Original Paper

Relationship of Malondialdehyde (MDA) Level in Seminal Plasma with Sperm Quality in Men Undergoing Fertility Investigation

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ABSTRACT

It has been proposed that malondialdehyde (MDA) plays an important role in male infertility. The aim of this study was to evaluate the relationship of seminal plasma levels of malondialdehyde with sperm concentration, morphology, motility and viability.

Samples were divided into four groups—Group 1: normozoospermia (n=48); Group 2: asthenozoospermia (n=20); Group 3: oligoasthenozoospermia (n=21); and Group 4: oligoasthenoteratozoospermia (n=27).

Seminal malondialdehyde (MDA) activity was measured by using spectrophotometer.

Mean values of sperm count, percentage motility, percentage normal sperm morphology and percent viability were found to decrease in order from group 1 to 4. Mean MDA concentrations in µmol/L were 30.42±8.99, 39.60±15.06, 47.75±11.52 and 48.51±10.51 ($p<0.001$), respectively in groups 1–4. There was negative correlation between MDA concentration with sperm concentration, morphology, motility and viability.

These results suggest a possible role of MDA in pathophysiology of male infertility.

Key Words: Male infertility; Oxidative stress; Malondialdehyde; MDA

Introduction

In recent years, the generation of reactive oxygen species (ROS) in male reproductive tract has become a real concern because of their potential toxic effects at high levels on sperm quality and function.[1] When levels of reactive oxygen species overwhelm the body's antioxidant system, oxidative stress (OS) occurs.[2] Excessive generation of ROS in semen by leukocytes as well as by abnormal spermatozoa could be a cause of infertility because it leads to injury to spermatozoa.[3] Moreover, the sperm plasma membrane contains large amounts of lipids in the form of polyunsaturated fatty acids which are vulnerable to attack by ROS. ROS in the presence of polyunsaturated fatty acids triggers a chain of chemical reactions called lipid peroxidation.[4] MDA (malondialdehyde) is the most widely used tool to assess lipid peroxidation. The hypoosmotic swelling (HOS) test can be used to assess the functional integrity of the sperm membrane by evaluating its reaction under hypoosmotic conditions.[5]

Considering all the above detrimental effects on various seminal parameters, the present study has been carried out to assess oxidative stress status in the male reproductive tract.

Materials and Methods

Semen samples: This study was carried out in the reproductive biology unit (infertility clinic) in the department of physiology, Jawaharlal Nehru Medical College, Sawangi, Wardha (MS), after taking approval from the

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institutional ethics committee. The patients were referred to semen analysis laboratory from the department of obstetrics and gynaecology, department of surgery, and sex and marriage counseling centre of the medical college. The subjects included for the study comprised 116 infertile men in the age group of 23–43 years. Following due consent of the subjects, their semen analysis was performed. They were all non-smokers, non-alcoholics and were all free from obvious genital tract abnormalities. Instructions regarding abstinence was given with regard to sexual intercourse as well as for masturbation, and night emissions for a period of 3–5 days for full maturation of sperms. Samples were obtained by masturbation or coitus interruptus method. Patients were advised not to use ordinary condoms, since they could contain spermicidal agents.

The samples were allowed to liquefy at least for 30 minutes protecting them from extremes of temperature, i.e., below 20°C and above 40°C. Semen analysis was carried out within one hour after liquefaction, as per the guidelines laid down by W.H.O (2000).[6,7] Microscopic examination was done taking into account the sperm count in millions per millilitre, motile sperm count in millions per millilitre, percentage motility, and percentage normal morphology (routine or conventional parameters of semen analysis).

Each sample was subjected to sperm function test, i.e., hypoosmotic swelling test (HOS). Hypoosmotic swelling test score of initial percentage of sperms with coiled tail was subtracted from the latter. If more than 60% spermatozoa, showed curling, it was considered as normal.[5]

Estimation of malondialdehyde (MDA): For estimation of malondialdehyde (MDA), liquefied samples were centrifuged at 3000 rpm for 5 minutes in cooling centrifuge machine. The supernatant was then withdrawn, transferred to Eppendorf tubes and stored at -40^{0}C until used. The seminal plasma was used for estimation of malondialdehyde (MDA) which is a thiobarbituric acid reactive substance by TCA-TBA method, a modified procedure of Satoh, 1978. Concentration of MDA was measured spectrophotometrically by using UV-VIS spectrophotometer (Systronics, model 117).[8]

Statistical analysis: The data obtained was analyzed by using mean ± standard deviation and student unpaired 't' test. The results were tested by using the SPSS (Version 14.0) statistical software.

