0.001, **: P< 0.01, *: P<0.05. One-way analysis of variance (ANOVA) was used to compare the parameters, Total cholesterol(TC), triglyceride(TG), high-density lipoprotein cholesterol(HDL-C), low-density lipoprotein cholesterol(LDL-C), very low-density lipoprotein cholesterol(VLDL-C), Atherogenic index (AIP), a: significant of PCOS & Unknown infertility in comparison to Male factor infertility. b: significant of PCOS with Unknown infertility

In Table [3], there were no statistically significant differences (P>0.05) in total protein level among the three groups. The mean level of MDA, total thiol, activity &specific activity of catalase in PCOS group were higher significantly (p<0.05) when compared with other groups.

The mean level of total thiol in Unknown infertility group showed non-significant difference (P>0.05) when compared with Male infertility group. While, the mean level of MDA, total thiol, activity &specific activity of catalase were higher significantly (p<0.05) when compared with Male infertility group.

Table 3: Mean (± SD) level of total protein, MDA, total thiol, activity &specific activity of catalase in the three groups

Parameters	**Group Male infertility (n=33)**	**Group Unknown infertility (n=32)**	**Group PCOS (n=33)**
Total protein g/dl	7.7 ± 0.6	7.8 ± 0.4	8 ± 0.6
MDA nmol/L	9.07± 1.67	11.16± 2.09**a	13.35± 3.73***a,**b
Total thiol nmol/L	0.2± 0.06	0.24± 0.1	0.3± 0.1***a,*b
Catalase activity U/L	7179.39±1887.3	4989.37±1011.98***a	3430.90±809.34***a,***b
catalase sp. activity U/mg	0.09±0.03	0.06±0.01***a	0.04±0.01***a,***b

***P< 0.001, **: P< 0.01, *: P<0.05. One-way analysis of variance (ANOVA) was used to compare the parameters, Malondialdehyde (MDA), a: significant of PCOS & Unknown infertility in comparison to Male factor infertility. b: significant of PCOS with Unknown infertility

In Table 4, the mean level of MAO-B activity of PCOS group was lower significantly (P <0.01) when compared with Male infertility group. While, the mean level of MAO-A activity of PCOS group was higher significantly when compared with two groups (Male infertility and Unknown infertility).

The mean level of MAO-B activity of Unknown infertility group was higher significantly (P<0.001) when compared with Male infertility group. The mean level of MAO-A specific activity of PCOS group was higher significantly when compared with other groups.

While, the mean level of MAO-B specific activity of PCOS group showed non-significant difference (P>0.05) when compared with that of Male infertility and Unknown infertility groups. The mean level of MAO-B of Unknown infertility group was higher significantly when compared with that of Male infertility group, but mean level of MAO-A specific activity showed non-significant difference (P>0.05) when comparing Unknown infertility group with that of Male infertility group. The comparison between mean levels of MAOs (MAO-A and MAO-B) measured in serum of three groups showed in Figure 1.

Table4: Mean (± SD) level of activity &specific activity of (MAO-B and MAO-A) in the three groups

Parameters	**Group Male infertility (n=33)**	**Group Unknown infertility (n=32)**	**Group PCOS (n=33)**
MAO-B activity U/L	309.06± 53.41	372.12± 61.37***a	351.79± 50.39**a
MAO-A activity U/L	373.30± 66.55	389.97± 70.73	450.48±62.31***a,**b
MAO B sp. activity U/mg	4.02±0.75	4.77±0.85***a	4.44±0.65
MAO A sp. activity U/mg	4.86±0.99	5±0.95	5.7±0.95**a,*b

***P< 0.001, **: P< 0.01, *: P<0.05. One-way analysis of variance (ANOVA) was used to compare the parameters, a: significant of PCOS & Unknown infertility in comparison to Male factor infertility. b: significant of PCOS with Unknown infertility. Monoamine oxidase (MAO)

