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Original Article

Oxidative Stress in Seminal Plasma Negatively Influences Sperm Quality in Infertile Males

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Abstract

Objective: To investigate the association between the malondialdehyde concentration in the seminal plasma of infertile men and sperm quality.

Methods: This case-control study included 60 male participants, ranging from 25-40 years old, with half of them were fertile and the other half were infertile. Semen analysis was performed as per the WHO standards, and spectrophotometric measurement of the seminal plasma malondialdehyde level was done.

Results: Results showed that infertile men had significantly a higher mean level of malondialdehyde in their seminal plasma than fertile men ($p<0.001$), which was inversely associated with sperm count and motility. Also, malondialdehyde was positively associated with abnormal sperm morphology.

Conclusions: Elevated malondialdehyde levels in the seminal plasma are associated with poor sperm quality. Malondialdehyde testing can, therefore, be used to diagnose and predict the outcome of male infertility. Antioxidants should also be administered to men with infertility to help counteract the effects of oxidative stress.

Keywords: Male infertility, malondialdehyde, sperm quality

Introduction

It has been estimated that fifteen percent of couples experience infertility due to various causes. Infertility is defined as the failure to achieve a spontaneous pregnancy after twelve (12) months or more of frequent, unprotected sexual intercourse.[1] Nearly half of all infertility cases can be traced back to male factors, which are just as important as the female factors.[2] The global prevalence of infertility ranges from 2.5% to 15%, which corresponds to at least 30 million infertile males.[3]

Numerous studies have pointed out that oxidative stress, which is a condition marked by an imbalance between the generations of reactive oxygen species (ROS) and antioxidant defense mechanisms, as a newly discovered cause of unexplained male infertility.[4,5] When kept within healthy limits, the ROS mediate vital physiological activities crucial to ensuring normal male reproductive functions, including sperm viability, maturation, hyperactivation, sperm capacitation, sperm motility, acrosome reaction, and oocyte interaction.[6,7] However, excessive amount of ROS can lead to infertility via various mechanisms, i.e., DNA damage, lipid peroxidation, enzyme inactivation, and protein oxidation in spermatozoa.[7] Spermatozoa are extremely vulnerable to oxidation because of a high concentration of unsaturated fatty acids found in their membranes and the absence of cytoplasmic antioxidant enzymes. As a result, oxidation has a negative impact on the quality and functionality of the sperm.[7,8] Thus, this study aimed to evaluate MDA as a marker of oxidative stress and investigate its relationship with the quality of sperm in infertile males.

Methods

The present case-control study was conducted at the Department of Biochemistry and In-vitro Fertilization (IVF) center of the MGM Medical College and Hospital, Aurangabad. This study was carried out from January 2013 through December 2013 after getting the approval from the Institutional Ethical and Research Committee (Reference No: EC/056/2012, dated 02 November 2012). The case group consisted of 30 infertile men with abnormal semen analysis between the ages of 25 and 40 years, whose wives had not conceived after a year of having regular, unprotected sex. Thirty healthy, fertile male volunteers in the same age range who were in good health and had normal semen parameters were used as controls.

Patients in the case group were excluded from the study if they had testicular damage, varicocele, leukocytospermia, hypogonadism, cryptorchidism, heart disease, tuberculosis, genital tract infections, renal disease, diabetes mellitus, or prolonged illness. Before starting the study, a written consent was obtained from both infertile males and healthy controls.

Semen samples for this study were collected from subjects and controls after a period of abstinence of three to four day . The specimens were obtained by masturbating into wide-mouth sterile plastic containers and analyzed within an hour of collection. After letting the semen liquefy for at least 30 minutes, it was analyzed to measure sperm concentration, motility, and morphology as described in the WHO guidelines.

MDA levels were analyzed by the method described by Nourooz-Zadeh *et al*.[9] In brief, the first semen sample was centrifuged after liquefaction to get the seminal plasma. Then, 100 µl of seminal plasma, 1,000 µl of 0.67% TBA (thiobarbituric acid), and 500 µl of 20% TCA (trichloroacetic acid) were added and incubated for 20 minutes at a temperature of 100^0C. After centrifugation at 12,000 rpm for 5 minutes, the optical density (OD) of the supernatant was taken at 532 nm using spectrophotometry. The MDA concentration was calculated using the molar extinction coefficient for the MDA-TBA complex of 1.56×10^5 $mol^{-1}Lcm^{-1}$.

Data collected were statistically analyzed using the IBM SPSS statistics version 20. Data were presented as mean±SD. The statistical differences between cases and controls were established by student-independent sample t-test. To understand how the variables related to one another, a Pearson correlation analysis was done. Values were considered statistically significant when $p<0.05$.

