

Evaluation of superoxide dismutase activity and its impact on semen quality parameters of infertile men

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Abstract: The evaluation of superoxide dismutase (SOD) activity, as one of the most important antioxidative defence enzymes, in seminal plasma of patients consulting for male infertility was presented in the article. The study included also the determination of its influence on selected human semen quality parameters. The material represents semen samples obtained from 15 men, which were divided into two groups: Group I (n=10) including patients consulting for infertility and Group II (n=5) containing healthy sperm donors as a control. All of the semen samples were cryopreserved and stored in liquid nitrogen. The frozen samples were thawed at the same time and then SOD activity was determined spectrophotometrically. The analysis of the investigations results indicates a significantly lower semen SOD activity detected in oligoasthenozoospermic patients, comparing to the activity found in normospermic men. The study showed a positive correlation between SOD activity in seminal plasma and semen quality parameters - sperm concentration and overall motility, which are regarded as the most important for normal fertilizing ability of the spermatozoa. Significantly lower SOD activity in seminal plasma of infertile patients, comparing to healthy sperm donors, as well as positive correlation and beneficial impact of SOD activity on human semen quality parameters seem to confirm the observations, that decreased seminal plasma scavenger antioxidant capacity, particularly in form of low SOD activity, can be responsible for male infertility. This trial shows that SOD activity survey in seminal plasma could be a useful tool for determining sperm fertilization potential and could improve the diagnosis of male infertility.

Key words: Superoxide dismutase - Semen quality parameters - Male infertility

Introduction

Reactive oxygen species (ROS) have beneficial as well as detrimental effect on sperm functions, depending on the nature and the concentration of the ROS involved. Hydrogen peroxide (H₂O₂) - a main toxic ROS for human spermatozoa in low concentrations causes sperm immobilization. High concentrations of H₂O₂ induce lipid peroxidation, which results in cell death. On the other hand, the superoxide anion (O₂⁻) appears to play an important role in the process of sperm hyperactivity and capacitation [1].

ROS in semen are generated mainly by neutrophils and also by abnormal spermatozoa. Human spermatozoa are rich in polyunsaturated fatty acids (PUFA) and are very susceptible to attack by ROS, that initiate peroxidation of PUFA in the sperm plas-

ma membrane [2,3]. It results in the inhibition of both sperm ATP production and sperm movement, particularly forward progression [2]. Excessive generation of ROS in semen could play a key role in the etiology of male infertility [1,6]. Several different studies have demonstrated that high ROS production was associated with oligoasthenozoospermia and poor sperm fertilization potential [6-8]. Zini *et al.* detected ROS in the semen of 40% of infertile men, whereas none was detected in semen from healthy sperm donors [8]. The other report demonstrated 72% of sperm preparations producing detectable amounts of ROS in patients consulting for infertility, compared to only 25% of preparations from a population of fertile men [6]. The Canadian authors additionally concluded that leukocytes are the predominant source of ROS in human sperm preparations. The ROS they produce have detrimental effects on the overall sperm motility, whereas the production of ROS by deficient spermatozoa is low and of no consequence to that semen quality parameter [9].

