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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 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 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 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

Fifteen percent of couples have infertility due to a variety of causes. Infertility is defined as the failure to achieve a spontaneous pregnancy after 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 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 researches pointed to oxidative stress, 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, ROS mediate vital physiological activities crucial to ensuring normal male reproductive functions including sperm viability, maturation, hyper activation, sperm capacitation, motility, acrosome reaction, and oocyte interaction.[6,7] However, excessive ROS can lead to infertility via a variety of mechanisms, including lipid peroxidation, DNA damage, enzyme inactivation, and protein oxidation in spermatozoa.[7] Spermatozoa are extremely vulnerable to oxidation because of the 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 sperm.[7,8] We, therefore, set out to evaluate MDA (as a marker of oxidative stress) and investigate how it relates to the quality of sperm in infertile males.

Methods

The present case-control study was carried out in the Department of Biochemistry and In-vitro Fertilization (IVF) center of MGM Medical College and Hospital, Aurangabad. The study was carried out from January 2013 through December 2013. The study was carried out after getting approval from the Institutional Ethical and Research Committee (Reference No: EC/056/2012, dated 02 November 2012). The case group comprised 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, genital tract infections, cryptorchidism, tuberculosis, diabetes mellitus, heart disease, renal disease, or prolonged illness. The written consent was taken from both infertile males and healthy controls.

From both patients and controls, semen samples were collected. After a period of abstinence of three to four days, 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 following 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. To 100 µl of seminal plasma, 1000 µl of 0.67% TBA (thiobarbituric acid) and 500 µl of 20% TCA (trichloroacetic acid) were added and incubated for 20 minutes at 100^0C. After centrifugation at 12,000 rpm for 5 minutes, the optical density (OD) of the supernatant was taken at 532 nm spectrophotometrically. MDA concentration was calculated using the molar extinction coefficient for the MDA-TBA complex of 1.56×10^5 $mol^{-1}Lcm^{-1}$.

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

Results

Table 1 shows the comparison of sperm characteristics and seminal plasma MDA between infertile and fertile males. The level of MDA in the seminal plasma of infertile men was significantly higher than that of the fertile control group. Subjects with infertility had significantly lower sperm counts and motility compared to those with fertile men. Additionally, abnormal sperm morphology was shown to be significantly higher in infertile males than in normal, healthy fertile males. 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

By modifying membrane fluidity and permeability and reducing sperm functional competence, oxidative stress negatively impacts sperm function. To evaluate the membrane damage, the quantity of MDA, the final product of lipid peroxidation, can

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

Parameters	Fertile males (n=30)	Infertile males (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

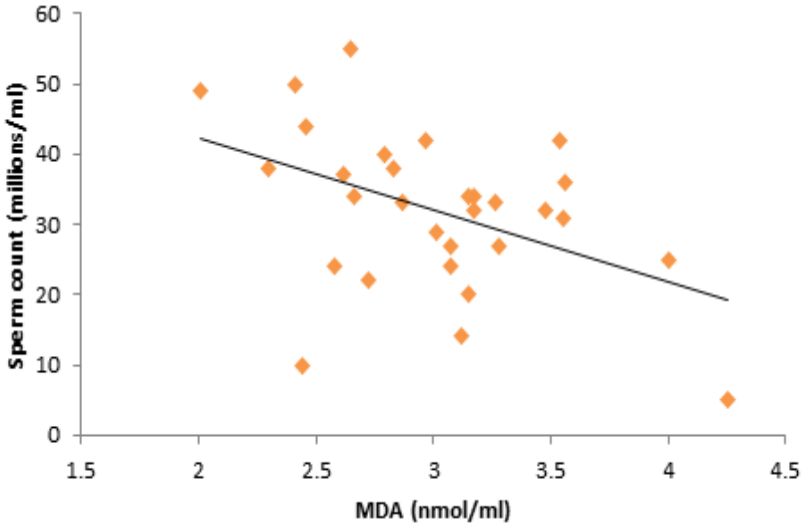

Fig. 1 Correlation between MDA and Sperm Count in Infertile Males (r=-0.447; p<0.05)

be taken into account.[10] The purpose of this study was to determine whether or not there is a connection between the amount of malondialdehyde found in the seminal plasma of infertile men and the quality of their sperm.

