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Association of Oxidative Stress Markers with Seminal Fluid Parameters in Men Attending Infertility Clinic in Benin City, Nigeria

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Abstract

Background: Although male infertility is highly prevalent in sub-Saharan African countries, minimal studies have been undertaken to identify its determinants, factors and root causes. We undertook this study to determine the association between oxidative stress markers and seminal fluid quality parameters among a cohort of Nigerian men attending an infertility clinic at the University of Benin Teaching Hospital in Southern Nigeria. ***Methods:*** This comparative cross sectional study involved 90 men attending the infertility clinic at the University of Benin Teaching Hospital, Benin City. The study consisted of 45 participants with abnormal semen parameters and 45 with normal semen parameters. Oxidative stress markers: superoxide dismutase and malondialdehyde levels in semen were assayed in both normal and abnormal semen group using the Adrenalin inhibition method by Misra and Fridovich for Superoxide dismutase and the Thiobarbituric acid assay by Buege and Aust for Malondialdehyde. Data were analysed using univariate, bivariate, and binary logistic regression to test the association between oxidative stress markers and the risk of abnormality in seminal fluid parameters, and Pearson's correlation to assess the direction of association. ***Results:*** The mean levels of superoxidase dismutase were higher in the normal group (1.37 u/g ± 0.34) as compared with the abnormal (0.78u/g ± 0.36, $p < 0.001$). The mean (SD) malondialdehyde level in the control group was (0.59mol/g ± 0.19) and was significantly lower than in the cases (1.38 ± 0.45mol/g, $P < 0.001$). There was a positive correlation between superoxide dismutase levels and a negative correlation between malondialdehyde levels and sperm concentration, motility and morphology in both cases and controls. However, after adjusting for cofounders in logistic regression, Superoxide dismutase had a statistically significant effect on motility, while Malondialdehyde had a substantial impact on motility and concentration. ***Conclusion:*** We conclude that levels of a marker of oxidative stress (Malondialdehyde) are higher in men with poor semen quality. In contrast, levels of the anti-oxidative stress marker (superoxide dismutase) were higher in men with better semen quality. Superoxide dismutase had a significant impact on sperm motility, while Malondialdehyde affected both sperm motility and concentration. We believe these results would serve as an adjunct when evaluating infertile males.

Keywords: Male infertility, Oxidative stress, Superoxide dismutase, Malondialdehyde, Seminal fluid.

INTRODUCTION

Infertility is a global challenge that affects the physical, emotional, psychological, and social lives of couples. Globally, about 15% of couples struggle with fertility.[1,2] It is even more problematic in Nigeria, where 10 – 30% of couples face challenges of conception.[3] It has been described as the most important reproductive health concern of Nigerian women [4] and the commonest presenting complaint in gynaecological clinics.[5] Male infertility contributes about 50%, whether as a single factor or when combined with a female factor.[6-8] Despite the high prevalence of male infertility in Nigeria, little research on the subject has been undertaken, especially in the West African sub-region.[6]

Available evidence indicates that male infertility may be associated with several factors, one of which is oxidative stress. [7-9] Oxidative stress results

from an imbalance between reactive oxygen species (ROS) and antioxidants in the body. Reactive oxygen species (ROS) are byproducts of normal intercellular and intracellular metabolism and have been implicated in the aetiology of many diseases across multiple organ systems, including the reproductive system.[9-11]

ROS are critical for normal cell functioning, but excessive levels harm cell survival and function.[10-12] In excess, they induce cellular damage by oxidising cellular and cell membrane components and cause DNA damage by modification of bases, deletions, frameshifts, and chromosomal relocations [12]. Usually, antioxidants are present in the plasma. They can be enzymes (Superoxide dismutase, Catalase, Glutathione peroxidase) and non-enzymes (Vitamin C, Vitamin E, beta carotenes, flavonoids, albumin, ferritin, and myoglobin) that are produced endogenously or exogenously [13, 14].

