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Original Research Article

The effects of stress biomarkers on sex hormones, sperm quality and quantity in infertile men in Abuja, Nigeria

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ABSTRACT

Background: Studies have shown the adverse effects of stress on infertility. It has been reported that 40% of infertility cases are attributed to the men. Therefore, this study is aimed at determining the effects of stress biomarkers on sex hormones, sperm quality, and quantity in men investigating infertility.
Method: A total of one hundred and fifty-two (152) participants were recruited into a case control study between September 2018 to August 2019. Prolactin, testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), salivary cortisol and salivary alpha amylase were estimated using both competitive and non-competitive enzyme linked immunosorbent assay (ELISA) techniques; semen was examined directly after liquefaction according to world health organization criteria.
Results: The mean and standard deviation of testosterone, FSH, sperm cells count and sperm activity (%) were significantly lower ($p<0.05$) in the men study group relative to the control group; while prolactin, salivary cortisol and alpha amylase were significantly higher ($p<0.05$) in the men study group relative to the control group. There was no significant difference in the value of LH in the men study group compared with the men control group ($p>0.05$).
Conclusions: Stress in men affects sex hormones, semen quality and quantity. Both conditions of oligospermia and azospermia were observed in the men study group, with associated hormonal abnormalities, decrease in sperm quality, quantity and elevated stress biomarkers.

Keywords: Men infertility, Salivary cortisol, Hormonal profile, Sperm cells count, Sperm cells active

INTRODUCTION

Male infertility, hormonal abnormalities, and stress are common all over the world affecting up to 14% of couples of childbearing age.[1,2] The prevalence of infertility is high in sub-Saharan Africa, ranging between 20-40%.[3] Although the Africa socio- cultural setting has before now focused on the female, fertility problems are obviously shared between both male and female sexes. Men infertility may account for up to 40% of infertility in couples.[4] Approximately one-third of the cases of infertility are equally attributable to man. Male infertility is established when identifiable female causes of infertility is excluded and when semen quantity and quality fails to meet WHO criteria.[5] Male infertility is referred to as male inability to cause pregnancy in the female who is fertile, male factor infertility accounts for about 45-50% of couple infertility.[6] In Nigeria, infertility is a growing health conditions, studies have shown that about 25% of couples are affected by infertility. Out of these numbers, the male factor of infertility accounts for 45-50% of the infertility cases.[7] A center for disease control and prevention (CDC) study analyzes data from the 2002 national survey of family growth and found that 7.5% of all sexually active men younger than 45 years,

were reported seeing a fertility doctor during their life-time; this is equal to 3.3-4.7 million men. Among men who sought for help in infertility, 20% were diagnosed with male related infertility problem, these includes sperm or semen abnormality (14%) and variococele (6%).[8]

The causes of male infertility could be pretesticular, testicular and post testicular.[9] The pretesticular and to some extent the testicular causes are mainly endocrine disorders originating from the hypothalamus-pituitary-gonadal- axis which have adverse effects on spermatogenesis and sex hormones. The main determinant of male potential is the quality and quantity of spermatozoa ejaculated during sexual intercourse.[10] Semen quality depends on factors such as lifestyle, environment, and sex hormone secretion.[11] Male fertility is critically dependent upon normal hormonal parameters. Evaluation of the sub fertile male requires a complete medical history, physical examination and specific laboratory investigation.[9] Several authors have suggested that the increased incidence of infertility in Africa is due to high prevalence of sexually transmitted disease.[9] It is observed that there are scares literatures on the effects of stress on sex hormones and sperm quality and quantity in infertile men in Northern Nigeria; this informed the bases of this research study.

METHODS

Study area

The study was carried out in the following major general hospitals: Asokoro, Garki, Gwarinpa, Maitama, Wuse, and departments of chemical pathology and microbiology laboratory of alpha royal medicals ltd, in federal capital territory (FCT), Abuja Nigeria.

Subject and sample

We carried out a case control study on the subjects who came for fertility clinic, between September 2018 to October 2019 one hundred and fifty-two participants were involved in the study, which consist of hundred infertile men and fifty two fertile men. Five milliliters of blood sample were drawn from each of the subject from the articular vein on their clinic visit days; the sample were allowed to clot after which; it was spin at 3000 rpm for 5 minutes; serum sample was then extracted from the clotted sample and then refrigerated at the temperature of 4-8 degree centigrade until analysis. Also, the saliva sample was collected into a universal container containing a preservative, Sodium benzoate. The Saliva sample is stable until analysis. The male participants were instructed to collect the semen sample after abstinence for 3-5 days, through withdrawal or masturbation into clean universal container, the semen samples were analyzed within 30-60 minutes after collection of specimens.