Results

Results of sperm characteristics and seminal plasma MDA comparison between infertile and fertile males are listed in Table 1. The level of MDA in the seminal plasma of infertile men was significantly higher than that of the fertile control group ($p<0.001$). Subjects with infertility had significantly lower sperm counts and motility compared to those with fertile men and the abnormal sperm morphology was demonstrated to be significantly higher in infertile males than in normal, healthy fertile males ($p<0.001$). MDA was significantly negatively correlated with sperm count and motility in infertile males and are presented in Fig 1 and 2, respectively. However, there was an insignificant positive correlation of MDA with sperm abnormal morphology in infertile males, which is presented in Fig. 3.

Discussion

Through the modification of the membrane fluidity and permeability, as well as reducing the sperm functional competence, oxidative stress can negatively impacts sperm functions. To be able to evaluate the membrane damage,

Table 1 Comparison of Sperm Characteristics and MDA between Fertile and Infertile Males

Parameter	Fertile male (n=30)	Infertile male (n=30)
Sperm count (millions/mL)	79.17±10.97	32.03±11.29*
Sperm motility (%)	74.57±5.67	32.66±8.18*
Sperm abnormal morphology (%)	17.93±3.77	35.4±4.90*
MDA	1.56±0.34	3.00±0.49*

*Highly significant ($p<0.001$); MDA: Malondialdehyde

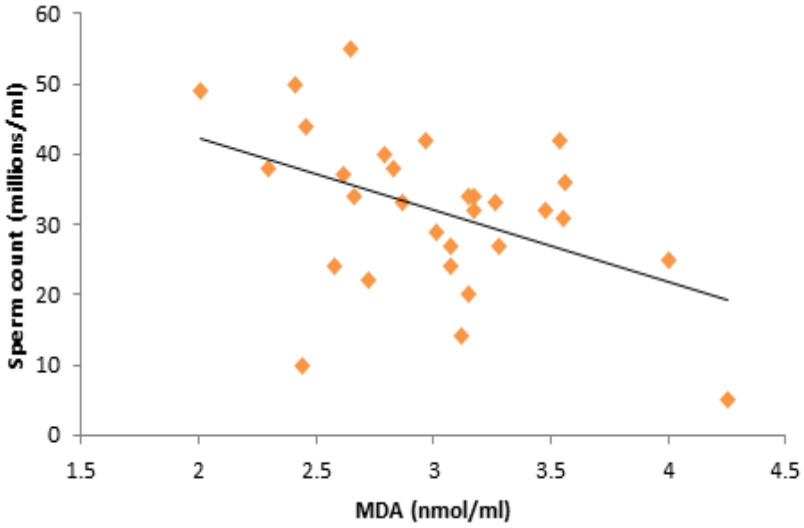

Fig. 1 Correlation between MDA and Sperm Count in Infertile Males (r=-0.447; p<0.05)

the quantity of MDA, the final product of lipid peroxidation, can be taken into account.[10]

In the present study, a significant increased level of MDA was observed in the seminal plasma of infertile males as compared to the fertile group. This is in accordance with the studies done by Atig *et al.*[11], More *et al.*[12], and Dorostghoal *et al.*[13] who also reported increased seminal MDA in infertile male patients. Similarly, a study conducted by Muley and Muley[14] has also demonstrated insignificant higher seminal plasma MDA level in asthenoteratozoospermic and azoospermic males as compared to normozoospermics.

However, a significant rise in MDA levels was observed in the oligoasthenoteratozoospermic

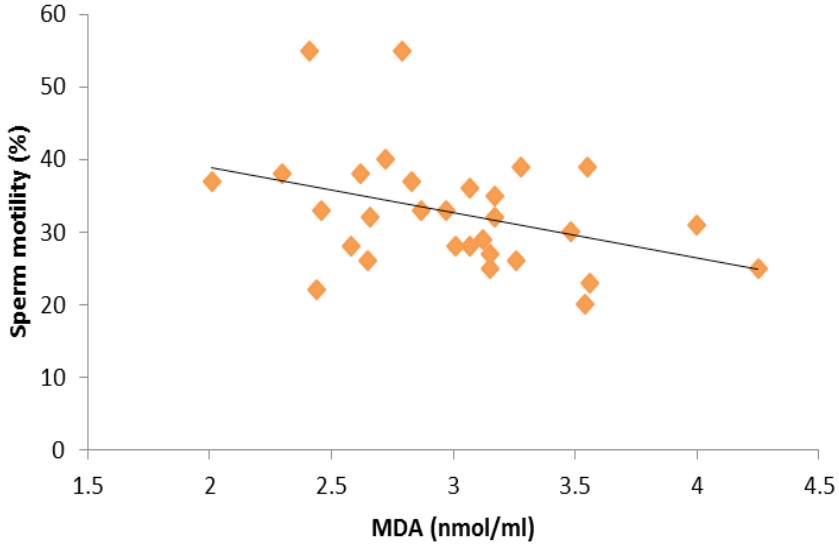

Fig. 2 Correlation between MDA and Sperm Motility in Infertile Males (r=-0.374; p<0.05)