Male infertility is mainly due to abnormality in the semen parameters, and seminal fluid analysis remains the backbone for the assessment of male fertilisation potential.[14,15] Free radicals emanating from physiological processes result in the modulation of sperm maturation, capacitation, and sperm-oocyte fusion.[10,12,16] Oxidative stress occurs when these free radicals exceed the inherent defensive capacity. This results in lipid peroxidation and DNA damage to the sperm.[13,14,17]

This study aims to evaluate the association between oxidative stress and semen quality parameters using Superoxide Dismutase (SOD) and Malondialdehyde (MDA) data, which could provide a platform for adjuvant evaluation of male infertility.

MATERIALS AND METHODS

Study Setting and Duration

This study involved men attending the fertility clinic at the Human Reproduction and Research Programme unit of the Obstetrics and Gynaecology Department at the University of Benin Teaching Hospital between September 2022 and February 2023.

Study Design

This was a comparative cross sectional study.

Participants

Included in the study were male partners of couples who presented at the fertility clinic during the study period and provided consent to participate. Excluded were men who had cryptorchidism, pyospermia, those who were on drugs such as steroids, antipsychotics, calcium channel blockers, antiepileptics, alpha-adrenergic blockers, anti-cancer drugs, anti-retroviral, anti-ulcer (cimetidine), and antioxidant therapy (for a period ≥3 months), which may affect semen parameters. The sample size of 90 was calculated using the Cochran formula.[15] Ninety men were recruited into the study, 45 in each arm (normal and abnormal SFA).

Variable

The semen parameters considered were concentration, morphology, and motility; those with all three parameters were recruited into the control group (normal SFA), while those with abnormal values for any of the three parameters were recruited into the case (abnormal SFA) group. Semen samples of participants were collected by masturbation after a recommended 3-5 days of abstinence from all participants. Seminal fluid analysis was performed according to the WHO 2010 semen criteria. [16] Malondialdehyde (MDA) activity was determined using the thiobarbituric acid assay by Buege and Aust, [17] and Superoxide Dismutase (SOD) was determined according to the methods of Misra and Fridovich. [18]

Data Collection

Data were collected using a structured questionnaire that included socio-demographic data, BMI, lifestyle, and drug history. A detailed history and examination (scrotal examination to rule out cryptorchidism and varicocele by the trained research assistants. The research assistants were trained in the details of the study, counselling, history-taking, examination, and data collection. Those with conditions listed in the exclusion criteria were not recruited, as these may affect semen quality.

Data Analysis

Data analysis was done using IBM SPSS version 25. Pearson's correlation and Logistic regression were used to test the association between oxidative stress markers and seminal parameters, and other cofounders. Statistical significance was set at $p < 0.05$, and the confidence interval was set at 95%. The results of the analysis were presented in frequency distribution tables and figures.

Ethical Considerations

Ethical approval for the study was obtained from the UBTH Ethical and Research committee with protocol number ADM/E 22/A/VOL. VII/14831279. The principles of ethics in biomedical research (Respect for persons, Beneficence, non-maleficence and Justice) were observed.

RESULTS

The 90 study participants were aged 30–39 years, with a mean age of 39.7 years ±6.64 SD, and most were married, Christians, with a tertiary level of education. The majority of them were from the Benin ethnic group and had an average body mass index (Table 1). The mean superoxide dismutase level was significantly associated with sperm concentration and motility and was considerably lower in those with oligospermia and asthenospermia (Table 2). Although the mean SOD level was higher in participants with

normal sperm morphology than in those with abnormal sperm morphology, this difference was not statistically significant (Figure 1).

Conversely, the mean Malondialdehyde level was significantly higher in participants with oligospermia, asthenospermia, and teratozoospermia when compared with those with normal seminal fluid parameters (Figure 2 and Table 3). When comparing the seminal fluid Superoxide dismutase and Malondialdehyde levels among the cases and the control groups, there was a statistically significant difference in the mean seminal fluid SOD and MDA levels among participants in the two SFA groups (Table 4). The mean SOD level is lower in the Case group. In contrast, the mean MDA level was higher in the Case SFA group than in the control group (Table 4).

