Impact of (Cu – Zn) Superoxide Dismutase activity and Malondyaldehyde concentration on Females Infertility

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Abstract

Oxidative stress (OS) induces infertility in women through a variety of mechanisms. Ovarian follicles experiencing OS can lead to direct damage to oocytes. Oocytes and spermatozoa can also experience direct damage, which can lead to impaired fertilization due to an environment of OS in the peritoneal cavity. Reactive oxygen species (ROS)-antioxidant imbalance is also implicated in luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy. The current study was designed to investigate the changes of OS in primary and secondary infertility of females with different etiology. To achieve the intended aim, a cross section study was conducted on 50 infertile females. Markers of OS, level of malonyldialdehyde (MDA) for free radicals and antioxidants (Cu – Zn) superoxide dismutase ((Cu – Zn) SOD) as scavenging enzyme was measured in blood of these females. The results indicated a significant ($p<0.03$) increase of MDA concentration and insignificant ($p<0.288$) decreases of (Cu – Zn) SOD activities in the infertile women with those of female factor when compared with those of male factor. The linear regression analysis demonstrated significant ($r = 0.215$, $p<0.022$) positive correlation for MDA levels with the duration of infertility. This study favored the significant role of free radicals injury in cases of female infertility and changes of oxidative stress were observed to be dependent on the etiology and the duration of infertility of the enrolled women.

Keywords: primary and secondary female infertility, (Cu–Zn) Superoxide dismutase, Malondyaldehyde

الخلاصة

الاجهاد االتاكسدي يدفع العقم لدى النساء من خلال مجموعة متنوعة من الآليات. جريبات المبيض التي تعاني من هذا الاجهاد يمكن أن تؤدي إلى ضرر مباشر على البويضات. البويضات والحيوانات المنوية التي تظهرالضرر المباشر الذي يمكن أن يؤدي إلى ضعف الإخصاب بسبب بيئة نظام التشغيل في التجويف البريتوني. ان اختلال الموازنة بين انواع الاوكسجبن التفاعلية ومضادات الاكسدة يسبب الانحدار الأصفري وعدم كفاية الدعم الهرموني الأصفري لاستمرار الحمل. وقد صممت الدراسة الحالية لتحري التغييرات الناتجة من الاجهاد التاكسدي في العقم الأولي والثانوي للاناث بمختلف الاسباب. لتحقيق الهدف المقصود، أجريت دراسة على 50 من الإناث المصابات بالعقم.. تم قياس مستوى المالوندايالديهايد كدليل عن الجذور الحرة من علامات الإجهاد التأكسدي.، وقياس السوبراوكسايد ديسميوتز كمؤشر لمضادات الاكسدة في امصال الاناث المدرجات في الدراسة. أشارت النتائج إلى زيادة معنوية في تركيز المالوندايالديهايد ونقصان غير معنوي في انزبم السوبراوكسابد ديسميوتز في النساء ذات المسبب الانثوي مقارنة مع ذات المسبب الذكري. كما اظهر تحليل الانحدار الخطي ارتباط موجب لزيادة المالوندايالديهايد مع مسبب وفترة العقم. هذه الدراسة اكدت الدور الكبير للجذور الحرة في حالات العقم عند النساء ولوحظ أن تغيرات الاكسدة تعتمد على المسببات ومدة العقم في النساء االمشاركين بالدراسة.

الكلمات المفتاحية: عقم النساء الاولي والثانوي، السوبراوكسايد ديسميوتز ، المالوندايالديهايد.

