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Evaluation of Malondialdehyde and Nitric Oxide and its Impact on Sperm Quality Parameters in Oligospermic Infertile Men

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Abstract: ***Male reproductive function is found to be defective in 50% of infertile couples. Oxidative stress induced lipid peroxidation is one of the potential cause of the male infertility. Present study was aimed to evaluate malondialdehyde and nitric oxide in oligospermic infertile and fertile men and to study correlation of malondialdehyde and nitric oxide with sperm parameters. The study is prospective, comprises 30 oligospermic infertile men and 30 fertile men. Seminal MDA and NO· levels were evaluated spectrophotometrically. The levels of MDA and NO· were found to be significantly increased in oligospermic infertile men as compared to fertile men. MDA and NO· levels were negatively correlated with sperm count and sperm motility. This suggests that oxidative stress induced lipid peroxidation can cause poor semen quality. Therefore assessment of MDA and nitric oxide will help to understand an etiology of infertile men and to decide treatment strategies.***

Keywords: Malondialdehyde (MDA), Nitric oxide (NO·), Oxidative Stress (OS), Oligospermia.

1. Introduction

Infertility is defined as there is no conception after at least 12 months of unprotected intercourse. By tradition female partner is held responsible for infertility. But now it's clear that, male reproductive function is found to be deficient in not less than 50% of infertile couples. Infertility could be attributed to a number of factors such as environmental, physiological and genetic factors.[1] Causes cannot be found in over 37–58% of infertile males and these cases are referred to as idiopathic infertility. [2]

Recent studies proposed that the imbalance between oxidant and antioxidant generates oxidative stress, which leads to metabolic and functional abnormalities in male germ cells in some types of infertility. A human spermatozoon is susceptible to oxidative attack because of its high content of polyunsaturated fatty acids and the little cytoplasmic antioxidants. Therefore oxidative stress induced lipid peroxidation leads to the loss of membrane integrity, increases cell permeability, inactivation of enzymes, structural damage of DNA and cell death.[3]

Recent reports have indicated that high levels of ROS are detected in semen samples of 25 to 40% of infertile men.[4] MDA measurements are relevant because major loss of sperm function, may occur with minimal damage to the membranes that envelop the sperm and/or divide key intracellular sperm compartments.[5]

Nitric oxide is one of the reactive oxygen species that has been implicated in a variety of physiologic cell signaling mechanisms in many tissues. NO· is a free radical generated from the oxidation of L-arginine to L-citrulline by 3 isoforms of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent NO synthases (NOS).[6]

Excessive production of ROS induced OS have been associated with impaired sperm motility, concentration and morphology. These parameters are the most important predictors of an individual's potential to produce viable sperm. [7] Therefore present study was aimed to assess level of lipid peroxidation and nitric oxide in infertile men with oligospermia and to study its relation with sperm quality parameters in infertile men.

2. Material and Methods

Study design

The present study was carried out in the Department of Biochemistry, Department of Obstetrics and Gynecology, MGM Medical College and Group of Hospitals, Navi Mumbai. The Institutional Ethical Committee clearance was obtained for the present study.

Thirty male subjects aged 21-45 years, whose partners had conceived within a year and having sperm count ≥ 20 million/ml with motility ≥ 50% in forward progression were selected from general population and considered as fertile (Control Group). Thirty infertile men with oligospermia (sperm count $<20x10^6$ sperm cells/ml) referred from Department of Obstetrics and Gynecology, aged 21-45 years, without any treatment, whose wives had not conceived after one year of regular, unprotected intercourse. The wives of infertile subjects included had no apparent causes of infertility like tubal blockage or ovulation disorders.

Subjects currently on any medication or antioxidant supplementation and azoospermia due to obstructive causes were excluded from study. The written consent was taken

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from fertile and infertile men. Samples were collected by masturbation in wide mouth sterile plastic container after minimum of three days of abstinence. After liquefaction, samples were processed by conventional analysis to determine sperm count, sperm motility and sperm morphology according to WHO criteria.

Assessment of MDA and Nitric oxide:

On centrifugation, seminal plasma was used for measurements of malondialdehyde by Satoh K method[8]. 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 napthyl-enediamine [9]

Statistical analysis

Statistical analysis of the data was carried out with SPSS, version 17; 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.

3. Results

Results were expressed as mean ± SD for each parameter. Statistically significant differences among infertile and fertile groups are indicated in Table No.1 along with their significant values. Seminal levels of MDA (4.7 ± 0.76) and nitric oxide (6.17 ± 0.79) were significantly high (p<0.001) in oligospermic infertile men than fertile men (1.62 ± 0.40) (2.32 ± 1.2) respectively.

Sperm count and sperm motility were significantly decreased whereas sperm abnormal morphology was significantly increased in oligospermic infertile men as compared to fertile men (p<0.001). Correlation coefficient of various parameters is indicated in Table No.2 along with their significant values. There was positive correlation of MDA and nitric oxide in oligospermic infertile men. MDA and nitric oxide have a negative correlation with sperm count, motility and positive correlation with sperm abnormal morphology.

