SEMINAL PLASMA OXIDATIVE STRESS AFFECTS SPERM MORPHOLOGY

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ABSTRACT:

Introduction: *According to the World Health Organization (WHO) infertility is defined as "no conception after at least 12 months of unprotected intercourse". Sperm morphology is an important index of fertility. The presence of many abnormal sperm forms indicate impaired fertility. The high levels of reactive oxygen species (ROS) as a result of oxidative stress have been associated with the peroxidation of cell membrane polyunsaturated fatty acids, thus it is hypothesised that the increased ROS level in seminal plasma due to oxidative stress may lead to sperm cell membrane defects which may cause morphological deformity in the sperm cells. Therefore, this study was undertaken with the aim to evaluate the sperm deformity index (SDI) and its relation with the ROS level in the seminal plasma of normozoospermic and oligoasthenoteratozoospermic subjects.*

Materials and Methods: *200 semen samples were studied (100 Normozoospermic and 100 oligoasthenoteratozoospermic cases). The samples were evaluated for sperm concentration, motility, morphology, sperm deformity index and level of oxidative stress.*

Results: *The SDI is inversely related sperm concentration, Percentage Motility, Percentage Normal Morphology. The linear relation was observed between SDI and level of reactive oxygen species (ROS) (MDA level).*

Conclusion: *The study concluded with the remark that high level of ROS may be responsible for the morphological deformities of the sperm cells. Further, SDI score is a useful tool in identifying infertile men with high seminal ROS in infertility clinics where facilities for measuring levels of seminal ROS are not available.*

Keywords: *Oxidative stress, Sperm morphology, Sperm Deformity Index, Reactive Oxygen Species*

INTRODUCTION

According to the World Health Organization (WHO) infertility is defined as "no conception after at least 12 months of unprotected intercourse", the time of 12 months is arbitrary, as the majority (approximately 85%) of the couples who achieve pregnancy spontaneously will do so within 12 months[1].

Moench [(2)] theorized that sperm morphology was more important index of fertility than cell density. Also the presence of many abnormal forms indicate impaired fertility and semen from infertile patients often contains higher abnormal forms than semen from fertile men [(3)].

The high levels of reactive oxygen species (ROS) as a result of oxidative stress have been associated with the peroxidation of cell membrane polyunsaturated fatty acids[(4)], thus it is hypothesised that the increased ROS level in seminal plasma due to oxidative stress may lead to sperm cell membrane defects which may cause morphological deformity in the sperm cells.

Therefore, this study was undertaken with the aim to evaluate the sperm deformity index (SDI) and its relation with the ROS level in the seminal plasma of normozoospermic and oligoasthenoteratozoospermic subjects.

MATERIALS AND METHODS

The study was conducted in the Reproductive Physiology Unit, Department of Physiology, MGIMS, Sevagram, Wardha, after clearance from institutional ethical committee.

The semen samples were collected from Male partners of the couples of primary or secondary infertility reporting to Reproductive Physiology Unit, Department of Physiology, MGIMS, Sevagram. The total number of subjects included in the study were 200 (100 Normozoospermic and 100 oligoasthenoteratozoospermic cases).

All semen samples were collected by masturbation after an abstinence period of 48–72 hours. After liquefaction, routine semen analysis was performed and subjects were classified into two main groups based on their semen analysis findings as follows:

Normal Group: **Normozoospermia**

1. Absence of abnormal findings in clinical history.
2. Semen containing 20 million spermatozoa / ml of semen or more.
3. Sperm motility is 50 % and above.
4. Normal sperm morphology in 30 % or more cells.

Infertile Group: Oligoasthenoteratozoospermia (OAT):

1. Sperm concentration less than 20 million / ml of semen.
2. Sperm motility below 50 %.
3. Normal sperm morphology in less than 30 % cells.

The volume of ejaculate was measured using a standard graduated glass tube after complete liquefaction of semen and the pH was measured immediately after liquefaction of semen using Elico's photoelectric digital pH meter.

The sperm concentration, motility and morphology was evaluated by Sperm Quality Analyzer (SQA-IIB) at the room temperature. [(5)]

Morphology was further studied for obtaining the sperm deformity index (SDI) after staining with modified Papanicoulou procedure for sperm according to the WHO manual (2000) [(1)]. A micrometer in the eyepiece of the microscope was used for routine measurements.

The sperm deformity index (SDI), described in 1996 by Aziz *et al* [(7)] is a method where the whole spermatozoon is assessed by the strict criteria and classified more than once if more than one deformity exists. Both normal and abnormal sperms are considered and the average number of deformities per sperm is determined to give a value to this index. This index reflects the balance between the prevalence of sperms with multiple structural deformities and the proportion of sperms with normal morphology in a semen sample.