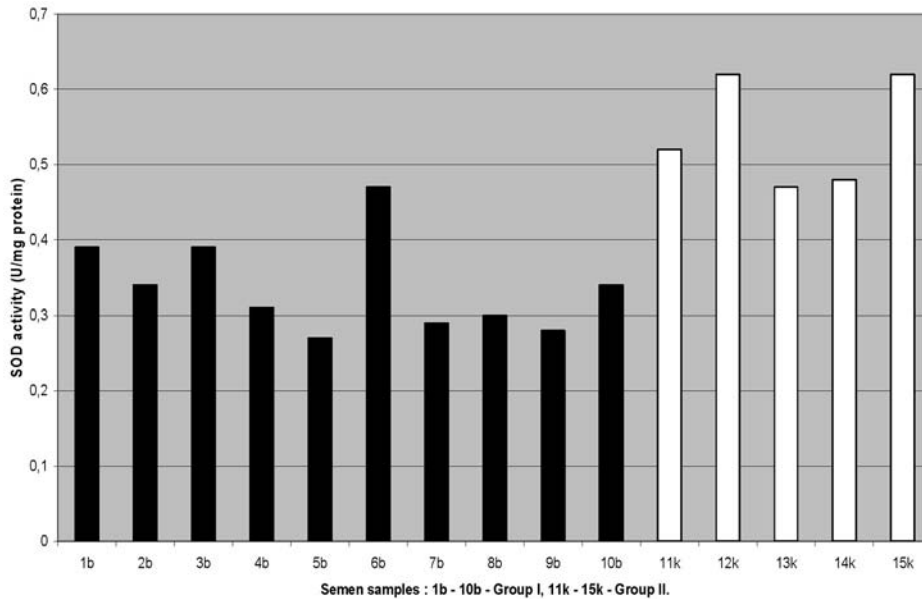


Fig. 1. Comparison of SOD activity within Group I and II

Testis germ cells as well as epididymal maturing spermatozoa are endowed with enzymatic and non-enzymatic scavenger systems to prevent lipoperoxidative damage. Also seminal plasma has a highly specialized scavenger system that defends the sperm membrane against lipoperoxidation. The system contains following main antioxidative defence enzymes: superoxide dismutase (SOD), catalase (CT) and glutathione peroxidase (GPx) [10,11]. Apart from the enzymatic scavenger antioxidant capacity, also α -tocopherol, ascorbic acid and zinc are regarded as the scavengers of excessive superoxide anions production, although simultaneously zinc was found to be a dose-dependent inhibitor of SOD-like activity in human spermatozoa [8].

It is considered that the ROS detected in semen are a reflection of the imbalance between ROS production and degradation [8]. In normal conditions a balance is maintained between the amount of ROS produced and that scavenged. When this equilibrium is disturbed through the systemic predisposition or a number of pathologies, the sperm cellular damage arises and the fertilizing ability of spermatozoa is jeopardized [2,3].

The purpose of this work was to evaluate SOD activity, as one of the most important antioxidative defence enzymes, in seminal plasma of patients consulting for male infertility. The study includes also determination the influence of SOD activity on selected human semen quality parameters.

Materials and methods

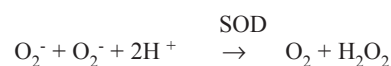
A study was performed in the years 2000-2002 in the I-rst Department of Gynecology and Obstetrics as well as Department of Medical Biochemistry of Medical University in Wrocław, Poland. The

material represents semen samples obtained from 15 men, which were divided into two groups: Group I (n=10) including patients consulting for infertility and Group II (n=5) containing healthy sperm donors as a control. All fresh semen samples were collected by masturbation into sterile containers after 48 to 72 hours of sexual abstinence. After complete liquefaction, the ejaculates were analyzed for selected semen quality parameters - sperm concentration and overall motility. They were measured with use of a haemocytometer, according to World Health Organization (WHO) guidelines. The study of semen analysis within Group I showed sperm pathological changes classified as oligoasthenozoospermia. All of the examined semen quality parameters in Group II were contained within the range of WHO norms. The samples of this Group were stated as normospermic.

All of the semen samples were then cryopreserved using the Test Yolk Buffer with Glycerol as a freezing medium, according to the protocol described in WHO guidelines (4-th edition, 1999). The cryo straws filled with frozen sperm were plunged in a liquid nitrogen container, where they were stored in a temperature -196°C . This method allowed a long-time sperm preservation without any risk of negative freezing impact on semen quality parameters.

The frozen semen samples at the same time were thawed by incubation for 30 minutes at room temperature, after removing the straws from liquid nitrogen storage tank. The equal 0.1 ml amounts of sperm were transferred into small conical tubes with caps and subjected to the biochemical examinations.

SOD activity was determined spectrophotometrically as the degree of inhibition of the competitive reaction using RANSOD kit (Randox Laboratories Ltd). The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical ($\text{O}_2^{\cdot-}$), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen.



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.

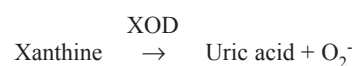
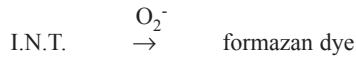


Table 1. SOD activity for particular semen samples, with M and SD values for Group I and II; case number: 1-10 - Group I; case number: 11-15 - Group II.