Results

Mean values of sperm count, percentage motility, percentage normal sperm morphology and percent viability were found to decrease in order from group 1 to 4 (**Table 1**). Mean MDA concentrations in micromol/L were 30.42±8.99, 39.60±15.06, 47.75±11.52 and 48.51±10.51 ($p<0.001$) respectively in groups 1 to 4 (**Table 2**). There was negative correlation between MDA concentration with sperm concentration, morphology, motility and viability (**Table 3**).

Discussion

Spermatozoa have been shown to be able to produce reactive oxygen species (ROS).[9-11] Many studies have demonstrated the association of sperm reactive oxygen species production with poor sperm quality.[10-13] However, recent evidence has emerged showing the beneficial role of low levels of ROS in different cells and tissues including spermatozoa.[14]

In the present study, sperm concentration exhibited decline transition from normozoospermic with motile sperm (group I) to oligoasthenoteratozoospermic group (group IV) (**Table 1**). The clinical significance of seminal oxidative stress has been suggested by several independent studies indicating a link between peroxidative damage to human spermatozoa and the incidence of male infertility.[15,16] The increased levels of MDA with decreasing sperm concentration could be due to peroxidative damage of spermatozoa (**Table 2**). When correlation was tested between seminal MDA level and sperm count in millions/ml in different groups, there was negative correlation (**Table 3**).

These data suggest that higher concentration of MDA in seminal plasma is associated with poor sperm quality. Lipid peroxidation may occur variably in different parts of human male reproductive tract, which could explain a decrease in sperm concentration. This oxidative damage is a probable cause of idiopathic male infertility involving disruption of spermatogenesis.[17] This can be attributed to the presence of one of the by-products of lipid peroxide decomposition, i.e., malondialdehyde (MDA). This by-product has been used in biochemical assays to monitor the degree of peroxidative damage in spermatozoa.[18] The peroxidative damage is mainly due to lipid peroxidation of sperm plasma membrane. The lipid composition of plasma membrane of mammalian spermatozoa is markedly different from mammalian somatic cells. They have very high levels of phospholipids, sterols, and saturated and polyunsaturated fatty acids. Therefore

Table 1 Mean values of sperm count, percentage motility, percentage normal sperm morphology and percent viability in different groups

	Group		Sperm concentration (millions/ml) (Mean ±SD)	Percentage motility of sperms (Mean±SD)	Percentage normal morphology of sperms (Mean±SD)
1	Normozoospermia with normal motility	>20 millions sperms/ml, >50% motility, >30% morphology	66.66±26.76	52.75±4.94	47.91±10.90
2	Normozoospermia with reduced motility	>20 millions sperms/ml, <50% motility, >30% morphology	56.00±24.14	39.95±5.60	44.00±17.59
3	Oligozoospermia with asthenozoospermia	<20 millions sperms/ml, <50% motility, >30% morphology	14.85±3.70	37.09±6.17	34.00±7.52
4	Oligoasthenoterato-zoospermia	<20 millions sperms/ml, <50% motility, <30% morphology	14.33±3.31	34.25±6.72	13.88±4.66
ANOVA Test			F-value	76.23	55.10
			p-value	0.000	0.000
			Remark	HS*, p<0.001	HS, p<0.001

*HS = Highly significant

sperm cells are particularly susceptible to the damage induced by excessive ROS release.[19]

Lipid peroxide content of human spermatozoa has been found to have a similar significant negative correlation with the concentration of spermatozoa in other studies.[20-24] Pasqualotto et al[20] reported that poor semen characteristics such as concentration, motility, morphology of spermatozoa, etc., are due to increased levels of oxidative stress. Fraczek et al[21] observed elevated seminal MDA concentration in patients with oligoasthenoteratozoospermia. Dandekar et al[22] studied MDA levels as an index of lipid peroxidation and found that MDA levels in normozoospermia (0.32±0.03 nmol/ml) were lower than mild oligozoospermia (0.52±0.28 nmol/ml) and azoospermia (1.25±0.28 nmol/ml) samples. They proposed that lipid peroxidation of human spermatozoa may result in decreased sperm count. Zarghami et al[23] reported significantly higher MDA concentration and lower sperm concentration in the seminal plasma of asthenozoospermic men than in normozoospermic males. Hsieh et al[24] observed that MDA levels correlated negatively with sperm motility and concentration, and suggested that the lipid peroxidation which was represented by the MDA activity might compromise sperm viability.

