International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: 10.7439/ijbr
CODEN: IJBRFA

Original Research Article

A study of variation in the levels of seminal plasma superoxide dismutase and glutathione peroxidase in normospermic and oligozoospermic men

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Abstract

Background: Infertility is one of the major concerns of humankind and the male cause of infertility contributes nearly a third for its condition. Amongst many a factor that influences sperm health, the seminal antioxidants are one among the vital determinants of male fertility.
Aim and objective: To estimate and compare the levels of seminal plasma superoxide dismutase (SOD) and glutathione peroxidase (GPx) between normospermic and oligospermic men and to correlate them with the seminal parameters of sperm count, motility and vitality of the seminal samples of the respective population.
Methodology: This pilot effort is a hospital –based study with the study population comprising of 10 normospermic men for the control group and 10 oligospermic volunteers for the case group. Superoxide dismutase and glutathione peroxidase were estimated in the seminal plasma to reflect the antioxidant status in the seminal sample.
Results: SOD levels in normospermic was 11.19± 1.22 (U/ml of seminal fluid) while in oligospermic was 9.17 ± 1.94 (U/ml of seminal fluid) andGPx level in normospermic was 24.46 ±7.39 (µg of GSH consumed /min/ml of seminal fluid) while in oligospermic was 17.23 ±8.27 (µg of GSH consumed/min/ml of seminal fluid). SOD is significantly reduced (P value 0.012 (<0.05)) and GPx reduced with P value 0.054 in oligospermic men, which correlates with linear decrease in the seminal parameters of sperm count, motility and vitality in the oligospermic samples .
Conclusion: The decreased levels of SOD and GPx in the seminal plasma of oligospermic men when compared to normospermic population signifies a reduced scavenging machinery in their seminal plasma which would have lead to the decrease in the sperm count due to oxidative stress thus leading to infertility.
Keywords: Glutathione peroxidase, Male infertility, Sperm count, Superoxide dismutase

1. Introduction

Amongst many an illness that affects mankind, infertility has a multi-faceted effect on life affecting it in many dimensions that includes social and psychological frames. Infertility (clinical definition) is defined as 1 year of unwanted non-conception with unprotected intercourse in the fertile phase of the menstrual cycles[1]. According to The World Health Organization (WHO) 60 to 80 million couples worldwide suffer from infertility with an overall prevalence of primary infertility between 3.9 and 16.8 per cent in India[2]. The male factor has been identified as a contributor to infertility in 40 %-50 % of infertile couples[3].

Infertility in men canresult from many causes, from gametogenesis to ejaculation, genetic abnormalities, infections, structural defects, hormonal imbalance, environmental causes etc[4]. Recently, the role of reactive oxygen species (ROS) in sperm damage is implicated in 30–80% of cases[5].

Within physiological limits the ROS produced in sperms assists in capacitation, acrosome reaction, and oocyte fusion. But when their formation

exceeds the scavenging capacity of the antioxidants endowed in sperms and seminal plasma, oxidative stress results leading to sperm damage[5]. Seminal plasma is endowed with an array of antioxidants, both enzymatic and nonenzymatic, that scavenge, dispose, and suppress the formation of ROS, or oppose their actions[6]. The male reproductive system has enzymatic scavengers to scavenge the ROS produced.

SOD is both extacellular and intracellular scavenger that causes the spontaneous dismutation of (O_2-) anion to form O_2 and H_2O_2 and H_2O_2 is disposed by being converted to O_2 and H_2O by catalase. The other alternative defence mechanisms present against reactive oxygen species is SOD and the glutathione peroxidase/ reductase pair (GPX/GRD)[7]. The H_2O_2 formed by SOD action gets disposed by glutathione peroxidase with glutathione as electron donor in the reaction. Glutathione disulphide(GSSG) formed from the above step gets converted to reduced glutathione by Glutathione reductase, which maintains the motility of sperms by scavenging peroxyl radicals as they abound in sperm mitochondria, an observation made in rats. Lipid peroxidation of sperm is also inhibited by GPx along with GSH[8].

