

**BJMHR**

British Journal of Medical and Health Research

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## **A Lipid Peroxidation Index Correlates with Sperm Indices in Oligospermic Male Subjects in Benin City, Nigeria.**

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### **ABSTRACT**

Male infertility is a relatively common condition affecting approximately 50% of couples. Male infertility has been associated with oxidative stress and lipid peroxidation, but calculated lipid peroxidation index is seldomly used to assess the extent of oxidative stress. The objective of this study was to evaluate the levels of lipid peroxidation index of oligospermic infertile males. A total of 120 subjects were recruited for the study which included 70 infertile males and 50 males of proven fertility as controls. Malondialdehyde (MDA) and total antioxidant status (TAS) were measured in the seminal fluid by spectrophotometric methods. Semen analysis was performed on the sample according to the World Health Organization criteria. The lipid peroxidation index was calculated as the ratio of MDA and TAS. The mean levels of measured sperm indices, MDA and TAS were compared between infertile and fertile subjects using students-t-test. The sperm count, the total motility and percentage morphology were significantly lower ( $p < 0.001$ ) in the infertile males compared with the controls. The mean level of MDA was significantly higher ( $p < 0.001$ ) while TAS was significantly lower ( $p < 0.001$ ) in infertile males than controls. The lipid peroxidation index was higher in oligospermic male subjects than control and correlates negatively with sperm count ( $r = -0.321; p < 0.01$ ), progressive motility ( $r = -0.292; p < 0.02$ ), percent morphology ( $r = -0.238; p < 0.05$ ). Lipid peroxidation index may be easy and better predictor of oxidative stress in oligospermia.

**Keywords:** Lipid peroxidation index, oligospermia, malondialdehyde and total antioxidant status.

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Received 05 March 2020, Accepted 22 March 2020

Please cite this article as: Emokpae MA *et al.*, A Lipid Peroxidation Index Correlates with Sperm Indices in Oligospermic Male Subjects in Benin City, Nigeria.. British Journal of Medical and Health Research 2020.

## INTRODUCTION

Infertility is a major clinical problem affecting people overtime all over the world both medical and psychosocially, causing marital disharmony and marital strain. In Nigeria and other African countries there are strong emphasis on child bearing, infertility poses a big challenge to such couples [1].

Infertility has been defined by the World Health Organization as the inability of sexually active couples to conceive after 12months of unprotected sexual intercourse without the use of contraceptive [2]. Overtime females have been held responsible for infertility in marriages especially in Africa countries including Nigeria due to our cultural settings and beliefs but, it is now known that abnormal male reproductive function is responsible for about 40- 50% of infertility cases [3].

Male factor infertility is seen as an alteration in sperm concentration, motility or morphology. Oligospermia refers to a sperm count less than 15million/milliter [4] and is the most frequent type of male infertility in Nigeria even though the prevalence varied from place to place. The prevalence of oligospermia as reported from several centres in Nigeria are 33% in Abakaliki [5], 25.6% in Ile Ife [6], 25% in Abraka [7] and 11.8% in Kano [8]. Infertility has been attributed to a number of factors however, the cause of 37-58% cases of male infertility cannot be identified and is termed 'idiopathic infertility' [9]. The underlying mechanism responsible for poor semen quality has been difficult to understand [10]. It has been explained that low sperm count might be as a result of prolonged exposure of the seminiferous tubules to reactive oxygen species which damages the seminiferous tubules [10] or infection [11].

The peroxidation of lipid rich spermatozoa by free radicals may cause damage to its membrane which leads to alteration in the morphology of the sperm and sperm count as well as the viability, mobility and DNA damage inducing strands breaks and other oxidative damages in spermatozoa leading to infertility [3,12]. The underlying mechanisms responsible for the poor semen quality has been quite difficult to understand, however recent studies have suggested that oxidative stress might have profound role in infertility [13] and has been implicated in the etiology of male reproductive dysfunction resulting from lipid peroxidation of the spermatozoa [14]. The calculated lipid peroxidation index is rarely used in the evaluation of oxidative stress and it might be a better predictor of oxidative stress than the evaluation of malondialdehyde (MDA) alone or total antioxidant status (TAS). This study therefore evaluates the level of seminal fluid MDA, TAS and calculated lipid peroxidation index in oligospermic infertile male subjects and also assesses the relationship between lipid peroxidation index and semen indices in infertile males.

## MATERIALS AND METHOD

## Study design

This is a cross sectional study of oligospermic male subjects evaluated for infertility.

## Subjects

A total of 120 subjects within the ages 25 -45 years were recruited for this study. Out of 120 subjects, 70 oligospermic infertile male partners of infertile couples who were visiting Central Hospital, Benin City, Edo State were recruited for the study while 50 apparently healthy males who have fathered a child within the last one year were recruited as control subjects.