Analysing the association between SOD, MDA, and semen quality, while adjusting for cofounders such as Age, obesity, alcohol, smoking, and previous groin surgery, showed that MDA and Age significantly impact sperm concentration, while SOD does not, even though the odds ratio is 1.7 (Table 5). Analysing the factors associated with sperm morphology shows that neither SOD nor MDA has a significant impact on morphology after adjustment for confounding factors**.** Previous groin surgery was noted to affect sperm morphology (Table 6). Analysing factors associated with sperm motility while adjusting for confounding factors showed that SOD, MDA, and obesity significantly affect sperm motility. Obese men are 52 times more likely to have abnormal sperm motility (Table 7).

Table 1: The study participants' socio-demographic characteristics and body mass index distribution.

Characteristics	Overall (n=90)	SFA group		p-value
		Normal(n=45)	Abnormal(n=45)	
Age group (years)*				
20-29	3 (3.3)	3 (6.7)	0 (0.0)	0.101
30-39	43 (47.8)	17 (37.8)	26 (57.8)	
40-49	37 (41.1)	20 (44.4)	17 (37.8)	
50-59	7 (7.8)	5 (11.1)	2 (4.4)	
Marital status				
Single	4 (4.4)	2 (4.4)	2 (4.4)	0.999
Married	86 (95.6)	43 (95.6)	43 (95.6)	
Religion				
Christianity	88 (97.8)	44 (97.8)	44 (97.8)	0.999
Others	2 (2.2)	1 (2.2)	1 (2.2)	
Educational level				
Primary	6 (6.7)	4 (8.9)	2 (4.4)	0.615
Secondary	25 (27.8)	11 (24.4)	14 (31.1)	
Tertiary	59 (65.5)	30 (66.7)	29 (64.4)	
Ethnicity				
Benin	50 (55.6)	22 (48.9)	28 (62.2)	0.418
Igbo	13 (14.4)	8 (17.8)	5 (11.1)	
Others	27 (30.0)	15 (33.3)	12 (26.7)	
Body Mass Index Category				
Normal	40 (44.4)	17 (37.8)	23 (51.1)	0.252
Overweight	27 (30.0)	17 (37.8)	10 (22.2)	
Obese	23 (25.6)	11 (24.4)	12 (26.7)	

***Mean (SD) = 39.7 (± 6.64) years**

Table 2: Relationship between mean superoxide dismutase and quality of seminal fluid parameters

Semen parameter	Overall Mean u/g (SD)	p-value
Sperm concentration		
Normal	1.15 (0.45)	0.003
Abnormal	0.81 (0.37)	
Sperm motility		
Normal	1.36 (0.36)	<0.001
Abnormal	0.86 (0.40)	
Sperm morphology		
Normal	1.09 (0.45)	0.329
Abnormal	0.93 (0.48)	

