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Effects of Oxidative Stress and Some Risk Factors Associated with Male Infertile Subjects in Jigawa, Nigeria

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***Abstract*: Male factor infertility is one of the discussed health issues globally in recent times. It accounts for about 20%-30% of infertility cases. A lot of factors involved significantly affect semen quality thereby contributing to male infertility. One hundred and twenty-six (126) participants were recruited in this study; 81 infertile and 45 control subjects. Socio-demographic characteristics of the participants were collected through interviewer administered questionnaire. Seminal fluid collected was analyzed using a computer-aided sperm analysis system. Serum sex hormone, 8-iso-PGF2α and antioxidant vitamins were accordingly determined using Elisa and colorimetric enzymatic techniques. Results obtained were compared among the groups. Significantly decreased levels of all antioxidant vitamins were observed among infertile subjects compared to controls. Results showed no significant change in FSH levels between the variables but elevated LH and a reduced testosterone values was observed among infertile. Furthermore, poor semen quality and elevated 8-iso-PGF2α was observed among infertile in comparison with control group. In conclusion, male infertility is a multi-factorial with smoking, alcohol consumption, oxidative stress, obesity and decreased vitamins playing a greater role in the course.**

***Keywords*: Male infertility, Oxidative stress, Risk-factors, Semen.**

1. Introduction

Infertility is one of the most important complications in gynecology and is defined as the inability to achieve pregnancy after one year of unprotected intercourse devoid of any contraceptive methods [1]. Male infertility factors particularly affect the normal sperm production with altered morphology and progressive motility [2].

In addition, factors such as obesity, smoking, nutritional deficiencies and oxidative stress may negatively contribute to reduce semen quality [3]. Recently, there are growing evidences and concerns regarding increase of male infertility worldwide with oxidative stress being one of the suspected culprit [4].

Reactive oxygen species (ROS) plays tremendous role in the development of sperm fertilization properties, promoting chromatin compaction in spermatozoa maturation, consequent hyperactivation, acrosome reaction and oocyte interaction [5].

Free radicals generated by the body naturally and through other unhealthy activities such as smoking, exposure to radiations etc., can consequently reduce body's antioxidant capacity causing an oxidative stress [6].

Oxidative stress occurs when the levels of free radicals produced surpasses the antioxidant substances and this can damage sperm DNA contributing to poor semen quality [2]. Semen quality is very important factor that reflects male reproductive health. It has been suggested that low semen quality may be a potential contributing factor in reducing fertility rates [7].

Epidemiological evidence suggests that antioxidant supplementation may play a key role in improving semen quality among infertile male. In this study, semen quality, sex hormones, oxidative stress status were estimated, in male infertile subjects and the results compared to fertile control group in Rasheed Shekoni Teaching Hospital Dutse, Jigawa, Nigeria.

2. Methods

This study was cross sectional analytical study. It was conducted in Gyanaecology clinic of Rasheed Shekoni teaching hospital (RSTH), Dutse. RSTH a tertiary teaching hospital located in Dutse, Jigawa state, Nigeria. The study population was clinically diagnosed 81 male infertile patients attending

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Gyanaecology clinic Rasheed Shekoni teaching hospital and 45 apparently healthy fertile control subjects.

Data and Specimens Collection: Demographic (data) information (such as age, infertility history, smoking and alcohol consumption) of the patients was collected through interviewer administered questionnaires. About 5mls of whole blood was collected from the study subjects using Vacutainer system. It was centrifuged at 15000 rpm for 5 minutes to obtain neat serum. Semen specimens were collected through masturbation after 2–7 days of sexual abstinence according to the 2010 WHO guidelines. Seminal fluid analysis was done using computer-aided (CFT-9201; Jiangsu Rich Life Science Instrument Co., Ltd., Nanjing, China).

Biochemical Parameters analysis: Vitamin A, C and E were determined using enzyme-linked immunoassay (ELISA) techniques. Assay principle of 8-iso-PGF2α was based on competitive enzyme-linked immunoassay (ELISA). While the serum levels of Testesterone, LH and FSH were assayed using high sensitive Eliza kits.

Statistical analysis: Values were expressed as mean ± standard deviation. The variables were analyzed statistically using one way analysis of variance (ANOVA). Differences were considered as significant when $P<0.05$. Pearson correlation product was conducted to find out the relationship between the parameters.

3. Results

Table 1
Socio-demographic characteristics of the study subjects

Parameters	Participants	
	Infertile (n=81)	**Controls (n=45)**
Mean Age (year)	35.7±5.4	35.9±5.6
BMI (kg/m^2)	25.5±4.1	22.6±3.0
Infertility type		
Primary	64 (79.0%)	
Secondary	36 (36.0%)	
Smoking		
Smokers	30(37.0%)	11(24.4%)
Non smokers	51(63.0%)	34(75.6%)
Alcohol consumption		
Non-Alcoholics	68(83.9%)	44(97.8%)
Alcoholics	13(16.1%)	01(2.2%)

One hundred and twenty-six (126) participants were recruited in this study, comprising of 81 infertile subjects and 45 apparently healthy controls. The mean age of infertile group is 35.7, and is not statistically different to the mean age of control group. Cases of primary infertility among the test subjects constitute about 79% with remaining 36% being the secondary type. About 37% and 24.4% of infertile and control subjects smokes respectively. Thirteen (13) out 81 infertile subjects consume alcohol while only person was found to have ever consumed alcohol among the control group.