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, in a study conducted by Muley and Muley,[14] insignificant higher seminal plasma MDA was observed in asthenoteratozoospermic and azoospermic

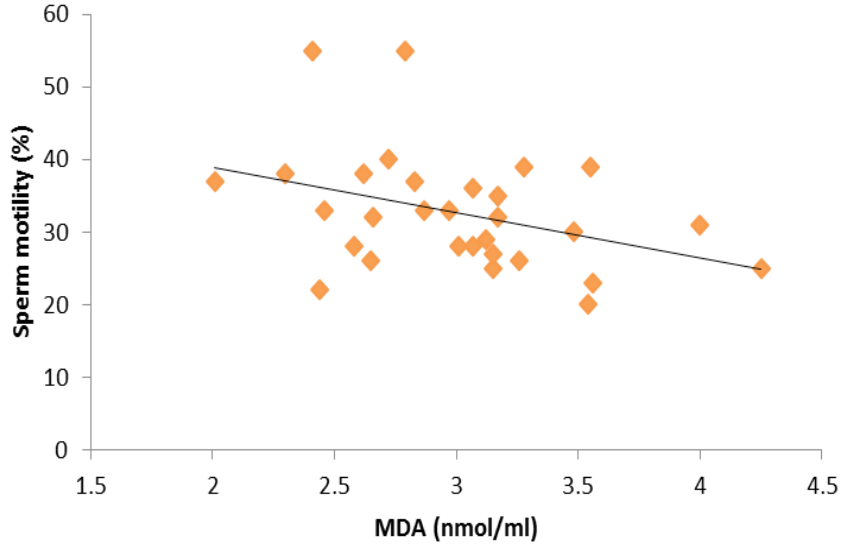

Fig. 2 Correlation between MDA and Sperm Motility in Infertile Males (r=-0.374; p<0.05)

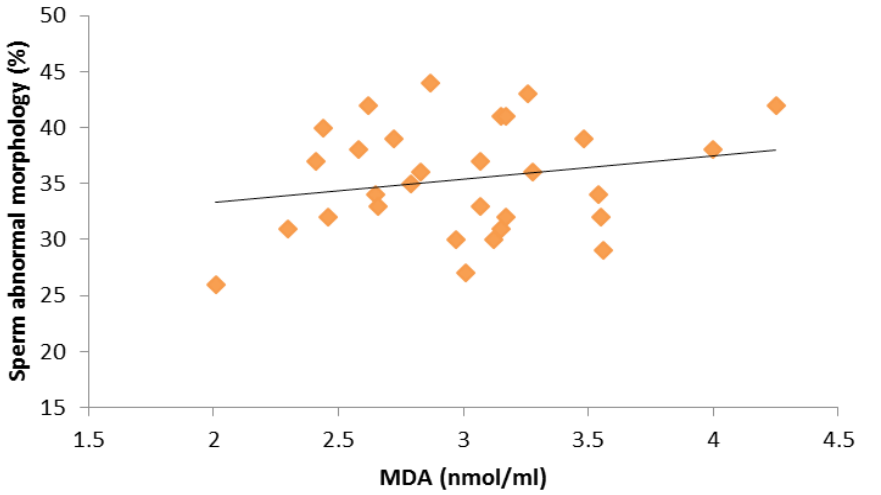

Fig. 3 Correlation between MDA and sperm abnormal morphology in infertile males (r = 0.208; p>0.05)

males as compared to normozoospermics.

However, a significant rise in MDA levels was observed in the oligoasthenoteratozoospermic group. In contrast, Palani *et al*.[15] did not find any significant difference in MDA levels between studied fertile and infertile males. The disparity between the results of the aforementioned research can be attributed, in part, to the variability of those studies with regard to patient selection, methodology, and oxidative stress assessment techniques, as well as genetic and racial characteristics.[15] Increased MDA levels in the seminal plasma of infertile males indicate excessive ROS is responsible for 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, peroxidation of the PUFAs in the membrane by excessive ROS disrupts the functions of the sperm membrane and decreases the ability of spermatozoa to fertilize.[12] It has been shown that both qualitative and quantitative sperm abnormalities exist in the semen of infertile males. The sperm count and motility were found to be significantly decreased in infertile men as compared to fertile ones. Also, abnormal morphology of sperm was reported to be significantly high in infertile males as compared to normal healthy fertile males. The findings of the present study indicate that 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]

In the current study, MDA showed a significant negative correlation with sperm count and motility. Also, a positive correlation was 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 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 increased mid-piece morphological defects with deleterious effects on sperm capacitation and acrosome reaction.[17,18] According to the findings of the research, 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, additional large-scale prospective investigations are necessary to strengthen the findings of this investigation. In conclusion, the results of the present study indicate that increased oxidative stress (reflected by

increased MDA levels) in seminal plasma is associated with poor sperm quality (reflected by decreased sperm count, decreased sperm motility and increased abnormal sperm morphology) in infertile males. Therefore, determining MDA levels in seminal plasma can help in the diagnosis and prognosis of male infertility. To verify these results, nevertheless, additional research with a large sample size is required.