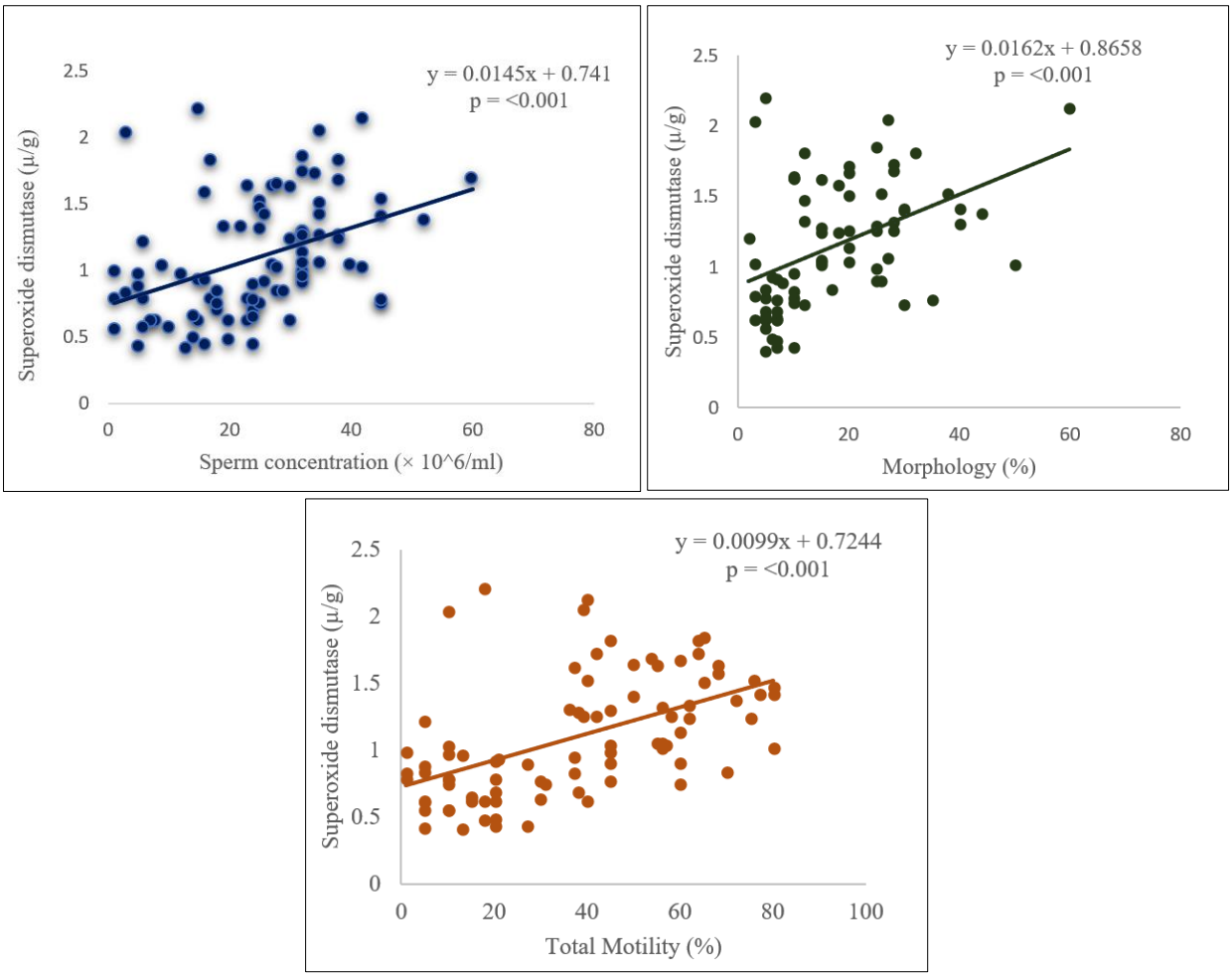

Figure 1: Correlation between Superoxide dismutase and seminal fluid parameters shown on scatter plot

Correlation between Superoxide dismutase and seminal fluid parameters

There was a positive correlation between SOD, sperm concentration, morphology, and motility (Figure 1). Thus, SOD was higher with higher sperm concentration, motility, and morphology. These were statistically significant.

Table 3: Relationship between mean Malondialdehyde and quality of seminal fluid parameters

Semen Parameter	Overall Mean mol/g (SD)	p-value
Sperm concentration		
Normal	0.82 (0.43)	<0.001
Abnormal	1.61 (0.36)	
Sperm motility		
Normal	0.58 (0.18)	<0.001
Abnormal	1.28 (0.50)	
Sperm morphology		
Normal	0.94 (0.51)	0.008
Abnormal	1.42 (0.48)	