As oxidative stress results due to defective antioxidant machinery, this study has been planned to compare the seminal parameters of oligozoospermic men with that of the normospermic men in reference to their seminal plasma concentrations of the enzymatic antioxidants superoxide dismutase (SOD) and glutathione peroxidase (GPx).

1.1 Aim &Objectives

To study the variations in the levels of seminal plasma antioxidants superoxide dismutase and glutathione peroxidase between normospermic and oligospermic seminal samples and correlate them with the seminal parameters of sperm count, motility, morphology and vitality of the respective population.

2. Materials and Methods

2.1 Methodology

This hospital based cross sectional study was carried out as a pilot effort at Sri Ramachandra University after obtaining Institutional Ethical Clearance. Men who visited andrology department for evaluation of infertility were recruited for the study (10 oligospermic and 10 normospermic men). The volunteers were selected based on their preliminary seminal analysis report particularly the sperm count and a screening questionnaire was administered to collect information about the exclusion criteria. Men between the age group of 20 and 35 were selected with sperm count of < 20 million/ml of seminal fluid for oligospermic and ≥ 20 million/ml for normospermic groups. Men on infertility treatment, on antioxidant therapy and those with active infections were excluded.

Informed written consent was obtained from the volunteers after recruitment and the purpose of the study was explained to them. The participants were requested to give a fresh seminal sample for the study, after a period of 48 hrs of abstinence. Samples were collected in sterile containers under aseptic precautions. Each sample was given an individual coding in respect to their groups. The samples collected were transferred to the lab immediately and centrifuged for 10 min at a rate of 3500 rpm at 4˚C. 2 ml of the supernatant of the centrifuged samples was transferred to an eppendorf and stored in deep freezer until analysis.

2.2 Laboratory Methods for the assessment of antioxidant status

The enzymes SOD & GPx were chosen for the assessment of antioxidant status.

2.2.1 Estimation of Superoxide dismutase (SOD)

Superoxide dismutase was assayed by taking 0.05ml of seminal fluid followed by addition of 0.3 ml of sodium pyrophosphate buffer (0.025M, PH 8.3, 0.025ml of PhenazoniumMetho Sulphate (186µM) and 0.075ml of Nitro Blue Tetrazolium (300µM in buffer of pH 8.3).The reaction was started by addition of 0.075 ml of Nicotinamide adenine dinucleotide (780µM in buffer of pH8.3). After incubation at 30°C for 90 seconds, the reaction was stopped by addition of 0.25ml glacial acetic acid. Then the reaction mixture was stirred vigorously and shaken with 2.0ml of n-Butanol. The mixture was allowed to stand for 10 minutes and centrifuged. 1.5 ml of n-butanol alone was served as blank. The colour intensity of the chromogen was read at 560 nm[9]. Enzymatic activity (1 Unit) = 50% inhibition /min.

2.2.2 Estimation of Glutathione peroxidase (GPx)

GPx was assayed by taking 200µl of Tris HCL Buffer (0.4mM), 0.4mM K.EDTA along with 100µl sodium azide (10mM) and 200 µl of seminal fluid and mixed well. Thereafter, 200µl of reduced glutathione solution (2mM) followed by 0.1 ml H_2O_2 were added. The overall reaction was arrested by adding 0.5ml of 10% Trichloroacetic acid. The precipitate was removed by centrifugation at 4000rpm for 10 min. The absorbance was read at 412nm using spectrophotometer. The non-enzymatic reaction rate was correspondingly assessed by replacing the enzyme sample by buffer. The results are expressed as nmoles / min / litre serum[10]. The activity of GPx expressed as microgram GSH consumed /min/ml.

2.3 Statistical Analysis

The data collected was analysed using SPSS 11.4 software. The two groups were compared using Student's t-test. The level of significance was taken at 5%.

3. Results

This cross sectional study was conducted among 10 oligospermic and 10 normospermic men to assess the antioxidant status in the seminal plasma. The variations in the age, sperm count, motility, morphology and vitality between the normospermic and the oligospermic groups are shown in the following descriptive table.

