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Comparison of Sperm Indices Selected Markers of Oxidative Stress and Sex Hormones among Males with Primary and Secondary Infertility in Osogbo, Nigeria

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Abstract

This study seeks to determine the frequency and the differences in the more accentuating factors between primary and secondary male infertility. Four hundred men evaluated for infertility were consecutively recruited for the study. The socio-demographic information was obtained by interviewer-administered questionnaires. After a medical history and physical examination, their semen was evaluated manually according to WHO guidelines. Serum gonadotrophin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), vitamin B_{12}, vitamin A, vitamin C, vitamin E, and glutathione (GSH) were determined. The results were compared by unpaired Students' t-test while categorical variables were compared using chi-square. The frequencies of secondary infertility 129(32.3%) was lower ($p<0.001$) than primary infertility in the study population. The secondary infertile men were older ($p<0.001$), had lower levels of sperm count ($p<0.046$), serum vitamin E ($p<0.033$), and testosterone ($p<0.001$) than the primary infertile men. Other independent associated variables were socioeconomic status and duration of the marriage. Serum vitamin E levels correlated negatively with sperm count ($r= -0.162;p<0.005$) while vitamin C correlated negatively with sperm count ($r=-0.136;p<0.02$) and morphology ($r= -0.144; p<0.04$). The mean serum concentrations of vitamin B_{12}, vitamin E, vitamin C, and GSH were not significantly different between primary and secondary infertile males. The more noticeable differences are age, duration of the marriage, levels of vitamin E and testosterone. The levels of vitamin E, testosterone, and sperm count were lower among men with secondary infertility than primary infertility.

Keywords: Male, spermatozoa, oxidative stress, gonadal steroid hormones

Introduction

The decline in fertility potential among humans over the decades has been attributed to several factors such as changes in lifestyle, environmental pollution, consumption of processed foods, stress toxins, and sexually transmitted infections (1, 2). Some have attributed the high rate of male factor infertility to increasing industrialization and the predisposition of individuals to hazardous chemicals, and electromagnetic waves in the environment. These could lead to oxidative stress due to increased generation of reactive oxygen species (ROS) in the body more than the capacity of the naturally available antioxidants to scavenge and have been associated with poor semen quality (3-5). The ability of spermatozoa to fertilize a functional ovum is considered the ultimate function of sperm cells. Male infertility is associated with the quality and quantity of spermatozoa in the seminal fluid. Abnormal sperm characteristics constitute up to 90% of cases of male infertility (6). Infertility can be classified into primary or secondary infertility. Primary infertility describes a man who has never been able to impregnate a fertile female partner after a minimum of 12 months of unprotected sexual intercourse while secondary infertility is the inability to achieve pregnancy in a couple who have had at least one successful pregnancy in the past (7). Male infertility may be due to abnormal sperm indices, hormonal abnormalities, age of couples, retrograde ejaculation, erectile dysfunction, complicated sexually transmitted infections, occupation and socio-economic status, lifestyle changes, and diet (8-10). Some authors have suggested that the malefactor is associated more with primary infertility than secondary infertility while female factor causes are associated more with secondary infertility (11, 12). Studies that relate vitamin deficiencies with male infertility are scarce in our setting. Vitamin C is a powerful antioxidant and contributes up to 65% of the seminal plasma antioxidant content which is 10 times more than the concentration in the plasma (13). Deficiency of vitamin C has been associated with low sperm count, morphology, and motility (14). Vitamin E is a lipid soluble chain-breaking antioxidant and is also involved in several physiological processes ranging from immune function, regulation of inflammation, gene expression and cognitive performance (15). Studies have shown that poor nutrition and high incidence of oxidative stressors including malaria and other infectious diseases predispose individuals in developing countries to vitamin E deficiency. Some authors have shown that men may be at higher risk for deficiency than women (15). It is not however known whether the concentrations of these