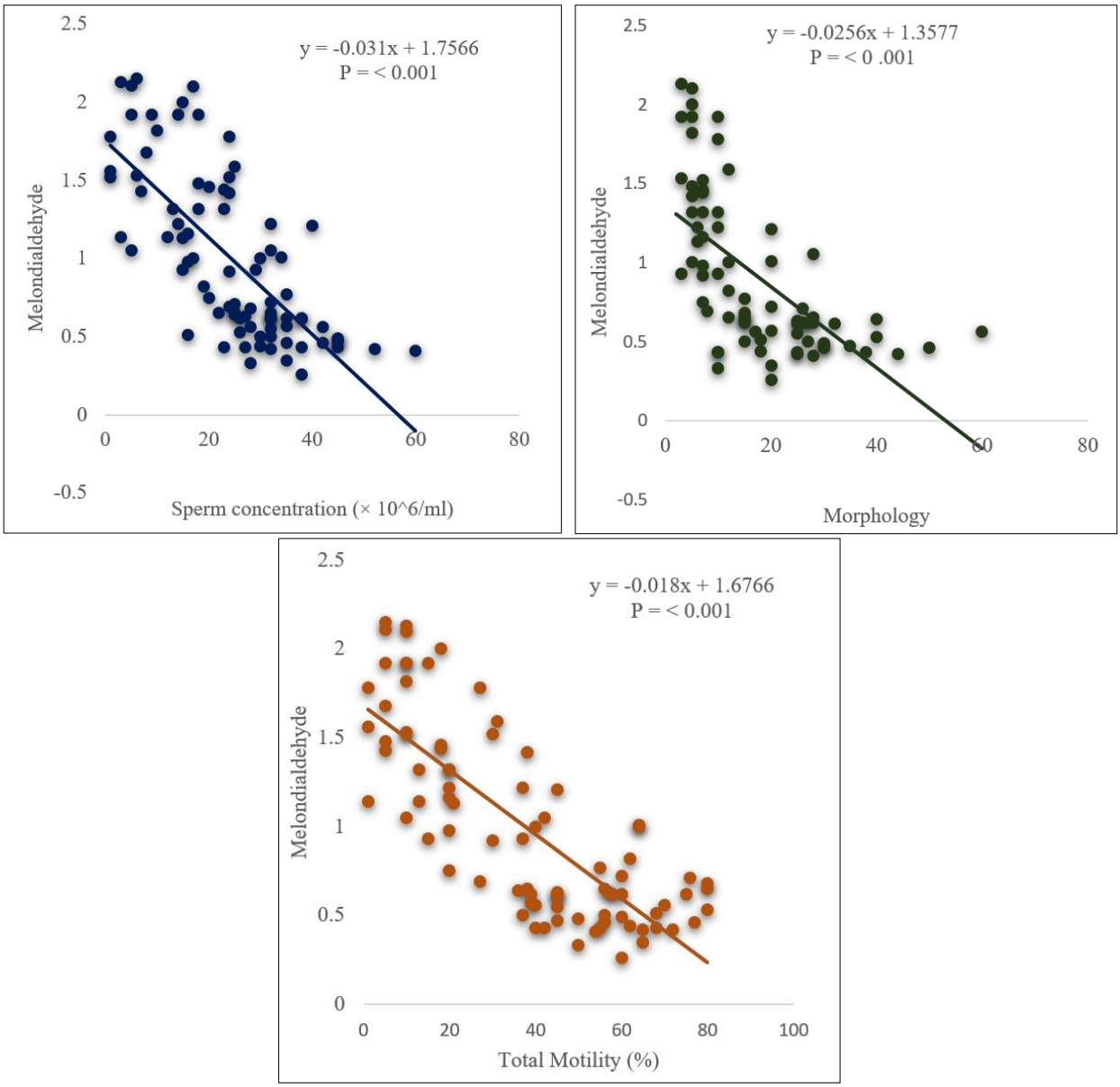

Figure 2: Correlation between Malondialdehyde and seminal fluid parameters

Malondialdehyde level was negatively correlated with sperm concentration, motility, and morphology. Statistically significant with a p-value of <0.001. (Figure 2).

Table 4: Comparing seminal fluid superoxide dismutase and malondialdehyde levels among case and control groups

Oxidative stress parameters	Overall (n=90)	SFA group		p-value
		Controls (n=45)	Cases (n=45)	
Superoxide dismutase*	1.07 (0.46)	1.37 (0.34)	0.78 (0.36)	<0.001
Malondialdehyde*	0.99 (0.53)	0.59 (0.19)	1.38 (0.45)	<0.001

*Mean (standard deviation)

Table 5: Binary logistic regression analysis of factors associated with sperm concentration among the study population

Variables	β Regression Coefficient	Odds ratio	95% CI for Odds ratio		p-value
			Lower	Upper	
Constant	-0.180	0.835			
Age	0.196	1.216	1.029	1.438	**0.022***
Obesity	-0.255	0.775	0.087	6.940	0.820
Alcohol	-1.272	0.280	0.043	1.815	0.280
Smoking	16.330	12365020.1	0.0001		1.000
Previous Groin surgery	-0.750	0.473	0.048	4.669	0.522
Superoxide dismutase	.570	1.768	.229	13.673	0.585
Malondialdehyde	-4.453	.012	.001	.118	**<0.0001***

