THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (September-October 2012) 62(5):546–550
DOI 10.1007/s13224-011-0070-3

ORIGINAL ARTICLE

Association of Oxidative Stress Biomarkers and Antioxidant Enzymatic Activity in Male Infertility of North-East India

Mahanta R. · Gogoi A. · Chaudhury P. N. B. · Roy S. · Bhattacharyya I. K. · Sharma P.

Received: 19 January 2011 / Accepted: 23 August 2011 / Published online: 14 April 2012

Abstract

Introduction Oxidative stress is a common pathology seen in approximately half of all infertile men. In a normal situation, the seminal plasma contains antioxidant mechanisms which are likely to quench these reactive oxygen species. However, during infertility complications these antioxidant mechanisms may downplay and create a situation which is called oxidative stress.

Objective The aim of the present study was to assess the levels of lipid peroxide (LPO), protein peroxide (PPO) and activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) in blood and semen samples of an infertile male population from North-East India.

Method We measured LPO, PPO, SOD and GPX in a total of 50 infertile individuals. For the study 20 fertile donors served as the control group.

Result Patients with male factor infertility had significantly higher LPO and PPO levels (60.84 ± 3.55 and 72.84 ± 3.66; $P < 0.001$) compared with controls (40.20 ± 4.33 and 59.93 ± 5.24) in blood. In semen also, the same trend was found with significantly higher LPO and PPO levels (200.27 ± 6.25 and 149.80 ± 11.47; $P < 0.001$) compared with controls (116.51 ± 5.49 and 59.10 ± 4.62). The SOD and GPX enzymes in blood (3.40 ± 1.06 and 0.16 ± 0.01; $P < 0.001$) and in semen (2.42 ± 1.32 and 0.24 ± 0.015; $P < 0.001$) showed a significantly lower activity when compared with their respective controls (4.85 ± 0.78; 0.36 ± 0.05 and 4.24 ± 0.89; 0.65 ± 0.03). The SOD and GPX activity when compared with the LPO and PPO values, showed a positive correlation.

Conclusion We conclude that oxidative stress is associated with male factor infertility. This assessment may help in the treatment of this male infertility by suitable antioxidants.

Keywords Oxidative stress · Antioxidants · Male infertility · Reactive oxygen species

Mahanta R., Principal Investigator ·
Gogoi A., Project Assistant ·
Chaudhury P. N. B., Project Assistant ·
Roy S., Project Assistant · Bhattacharyya I. K.,
Co-investigator Cotton College, Guwahati, Assam, India

Sharma P., Co-investigator
Pratiksha Hospitals, Guwahati, Assam, India

Chaudhury P. N. B. (✉), Project Assistant
Department of Zoology, Cotton College, Guwahati 781001, India
e-mail: purnali.nath@gmail.com

Introduction

There is a growing body of evidence to indicate that a significant factor in the aetiology of male infertility involves loss of sperm function as a consequence of oxidative stress which triggered intense interest in this field of andrology.

Infertility affects approximately 15% of all couples trying to conceive. Male factor infertility is the sole or contributing factor in roughly half of these cases [1]. The north eastern region with a mosaic tribal population contains more than 25 hill and plain tribes. Reports available from local infertility clinic indicate that male infertility is a major health problem among this population.

In recent years, the generation of reactive oxygen species (ROS) in the male reproductive tract has become a real concern because of their potential toxic effects at high levels on sperm quality and function [2].

Cells living under aerobic conditions constantly face the oxygen (O_2) paradox. Oxygen is required to support life, but its metabolites such as ROS can modify cell functions, endanger cell survival, or both [3]. Seminal oxidative stress develops as a result of an imbalance between ROS generating and scavenging activities [4, 5].

Oxidative stress attacks not only the fluidity of the sperm plasma membrane, but also the integrity of DNA in the sperm nucleus [6]. Sperm DNA damage is significantly increased in men with idiopathic and male factor infertility. Such an increase may be related to high levels of seminal oxidative stress [7].

Mammalian spermatozoa membranes are rich in high unsaturated fatty acids and are sensitive to oxygen induced damage mediated by lipid peroxidation. Assessment of such oxidative stress status (OSS) may help in the medical treatment of this male factor infertility by suitable antioxidants [8].

An increase in oxidative damage to sperm membranes, proteins and DNA is associated with alterations in signal transduction mechanisms that affect fertility. Recent evidence suggests that spermatozoa and oocytes possess an inherent but limited capacity to generate ROS to aid in the fertilization process [9]. Moreover data have recently been obtained to indicate, although excessive exposure to ROS may be harmful to spermatozoa, in physiological amounts these molecules are of importance in the control of normal sperm function [10].

