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CORRELATION BETWEEN SEMINAL FLUID ANALYSIS AND LEVEL OF FSH, PROLACTIN AND TESTOSTERONE IN SUDANESE INFERTILE PATIENTS

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ABSTRACT

Background: Infertility, defined as the inability to conceive after at least one year of unprotected intercourse. The management of infertility problems has become an increasingly important part of health services during the past two decades in most countries. Recent studies have focused on semen quality of men in the general population. However, semen analysis and hormonal evaluation are prerequisite in the assessment of infertile men and level of hormones has direct effects on testicular functions and spermatogenesis. The general objective of this study is to correlate seminal fluid analysis and level of FSH, prolactin and testosterone in infertile Sudanese patients and specifically to measure the levels of these hormones and effect on parameters of seminal fluid analysis (SFA) and depicts if there is any correlation between level of serum FSH, prolactin and testosterone in healthy men and infertile patient. **Methodology:** One hundred semen samples, of Sudanese males attending reproductive health care centers, namely Ashmaig Center and AL Sirabuolhasan Center, were collected by masturbation after 3-5days prior abstinence from sexual intercourse. Of the total number, 80 infertile patients (age range:25-50years); classified into four categories, azoospermia, oligoazoospermia, asthenoazoospermia, and teratoazoospermia, and twenty apparently healthy subjects as controls (age range:27-40years). Levels of follicular stimulating hormone, prolactin, and testosterone were assessed, in addition to examination of seminal fluid analysis. Macroscopic and microscopic parameters of semen specimens were determined, as wet preparation for sperm motility, morphology and count. **Results:** The follicle stimulating hormone (FSH) level was markedly increased ($p<0.05$) in azoospermia and oligospermia, the testosterone level was found significantly decreased ($p<0.05$) in the infertile patients and decrease was clear in azoospermic patients. Prolactin level was significantly increased ($p<0.05$) in azoospermia and significantly increase in other infertile patients as well. Highly positive significant correlation was noticed between the FSH and sperm count in teratoazoospermia($p<0.005$),and there is no significant correlation between the prolactin, follicular stimulating hormone, and testosterone with semen volume in infertile groups. **Conclusion:** In the infertile study groups testosterone level was decreased whereas FSH and prolactin levels were increased. This indicates an inverse relationship between testosterone level and the other reproductive hormones. The paper recommends that further study is needed to investigate the effect of Luteinizing hormone (LH) as it is produced by pituitary gland like FSH and may be linked to factors regulating spermatogensis.

KEYWORDS: infertile males, semenquality, reproductive hormones.

INTRODUCTION

Infertility, defined as the failure of a couple to become pregnant after at least one year of regular unprotected intercourse. It is reported that the infertility affects about 812% of couples in the world.[1] Abnormal hormone production has been noted as a male causative factor.[2] The male reproductive system creates sperm that is manufactured in the seminiferous tubules within each testicle. The head of the sperm contains the DNA, when combined with the egg's DNA, will create a new individual. The tip of the sperm head is the portion called the acrosome, which enables the sperm to penetrate the egg. The mid-section contains mitochondria, which supply the energy to the tail needs to move. The tail moves with whip-like movements back and forth to propel the sperm towards the egg. The sperm have to reach the uterus and the fallopian tube in order to fertilize a woman's egg.[3] Male fertility depends on the proper function of a complex system of organs and hormones:1) The process begins in the area of the brain called the *hypothalamus-pituitary axis*, a system of glands, hormones, and chemical messengers called neurotransmitters, all of which are critical for reproduction; 2) The first step in fertility is the production of *gonadotropin-releasing hormone (GnRH)* in the hypothalamus, which prompts the

pituitary gland to manufacture *follicle-stimulating hormone (FSH)* and *luteinizing hormone (LH)*; 3)FSH maintains sperm production, and LH stimulates the production of the male hormone *testosterone*; 4)Both sperm and testosterone production occurs in the two testicles, which are contained in the scrotal sac- the scrotum[4]; and5)Excess prolactin is associated with gynecomastia and impotence.[5]

- **Sperm**

Sperm are made in hundreds of microscopic tubes, known as *seminiferous tubules*, which make up most of the testicles. Surrounding these tubules are clumps of tissue containing Leydig *cells*, which produce testosterone when stimulated by luteinizing hormone (LH). The ability of a sperm to move forward rapidly and straight is probably the most significant determinant of male fertility.