CI – Confidence Interval, * Statistically Significant

Table 6: Binary logistic regression analysis of factors associated with sperm morphology among the study population

Variables	β Regression Coefficient	Odds ratio	95% CI for Odds ratio		p-value
			Lower	Upper	
Constant	32.584				
Age	-0.025	0.975	0.716	1.329	0.873
Obesity	-20.4	0.0001	0.0001		
Alcohol	-2.276	0.103	0.002	6.216	0.277
Smoking	14.936	3066447.4	0.0001		1.000
Previous Groin surgery	-3.848	0.021	0.001	0.595	**0.023***
Superoxide dismutase	-1.711	0.181	0.007	4.812	0.307
Malondialdehyde	-2.867	0.057	0.003	1.194	0.065

CI – Confidence Interval, * Statistically Significant

Table 7: Binary logistic regression analysis of factors associated with sperm motility among the study population

Variables	β Regression Coefficient	Odds ratio	95% CI for Odds ratio		p-value
			Lower	Upper	
Constant	5.113				
Age	0.001	1.001	0.823	1.217	0.993
BMI					
Obesity	3.956	52.24	1.442	1892.8	**0.031***
Alcohol	-2.344	0.096	0.003	2.979	0.181
Smoking	19.91	441474846.7	0.0001		0.999
Previous Groin surgery	-0.440	0.644	0.006	64.89	0.852
Superoxide dismutase	3.565	35.33	1.386	900.5	**0.031***
Malondialdehyde	-8.204	0.0001	0.0001	0.023	**<0.0001***

CI – Confidence Interval, * Statistically Significant

DISCUSSION

Oxidative stress has been implicated in many disease conditions, including infertility. This study evaluated the association between oxidative stress and semen quality, focusing on sperm concentration, motility, and morphology, by assaying the oxidative stress markers Superoxide dismutase and Malondialdehyde in the seminal fluid of men with normal and abnormal semen parameters.

The study participants consisted mainly of men aged 30 to 39, with a few spilling into the sixth decade. However, there was no significant difference in the Age of participants between the two groups in the study, those with normal and abnormal semen parameters. However, Age was noted to affect sperm concentration.

The key findings were a significantly higher mean SOD level noted in men with normal seminal fluid parameters compared to those with abnormal semen parameters. There was a positive correlation between SOD levels and sperm concentration, motility, and morphology. Unlike the SOD, the mean Malondialdehyde level was lower in those with normal semen parameters than in the abnormal SFA group. Also, Malondialdehyde showed a significantly strong negative correlation for sperm concentration, motility, and morphology. Superoxide dismutase had a statistically significant effect on sperm motility alone, whereas Malondialdehyde affected both sperm concentration and motility.

Superoxide dismutase is an essential first-line endogenous enzymatic antioxidant defence and plays a vital role in protecting spermatozoa from oxidative damage.[19] Tissue metabolism generates superoxide free radicals, which SOD converts to hydrogen peroxide (H_2O_2) and free oxygen (O_2). The (H_2O_2) molecules are further converted to valuable water and oxygen molecules by catalase enzymes, curtailing oxidative stress.[18, 19] Hence, it is not surprising to find higher levels of superoxide in the participants with normal semen parameters and lower levels in participants with poor semen parameters.

Superoxide dismutase activity in this study was positively correlated with sperm concentration, motility, and morphology. This may likely explain the finding of high SOD levels in the men with normal semen parameters when compared to those with oligospermia, asthenospermia and teratozoospermia, corroborated by Wdowiak *et al.,* [20] and Yan *et al.,* [21] who noted an increase in sperm concentration, motility, and normal morphology with increased SOD activity. Yan *et al.,*[21] reported, in addition to normal sperm parameters, reduced DNA fragmentation, with an overall impression of an association between SOD and semen quality. Also, Garrat *et al.,*[22] reported poor motility and functional dysfunction in mouse semen with SOD deficiency, in consonance with our findings. Although the study was in mice, their finding may also apply to humans. Adjusting for confounders in logistic regression showed that SOD was not statistically associated with sperm concentration and morphology, but was significantly associated with motility (p=0.031). In contrast, Kurkowska *et al.,* [23] did not find any association between SOD and sperm motility, perhaps because motility was measured over time and in healthy men.