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antioxidants are different among males with primary infertility from those with secondary infertility. This is particularly important because a study that evaluated semen parameters, serum testosterone, and oxidative stress in primary and secondary infertile men showed that the mean seminal total antioxidant capacity, malondialdehyde, and serum testosterone were not significantly different between males with primary and secondary infertility. In the same vein, semen volume, sperm count, sperm motility, and sperm morphology were also non-significantly different (16). The objective of this study was to determine whether there are differences in the sperm indices, serum concentrations of vitamin C, vitamin E, GSH, gonadotrophin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone between primary infertile males and secondary infertile males. It also seeks to correlate the levels of serum vitamin C, vitamin E with sperm count, motility, and morphology among males investigated for infertility.

Materials and Methods

This is a cross-sectional study of men who were evaluated for infertility. They were consecutively recruited from among men who were investigated for infertility at fertility clinics of tertiary and secondary health facilities in Osogbo, Osun State. Four hundred volunteers infertile males (test group), aged 20-60 years were recruited and further grouped as primary and secondary infertility as appropriate by the attending physicians. Two hundred and seventy-one of the men had primary infertility while 129 of them had secondary infertility. One hundred men of proven fertility, aged 21-59 years were used as controls. The protocol of the study was approved by the Health Research Ethics Committee of Osun State Ministry of Health, Abere, Osogbo, Osun State (Ref. OSHREC/PRS/569/149) dated 30th November 2017. All study participants were enlightened on the nature of the study and informed consent was given before specimens were collected.

Inclusion and Exclusion Criteria

Only the male subjects who gave consent to be enrolled without physical abnormalities or chronic illnesses were included in the study. Individuals with known pathological or congenital conditions such as hypertension, diabetes mellitus, sexually transmitted diseases, testicular varicocele, and genital warts were excluded. In addition, individuals currently on antioxidant supplementation, smokers, and alcoholics were also excluded.

Sample Collection

Semen samples were collected in a sterile container by self or assisted masturbation after at least 72 hours of sexual abstinence (without the use of spermicidal lubricants). The specimens were delivered to the laboratory within 30 minutes of ejaculation. Two specimens were collected at different visits within two months for analysis and the mean value of the determinations was used.

Blood/Serum

Five milliliters of venous blood sample was collected in the morning from subjects aseptically by veinpuncture into vacutainer plain tubes (bottles) with minimum stasis. The blood was allowed to clot, retracted, and was thereafter centrifuged at 3000rpm for 10 minutes. The supernatant was separated into a vacutainer plain tube and stored at -80℃ until biochemical Analyses were done.

Laboratory Analysis and Techniques

Routine Semen Analysis

After the liquefaction, the semen specimens were assessed for volume, appearance, pH, and viscosity. Routine semen analysis was performed microscopically according to the WHO criteria (17) with a special interest in the sperm concentration, percentage motility, and percentage morphology. Serum vitamin C and vitamin E were assayed by colorimetric method using reagents supplied by Kamiya Biomedical company, Copenhagen and Elabscience, China respectively. Appropriate calibrators and quality control sera were used to ensure the accuracy of analytes.

Determination of serum vitamin C.

The enzymatic method quantifies serum vitamin C based on the oxidation of L-Ascorbate (vitamin C) by Ascorbate Oxidase to produce hydrogen peroxide. Peroxidase catalyzes the hydrogen peroxide to water and oxygen. The oxygen reacts with the unique chromogen to form blue color.

Determination of serum vitamin E

Serum vitamin E reduces Fe^{3+} to Fe^{2+} in the presence of ferroin. The Fe^{2+} reacts with phenanthroline to form a pink color. This color is then read using a spectrophotometer.

Determination of serum vitamin A and vitamin B_{12}

Serum vitamin A and vitamin B_{12} were determined by ELISA technique using reagents supplied by Lifespan Biosciences, Seattle, USA, and Eagle Biosciences respectively. The essential reagents required for an enzyme immunoassay include an antibody, enzyme–antigen conjugate, and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the micro well occurs. This affects the separation of the antibody-bound fraction after decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose-response curve was generated from which the antigen concentration of the unknown was determined.