- **Causes of Male Infertility**

More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. The remaining cases of male infertility can be caused by a range of conditions including anatomical problems, hormonal imbalances, and genetic defects as well as the following:

1. Sperm Abnormalities
2. Retrograde Ejaculation
3. Structural Abnormalities
4. Hormonal Deficiencies
5. Genetic Disorders

Risk Factors

Risk factors for male infertility include: Varicocele, aging, sexually transmitted disease, lifestyle factors, long-term or intensive exposure to certain types of chemicals, toxins or medications. Several researchers reported that disturbance in gonadotropin ratios may cause male infertility since these hormones act synergistically in the stimulation and maintaining healthy spermatozoal production. In infertile men, higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage, and was shown to be associated with azoospermia and severe oligoazoospermia as reported by Sheikh MA; Khan MS and Danyal A.[6] Also study conducted at Nigeria[7] reported that infertile men have abnormalities in FSH level (IMOH, AA, et. al, 2012). FSH secretion is regulated by the negative feedback of the testosterone and inhibin, a protein-type substance produced in Sertoli cells. The actual inhibin production reflects the extent of the alteration of spermiogenesis; a considerable oligozoospermia results in decreased inhibin synthesis, which leads to an increased FSH productionas reported by Turner, CD. and BagnaraJT[8] Zabul. J., Mierzejewski, W. and Rogoza N statedthat the suppression of testosterone secretion could be due to deficiency of hypothalamic GnRH resulting in impairment of gonadotropinsecretion from pituitary[9] The low levels of serum testosterone was observed in normozoospermic males and directly associated with infertility due to loss of germinal epithelium and disturbance of seminiferous tubules function probably due to deficient secretion of inhibin and sex steroids, but Leydig cells of testes remain intact (Reyes-fuentesA,Chavarriaa M and carrerA.) in[10] and Babu,R.,et.al,2004.[11]

MATERIALS AND METHODS

Case study: Case study control

One hundred men were enrolled in this study during their attendance to reproductive health care centers (Ashmaig Center) and ALsirAbuolhasancenter, in Khartoum State during March -April 2017.These patients were selected from men who consulted the infertility clinics after being unable to achieve pregnancy for at least 2 years and from whom their female partners have shown no diagnosed causes of infertility, or any disorder of ovulation.

Seminal fluid analysis (SAF)

Samples of seminal fluid from both tests and control groups were collected by masturbation into sterile polystyrene containers after 3-5 days of abstinence. The container was labeled with the necessary information including name, age, abstinence period and time of sample collection. After liquefaction the samples were analyzed at room temperature and macroscopic and microscopic examinations were performed according to WHO methodology mentioned in details in WHO manual[12]

Reproductive hormones analysis

Venous blood (5ml) from both control and infertile males was collected in an anticoagulant – free tube for analyzing the reproductive hormones and the serum was separated from the blood cells by twice centrifugation at 2000 rpm at room temperature, then stored at - 20°C,and assessed within one week using the TOSHO machine. Statistical analysis of data was performed with the help of SPSS and the results were expressed in terms of one-way variance (ANOVA), standard error, and standard deviation.

RESULTS

The result of this study appeared according to the parameter of SFA in patients that were classified into 20 controls (age range: 27-40years) and 80 infertile patients(age range:25-50years) with abnormal semen significant impairment at least one parameter of their SFA. Therefore, infertile patients were divided into: Azoospermic patients (n=20) without sperms in their semen after centrifugation, oligospermic patients (n=20) with sperm density< 20X10 /ejaculation, Asthenosperm motility patients (n=20) with sperm motility <30% and teratospermia patients (n=20) with abnormal morphology>35% control fertile subjects were restricted to 20 men who had fathered a child during the last 2 years. The mean values(table 1) of prolactin in azoospermia and control groups were (290 MIU/ML±506) and(164MIU/ML±87), respectively; and

in teratospermia and control groups were (289MIU/ML±637) and (164MIU/ML±87); and in oligoazoospermia and control groups were (272MIU/ML±213),(164MIU/ML±87); and in asthenospermia and control groups were (216MIU/ML±164),(164MIU/ML±87). Therefore ,is no significant difference between control and infertile patients in prolactin concentration with level of significance (P=0.804).The mean values of FSH in azoospermia and control groups were respectively (30MIU/L±18) and (5MIU/ML±3); and in teratozospermia and control groups(5MIU/ML±7) and (5MIU/ML±3); and in oligospermia and control groups (24MIU/ML±51) and (5MIU/ML±3); and in asthenospermia and control groups(5MIU/ML±3) and (5MIU/ML±3). Thus there were significant differences between control and infertile patients in FSH concentration with (p=0.001).

The mean values of testosterone in azoospermia and control groups were respectively (15NMOL/L±8) and (19NMOL/L±7); in teratozospermia and control groups (16NMOL/L±6) and (19NMOL/L±7); in oligospermiaand control groups (16NMOL/L±7) and (19NMOL/L±7); in asthenospermia and control groups(17NMOL/L±8) and (19NMOL/L±7). Therefore, there is no significant differences between controls and infertile patients in testosterone concentration with (p=0.610).The mean values of sperm Count/mL in teratozospermia and control groups were respectively (88/ML±60) and (104/ML±71); in oligospermia and control groups (9/ML±5) and (104/ML±71). Thus there were significant differences between control and infertile patients in sperm count/ML with (p=0.001). Similarly, there were significant differences between controls and infertile patients in semen volume in mL with (p=0.021), abnormal morphology with (p=0.001) and sperm motility with (p=0.001).