Similar to the sperm concentration in the present study, mean percentage of motility also exhibited decline transition from normozoospermic with motile sperm group (group I) to oligoasthenoteratozoospermic group (group IV) (**Table 1**). Analysis of variance for percentage motility between different groups was statistically highly significant (ANOVA test, $F=76.23$, $p<0.001$), thus showing good relationship between sperm count and percentage sperm motility. Impairment of motility could be due to increased formation of ROS, which has been correlated with a reduction of sperm motility.[18] The link between ROS and reduced motility may be due to a cascade of events that results in a decrease in axonemal protein phosphorylation and sperm immobilization, both of which are associated with a reduction in membrane fluidity that is necessary for sperm-oocyte fusion.[25] Another explanation could be that hydrogen peroxide can diffuse across membranes into the cells and inhibit activity of some enzymes such as glucose-6-phosphate-dehydrogenase (G_6PD). This enzyme controls the rate of glucose flux

Table 2 Seminal MDA level along with analysis of variance

	Groups	No. of subjects	MDA level (micromol/ml) Mean±SD	ANOVA test	Remarks
1	Normozoospermia with normal motility	48	4.33±1.90	'F'value	0.000 p<0.001 HS*
2	Normozoospermia with reduced motility	20	7.11±1.60	58.28	
3	Oligozoospermia with asthenozoospermia	21	9.34±2.90		
4	Oligoasthenoteratozoospermia	27	9.74±2.17		
Total		116	8.30±3.95		

*HS = Highly significant

through the hexose-monophosphate shunt, which in turn, controls intracellular availability of nicotinamide-adenine dinucleotide phosphate (NADPH). The latter is used as a source of electrons by spermatozoa to fuel the generation of ROS by an enzyme system known as NADPH oxidase.[26] Inhibition of G_6PD leads to decrease in the availability of NADPH and a concomitant accumulation of oxidized glutathione and reduced glutathione. This can reduce the antioxidant defenses of spermatozoa and increase peroxidation of membrane phospholipids.[27] Similar findings were observed in studies undertaken by other investigators.[13,18,23,28] Aziz et al[13] found that there was significant negative correlation between sperm ROS production and sperm concentration and percentage of sperm motility. Aitkin et al[18] observed a significantly reduced level of sperm motility in association with marked increase in the generation of reactive oxygen species, and this production of reactive oxygen species was associated with a significant increase in the levels of malondialdehyde production. Zarghami et al[23] found that MDA levels were higher in asthenozoospermic subjects than in control subjects ($p<0.05$). Twigg et al[28] found that high ROS generation leads to significant peroxidative damage and a drastic loss of sperm motility.

In the present study, increased MDA level was associated with increase in abnormal sperm morphology. This may be because whenever spermatogenesis is impaired, the cytoplasmic extrusion mechanisms are defective; spermatozoa are released from the germinal epithelium carrying surplus residual cytoplasm. Under these circumstances, the spermatozoa that are released during spermiation are believed to be immature and functionally defective.[29] Retention of residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanisms that may be mediated by the cytosolic enzyme G_6PD.[30]

Table 3 Correlation of MDA with sperm concentration, %motility, %morphology, %HOS & age (yrs)

Correlation with MDA	Correlation (r)	p value
Sperm concentration	-0.642	0.000 Highly significant p<0.001
% Motility	-0.555	0.000 Highly significant p<0.001
% Morphology	-0.615	0.000 Highly significant p<0.001
% HOS	-0.695	0.000 Highly significant p<0.001
Age	0.135	Not significant p>0.05

Similar findings were reported earlier by other studies.[13,17,31,32] Aziz et al[13] found significant negative correlation between sperm ROS production and proportion of sperm with normal morphology and borderline morphology. Tavilani et al[17] found that abnormal forms of spermatozoa were higher in asthenozospermic group than normozoospermic groups. The ROS production was positively correlated with various sperm morphological defects. Gil–Guzman et al[31] showed that levels of ROS production were negatively correlated with teratospermia and spermatozoa developmental stages. ROS production was studied in ejaculated sperms, which also contained sperm with abnormal head morphology and cytoplasmic retention. Aksoy et al[32] reported that the production of NO is increased in patients with varicocele, and the high NO concentration is negatively correlated

with morphology. Contrast studies were performed by Donnelly et al and Huang et al.[33,34]

In the present study, the mean level of MDA in normal HOS score (%) was 4.94±2.11 and in abnormal HOS score (%) it was 8.52±3.00, which was statistically highly significant by student 't' test ($t=7.18$, $p<0.0001$) (Table 3). There was increase in levels of MDA with increase in abnormality of HOS score (%). So decreasing HOS % score with increasing MDA concentration showed that there was loss of sperm membrane integrity, probably because of lipid peroxidation of sperm plasma membrane. Peroxidation may result in hyperpermeability which in turn may lead to decreased HOS (%) score.[22]

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