Lipid Peroxidation, Sperm DNA Fragmentation Total Antioxidant Capacity and Semen Quality in Male Infertility

1Kavita More, 2ZG Badade, 3JG Narshetty, 4DS Joshi, 5S Mukherjee, 6AD Deepak, 7VZ Badade

ABSTRACT

Infertility is a major clinical problem, affecting people medically and psychosocially. Male factor plays a significant role in about 50% of infertile couples. Recent reports indicate that increasing male infertility could be due to genomic abnormalities. The etiology of sperm DNA damage is multi-factorial but compromised due to nuclear defects, protamine deficiency and oxidative stress. The present study was aimed to evaluate sperm DNA integrity and oxidative stress in infertile men.

The study is prospective, comprises 96 infertile patients and 30 fertile controls. Sperm DNA integrity was assessed by flowcytometry. MDA and TAC were evaluated spectrophotometrically. The percentage of DNA Fragmentation Index and MDA were found to be significantly increased while TAC was significantly decreased in infertile men as compared to control. DFI and MDA were negatively correlated with TAC levels. Present study indicates significant increases in seminal MDA and sperm DNA damage in infertile men. Seminal MDA was significantly correlated with sperm DNA damage, TAC and standard sperm parameters. The elevated levels of seminal OS observed in these infertile patients could be responsible for poor sperm quality and sperm DNA fragmentation. Hence evaluation of DFI, MDA and TAC can be used for diagnosis, prognosis of male infertility in addition to routine semen parameters to decide the treatment strategies.

Keywords: DNA fragmentation index (DFI), Malondialdehyde (MDA), Oxidative stress (OS), Total antioxidant capacity (TAC).

How to cite this article: More K, Badade ZG, Narshetty JG, Joshi DS, Mukherjee S, Deepak AD, Badade VZ. Lipid Peroxidation, Sperm DNA Fragmentation Total Antioxidant Capacity and Semen Quality in Male Infertility. MGM J Med Sci 2014;1(1):1-6

INTRODUCTION

Infertility is a major clinical problem, affecting people medically and psychosocially. Its prevalence in Western countries has been estimated as 20% and in India as 15%. Male factor plays a significant role in about 50% of infertile couples.1-3 Male infertility could be due to genomic abnormalities4 and assessed by semen analysis. These factors are generally modest predictors of reproductive outcomes that are unable to focus on sperm nuclear and chromatin effects.5 Men with normal semen analysis can be infertile; sometimes infertile men could not get answer for their decrement semen quality, the cause could be related to abnormal sperm DNA.6

The etiology of sperm DNA damage is multi-factorial but compromised due to condensation or nuclear maturity defects, DNA breaks or DNA integrity defects, protamine deficiency, apoptosis and oxidative stress, etc. Oxidative damage to spermatozoa is very common,7 affecting between 30 and 80% of infertile men, and is due to a number of predisposing factors. There are various sources for generation of oxidative stress in spermatozoa and seminal plasma. First of all, infertile men’s semen often contains more morphologically abnormal, immature spermatozoa that have an increased capacity to produce ROS compared with mature spermatozoa. Secondly, sperm plasma membranes contain large quantities of polyunsaturated fatty acids (PUFAs), and their cytoplasm and seminal plasma contains low concentrations of scavenging antioxidants, which particularly makes them susceptible to the damage induced by excessive ROS.8

Seminal plasma malondialdehyde (MDA) is stable peroxidation product; its estimation is simple and helps to evaluate the effect of peroxidation on sperm. Number of studies has shown that lipid peroxidation affects sperm quality.9 Antioxidants protect spermatozoa from excess concentration of ROS. The most common antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), α-tocopherol, ascorbic acid, β-carotene, zinc and altogether represent the total antioxidant capacity.10

There is evidence to show, infertile men possess substantially more sperm DNA damage than fertile men.
and this adversely affect reproductive outcomes.\textsuperscript{11} Sperm DNA damage assessment has been recommended as a complementary test in male infertility work-up by some authors. However, it is still unclear whether sperm DNA damage assessment should be introduced as a routine test in infertile men or only applied in selected cases.