Ethical approval

Ethical approval was sought and obtained from the ethical clearance committee of health research ethics committee Abuja with reference number FHREC/2018/01/97/21-08-18 dated August 21, 2018.

Exclusion and inclusion criteria

Infertile male due to vasectomy, those who were less than 18 years and above 45 years and with chronic diseases were excluded, while those within 18-45 years and without any known use of contraceptive were included.

Informed consent

The purpose and protocol of the study were clearly explained to each patient and all participants were requested to voluntarily sign the consent forms in their own handwriting as proof of willingness to provide samples for the research work.

Data collection

Prior to specimen collection, demographic information of the participants were obtained through administration of prepared questionnaires. Interpreter was provided for translation where it was necessary. Each questionnaire had a unique participant identification number (PIDN). The first part of the questionnaires contained the bio data of the patients e.g. sex, age etc. The second part consists of duration of the condition of infertility. For reason of privacy, all data were kept confidential in accordance with world medical association declaration of Helsinki (WMA, 2008). For each participant, only the PIDN was recorded on the laboratory forms (no names). All the filled questionnaires were destroyed after data entry had been completed.

Hormonal assay and semen analysis

The serum LH, FSH, prolactin, testosterone, salivary cortisol and salivary alpha amylase were measured spectrophotometrically by the microwell ELISA technique based on the noncompetitive sandwich principle, in accordance with the methods provided by diagnostic reagent kit supplied by Darlez Nig Ltd.

Semen was obtained by masturbation and examined directly after liquefaction according to the 2010 world health organization criteria. Prior to the semen collection, the patients were asked to maintain a four days abstinence from sex and alcohol.

Statistical analysis

The demographic characteristics of the participants were expressed as mean values and standard deviation. Differences in serum hormones levels, salivary cortisol levels, salivary alpha amylase levels, sperm cells count

and sperm cell activity (%) levels between the study group and the control group were tested by student t test. P<0.05 considered statistically significant. Statistical package for social science (SPSS) window version 20.0 was used for all calculation and data analysis.

RESULTS

In these study sex hormones, some stress biomarkers, and semen analysis were determined in all the participants. The mean age of the participants are presented in Table 1.

The mean standard deviation (SD) of sex hormones, stress biomarkers and sperm parameters in the men study and control groups are presented in Table 2. Comparisons of measured hormones and stress biomarkers according to semen quality and quantity of the men category are presented in Table 3. Multiple comparisons of semen quality and quantity of measured variables among men are presented in Table 4 and Figures 1 and 2 respectively.

The mean age of the men subjects of the study group was (33.07±4.56) while that of the control group was (32.25±4.14) as shown in Table 1. They have been in infertility for a period of 4-5 years.

The mean (SD) of the FSH concentration of the men study group (4.50±2.20 MIU/ml l) was significantly lower (p<0.05) relative to that of the men control group (5.91±1.66 MIU/ml); mean value of the LH of the men study group (5.64±2.26 MIU/ml) show no statistical significance (p>0.05) to that of the men control group (5.43±1.66 MIU/ml); mean value of the prolactin of the men study group (18.01±11.56 ng/ml) was significantly higher (p<0.05) than that of the men control group (6.98±3.34 ng/ml). The level of testosterone in the men study group (3.44±2.35 ng/ml) was significantly lower (p<0.05) relative to those of the control group (5.86±1.55 ng/ml); the salivary cortisol level was equally significantly higher (p<0.05) in the study group (449.75±106.81) compared to the control group (340.65±72.53). Salivary alpha amylase level was significantly higher (p<0.05) in the study group (13.12±4.39) compared with the control group (8.45±3.01); the Sperm cell count ($\times 10^6$) was significantly lower (p<0.05) in the study group (19.42±26.08 cells/ml) compared with that of the control group (53.80±11.74 cells/ml); percentage of active sperm cells (%) was significantly lower (p<0.05) in study group (33.99±26.07) compared with the control group (49.10±14.80) as shown in table 2; the sperm cells viability was significantly lower in the study group relative to the control group (p>0.05). The mean ejaculate volume was also higher in the control group (p<0.05) when compared with the study group, (5.6 ml) versus (3.3 ml), as shown in Table 2.

Of the 100 men (study group) examined, 54 (54%) were normospermia, 33 (33%) were oligospermia, while 13 (13%) were azoospermia as shown in Table 3. There was statistical significant difference (p<0.05) in the hormones, biomarkers and semen quality measured across control group, normospermia, oligospermia, and azoospermia groups respectively as shown in Table 3.

