

Oxidative stress adversely affects spermatogenesis in male infertility'

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Abstract

Oxidative stress is one of the factors which affects fertility potential of spermatozoa by lipid peroxidation which may lead to sperm dysfunction. Sperm count and sperm motility are prime parameters that determine the functional ability of spermatozoa. The present study was aimed to study adverse effect of oxidant on spermatogenesis in idiopathic male infertility. The study includes total 60 subjects, Normospermic (n=30) and Oligoasthenospermic (n=30). Seminal plasma malondialdehyde, nitric oxide, zinc and SOD were estimated by spectrophotometric methods and correlated with sperm count and sperm motility. Seminal levels of malondialdehyde and nitric oxide were significantly higher ($p<0.001$) while zinc and SOD were significantly lower ($p<0.001$) in oligoasthenospermic than normospermic men. There was significant negative correlation between malondialdehyde and nitric oxide with sperm count and motility in oligoasthenospermia. The increase in seminal malondialdehyde, nitric oxide and decrease in zinc and superoxide dismutase levels in oligoasthenospermia may be responsible for disruption in the membrane integrity of spermatozoa and have role in reduction of sperm DNA integrity and DNA damage. The positive correlation of zinc with sperm count and sperm motility indicates an important role of zinc in spermatogenesis. Thus these parameters could be useful for determining sperm fertilization potential further in diagnosis, prognosis and treatment of male infertility especially in idiopathic male infertility.

Key Words: Oxidants, antioxidants, zinc, oligoasthenospermia.

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Introduction

Human male infertility is the major health problem worldwide [1]. In last ten years there has been tremendous scientific growth in the field of reproductive medicine. Its prevalence in Western countries has been estimated with 20%. As per WHO study, incidence of infertility in India is 10 to 15%. In 15% infertile couples, about 30% cases are due to males only and in another 20% both partners have detectable abnormalities. Thus a male factor plays a significant role in about 50% of infertile couples [2-3]. Infertility is defined as failure to conceive after one year of regular unprotected intercourse with the same partner. Infertility has been attributed to number of factors such as anatomic defects, endocrinopathies, immunologic problems, gene mutation, radiation exposure, chemotherapy, ejaculatory failures and environmental exposures. Causes cannot be found in over 25% of infertile males, and these cases are referred to as idiopathic infertility [4]. In many such cases oxidative stress has been implicated as a major causative factor.

Oxidative stress (OS), results from accumulation of excessive reactive oxygen species (ROS), during spermatogenesis epididymal sperm maturation as well as from exposure of toxic chemicals, environmental pollutants etc. ROS change lipid/protein ratio of membranes by affecting polyunsaturated fatty acids and lipid peroxidation (LPO) causes functional irregularities of several cellular organelles [5,6]. It is therefore essential to understand role of such free radical mediated sperm damage mechanisms that could induce male infertility. Scavenging of these free radicals during spermatogenesis by means of antioxidants could provide a promising approach to suppress such damage.

Generally male infertility is assessed by semen analysis which provides a basic data regarding reproductive male function [7]. Sperm count and sperm motility are first and most important predictors of fertility [8]. Seminal plasma malondialdehyde is the stable peroxidation product; its estimation is a simple to evaluate the effect of peroxidation on sperm [9]. The nitrogen derived free radicals, nitric oxide (NO·) and peroxy nitrite anion (ONOO·) also play significant role in the reproduction and fertilization.

High amounts of NO· have detrimental effects on sperm [10]. Zinc also plays an important role in normal testicular development, spermatogenesis and sperm motility [7]. With a view of understanding the potential role of oxidative stress in idiopathic male infertility, the present study was undertaken. We aimed to assess seminal plasma levels of malondialdehyde, nitric oxide, zinc and SOD activity in idiopathic oligoasthenospermic patients and normospermic group and to find out their correlation with sperm count and sperm motility.

Materials and Methods

Present study was carried out in the Department of Biochemistry, Department of Obstetrics and Gynecology, MGM Medical College, Kamothe, Navi Mumbai. Thirty primary infertile male subjects aged 21-45 years, without any treatment, who had regular unprotected intercourse for at least 12 months without conception with their partner, were selected. The institutional ethical committee clearance was obtained for the present study. Cases with leukocytospermia, Varicocele, hypogonadism, history of smoking, alcohol and prolonged illness were excluded from the study. Wives of the infertile subjects included had no obvious causes of infertility like tubal blockage or ovulation disorders. At first clinic attendance, a detailed background history and physical examination were done on both husband and wife.