In view of etiology of sperm DNA damage by oxidative stress, the objectives of our study was to examine levels of sperm DNA damage and oxidative stress in infertile men and determine the correlation of the sperm DNA damage with seminal MDA and standard sperm parameters.

**MATERIALS AND METHODS**

The present study was carried out in Department of Biochemistry, Department of Obstetrics and Gynecology, MGM Medical College and Group of Hospitals, Navi Mumbai and National Institute for Research in Reproductive Health, Mumbai. The institutional ethical committee clearance was obtained for the present study.

Thirty male subjects aged 21 to 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). Ninety six infertile male (Study Group), referred from Department of Obstetrics and Gynecology, aged 21 to 45 years, without any treatment, whose wives had not conceived after 1 year of regular, unprotected intercourse. The wives of infertile subjects included had no obvious causes of infertility like tubal blockage or ovulation disorders.

In Study Group, patients who had varicocele, hypogonadism, prolonged illness, leukocytospermia, genital tract infections, cryptorchidism, parotitis, mumps orchitis, tuberculosis, diabetes mellitus, testicular injury, azoospermia due to obstructive causes were excluded from the study. At first clinic attendance, a detailed background family and personal history was taken as per written proforma in the form of questionnaire. Detailed physical examination was also done on both husband and wife. The written consent was taken from healthy volunteers and infertile males.

Samples were collected by masturbation in wide mouth sterile plastic container after minimum of 3 days of abstinence. After liquefaction, samples were processed by conventional analysis to determine sperm count, sperm motility and sperm morphology according to WHO criteria. On centrifugation, seminal plasma was used for evaluation of lipid peroxidation in terms of malondialdehyde by Satoh K method.\textsuperscript{12}

Seminal plasma TAC was determined by using a novel automated method developed by O Erel.\textsuperscript{13} In this method, the hydroxyl radical, the most potent radical, is produced via Fenton reaction and consequently the colored diansidinyl radical cations, which are also potent radicals, are produced in the reaction medium of the assay. Antioxidant capacity of the added sample against these colored potent free radical reactions measured the total antioxidant capacity. The results were expressed as millimoles of Trolox equivalent per liter.

Sperm DNA integrity was assessed by the Sperm Chromatin Structure Assay (SCSA) by flow cytometry by Evenson et al method.\textsuperscript{14} (FACSDiva Version 6.1.3 flow cytometer (BD Biosciences, USA). The assay is based on the metachromic properties of DNA binding fluorescent dye, acridine orange (AO). AO intercalates with DNA and emits green fluorescence when bound to intact, double strand DNA and red fluorescence when bound to single strand, fragmented DNA.

The SCSA however, quantifies the fluorescent signals by flow cytometry. Upon excitation by laser light, the emitted red and green fluorescent signals from individual cells are detected by photomultiplier tube. The green fluorescence (FL1) was collected through a 515 to 545 nm bandpass filter and the red fluorescence (FL3) was collected through a 650 nm longpass filter. Through a specific SCSA software a scatter plot is created, showing the ratio of green and red sperm. The percentage of red sperm is called DNA fragmentation index, visualized by a histogram.

**STATISTICAL ANALYSIS**

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

RESULTS

Results were expressed as mean ± SD for each parameter. Statistically significant differences among infertile and fertile men are indicated in Table 1 (Graphs 1 and 2) along with their significant values. The DFI, MDA and sperm abnormal morphology were significantly increased (p < 0.001) whereas TAC, sperm count and sperm motility were significantly decreased (p < 0.001) in infertile men as compared to control group.

Correlation coefficient of various parameters indicated in Table 2 along with their significant values. Seminal plasma malondialdehyde and DFI showed negative correlation with sperm count, motility and noted positive correlation with abnormal morphology. Seminal plasma TAC levels were positively correlated with sperm count, motility and have negative correlation with abnormal sperm morphology in infertile men. There was negative correlation between malondialdehyde and DFI with TAC in infertile men. We found the same correlations in control group (Table 3) but most of the correlations are weak and not significant in fertile controls.