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SOD activity (U/mg protein)	0.39	0.34	0.39	0.31	0.27	0.47	0.29	0.30	0.28	0.34	0.52	0.62	0.47	0.48	0.60
M	0.338										0.542				
SM	0.063										0.074				



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

Statistical analysis. All statistical calculations were performed using the Instat ver. 2.0 program from GraphPad Software. The numerical data were subjected to arcsine transformation and are expressed as mean (M) and standard deviation of the mean (SD). The data were evaluated statistically by one-way analysis of variance and statistical significance ($p < 0.001$) was assessed by Tukey-Kramer multiple comparisons test. When two means were compared, statistical analysis was carried out using the Student's t-test for incoherent trials.

Results

The results obtained in the study, comparing the SOD activity within both investigated groups were presented graphically in Fig. 1.

The numerical data concerning SOD activity (in U/mg protein) for particular semen samples, with M and SD values for both investigated groups were placed in Table 1.

Mean value of SOD activity within Group I was calculated as 0.338 ± 0.063 , whereas within Group II was evaluated as 0.542 ± 0.074 . There was statistically significant difference ($p = 0.000083$) between SOD activity within Group I and Group II. SOD activity is distinctly decreased in semen of infertile men comparing to sperm of healthy donors.

Discussion

The analysis of the results of our investigations indicates a significantly lower semen SOD activity detected in oligoasthenozoospermic patients, comparing to the activity found in normospermic men. The statistical difference in the activity of this enzyme between two evaluated groups of semen samples was calculated on the level of significance $p < 0.001$. This outcome confirms previously published observations of the other authors [5,13,14].

Our study showed a positive correlation between SOD activity in seminal plasma and semen quality parameters - sperm concentration and overall motility, which are regarded as the most important for normal fertilizing ability of the spermatozoa. However, some

investigators suggest that the beneficial impact of SOD activity concerns only sperm movement, whereas no influence on sperm count has been noticed [15,16]. In contrast, some authors reported a significantly higher SOD activity detected in spermatozoa from infertile men, compared to the activity found in normospermic samples. In these trials the SOD activity level was found to be increased in cases with an elevated production of ROS. The over expression of SOD may reflect a defect in the development or maturation of spermatozoa, as well as sperm cellular damage, resulting in decreased sperm fertilization potential [7,17,18]. No significant difference in the SOD activity measured in seminal plasma from normospermic samples and from infertile men (that did produce ROS) was observed by Zini *et al.* [8]. Also no significant correlation between SOD activity in seminal plasma and semen quality was found by Chinese authors [19].

SOD as an important element of seminal plasma superoxide anion scavenging capacity plays an essential role in maintaining the balance between ROS generation and degradation. Decrease of the capacity can result in abnormal sperm motility determined as sperm hyperactivation [20]. Several toxic substances including chemical reagents, drugs, heavy metal ions or nicotine decrease semen quality as well as SOD levels in seminal plasma. It was demonstrated that all of these parameters were negatively correlated with the amount and duration of cigarette smoking [21,22].

The essential role of SOD as antioxidative defence enzyme is inferred from the observation that complete loss of motility of a sperm sample is directly proportional to the SOD activity of that sample [14].

In 1998 three Japanese scientists reported that some antioxidants can reverse the decrease in sperm motility in the seminal plasma of infertile men. They started to treat them with oral administration of antioxidant - AOA, a natural medicinal product, achieving marked improvement in approximately 50% of cases [23].

In conclusion, the preliminary study demonstrates that increased ROS production and decreased seminal plasma scavenger antioxidant capacity, particularly in form of low SOD activity, can be responsible for male infertility [5]. Significantly lower SOD activity in seminal plasma of infertile patients, comparing to healthy sperm donors, as well as positive correlation and beneficial impact of SOD activity on human

semen quality parameters seem to confirm these observations. This trial shows that SOD activity survey in seminal plasma could be a useful tool for determining sperm fertilization potential and could improve the diagnosis of male infertility.