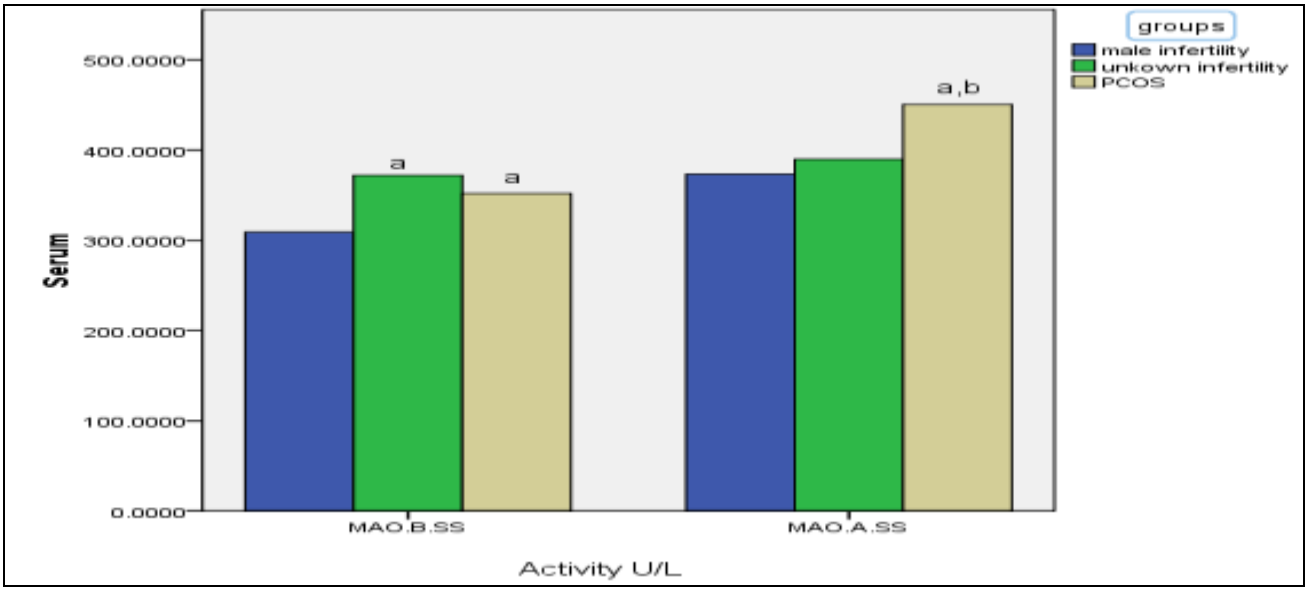

Figure 1: The mean level of (MAO-A and MAO-B) activities measured in serum from (PCOS, Unknown infertility, Male infertility) groups [a: significant of PCOS & Unknown infertility in comparison to Male factor infertility. b: significant of PCOS with Unknown infertility]

Discussion

In the present cross-sectional study, the results showed infertile women especially women with PCOS suffer from dyslipidemia. The defect in lipid profile levels where higher in PCOS patients mainly TG & LDL mean levels and lower HDL-C.

These results were in agreement with the data in [17, 18].A highly significant increase in LH, and LH/FSH ratio were found in PCOS in comparison with other groups. This result is in agreement with other studies [19, 20]. The raised levels of LH are one of main important endocrinological disturbances in patients with PCOS [21]. Some studies have linked hyperandrogenemia to dyslipidemia that usually found in PCOS patients [17, 18].

Another study found high LH levels in PCOS patient that are associated with increase in ROS production [22]. When comparing ROS levels of women with infertility and those with PCOS, women with PCOS were found to have higher mean levels of ROS [23, 24], this point lead to explore the role of monoamine oxidase enzymes in PCOS pathophysiology, hence researchers have associated the increased activity of serum MAO with the development of oxidative stress [25].

The MAO enzymes responsible of metabolism of neurotransmitters like Dopamine (DA). The ability of DA to produced ROS was already found, for example, in brain cells (in astrocytes) by stimulating the production of H_2O_2 and caused lipid peroxidation, leading to change Ca^{+2} signaling, and increased DA metabolism can cause elevated production of ROS [26]. Monoamine oxidase activity has come forward as an important tool to assess mitochondrial sources of oxidative stress [27]. A previous study found that increase MAO activity is contributing to free radical-mediated lipid peroxidation [28]. It is important to mention that neurotransmitters, independently or in combination with one another, regulate GnRH release.

The alteration in neurotransmitters are likely to be responsible for the increased GnRH and LH pulsatility in PCOS condition [9], which lead to suggest that there is a complex relationship between MAOs activities levels , neurotransmitters and problems existed in PCOS patients such as defect in hormones level and increase ROS production. The evidence for alterations in catecholamine metabolism, together with altered mitochondrial functions in human with PCOS was observed in other studies [29, 30].

In this study PCOS and Unknown infertility groups have higher MDA levels and MAOs activities as well as lower antioxidant enzyme activity (catalase)leading to the conclusion that they have significant deficiency in antioxidant defenses and oxidative increase that causes OS.

Conclusion

This study revealed the presence of an

imbalance between oxidant and antioxidant status in serum from infertile women, which MAOs enzymes one of the participants, indicating the presence of oxidative stress in PCOS.

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