The 60 semen samples were divided into two groups: Group I - Normospermic (n=30), Group II – idiopathic Oligoasthenospermic (n=30). The subjects having sperm count less than 20 million/ml and sperm motility less than 50% were considered as oligoasthenospermic, while thirty fertile males aged 21-45 years, whose partners had conceived within a year and having sperm count more than 20 million/ml with motility more than 50% in forward progression were selected from general population and considered as normospermic control group.

Semen samples were analyzed according to WHO criteria. Samples were collected by masturbation in wide mouth sterile plastic container after minimum of three days of abstinence. On due orientation about the nature of study, a written consent was obtained from healthy individuals &

Table 1. The mean values of sperm count, sperm motility, seminal MDA, nitric oxide, SOD and zinc in normospermic and oligoasthenospermic men.

Parameters	Normospermic (n=30)	Oligoasthenospermic (n=30)	p-value
Seminal malondialdehyde conc.(nmol/l)	1.67 ± 0.56	5.34 ± 0.95	<i>p</i> <0.001
Seminal nitric oxide conc. (µmol/l)	2.56 ± 1.3	10 ± 2.5	<i>p</i> <0.001
Seminal zinc conc. (mg/dl)	21.15 ± 2.9	13.97 ± 3.71	<i>p</i> <0.001
Seminal SOD activity (U/ml)	21.9±3.13	12.53 ± 3.72	<i>p</i> <0.001
Sperm count (millions/ml)	71.38 ± 9.32	10.78 ± 3.4	<i>p</i> <0.001
Motility (%)	73.55 ± 8.36	30.39 ± 3.61	<i>p</i> <0.001

(Mean ± S.D., Comparison with control: **p*<0.001)

infertile male subjects. After liquefaction, samples were processed by conventional analysis to determine sperm count, sperm motility according to WHO criteria.

On centrifugation, seminal plasma was used for measurements of malondialdehyde by Satoh K method [11]. Nitric oxide was estimated by Griess reaction. In this kinetic method, nitrate is reduced to nitrite by copper coated cadmium granules. This nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylenediamine [12]. Zinc was estimated by spectrophotometric method (kit by Coral Clinical systems); zinc in alkaline medium reacts with nitro-para amino phospho sulphate to form purple colored complex. The color intensity is directly proportional to the amount of zinc present in the sample. SOD was measured according to the method of Marklund and Marklund [13]. Superoxide anion radical is involved in the auto-oxidation of pyrogallol. At alkaline pH, SOD dismutates superoxide, thereby inhibiting the auto oxidation of pyrogallol.

Statistical analysis

Statistical analysis of the data was carried out with SPSS, version 16; Data was reported as mean ± SD. The comparisons between two groups were tested by unpaired t-test. A 95% confidence interval was used. P values less than 0.05 were considered statistically significant. Correlation between two continuous outcomes was evaluated using Pearson correlation coefficients.

Results

Results were expressed as mean ± SD for each parameter. Statistically significant differences among oligoasthenospermic and normospermic groups are indicated in Table No.1 along with their significant values. Seminal levels of malondialdehyde (5.34 ± 0.95 nmol/l) and nitric oxide (10 ± 2.5 µmol/l) were significantly high (*p*<0.001) in oligoasthenospermic patients than normospermic men (1.67 ± 0.56, 2.56 ± 1.3 respectively). Seminal levels of zinc (13.97± 3.71mg/dl) and SOD (12.53 ± 3.72 U/ml) were significantly lower (*p*<0.001) in oligoasthenospermic men than normospermic men (21.15 ± 2.9, 21.9±3.13 respectively).