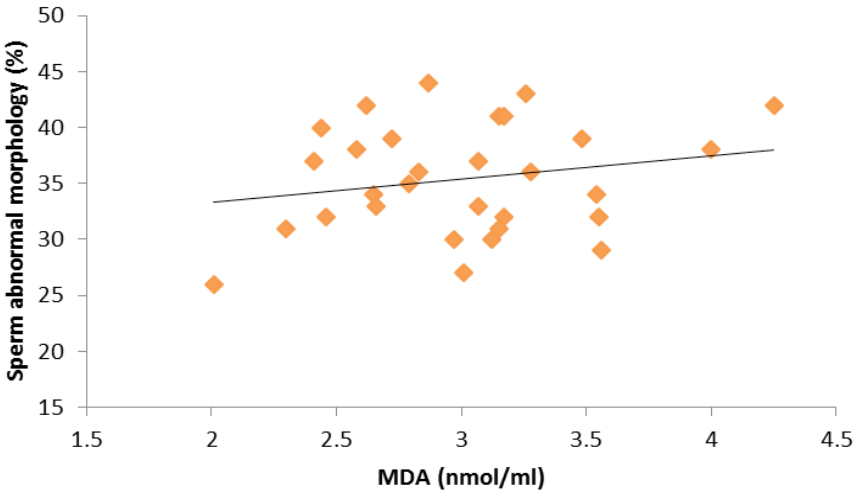

Fig. 3 Correlation between MDA and Sperm Abnormal Morphology in Infertile Males (r = 0.208; p>0.05)

group. In contrast, Palani *et al*.[15] did not find any significant differences in the MDA levels between fertile and infertile males in their study. The disparity between the results of the aforementioned research can be attributed, in parts, to the variability of those studies with regard to patient selection, methodology, and oxidative stress assessment techniques, as well as the genetic and racial characteristics.[15] Increased MDA levels in the seminal plasma of infertile males indicating excessive ROS is responsible for the lipid peroxidation of the membrane lipids. Because the sperm plasma membrane contains a high concentration of polyunsaturated fatty acids (PUFAs), which are essential for ion transport and membrane fluidity, the peroxidation of the PUFAs in the membrane by excessive ROS disrupts the functions of sperm membrane, thus decreasing the ability of spermatozoa to fertilize.[12] It has been demonstrated that both qualitative and quantitative sperm abnormalities exist in the semen of infertile males. The sperm count and motility significantly decreases among infertile men when compared to the fertile ones. Also, the abnormal morphology of sperm has been reported to be significantly high among infertile males when compared to normal healthy fertile males. The findings of this present study indicate that the elevated oxidative stress in the seminal plasma of infertile males is associated with poor sperm quality. These results are well supported by the findings of previous studies.[12,14]

The MDA has been demonstrated to have a significant negative correlation with sperm count and motility in this study. A positive correlation was also observed between seminal MDA and sperm abnormal morphology. These results are in line with the findings of More *et al*[12] and Mehrotra *et al*.[16] Dorostghoal *et al*.[13] also reported the presence of significant negative correlations between MDA levels and sperm motility and normal morphology. An excessive amount of ROS can decrease sperm motility, most likely through a rapid loss of intracellular ATP leading to axonemal damage, decrease sperm viability, and increase mid-piece morphological defects with deleterious effects on sperm capacitation and acrosome reaction.[17,18] According to the findings of this study, the ROS are responsible for inducing base modification, DNA strand breakage, and chromatin cross-linking, all of which are detrimental to the integrity of the DNA in the sperm nucleus.[19] On the other hand, DNA damage caused by high levels of ROS may hasten the process of germ cell apoptosis, resulting in a drop in sperm counts associated with male infertility.[20] Due to the small sample size and lack of follow-up of patients in the present study, among other limitations, large-scale prospective investigations are needed to strengthen the findings. In conclusion, it is indicated that increased oxidative stress, as reflected by increased MDA levels in seminal plasma, is associated with poor sperm quality, as reflected by decreased sperm count,

decreased sperm motility, and also increased abnormal sperm morphology as observed in infertile males. Therefore, determination of the MDA levels in seminal plasma can help the diagnosis and prognosis of male infertility. To verify these results, nevertheless, additional research with a large sample size is required.

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