The multiple comparisons of measured parameters of the different semen quality and quantity state and the control group. The prolactin, salivary alpha amylase and salivary cortisol level in the control were significantly higher than those of normospermia group (p<0.05), oligospermia (p<0.05) and azoospermia group (p<0.05); the LH level of the control group show no statistical significant difference from those of normospermia group (p>0.05) and oligospermia (p>0.05), but show significant difference (p<0.05) in azoospermia; there was no significant difference (p>0.05) in LH level of normosperima and azoosperima, it was however significantly different (p<0.05) in normospermia and azoospermia and also in oligospermia and azoospermia respectively. The FSH and testosterone level of the control group show no statistically significant difference from those of normospermia group (p>0.05) but show significant difference (p<0.05) in oligospermia and in azoospermia respectively; there was significant difference (p<0.05) in FSH and testosterone levels of normospermia, oligospermia, and azoospermia.

The sperm cells count ($\times 10^6$) and active sperm cell (%) of the control group show no statistical significant difference to those of normospermia group (p>0.05), but show significant difference (p<0.05) in oligospermia and in azoospermia respectively, there was significant difference (p<0.05) in sperm cells count and active sperm cell level of normosperima and oligospermia, normospermia and azoospermia and in oligospermia and azoospermia respectively as shown in Table 3.

The multiple comparisons of measured parameters of the different semen quality state of study and control groups. The prolactin, salivary alpha amylase and salivary cortisol level in the control were significantly different from those of normospermia group (p<0.05), oligozoospermia (p<0.05) and azoospermia group (p<0.05), the LH level of control group was not statistical significant difference from those of normospermia group(p>0.05) and oligozoospermia (p>0.05), but significant difference (p<0.05) in azoospermia, there was no significantly difference (p>0.05) in LH level of normosperima and azoosperima, it however significantly different (p<0.05) in normospermia and azoospermia and also in oligozoospermia and azoospermia respectively. FSH and testosterone level of the control group were not statistically significant difference from those of Normospermia group (p>0.05) but was significant difference (p<0.05) in oligozoospermia and in azoospermia respectively, there was significantly difference (p<0.05) in FSH and testosterone level of normosperima and oligozoospermia, and normospermia and azoospermia, it was however insignificantly different (p>0.05) in oligozoospermia and azoospermia.

The sperm cells count, and active sperm cell of control group not statistical significant difference from those of normospermia group (p>0.05), but significant difference (p<0.05) oligozoospermia and azoospermia respectively, there significant diff. (p<0.05) in sperm cells count and active sperm cell level of normosperima and oligozoospermia, normospermia and azoospermia and oligozoospermia and azoospermia (Table 4).

Table 1: Mean age of study group and the control group in the subjects.

Parameters (Years)	Study group, mean ± SD	Control group, mean ± SD	T student test	P value
Age	33.07±4.56	32.25±4.14	1.064	>0.201
Duration of infertility	4-5			

Table 2: Mean hormones, biomarkers and sperm quality in the study and control group in the male's category.

Parameters	Study group, mean ± SD (n=100)	Control group, mean ± SD (n=52)	Normal values	P value
FSH (MIU/ml)	4.50±2.20	5.91±1.66	2.0-14.0	<0.001
LH (MIU/ml)	5.64±2.26	5.43±1.66	2.0-14.0	0.560
Prolactin (ng/ml)	18.01±11.56	6.98±3.34	4.0-12.0	<0.001
Testosterone (ng/ml)	3.44±2.35	5.86±1.55	2.5-10.0	<0.001
S. cortisol (micromole/L)	449.75±106.81	340.65±72.53	221-552	<0.001
S. amylase (U/L)	13.12±4.39	8.45±3.01	1-15	<0.001
Sperm cells count (x10^6)	19.42±26.08	53.80±11.74	>20×10^6	<0.001
Sperm cells active (%)	33.99±26.07	49.10±14.80	> 50%	<0.001
Viability (%)	60.7±13.12.9	74.8±14.71	-4.712	<0.001
Semen volume (ml)	3.25±1.97	5.62±2.0	-6.077	<0.001

LH=Luteinizing hormone; FSH=Follicle stimulating hormone; S=Salivary.

Table 3: Comparison of hormones and biomarkers according to semen quality of the male.

