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Impact of Malondialdehyde (MDA) Level on Semen Plasma In Male Infertility

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Abstract

Background: Male factor infertility contributes to 40% of couples seeking fertility treatment, and about 50% of such couples require assisted reproduction technology as a mainstay treatment modality. Evidence has shown that a significant amount of sperm damage is orchestrated by reactive oxygen species (ROS). The imbalance phase between the oxidant and antioxidant can lead to various pathological conditions culminating in abnormal sperm quality. MDA as a biomarker for lipid peroxidation has been studied. However, the impact of its concentration on sperm quality and function is yet to be demonstrated. Despite the perceived association with male infertility.
Materials and Method: A total of 90 men were recruited. Forty-five of them were classified as fertile. They consisted of 25 sperm donors of proven fertility and 20 men in an infertile relationship of purely female aetiology. In contrast, the remaining 45 participants had at least one defect in routine sperm parameters and were considered as having male infertility. Semen samples were produced by masturbation after a period of 3–5 days of abstinence and then analyzed for sperm count, motility, and morphology as per WHO guidelines. Semen MDA was determined using the thiobarbituric acid assay (Buege and Aust, 1978).
Result: Sperm count, sperm total motility and sperm morphology were significantly lower ($p<0.01$) in infertile males compared to healthy fertile males. The level of oxidative stress to which the participants' spermatozoa were exposed was determined by the levels of MDA in the seminal plasma. MDA level was significantly ($p<0.01$) elevated in infertile males compared to healthy fertile males.
Conclusion: The association between oxidative stress and male infertility is established. MDA, a valuable biomarker of oxidative stress, could serve as an adjunct investigation when evaluating infertile males.

Keywords: Male infertility, seminal fluid analysis, malondialdehyde, MDA

Introduction

Male factor infertility contributes to 40% of couples seeking fertility treatment, and about 50% of such couples require assisted reproduction technology as the mainstay treatment modality.[1] Evidence has shown that a significant amount of sperm damage is orchestrated by reactive oxygen species (ROS).[2,3] Reactive Oxygen Species are products of cellular physiological activities, especially amongst the immature spermatozoa and seminal leucocytes in the semen.[4] It results from the enzymatic reduction of Oxygen to produce energy and eventual production of Oxygen-derived free radicals.[5,6] Excessive production of the ROS could cause harmful effects on the cells.[7,8] As a result, cells are endowed with a certain defensive mechanism known as antioxidants to forestall such catastrophes.[9]
Antioxidants are present at low concentrations and

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neutralize the destructive effect of ROS by preventing its formation, scavenging for interception and resuscitation of target molecules.[7,10] Furthermore, antioxidants are organized into enzymatic and non-enzymatic.[11-13] Enzymatic antioxidants constitute the foremost cellular defensive approach. These include superoxide dismutase (SOD), catalase and glutathione peroxidase.[14,15] While the non-enzymatic components are made up of some low molecular weight substances like α-tocopherol, ascorbic and bilirubin.[16,17] They complement the enzymatic counterpart by directly scavenging the ROS and, to some extent, interfering with the availability of active redox metals like Iron and Copper required for its production.[18] Oxidative stress results when the ROS overwhelms the inherent antioxidant scavenging status.[19]

The impact of oxidative stress on the relevant tissues may be ascertained by evaluating its peculiar biomarkers extracted from the tissues and the biological fluid.[8,20] Biomarkers are natural molecules characterized by their objective means of assessing physiological and pathological processes. Several oxidative stress biomarkers have been identified in vitro. Several have limited value in vivo concerning their sensitivity and specificity.[8,21] However, the number of ROS has been detected in-vitro by electron spin resonance and chemiluminescence R8.[22] They were of limited value for clinical purposes due to their associated instability and the need for expensive equipment's.[18] Considering this, a more stable molecular product of ROS or oxidation target products of lipid peroxidation in the form of biomarkers is measured as an indirect index of oxidative stress.[23] These include Malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and 2-propenal (acrolein).[24]

Evidence has shown that Oxidative stress could result in male infertility[23] through its damage effect on the sperm membrane by lipid perodixation.[25] Thus, it compromises its motility and potential to fuse with the oocyte for fertilization.[15,22] The susceptibility of the human sperm to oxidative stress stems from its endowed high concentration of polyunsaturated fatty acid (PUFA) essential for plasma membrane fluidity and ion transport.[24,26] Oxidation of the PUFA results in lipid peroxidation with consequent loss of sperm function and formation of MDA as biomarker.[14,27] Furthermore, ROS has a direct negative impact on the sperm DNA integrity and the paternal genome in the resulting embryo, which may result in miscarriage.[6,22]