Antioxidants act as free radical scavengers to protect spermatozoa against ROS. These antioxidants are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX). In addition, semen contains a variety of nonenzymatic antioxidant molecules such as vitamin C, vitamin E, pyruvate, glutathione, and carnitine [11]. These antioxidants compensate for the loss of sperm cytoplasmic enzymes as the cytoplasm is extruded during spermiogenesis, which in turn, diminishes endogenous repair mechanisms and enzymatic defenses [12].

Assessment of such OSS along with the role of antioxidants may help in the medical treatment of this male factor infertility. The aim of the present investigation is to study and correlate the extent of lipid peroxidation and protein peroxidation in infertile males along with the role of antioxidants.

Materials and Methods

Prior to the study approval was taken from the institutional ethics committee and informed consent was taken from all the participating subjects. All samples were obtained from a local infertility clinic.

Patients

Men with proven fertility (who had normal semen profile) were taken as controls.

Blood and semen samples were collected from 50 men with idiopathic infertility aged between 25 and 45 years. The blood and semen samples were divided according to the World Health Organization [13] classification and by Kruger's strict criteria [14]. 20 normal fertile men (normozoospermia, $>40 \times 10^6$ sperms/ml) were taken as controls and 50 infertile males: 20 severe oligozoospermic; $<10 \times 10^6$ sperms/ml, 20 asthenoteratozoospermic; morphology <14% and motility −a + b grade sperms <50% and 10 azoospermic (no sperms) were included in the infertile group (Table 1).

Seminal Plasma Isolation

After 30 min of liquefaction at room temperature, spermatozoa from all samples were separated from seminal plasma by centrifugation at $500 \times g$ for 10 min. The seminal plasma was used for all the enzymatic estimations.

Lipid Peroxidation

Lipid peroxidation was estimated according to the method of Ohkawa et al. [15]. The amount of malondialdehyde (MDA) produced was used as an index of lipid peroxidation. The samples were treated with 600 µl of 10% TCA solution, mixed properly and centrifuged for 10 min at 5,000 rpm. After 5 min 150 µl of TBA reagent was added to 200 µl of the supernatant and again mixed with 100 µl

Table 1 Percentage distribution of the samples

Types of samples	No. of samples	% distribution
Normozoospermic	20	28.57
Oligozoospermic	20	28.57
Asthenoteratozoospermic	20	28.57
Azoospermic	10	14.28
Total no. of samples	70	

of deionised water. A blank tube without the sample was used as reference. Both the sample and the blank tubes were placed in a boiling water bath for 10 min and allowed to equilibrate with room temperature. Absorbance of the samples and blank were measured at 532 nm. All values were expressed as nmoles of MDA/ml present in it.

Protein Peroxidation

Protein peroxidation was estimated according to the method of Ohkawa et al. [15]. 10 µl of samples were treated with 600 µl of 10% TCA solution, mixed properly and centrifuged for 10 min at 5,000 rpm after 5 min. The precipitate was washed two times with distilled water and then dissolved in 0.6 ml of 0.5 N NaOH solution. 200 µl of this sample solution is again mixed with 150 µl of TBA reagent and 100 µl of deionised water. A blank tube without the sample was used as reference. Both the sample and the blank tubes were kept for equilibration similar to lipid peroxidation and absorbances were measured at 532 nm. The protein peroxide is expressed as nanomoles of thiobarbituric acid reactive substance producing MDA per ml of sample. The content of MDA is calculated by using the molar extinction coefficient for MDA at 530 nm.

Determination of SOD Activity

SOD activity was estimated using Ransod Superoxide Dismutase diagnostic kit (RANDOX Laboratories Ltd, United Kingdom) according to the manufacturer's instructions. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye.

The superoxide dismutase activity is then measured by the degree of inhibition of this reaction [16].

Determination of GPX Activity

GPX activity was estimated using Ransel Glutathione Peroxidase diagnostic kit (RANDOX Laboratories Ltd, United Kingdom) according to the manufacturer's instructions. This method is based on that of Paglia and Valentine [17]. GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

All data is presented as mean ± standard deviation, followed by the students '*t*' test.

Results

We studied a total of 70 samples. Of these, 20 were normozoospermic, 20 oligozoospermic, 20 asthenoteratozoospermic and 10 azoospermic. The normozoospermic samples were taken as controls.