Serum levels of antioxidant vitamins A, C and E were evaluated among the study subjects, values for all the vitamins of infertile were significantly different with control group. 8-iso-PGF2α was determined as a marker of oxidative stress and the values in infertile subjects is statistically higher than in normal persons.

Table 2
Serum antioxidant vitamins and oxidative stress marker in infertile and control subjects

Parameters	Study subjects	
	Infertile (n=81)	**Controls (n=45)**
Vitamin A (μg/dL)	20.0±2.0	28.3±2.7
Vitamin C (mg/dL)	0.31±0.04	0.48±0.08
Vitamin E (mg/dL)	0.45±0.06	0.65±0.05
PGF (pg/mL)	100.6±4.6	69.9±10.9

n=number of subjects

Table 3
Serum levels of some sex hormones among the study subjects

Subjects	Sex Hormones		
	LH (ng/mol)	**FSH (ng/mol)**	**Testosterone (ng/mol)**
Infertile (n=81)	6.8±0.5	11.2±1.6	3.5±2.6
Controls (n=45)	6.9±6.6	11.8±1.7	8.3±2.2

LH=Luteinizing hormone; FSH=Follicle stimulating hormone; n=number of subjects

No significant difference was found in the levels of luteinizing hormone and follicle stimulating hormone between infertile and control subjects, however, serum testosterone levels in infertile and control subjects were 3.5±2.6 and 8.3±2.2 respectively. Majority (60%) of infertile subjects have testosterone levels below normal range.

Seminal fluid analysis (SFA) was conducted on the total of 126 participants. Out of 81 infertile subjects, 8.6% have semen volume below 1.5mls, 87.7% have values ranging between 1.5-

Table 4
Comparison of some seminal fluid characteristics of fertile and infertile subjects

Parameters		Study subjects	
		Infertile (n=81)	**Controls (n=45)**
Seminal fluid volume (mls)	Less than 1.5	7(8.6%)	2(4.4%)
	1.5-4.0	71(87.7%)	26(57.8%)
	4.1-7.6	3(3.7%)	15(33.3%)
	Above 7.6	0	2(4.4%)
		2.4±0.9	3.7±1.4
Sperm Count X10^6	Less than 1M	09(11.1%)	0(0%)
	1M-9M	24(29.6%)	09(20%)
	10M-20M	48(59.3%)	34(75.6%)
	Above 20M	0(0%)	02(4.4%)
		11.2±3.4	12.2±3.6
Sperm Motility	Progressive	54(66.7%)	38(84.4%)
	Non progressive	20(24.7%)	04(8.9%)
	Non-Linear	05(6.2%)	02(4.4%)
	Immotile	02(2.4%)	01(2.2%)

4.0mls and only 3 persons have volume of 4.1-7.6mls. Most of the infertile subjects (59.3%) have normal distribution of sperm count ranging from 10-20 million, while 11% of them were found to have a sperm count of less than 1million.

A Pearson product-moment correlation was conducted to examine the relationship between risk-factors such as smoking, alcohol consumption, BMI and levels of 8-isoPGF2 alpha among male with infertility. PGF2a strongly negative association alcohol consumption, BMI and smoking = -.0580, -.097 and -.0292 n=81, $p < .001$, respectively.

4. Discussion

Male-factor infertility is one of the well-known health issues reported all over the world including Nigeria and other developing countries. Our study documented 64% of primary infertility cases among the study population; this corresponds to the earlier research of [8] who reported 68.4% and 31.6% of primary and secondary infertility cases respectively. In another study on epidemiology of infertility by [9] it is reported that male factor is associated with a greater percentage of primary cases rather than secondary infertility.

Cigarette smoking is an important variable that affects different semen parameters which leads to decrease concentration, morphology and motility of sperms [10]. Earlier study of [11] provided results concerning the effects of smoking on semen parameters of male infertile subjects. Present study constituted of about 30 (37.0%) smokers and 51 (63.0%) non-smokers, it is further shown in this research that decreased circulating levels of LH, FSH and testosterone exists among the smokers compared to non-smokers and this can negatively impact spermatogenesis [12]. reported a 19% lower sperm count among heavy infertile smokers. Furthermore, it is imperative to note that single cigarette is reported to have contained 1.5µg of cadmium and this may consequently lead to asthenoteratozoospermia.

According to our results, 16.1% among infertile patients are alcoholics and the evidence is accumulating regarding the excess free radicals generation among the alcoholic consumers of existing condition, In another study on the effect of alcohol on male reproductive system by [13] alcohol consumption causes hypogonadism and testicular failure which in turn may cause infertility. However, alcoholic women are reported to experience high rates of ovulation disorders, abortions and endometriosis as well infertility than non-alcoholics [14].