## Inclusion and Exclusion criteria

Oligospermic male partners of infertile couples without chronic illnesses attending the infertility clinics and the control subjects made up of fertile apparently healthy subjects with sperm count above 15million sperm per millilitre were included in the study. On the other hand, Individuals on antioxidant supplements, had hepatic, renal, endocrine diseases and any other systemic disease that can cause impaired reproductive capability were excluded. In addition, subjects with genital tract infections both sexual and non-sexually transmitted infections, having other specific genital causes such as undescended testis(cryptorchidism) and those that consume narcotics such as cigarettes, tobacco, and excessive alcohol were also excluded.

## Ethical Consideration:

The purpose and nature of the study of the study was discussed with the study participants. Informed consent was obtained before specimens were collected. Ethical approval was sought and obtained from the Edo State Ministry of Health, Benin City (Reference number: HM.1208.355; dated: 26<sup>th</sup> October, 2017).

## Specimen collection and Analyses

The semen was collected by self or assisted masturbation into a wide mouthed container after 3 – 5days of sexual abstinence. The semen was submitted to the laboratory within 1 hour of collection for analyses. The semen was ejaculated into the sterile container without the use of condom or spermicidal agent. The semen analyses was done using microscopic technique according to World Health Organization criteria [4]. The liquefied sample was centrifuged at 1000g for 5minutes to obtain seminal plasma. The seminal plasma was kept frozen -20°C until total antioxidant status and malondialdehyde was evaluated using spectrophotometric method. Lipid peroxidation index was calculated as the ratio of MDA to TAS in the same unit.

## Total Antioxidant Status [Trolox Equivalent Antioxidant Capacity (TEAL) Assay]

### Test principle

The ability of antioxidant species to scavenge for 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS<sup>+</sup>) gives an overall idea of the total antioxidant capacity in a particular specimen. The ABTS radical cation was produced by reacting ABTS solution

with potassium persulphate in the dark. This radical cation is a blue-green chromophore with maximum absorption at 734nm which decreases in its intensity in the presence of antioxidants. Upon addition of the specimen, available antioxidant species present scavenge for  $ABTS^+$ , after a certain reaction time, the amount of  $ABTS^+$  remaining is measured and expressed a Trolox equivalent.

### **Malondialdehyde**

#### **Test Principle**

Malondialdehyde is a product of lipid peroxidation which reacts with thiobarbituric acid under heat to form a MDA–TBA2 adduct, a pink coloured complex is measured at 532nm.

#### **Statistical analysis**

The data collected was statistically analysed using the Statistical Analysis for Social Sciences (SPSS) version 16.0. Values obtained were represented as mean and standard error of mean (SEM) for both tests and controls. Student's t test was used to compare the means while correlation was done using Pearson correlation coefficient. Values were considered statistically significance at ( $p \leq 0.005$ ) using 95% confidence interval.

### **Results**

Table 1 shows the mean levels of sperm count, total motility, percentage morphology and volume in oligospermic male and control subjects. The sperm count, total motility, and percentage morphology of oligospermic males were significantly lower than the control group ( $P < 0.001$ ). The mean semen volume was slightly lower in oligospermic males but no statistically significant when compared to control group. The mean total antioxidant status was significantly lower in oligospermic subjects than the control group ( $P < 0.001$ ). On the other hand, the mean level of seminal fluid malondialdehyde was significantly higher in oligospermic subjects than controls. The calculated lipid peroxidation index was significantly higher in oligospermic subjects than controls ( $P < 0.001$ ) (table 2). Table 3 shows the association between lipid peroxidation index, MDA and TAS with measured sperm indices. Sperm count ( $r = -0.321$ ;  $p < 0.01$ ), progressive motility ( $r = -0.292$ ;  $p < 0.02$ ), percent morphology ( $r = -0.238$ ;  $p < 0.05$ ) and volume ( $r = -0.118$ ;  $p < 0.32$ ) were negatively associated with lipid peroxidation index. Malondialdehyde correlated negatively with sperm count ( $r = -0.240$ ;  $p < 0.05$ ), progressive motility ( $r = -0.218$ ;  $p < 0.04$ ), percent morphology ( $r = -0.242$ ;  $p < 0.05$ ) and volume ( $r = -0.074$ ;  $p < 0.06$ ). Total antioxidant status also correlated positively with sperm count ( $r = 0.239$ ;  $p < 0.05$ ), progressive motility ( $r = 0.241$ ;  $p < 0.05$ ), percent morphology ( $r = 0.240$ ;  $p < 0.05$ ) and volume ( $r = 0.050$ ;  $p < 0.80$ ).