Introduction

Infertility is a disease defined as the inability to conceive following 12 or more months of unprotected sex (Al-Azzawie *et al*., 2014). In general, an estimated 84% of couples conceive after 1 year of intercourse, and 92% of the couples conceive after 2 years (Yu SL *et al*., 2003). It is classified as primary infertility in which a couple has unable to have their own biological child and secondary infertility happens when a couple previously had a child, but can’t get pregnant again. Infertility can be caused by problems in the woman, the man, or both (World Health Organization, 2013). A primary diagnosis of male factor infertility is made in 30% of infertile couples (Lucky *et al*., 2010). While combined female and male factor infertility is responsible for 20%–30% of cases. If the results of a standard infertility examination are normal, a diagnosis of unexplained or idiopathic infertility is assigned, oxidative stress has a well-established role in pathogenesis of unexplained infertility, which is seen to affect 15% of couples (DeCherney *et al.*, 2003). Although the frequency and origin of different forms of infertility varies, 40%–50% of the etiology of infertility studied is due to female causes (Duckitt, 2003). Females factors include endometriousis, polycystic ovary syndrome, tubal factors, premature ovarian failure, oocyte aging, and unexplained infertility (Group REA-SPCW, 2003). In male conditions affecting the sperms, such as azospermia, damaged DNA, and varcosile may contribute to infertility (Agrval *et al*., 2004). Oxidative stress (OS) is a common condition caused by biological systems in aerobic conditions such that antioxidants cannot scavenge the reactive oxygen species (ROS). This causes an excessive generation of ROS, which damages cells, tissues, and organs (Al-Asady *et al*., 2014). Over the years oxidative stress has been increasingly thought to exert adverse effect on the reproductive ability of both men and women (Agrval *et al*., 2004).

Reactive oxygen species are formed endogenously during aerobic metabolism and as a result of various metabolic pathways of oocytes and embryos or as part of the body's defense mechanisms, ROS also can arise from exogenous sources, such as alcohol, tobacco, and various environmental pollutants (Agarwal *et al*., 2008). In vivo the damaging effects of oxygen radicals are usually prevented or limited by endogenous antioxidants/scavengers of free radicals. Several biomarkers indicative of redox status, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), lipid peroxides, and nitric oxide, have been identified within the ovary, endometrium, fallopian tubes, embryo, placenta, and the peritoneal fluid of women. The SOD enzyme exists as three isoenzymes SOD 1, SOD 2, and SOD 3. SOD 1 contains copper (Cu) and zinc (Zn) as metal co-factors and is located in the cytosol. SOD 2 is a mitochondrial isoform containing manganese (Mn), and SOD 3 encodes the extracellular form (Agarwal *et al*., 2012).

Under normal conditions, antioxidants act to oppose ROS production, scavenge existing free radicals, and promote the repair of ROS-induced damage to cell structures (Agarwal *ey al*., 2004). However, when ROS increase to pathological levels, they are capable of inflicting significant damage to cell structures. OS induces infertility in women through a variety of mechanisms. Ovarian follicles experiencing OS can lead to direct damage to oocytes. Oocytes and spermatozoa can also experience direct damage, which can lead to impaired fertilization due to an environment of OS in the peritoneal cavity. ROS-antioxidant imbalance is also implicated in luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy (Agarwal *et al*., 2004). While in male Excessive production of free radicals or reactive oxygen species (ROS) can damage sperm, and ROS have been extensively studied as one of the mechanisms of infertility. Superoxide anion, hydroxyl radical and hydrogen peroxide are some of the major ROS present in seminal plasma (Wasan *et al*., 2012). In contrast, the role of antioxidants and ROS in relation to female reproduction has received relatively little attention, despite the evidence of both physiological and pathological effects (Veena *et al*., 2008).

Therefore, it was of interest in present study to measure the concentrations of oxidative stress marker- lipid hydroperoxides (MDA), along with a marker of antioxidant (Cu – Zn) SOD in serum of women attending the infertility clinic, and to investigate the changes of oxidative stress (OS) in primary and secondary infertile females.

