

CORRELATION AMONG ROUTINE SEMEN PARAMETERS,
SPERM VIABILITY AND MALONDIALDEHYDE
LEVELS IN HUMAN SUBJECTS WITH
DIFFERENT FERTILITY POTENTIAL

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Abstract : Percentage of viable sperms has good potential to reflect male fertilizing potential. In the present study, an attempt was made to find out the percentage of viable sperms in normal and abnormal ejaculates and influence of reactive oxygen species in affecting the viability of sperm. Malondialdehyde (MDA) in seminal plasma of normal and abnormal ejaculates was measured by Thiobarbituric Acid method and percentage of viable sperms was assessed by Eosin-Nigrosin staining. The results revealed that the abnormal semen samples have significantly lower number of viable spermatozoa ($59.06 \pm 9.63\%$ vs. $68.33 \pm 5.46\%$, $P < 0.05$) and higher levels of MDA (2.53 ± 0.66 vs. 1.66 ± 0.37 nmol/ml, $P < 0.05$) as compared to normal. A significant negative correlation was found between seminal MDA level and sperm viability. Based on the results obtained from the present study, we suggest that viability of sperm is an authentic parameter to assess male fertility potential and it can be affected significantly by oxidative stress.

Key words : fertilizing potential
semen

oxidative stress
spermatozoa

INTRODUCTION

Oxidative stress is the effect of imbalance between Reactive Oxygen Species (ROS) and antioxidant forces operating in any given system. Reactive oxygen metabolite like super oxide anion, hydroxyl radical, singlet oxygen, hydrogen peroxide etc. are generally

considered to be cytotoxic because of their ability to induce Lipid Peroxidation within the cell membrane (1) which produces metabolites like MDA that is lethal for the cell. Sperms like all other cells living in aerobic environment are very much susceptible to ROS which can modify cell function endanger cell survival or both (2).

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Moreover, susceptibility of sperm to oxidative damage is much more than other cell type as they contain large quantity of poly unsaturated fatty acids in their plasma membrane and very low content of scavenging enzymes in their cytoplasm (3). This excess lipid peroxidation occurring in sperm cell membrane can lead to loss of membrane integrity rendering the sperms nonfunctional (4). Thus it appears that oxidative stress is a major factor for the pathogenesis of male infertility as it affects many of the structural and functional aspects of spermatozoa (5).

Viability of sperm which is not routinely done during semen analysis seems to have good potential to assess semen quality. It was found to have positive correlation with sperm motility (6). Moreover, study about the association between oxidative stress and sperm viability is generally thin. So in the present study, viability of sperm was evaluated in human subjects with different fertility potential and its correlation with overall semen quality was tested. Oxidative stress was assessed by measuring MDA (Malondialdehyde-end product of lipid Peroxidation.) levels of the seminal plasma of the same subjects and correlation between MDA level and viability of sperm was evaluated.

MATERIALS AND METHODS

Semen samples were obtained from 60 male patients of 21–50 years of age attending the Reproductive Biology Unit, MGIMS, Sevagram, with complaints of infertility (both primary and secondary infertility cases). Detailed history of present and past illness

as well as medical and surgical management was taken. Selected male partners underwent thorough surgical examination of genito-urinary system to rule out the exclusion criteria. Only subjects with normally developed genito-urinary organs were included in the study. All the tests were done with due permission of the ethical committee of the Institute and with written consent from the subjects. Specimens of semen were collected by masturbation after 3 days of sexual abstinence. After complete liquefaction, samples were analyzed by SQA II B sperm quality analyzer (M.E.S. Ltd., Israel) for sperm concentration, motility, morphology and according to WHO guideline (7) grouped into following two groups:

1. Group I – showing normozoospermia, (n=20): sperm count >20 million/ mL, motility >50%, normal morphology in >30% sperms, labelled as ‘Normal’.
2. Group II – showing oligoasthenoterato or asthenoteratozoospermia, (n=40): sperm count <20 million/mL, motility <50%, normal morphology in <30% sperms, labelled as ‘Abnormal’.

Except for the normozoospermics, all other subjects with abnormal seminogram profile were asked to come for repeat semen analysis after 1 month. If the second report was also abnormal then only they were included in the abnormal groups and the semen samples were utilized for further physio-chemical assessment.

Subjects with varicocele, hydrocoele, undescended testes or any other structural

abnormality or any history of surgical intervention in the genitourinary tract which may interfere with male fertility were excluded from the study. Subjects with any acute febrile ($>38^{\circ}\text{C}$) illness or a history of similar episode in last six month or treatment history with drugs like cancer chemotherapy, nitrofurantoin, niridazole, colchicine or any hormonal preparation which may directly suppress the spermatogenesis were also excluded from the study. According to the semen analysis report, those who had <5 millions sperm/ml of semen were also excluded from the study.

Viability of sperms was assessed by eosin-nigrosin staining as per the method of Eliasson (8). For this, semen samples were kept at 37°C for 30 minutes after collection and well mixed before analysis. Slides were prepared by placing one drop of semen on a microscopic slide, adding two drops of 1% Eosin solution to it and waiting for 30 seconds, thereafter adding 3 drops of 10% Nigrosin solution to it and again waiting for 30 seconds. All the three were well mixed and smears were prepared. After drying, slides were seen with 100x oil immersion lens of light microscope to get the percentage of unstained live sperms. 200 cells were examined for each sample.