Variables	Control group (Mean ± SD)	Study group (Mean ± SD)			P value	Sig
	Control, n=52	Normospermi, n=54 (54%)	Oligospermia, n=33 (33%)	Azoospermia, n=13 (13%)		
FSH (MIU/ml)	5.91±1.66	5.53±1.96	3.22±0.87	1.80±0.48	0.0001	S
LH (MIU/ml)	5.43±1.66	6.46±1.97	5.12±2.03	2.90±1.01	0.0001	S
Prolactin (ng/ml)	6.98±3.34	12.94±6.05	18.82±7.50	37.73±11.45	0.0001	S
Testosterone (ng/ml)	5.86±1.55	5.75±1.65	2.73±0.81	1.04±0.75	0.0001	S
S. cortisol (micromole/L)	340.65±72.53	397.5±79.95	505.33±73.76	598.46±46.52	0.0001	S
S. amylase (U/L)	8.45±3.01	11.19±2.94	14.13±3.20	19.85±3.53	0.0001	S
Sperm cells count (×106)	53.80±11.74	46.40±19.43	5.73±3.41	0.00±0.00	0.0001	S
Sperm cells active (%)	49.10±14.80	48.92±18.97	10.67±6.78	0.00±0.00	0.0001	S

LH=Luteinizing hormone; FSH=Follicle stimulating hormone; S=Salivary.

Table 4: Showing multiple comparisons of semen quality of measured variables among the male groups.

Group	LH (MIU/ml)	FSH (MIU/ml)	Prolactin (ng/ml)	Testosterone (ng/ml)	S. cortisol	S. amylase	Sperm cells count/ml	Sperm cells active (%)
AvB	0.38	0.56	0.01	0.87	0.02	0.002	0.09	0.17
AvC	0.97	0.01	0.01	0.01	<0.001	<0.001	<0.001	<0.001
AvD	0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BvC	0.35	0.03	0.01	0.01	<0.001	0.02	<0.001	<0.001
BvD	0.003	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
CvD	0.002	0.09	<0.001	0.26	0.03	0.02	<0.001	<0.001

A-Control, B-Normospermia, C-Oligozoospermia, D-Azospermia.

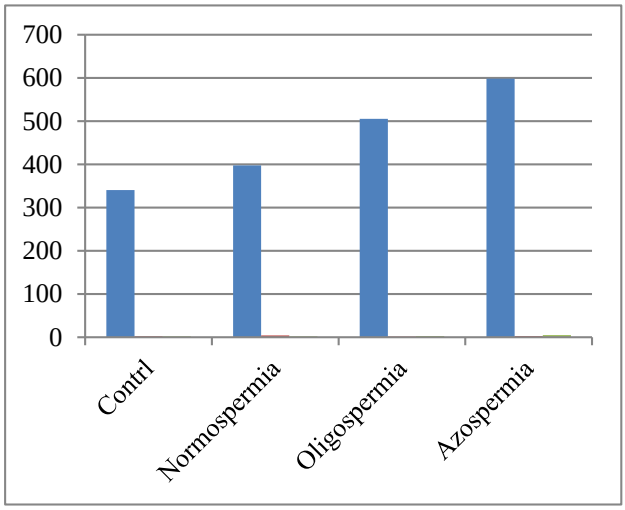

Figure 1: The mean level of S. cortisol between control, normospermia, oligospermia and azoospermia.

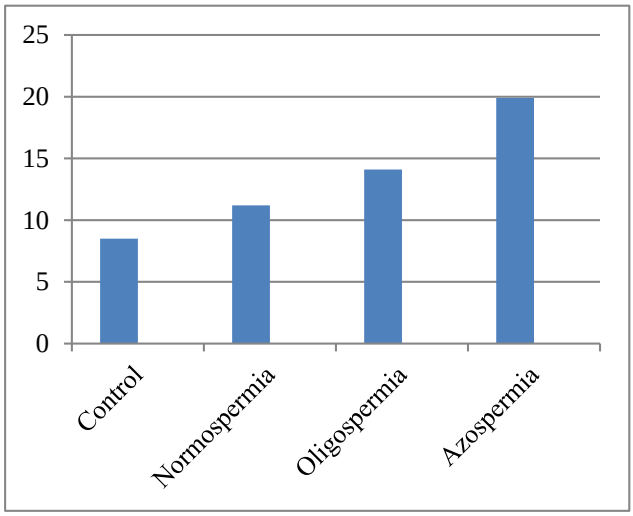

Figure 2: The mean level of S. AL amylase between control, normospermia, oligospermia and azoospermia.

