Evaluation of some individual antioxidants in seminal plasma of fertile & infertile men.

Dr. Hathama Razooki Hasan* (Biochemist)
Enam J. Ena *

Summary:

J Fac Med Baghdad
2005; vol.47 No. 2
Received: Sep. 2002
Accepted: Aug. 2003

Background: *infertility is defined as the inability of a couple to conceive after one year of a regular unprotected intercourse with the same partner. Many factors seem to play a role in male infertility.*

Aim: *to evaluate the role of some seminal plasma biochemical components in male infertility.*

Methods: *seminal plasma of 100 infertile men & 25 fertile men were chosen as a subject of this study.*

Different biochemical parameters that are related to oxidative stress were measured. These included Glutathione Peroxidase (GSHPX),superoxide dismutase (SOD),cerulopasmin (CP) scavenging activity, albumin & total sulfhydral (T-SH) & its individual parts (protein & non protein binding sulfhydral (PB-SH & NP-SH)).

Results:- *a significant decrease in GSHPX activity ($P<0.05$) , T-SH level ($P<0.05$), BP-SH ($P<0.05$) & SOD scavenging activity ($P<0.01$) was observed in infertile men in comparsion to that in the control group. While no significant differences were observed in albumin & CP scavenging activity between the two studies groups.*

Conclusion:- *the result of this study indicate the presence of imbalance between pro/antioxidant, which in turn lead to increase in the production of reactive oxygen species in seminal plasma & ultimately cause defect in sperm function.*

Introduction

Multicenter studies carried by the World Health Organization during (1982-1985) on infertility, found that 20% of cause of the problem were predominately male (1). In general, infertility is defined as the inability of a couple to conceive after one year of a regular unprotected intercourse with the same partner (2). Many factors seem to play a role in male infertility among them is oxidative stress. This oxidative stress in the biological systems results from an imbalance between the production and removal of reactive oxygen species (ROS) Such imbalance leads to an increase in the reactive intermediate formation in the body which , in turn lead to cellular damage (3,4) . Reactive oxygen species include the superoxide anion (O_2), hydrogen peroxide (H_2O_2), and the highly potent oxidant, the hydroxyl radical (OH). Previous reports indicated that the excessive production of reactive oxygen species by spermatozoa could be a cause for idiopathic infertility, since the increased ROS levels in semen lead to biochemical or physiologic abnormalities, with subsequent sperm dysfunction or cell death (1,5). Spermatozoa and seminal plasma contain a battery of ROS scavengers, including enzymes such as superoxide dismutase (SOD,EC,1.15. 1.1) , Ceruloplasmin (Cp; EC, 1.12.3.1) and glutathione peroxidase (GSHPX; EC ,1.11.1.9) that in association all together , control the level of reactive oxygen species (superoxide anion , hydrogen peroxide, and hydroxyl radicals) .SOD and Cp disproportions superoxide to hydrogen peroxide , which is further metabolized by selenium-dependent GSHPX (Se- GSHPX) . Also a variety of substances such as albumin and glutathione that are present in spermatozoa and seminal plasma, have SOD like activity (6). The aim of the present study is to evaluate the scavenger enzymatic activities of superoxide dismutase, cerulopasmin and glutathione peroxidase in addition to thiol, and albumin in seminal

* *Dep. of Chemistry / College of Science / University of Baghdad.*

plasma of fertile and infertile men.

Subjects and Methods:-

One hundred (non-smoker, and non-alcoholics) infertile patients aged range between (20-45) years, were selected among patients during their attendance at Kamal-Al-Samerae, hospital. These patients were examined by Dr. Ziad Tark for their general physical condition, signs of defective androgenization, and testicular function, including consistency, tenderness, and varicoceles.Those individuals who had urogenital disorders were excluded from the present study. As a control twenty-five healthy individual volunteers (aged matched) were subjected to this study. These volunteers were checked according to the same parameters as above.

Seminal plasma preparation: Semen sample was produced by masturbation after (3 to 5 days) of sexual abstinence. Samples were left for 30 minutes for liquefaction to occur, then semen quality was evaluated by using two parameters:- Macroscopically and microscopic examination .Seminal plasma was obtained as soon as possible by centrifugation of semen sample at 3000 rpm for 15 minutes using bench centrifuge.