As long as one spermatozoon exhibits more than one morphological anomaly, it was initially registered as pathological, and then each anomaly was registered separately. In this manner, the normal and pathological spermatozoa were determined, as well as their total morphological anomalies per 100 spermatozoa. The SDI was derived from the formula:

$$SDI = \frac{\text{Total no. of morphological anomalies}}{\text{Total no. of sperms investigated.}}$$

For each specimen 100 sperms were studied

The levels of reactive oxygen species (ROS) i.e. Malonaldehyde (MDA) were measured by an absorbance method at 535nm. Liquefied semen was centrifuged at 3000 rpm for 10 minutes, and the seminal plasma separated. 1 ml of supernatant was mixed with 2 ml of Thiobarbituric acid (TBA) reagent the mixture was boiled in water bath for 15 minutes and after cooling was centrifuged at 1000 rpm for 10 minutes. The absorbance of the supernatant was measured at 535 nm with distilled water as blank. [(8)]

The statistical analysis was done using the "Z" test, for calculating the "P"values to determine the significance of difference observed in the values obtained for different parameters studied in various groups and the Coefficient of correlation (***r***) was calculated to find out the correlation between the different parameters.

OBSERVATION AND RESULTS

The mean age of the subjects studied was 31 years in normozoospermic group and 32 years in OAT group. The mean volume of semen in normozoospermic group was 2.26 $\pm$ 0.75 ml. and in oligoastenoteratozoospermic group it was 2.72 $\pm$ 1. 11 ml there was no significant difference in both the groups. In Normozoospermic group the pH was 7.5 $\pm$ 0.09 and in OAT group it was 7.7 $\pm$ 0.25 there was no significant difference in both the groups.

In normozoospermic group mean count was 114 $\pm$ 40.07 millions per ml and in OAT group the sperm count was 18 $\pm$ 12.63 millions per ml ($P<0.05$). In normozoospermic group percent motility was 63% $\pm$ 10.82% and in OAT group percent motility was 21% $\pm$ 8.53% ($P<0.05$). In normozoospermic group percentage normal morphology was 44% $\pm$ 11.52% and in OAT group percentage normal morphology was 17% $\pm$ 3.61% ($P<0.05$). The reactive oxygen species (Malonaldehyde- MDA) levels were 2.96 $\pm$ 0.47 n mole/ml in normozoospermic group and 4. 46 $\pm$ 0.56 n mole/ml in OAT group ($P<0.05$) (Fig. no. 1).

In normozoospermic group the SDI was 0.74 $\pm$ 0.13 and in OAT group the SDI was 1.26 $\pm$ 0.26 ($P<0.05$). In Normozoospermic group the SMI was 300.59 $\pm$ 121.79 and in OAT group it was 56 $\pm$ 25.87($P<0.05$) (Fig. no. 2).

The SDI is inversely related sperm concentration (Coefficient of correlation ((*r*) -0.75) (Fig. no. 3), Percentage Motility (*r*) -0.83) (Fig. no. 4), Percentage Normal Morphology ((*r*) -0.76) (Fig. no. 5). The linear relation was observed between SDI and level of reactive oxygen species (ROS) (MDA level) ((*r*) 0.91) (Fig. no. 6).

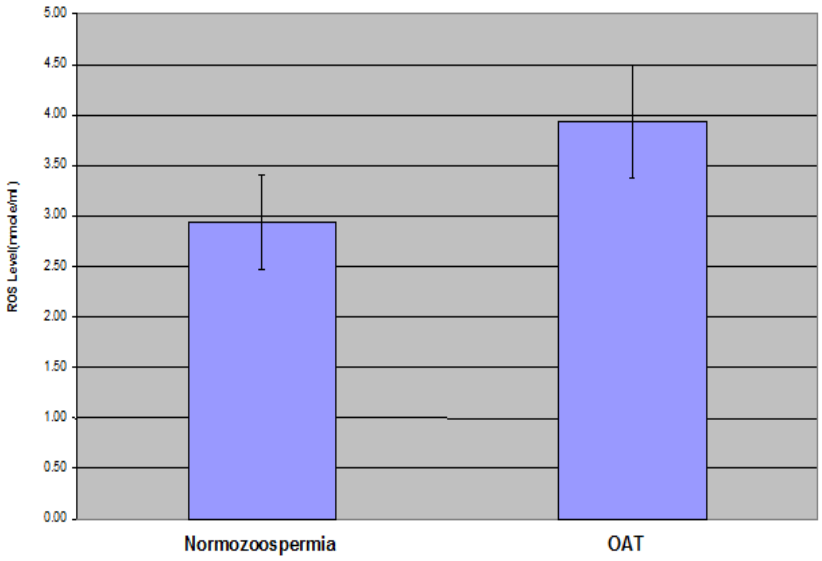

Fig. 1: Level of Reactive Oxygen Species (ROS)