Table 1: Descriptive of the study subjects

Parameters	Type	Mean	Std. Deviation	P value
Age in years	Normal	30.20	2.74	0.934
	Oligospermia	30.10	2.56	
Sperm count (millions/ml)	Normal	49.40	20.88	< 0.001
	Oligospermia	7.80	7.31	
Motility (%)	Normal	55.00	10.80	<0.001
	Oligospermia	16.50	14.92	
Morphology (%)	Normal	5.60	2.55	<0.001
	Oligospermia	1.60	1.07	
Vitality (%)	Normal	78.40	10.54	0.015
	Oligospermia	47.60	34.63	

The two groups did not differ by age but the seminal parameters are significantly reduced in the oligospermic group. (<0.05)

Figure 1: Graph showing Comparison of sperm count levels between Normospermic and Oligospermic groups

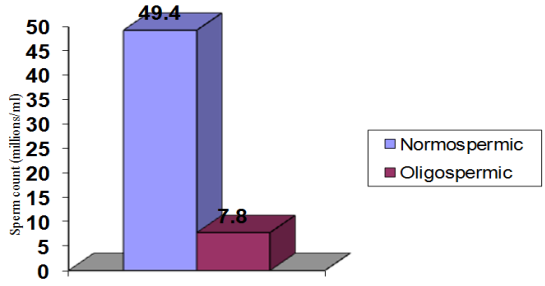


Figure 1 shows the variation in the levels of sperm count between the normospermic and oligospermic groups. The sperm count of the normospermic group is 49.40 ± 20.88 millions/ml of seminal fluid while that of the oligospermic group is 7.80 ± 7.31 millions/ml of seminal fluid. The count is significantly reduced in the oligospermic men with a p value <0.001 which is statistically significant.

Figure 2: Graph showing Comparison of SOD levels between Normospermic and Oligospermic groups

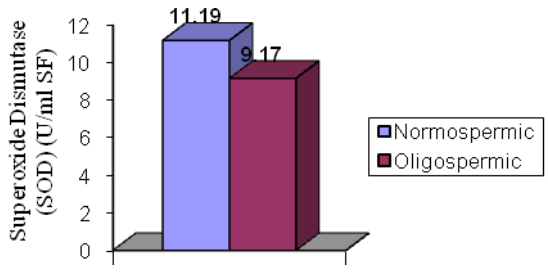
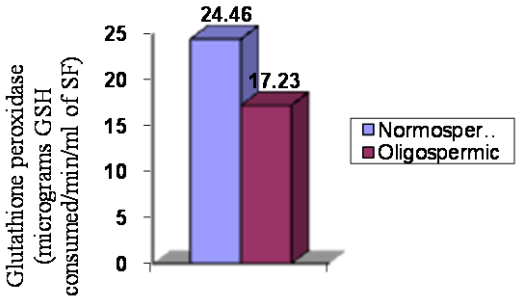


Figure 2 shows the difference in the concentrations of seminal plasma Superoxide dismutase (SOD) between the normospermic and the oligospermic groups.

The level of SOD in normospermic men is 11.19±1.22 (U/ml of seminal fluid) while in oligospermic is 9.17±1.94 (U/ml of seminal fluid) and the reduction in the oligospermic group is significant with a p value 0.012.

Figure 3: Graph showing Comparison of GPx levels between Normospermic and Oligospermic groups



The comparison between the normospermic and oligospermic levels of glutathione peroxidase (GPx) is shown in the above bar plot (graph 3).

The mean level of GPx in normospermic is 24.46 ± 7.39 (micrograms GSH consumed /min/ml) while that of oligospermic is 17.23 ± 8.27 (micrograms GSH consumed /min/ml). The level of glutathione peroxidase is reduced in oligospermic men with a p value 0.054.

4. Discussion

The results of our study showed that SOD was reduced significantly and GPx also reduced in the oligospermic seminal fluid samples reflecting a decline in seminal plasma antioxidant status. The parameters of sperm count, motility, morphology and vitality were also found to be significantly reduced in oligospermic men when compared to normospermic subjects reflecting the consequence of derangement of seminal antioxidant machinery which has led to oxidative stress.

