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Role of Oxidative Stress and Some Antioxidant Enzymes in Male Infertility

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Abstract: More than half of all infertile male suffer from oxidative stress. Seminal plasma have antioxidant mechanisms to suppression the reactive oxygen species. The aims of the project was to evaluate the levels of protein peroxidase, lipid peroxidase and antioxidant enzymes such as glutathione peroxidase, superoxide dismutase in blood sample of infertile male and control group. Samples were collected from 50 infertile men and 25 apparently healthy men to measure the levels of oxidative stress biomarkers: protein peroxidase (PPO), lipid peroxidase (LPO) and antioxidant enzymes: glutathione peroxidase (GPX), superoxide dismutase (SOD). Results show highly significant differences in patients with male factor infertility in PPO and LPO (76.48±9.51; 58.22±5.33) compared with control group (54.72±8.63; 39.05±4.74) respectively. While GPX and SOD enzymes levels show significant differences in patients with infertility (8.01±1.04; 12.26±2.17) compared with control group (11.23±1.74; 18.26±3.24) respectively. In conclusion oxidative stress was associated with infertility and this may help for treatment of male factor infertility by using proper antioxidants such as vitamin C, vitamin A, vitamin E and selenium.

Key words: Oxidative stress, antioxidant enzymes, male infertility.

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Introduction

One of the important causes of male infertility is the excessive production of free radicals (FR) or reactive oxygen species (ROS) that can damage sperm, and ROS have been extensively studied as one of the mechanisms of infertility. Any excess ROS must be continuously inactivated in order to maintain normal cell function. This function is taken up by the antioxidants present in the seminal plasma. When there is an excessive production of ROS or impaired antioxidant defense mechanisms, oxidative stress (OS) occurs, which is harmful to spermatozoa (1,2).

Free radicals have important role in life and death of cells. The unstable single electrons may become more reactive. Reactive oxygen species occurs from molecular oxygen/nitrogen through Electron Transport Chain (ETC), cytochrome P450, and other cellular and sub-cellular functions. ROS have beneficial effect on cellular processes and metabolism and play important role in pathological conditions of the body (3).

The normal balance is by endogenous antioxidant system, while imbalances in

redox status may develop cellular oxidative stress. If the endogenous antioxidants fail to overcome the reactive metabolites production, then exogenous antioxidants would be necessary to balance redox status. Dietary sources, including plants, herbs, spices, vitamins and herbal extracts, play an important role in this regard (4). Antioxidants are define as substances that have important role to prevent oxidative damage in base molecules, and can be classified to enzymes or protein non-enzymatic molecules depend on structure of molecules, and can be classified depend on source of molecules to endogenous and exogenous antioxidants. Organisms have many enzymes act as antioxidants such as: glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase, cytochrome oxidase and vitamins C and E (**5**). The role of SOD to reduce the O_2 level by catalase into H_2O_2 and O_2. While the GPX decrease H_2O_2 and organic hydro-peroxidase. Vitamin C is an important component of the cellular defense against, lipid peroxidase (LPO) and O_2 toxicity caused by free radicals. It suppresses O_2, OH and singlet oxygen, and reacts with the tocopheroxyl radical to re-form tocopherol, and thus prevent LPO (6).

Materials and Methods

Patients and Samples Collection

This study was carried out in the laboratories of Biotechnology Research Center, AL-Nahrain University, Baghdad/Iraq. Patients were selected according to clinical and laboratory examination. Questionnaire form was filled for each patient and includes: name, age, family history, diagnosis of idiopathic infertility.

In this study, 50 infertile and 25 fertile men were used, and blood samples were collected to measure the levels of oxidative stress biomarker: protein peroxidase (PPO), lipid peroxidase (LPO) and antioxidant enzymes: glutathione peroxidase (GPX), superoxide dismutase (SOD).

Oxidative Stress Biomarkers and Antioxidants Enzymes Measurement

Lipid Peroxidation

The estimation of lipid peroxidation was according to the method of Ohkawa et al. (**7**):

1. The amount of malondialdehyde (MDA) produced was used as an index for lipid peroxidation.
2. The samples were treated with 600µl of 10% Trichloroacetic Acid (TCA) solution, mixed gently and centrifugation at 5000 rpm for 10 min.
3. After 5 min, 150µl of Thiobarbituric acid (TBA) reagent were added to 200µl of the supernatant and mixed with 100µl of distilled water.
4. A blank tube without the sample was used as reference.
5. Both the sample and the blank tubes were placed in a boiling water bath for 10 min and allowed to equilibrate with room temperature.
6. Absorbance of the samples and blank were measured at 532 nm. All values were expressed as nmoles of MDA/ml.