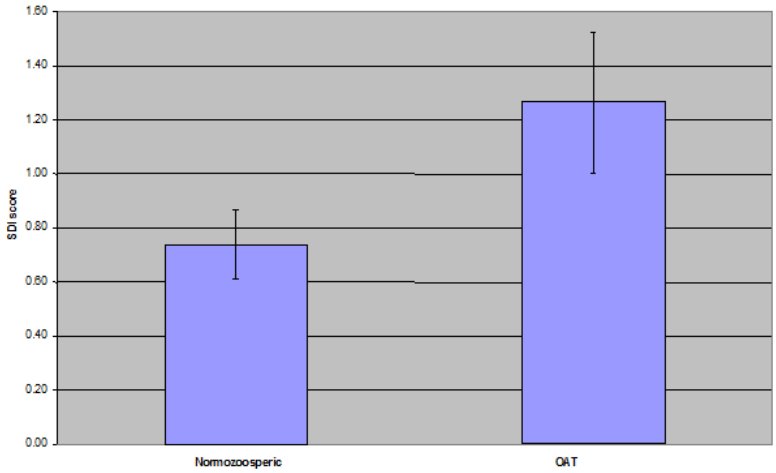

Fig. 2: Sperm Deformity Index (SDI) score

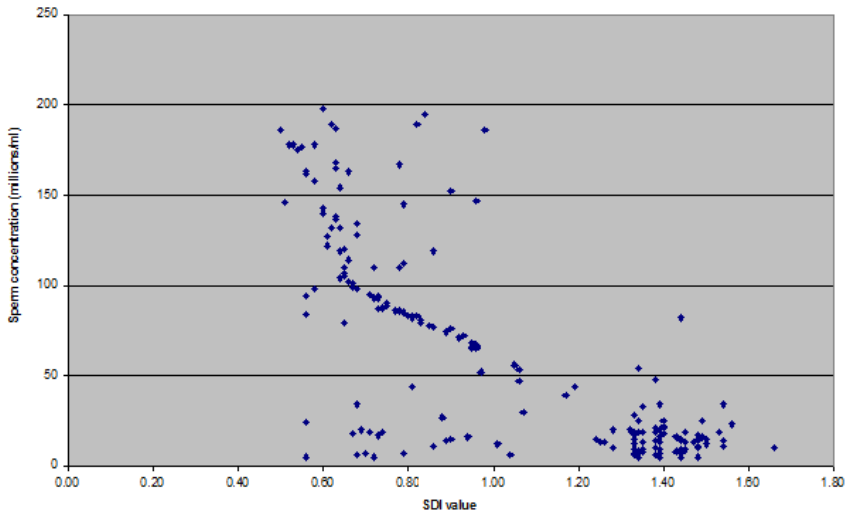

Fig. 3: Relation between SDI and Sperm concentration

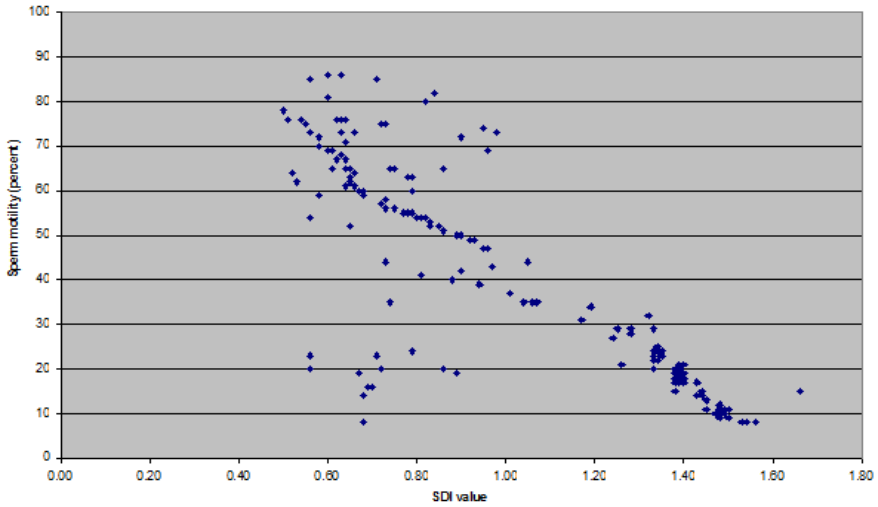

Fig. 4: Relation between SDI and Sperm motility

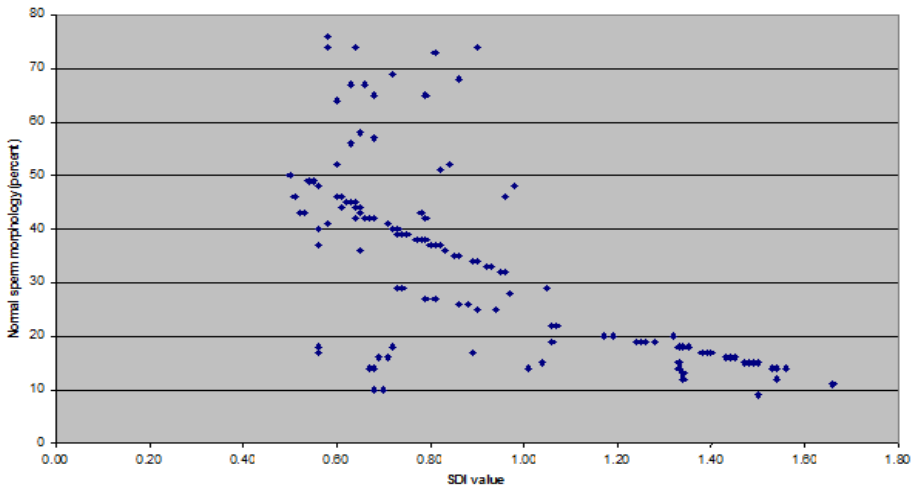

Fig. 5: Relation between SDI and Sperm morphology

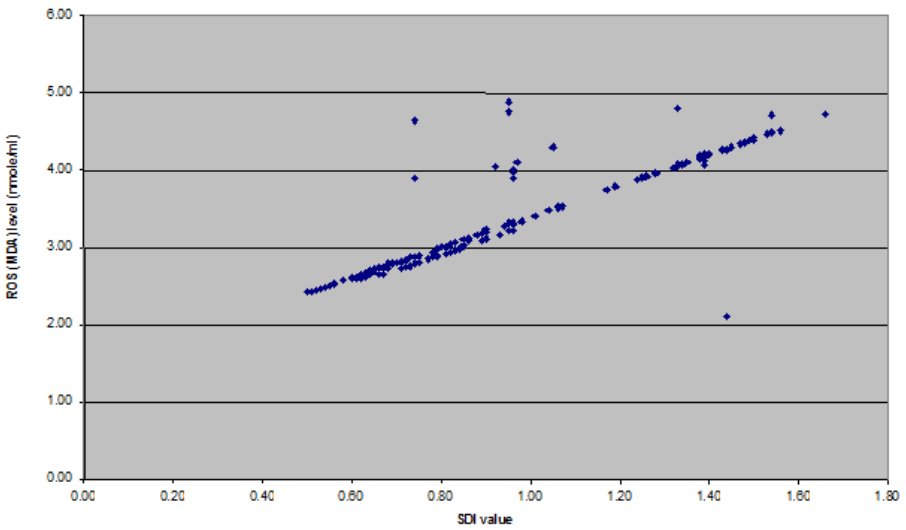

Fig. 6: Relation between SDI and Seminal ROS level

DISCUSSION

In the present study the Sperm Deformity Index (SDI) in normozoospermic group was 0.74 ± 0.13 and in OAT group the SDI was 1.26 ± 0.26 ($P<0.05$) which was in accordance with the reported value by Panidis *et al* who confirmed the cut off value of SDI for fertile subjects less than 0.93. In the present study in the normozoospermic group 15% of the population was found to have SDI value > 0.93. In the OAT group 17% subjects had SDI value <0.93. According to Panidis *et al* the SDI values (sensitivity 98% and specificity 97%) were better in terms of sensitivity and specificity than the percentage of spermatozoa with normal morphology (sensitivity 82% and specificity 95%). The values of SDI facilitate diagnosis of almost all potentially infertile men (97% specificity) who are thus eligible for ART to increase their chances for successful fertilization. In addition the high negative diagnostic value of this index minimizes the number of men who are classified as potentially infertile[(9)].

As the linear relation was observed between SDI and level of reactive oxygen species (ROS) (MDA level) ((*r*) 0.91) (Fig. no. 6) which indicates that high level of ROS may be responsible for the morphological deformities of the sperm cells.

CONCLUSIONS

The study concluded with the remark that high level of ROS may be responsible for the morphological deformities of the sperm cells. Further, SDI score is a useful tool in identifying infertile men with high seminal ROS in infertility clinics where facilities for measuring levels of seminal ROS are not available.

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