Various causative conditions of infertility could have reduced SOD, GPx and other endogenous antioxidants leading to the onset of lipid peroxidation[11]. In accord with our study are the studies of Murawski *et al* and Nissen *et al*, who had similar observation in oligoasthenozoospermic patients, found a positive correlation between SOD activity in seminal plasma and seminal parameters of sperm concentration and overall motility thus establishing a liner relation between SOD concentration and sperm motility by prevention of lipid peroxidation[12][13]. The positive correlation between decreased SOD level and sperm count could

be due to abortive apoptosisand DNA damage [14]-[17]. SOD prevents spontaneous O_2 toxicity and lipid peroxidation (LPO) of spermatozoa as well prevents premature hyperactivation and capacitation induced by superoxide radicals before ejaculation[6].

In contrast, significant higher SOD activity was reported in spermatozoa from infertile men by Zalata *et al* and Sinha *et al*; which is attributed to a defective maturation or development of sperm, as well as damage to sperm leading to infertility[18][19]. Non significant relation between SOD concentration and semen quality was also reported by Zini *et al* and Hsieh *et al*[20][21].

Our study demonstrated a reduction in the seminal plasma glutathione peroxidase with p value 0.054. The reduction in their levels among the oligospermic population correlated positively with the sperm count and motility. The reduction in sperm count in oligospermic men is attributable to decreased GPx levels[22][23].

In contrast, non-difference of GPx activities between normo and hypomotile human sperm samples and non association of the GPx activities with the sperm motility or concentration were also reported by Tramer *et al* and Yao-Yuan Hsieh *et al*[23][24].

The decreased seminal parameter that was reported in our study could be the result of imbalance between ROS generation and scavenging activities. Levels of antioxidants in seminal plasma from infertile men were significantly low.It is thus inferred from our study that the antioxidant concentration of the seminal plasma of oligospermic men is significantly reduced when compared to normospermic men. This significant reduction in the scavenging capacity would have lead to the precipitation of oxidative stress in the seminal plasma of oligospermic men leading to decreased sperm count, impaired motility & vitality.

5. Summary and Conclusion

In this study, the antioxidant concentration of the seminal plasma which is measured by assessing the levels of SOD and GPx ,with reduction in the former with significance and the latter comparatively less in the oligospermic subjects when compared to the normospermic men, make us infer that the seminal scavenging capacity is declined in the oligospermic group which could have lead to the occurrence of oxidative stress in the oligospermic population which is reflected by the significant reduction in the semen quality in terms of sperm count, motility, morphology and viability in the same category of people.

Limitations

- Sample size of this study is less; nevertheless this pilot effort serves to make an emphasis on the role of antioxidants on fertility.
- As it is a self-funded project the total antioxidant capacity (TAC) estimation of the seminal plasma was not done, which could have added more insight into the antioxidant status.

References

[1] Gnoth C., E. Godehardt, P. Frank-Herrmann, K. Friol, Jurgen Tigges and G. Freundl Definition and prevalence of subfertility and infertility.*Hum. Reprod*. 2005; 20(5): 1144-1147.

[2] Paul C. Adamson, Karl Krupp , Alexandra H. Freeman, Jeffrey D. Klausner, Arthur L. Reingold & Purnima Madhivanan Prevalence & correlates of primary infertility among young women in Mysore, India. *Indian J Med Res* 2011; 134; 440-446.

[3] Rajvi H. Mehta, Sanjay Makwana, Geetha M. Ranga, R. J. Srinivasan, S. S. Virk. Prevalences of oligozoospermia and azoospermia in male partners of infertile couples from different parts of India. *Asian J Androl* 2006; 8 (1): 89-93.

[4] Olooto, W.E. Infertility in male; risk factors, causes and management- A review. *J. Microbiol. Biotech. Res.*, 2012; 2(4):641-645.

[5] Kelton Tremellen. Oxidative stress and male infertility—a clinical perspective. *Human Reproduction Update* 2008; 14(3):243-258.

[6] Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Front Biosci* 1996; 1:78-86.

[7] Irvine DS Glutathione as a treatment for male infertility. *Reviews of Reproduction*1996; 1: 6-12.