MDA as a biomarker for lipid peroxidation has been studied.[22-24,26] However, the impact of its concentration on sperm quality and function is yet to be demonstrated.[24] Despite the perceived association with male infertility,[1,28] it's often not considered when evaluating infertile males. Furthermore, oxidative stress is common and can be treated with the possibility of achieving spontaneous conception.[26,29] Instead, the emphasis seems placed on mechanical interventions such as intracytoplasmic sperm injection (ICSI) and intrauterine insemination (IUI). In most cases, this is beyond the reach of many in low resource settings.[2,7,30]

Materials and methods

Participants were recruited from men undergoing infertility assessment or fertile sperm donors at the Human Reproduction and Research Program (HRRP) of the University of Benin Teaching Hospital, Benin City, between January 2019 and December 2020. The only entry criteria for participants were the requirement to have a minimum sperm concentration of 2 x 106 ⁄ mL, as this, in our experience, was the minimum amount of sperm needed to perform the various sperm assays reliably. Men with proven past paternity or men in an infertile relationship but who had normal semen parameters (count, motility morpho- logy) and their partner who had a well-defined female cause for their infertility (anovulation, endometriosis, tubal obstruction and diminished ovarian reserve) were considered 'fertile'. Those men in an infertile relationship who had at least one defect in either count, motility or morphology were supposed to have male factor infertility ('infertile group').

Exclusion Criteria: Those male subjects having one or more of the following criteria were excluded from this study: Acute illness within the last three months, Chronic illness like- neoplasm, e.g. seminoma, cryptorchidism, varicocele, trauma, hydrocele. Mumps; testicular dysgenesis syndrome and history of genital tract infections; Persons under drugs for infertility that may affect sperm count within the last three months. Hypogonadism-

hypogonadism subjects were excluded by testing serum testosterone, FSH, LH and prolactin—obstruction of vas deferens, Hypospadias and Retrograde ejaculation.

A total of 90 participants were enrolled in the study. Forty-five men were classified as fertile, and they consisted of 25 sperm donors of proven fertility and 20 men in an infertile relationship of purely female aetiology. In contrast, the remaining 45 participants had at least one defect in routine sperm parameters and were considered as having male infertility. The Hospital's Ethics Committee prospectively approved the study, with all participants giving written informed consent for their involvement.

The participants produced semen samples by masturbation after a period of 3–5 days of abstinence and then analyzed for sperm count, motility, and morphology as per WHO guidelines. Semen samples were centrifuged, and seminal plasma was separated to determine MDA concentrations using the thiobarbituric acid assay. Data were analyzed by using SPSS 12.0. Mann-Whitney U and unpaired t-tests were done to find significant differences between groups. Statistical significance was set at $p<0.05$.

Results

The mean age of the participants in the study was 39.5 + 6.2 years and 39.9 + 7.2 years, respectively. In comparison, the difference between the mean ages of the participants with normal and abnormal Seminal Fluid Analysis (SFA) (p-value = 0.789) was not statistically significant (Table 1). The seminal fluid analysis of the participants showed sperm count, sperm total motility and sperm morphology were significantly lower ($p<0.01$) in infertile males compared with healthy fertile male subjects. The concentration of MDA in the seminal plasma measures the oxidative stress level, and the Mean MDA level was 0.6 + 0.2 and 1.4 + 0.5nmol/ml in participants with normal SFA and participants with abnormal SFA, respectively. MDA level was significantly ($p<0.01$) elevated in infertile males compared to healthy fertile males.

Using Spearman's rank correlation coefficient, MDA predicts abnormal sperm motility, sperm count and sperm morphology. More likely to cause abnormal motility, abnormal count and abnormal morphology by rho 0.776, 0.760, and 0.720, respectively (Table 2 and Figures 2,3 and 4) irrespective of age (Table 2 and Figure 1).

Table 1: Association between age, sperm characteristics and respondents' category

	Normal (mean±sd)	Abnormal (mean±sd)	Test statistics	p value
Age	39.5±6.2	39.9±7.2	0.268*	0.789
MDA	0.6±0.2	1.4±0.5	10.64*	<0.01***
Morphology	24.5±10.9	6.4±2.5	8.94*	<0.01***
Total motility	56.4±13.0	16.2±10.9	11.5**	<0.01***
Sperm conc.	33.0±8.8	14.6±8.7	107.5**	<0.01***

