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SEMINAL MALONDIALDEHYDE LEVEL: AN OXIDATIVE STRESS MARKER IN INFERTILE MALES IN NORTH CENTRAL PART OF NIGERIA

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ABSTRACT

Aim: To evaluate the association between oxidative stress and male infertility with sperm quality and malondialdeyde concentration. **Background:** Infertility is a major problem affecting approximately 30% of the male population. Apart from known factors, it becomes very important to identify new and nonconventional factors that may play significant roles in male infertility. Oxidative stress has recently been identified as an underlying mechanism of numerous chronic diseases of which male infertility has been claimed to have a relationship. **Materials and methods**: A cross sectional prospective study of 80 (30 fertile and 50 infertile) men with age range between 25-50 years were enrolled in the study. WHO guidelines was used in seminalysis and thiobarbituric acid method for malondialdeyde analysis. **Results:** The mean ± SD malondialdehyde (MDA) concentration in seminal plasma of infertile men (3.3 ± 0.4 nmol/ml) was significantly higher than fertile men (2.4 ± 0.2nmol/ml) (P-value < 0.05) and had a negative relationship with sperm count. **Conclusion:** This study revealed that high level of MDA was associated with increase in lipid peroxidase with sperm membrane destruction.

KEYWORDS: Seminal plasma, Male infertility, Sperm count, Lipid peroxidase, Malon dialdehyde (MDA).

INTRODUCTION

Infertility is a major health problem affecting approximately 5-15% of all married couples worldwide.[1] Infertility impacts great physiological and social stigma mostly directed against the female partner in spite of the fact that actual causes of infertility include a male factor (30%), a female factor (35%) and finally an unexplained idiopathic infertility (15%).[2] Infertility can be defined as one or more abnormalities within semen analysis (i.e sperm concentration, volume, motility, and morphology).[3] The actual pathological basis of qualitative and quantitative defects of sperm leading to defects in fertilization is not clear in many cases. Since oxidative stress is said to be involved in many chronic physiological conditions, the current study was aimed at evaluating if there is any association that exists between male infertility and oxidative stress., 15% of couples suffer from infertility in the United States[4] while in India it has also been reported that one in every six couples suffers from infertility[5], unfortunately, such data is not available in this region.

There are three main factors that can affect the fertility of a male gender namely pre testicular, testicular and post-testicular.[6] The pre-testicular are related to hormonal imbalance and poor general health. The most important causes are hypogonadism, drugs, alcohol and smoking,[7] physiological stress, genetic abnormalities, chemotherapy and different types of medications such as cimetidine, phenytoin and nitrofurantoin. The testicular factors are conditions where the testis produces semen of low quality and/or poor quantity despite adequate hormonal support, the causes could include age, chromosomal abnormality, seminoms, oligosperms, hydrocele and mumps.[8] The post-testicular factors decreases male infertility by affecting the male genital systems, testicular sperm production and include defects of the genital tract as well as problems with ejaculation. With all these, identifying the exact factor responsible for male infertility is difficult and the mechanisms of the defects of poor semen quality remain uncertain. Therefore, potential non-conventional factors for the pathogenesis of male infertility has been looked for, and recent studies suggests that oxidative stress must have its unique role in male infertility.[9]

Oxidative stress in conventionally defined as an imbalance between pro oxidant stress and anti-oxidant defense. Reactive oxygen species (ROS), especially superoxide anion (O_2), hydrogen peroxide (H_2O_2) and

hydroxyl radical (OH) are highly reactive oxidizing agent that belongs to the class of free radicals.[10] They have one or more unpaired electron and react with macromolecules for compensation of their deficit electron.[11] Nevertheless they have physiological roles such as acrosome induction and sperm capacitation in low concentration, but they have pathological effect on macromolecules such as polyunsaturated fatty acids, amino acids and sugars in high levels.[12] Lipid peroxidation (LPO) is one of the pathological effects from ROS that is associated with oxidation of membrane polyunsaturated fatty acids (PUFA).[13] It attacks the fluidity of sperm plasma membrane with subsequent loss of the ability of oocyte fusion and fertilization.[13] Human sperm cells in contrast with other cells are particularly susceptible to oxidation of their plasma membrane due to the existence of a high concentration of PUFA in the membrane malondialdehyde (MDA) is a stable peroxidation product of PUFA, usually cross-linked to proteins. It is a diagnostic tool for lipid peroxidation and the analysis of etiology of male infertility.[14] The aim of the present study is to evaluate the association between oxidative stress and male infertility investigated with semen analysis and malondialdeyde as a tool for estimating oxidative stress.