Oxidative Stress Status

The levels of lipid peroxides in the blood of the infertile group were significantly higher than those of the fertile group ($P < 0.001$). In semen samples also, significantly high levels of lipid peroxides were observed ($P < 0.001$).

Protein peroxidation scores in blood and semen samples of the infertile group was significantly increased ($P < 0.001$) compared with that of the normal control group (Table 2).

Antioxidants Status

In the infertile group, the activity of SOD and GPX in blood was significantly lower ($P < 0.001$) compared with the fertile donors.

Similar to the results from blood, the SOD and GPX activities in seminal plasma showed significantly decreased levels in the infertile group (Table 3).

The lipid peroxide and protein peroxide levels correlated positively with the superoxide dismutase and glutathione peroxidase activity in blood and seminal plasma of infertile males.

Discussion

The over-production of ROS in the male reproductive tract has become a real concern in recent times.

ROS may cause a defect in sperm function through lipid peroxidation [4]. A major cause of oxidative stress appears to be the high rate of reactive oxygen species generation associated with the retention of excess residual cytoplasm in the sperm midpiece. Other possible causes include the redox cycling of xenobiotics, and antioxidant depletion or apoptosis. Oxidative stress induces peroxidative damage in the sperm plasma membrane and DNA damage in both the mitochondrial and nuclear genomes.

However, it has been suggested that small amounts of ROS are essential for regulation of normal sperm functions like sperm capacitation, acrosome reaction and oocyte fusion [18], but high levels have potential toxic effects on sperm quality and function. Lipid peroxidation and levels of antioxidants have been implicated in disturbances of sperm function.

Table 2 Mean, standard deviation and standard error of mean of lipid peroxidase (nmole/ml) and protein peroxidase (nmole/ml) content in blood and semen samples of infertile males and controls

Groups		Blood		Semen	
		LPO	PPO	LPO	PPO
Normal fertile males (n = 20)	Mean	40.20	59.93	116.51	59.10
	SD±	4.33	5.24	5.49	4.62
	SEM±	0.96	1.17	1.22	1.03
Infertile males (n = 50)	Mean	60.84*	72.84*	200.27*	149.80*
	SD±	3.55	3.66	6.25	11.47
	SEM±	0.50	0.52	0.88	1.62

*$P < 0.001$

Table 3 Mean, standard deviation and standard error of mean of superoxide dismutase (units/ml) and glutathione peroxidase (units/ml) activity in blood and semen samples of infertile males and controls

Groups		Blood		Semen	
		SOD	GPX	SOD	GPX
Normal fertile males (n = 20)	Mean	4.85	0.36	4.24	0.65
	SD±	0.78	0.05	0.89	0.03
	SEM±	0.17	0.01	0.19	0.006
Infertile males (n = 50)	Mean	3.40*	0.16*	2.42*	0.24*
	SD±	1.06	0.01	1.32	0.015
	SEM±	0.15	0.001	0.18	0.002

*$P < 0.001$

ROS is produced by a variety of semen components including immotile or morphologically abnormal spermatozoa, leukocytes, and morphologically normal but functionally abnormal spermatozoa [19].

Normally, a balance is maintained between the amount of ROS produced and that scavenged by a cell. Cellular damage arises with the disturbance of this equilibrium, especially when the cellular scavenging systems cannot eliminate the increase in ROS and correlate with idiopathic infertility.

The SOD level in spermatozoa is positively correlated with sperm motility [20]. Similarly, a selenium-containing glutathione enzyme scavenging system exists in the sperm of several mammalian species; including human beings [21]. This system may act directly as an antioxidant and an inhibitor of LPO. Recent reports have indicated that high levels of ROS can be detected in semen samples of 25–40% of infertile men [22].

It has been reported that seminal plasma from fertile men has a higher total antioxidant capacity (TAC) than seminal plasma from infertile men [23].

Therefore, in this prospective, controlled study, we assessed the levels of lipid peroxide and protein peroxide production in infertile patients and compared it to normal fertile men.

The findings of present investigation are in agreement with the findings of previous reports. Our data revealed significantly higher ($P < 0.001$) levels of ROS in semen and blood of infertile patients than the control groups. The SOD and GPX activities in semen of infertile men are significantly lower ($P < 0.001$) than the normal fertile men. The results also suggest significantly lower antioxidant activity in blood of infertile males when compared with the normal fertile males. However, pathological levels of ROS detected in semen from infertile men are more likely a result of increased ROS production rather than reduced antioxidant capacity of the seminal plasma [24].