There is no significant correlation between the prolactin, follicular stimulating hormone and testosterone with semen volume in infertile groups; and also there is no significant correlation between the prolactin and testosterone in infertile groups with sperm count. However, there is positive significant correlation between the FSH and teratoazoospermia regarding the sperm count (table 2).

Table (1): levels of FSH, Prolactin and testosterone hormones in the serum and semen analysis in control and different groups
The values are mean (±) SD

Parameter	Groups					Sig
	CONTROL	AZOOSPERMIA	TERATOZOSPERMIA	OLIGOSPERMIA	ASTHENOSPERMIA	
S, PRL MIU/ML	164±87	290±506	289±637	272±213	216±164	.804
S, FSH MIU/ML	5±3	30±18	5±7	24±51	5±3	.001
S, TESTOSTERONE IN NMOL/L	19±7	15±8	16±6	16±7	17±8	0.000
PROGRESSIV. MOTILE SPERM	2±0.5	0	2±0.7	1±0.5	1±0.6	0.000
SPERM COUNT IN MILLIONS/ML	104±71	0	88±60	9±5	75±63	0.000
LIQUIFACTION TIME IN MINUTES	21±10	24±12	22±9	25±22	22±9	0.886
VOLUME IN ML	2.2±0.9	1.6±0.8	2.4±1	2.6±0.7	2.2±1	0.021
ABNORMAL MORPHOLOGY IN %	28±6	0	42±6	30±6	38±6	0.000
20 MIN %ACTIVE MOTILITY	65±9	0	58±12	75±9	37±15	0.000
60 MIN %ACTIVE MOTILITY	60±9	0	53±12	61±9	32±15	0.000
120 MIN %ACTIVE MOTILITY	55±9	0	48±12	56±9	27±15	0.000
20 MIN %NONMOTILE	27±8	0	34±11	26±7	55±16	0.000
60 MIN %NONMOTILE	32±8	0	39±11	32±8	60±17	0.000
120 MIN %NONMOTILE	37±8	0	44±11	37±8	62±20	0.000

Table 2: Correlation of semen volume, sperm count with serum FSH, prolactin and testosterone levels in control and different groups.

Hormones	S. VOL									
	Cont		Azoo		Tera		Oligo		Astheno	
	R	P	R	P	R	P	R	P	R	P
PRL	-.434	.056	-.117	.624	.104	.661	.153	.520	-.338	.145
FSH	-.035	.885	-.147	.535	-.028	.905	.274	.242	-.133	.575
TESTO	-.065	.786	-.186	.432	.132	.578	.014	.955	042-.	.860
	S Count									
PRL	.032	.892	0	0	-.010	.967	-.325	.163	-.101	.663
FSH	-.283	.227	0	0	.503*	.024	-.338	.145	.000	.998
TESTO	.035	.884	0	0	.022	.926	-.265	.259	-.103	.656

Significant <0.05 non-significant >0.05

DISCUSSION

In this study the FSH level in infertile patients was found significantly increased and it is higher with azoospermia and oligospermia groups. These findings are in agreement with the study of(IMOH,A. A. et. al, 2012)and (Sheikh MA, Khan MS. and Danyal A2005). Testosterone was reduced in azoospermia and slightly lower in oligo and tereto; and these findings were in agreement with the study of Reyes-fuentesA,Chavarriaa M and carrerA, 1996 and Babu,R,et al, 2004. Testosterone is essential for all steps in spermatogenesis, so reduction in such hormone concentration may affect sperms count and semen quality(13).Testosterone and FSH receptors are located on the Sertoli cells not on the germ cells. They exert independent actions, but they are likely to exhibit synergism. Thus disruption of Sertoli cells function may affect the semen parameters, since these cells nurse and support the germ cells. This condition leads to excessive prolactin production by the pituitary gland and this disruption was detected among infertile patients. The prolactin level was significantly increased, and this increase was demonstrated in infertile patients with azoospermia, teretospermia and oligospermia. The liquefaction time is also slightly increased in oligospermia and azoospermia .Likewise there is significant increased reduction in seminal volume in azoospermia groups. For the teretospermia and asthenospermia the result indicates significant increase of abnormal morphology and significant reduction of motility.

CONCLUSION

In the infertile study group testosterone level was decreased while the FSH and prolactin level were increased .This indicates an inverse relationship between the testosterone level and the other reproductive hormone. The authors suggest that further study may be needed to investigate the effect of Luteinizing hormone (LH) as it is produced by pituitary gland like FSH and may be linked to factors regulating spermatogensis.

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