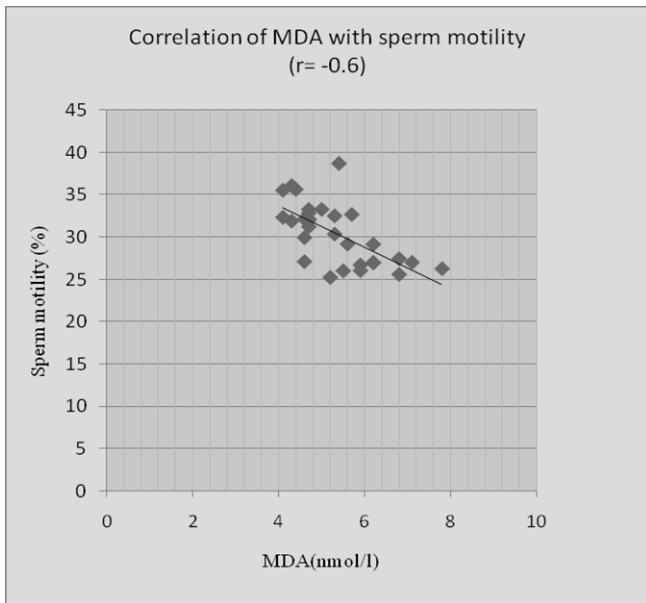
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Table 2. Correlation coefficient of various parameters studied in oligoasthenospermic men

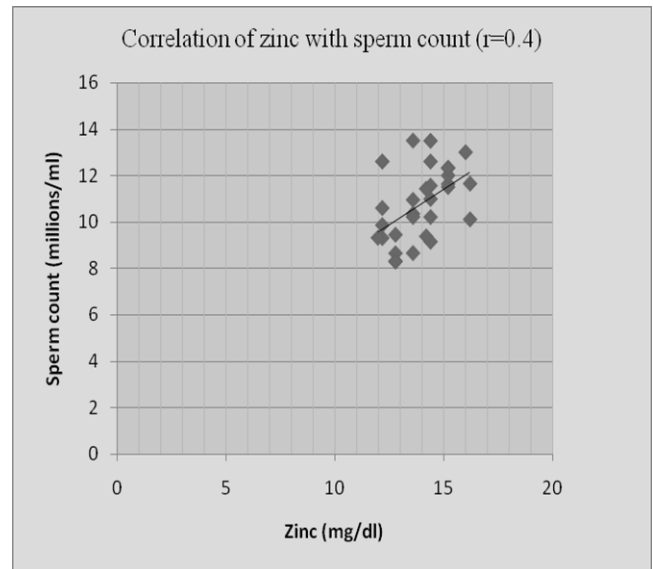
Parameters		Nitric oxide	Zinc	SOD	Sperm count	Sperm motility
MDA	r -value	0.229*	-0.516**	-0.367**	-0.554**	-0.646***
Nitric oxide	r- value	-	-0.390**	-0.531**	-0.463**	-0.504**
Zinc	r -value	-	-	0.786***	0.489**	0.688***
SOD	r -value	-	-	-	0.370**	0.580***
Sperm count	r -value	-	-	-	-	0.678***

r = Pearson's correlation co-efficient.

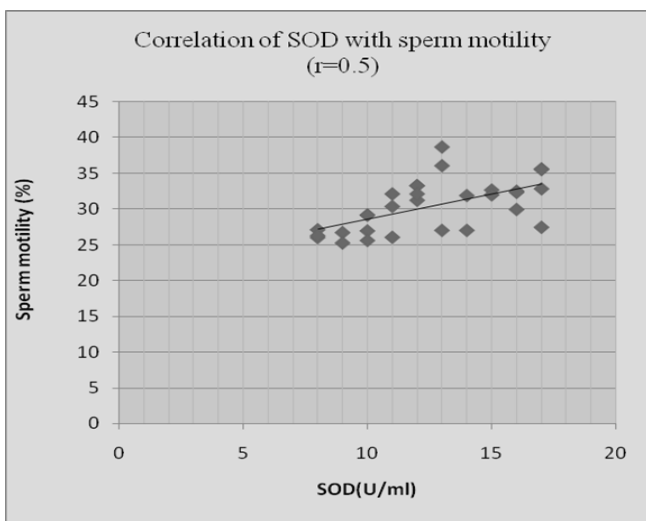
*** Highly significant ($p < 0.001$), **Significant ($p < 0.05$), * Not significant ($p > 0.05$).