Grouping of subjects: The subjects were classified according to the results of routine semen analysis using World Health Organization Criteria (7): into two groups as follows: - Fertile (control) group:-

Sperm count > 20 million / ml, progressive motility >50% and morphology >50% infertile group:-

Sperm count < 20 million / ml, progressive motility <40% and sperm morphology > 50%.

Antioxidant Assays Determination of GSHPX:-

GSHPX activity was determined in seminal plasma according to Burke et al (8). The reaction mixture containing (1 mM) reduced glutathione, (60 ug/ ml) of glutathione reductase, (0.2 mM) NAPH, (ImM) sodium azide, (ImM) EDTA and 50 mM phosphate buffer pH 7.0 as a final concentration.

The mixture was incubated at 25 C for 10 minutes and the reaction was initiated by adding (1.5 mM) H_2O_2 to the reaction mixture. The conversion of NADPH to NADP was followed by continues recording of the change in absorbance of the system at 340 nm for 5 minutes, after initiation of the reaction. Enzyme units (e.u) were defined as the number of micromoles of NADPH oxidized per minute and was calculated on the bases of molar absorptivity for NADPH at 340 nm. of 6,22x 10-6.

Determination of Albumin : Albumin was determined in seminal plasma by using Randox kit. The method is based on the quantitative binding of albumin to the indicator of the sulphonphalein. The absorbance of the produced green complex was measured using a wavelength of 578 nm.

Determination of sulfhydryl groups : These including total sulfhydryl groups and its individual parts (protein binding sulfhydryl group (BP-SH), and non-protein binding sulfhydryl group (NP-SH). Thiol groups were determined in seminal plasma according to Sedlok & Lind method (9). Where the sulfhydryl groups react with 5, 5 di- thio- bis-(2-nitrobenzoic acid) to form a colored product that can be measured spectrophotometrically at 412 nm. Calculation of sulfhydryl group level was based on the molar extinction coefficient for both (T-SH) and (NP-SH) at 412 nm which is equal to 13.000 M-l cm-1. The concentration of (PB- SH) was calculated by subtracting the (NP-SH) concentration from (T-SH) concentration.

Determination of SOD & Cp scavenging activity : SOD and Cp. scavenging activities for Superoxide anion radical were measured in seminal plasmas using the nitro-blue tetrazolium (NBT) method (10), with some modifications that include using of sodium azide as an inhibitor for cerulopasmin ability for Superoxide anion scavenging (11) .The mixture reaction contained NBT (0.057 mM) , L-methionine (9.9 mM) ; triton x-100 (0.025 % [w/v]) and riboflavin (0.044 M) which should be added last to initiate the reaction , then the color was measured at 560nm . Enzymatic unit was defined as the amount of the enzyme that caused 50% inhibition of NBT reduction. In order to determine the enzyme unit, serial volumes of seminal plasma were
used as a source of the enzyme then, the percent inhibition of the enzymatic reaction was plotted against the volumes of seminal plasma. Where one unit of SOD activity in seminal plasma was found to equal to 12.5% inhibition, while that of Cp. scavenging activity was found to equal to 20 % inhibition. The specific activity of the enzyme is expressed as the enzyme activity per milligram of protein.

Statistical analysis:

Statistical analysis was performed using a statistical software package for descriptive statistics; mean value ± standard deviation is given. Student t-test was employed to examine the difference between fertile and infertile for various biochemical parameters. Probability value of ($p<0.05$) was considered to be significant.

Results

Glutathione peroxidase activity was measured in seminal plasma of fertile group and the results are listed in table (1-1) .It is clear from this table that there is a significant decrease in GSHPX activity and its specific activity in infertile group when compared with that of fertile group ($p<0.05$) . Also a significant decrease in SOD activity ($p<0.001$) and its specific activity ($p<0.05$) in infertile group in comparison with the fertile group was observed, as shown in table (1-2) . On the other hand, it is clear from table (1-3) that there is no significant difference in Cp-scavenging activity between fertile and infertile group. Total sulfhydral group and its individual

parts (protein binding and non-protein binding sulfhydryl) were measured in seminal plasma and the results are listed in table (l-4).It is clear from this table that there is a significant decrease-in total sulfhdryl and protein binding sulfhydryl group levels in infertile group 1 when compared with that of fertile group, while no significant difference in non- protein binding sulfhydryl group level was observed between the two studied groups. Level of seminal plasma albumin was measured using Randox kit, and table (1-5) shows that there is no significant difference in seminal plasma albumin of fertile and infertile group ($p>0.05$).