**Table 1: Mean levels of measured sperm indices in Oligospermic Males and Control Subjects**

Measured Variables (units)	Oligospermia n=70 Mean $\pm$ SD	Control subjects n=50 Mean $\pm$ SD	P value
Sperm Count( $\times 10^6$ cells/ml)	10.72 $\pm$ 4.1	95.40 $\pm$ 4.8	0.001
Total Motility (%)	40.81 $\pm$ 1.72	58.59 $\pm$ 2.89	0.001
Percentage Morphology (%)	40.36 $\pm$ 1.42	67.12 $\pm$ 1.60	0.001
Semen Volume (mL)	3.01 $\pm$ 0.10	3.38 $\pm$ 0.18	0.99

**Table 2: Seminal fluid Levels of Malondialdehyde, total antioxidant status and calculated Lipid Peroxidation Index (Mean  $\pm$ SD)**

Biomarkers of lipid peroxidation	Oligospermic Males n=70	Control subjects n=50	P value
Malondialdehyde (nmol/mL)	43.36 $\pm$ 1.57	36.10 $\pm$ 2.10	0.001
Total Antioxidant Status (nmol/ $\mu$ L)	3.60 $\pm$ 0.81	4.90 $\pm$ 0.30	0.001
Lipid Peroxidation index	12.16 $\pm$ 1.82	7.29 $\pm$ 1.02	0.001

**Table 3: Correlation of lipid peroxidation index,MDA and TAS with sperm indices in oligospermic male subjects**

Correlation	R-value	P-value
Lipid peroxidation index/Sperm count	-0.321	0.01
Malondialdehyde/Sperm count	-0.240	0.05
Total antioxidant status/Sperm count	0.239	0.05
Lipid peroxidation index/progressive motility	-0.292	0.02
Malondialdehyde/progressive motility	-0.218	0.04
Total antioxidant status/progressive motility	0.241	0.05
Lipid peroxidation index/percent morphology	-0.238	0.05
Malondialdehyde/percent morphology	-0.242	0.05
Total antioxidant status/percent morphology	0.240	0.05
Lipid peroxidation index/semen volume	-0.108	0.32
Malondialdehyde/semen volume	-0.074	0.60
Total antioxidant status/semen volume	0.050	0.80

## DISCUSSION

Lipid peroxidation has been implicated in male infertility. The extent of peroxidation varies and may depend on the aetiology of disorder. The aim of this study was to evaluate the lipid peroxidation levels via measurement of MDA, TAS and calculated lipid peroxidation index to measure the level of oxidative stress and associated with semen parameters in oligospermic males. The findings in this study showed that the significantly higher level of seminal fluid MDA and significantly lower levels of TAS leading to higher lipid peroxidation index in infertile men might have contributed to oligospermia. This is partly consistent with previous studies [15,16]. The authors reported significantly higher level of MDA in oligospermic males than fertile controls. The calculated lipid peroxidation index might be a better predictor of oxidative stress than MDA or TAS [17]. The mean lipid peroxidation index was significantly

higher ( $P < 0.001$ ) in the infertile study group compared to the controls. This shows that the level of lipid peroxidation in the seminal plasma of the oligospermic males is higher than the capacity of the non-enzymatic antioxidants present. Lipid peroxidation index corrected better than MDA and TAS with measured sperm indices in this study.

The level of MDA reflects the extent of peroxidation of membrane polyunsaturated fatty acids which may be responsible for abnormal sperm count, sperm motility as well as inhibition of other semen parameters. High levels of MDA was previously reported to cause cellular damage to the developing germ cells and mature spermatozoon and was thought to initiate a loss of motility, reduction in sperm concentration and abnormal morphology [18]. This has confirmed that a high level of MDA may have a diminishing effect on the semen parameters resulting in infertility which is in line with previous studies, MDA was reported to correlate negatively with sperm count, sperm motility and sperm morphology [16,19-21]. However, this study has shown no significant difference in the volume of seminal fluid between fertile and infertile groups and this is consistent with previous studies [20,22]. Sperm count and sperm motility are fundamental parameters that ascertain the functional ability of the spermatozoa [16]. The negative correlation of sperm indices with lipid peroxidation index may indicate that lipid peroxidation adversely affects the membrane fluidity, mobility of the spermatozoa thus affecting the functional competence and fertilizing potential of the spermatozoa [18].

Total antioxidant status measures the capacity of the non-enzymatic antioxidants present in a biological fluid to scavenge free radicals generated within the fluid or the ability to inhibit the oxidation of biomolecules. In this study, the mean value of the TAS was observed to be significantly higher in the control group compared to the study group indicating that there was a significantly lower TAS in the oligospermic infertile males. This is also consistent with studies elsewhere [18,23,24]. The seminal fluid contains some levels of antioxidants; both enzymatic and non-enzymatic that acts as free radical scavengers to dispose, scavenge and inhibit oxidative stress and protect against lipid peroxidation. This study shows that low level of TAS correlated with poor semen parameters, thus suggesting that decreasing TAS may significantly impair semen parameters.

## CONCLUSION

The study has shown that seminal plasma MDA was significantly higher while the TAS was significantly lower in the oligospermic male subjects than in the controls, which is an indication of higher lipid peroxidation index. It is suggested that lipid peroxidation index may be an easy and a better predictor of lipid peroxidation than either MDA or TAS in oligospermia.

## ACKNOWLEDGEMENTS:



We appreciate the contributions of all Clinicians, Nurses and Medical Laboratory Scientists at the Central Hospital, Benin city towards the completion of this study.

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