Table 1: The mean values of sperm count, sperm motility, sperm abnormal morphology, seminal MDA and seminal nitric oxide in fertile and oligospermic infertile men.

Parameters	Fertile men (n=30)	Oligospermic Infertile men (n=30)	p-value
Malondialdehyde (nmol/l)	1.62 ± 0.40	4.7 ± 0.76	p<0.001
Seminal nitric oxide (µmol/l)	2.32 ± 1.2	6.17 ± 0.79	p<0.001
Sperm count (10^6millions/ml)	69.72 ± 13.47	9.35 ± 5.27	p<0.001
Sperm Motility (%)	72.21 ± 13.11	63.57 ± 12.77	P<0.05
Sperm Abnormal Morphology (%)	18.76 ± 4.74	35.92 ± 13.94	p<0.001

Table 2: Correlation coefficient of various parameters studied in oligospermic infertile men.

Parameters		MDA	$NO^{\cdot}$	Sperm count	Sperm motility	Morphology
MDA	r -value	1	0.306**	-0.321*	-0.420**	0.260*
$NO^{\cdot}$	r- value	-	1	-0.301*	-0.523**	0.315*
Sperm count	r -value	-	-	1	0.311*	-0.278*
Sperm Motility	r -value	-	-	-	-	-0.253*

r = Pearson's correlation co-efficient.
tnacifingiS* (p<0.05) ** Highly significant (p<0.001)

4. Discussion

Our study revealed that the infertile men with oligospermia have significant increase in lipid peroxidation and nitric oxide than fertile control. It has been studied that oxidative stress adversely affects on spermatogenesis in male infertility.[10] More than half (55%) of the oligozoospermic patients who display a spermatozoa–oocyte penetration rate lower than 25% have an elevated ROS production.[11] Furthermore, spermatozoa of oligozoospermic patients have been confirmed as a very important source of ROS.[12]

Oligozoospermia is a frequent presentation in male infertility. Oligozoospermia can be due to high fever, smoking, alcohol and/or use of recreational drugs. Other causes could be varicocele, obstruction, testicular trauma, secondary testicular failure, chromosomal disorders and sexually transmitted diseases. Hormonal disorders varicocele, obesity and malnutrition can also result in oligozoospermia. Both poor motility and morphology are surrogate markers of poor sperm function.[13]

Oligozoospermia and ROS have a strong association with infertility.[14,15] Kullisaar et al. showed that $NO^{\cdot}$ and H_2O_2 show high levels in patients with mild inflammation and non-inflammatory oligospermia and showed a negative correlation with sperm count and motility.[15]

Du Plessis et al.[16] reported that human spermatozoa incubated and exposed to artificial H_2O_2, showed detrimental effects on sperm motility and results into significant increase in the ROS and $NO^{\cdot}$ concentration. In addition, ROS generated by leukocytes or granulocytes have also showed harmful effects on human spermatozoa, that leads to marked loss of sperm motility and morphology and which reduces hyperactivation and oocyte penetration.[17]

Amiri et al.[18] found that the mean concentration of $NO^{\cdot}$ in infertile males was significantly higher than control men.

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Giancarlo et al.[19] demonstrated same findings in idiopathic asthenospermia and found significant negative correlation between $NO^{\cdot}$ concentration and sperm motility. Garg Vidya et al.[6] also reported negative correlation between $NO^{\cdot}$ concentrations with sperm concentration and sperm morphology.

Infertile men have higher concentrations of $NO^{\cdot}$ may be due the male genital tract disease and associated factors, such as inflammation and infection, which can lead to $NO^{\cdot}$ overproduction or occupation, lifestyle or other environmental exposures, habits like smoking, excess alcohol intake which can also increase the $NO^{\cdot}$ concentration.

Low sperm count might be caused due to prolonged exposure of seminiferous epithelium to high levels of ROS producing spermatozoa, which damages the seminiferous tubules leading to testicular atrophy and reduction of total sperm production.[6] In addition Oxidative stress-induced damage to sperm mediated by lipid peroxidation can causes reduction of sperm motility.

In this study, increased MDA and NO levels were found to be associated with increase in abnormal morphology, it has been reported that spermatozoa with abnormal morphology have the capacity to generate high levels of ROS and if it is more than critical levels, can cause oxidative stress[20]

5. Conclusion

The levels of MDA and Nitric oxide were increased significantly in oligospermic infertile men and showed negative correlation with sperm count and sperm motility. Sperm abnormal morphology showed positive correlation with MDA and nitric oxide. This suggests oxidative stress induced lipid peroxidation can cause poor semen quality. So assessment of MDA and nitric oxide will help to understand an etiology of infertile men to decide treatment strategies.

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