Materials and methods

This cross section of study was included a total of 50 females that were enrolled in the study from March 2013 to June 2013. These females attended to infertility clinic in Al-Sadder Teaching Hospital. The cause of infertility on females factor was ovulatory cause have been diagnosed by Dr. Bassima Shamki. All of them were recruited according to the following criteria: Non-smokers; Free from hepatitis and HIV(by screening test); absence of any metabolic or endocrine system-associated diseases, or any other associated condition which could alter the level of free radicals like malignancy and antioxidant therapy. This entire group was divided into two groups based on the etiology of infertility as- infertility due to female factor (N=24), and infertility due to male factor (N=26), The females further classified into group1(G 1) included those with primary infertility (N=36) and the group2 (G2) included those with secondary infertility(N=.14).

Blood sample was collected from all females then allowed to clot for 10-15 minutes and serum was obtained by centrifugation at 1500 $_X$g for approximately 10-15 minutes**.** The serum samples were placed into new clean disposable plain tubes to measured (Cu– Zn) SOD and MDA level.

Measurement of MDA, a secondary product of lipid peroxidation, was based on the calorimetric reaction with thiobarbituric acid (TBA) to form a pink color product, which could be measured by spectrophotometer (Yagi 1984).

(Cu-Zn)SOD activity was determined by using a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. The autoxidation of pyrogallol in the presence of EDTA in the pH 8.2 is 50%. The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\bar{}}$ and the dismutation of this radical by (Cu-Zn) SOD. (Cu-Zn) SOD activity are expressed as unit/ml. one unit of (Cu-Zn) SOD activity being defind as amount of enzyme required to cause 50%inhibition of pyrogallol autoxidation (Luc *et al*., 2000).

Statistical analysis

Statistical analysis was performed in this study using SPSS (Statistical Package for Social Science; Version 17) program. Independent *t*-test was used to estimate differences between groups in continuous variables. Pearson's correlation analysis was used for correlation. Results are reported as (mean ±SD). P<0.05, was considered statistically significant.

Results

The baseline characteristics of the studied groups are summarized in the table (1). On statistical analysis, serum MDA concentration was comparatively high and statistically significant in infertile women with female factor than that of male factor, while the serum (Cu – Zn) SOD activity shows insignificant difference (p>0.05) between women with female and male factor.

Table (1): (Cu – Zn) superoxidedismutase activity and malondyaldyhyde concentration in sera of women with infertility due to various etiologies.

Parameters	Cause	Number	Mean	SD	P value	95% Confidence interval
Age	Male	26	31.57	5.55	0.845	-3.79946 - 2.69
	Female	24	31.26	5.653		
BMI	Male	26	26.43	4.53	.318	-4.07 -1.54
	Female	24	27.72	4.51		
MDA	Male	26	2.60	1.39	.033	-1.87- 48333
	Female	24	3.92	2.70		
(Cu – Zn) SOD	Male	26	1.53	.59	.288	-.14- .38
	Female	24	1.35	.61		

BMI-body mass index, MDA- malondyaldyhyde, (Cu – Zn) SOD – superoxidedismutase, SD- standard diviation

On the other hand, the present study showed the age of females was positively correlated with BMI (r = 0.399, P < 0.006) while insignificant negative correlation between MDA, and (Cu – Zn) SOD level was noticed (r = -.259-, P < 0.07). In addition to that duration of disease positively correlated with MDA (r= .330, P<.022) as shown in table (2).

Table(2). Correlation factors of biochemical and clinical characteristics parameters of the studied subjects.

		Age	BMI	Duration	MDA	(Cu – Zn) SOD
Age	r	1	.387	.090	-.098-	-.061-
	P value		.006	.535	.499	.672
	Number	50	50	50	50	50
BMI	r	.387	1	.135	-.167-	-.278-
	P value	.006		.349	.245	.051
	Number	50	50	50	50	50
Duration	r	.090	.135	1	.215	.221
	P value	.535	.349		.022	.124
	Number	50	50	50	46	50
MDA	r	-.098-	-.167-	.215	1	.259
	P value	.499	.245	.022		.070
	Number	50	50	46	50	50
(Cu – Zn) SOD	r	-.061	-.278-	.211	.259	1
	P value	.672	.051	.124	.070	
	Number	50	50	50	50	50

BMI-body mass index, MDA- malondyaldyhyde, (Cu – Zn) SOD - superoxide dismutase

The diagnosis of primary infertility was made in 72%, and the secondary infertility was present in 18% among studied groups as figure (1) revealed.