[8] Maneesh M and H Jayalekshmi. Role of reactive oxygen species and antioxidants on pathophysiology of male reproduction. *Indian Journal of Clinical Biochemistry*, 2006; 21(2): 80-89.

[9] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind J BiochemBiophys*1984; 21:130-132.

[10] Rotruck, J. T., Pope, A. L., Ganther, H. E.,Swanson, A. B. & Hoekstra, D. G.Selenium: Biochemical role as a component of glutathione peroxidase. *Science*1973; 179, 588-590.

[11] Aitken RJ and Shaun D.Roman. Antioxidant systems and Oxidative stress in the testis. Bookshelf, Madame Curie Bioscience Database, *Reproductive Biology (Internet)*, 2000.

[12] Murawski M, Saczko J, Marcinkowska A, Chwikowska A, Grybooe M, Banaoe T Evaluation of superoxide dismutase activity and its impact on semen quality parameters of infertile men. *Folia Histochem Cytobiol* 2007; 45:123–126.

[13] Nissen H.P. and H.W. Kreysel. Superoxide Dismutase in Human Semen. *Klin Wochenschr* 1983; 61:63-65.

[14] Bayard T. Storey Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. *Molecular Human Reproduction*1997; 3(3): 203–213.

[15] Shamsi, M B, Venkatesh, S, Kumar, R, Gupta, N P, Malhotra, N, Singh, N, Mittal, S *et al* Antioxidant levels in blood and seminal plasma and their impact on sperm parameters in infertile men. *Indian Journal of Biochemistry & Biophysics* 2010; 47:38-43.

[16] Samant Parineeta. M., Badade Zunjarrao. G., Kate Madhuri. S. & Deepak. A.D. Seminal Malondialdehyde Concentration and Superoxide Dismutase, Catalase Activity in Male Infertility. *The Internet Journal of Health.* 2010; 11(2).

[17] Hong Chen, Pak Ham Chow,So Kwan Cheng, Annie L. M. Cheung, Lydia Y. L. Cheng and Wai-Sum O. Male Genital Tract Antioxidant Enzymes: Their Source, Function in the Female, and Ability to Preserve Sperm DNA Integrity in the Golden Hamster. *J Androl* 2003; 24:704–711.

[18] Zalata A, Hafez T, Comhaire F. Evaluation of the role of reactive oxygen species in male infertility. *Hum Reprod* 1995; 10: 1444–51.

[19] Sinha S, Pradeep KG, Laloraya M, Warikoo D. Over-expression of superoxide dismutase and lack of surfacethiols in spermatozoa: inherent defects in oligospermia. *Biochem Biophys Res Commun*. 1991; 174(2):510-517.

[20] Zini A, de Lamirande E, Gagnon C. Reactive oxygen species in semen of infertile patients: levels of superoxide dismutase and catalase like activities in seminal plasma. *Int J Androl*1993; 16: 183-8.

[21] Hsieh YY, Sun YL, Chang CC, Lee YS, Tsai HD, Lin CS. Superoxide dismutase activities of spermatozoa and seminal plasma are not correlated with male infertility. *J Clin Lab Anal.* 2002; 16(3):127-31.

[22] Dandekar SP, Nadkarni GD, Kulkarni VS, Punekar S. Lipid peroxidation and antioxidant enzymes in male infertility. *J Postgrad Med*. 2002; 48:186-90.

[23] Hsieh, Y.Y.; Chang, C.C.; and Lin CS. Seminal malondialdehyde concentration but not glutathione Peroxidase activity is negatively correlated with seminal concentration and motility. *Int J Biol Sci*. 2006; 2: 23-29.

[24] Tramer F, Caponecchia L, Sgro P, Martinelli M, Sandri G, Panfili E, Lenzi A, Gandini L. Native specific activity of glutathione peroxidase (GPx-1), phospholipid hydroperoxide glutathione peroxidase (PHGPx) and glutathione reductase (GR) does not differ between normo- and hypomotile human sperm samples. *Int J Androl* 2004; 27: 88 93.