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Studies on Fertility Hormone in Azoospermia Men Attending Imo State Specialist Hospital, Owerri

Ukamaka Edward[1], Marvis U. Okehie[1], Emmanuel Chinedu Onuoha[2] and *Emmanuel Ifeanyi Obeagu[3]

[1]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.
[2]Federal University Otuoke, Bayelsa State, Nigeria
[3]Department of Medical Laboratory Science, Kampala International University, Uganda,
***Corresponding Author:obeagu.emmanuel@kiu.ac.ug,ORCID:0000-0002-4538-0161**

ABSTRACT

Azoospermia, a condition characterized by the absence of sperm in a man's semen, affects approximately 1% of the male population and accounts for about 10 to 15% of male infertility cases. This study aimed to assess the fertility hormone levels in Azoospermia patients in Owerri. A cross-sectional study included twenty-five Azoospermia patients attending the fertility clinic at Imo Specialist Hospital and twenty-five healthy individuals as a control group. Venous blood samples were collected and analyzed for serum testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) using the ELISA method. The results indicated that infertile subjects had significantly higher levels of FSH (9.91 ± 3.69 IU/L) compared to control subjects (5.54 ± 0.90 IU/L) (P = 0.0001). Similarly, infertile subjects showed significantly higher levels of LH (8.50 ± 2.63 IU/L) compared to control subjects (4.75 ± 1.15 IU/L) (P = 0.0001). In contrast, the testosterone levels in infertile subjects (2.56 ± 0.97 IU/L) were significantly lower than those in control subjects (4.80 ± 1.19 IU/L) (P = 0.0001). In conclusion, the findings of this study demonstrate a significant increase in gonadotropins (FSH and LH) and a significant decrease in testosterone levels in males with Azoospermia.

Keywords: fertility, hormone, azoospermia, men

INTRODUCTION

Infertility is typically defined as the inability to conceive after at least one year of regular, unprotected sex. This affects 15–20% of couples [1]. A male factor is estimated to be present in about 50% of cases, with sole responsibility in 30% of cases and a co-contributing female factor in 20% of factors [2]. Male infertility is associated with significant psychosocial and marital stress [3-4]. Azoospermia is the medical condition of a man whose semen contains no sperm [5-6]. It is associated with infertility, but many forms are amenable to medical treatment. In humans, azoospermia affects about 1% of the male population [5] and may be seen in up to 20% of male infertility situations in Canada [7].The production of sperm and androgens by the testis is under endocrine control. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are glycoprotein hormones secreted by the pituitary gland. They control development, maturation and function of the gonad. It is assumed that either testosterone or FSH alone can initiate, maintain and reinitiate spermatogenesis [8-9]. Understanding these hormonal interactions have significant clinical consequences in the evaluation and treatment of azoospermic men.

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Testicular biopsy was the main investigative modality for assessing men with azoospermia and normal testicular volume however with the advent of in-vitro fertilization (IVF), there is a growing trend towards therapeutic testicular biopsy/ sperm retrieval and sperm banking in the management of azoospermic men [10]. The current emphasis with assisted reproductive technologies (ARTs) is on identifying motile sperm in the semen, or of elongated spermatids in testicular tissue during therapeutic biopsy or testicular sperm extraction (TESE) for intracytoplasmic sperm injection (ICSI) [11]. Hormones evaluation is an essential parameter in giving a definitive diagnosis in infertile males [12]. Abnormal hormone production has been noted as a male causative factor in male infertility and hormonal replacement could play a corrective role [12]. The most essential hormones to be evaluated include, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (Schlegel, 2004). It is suggested that decrease in sperm count is associated with low testosterone level [12]. Changes in FSH and LH levels could result in abnormalities of spermatogenesis in patients with low sperm counts [13].

MATERIALS AND METHODS

Study Area

The study was carried out at Imo Specialist hospital, Owerri, Imo State in South East Nigeria.

Ethics, Advocacy and Pre-survey Contacts

A letter of introduction was obtained from the head of Department of Medical Laboratory Science, Imo state University. The letter was submitted to the ethical committee of the hospital. An ethical approval was obtained before collection of samples from study subjects. Consent form and questionnaires were collected from the study participants. The modalities for the survey were reached and date fixed for blood sample collection at appropriate clinic days.

Study Population

A total of 50 subjects were recruited. Twenty-five were male subjects who had azoospermia attending the fertility clinic at Imo Specialist hospital, Owerri were selected and served as the test and twenty-five who were apparently healthy individual that served as control subjects.

Selection Criteria

A. Inclusion Criteria

1. Subject between the ages of twenty and fifty years old.
2. Male subjects who were changed with Azoospermia. They were between the ages 25 to 50 years old.
3. Apparently healthy individuals that served subjects whom were as control subjects Induced subjects who were apparently healthy and were used as control subjects. Informed consent were obtained.

B. Exclusion Criteria

The following subjects were excluded from the study

1. Men who were below the years and above the age 50.
2. Those who informed consent could not be obtained.
3. Subjects who had complicated diseases.

Sample Collection

Five milliliters of blood were collected aseptically from the ante- cubital vein by venipuncture from each subject. The blood samples were then dispensed into plain tubes and were immediately labelled for proper identification and allowed to clot. The clotted samples were spun at 5000rpm for 5 minutes. The serum was separated using Pasteur pipette and transferred into sterile sample tubes and stored at -20° C prior to testing.