Determination of serum Testosterone, GnRH, FSH, and LH

Serum GnRH, FSH, LH, and testosterone were assayed by Cobas e411 autoanalyzer, Roche diagnostics, Germany. The analyzer uses the principle of the electrochemiluminescence immunoassay technique.

Statistical Analyses

The data generated from the study were compared between the groups using unpaired Students-t-test by statistical software SPSS version 21 (SPSS Inc, Chicago, IL, USA). Categorical data were compared using Chi-square. A p-value ≤ 0.05 was considered statistically significant.

Results

Figure 1 shows the pie chart of males investigated for infertility and it indicates that 271(67.7%) were suffering from primary infertility while 129 (32.3%) had secondary infertility. Table 1 shows the comparison of measured parameters between primary and secondary infertile men. The mean age, socio-economic status, and duration of marriage were significantly different between primary and secondary infertile males.

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Serum vitamin E ($p<0.033$) and testosterone ($p<0.001$) were significantly lower among men with secondary infertility than those with primary infertility.

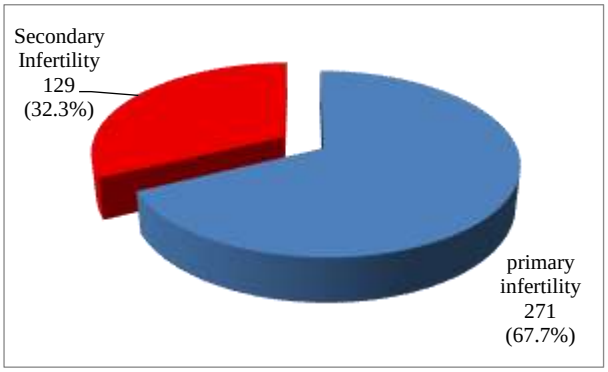

Figure 1: Pie chart of males with primary and secondary infertility

Even though serum vitamin C, vitamin B_{12}, GSH, GnRH, and LH were lower among men with secondary infertility than primary infertility, the differences were not statistically significant. Serum FSH was higher among secondary infertile than primary infertile males but the difference was not significant. Table 2 indicates that sperm count was significantly lower ($p<0.04$) among secondary infertile men than primary infertile men. There were no significant differences between the mean levels of pH, volume, percentage motility, and percentage morphology. Generally, serum vitamin E levels correlated negatively with sperm count ($r= -0.162$; $p<0.005$) while vitamin C correlated negatively with sperm count ($r=-0.136$; $p<0.02$) and morphology ($r= -0.144$; $p<0.04$). The mean serum concentrations of vitamin B_{12}, vitamin E, vitamin C, and GSH were significantly lower among infertile males than fertile male control subjects (Table 4).

Table 1: comparison of measured parameters between primary and secondary infertile males (mean±SD)

Variables	Primary infertility (n=271)	Secondary infertility (n=129)	P-value
Age (Years)	35.4±3.8	40.2±2.4	0.001
Socio-economic status			
Low	164 (60.5%)	73(56.6%)	0.001
Average and High	107 (39.5%)	56(43.4%)	0.001
Duration of Marriage			
<5years	186 (68.6%)	29 (22.5%)	0.001
>5years	85 (31.4%)	100 (77.5%)	0.001
Vitamin E (µmol/L)	2.12 ± 0.06	1.91 ± 0.07	0.033
Vitamin C (µmol/L)	7.14±0.13	7.15±0.00	0.963
Vitamin B12(pg/mL)	371±10.2	368±11.2	0.195
GSH (µmol/L)	0.40 ± 0.01	0.39 ± 0.01	0.772
GnRH (pg/mL)	25.81 ± 1.03	23.58 ± 1.18	0.189
FSH (mIU/mL)	5.56 ± 0.29	6.16 ± 0.45	0.243
LH (mIU/mL)	5.59±0.17	5.54±0.27	0.243
Testosterone (ng/mL)	4.85±0.19	4.16±0.10	0.001

GSH: glutathione; GnRH: gonadotropin releasing hormone; FSH: follicle stimulating hormone; LH: luteinizing hormone.

