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Research Article

Assessment of Reproductive Hormones among Infertile Sudanese Males in Khartoum State, 2019

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Abstract

Male infertility is a complex condition and overlaps many factors and affects infertility in about 8 - 5% of the population in the world and the man is responsible for 40% of these cases.

The present study was designed to analyze the level of reproductive hormone among different groups of infertile patients serum testosterone, FSH, LH and Prolactin were measured were measured in 160 samples from normozoospermic, oligoathenospermic and non-obstructive azoospermic men using by using full automated tosohAIA360 analyzer, data was analyzed using the statistical software package SPSS version 17.

Serum sample analysis showed significant elevation in prolactin level among patient with non-obstructive azoospermia and oligo-asthenospermia as compared to control group, p-value (0.000), (0.001) respectively, significant elevation in FSH level among patient with non-obstructive azoospermia and oligoasthenospermia as compared to control group, p-value (0.000), (0.003) respectively. Serum sample analysis showed high LH level in patients with non-obstructive azoospermia as compared to control group with p-value (0,000). We conclude that reproductive hormones FSH level and LH level were significantly increased among patients with non-obstructive azoospermia (NOA) as compared with patients with oligoasthenospermia with p-value (0.002) and (0,007) respectively. Additional study is recommended, including other biochemical parameter such as Fructose, Estradiol hormone, B Inhibin Hormone and volume and motility.

Keywords: *Reproductive Hormones; Infertile Males*

Background

Infertility is a complicated condition and has several etiologies and consequences relining on the gender, sexual history, lifestyle and cultural background [1]. Infertility influences about 8% to 12% of the world's population and in about half of cases; men are either the single cause or contribute to couple's infertility [2]. Seminal plasma is very significant for sperm metabolism, role, viability, and transfer in the women genital tract.

Follicle-stimulating hormone (FSH) is a gonadotropin, a glycoprotein_polypeptide_hormone. It is formed within the gonadotropic cells of the anterior pituitary gland, and manages the development, growth, pubertal maturation and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system [3].

Follicular stimulating hormone activates principle spermatocytes to experience the initial division of meiosis, to shape resulting spermatocytes, also improve the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes [4] and is critical for the initiation of spermatogenesis.

Low level of FSH or Diminished secretion of FSH can result in failure of gonadal function (hypogonadism). This state is characteristically manifested in men as failure in production of normal numbers of sperm.

Luteinizing hormone, identified as lutropin and sometimes lutropin) [5], is a hormone produced by gonadotropic cells in the anterior pituitary gland. In women, an acute rise of LH triggers ovulation and progress of the corpus luteum. In males, where LH had also been called interstitial cell-stimulating hormone (ICSH) [6], it stimulates Leydig cell production of testosterone. It acts synergistically with FSH.

LH works upon the Leydig cells of the testis and is managed by gonadotropin-releasing hormone (GnRH) [7]. Leydig cells create testosterone (T) in the power of LH, which manages the expression of the enzyme 17β-hydroxysteroid dehydrogenase that is used to convert androstenedione, the hormone produced by the testes, to testosterone. The beginning of puberty is restricted by two major hormones: FSH initiates spermatogenesis and LH signals the production of testosterone. An androgen that exerts both endocrine action and intratesticular activity on spermatogenesis.

Prolactin is a protein recognized for its function in enable mammals, frequently females, to produce milk. It is significant in over 300 separate mechanisms in a variety of vertebrates, including humans [8]. Prolactin is produced from the pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. It is secreted in pulses in between these events. Prolactin has a significant role in metabolism, regulation of the immune system and pancreatic development.

High levels of prolactin reduce the levels of sex hormones-estrogen in women and testosterone in men [9]. The influences of mildly high levels of prolactin are much more changeable, in women, substantially increasing or decreasing estrogen levels.

Testosterone is the main men sex hormone and an anabolic steroid. It has a key role in the growth of male reproductive organs such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair loss.