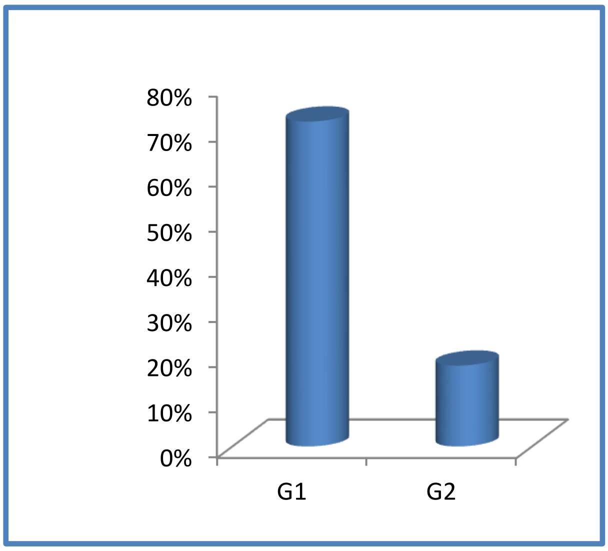

Figure (1): Distribution of infertile subgroups.
G 1- primary infertility G 2- secondary infertility

In addition to that, table (3) revealed that insignificant differences observed between group I and 2 for both MDA and (Cu – Zn) SOD levels, while age of females with secondary infertility increase significantly(p<0.01) when compared to primary infertility group.

Table (3): Clinical characters, (Cu – Zn)superoxidedismutase activity and malondyaldyhyde concentration in sera of women with primary and secondary infertility.

		Age	BMI	Duration	MDA	(Cu – Zn) SOD
G1	Mean	30.19	27.00	9.08	3.44	1.41
	N	36	36	36	36	36
	SD	5.49	4.84	4.36	2.47	.50
G2	Mean	34.59	27.19	6.37	2.72	1.52
	N	14	14	14	14	14
	SD	4.43	3.73	3.35	1.21	.81
	P value	.010	.896	.042	.302	.579

BMI- body mass index, MDA- malondyaldyhyde, (Cu – Zn) SOD- superoxide dismutase
G1-primary infertility, G2- secondary infertility, SD- standard deviation

Discussion

Successful pregnancy results from an interaction between myriad physiological processes in both men and women. Any disruption to this interactive system, whether in a man or woman, can result in an inability to have a biological child called infertility (Agarwal *et al*., 2004). ROS exert their cytotoxic effects by causing peroxidation of membrane phospholipids, which results in an increase in membrane permeability, loss of membrane integrity, enzyme inactivation, structural damage to DNA and cell death (Halliwell *et al*., 1994).

In the present study, the various causes of female infertility and the role of ROS and OS on various etiologies of female infertility was examined. It has also been emphasized that free radicals have important physiological functions in the female reproductive tract and excessive free radicals may precipitate female reproductive tract pathologies (Veena *et al*., 2008). The present study also emphasizes on an increase in ROS, as indicated by elevated MDA in serum of infertile women with those of female factor than in male factor group. This is supported by the lack of similar increase in antioxidant levels- in the form of (Cu – Zn) SOD, thus providing way for oxidative damage.