Mean values of BMI among the infertile male are statistically higher than the control group, this finding agrees with previous studies of [15], [16] who both reported high values of BMI in infertile patients. Sedentary life style in conjunction with some genetic factors might be a predisposing factor to obesity which may consequently causes male impotency [17] reported that overweight infertile group had a slightly decreased sperm concentration and sperm count with altered sex hormone profile compared with normal BMI group. Elsewhere, a cross-sectional study conducted in Australia on 225 infertile male concluded that obesity was significantly related to reduce sperm counts. [18] Obese men are more likely to be infertile, obviously due to obesity-induced endocrine derangements and its deleterious effects on spermatogenesis.

Serum levels of antioxidant vitamins A, C and E were determined among the study subjects, our results showed significantly lower levels of vitamins among infertile group compared with control group. Results of the current study correspond to the earlier researches by [19]-[21] who found deficient vitamin C level among infertile male. Decreased vitamin C is associated with increased free radicals that can interfere with normal spermatogenesis [120] further demonstrated that synergistic effect of vitamin C and E can shield spermatozoa against peri oxidative action and DNA fragmentation. Similarly, in another study by [22] in which a combined therapy of vitamin E and selenium were administered for six months, there was a statistical increase in sperm motility and a reduced percentage of defective spermatozoa compared to pre-supplementation period. However, this is contrast with recent findings of [23] involving 822 couples in a randomized clinical trial which reported that antioxidant treatment does not improve semen parameters or DNA integrity in infertile males.

8-isoPGF2α was assayed as an oxidative stress marker to ascertain oxidative stress status of the study groups. 8-isoPGF2α is the most sensitive, specific and stable by-product formed as a result of free radicals action on PUFA. According to our results, 8-isoPGF2α levels was found to be excessively high in infertile compared with fertile control group. Appropriate balance of antioxidants and ROS is critical in acrosome reaction, motility as well as capacitation of sperm cells [24] however, when the levels of free radicals outweighed antioxidant system can result in DNA damage and destruction of spermatozoa leading to infertility. Previously, growing evidences have indicated the role of total antioxidant capacity (TAC) in male infertility [25]-[28] who agrees with our recent findings that oxidative stress is inversely correlated with normal motility, counts as well as overall function of sperm and male infertility. Furthermore, [29] investigated the correlation of antioxidant biomarker specifically malondialdehyde (MDA) regarding male reproductive potential among infertile and the results are consistent with our findings of increased 8-isoPGF2α and decreased antioxidant substances.

Some serum sex hormones (LH, FSH and testosterone) were assayed in this study across the subjects because of their roles in spermatogenesis and sperm maturation. Our results showed no significant change in FSH levels between the variables but elevated LH and reduced testosterone was observed among infertile compared with non-fertile. Findings of this research work are in agreement with that of [30] who in addition revealed negative correlations of LH and FSH with sperm concentration, motility, and morphology among infertile men, this is in contrast with work of [31] which reported that only FSH levels had a negative correlation with semen parameters, while LH and testosterone levels did not. Furthermore, [32] found that LH, FSH and TT levels were all inversely associated with sperm motility among infertile male in China. Decreased circulating testosterone levels recorded among infertile patients and slightly raised LH may negatively impact normal spermatogenesis and in turn affects male fertility.

Many factors may be related to male infertility rather than

abnormal semen parameters; however low sperm counts, abnormal motility and morphology are tantamount to male impotency. In this study, a low seminal fluid volume and sperm count was observed among infertile group compared with control group. This is in line with previous work of [33] which reported reduced sperm counts of 74.93±40.16×10^6/mL. In another study on impact of oxidative stress on semen parameters in infertile men, sperm counts and seminal volume of 249.9±193.2 and 3.9±1.4 respectively was recorded among infertile men. Similarly, in another study conducted by [34] on the semen analysis of male partners of infertile couples in Nigeria, 42.5% of the subjects were reported to have sperm count of less than 20 million per ml. According to the values obtained in this research, normal semen analysis was present only in 36% among infertile patients of 29 out of 81, with sperm count 305.9±40.16×10^6/mL, progressive motility of 66%. Normal semen parameters do not guarantee fertility, however, the chances lies in physiological spermatogenesis and sperm maturation.

This study has several strengths such as inclusion of various factors, determination of oxidative stress using the most stable biomarker etc. however, can be limited to relatively small sample size. Secondly, only LH, FSH and testosterone were assayed with single measurement of circulating hormones which could not represent long-term levels. Additionally, one semen sample collection was used in this study as it may not well reflect a man's long-term values. Although, previous researches revealed no significant difference.

5. Conclusion

In conclusion, male infertility is a multi-factorial. Current study evaluated effect of obesity, Smoking, antioxidant vitamins, oxidative stress marker, sex hormones and semen parameters among infertile subjects and their relationship. Our findings suggest that oxidative stress and could result in sperm DNA damage affecting physiological spermatogenesis and in turn male fertility. Elevated BMI and deranged sex hormones noted among infertile group may as well contribute negatively to low semen quality.

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