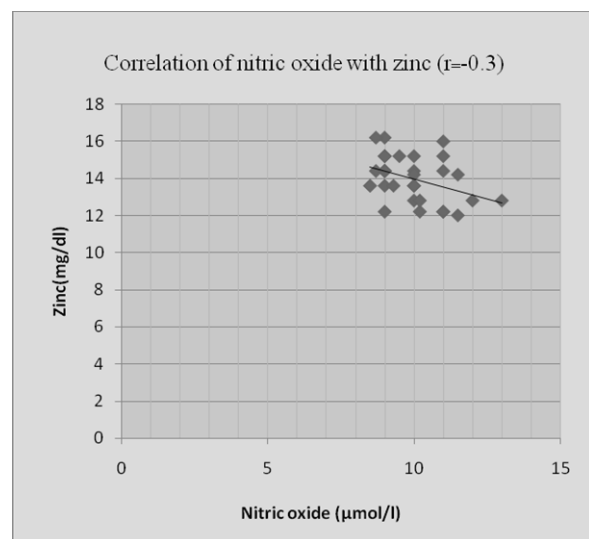
Graph 1: Correlation between MDA and Sperm motility in oligoasthenospermic men



Graph 3: Correlation between zinc and sperm count in oligoasthenospermic men



Graph 2: Correlation between SOD and sperm motility in oligoasthenospermic men



Graph 4: Correlation between Nitric oxide and Zinc in oligoasthenospermic men

Discussion:

Impairment of spermatogenesis and sperm function is the most common causes of male infertility. Abnormal sperm function is difficult to evaluate and treat. There is a lack of understanding of the factors contributing to normal and abnormal sperm function leading to infertility [14]. Present routine investigations are insufficient to predict exact cause of infertility. Recent studies showed oxygen-derived free radicals, induce damage to spermatozoa. Several studies reported that Idiopathic male factor infertility has been linked with oxidative stress [15, 16] Pasqualotoo et al reported men with idiopathic infertility have higher levels of ROS and lower antioxidant properties than healthy controls [17]. The main cause for this connection is the observation of morphologically abnormal sperm, commonly identified in the idiopathic infertile male population and a reduced antioxidant capacity [18]. Mammalian spermatozoa membrane is rich in polyunsaturated fatty acids. This makes them very susceptible to oxygen-induced damage, which is mediated by lipid peroxidation. In a normal situation, the antioxidant mechanisms present in the reproductive tissues are likely to quench these reactive oxygen species (ROS) and protect gonadal cells and mature spermatozoa from oxidative damage [14].

Unlike somatic cells, mature spermatozoa lack cytoplasm. Since cytoplasm is the major source of anti-oxidants, lack of cytoplasm in the mature spermatozoa causes deficiency in both oxidant defense and endogenous repair mechanism, however naturally the deficiency of antioxidant system is compensated by the enzymatic and non-enzymatic antioxidants in seminal plasma [19]. So decreased antioxidant enzyme or increased ROS level disrupts the physiological functions of the spermatozoa and impairs sperm motility and process of fertilization [20].

The purpose of our study was to evaluate the adverse effect of oxidant on spermatogenesis in idiopathic male infertility and to find out their correlation with sperm count and sperm motility.

Previous studies reported that lipid peroxidation plays significant role in impairing sperm functions and semen quality especially sperm count, motility and morphology. Excess ROS and low antioxidant levels in semen enhanced susceptibility of sperm DNA to denaturation and might cause mitochondrial DNA mutations which impairs the fertilizing capacity of spermatozoa [21, 22].

In present study, we found high levels of MDA in oligoasthenospermic patients as compared to normospermic and it is negatively correlated with sperm count and sperm motility. Our results of MDA are on par with studies by Hsieh YY et al. [23] and Fraczek et al. [24]. Frac-

zek et al. [24] reported elevated seminal MDA concentration in patients with oligoasthenoteratozoospermia. Our results are in contrast with Suleiman et al. [25]. They demonstrated that MDA concentration in the seminal plasma was not related with the sperm concentration and motility.

Amiri et al. [10], Sheikh et al. [26] reported significantly higher values of seminal nitric oxide in infertile males as compared to fertile males. Giancarlo et al. [27] demonstrated same findings in idiopathic asthenospermia. Further they observed significant negative correlation between NO[•] concentration and sperm motility. In contrast, Revelli et al. [28] observed that NO[•] concentration in the seminal plasma was not correlated with sperm concentration and motility. We observed the negative correlation of the NO[•] concentration with sperm count and motility.

Nitric oxide is a biological messenger. It is produced by one of the essential amino acids L-arginine, by the catalytic action of enzyme NO synthase (NOS). It's overproduction in seminal plasma may be due to induced genital tract cells such as Leydig cells, epididymal or vas deference, epithelial cell or spermatozoa itself and also observed in sub infectious or inflammatory diseases of male genital tract and by induced leucocytes [10]. In the present study, source of NO[•] is unknown and it may be produced by macrophages in response to infection, or from steady secretion from multiple sources such as testis and structures of male reproductive tract [29].