Oxidative stress due to free radicals precipitates a range of pathological changes that affect the reproductive functions in both men and women. Said *et al* supported the role of NADPH in oxidative stress mediated DNA damage in abnormal sperm morphology (Said *et al*., 2005). Oxidative stress also, can have detrimental effects on female fertility by affecting ovulation, fertilization, embryo development, and implantation (Bedaiwy *et al*., 2003). Thus, OS is considered a cause of female infertility. This is particularly clear in cases of endometriosis (Agarwal *et al*., 2004). It is suggested that OS is caused by ROS overproduction rather than antioxidant depletion (Veena *et al*., 2008). An elevated lipid peroxide concentration and decreased SOD concentrations have been reported in human endometrium in the late secretory phase, and these changes may be responsible for the breakdown of the endometrium, implicating the involvement of OS in the process of menstruation (Rosselli *et al*., 1998).

The overall result was somewhat closely comparable to previous reports, (Hussein *et al*., 2013) have found significantly higher concentration of MDA and decreased serum levels of GST and CAT as compared to fertile women. (Veena *et al* .,2008) and (Edan *et al*., 2009) h*ave* found significantly higher concentration of MDA in serum of infertile women than in fertile women. Also, (Savita *et al*., 2009) have shown significantly high plasma levels of MDA in infertile women when compared to parous women and this is noticed from there dictions of levels of eicosapentaenoic acid (EPA), and more so indocosahexaenoic acid (DHA), they suggested that these changes are consequence of increased oxidative stress that mediate lipid per oxidation Product , i.e. MDA. In addition to that, the OS markers such as (SOD), glutathione peroxidase (GSH) and Catalase have been identified to be low while (MDA) concentration is high within the peritoneal fluid of oxidative stressed women inflicting significant damage to reproductive cells structures(Bakare *et al*., 2014). A positive association between infertility related to endometriosis, advanced disease stage and increased serum hydroxyperoxide levels were demonstrated suggesting an increased production of reactive species in women with endometriosis (Barcelos *et al*., 2014).

The analysis of our results indicated the elevated MDA evident as the duration of infertility was prolonged in the enrolled infertile women. The results suggest that prolonging of the duration of infertility exaggerate the implication of oxidative stress in the impairment of female infertility. This result is agreements with (Hussein *et al*.,

2013) and disagreement with the result obtained by (Edan *et al*., 2009) how fined the MDA concentration of infertile women with unexplained infertility correlated insignificantly with duration of infertility , the difference could be attributed to the cause of infertility, dietary regimen and therapeutic drugs of females enrolled in this study.

On the other hand, in this study, 72% of the infertile females had primary infertility and 18% had secondary infertility. This result was in agreement with (Mokhtar *et al*., 2006) that reported 80% and 20% had primary and secondary infertility respectively, while in the United States, (Mosher *et al*., 1998) reported that 65% of the infertile females had primary infertility and 35% had secondary infertility. It is worth mentioning females enrolled in this study suffering from ovulatory disturbance. The high incidence may refer to the common risk factors present such as polycystic ovary, infections, family history, menstrual irregulation and others (Mokhtar *et al*., 2006). On the other hand, present result did not show any significant changes in MDA concentration and (Cu – Zn) SOD between primary and secondary infertility. The consequences appear to be equal precipitation in the pathophysiology of their productive systems in infertile patients. These evidences suggest that oxidative stress is an independent on type of female infertility. Such independency may relate to the activation of macrophages which are a source of generation of ROS (Hussein *et al*., 2013).

Moreover, age of present females play significant role in developing secondary infertility. It is widely accepted that during the last twenty years, the average age of having children has increased and this is a key factor for infertility. As the age of giving birth is increased, the reproductive capacity is decreased, the ovary becomes less efficient, the frequency of sexual intercourse is decreased and the possibility of chromosomal abnormalities and miscarriage is increased (Roupa *et al*., 2009).

Conclusion: Our study supports etiological role of free radicals in female infertility. It also shows that damage is more pronounced at the progress duration. Furthermore, these results suggest that oxidative stress is involved in the pathophysiology of primary as well as secondary infertility.

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