References

- [1] de Lamirande E, Gagnon C. Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. *Hum Reprod.* 1995;10(Suppl 1):15-21.
- [2] Dandekar SP, Nadkarni GD, Kulkarni VS, Puneekar S. Lipid peroxidation and antioxidant enzymes in male infertility. *J Postgrad Med.* 2002;48(3):186-189.
- [3] Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. *Urology.* 1996; 48(6): 835-850.
- [4] Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. *Free Radic Biol Med.* 1999;26(7-8): 869-880.
- [5] Alkan I, Simsek F, Haklar G, Kervancioglu E, Ozveri H, Yalcin S, Akdas A. Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: relationship to seminal plasma antioxidants. *J Urol.* 1997;157(1): 140-143.
- [6] Ford WC, Whittington K, Williams AC. Reactive oxygen species in human sperm suspensions: production by leukocytes and the generation of NADPH to protect sperm against their effects. *Int J Androl.* 1997;20(Suppl 3):44-49.
- [7] Zalata A, Hafez T, Comhaire F. Evaluation of the role of reactive oxygen species in male infertility. *Hum Reprod.* 1995; 10(6):1444-1451.
- [8] 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 and spermatozoa. *Int J Androl.* 1993;16(3):183-188.
- [9] 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(2):387-393.
- [10] Lenzi A, Gandini L, Lombardo F, Picardo M, Maresca V, Panfili E, Tramer F, Boitani C, Dondero F. Polyunsaturated fatty acids of germ cell membranes, glutathione and glutathione-dependent enzyme-PHGPx: from basic to clinic. *Contraception.* 2002;65(4):301-304.
- [11] Lenzi A, Gandini L, Picardo M, Tramer F, Sandri G, Panfili E. Lipoperoxidation damage of spermatozoa polyunsaturated fatty acids (PUFA): scavenger mechanisms and possible scavenger therapies. *Front Biosci.* 2000;1;5:E1-E15.
- [12] Gavella M, Lipovac V, Vucic M, Sverko V. In vitro inhibition of superoxide anion production and superoxide dismutase activity by zinc in human spermatozoa. *Int J Androl.* 1999;22(4):266-274.
- [13] Siciliano L, Tarantino P, Longobardi F, Rago V, De Stefano C, Carpino A. Impaired seminal antioxidant capacity in human semen with hyperviscosity or oligoasthenozoospermia. *J Androl.* 2001;22(5):798-803.
- [14] Storey BT. Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. *Mol Hum Reprod.* 1997;3(3):203-213.
- [15] Kurpisz M, Miesel R, Sanocka D, Jedrzejczak P. Seminal plasma can be a predictive factor for male infertility. *Hum Reprod.* 1996;11(6):1223-1226.
- [16] Sanocka D, Miesel R, Jedrzejczak P, Kurpisz MK. Oxidative stress and male infertility. *J Androl.* 1996;17(4):449-454.
- [17] Gavella M, Lipovac V, Vucic M, Rocic B. Relationship of sperm superoxide dismutase-like activity with other sperm-specific enzymes and experimentally induced lipid peroxidation in infertile men. *Andrologia.* 1996;28(4):223-229.
- [18] Sinha S, Pradeep KG, Laloraya M, Warikoo D. Over-expression of superoxide dismutase and lack of surfactethiols in spermatozoa: inherent defects in oligospermia. *Biochem Biophys Res Commun.* 1991;174(2):510-517.
- [19] 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):127-131.
- [20] de Lamirande E, Gagnon C. Human sperm hyperactivation in whole semen and its association with low superoxide scavenging capacity in seminal plasma. *Fertil Steril.* 1993;59(6): 1291-1295.
- [21] Zhang JP, Meng QY, Wang Q, Zhang LJ, Mao YL, Sun ZX. Effect of smoking on semen quality of infertile men in Shandong, China. *Asian J Androl.* 2000;2(2):143-146.
- [22] Zhang JP, Meng OY, Zhang LJ, Mao YL, Sun ZX. Study of correlation and effect of smoking on semen quality of men. *Zhonghua Nan Ke Xue.* 2002;8(1):35-37.
- [23] Niwa Y, Tominaga K, Yoshida K. Successful treatment of severe atopic dermatitis - complicated cataract and male infertility with a natural product antioxidant. *Int J Tissue React.* 1998;20(2):63-69.