Discussion : It has been shown that human spermatozoa have the capacity to generate reactive oxygen species (12). De- Lamirande and Gagnon (13) demonstrated the physiological role of reactive oxygen species in spermatozoa hyper activation and acrosome reaction. On the other hand, peroxide damage induced by hyperactive oxygen species has been reported as a major cause of defective spermatozoa function. Aitken el. al (14) observed that hydrogen peroxide was the most oxygen species that was toxic to human spermatozoa . They showed that spermatozoa capability for oocyte fusion was impaired upon the addition of hydrogen peroxide. This toxicity was eliminated with glutathione peroxidase, therefore, glutathione peroxide activity and specific activity were measured and the results show a significant decrease in GSHPX activity in seminal plasma of infertile group. These results agree with those of Alkan et al (15). The decrease in GSHPX activity that was observed in the present study may be attributed to the defect in GSHPX production rather than regulation of its activity. This conclusion arises from the observed decrease in GSHPX activity in this study, which is concomitant with a decrease in its specific activity in seminal plasma of infertile men, taking into account that no significant difference between the two groups was observed in non- protein sulfhydryl level (Table 1-4). SOD and Cp are the primary antioxidant enzymes that catalyze the dismutation of superoxide anions in mammalian cells (16). The significant decrease in SOD activity in seminal plasma of infertile men that was obtained in this study agree well with that reported by Zini et al (17), Alkan et al (15), and Alvarez et al (18), who suggested that SOD plays a role in protecting spermatozoa and hence might be used in sperm preparation .On the other hand, these results disagree with the findings of Aitken et al (20), and Gavella et al (21), who proposed that the increase in SOD activity may have deleterious effect on spermatozoa, due to an increase in dismutition of O_2 and enhanced production of H_2O_2. The discrepancies between their results was explained by the effectiveness and harmony of other enzymes such as catalase and / or glutathione peroxidase, which are associated with SOD, in order to avoid peroxide accumulation, which could determine the final balance between the generation and elimination of H_2O_2 and thus protection of the human spermatozoa against H_2O_2 effect .The observed decrease in SOD scavenging activity in this study may result from synthetic defect or inactivation of the enzyme by the increase in H_2O_2 production .

The decline in GSHPX activity that was observed here in the seminal plasma of infertile men may lead to hydrogen peroxide accumulation to a concentration that might suppress SOD activity (22). It seems that the main superoxide anion scavenger in plasma is SOD, since that when Cp scavenging activity was measured, no significant differences was observed ($p> 0.05$) between the two studied groups. Total sulfhydryl level and its individual parts protein and non-protein sulfhydryl levels were measured in seminal plasma as a non-enzymatic antioxidant. The results in table (1-4) show a significant decrease in (T-SH) & (PB-SH). These results are consistent with that reported by others, who found that a significant elevation in ROS level was associated with a significant reduction in antioxidants level including total sulfhdryl groups' content in seminal plasma of infertile men when compared to fertile men (23,24,15). Potts et al (25) throughout their study on vasectomized men suggested that certain antioxidant, such as thiol containing compounds accumulate in the human epididymal, where they act to protect the sperm against the adverse effects of ROS. Thiol group is considered as one of the important mechanisms that protect the cellular components against the free radicals, this mechanism involves the ability of the reducing thiol to react with radicals leading to its conversion to inactive oxidized form (26). According to the above reasons, the low level of sulfhydry group that was observed here seems to contribute to the oxidation reduction imbalance that present in seminal plasma. Another non-enzymatic parameter that was measured in seminal plasma was albumin. It acts through its role in inhibition the generation of free hydroxyl radicals from systems containing copper ions and hydrogen peroxide. This mechanism could be explained according to the ability of copper ions to bind to albumin, and thus become less available for oxidation-reduction reaction; however, these radicals immediately attack the albumin molecule itself and so they are no longer be free in the solution (27). The albumin will itself be damaged (6,27-28) , but there is so much albumin in seminal plasma , as well as, it is quickly replaced by newly made of albumin, that such damage will be biologically insignificant. In conclusion, the results of the present study shows those seminal plasma antioxidant activities were significantly lower in infertile patients, when compared with that of control group. This may reflect the presence of the imbalance between pro/ antioxidant, which in turn lead to increase in the production of reactive oxygen species in seminal plasma and ultimately cause defect in sperm function.