Laboratory Procedures

All reagents were commercially procured and the manufacturers standard operational procedures were strictly followed.

Determination of Serum Testosterone

Procedure

The desired number of coated wells were Secured in the holder. 10ul of standards, specimens and controls were Dispensed into appropriate wells. 100ul of testosterone-HRP conjugate reagent was dispensed into each well. 50ul of rabbit anti-testosterone reagent was Dispensed into each well. Thoroughly mixed for 30 seconds. It was Incubated at 37°C for 90 minutes. After incubation, the incubation mixture was removed by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with deionized or distilled water. The microtiter was used. The wells were sharply stricken onto absorbent paper or paper towels to remove all residual water droplets. 100ul of TMB reagent was dispensed into each well and mixed gently for 5 seconds. The mixture was Incubated at room temperature for 20 minutes. The reaction was stopped by adding 100ul of stop solution to each well. Gently mix for 30 seconds. Absorbance was read at 450nm with a microtiter well reader within 15 minutes.

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Determination of Serum FSH

Procedure

The desired number of coated wells were secured in the holder. 50ul of standard, specimens and controls were dispensed into appropriate wells. 100ul of Enzyme conjugate reagent was dispensed into each well. It was thoroughly mixed for 30 seconds. It is very important to have a complete mixing in this setup. Incubate at room temperature for 45 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The microtiter wells were rinsed and flicked 5 times with distilled or deionized water. The wells were stricken sharply onto absorbent paper or paper towels to remove all residual water droplets. 100ul TMB reagent was dispensed into each well and mixed gently for 10 seconds. Incubate at room temperature in the dark for 20 minutes. The reaction was Stopped by adding 100ul of stop solution to each well. Optical density was read at 450nm with a microtiter plate reader within 15 minutes.

Determination of Serum LH

Procedure

The coated wells were secured in the holder. 50ul of standard, specimens and controls were dispensed into appropriate wells. 100ul of Enzyme conjugate reagent was dispensed into each well. Gently Mix for 30 seconds. It is very import to have a complete mixing in this setup. Incubated at room temperature for 45 minutes. Incubation mixture was removed by flicking plate contents into sink. Microtiter well was rinsed and flicked 5 times. The wells were Striken sharply onto absorbent paper or paper towels to remove all residual water droplets. 100ul TMB reagent was dispensed into each well mixed gently for 10 seconds. Incubate at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100ul of stop solution to each well. Gently mix for 30 seconds. Absorbance was read at 450nm.

Statistical Analysis

The data obtained was analysed by Statistical package for social science (SPSS) version 21 using student independent T-test. The result obtained was expressed as mean ± standard deviation in tables. The probability $P < 0.05$ was considered statistically significant.

RESULTS

The result in table 1 showed that the mean value of follicle stimulating hormone in the infertile subjects (9.91 ±3.69 IU/L) was higher when compared with the control subjects (5.54 ±0.90 IU/L) and was statistically significant (P = 0.000l). The mean value of Luteinizing hormone in the infertile subjects (8.50 ±2.63 IU/L) was higher when compared with control subjects (4.75 ±1.15 IU/L) which was statistically significant (P = 0.000l). The mean value of Testosterone hormone in the infertile subjects (2.56 ±0.97 IU/L) was higher when compared with the control subjects (4.80 ±1.19 IU/L) which was statistically significant (P = 0.000l).

Table 1: hormones of infertile subjects

Parameters	Infertile subjects	control	P-Value
FSH (IU/L)	9.91 ±3.69*	5.54 ±0.90	0.0001
LH (IU/L)	8.50 ±2.63*	4.75 ±1.15	0.0001
Testosterone (IU/L)	2.56 ±0.97*	4.80 ±1.19	0.0001

DISCUSSION

Approximately 1% of all men in the general population suffer from azoospermia, and azoospermic men constitute approximately 10 to 15% of all infertile men. FSH, LH and testosterone evaluation is useful in the management of male infertility [14]. In the present study, the mean value of FSH was significantly higher in azoospermia men when compared to control subjects. The high level of FSH might be as a result of the low sperm level the increase in the levels of FSH might have disrupted the spermatogenic process leading to infertility. FSH acts directly on the seminiferous tubules and provides indirect structural and metabolic support for development of spermatogonia into mature spermatids via its membrane-bound receptor in Sertoli cells. FSH also plays a crucial role in determination of the number of Sertoli cells and thus their capacity to maintain spermatogenesis **[15].** This result is in agreement with the study carried out by de Kretser, (2014) who reported an elevated level of serum FSH with increasing severity of seminiferous epithelial destruction. These results are in accordance with the studies of Sulthan *et al.* [16]. FSH, LH and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH LH and testosterone [17]. FSH acts directly on the seminiferous tubules whereas luteinizing hormone stimulates spermatogenesis indirectly via testosterone. FSH plays a key role in stimulating mitotic and meiotic DNA synthesis in spermatogonia [18].

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CONCLUSION

In conclusion the overall results clearly indicate a significant increase in gonadotropins (FSH and LH) and a significant increase in testosterone level in azoospermia males.

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