Protein Peroxidation

The estimation of protein peroxidation was according to the method of Ohkawa *et al*. (**7**):

1- 10µl of blood samples were treated with 600 µl of 10% Trichloroacetic Acid (TCA) solution, mixed gently and centrifugation for 10 min at 5,000 rpm after 5 min.
2- The precipitate was washed two times with distilled water and then dissolved in 0.6 ml of 0.5 N NaOH solution.
3- 200µl of this sample solution is again mixed with 150µlof Thiobarbituric acid (TBA) reagent and 100µl of distilled water.
4- A blank tube without the sample was used as reference.
5- Both the sample and the blank tubes were kept for equilibration similar to lipid peroxidation and absorbance were measured at 532 nm.
6- The protein peroxide is expressed as nanomoles of thiobarbituric acid reactive substance producing MDA per ml of sample.

Determination of SOD

Estimated activity of SOD was done by using superoxide dismutase diagnostic ELISA kit (Biovision, lnc. USA) according to the manufacturer's instructions.

Determination of GPX Activity

Estimated activity of GPX was done by using glutathione peroxidase diagnostic ELISA kit (Biovision, lnc. USA) according to the manufacturer's instructions.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA- test), data are presented as means ± SD. The level of significance was $P < .05$.

Results and Discussion

Results show highly significant differences in patients with male infertility in PPO and LPO (76.48±9.51; 58.22±5.33) compared with the control group (54.72±8.63; 39.05±4.74) respectively (Table 1). While GPX and SOD enzymes levels show lower significant differences in in patients with infertility(8.01±1.04; 12.26±2.17) compared with the control group (11.23±1.74; 18.26±3.24) respectively (Table 2).

Table 1. Levels of oxidative stress biomarker: protein peroxidase (PPO), lipid peroxidase (LPO) in patients and control

Groups	PPO (nmole/ml)) mean±SD	LPO((nmole/m) mean±SD
Infertile males	A 76.48±9.51	A 58.22±5.33
Control	B 54.72±8.63	B 39.05±4.74

Differences A, B are significant (P<0.05) to compression rows

Table 2. Levels of antioxidant enzymes: glutathione peroxidase (GPX), superoxide dismutase (SOD) in patients and control

Groups	GPX (U/ml) mean±SD	SOD(U/ml)mean±SD
Infertile males	A 8.01±1.04	A 12.26±2.17
Control	B 11.23±1.74	B 18.26±3.24

Differences A, B are significant (P<0.05) to compression rows

ROS have important beneficial effects on spermatozoa activity based on nature and ROS concentration in addition to position and length of exposure (8). When transit through epididymal tube, sperm get the ability to move progressively, these assist spermatozoa to acquire ability to fertilized oocytes in the female tract through a series of physiological changes called capacitation (8). During Capacitation, spermatozoa output small amounts of ROS, which are needed for capacitation and acrosomal reaction, hyper activation, motility and fertilization (7,9).

Griveau *et al.,* (10) reported that the small amounts of ROS are necessary to maintain and regulate of the function of sperms include: capacitation of sperm, reaction of acrosome and oocyte fusion. When spermatozoa incubation with low concentration of H_2O_2 has been shown to stimulate sperm capacitation, hyper-activation, acrosome reaction and oocyte fusion (11,12) While the highly level of ROS have toxic effect on motility and function of sperms(10).

There are positive relationship between SOD and motility of sperms. (13). Agarwal *et al.*,(14), suggested that the plasma of seminal fluid collected from fertile men which have higher total antioxidant capacitation (TAC) than seminal plasma from infertile men.

This study aimed to determine the role of oxidative stress and antioxidants enzymes and it was found a negative correlation between OS biomarkers and Antioxidant enzymes, and these results agree with other studies (15,16), the results of the present study showed data reveled to a significant increase in ROS levels in blood and semen of infertile patients compared with fertile men. While SOD and GPX levels show a significant decrease in infertile men compared with control group, these results also suggested pathological levels of ROS increase causing reduce antioxidant capacity in blood and seminal plasma (16).

Other studies suggested that the positive correlation between ROS and antioxidants enzymes activity may indicate that the scavenger system are inadequate or defected in men with infertility which lead to reduce fertilization potential of sperm. Patients with infertility problem because of inadequate or defect in oxidative property can be treated with different antioxidant by oral administration of vitamin C or E which have been show improve the sperm penetration to zona pellucida during *in vitro* fertilization (3).

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