Testosterone is essential for normal sperm progress. It stimulates genes in Sertoli cells, which encourage differentiation of spermatogonia. It manages acute HPA (hypothalamic-pituitary-adrenal axis) reaction under dominance challenge [10]. Androgen together with testosterone promotes muscle growth. Testosterone moreover regulates the population of thromboxane A_2 receptors on megakaryocytes and platelets and therefore platelet aggregation in humans [11,12].

Adult testosterone influences are more obviously provable in males than in females but are likely significant to both. A few of these influences may decrease as testosterone levels might reduce in the later decades of adult life [13].

Rationale

The determination of spermatozoa concentration, morphology and motility remains the primary clinical tool for the assessment of male infertility. Reproductive hormone profiles (total testosterone, prolactin, LH and FSH) in addition to semen parameters are typically used to assess male reproductive function. Development of clinical assays to delineate new parameters that better reflect sperm fertilizing competence are needed.

There are So many studies performed about obesity, it's correlation with sperm analysis, but the studies concerning its correlation with both sperm count and serum hormone levels of LH, FSH and Prolactin are generally few.

In Sudan little is known about the link between hormones and sperm count and motility. Accordingly, the present study will be con-

ducted to assess the level of FSH, LH and Prolactin in oligoasthenospermia and azoospermia to make a correlation between these hormones and other variables such as age and mass index.

Objectives of the Study

General objective

To assess the reproductive hormones among infertile Sudanese males in Khartoum state.

Specific Objectives

1. To estimate serum FSH, LH, prolactin and total testosterone levels among infertile males.

2. To assess serum FSH, LH, prolactin and total testosterone levels among infertile males.

Materials and Methods

Study design

This is a case control hospital based study.

Study area

Reproductive care center in Almuk Nemer Street in Khartoum state.

Study duration

The study was carried out during the period from December 2016 to September 2019.

Study population

Sudanese male patients referred by various fertility centers and hospitals in Khartoum state during study period.

Inclusion criteria

Men with oligoasthenospermia and azoospermia as test group and normal males (age group 24 - 78 years) belonging to the same socio-economic status were selected as control group.

Exclusion criteria

Patients under hormonal treatment and diabetic patients.

Sample size and study population

One hundred and sixty blood samples were collected in this study.

Case group: 80 infertile male patients (oligoasthenospermia and azoospermia).

Control group: 80 apparently healthy individuals.

Blood sample collection and analysis

Before collection, a local antiseptic (70% alcohol) was used to clean the skin, venous blood (about 4 ml) was taken from each participant in plain container, then the centrifugation was done at 3000 rpm for 3 - 5 minute to obtain serum and kept in -10°C and -20°C until used for measurement of FSH, LH, Prolactin, and Total testosterone.

Hormonal parameters were be measured by Tosoh auto analyzer instrument.

Collection of semen samples and analysis

Semen was collected by masturbation into a sterile plastic specimen container at the hospital. Subjects were instructed to abstain from ejaculation for at least 72 hours prior to producing the semen sample. The sample was liquefied for at least 20 minutes, but no longer than 1 hour prior to performing a routine semen analysis, which included measurements of volume, pH, sperm concentration, sperm motility as well as morphology and direct microscopic examination.

Estimation of sperm counting will be done using the Neubauer chamber. Sperm analysis was carried out according to the World Health Organization guidelines. Based on the sperm concentration the infertile subjects were classified as follows:

- Normozoospermia (> 20 million sperm/ml and normal semen profile).
- Oligoasthenospermia (<20 million sperm/ml and motility grade Cor D).
- Azoospermia (no spermatozoa).

In proven fertile controls, the sperm count ranged from 20 - 120 million sperm/ml.

Seminal plasma collection and analysis

Sample preparation

Seminal plasma was diluted 1:10 with 0.5% v/v HNO_3 to determine the concentrations of zinc and copper.

Instrument

Atomic absorption spectrophotometer (Buck Scientific, model 210 VGP).

