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Okafor Chidimma Donatus
Department of Chemical Pathology Nnamdi Azikiwe University, Nnewi campus, Nnewi, Anambra State, Nigeria

Meludu Samuel Chukwuemeka
Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi campus, Nnewi, Anambra State, Nigeria

Nwaefulu Kester
Department of Human Physiology Nnamdi Azikiwe University, Nnewi campus, Nnewi, Anambra State, Nigeria

Ikechebelu Joseph Ifeanyichukwu
Department of Obstetrics and Gynaecology, Nnamdi Azikiwe University, Nnewi campus, Nnewi, Anambra State, Nigeria

Dioka Chudi Emmanuel
Department of Chemical Pathology Nnamdi Azikiwe University, Nnewi campus, Nnewi, Anambra State, Nigeria

Obi Ugochukwu
Department of Chemical Pathology, Nnamdi Azikiwe University, Nnewi campus, Nnewi, Anambra State, Nigeria

Correspondence:
Okafor Chidimma Donatus
Department of Chemical Pathology Nnamdi Azikiwe University, Nnewi campus, Nnewi, Anambra State, Nigeria

Comparison of serum oxidative stress markers among male partners of fertile and infertile couple at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria

Okafor Chidimma Donatus, Meludu Samuel Chukwuemeka, Nwaefulu Kester, Ikechebelu Joseph Ifeanyichukwu, Dioka Chudi Emmanuel, Obi Ugochukwu*

Abstract

Infertility is a common cause of marital disharmony in Nigeria because of the high premium placed on child bearing. Male factor is a significant contributor and declining male fertility rate has been observed over the past decade. Oxidative stress has been implicated in the aetiology of male infertility. The objective of the study was to compare the serum levels of oxidative stress markers (TAC – Total Antioxidant Capacity, MDA - Malondialdehyde and SOD – Superoxide dismutase) in male partners of fertile and infertile couples. This was a case controlled study carried out over a period of 8 months on male partners of infertile couple. Thirty-six were male partners of fertile couple with normal semen parameters and no history of infertility while 73 were male partners of infertile couple which consists of 28 males with oligospermia, 28 with oligoasthenospermia and 17 with asthenospermia.. Serum markers of oxidative stress (TAC, MDA and SOD) were evaluated and compared among the group. The male partners of infertile couple showed significantly low levels of TAC (P = 0.000), SOD (P = 0.000) and significantly high levels of MDA (P = 0.000) when compared with male partners of fertile couple (750.93±146.37 versus 1269.20±330.79, 11.22±3.33 versus 13.96±1.38 and 2.48±1.20 versus 1.51±0.19). Oxidative stress may be a major contributor to male infertility. Its assay may be of importance in prevention and treatment of male infertility.

Keywords: Oxidative stress markers, Oligospermia, Asthenospermia, Male infertility.

INTRODUCTION

Infertility is considered a serious problem in developing countries due to the premium placed on child bearing [1, 2]. It has been defined as the inability of a couple to conceive after twelve months of contraceptive-free sexual intercourse [2]. Approximately 8-15% of couples attempting their first pregnancy are unable to achieve it within one year of unprotected sexual intercourse [3, 4]. Research has shown that in approximately 30% of cases, pathology is found in the male partner alone and in another 20% abnormalities are found in both partners [5]. A Study on prevalence of male infertility done in Nnewi and Awka showed that male factor alone accounted for 42.4% of the infertile couple studied [6], therefore, male factor has been found to be partly responsible in at least 50% of infertile couples [1, 5, 7].

One of the important causes of male infertility is the excessive production of free radicals or reactive oxygen species (ROS) that can damage sperm. ROS has been implicated in various studies as one of the mechanisms of infertility [8].