DISCUSSION

It is established that sperm DNA quality is important in maintaining the reproductive potential of sperm. The fertilizing potential of sperm depends not only on the functional competence of spermatozoa but also on sperm DNA integrity. Sperm DNA integrity has an important role not only for fertilization but also for normal embryo and fetal development, hence any abnormality in sperm DNA integrity could affect any of this process.6

Methods of evaluating sperm DNA integrity include the single cell gel electrophoresis assay, i.e. COMET assay, terminal deoxynucleotidyl transferase mediated nick end labeling (TUNEL); in situ nick translation (NT) and SCSA. We assessed sperm DNA integrity by SCSA and the advantages of the flowcytometric (FCM) technique, such as automation, standardization, objectivity, velocity, precision, reproducibility and statistical robustness.14

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (30) Mean ± SD</th>
<th>Study group (96) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (10^6 millions/ml)</td>
<td>72.74 ± 14.92</td>
<td>34.78 ± 29.55*</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>69.16 ± 8.10</td>
<td>34.42 ± 21.45*</td>
</tr>
<tr>
<td>Sperm abnormal morphology (%)</td>
<td>18.76 ± 4.74</td>
<td>31.07 ± 13.43*</td>
</tr>
<tr>
<td>MDA (nmol/l)</td>
<td>1.71 ± 0.50</td>
<td>5.19 ± 1.31*</td>
</tr>
<tr>
<td>TAC (mmol trolox Eq/l)</td>
<td>22.17 ± 2.85</td>
<td>13.79 ± 1.68*</td>
</tr>
<tr>
<td>DFI (%)</td>
<td>11.65 ± 3.38</td>
<td>48.90 ± 13.90*</td>
</tr>
</tbody>
</table>

p-value: *Highly significant (p < 0.001)
Numbers of studies have shown that sperms of infertile men contain more DNA damage than fertile men and may have negative effect on fertility potential and semen quality of these patients. It has been suggested that sperm DNA integrity may be a better predictor of male fertility than routine semen analysis.14-16 High levels of sperm DNA damage often correlates with poor seminal parameters, such as reduced count, sperm motility and abnormal sperm morphology.15,17

In present study, percent DNA fragmentation Index (DFI) was significantly increased in infertile men (48.90 ± 13.90) (p < 0.001), and showed significant negative correlation with sperm count (r = – 464) (p < 0.001), motility (r = – 485) (p < 0.001) and positively correlated with abnormal sperm morphology (r = 0.143). Our results are supported by Evenson DP et al.14 Spano et al.16 Sahel et al.18

Saleh et al.18 reported that sperm DNA damage was significantly increased in men with idiopathic and male factor infertility and in men who failed to initiate a pregnancy after assisted reproductive techniques whereas Irvine et al.19 also demonstrated a significant proportion of infertile men have elevated levels of DNA damage in their ejaculated spermatozoa and noted highly significant negative correlations between DNA fragmentation and semen quality, particularly sperm concentration.

Sperm DNA damage may be caused by number of factors. Recently it was suggested that aberrant spermatogenesis could lead to alterations in chromatin packaging and deficiency in protamination which would make sperm DNA more susceptible and vulnerable to variety of stressors, mostly ROS.

Mean levels of MDA in seminal plasma was found to be significantly increased in infertile men as compared to control group. Our results are compatible with Kumar et al.20 Kobayashi et al.21 Nabil et al.22 Piyali Das et al.23 and Badade et al.24

MDA showed a significant negative correlation with sperm count (r = –0.441) (p < 0.001) and motility (r = –0.484) (p < 0.001) which was compatible with the findings of Nabil et al.22 Fraczek et al.25 We noted positive correlation between sperm abnormal morphology (r = 0.179 and MDA which was supported by Shamsi et al.26

Recently, it has been reported that percentage of immotile spermatozoa correlate positively with seminal plasma MDA concentrations (r = 0.50, p < 0.01), while sperm concentration displays a significant negative correlation (r = –0.63, p < 0.001). On the contrary, a decrement of MDA corresponds to an increase of the pregnancy rate.27

Piyali Das et al.23 also found significantly higher levels of MDA in the seminal plasma of the abnormal groups (including oligoasthenoteratozoospermia and asthenoteratozoospermia).