Principle of atomic absorption spectrophotometer

Atomic absorption spectrophotometer utilizes the phenomenon that atoms absorb, radiation of particular wavelength. When the light from a hollow cathode lamp shines into the flame, ground state atoms of the element wanted to be measured absorb some of the light resulting in a decrease of the light (atomic absorption).

Methodology

Instrument

Full automated chemistry analyzer TOSOH A1A360.

Principles

FSH

The ST-pack FSH is two-site immunoenzymemometric assay, which is performed entirely in the AIA-pack. FSH present in the test sample is bound with monoclonal antibody immobilized on magnetic solid face and enzyme-labeled monoclonal antibody in the AIA-pack. The magnetic beads are washed to remove unbound enzyme labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methyleumbelliferyl phosphate (4mup) the amount of enzyme- labeled monoclonal antibody that binds to the beads is directly proportional to the FSH concentration in the test sample. Standard curve is constructed and unknown sample concentrations are calculate using curve.

LH

The ST -pack LH is two-site immunoenzymemometric assay, which is performed entirely in the AIA-pack. LH present in the test sample is bound with monoclonal antibody immobilized on magnetic solid face and enzyme -labeled monoclonal antibody in the AIA-pack. The

magnetic beads are washed to remove un bound enzyme labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methyleumbelliferyl phosphate (4mup) the amount of enzyme- labeled monoclonal antibody that binds to the beads is directly proportional to the LH concentration in the test sample. Standard curve is constructed and unknown sample concentrations are calculated using curve.

Prolactin

The ST -pack PRL is two-site immunoenzymemometric assay, which is performed entirely in the AIA-pack. Prolactin present in the test sample is bound with monoclonal antibody immobilized on magnetic solid face and enzyme-labeled monoclonal antibody in the AIA-pack. The magnetic beads are washed to remove unbound enzyme labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methyleumbelliferyl phosphate (4mup) the amount of enzyme- labeled monoclonal antibody that binds to the beads is directly proportional to the prolactin concentration in the test sample. Standard curve is constructed and unknown sample concentrations are calculated using curve.

Testosterone

The ST-pack testosterone is a competitive immunoenzyme mimetic assay, which is performed entirely in the AIA-pack. testosterone present in the test sample compete with enzyme labeled testosterone for a limited number of binding site on testosterone specific monoclonal antibody immobilized on a magnetic solid face. The magnetic beads are washed to remove un bound enzyme labeled testosterone and are then incubated with a fluorogenic substrate, 4-methyleumbelliferyl phosphate (4mup) the amount of enzyme- labeled testosterone that binds to the beads is inversely proportional to the testosterone concentration in the test sample. Standard curve is constructed and unknown sample concentration s are calculated using curve.

Data collection

Direct questionnaire was done to obtain clinical data for each participant and sample.

Data analysis

Data was analyzed using Statistical Package for Social Science Software (SPSS).

Ethical considerations

This study was approved by the research committee of the College of Medical Laboratory Sciences - Shendi University. Informed consent was obtained from each participant before taking the samples.

Results

This is a case control hospital based study conducted in Khartoum state in the Reproductive Care Center during the period from December 2016 to September 2019. This study included 160 samples, 80 from these samples were collected from infertile males as case group (40 of them collected from Azoospermia 25% and the rest from Oligoathenospermia 25%) and the rest of the samples collected from normal male (Normozoospermia 50%) as control group.

Discussion

The level of serum Testosterone was insignificantly decreased in patients with non-obstructive azoospermia when compared with control group, with p-value (0.88) and the level of serum Testosterone showed insignificant variation between patients with Oligoasthenospermia and control group, p-value (0.129).

Serum sample analysis showed significant elevation in prolactin level among patients with non-obstructive azoospermia and oligoasthenospermia as compared to control group, p-value (0.000), (0.001) respectively.