DISCUSSION

It is observed in this research that the men in the study group presented with significantly low serum FSH level, testosterone level, sperm cells count and Active sperm cells (%), ($p<0.05$) relative to the control group; this findings support the report of Gurunat, who reported low sperm quality, low sperm quantity and hormone abnormalities, as causes of infertility in male of child bearing age.[12] The sperm count in this study is categorized into normospermia (normal sperm cell count), oligospermia (low sperm cell count) and azospermia (no sperm cell); and 54% of the male study subjects presents with the condition of normospermia, 33% present with oligospermia, while 13% presents with azospermia. In this study the hormonal abnormalities were pronounced in the azospermic condition, but less in oligospermic condition. Emokpae reported hormonal abnormalities in male infertility in Kano, Nigeria in both conditions of oligospermia and azospermia.[13] Also, Lieberman reported that abnormal testosterone and FSH levels can impair the mechanisms of spermatogenesis; furthermore, low Testosterone concentration is a marker of H-P-A activation; one factor that can deregulate testosterone and FSH secretion is chronic anxiety and depression.[14] The low values of testosterone and FSH in the male with azospermia is suggestive that, the cause is secondary to anterior pituitary failure. In men, stress adversely affect semen quality and can inhibit GnRH secretion through H-P-axis activation, stress-induced spermatogenesis impairment is typically manifested in decreased sperm count and motility (asthezospermia) and increased percentage of morphologically abnormal sperm.[15,16] An increase in stress hormone levels i.e., cortisol can impair the conversion of androstenedione to testosterone in the Leydig cells. This disrupts the hormonal transformation cycle required for testosterone secretion, leading to lower average values of semen volume and sperm quality.[16]

However, there was no significant difference in the LH values between the male study group and the control group; but there is a statistically significant difference in the values of prolactin ($p<0.05$) in the male study group relative to the control group, this finding agrees with that of Wdowiak who reported high prolactin concentration in the males with infertility.[17] The findings in this study suggest that most of the infertility cases experienced by couples, about 40% could be attributed to the male subjects; this is due to the fact that without a viable active sperm cells during the process of sexual intercourse, there will be no fertilization of the ovum, resulting in infertility.

In this study, the mean concentration of both salivary cortisol and salivary alpha amylase were significantly higher in the study group when compared with the control group ($p<0.05$).

However, in the male study subjects 13 (13%) showed azoospermic condition, the biomarkers were significantly higher ($p<0.05$) when compared with the normospermic and oligospermic conditions. The findings in this study support the reports of Morgan, lynch who reported high level of cortisol as the adverse effect of stress on fertility, and opined that 30% cases of infertility are attributed to stress; that when stress reducing measures are applied, those couples who could not get pregnant before got pregnant.[18,19] Since stress disrupts the GPA-axis, it could have contributed to the low quality and quantity of sperm cells in this study leading to infertility in men.

This result confirms the impacts of stress on sperm quality, in line with those reported by other authors.[20,21]

Although, some researchers have disclaimed the effect of stress on fertility, Lovely could not see any obvious link

of stress and infertility.[22] However, the elevated values of salivary cortisol and salivary amylase in this study suggest and support the adverse negative impact of stress on sex hormones and semen quality and quantity in some male of child bearing age, as also reported by Bhongade, that male patients with anxiety and depression were found to have lower testosterone levels and low sperm quality.[23] Thus, stress can compromise every aspect of fertility including libido and sperm quality, in men.[23,24] High cortisol level in this study suggests an indication of chronic stress; where the stress neuroendocrine are stimulated via the hypothalamus- pituitary-adrenal axis, which in turn affects the activities of the gonadotrophic releasing hormones (GnRH).[25] The stress hormones inhibit and decrease the pulsatility of the GnRH which is responsible for the stimulation and production of the gonadotropins (FSH and LH), these suggest the reason for low FSH value obtained in this study. High cortisol level, high alpha amylase and hyperprolactinemia may occur primarily as a result of physiological changes in hypothalamus-pituitary gland due to stress or any disease affecting them.[25] The reports in this study agree to the effect or adverse impact of stress biomarkers on sex hormones and semen quality and quantity, as observed by other researchers.

Our study had a couple of set- back such as; some subjects investigating infertility were already on treatment, We did not categorize them to those on treatment or not. Considering that there are diverse views in this area of study; therefore, further studies in this area are required in order to validate the effects of stress biomarkers on sex hormones, sperm quality and quantity.

CONCLUSION

Some men in infertility have higher values of stress biomarkers (Cortisol and Amylase). Some men suffering infertility show lower levels of testosterone, FSH, sperm quality and, quantity, and higher values of prolactin. Decreased semen quality and quantity is observed in subjects with higher values of stress biomarkers. Men investigating for infertility are found to have high stress biomarkers than those confirmed to be fertile. Men investigating for infertility with increased stress biomarkers show sex hormone abnormalities.

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