MATERIALS AND METHODS

This case study was done in the departments of chemical pathology and obstetrics and gyneacology of Jos University Teaching Hospital, Jos, Plateau State, North - central part Nigeria. 50 diagnosed infertile male patients (having normal female partner) who attended the infertility unit of obstetrics and gynaecology department of JUTH and 30 age-matched fertile control subject were recruited into the study.

Before semen analysis, a questionnaire was distributed to obtain information biodata on male risk factors for infertility including smoking habits, alcohol use, use or abuse of other substances and drugs and history of orchitis, testicular trauma, sexually transmitted disease, varicocoele, singuinal hernia operation and cryptorchidism. Ethical clearance was obtained from research and ethical committee of Jos University Teaching Hospital, Jos, Plateau state. Nigeria. Consent was gotten from each participant.

Laboratory Method

From each participant, semen samples were obtained by masturbation into a clean wide mouthed glass container after 3 days of abstinence from sexual intercourse. For internal quality control of semen analysis, spermiograms were carried out by the trained observer, according to the World Health Organization guidelines.[14] Spermiograms included semen volume (ml), sperm density ($x10^6$ per ml), sperm motility (%) and abnormal morphologic features (%). Semen samples were centrifuged for 5minutes at 3000g and then seminal plasma was separated for determination of MDA concentration.

Measurement of malondialdehyde (MDA) levels

Seminal MDA levels were analyzed according to Rao et at (1989). MDA was assessed using the thiobarbituric acid method. 10ul of seminal plasma (supernatants) was added to 900ul of distilled water into glass tubes. To each tube, 500ul of thiobarbituric acid reagent (0.67 of 2-thiobarbituric acid dissolved in 100ml of distilled water with 0,5g NaOH and 100ml of glacial acetic acid added) was added and then heated for 1hr in a boiling water bath. After cooling temperature, each tube was centrifuged for 10 minutes at 4000g and the supernatant absorbance of these was read in a spectrophotometer at 534nm.

Statistical Analysis

All the data were recorded systematically in a preformed data collection sheet. Data were analyzed using SPSS 12.0 for windows. Mann-Whitney U test and unpaired t-test were done to find significant difference between groups. Statistical significance was set at $P<0.05$.

RESULTS

The parent study was conducted in Chemical Pathology Department of Jos University Teaching Hospital, Jos, Plateau state, North- Central Nigeria. During this period 50 infertile and 30 fertile male subjects were enrolled as case and control respectively. The mean ages of case and control were 30.94 ± 6.94 and 3.40 ± 6.89 years respectively. As shown in table 1. There was no significant difference of age between the case and control groups.

The Sperm analysis of both the infertile men (case) and fertile men (control) was done for sperm count, sperm motility and sperm morphology. The semen volume was also evaluated.

As results shown table II sperm count was found to be significantly lower ($p<0.05$) in infertile men compared with fertile men.

In the case of sperm motility in table III, 'Z' proportion test was found to show significant ($p<0.05$) difference between the fertile men and the infertile men. The same thing is applicable in the area of sperm morphology as seen table IV. Semen volume was also found to be lower in infertile men compared with fertile men as shown in table V. the level of significance is $p>0.05$ when the data are presented in mean ± SD and the unpaired t-test was done as the test of significance.

To determine the oxidative stress to which the spermatozoa of the study participants were exposed to, the level of MDA in the seminal plasma were measured both in infertile (case) and fertile (control) groups. MDA in the seminal plasma was found to be 3.3 ± 0.4 nmol/ml and 2.4 ± 0.2 nmol/ml in infertile and fertile males respectively. As shown in table 2, the seminal plasma MDA level was found to be significantly ($p<0.05$) elevated in infertile males compared with healthy fertile male subjects.

Table 1: Age Distribution of the Study Subjects

Groups	n	Ages(yrs)mean ±SD	p-value
Infertile male(case)	50	30.94 ± 6.94	< 0.05
Fertile male(control)	30	31.40 ± 6.89	

Table II: Distribution of Sperm Count Concentration In Infertile Men And Fertile Men.