Oxidative stress is a condition where the production of reactive oxygen species overwhelms antioxidant levels, has been considered as one of the major factors believed to be involved in idiopathic male infertility. It has been postulated that oxidants interfere with normal sperm plasma function via peroxidation of unsaturated fatty acids in the sperm plasma membrane which results in sperm dysfunction. In addition, ROS are known to attack DNA, inducing strand breaks and other oxidative based damage in spermatozoa. High levels of ROS endanger sperm motility, viability and increased midpiece sperm defects that impair sperm capacitation and acrosome reaction. The fertilizing ability of human spermatozoa is inversely related to the sperm ROS production [9, 10].

Scientifically, causes of male infertility abound, and the role of oxidative stress and reproductive hormones in the aetiology cannot be over emphasized [9, 10, 11]. Male infertility is associated with a reduction in the quality of sperm. It has also been found that decrease in sperm density, eventually leading to severe oligospermia and azoospermia is associated with raised FSH, LH and low testosterone level in gonadal dysfunction and reduced FSH and LH levels in pituitary lesion and oxidative stress [4, 9, 12].

Evidence now suggests that reactive oxygen species (ROS)-mediated damage to sperm is a significant contributing pathology in 30–80% of cases. ROS, at high levels, are potentially toxic to sperm quality and function. Also, additional reports have indicated that high levels of ROS are detected in semen samples of 25% to 40% of infertile men [12].

The assessment of seminal oxidative stress and sperm DNA damage, together with sperm parameters and hormone analysis may play an important role in the diagnosis and treatment of male infertility [13].

The objective of this study is to compare the serum levels of oxidative stress markers among fertile and infertile male partners.

SUBJECTS AND METHODS

Study design

This was a case control study designed to evaluate and compare the levels of SOD, TAC and MDA between male partners of infertile couple with abnormal semen parameters and male partners of fertile of fertile couple with normospermia.

Study population

The study population involved male partners of infertile couples with abnormal semen parameters (oligospermia and asthenospermia) being evaluated for infertility at Gynaecology Department of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria and eligible healthy male partners of fertile couple with normospermia and no history of infertility. The result obtained was compared among the group. Inclusion criteria for the partners with abnormal semen parameters (test group) was history of infertility. The controls were male partners of fertile couple of comparable age limit with no history of infertility. Exclusion criteria included male partners with history of unprotected sexual intercourse of less than one year duration, chronic illness like diabetes mellitus and malignancies, chronic chain smokers, recent acute illness, varicocele, genital tract infection and anatomical abnormalities like obstruction of vas deferens including previous vasectomy. The tests and controls were sampled with a well-designed questionnaire, administered by the researcher.

Blood sample collection

Using a sterile syringe and needle, blood was drawn from the cubital vein into an appropriate tube. The samples in the plain tubes were allowed to clot undisturbed and serum were separated by centrifugation for 10min at 4000rpm into plain tubes prior to analysis.

Biochemical analysis

MDA, SOD, TAC were determined using Gutteridge and Wilkins (1982), Misra and Fredovich (1972), and Benzie and Strain (1996) methods respectively [14, 15, 16].

Principle

MDA assay was based on the intensity of colour produced when thiobarbituric acid (TBA) is heated under alkaline condition. The intensity of colour generated is directly proportional to the concentration of MDA in the sample.

SOD assay is based on the ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2.

Total antioxidant activity (TAC) was estimated by Ferric Reducing Ability of Plasma (FRAP) method.

Statistical analysis

The statistical package for social sciences (SPSS) software package version 21 was used for analysis. Values obtained from this study were expressed as mean and standard deviation when compared using independent 't' test.

Ethics Review and Approval

This research work was reviewed and approved by Nnamdi Azikiwe University Teaching Hospital Ethics Committee.

RESULTS

The study population consisted of male partners of fertile couple (control group) and male partners of infertile couple (test group). Thirty-six were male partners of fertile couple with normal semen parameters and no history of infertility while 73 were male partners of infertile couple consisting of 28 males with oligospermia, 28 with oligoasthenospermia and 17 with asthenospermia.