Table 2: Comparison of Sperm indices between males with Primary and Secondary infertility (Mean±SD)

Parameters	Primary Infertility	Secondary Infertility	P-Value
pH	8.11 ± 0.03	8.13 ± 0.05	0.684
Volume (mL)	3.04 ± 0.09	2.99 ± 0.17	0.826
Sperm Concentration($x10^6$/mL)	38.42±3.02	28.74±2.99	0.046
Motility (%)	36.63+0.93	36.13±1.31	0.761
Morphology (%)	33.63 ± 0.95	33.17 ± 1.39	0.786

Comparison of parameters between primary and secondary infertility shows that only the sperm concentration shows a significant difference between the two types of infertility, sperm concentration in primary infertility (38.42±3.02) was higher than secondary infertility (28.74±2.99) with p=0.046.

Table 3: Correlation of Vitamins E, C and sperm indices

Correlation	Vitamin E		Vitamin C	
	R	P	R	P
Sperm Count	-0.162	0.005	-0.136	0.02
Motility	-0.041	0.410	-0.094	0.061
Morphology	-0.044	0.377	-0.114	0.04

Table 4: Comparison of Oxidative Stress Levels between Subject and Controls (Mean±SD)

Stress Biomarkers	Test Mean ± *SD* (*n*=400)	Control Mean ± *SD* (*n*=100)	*P-value*
Vitamin B_{12}(pg/ml)	376±14.26	465.85±14.26	0.001
Vitamin E(µmol/L)	2.05±0.92	23.44±9.05	0.001
Vitamin C(µmol/L)	7.15±2.22	39.012.23	0.001
GSH	0.40±0.04	0.80±0.25	0.001

GSH= Glutathione

Discussion

Studies have reported beneficial effects of vitamin E, vitamin C and GSH on the prevention of lipid peroxidation of sperm membrane, on sperm DNA integrity, and on the capacity of spermatozoa to navigate the female reproductive tract, bind and penetrate the oocyte in human studies (18-20). It is not clear whether deficiencies of these antioxidants that occur in male infertility are more severe among individuals with primary infertility than secondary infertility. The rates of primary and secondary male infertility were 67.7% and 32.3% respectively. Secondary infertility was the predominant type of male infertility in this study. The result is consistent with the 62.9% rate of primary infertility reported in Lagos, Nigeria (21) but slightly lower than the 59% rate of primary infertility reported in Nnewi, southeast Nigeria (22). The rates were lower than rates reported elsewhere outside Nigeria (23-25). Whereas 78.9% prevalence of primary male infertility was reported among infertile males in Saudi Arabia (23), Abdalla (24) reported a prevalence of primary male infertility of 77.4% in Sudan, and a rate of 77.4% primary male infertility was observed among subjects evaluated for infertility medical care in Turkey (25).

The mean age of subjects with secondary infertility was higher ($p<0.001$) than those with primary infertility. This observation is consistent with previous studies (23, 24). It was observed that subjects with primary infertility were younger than those with secondary infertility and the duration of marriage was less than 5 years. This is an indication of possible delay on the part of the subjects with primary infertility in seeking medical assistance. There could be several reasons for this, but the African socio-cultural setting has before now focused on the female as the source of infertility in marriages (26) and fertility challenges are shared by both male and female sexes. The men are less likely to seek fertility evaluation than females in our setting. The need for quick medical evaluation and assistance cannot be over-emphasized. Early diagnosis of male infertility is important to enable quick and effective treatment. The difference between primary and secondary male infertility may be associated with the following independent variables; age (RR=1.13), socio-economic status (RR=1.5), duration of marriage (OR=7.5; 2.6-58.2), vitamin E (RR=1.11), and testosterone (RR=1.17). This is partly similar to the report from Morocco (27). In the study of the difference between primary and secondary infertility in Morocco, three independent variables such as the duration of marriage (OR=12.263; 2.289-65.685), the age of women (OR=1.268), and socio-economic status (OR=3.83) were observed to be associated with male infertility (27). The age of female partners of the participants was not determined in this study.