References: -

1- De-Kretser, D.M.; (1997): Male infertility. Lancet; 349; 15: 787-790.

2- Irvine, D.S.; (1996): Assessment of Spermatogenesis Obst. Gyn .j.; p.20.

3- Halliwall, B.; (1994): Free Radical, Antioxidants and Human Disease: Curiosity, Cause or Consequence. Lancet, 344, 10:721-724.

4- Thomas, E.; and Andreoil, M.D.; (2000): free radical and oxidative stress. Am.J.Med; 108:650-651.

5- Comhaire, F.H.; Schoojnans; F.A.; Rombaut; E.et al.; (1988): The History of Male Infertility and Computer Assisted Management of the infertile Male. In: Barrett, C.I.; Cook, I.D. (Eds.): Advances in Clinical Andrology. Lancaster: MTP press limited: 5-13.

6- Tauber, P.F.; Zaneveld, L.J.D.; Propping, D.; and Schumicher 'G.F.B. (1975): components of Human split Ejaculate. Spermatozoa, Fructose, Immunoglobulin, Albumin, Lactoferin, Transferring, and other proteins. J.Reprod Fertil.43, p.249.

7- World Health Organization. WHO (1993): Laboratory Manual for Examination of Human Semen and semen cervical Mucus Interaction, 3 ed., Cambridge, UK: The press syndicate of the university of Cambridge.

8- Burk, F.R.; Lawrence, A.R.; and Lane, M.J.; (1980) Liver Necrosis and lipid peroxidation in the Rat as the Result of paraquat and Diquat Administration. Clin. Invest; 65:1024-1037.

9- Sedlok J. And Lind Say H.R; (1968): Estimation of Total protein - Bound, and Nonprotein Sulfhydryl Groups in tissue With Ellman's Reagent. Anal, Biochem.25: 192-205.

10- Beyer, W.F. and Fridovich, I., (1987): Assaying for speroxide dismutase activity: Some Large Consequences of Minor in Condition. Anal. Biochem; 161:559-566.

11- Friden, E., CP; 1980: Multifunctional Metalloprotein of vertebrate plasma in Biological Roles of copper, Ciba foundation symp. 79 Escerrpta, Medica Elsevier Broth Holland, Amsterdam.

12- De-Lamirande, E.; Tiang, H.; Zini, A., and Gagnon, C.; (1997): Reactive Oxygen Species and Sperm Physiology. Reviews of Reproduction, 2,48:54.

13- De-Lamirande, E.and Gagnon, C.; (1993): A positive Role for the Superoxide Anion in Triggering Hyper activation and Capacitation of Human Spermatozoa. Int. J.Andro. 61:21-25.

14- Aitken; J.R.; Buckingham and west, M.K. ;(1992): Reactive Oxygen Species and Human spermatozoa: Analysis of the cellular Mechanisms Involved in Luminol-and lucigenin - dependent chemiluminescence's J.Cell. Physiology .151:466.

15-Alkan, I.; Simsek, F., Haklar, G.; Kervancioglu, E.; Ozveri, H.; Yalcin, S.; And Akdas, A.; (1997) ROS production by the spermatozoa of patients with Idiopathic Infertility: Relationship to Seminal Plasma Antioxidant. J. Urol,. 157:140-143.

16- Crap, J.D., Oury, I.; Raboville, C.and et al.; (1992): Copper, Zinc-SOD Inprimarly Aacytosolic Protein In Human cells, Proc Nat Acad Sci: USA; 89:10415-10409.

17- Zini, A.; de-Lamirande, E.; and Gagnon, C.; (1993): ROS In Semen Of Infertile Patients: level of spermatozoa. Int.J. Androl.; 16:183-188.

18- Alvarez, J .G.; Touchtone, J.C.; Basco, L.; and Storey, B.T.; (1987): Spontaneous Lipid Peroxidation And Production of Hydrogen peroxide And Superoxide In Human Spermatozoa: SOD as Major Enzyme protectant Against Oxygen Toxicity J. Androl; 8,338-348.