The study also found that men with abnormal semen parameters had higher mean MDA levels compared to those with normal semen parameters. Sperm cells are particularly vulnerable to lipid peroxidation because they contain large amounts of polyunsaturated fatty acids in their plasma membrane. The process of lipid peroxidation produces lipid peroxides, which accumulate in spermatozoa, creating a variety of harmful byproducts, such as Malondialdehyde, 4-hydroxynonenal, and isoprostanes, which can be measured as indicators of oxidative stress.[21] This lipid peroxidation causes a loss of intracellular adenosine triphosphate, resulting in axonemal damage and decreased sperm viability and motility. Malondialdehyde, being a product of lipid peroxidation, is a good measure of lipid peroxidation and oxidative stress

The negative correlation between MDA and sperm concentration, sperm motility, and sperm morphology in this study is consistent with previous studies.[24-27] Emokpae *et al.,,*[26] noted that the lipid peroxidation index correlates with sperm indices; they found that MDA levels negatively correlate with sperm motility and morphology, and higher levels of MDA were observed in oligospermic males compared to normozoospermic males. However, regression analysis showed that MDA was not significantly associated with sperm morphology, while MDA was strongly associated with sperm concentration and motility. Similarly, Jannatifar *et al.,* [25] found a higher mean level of MDA in infertile men with asthenoteratozoospermia compared to fertile men. In contrast, Suleiman *et al.,*[28] *found no correlation between sperm concentration and motility, despite a higher mean MDA level among men with asthenoteratozoo*spermia. Palani, in his study, also noted no significant difference in MDA levels among men with normal and abnormal semen. This finding may be attributable to the small sample size used in the study and possibly due to racial and genetic differences.[29]

This study showed a statistically significant difference in mean oxidative stress markers between those with normal and abnormal seminal parameters. Superoxide dismutase showed a positive correlation with semen quality; however, it was only significantly associated with sperm motility. Malondialdehyde, on the other hand, showed a negative correlation with sperm quality and was significantly associated with sperm concentration and motility.

Strength of the study

Testing for two oxidative stress markers, one which is a product of oxidative stress and the other an antioxidant, helped reinforce evidence of the association between oxidative stress and seminal fluid parameters.

Aside from oxidative stress markers, which are being investigated in this study, other predictors of abnormal SFA, such as obesity, Age, previous groin surgeries, alcohol consumption, and smoking, were assessed; Age, obesity, and previous groin surgery were associated with sperm quality.

Superoxide dismutase is a first-line enzymatic antioxidant defence, while MDA is a byproduct of lipid peroxidation. Both are strong markers of oxidative stress and are relatively affordable in low-resource settings compared to direct assays. Both SOD and MDA were significantly associated with motility, the most essential semen parameter. Hence, it is a good tool for evaluating oxidative stress in infertility in low-resource settings.

Limitations of the study

This study tested only for association; causal inference to establish that oxidative stress caused abnormal semen parameters or male infertility cannot be made. Also, this is a hospital-based study with some bias in the recruitment process; hence, it may not be objectively representative of the populace.

CONCLUSION

The study has established an association between oxidative stress markers (SOD and MDA) and sperm quality in the West African Sub-region, which is a domesticated region. Thus, it is a possible cause of male infertility. Superoxide dismutase shows a positive correlation with semen quality and is significantly associated with sperm motility, whereas Malondialdehyde shows a negative correlation with semen quality and is significantly associated with sperm concentration and motility. These biomarkers could serve as an adjunct when investigating males while evaluating couples for infertility. The antioxidants emanating from this study can play a therapeutic role when managing couples with infertile males, instead of the use of advanced methods that are beyond the reach of most couples in our poor resource setting.

What is known about this topic

Male infertility contributes significantly to the problem of infertility amongst couples. Oxidative stress can cause abnormal sperm parameters by promoting lipid peroxidation.

What this study adds

Oxidative stress is associated with male infertility, and its biomarkers could serve as an adjunct when evaluating an infertile male.

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Authors' Contribution: The authors contributed equally to the development of the manuscript.

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