The discrepancy in the levels of lipid peroxidation in azoospermia, in spite of the lack of sperm and leucocytes, may be due to the lower antioxidant levels found in infertile men. The lipid peroxide in the seminal plasma does not arise from the spermatozoa only, since it was detected from the patients with azoospermia also [25].

From our study, it can be concluded that the ROS levels in blood and seminal plasma of infertile males are positively correlated with the antioxidant capacity of the same. It appears that the scavenging systems are defective or inadequate in male factor infertility and perhaps the ROS generated significantly decrease the fertilizing potential of the sperm.

Patients having infertility problem due to defective or inadequate oxidative property of the seminal fluid can be treated with different antioxidants as indicated by different researchers at various times. High levels of alpha-tocopherols in serum achieved upon oral administration of vitamin E have been shown to improve the performance of spermatozoa in the zona binding test [26]. The effect of vitamin E supplementation in combination with in vitro fertilization (IVF) techniques is therefore a worthy notion. Other antioxidants could be vitamin C, vitamin A, L-carnitine, selenium etc. However, in the area of reproduction, the role of oxidative stress and its therapy with antioxidants remain in their initial stages and thereby warrant additional exploration.

Acknowledgment This study was supported and funded by Department of Science and Technology, Govt. of India.

References

1. Sharlip ID, Jarow JP, Belker AM, et al. Best practice policies for male infertility. Fertil Steril. 2002;77:873–82.
2. Aitken RJ. Molecular mechanisms regulating human sperm function. Mol Hum Reprod. 1997;3:169–73.
3. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003;79:829–43.
4. Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. Urology. 1996;48:835–50.
5. Sikka SC. Relative impact of oxidative stress on male reproductive function. Curr Med Chem. 2001;8:851–62.
6. Aitken RJ. The human spermatozoon—a cell in crisis? J Reprod Fertil. 1999;115:1–7.
7. Saleh RA, Agarwal A, Nada AE, et al. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril. 2003;79:1597–605.
8. Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Front Biosci. 1996;1:78–86.
9. Sikka SC, Rajasekaran M, Hellstrom WJ. Role of oxidative stress and antioxidants in male infertility. J Androl. 1995;16:464–81.
10. Aitken RJ. Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev. 1995;7:659–68.
11. Agarwal A, Saleh RA. Role of oxidants in male infertility: rationale, significance, and treatment. Urol Clin North Am. 2002;29:817–27.
12. Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol Reprod. 1989;41:183–97.
13. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge, UK: Cambridge University Press; 1999.
14. Kruger TF, Acosta AA, Simmons KF, et al. New method of evaluating sperm morphology with predictive value for human in vitro fertilization. Urology. 1987;30:248–51.
15. Ohkawa M, Ohisi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt Biochem. 1979;95:357–8.
16. Woolliams JA, Wiener G, Anderson PH. et al. Res Veter Sci. 1983;34:253–6.
17. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158–69.
18. Griveau JF, Le Lannou D. Reactive oxygen species and human spermatozoa. Int J Androl. 1997;20:61–9.
19. Plante M, de Lamirande E, Gagnon C. Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect normal sperm motility. Fertil Steril. 1994;62:387–93.
20. Alvarez JG, Touchstone JC, Blasco L, et al. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa: superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl. 1987;8:338–48.
21. Li TK. The glutathione, thiol content of mammalian spermatozoa and seminal plasma. Biol Reprod. 1975;12:641–6.
22. Hafeman DJ, Sunde RA, Hoekstra WG. Effect of dietary selenium in erythrocyte and liver glutathione peroxidase in rates. J Nutr. 1974;104:580–7.
23. Lewis SEM, Boyle PM, McKinney KA, et al. Total antioxidant capacity of seminal plasma is different in fertile and infertile men. Fertil Steril. 1995;64:868–70.
24. Zini A, de Lamirande E, Gagnon C. Reactive oxygen species in the semen of infertile patients: levels of superoxide dismutase- and catalase-like activities in seminal plasma. Int J Androl. 1993;16:183–8.
25. Kobayashi J, Miyazaki T, Natori M, et al. Protective role of superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod. 1991;6:987–91.
26. Kessopoulou E, Barratt CLR, Powers HJ, et al. Rational treatment of reactive oxygen species associated male infertility with vitamin E: a double blind, randomized, placebocontrolled trial. In: 2nd International meeting of the British Fertility Society, Glasgow, U.K.; 1994. p. 7–8.