19- Aitken, J.R. and Fisher, H.; (1994): Reactive Oxygen Species Generation And Human Spermatozoa: The Balance of Benefit And Risk. Bioassays, 16:259-267.

20- Zalata, A.; Hafez, T.and Comhair, F.;(1995): Evaluation of the Role of Reactive Oxygen Species In Male Infertility .Hum-Repord.; 10; 1444-1451.

21- Gavella, Mm.; Lipovac, V.; Vucic and Rocic, B.; (1995): Relationship of Sperm SOD-like Activity with Other Sperm-Enzyme and Experimentally Induced Lipid Peroxidation in Infertile Men. Ander.28: 223-229.

22- Hodgson, E.K. and Fridovich, I.; (1975): The Interaction Of Bovine Erythrocyte Superoxide Dismutase With Hydrogen Peroxide: Inactivation of the Enzyme Biochem.14, 24,5294-5298.

23- Sannocka, D.; Miesel; R.; Jedrzijczak, P.; and Kurpisz, M., K. .; (1996) : Oxidative Stress And Male Infertility J.Androl; 17;449-454.

24- Lewis, S.E.M; Boyle, P.M.; Me Kinney, K.A.; Young, I.S., and Thompson, W.; (1995): Total Antioxidant Capacity Of Seminal Plasma Is Different in Fertile And Infertile Men .Sterile. 64:868:870.

25- Potts, R.J; Jeffries, M.T, and Nortarianni, J, L.; (1999): antioxidant Capacity of the Epidiymis. Hum.Repord. 14, 10:2513-2516.

26- Munday, R.and winterboun, C.C.; (1989): Reduced Glutathione in Combination with Superoxide Dismutase As An Important Biological Antioxidant Defense Mechanism Biochem.Pharma. 38, 24:4349-1352.

27- Hinton, B.T.;(1980): the Epididmal Microenvironment .Invest.Urol., 18:1.

28- Mann, T.;(1946): Studies on Metabolism of Semen; Fructose as a Normal Constituent of seminal plasma, Site of formation And Function of fructose in semen .Biochem. J., 40:481.

Table (1-1): Biostatistical calculations of students' t-test for seminal plasma Gpx Activity and specific activity in fertile and infertile group

Group	Sample Size (n)	Activity Mean S.D (m.unit)	Activity Range (m.unit)	P value	Specific Activity Mean S.D m.unit/mg	Specific Activity Range m.unit/mg	P value
Fertile	18	53.7±7.02	45.8±73.9		1.14±0.37	0.98-1.58	
Infertile	66	33.9±6.6	29.6±55.0	<0.05	0.71±0.11	0.62-1.35	<0.05

Group	Sample Size	Activity (unit/mg)	P value	Specific activity (unit/mg)	P value
Fertile	25	9.33±1.61	-	0.199±0.062	-
Infertile	100	5.33±0.86	0.001	0.111±0.006	<0.05

Table (1-2): Biostatistical calculations of students' t-test for seminal plasma SOD scavenging activity and specific activity in fertile and infertile group

Table (1-3): Biostatistical calculations of students' t-test for seminal plasma Cp. scavenging activity in fertile and infertile group

Group	Sample Size (n)	Activity mean value (unit/ml)	S.D	Activity range (unit/ml)	P value
Fertile	25	31.21	7.68	23.2-37.0	-
Infertile	100	27.92	6.63	21.2-30.1	<0.05

Table (1-4): Biostatistical calculations of students' t-test for seminal plasma (T-SH), (BP-SH) and (NP-SH) level in fertile and infertile group

Group	Sample Size	T-SH		BP-SH		NP-SH	
		Mean value S.D (mole/L)	P value	Mean S.D (mole/L)	P value	Mean S.D (mole/L)	P value
Fertile	25	288.89±20.9	-	190.07±13.6	-	38.82±6.20	-
Infertile	100	188.0±28.1	<0.05	155.5±20.3	<0.05	32.5±4.6	<0.05

Table (1-5): Biostatistical calculations of students' t-test for seminal plasma albumin level in fertile and infertile group

Group	Sample Size (n)	Mean (Gm/100ml)	S.D	Range (Gm/100/ml)	P value
Fertile	25	0.68	0.19	0.41-1.2	-
Infertile	100	0.65	0.21	0.19-1.32	<0.05