Table 1: There was no statistical significant difference (P=0.088) in the mean ages between the control group and the test group (37.11±9.56 years versus 40.25±8.63 years). The mean sperm count for test group was 13.20±6.11x10^6cells/ml and 46.46±18.82x10^6cells/ml for controls, P=0.000 (t-test). The sperm motility for the test group was 25.60±23.73% and 59.61±15.16 for control group, P=0.0000 (t-test).

Table 1: Mean age, sperm count and motility in fertile and infertile group

Parameters	Infertile (Test) group	Fertile (Control) group	t-test	P-value
Age (Years)	40.25±8.63	37.11±9.56	1.721	0.088
Sperm Count (x10^6cells/ml)	13.20±16.11	46.46±18.82	-9.580	0.000
Sperm Motility (%)	25.60±23.73	59.61±15.16	-7.835	0.000

*****Result is significant at P<0.05

Table 2: Serum TAC was significantly higher in control group than in test group (1269.20±330.79 vs 750.93±146.37 umol/l. P=0.000) (t-test). Serum MDA levels was significantly higher in test when compared to control group (2.48±1.20 vs 1.51±0.19 nmol/ml. P=0.000) respectively. Serum SOD was significantly lower in the test group when compared to control group (11.11±3.33 vs 13.96±1.38 U/ml. P=0.000).

Table 2: Comparison of TAC, MDA and SOD Serum Levels in Infertile and Fertile Male Partners (Mean±SD).

Parameters	Infertile Partners (test) n=73	Fertile Partners (control) n=36	t-test	P-value
TAC (umol/l)	750.93±146.37	1269.20±330.79	-11.357	0.000
MDA (nmol/ml)	2.48±1.20	1.51±0.19	4.811	0.000
SOD (U/ml)	11.22±3.33	13.96±1.38	-4.723	0.000

*Result is significant at P<0.05

KEY: TAC – Total Antioxidant Capacity, MDA – Malondialdehyde, SOD – Superoxide Dismutase

DISCUSSION

This study was carried on age comparable groups and it showed that the mean serum level of TAC was significantly lower in male partners of infertile couple with abnormal semen parameters when compared to fertile male partners with normal semen parameters. Similar finding was reported by Pranjali *et al.,* 2013 [17]. Work done by Fazeli *et al*., in 2016 showed that there is no significant difference in the mean level of TAC in oligospermic males when compared to fertile males while asthenospermic males showed significant lower mean level of TAC when compared with fertile males [18]. Appasamy *et al.,* 2007 reported a significant lower mean blood concentration of TAC in oligospermic men when compared to normospermic men [19]. The study also showed that the mean serum level of MDA was significantly higher in male partners of infertile couple with abnormal semen parameters when compared to fertile male partners with normal semen parameters. This is similar to the work done by Fazeli *et al* in 2016 where mean levels of MDA was found to be higher in male partners with oligospermia and asthenospermia when compared to the fertile men [18]. Similar findings were reported by Naher *et al.,* 2011 and Bayejid *et al*., 2015 [13, 20]. SOD in this study was found to be significantly lower in male partners of infertile couple with abnormal semen parameters when compared to fertile male partners with normal semen parameters. Similar finding was reported by Bayejid *et al*., 2015 and Hasan *et al*., 2014 [10, 13]. The work done by Ashraf *et al.,* 2012, reported that the mean level of SOD was significantly lower in oligospermic when compared to normospermic patients [9]. This shows oxidative stress may have contributed to the oligospermia and asthenospermia found in the male partners of infertile couple with abnormal semen parameters, therefore responsible for their infertility

CONCLUSION

The infertile group showed abnormal levels of MDA TAC and SOD when compared to fertile group. This suggest that oxidative stress may be responsible for their infertility and thus its (TAC, SOD and MDA) assays may be useful in the evaluation and treatment of male infertility. In addition, these markers may provide a guide to antioxidant therapy.

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Conflict of Interest

We declare no conflict of interest

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