The mean levels of vitamin E were lower ($p<0.033$) among men with secondary infertility than primary infertility in this study. Vitamin E is one of the most powerful antioxidants that scavenge free radicals in order to protect biomembranes and lipoproteins from oxidative damage. Lipid hydroperoxides (ROOH) are oxidized to peroxyl radicals ($ROO^{\cdot}$) in the presence of free metals such as iron or copper. The peroxyl radicals react more readily with vitamin E than with polyunsaturated fatty acids, hence preventing further oxidation (15). Again, tocopheroxyl radical then interacts with hydrogen donors such as vitamin C and gluthathione to return vitamin to its reduced state. This probably indicates that more vitamin E is utilized in secondary infertile male than primary infertile male or the generation of free radicals is higher in secondary than primary infertile males. Serum levels of vitamin E is widely used as a biomarker of oxidative stress, but the levels in the circulation may be affected by some confounding factors such as age, concentrations of plasma lipids, use of lipid-lowering drugs, and smoking (28). Most of these factors were adequately controlled in design in this study. It has been established that vitamin E deficiency is accompanied by low levels of other antioxidants (15). Some authors have suggested that vitamin E deficiency may be due to inadequate intake and greater oxidative stress. Among adults, obesity and male gender may predispose individuals to vitamin E deficiency (29-31). Sperm count and morphology correlated inversely with vitamin E and vitamin C, indicating that they had adverse effects on spermatozoa. Low levels of antioxidants have been associated with male infertility.

The observation of significantly lower levels of serum testosterone among secondary infertile males than primary infertile males is not consistent with a study of semen parameters, serum testosterone, and oxidative stress among primary and secondary infertile males with varicocele (16). The authors observed that total testosterone level was insignificantly lower among secondary infertile males than primary infertile counterparts but serum free-testosterone was significantly lower in the secondary than primary infertility (16). It has been established that serum testosterone undergoes a gradual decline after the age of 40 years. The effect of age on circulating testosterone is exacerbated by chronic illnesses, prescription medication, obesity, and alcoholism (32) among humans.

The observed significantly lower sperm count among men with secondary infertility disagreed with the previous report in which individuals with primary infertility had significantly lower levels of sperm count than those with secondary infertility (33). Other authors reported non-significant differences in semen volume, sperm count, sperm motility, and sperm morphology (16).

Conclusion

The overall rates of primary and secondary male infertility are 67.7% and 32.3%. Lower levels of serum vitamin E and testosterone were observed among secondary infertile males than primary infertile males. The more noticeable differences are age, duration of the marriage, levels of vitamin E and testosterone. The levels of vitamin E, testosterone, and sperm count were lower among men with secondary infertility than primary infertility.

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Conflicts of Interest

None declared

Authors contributions

This work was conducted and approved in collaboration between all the authors. MAE designed the study; MAM sourced for funding; MAE, MAM wrote the protocol; MAE, MAM contributed in literature search; MAM did the experiments; MAE, MAM did statistical analysis; MAM drafted the manuscript; MAE supervised the study; MAE Wrote the final manuscript; MAE proofread the manuscript.

Ethical issue

This prospective cross-sectional study was conducted in a tertiary health institution with permission from the Health Research Ethics Committee of Osun State Ministry of Health, Abere, Osogbo, Osun State (Ref. OSHREC/PRS/569/149) dated 30th November 2017. This cross-sectional study involving human participants was under the ethical standards of the institutional and national health research committee and with the 1964 Helsinki Declaration as amended. Informed consent was given by all participants in the study.

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