Subjects	N	Sperm concentration x 10^6/ml Mean ± SD
Fertile men	30	68.9 ± 9.9
Infertile men	50	27.9 ± 13.9
p-Value		< 0.05

Table III: Distribution of Sperm Motility In Infertile Men and Fertie Men.

Motility	Infertile men n = 50		Fertile men n = 30		n value
	n	%	n	%	
Normal	19	38	29	96.7	< 0.05
Abnormal	31	62	1	3.3	

Table IV. Distribution of Sperm Morphology in Infertile Men and Fertile Men.

Morphology	Infertile men n = 50		Fertile men n = 30		p value
	n	%	n	%	
Normal	20	40	28	93.3	< 0.05
Abnormal	30	60	2	6.7	

Table V: Distribution of Semen Volume in Infertile Subjects and Fertile Subjects.

Subjects	n	Volume(mls) Mean ± SD
Fertile men	30	4.41 ± 1.2
Infertile men	50	2.34 ± 1.4
p value		< 0.05

Table VI: MDA Level of Seminal Plasma in Infertile and Fertile male Groups.

Variable	Infertile n=50	Fertile n=30	n
MDA(mean ± SD) (nmol/ml)	3.3 ± 0.4	2.4 ± 0.2	< 0.05

DISCUSSION

Infertility is a problem affecting approximately 18% of couple who make attempt to conceive a baby. In the past the problems were attributed to the woman. It is now known that male factors play a role in almost half of cases. Before a man can be said to be fertile, it means there is production of normal sperm and delivery of it to the female partner's reproductive tract. Most of the time male infertility arises when the male counterpart is unable to produce or deliver fully functioning sperm. Many factors have also been identified that can lead to male infertility, however, the main reason that causes both qualitative and quantitative defect of the spermatozoa are still yet to be understood in several cases of male infertility.

All cellular components including lipids, proteins, nucleic acid and sugars are potential targets for ROS.[16] ROS induces the oxidative stress which has recently been linked with a number of chronic and inflammatory diseases. Free radicals within physiological concentration regulate sperm maturation, capacitation and hyper-activation, the acrosome reaction and sperm-oocyte fusion.[17] However oxidative stress resulting from excessive generation of reactive species compound with antioxidant defense capacity can cause lipid peroxidation, DNA damage and apoptosis of the sperm.[18] A number of studies have shown that lipid peroxidation affects the sperm concentration, motility, morphology and untimately leads to poor sperm quality.[19]

From the aim of this study, to evaluate the role of oxidative stress in the pathogenesis of male infertility and as a marker of oxidative stress-induced lipid peroxidation, the study shows that seminal plasma MDA levels were minimal in fertile subjects (2.4nMol/L) but higher in infertile male subjects (3.3nMol/L).

In this present study MDA levels were found to be markedly elevated in seminal plasma in infertile subjects compared with fertile control subjects which simply means that the infertile subjects suffer from oxidative stress induced lipid peroxidation. This was also confirmed in the work of several studies which shows clearly an elevated MDA level in seminal plasma of infertile groups compared with healthy fertile groups[20], which is actually similar to our present findings. It could also be said that the high level of MDA in the semen of infertile subjects could indicate that the spermatozoa of infertile subjects were exposed to oxidative stress. The

sperm count, sperm morphology and even motility and the volume were found to be significantly lower in infertile subjects compared to fertile subjects. This suggests that that the semen analysis report showed both quantitative and qualitative defects on the sperm obtained from the infertile patients. It is also suggestive that the increased oxidative stress in the seminal fluid has a negative impact on semen analyzed. This is supported by several previously published studies. Some studies have suggested that ROS attack the integrity of DNA in sperm nucleus by causing base modification, DNA strands break and chromatin cross-linking.[21] The damage to the DNA by excessive levels of ROS could accelerate the process of germ cell apoptosis, leading to the decline in sperm counts which is associated with male infertility.[22] The peroxidase damage to the membrane of the sperm and the axonemal proteins appear to be the cause of permanent impairment in sperm motility.[22] The increase in ROS causes ATP to deplete rapidly resulting in decreased phosphorylation of axonomal proteins and cause transient impairment of sperm motility.

CONCLUSION

The findings of this index study shows an association between oxidative stress and infertility. It also suggest that oxidative stress was involved in low sperm quality and the etiology of male infertility. The measurement of MDA could be a useful diagnostic tool for estimation of oxidative stress in the infertile male.

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