* t-test ** Man-Whitney U Test *** Significant

Table 2: Correlation between MDA and other sperm parameters

	Rho
Age	0.014
Total motility	-0.776
Sperm concentration	-0.760
Morphology	-0.726

rho - Spearman's rank correlation coefficient constant

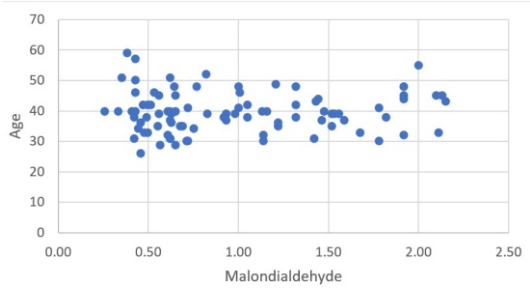

Fig 1: Simple scatter of age and MDA

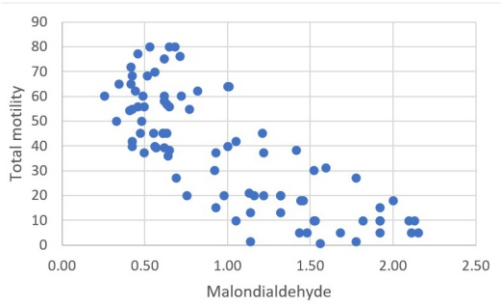

Fig 2: Simple scatter of total motility and MDA

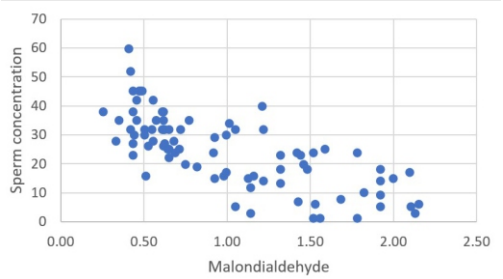

Fig 3: Simple scatter of sperm concentration and MDA

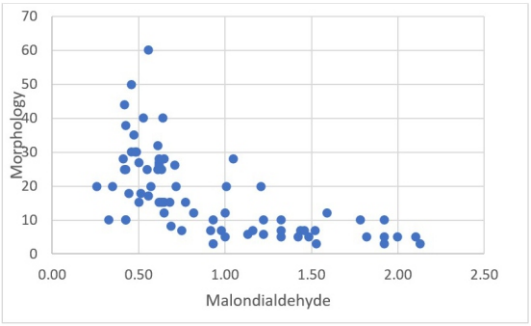

Fig 4: Simple scatter of morphology and MDA

Discussion

The imbalance phase between the oxidant and antioxidant can lead to various pathological conditions culminating in abnormal sperm quality.[33] One of the pathological processes of oxidative stresses is lipid peroxidation and the subsequent release of Malondialdehyde (MDA), used as a biomarker. This study looked at the impact of oxidative stress using MDA as a biomarker of sperm quality. MDA was significantly increased in subjects with abnormal sperm when compared with subjects with normal sperm ($p<0.01$), corroborated by other studies.[34-36] Suggesting that the subjects with abnormal sperm were exposed to oxidative stress with significant lipid peroxidation. Furthermore, the seminal fluid analysis for both subjects showed a significant decrease in sperm morphology, motility, and count. ($p<0.01$) in subjects with abnormal sperm compared to subjects with normal sperm, similar to previous studies 37). It suggests that lipid peroxidation caused by oxidative stress negatively impinges sperm quality.

Like other studies,[33,36-38] our study showed that MDA level negatively correlates with sperm motility, sperm morphology and sperm count. However, these findings contradict Gharagozloo et al,39 who observed no correlation between sperm count and motility. The study also noted that the age of the subjects did not influence the negative correlation, as indicated in similar studies.[33,36] Overall, oxidative stress was responsible for the abnormality observed in the sperm count, motility, and morphology in this study, demonstrating that oxidative stress could cause male infertility.

Furthermore, the oxidative stress biomarker MDA used in this study could play a prognostic role in Assisted Reproductive technology (ART), as oxidative stress-induced DNA damage could negatively impact ART outcomes.[40,41] The determination of its benchmark could make it a veritable tool when evaluating infertile male. However, oxidative stress induces DNA damage, which could not be demonstrated in this study for financial and technical reasons. Substantially, our findings, like other studies,[36,41-43] have established the adverse impact of lipid peroxidation orchestrated by oxidative stress on sperm count, motility, and morphology, culminating in poor sperm quality.

Conclusion

The association between oxidative stress and male infertility has been established. MDA, a valuable biomarker of oxidative stress, could serve as an adjunct investigation when evaluating infertile males. A follow-up study on the impact of antioxidants on MDA could modify treatment protocol for infertile males. Findings from such a study may establish benefits from a wide range of antioxidants instead of undergoing intracytoplasmic sperm injection (ICSI), which is beyond the reach of most infertile couples in our low-resource setting.

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