MDA levels were analyzed according to Thiobarbituric Acid (TBA) method (9). Briefly, first semen sample was centrifuged at 3000 rpm for 10 minutes after liquefaction to get the seminal plasma. Then 0.1 mL of seminal plasma was added to 0.9 mL of distilled water in a glass tube, to it 0.5 mL of TBA reagent (0.67 gm of 2- Thiobarbituric

acid dissolved in 100 mL of distilled water with 0.5 gm of NaOH and 100 mL of glacial acetic acid) was added and then heated for 1 hour in a boiling water bath. After cooling the tube was centrifuged for 10 minutes at 4000 rpm and the supernatant absorbance was read on a spectrophotometer at 534 nm.

The viability of (the percentage of viable sperms) in normal and abnormal ejaculates were compared to find the significance of difference using Student *t*-test (P-value <0.05 was considered statistically significant). The relationship between percentage of viable sperms and MDA level of seminal plasma was tested by measuring coefficient of correlation (*r* value).

RESULTS

The average percentage of viable sperms in normal group was found to be $68.33\pm 5.46\%$ and that in abnormal group was found to be $59.06\pm 9.63\%$. Viability decreased significantly from normal to abnormal group, $P<0.01$.

The lipid peroxidation as expressed by MDA level in seminal plasma was found to be significantly increased in abnormal group than in normal group. Mean MDA level in abnormal group was found to be 2.53 ± 0.66 nmol/mL, whereas the level in normal group was 1.66 ± 0.37 nmol/mL, $P<0.01$. A negative co-relation was found between viability of sperm (%) and MDA level of seminal plasma (nm/ml). ($r = -0.8$, $P<0.01$) (Fig. 1). The findings of the physio-chemical analysis of semen samples are summarized in Table I.

TABLE I: Summary of semen analysis.

Group	Mean Sperm count (millions/ml)	Mean Motility (%)	Mean Morphology (%)	Mean Viability (%)	Mean MDA level (nmol/ml)
Normal (n=20)	92.95±29.15	58±8.02	40.12±6.29	68.33±5.46	1.66±0.37
Abnormal (n=40)	27.55±18.37	28.25±12.11	20.02±4.97	59.06±9.63*	2.53±0.66*

Values are shown as means±SD. *P<0.01 compared with normal group.

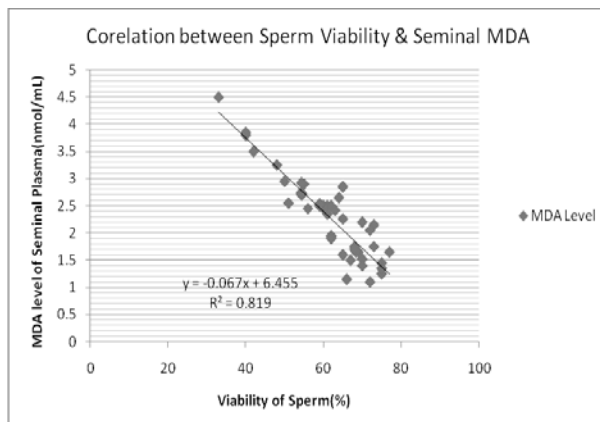


Fig. 1: Correlation of sperm viability & MDA level of seminal plasma.

DISCUSSION

The causation of male infertility may be due to various reasons and its management depends on proper diagnosis of the etiology. Till date, clinicians mostly depend on routine parameters of semen analysis (e.g. sperm concentration, motility, morphology) for the evaluation of semen quality. But viability of sperm can also be an authentic parameter as it reflects the integrity of sperm membrane (10), capacity of the sperm to withstand the diversities in the female genital tract and thereby its overall fertilizing potential. Our study showed that semen samples containing higher percentage of viable sperms were mostly with normal physical profile (Normozoospermia) and otherwise abnormal

semen samples (Oligasthenoterato and Asthenoteratozoospermia) were having poor viable sperm count. Thus, viability of sperm may be considered as an authentic and handy tool to assess male fertilizing potential specially when facility for other sophisticated techniques are not available.

In recent years, it became evident from different studies that oxidative stress has a definite role in causation of male factor infertility but there are controversies regarding the extent of lipid peroxidation as reflected by seminal MDA level in subjects with different fertility potential. Kobayashi et al (11) demonstrated that higher MDA level of semen was significantly related to the number of immotile spermatozoa. Elevated MDA level was also noted by Fraczek et al (12) in Oligoasthenoteratozoospermia. In contrast, Suleiman et al (13) demonstrated that MDA concentration in seminal plasma was not correlated with the sperm concentration and motility. In the present study, we found significantly higher level of MDA in the seminal plasma of the Abnormal group (including oligoasthenoterato and asthenoteratozoospermics) which is corroborative of the findings of previous report (9). The finding may be supportive of the fact that major source of MDA in seminal plasma are abnormal spermatozoa as well as infiltrating leukocytes that are

preponderant in abnormal ejaculates (14).

Different pathological condition associated with high lipid peroxidation can cause damage to the cell membrane, loss of cytosolic components and cell death (15). Excess ROS can decrease sperm motility presumably by a rapid loss of intracellular ATP leading to axonemal damage, decrease sperm viability and increased mid-piece morphological defects with deleterious effects on sperm capacitation and acrosome reaction (16). Moreover oxidative damage can also cause DNA fragmentation, base degradation and cross linking of proteins with loss of function in sperms (14). Overall effects lead to increase apoptosis (Programmed cell death) in sperms with excess oxidative damage (17).

Negative correlation as found in our study between seminal MDA and sperm viability supports all the detrimental

effects of excess lipid peroxidation on sperm survival that can hamper the male fertility potential.

From the present study it can be concluded that viability of sperm may be considered as an authentic parameter to assess male fertility potential and it can be affected significantly by oxidative stress. Higher MDA level may reflect greater extent of oxidative damage to the sperm thus and a significant probability of associated poor semen quality.

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