The high concentration of NO[•] may react with superoxide ion and hydrogen peroxide resulting in the formation of peroxynitrite, hydroxyl radical which cause oxidation of sperm membrane lipids and thiol-proteins [30]. It can cause reduction of sperm DNA integrity, sperm DNA damage and fragmentation of sperm DNA, which are main factors in male infertility. NO[•] may also inhibit cellular respiration by nitrosylation of heme in mitochondrial enzymes, aconitase and glyceraldehyde phosphate dehydrogenase leading to depletion of adenosine triphosphate (ATP) and consequent loss of motility by spermatozoa [27].

Oligoasthenospermia is associated with high levels of MDA and nitric oxide. It suggests that lipid peroxidation of the membrane lipid may disturb the functions carried out by the sperm membrane. There was significantly negative correlation of MDA and Nitric oxide with sperm motility suggesting their role in inhibition of mitochondrial function and reduction of membrane fluidity which is necessary for sperm-oocyte fusion.

Omu et al. [31] had demonstrated that zinc therapy results in significant improvement in sperm quality with increase in sperm density, progressive motility and improve conception and pregnancy outcomes. It appears to be a potent

scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation [32]. Insufficient intake of zinc can impair antioxidant defenses and may be an important risk factor in oxidant release, compromising the mechanism of DNA repair and making the sperm cell highly susceptible to oxidative damage. In the absence of Zn, the possibility of increased oxidative damage exists that would contribute to poor sperm quality. Decrease of seminal Zn and poor Zn nutrition can be a risk factor for sperm abnormality and idiopathic male infertility [33].

Chia et al. [34] reported significant positive correlation of zinc concentration with sperm density, motility and viability. Fuse H et al. [35] demonstrated lower levels of zinc in oligoasthenospermic male which was a positively correlated with sperm concentration and sperm motility. Wong WY et al. [36] reported no correlation of zinc concentration with sperm quality or with male infertility. In present study, we observed significantly low levels of zinc in oligoasthenospermic patients and showed significantly positive correlation with sperm count and sperm motility. Zinc act as an essential element for spermatogenesis, motility and scavenger of superoxide anion. The low zinc levels may contribute to low sperm concentration and poor motility.

SOD is an important antioxidant of seminal plasma which has superoxide scavenging capacity and plays an essential role in maintaining the balance between ROS generation and degradation [37]. Several toxic substances including chemical reagents, drugs, heavy metal ions or nicotine decrease semen quality as well as SOD activity in seminal plasma [38].

We found significantly lower seminal SOD activity in oligoasthenospermic compared to normospermic. Our results are in accordance with Murawski et al. [38], Siciliano et al. [39]. In present study, SOD activity showed significantly positive correlation with sperm count and sperm motility which was compatible with Murawski et al [38]. Murawski et al. [38] got same findings in oligoasthenospermia. Storey BT et al. [40] demonstrated complete loss of motility in the sperm sample and it was directly proportional to the SOD activity. This strongly suggests SOD activity is insufficient to cope with the excessive amount of ROS and Reactive nitrogen species.

There was negative correlation between malondialdehyde and nitric oxide with zinc and SOD in oligoasthenospermia, indicating oxidative stress can cause male infertility by affecting the process of conception at various stages, like damaging the sperm membrane, DNA and protein. It may impair normal sperm function as well as sperm capacitation and acrosome reaction, which are essential for fertilization.

Thus our study suggests evaluation of oxidative stress and antioxidant status can be considered as additional parameters of male infertility especially in idiopathic cases. The threshold ROS levels above which antioxidants can be used for the treatment of male infertility can be determined, which will be helpful in the management of infertility.

Conclusion

The increase in malondialdehyde, nitric oxide and decrease in zinc and superoxide dismutase levels in oligoasthenospermic men may cause disruption in the membrane integrity of spermatozoa and may have role in reduction of sperm DNA integrity. There was a positive correlation of zinc with sperm count and sperm motility, indicating zinc plays an important role in spermatogenesis. Thus estimation of seminal malondialdehyde, nitric oxide, zinc and SOD can be useful tool for determining sperm fertilization potential. These parameters could assist in diagnosis and treatment of male infertility especially in idiopathic cases.

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