References

1. Aitken RJ. Impact of oxidative stress on male and female germ cells: implications for fertility. Reproduction. 2020;159(4):R189–R201.
2. Wagner H, Cheng JW, Ko EY. Role of reactive oxygen species in male infertility: An updated review of literature. Arab J Urol. 2017;16(1):35–-43.
3. Agarwal A, Ahmad G, Sharma R. Reference values of reactive oxygen species in seminal ejaculates using chemiluminescence assay. J Assist Reprod Genet. 2015;32:1721–9.
4. Agarwal A, Ahmad G, Sharma R. Reference values of reactive oxygen species in seminal ejaculates using chemiluminescence assay. J Assist Reprod Genet. 2015;32(12):1721–9.
5. Cito G, Becatti M, Natali A, et al. Redox status assessment in infertile patients with non-obstructive azoospermia undergoing testicular sperm extraction: A prospective study. Andrology. 2020;8(2):364–71.
6. Du Plessis SS, Agarwal A, Halabi J, Tvrda E. Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. J Assist Reprod Genet. 2015 Apr;32(4):509–20.
7. Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. Cell Mol Life Sci. 2020 Jan;77(1):93–113.
8. Agarwal A, Roychoudhury S, Sharma R, Gupta S, Majzoub A, Sabanegh E. Diagnostic application of oxidation-reduction potential assay for measurement of oxidative stress: clinical utility in male factor infertility. Reprod Biomed Online. 2017;34(1):48–57.
9. Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP. Elevated levels of authentic plasma hydroperoxides in NIDDM. Diabetes. 1995;44(9):1054–8.
10. Collodel G, Moretti E, Micheli L, Menchiari A, Moltoni L, Cerretani D. Semen characteristics and malondialdehyde levels in men with different reproductive problems. Andrology. 2015;3(2):280–6.
11. Atig F, Raffa M, Ali HB, Abdelhamid K, Saad A, Ajina M. Altered antioxidant status and increased lipid per-oxidation in seminal plasma of tunisian infertile men. Int J Biol Sci. 2012;8(1):139–49.
12. More K, Badade ZG, Narshetty JG, Joshi DS, Mukherjee S, Deepak AD, et al. Lipid peroxidation, sperm DNA fragmentation, total antioxidant capacity and semen quality in male infertility. MGM J Med Sci. 2014;1(1):1–6.
13. Dorostghoal M, Kazeminejad SR, Shahbazian N, Pourmehdi M, Jabbari A. Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. Andrologia. 2017;49(10):e12762.
14. Muley PP, Muley PA. Oxidative stress in seminal plasma and its relation to fertility potential of human male subjects. J Datta Meghe Inst Med Sci Univ. 2020;15:172–5.
15. Palani A, Alahmar A. Impact of oxidative stress on semen parameters in normozoospermic infertile men: a case–control study. Afr J Urol. 2020;26:50.
16. Mehrotra A, Katiyar DK, Agarwal A, Das V, Pant KK. Role of total antioxidant capacity and lipid peroxidation in fertile and infertile men. Biomed Res. 2013;24(3):347–52.
17. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014;32(1):1–17.
18. Fatima, S. Role of Reactive Oxygen Species in Male Reproduction. In: Atukeren, P., editor. Novel Prospects in Oxidative and Nitrosative Stress [Internet]. London: IntechOpen; 2018 [cited 2023 Jan 15]. Available from: https://www.intechopen.com/chapters/59757 doi: 10.5772/intechopen.74763 .
19. Alahmar AT. Role of oxidative stress in male infertility: an updated review. J Hum Reprod Sci. 2019;12(1):4–18.
20. Agarwal A, Allamaneni SSR. Oxidants and antioxidants in human fertility. Mid East Fert Society J. 2004;9:187–97.