We found high levels of MDA in infertile patients that indicate excessive ROS is responsible for lipid peroxidation of the membrane lipids. Sperm plasma membrane has a high concentration of polyunsaturated fatty acids (PUFAs) which play an important role in ion transport and sperm membrane fluidity, therefore peroxidation of membrane PUFAs by excessive ROS disturbs the functions, carried out by the sperm membrane and impairs the fertilizing capacity of spermatozoa.

We found significantly lower seminal TAC activity in infertile men (p < 0.001 as compared to control group and showed positive correlation with sperm count (r = 0.466) (p < 0.01) and sperm motility (r = 0.284) (p < 0.001) and negative correlation with abnormal sperm morphology.
Lipid Peroxidation, Sperm DNA Fragmentation Total Antioxidant Capacity and Semen Quality in Male Infertility

MGM Journal of Medical Sciences, January-March 2014;1(1):1-6

MGMJMS

(r = –0.223) (p < 0.05). Our results are compatible with the Khosrowbeygi A et al., Koca et al., Pasqualotto et al., Pasqualotto et al, Moein et al., and Variet et al., Badade ZG et al. We also noted negative correlation of TAC with MDA (r = –0.295) (p < 0.01), DFI (r = –0.578) (p < 0.001) (Graph 3).

Antioxidants are important in maintaining the oxidant-antioxidant balance in tissues which have a significant role in protecting the sperm against peroxidative damage. Depressed seminal antioxidant capacity has been implicated in male subfertility. TAC levels have been shown to be lower in the semen of subfertile men as compared with fertile men. Nasrin S et al reported DNA damage was significantly correlated with nitric oxide concentration in infertile men and low levels of TAC. Variet et al also demonstrated significant low TAC in male factor subfertility group as compared to fertile control but not reported significant difference in idiopathic infertile group. We found lower TAC levels in infertile patients; this strongly suggests that total antioxidant capacity was insufficient to cope with the excessive amount of ROS.

In this study, elevated levels of sperm DNA damage in subgroups of infertile men may be caused by the high levels of seminal oxidative stress. Another explanation for the link between seminal oxidative stress and sperm DNA damage may be related to defect in spermiogenesis that causes the release of spermatozoa that are immature and have abnormal chromatin structure/high DNA damage and abnormal morphology. Spermatozoa with abnormal morphology have been shown to have a capacity to generate high levels of ROS that, on exceeding critical levels, can cause oxidative stress.

Therefore, results of our study clearly showing that significant elevation of MDA and significantly low levels of TAC in infertile group as compared to fertile men, signify imbalance between oxidants and antioxidants levels which is an indication of increased oxidative stress. This leads to excessive exposure of spermatozoa to oxidative stress that initiate peroxidation of PUFA in sperm plasma membrane. This disrupt sperm membrane fluidity and integrity, as a result of which the spermatozoa lose their competence to participate in the membrane fusion events associated with fertilization, which ultimately causes decreased sperm motility, viability, increased midpiece morphology defects. Further insufficient antioxidants present in seminal plasma makes infertile men’s spermatozoa more susceptible to oxidative attack and leads to DNA damage leading to male infertility.

CONCLUSION

Our study indicates significant increases in seminal MDA and sperm DNA damage in infertile men. Seminal MDA was significantly correlated with sperm DNA damage, TAC and standard sperm parameters. The elevated levels of seminal OS observed in these infertile patients could be responsible for poor sperm quality and sperm DNA fragmentation. Hence evaluation of DFI, MDA and TAC can be used for diagnosis, prognosis of male infertility in addition to routine semen parameters to decide the treatment strategies.

ACKNOWLEDGMENT

This work was supported by MGM Medical College, MGM Institute of Health Sciences (MGMIHS), Kamothe, Navi Mumbai, and NIRRH, Mumbai, Maharashtra, India. We acknowledge Mr Pandurang Thatkar, Statistician for helping us in data analysis. Also we acknowledge Dr RP Dixit, University Librarian, MGMIHS who has helped us in citing the references appropriately.

REFERENCES