	Azoospermia (NOA)		Oligoasthenospermia	
	Case	Control	Case	Control
No	40	80	40	80
Mean (mIU/ml)	12.5	12.6	14.0	12.6
Std. Dev	7.22	3.40	6.67	3.40
P-Value	0.88		0.129	

Table 1: *Independent sample T. test showed Mean of Testosterone level among case (Non-Obstructive Azoospermia (NOA) and Oligoasthenospermia) group and Control group. P-value ≤ 0.05 is considered significant.*

	Azoospermia (NOA)		Oligoasthenospermia	
	Case	Control	Case	Control
No	40	80	40	80
Mean (mIU/ml)	242.5	162.4	244.08	162.48
Std. Dev	123.5	88.6	172.102	88.643
P-Value	0.000		0.001	

Table 2: *Independent sample T. test showed Mean of Prolactin level among case (Non-Obstructive Azoospermia and Oligoasthenospermia) group and Control group. P-value ≤ 0.05 is considered significant.*

	Azoospermia (NOA)		Oligoasthenospermia	
	Case	Control	Case	Control
No	40	80	40	80
Mean (mIU/ml)	18.1	6.7	9.9	6.7
Std. Dev	12.67	3.42	8.2	3.42
P-Value	0.000		0.003	

Table 3: *Independent sample T. test showed Mean of FSH level among case (Azoospermia and Oligoasthenospermia) group and Control group. P-value ≤ 0.05 is considered significant.*

	Azoospermia (NOA)		Oligoasthenospermia	
	Case	Control	Case	Control
No	40	80	40	80
Mean (mIU/ml)	7.6	4.7	5.2	4.7
Std. Dev	4.65	1.70	2.05	1.70
P-Value	0.000		0.232	

Table 4*: Independent sample T. test showed Mean of LH level among case (Azoospermia (NOA) and Oligoasthenospermia) group and Control group. P-value ≤ 0.05 is considered significant.*

Paired Group	Mean (mIU/ml)	N	Std. Deviation	P-Value
Testosterone level (AZO)	12.1	40	7.22	0.227
Testosterone level (OAS)	14.0	40	6.67	

Table 5: *Paired sample T. test showed Mean of plasma Testosterone level among Oligoasthenospermia and non-obstructive azoospermia. P-value ≤ 0.05 is considered significant.*

Paired Group	Mean (mIU/ml)	N	Std. Deviation	P-Value
Prolactin level (AZO)	242.5	40	123.5	0.959
Prolactin level (OAS)	244.1	40	172.1	

Table 6: *Paired sample T. test showed Mean of plasma Prolactin level among Oligoasthenospermia and non-obstructive azoospermia. P-value ≤ 0.05 is considered significant.*

Paired Group	Mean (mIU/ml)	N	Std. Deviation	P-Value
LH level (AZO)	7.6	40	4.65	0.007
LH level (OAS)	5.2	40	2.05	

Table 7: *Paired sample T. test showed Mean of plasma LH level among Oligoasthenospermia and non-obstructive azoospermia. P-value ≤ 0.05 is considered significant.*

The displayed results in table 4. Showed significant elevation in FSH level among patient with azoospermia (non-obstructive) and oligoasthenospermia as compared to control group, p-value (0.000), (0.003) respectively.

Serum sample analysis showed high LH level in patients with non-obstructive azoospermia as compared to control group with p. value (0,000), while level showed insignificant increase in patients with oligoasthenospermia. P-value (0.232).

Conclusion

- The level of serum Testosterone was insignificantly decreased in patients with non-obstructive azoospermia when compared with control group, and the level of serum Testosterone shows insignificant variation between patients with Oligoasthenospermia and control group.
- FSH level showed elevation in patient with azoospermia (non-obstructive) and oligoasthenospermia as compared to control group.
- Serum sample analysis showed significant elevation in prolactin level among patients with non-obstructive azoospermia and oligoasthenospermia as compared to control group.
- Serum sample analysis showed high LH level in patients with non-obstructive azoospermia as compared to control group, while its level is insignificantly increased in patients with oligoasthenospermia.

Recommendations

Further study is to be conducted including other biochemical parameter such as Fructose and Estradiol hormone and B Inhibin Hormone and volume and motility.

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