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FSH, LH and Testosterone status and Categorization of Infertile Azoospermic Males – a Single Centre Cross Sectional Study

Lubna Naznin[1], Tabassum Parveen[2], Mimi Parvin[3], Susane Giti[4], Arif Ahmed Khan[5], Sarmin Sultana[6], Md Rafiqul Islam[7]

Abstract

Background: Male factor, alone or in addition to female partner contributes in about 50% of infertile couples. FSH, LH and testosterone are the prime regulators of germ cell development.

Objective: The aim of this study was to find the status of serum gonadotropin and total testosterone levels and to compare the findings between azoospermic primary infertile males and normozoospermic fertile males.

Materials and Methods: We had an analytical cross-sectional study in the department of Biochemistry, AFIP from Jan 2019 to Dec 2019. The study population was 50 azoospermic primary infertile males and 50 age matched normozoospermic males of proven fertility. All selected study subjects were then subjected to evaluation for serum hormonal status of FSH, LH and Testosterone levels.

Result: Mean ± SE of the FSH, LH & testosterone levels in azoospermic infertile males were 18.50 ± 2.89 mIU/mL, 10.01 ± 1.11 mIU/mL, & 5.48 ± 0.35 ng/ml respectively versus in the age matched fertile normozoospermic males were 4.26 ± 0.34 mIU/mL, 4.31 ± 0.20 mIU/mL & 5.48 ± 0.35 ng/mL respectively. FSH and LH levels were found significantly elevated ($p<0.001$) in azoospermic infertile males in contrast to normozoospermic males but there was no significant ($p>0.05$) difference for testosterone level in between the two groups. Majority (48%) among the azoospermic patients was found to have primary gonadal failure and second commonly (34%) had non-specific abnormality or other endocrinopathy. In contrast, none of the normozoospermic fertile males had abnormality in hypothalamo-pituitary-gonadal axis and had normal FSH, LH & Testosterone.

Conclusion: It is not necessary to measure FSH, LH and testosterone in normozoospermic males.

Key words: Male infertility, Azoospermic, Normozoospermic, FSH, LH, Testosterone

Introduction:

Approximately 15% of couples attempting their first pregnancy meet with failure. Most authorities define these patients as primarily infertile if they have failed to conceive after one year of unprotected intercourse. Data available over the past twenty years revealed that in approximately 30% of cases, pathology was found in the men alone and in another 20% both the men and women were abnormal. Therefore, the male factor is at least partly responsible in about 50% of infertile couples.[1] This makes the evaluation and treatment of the male extremely important.

At present in addition to history and physical examination, the armamentarium for evaluation of male infertility includes semen analysis, biochemical examination, hormonal assay, immunological and radiological investigations, cytogenetics and testicular biopsy.

Gonadotrophins (FSH, LH) and testosterone are the prime regulators of germ cell development. The successful and complete male germ cell development depends on the balanced endocrine interplay of hypothalamus, pituitary and the testis. Episodic secretion of Gonadotrophin Releasing Hormone (GnRh) by the hypothalamus elicits the pulsatile release of gonadotrophins i.e. follicle stimulating hormone (FSH) and lutenizing hormone (LH).[2,3] LH and FSH are the important regulators of steroidogenesis in the gonads.

1. Classified Specialist in Pathology, AFIP, Dhaka Cantonment.
2. Professor, Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh.
3. Classified Specialist in Pathology, AFMC, Dhaka Cantonment.
4. Classified Specialist in Pathology, Commandant, AFIP, Dhaka Cantonment.
5. Classified Specialist in Pathology, AFIP, Dhaka Cantonment.
6. Classified Specialist in Pathology, AFMC, Dhaka Cantonment.
7. Graded Specialist in Pathology, AFIP, Dhaka Cantonment.
Corresponding Author: Colonel Lubna Naznin, Classified Specialist in Pathology, AFIP, Dhaka Cantonment. E-mail: lubna101000@gmail.com

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In the testes, LH is the exclusive steroidogenic hormone acting only on the interstitial cells of Leydig, whilst FSH acts exclusively on the Sertoli cells. FSH binds with receptors in the sertoli cells and stimulate spermatogenesis. LH stimulates the production of testosterone in Leydig cells. Testosterone, in turn acts on the Sertoli cells and peritubular cells of the seminiferous tubules and stimulates spermatogenesis.[3]

Testosterone, estradiol and inhibin control the secretion of gonadotropins.[4] Testosterone, the major secretory product of the testes, is the primary inhibitor of LH secretion in males. Testosterone can be metabolized in peripheral tissue to the potent androgen, dihydrotestosterone or the potent estrogen, estradiol. These androgens and estrogens act independently to modulate LH secretion. Inhibin, a sertoli cell product, regulates the feedback control of FSH. Reduction in spermatogenesis is accompanied by decreased production of inhibin, causes reciprocal elevation in FSH level. Isolated rise in FSH is an important and sensitive marker of the state of the germinal epithelium. The increased FSH level in men with azoospermia or severe oligozoospermia (< 5million sperm/ml) is indicative of damaged seminiferous tubule.[5] The failure of pituitary to secret FSH and LH will result in disruption of testicular function also leads to infertility. Thus, hypogonadotropic hypogonadism cause decreased sperm counts and a state of infertility.[6]

With advancement of science, modern techniques have opened a horizon for the diagnosis and management of infertile couple, in which male partner contributes a large part. In Bangladesh few studies have been carried out on hormonal levels in different subgroups of infertile males. Hence, an attempt has been made to estimate the gonadotropins (FSH, LH), testosterone levels in primary infertile males with azoospermia.

Materials and Methods

Study design: Analytical Cross-Sectional study.
Place of study: Department of Biochemistry, AFIP.
Period of study: From Jan 2019 to Dec 2019.
Sample size: This study included 100 male subjects.
Study subjects and Grouping: The study population was 50 azoospermic primary infertile male cases, and 50 age matched normozoospermic males of proven fertility.

Sample technique: Convenient random sampling.
Inclusion criteria of Azoospermia:
1. Age: 24 years to 45 years.
2. Male partner of primary infertile couple of more than 1 year.
3. Azoospermia evident by semen analysis.

Inclusion criteria of Normozoospermia:
1. Age: 24 years to 45 years.
2. Male partner of fertile couple.
3. Normozoospermia evident by semen analysis.

Exclusion criteria:
1. Inadequate specimen.
2. Oligozoospermia
3. Coexisting genitourinary tract infection
4. Coexisting systemic illness e.g., Diabetes Mellitus,
5. Chronic renal disease, Heart disease, Brain Tumor etc.
6. Thyroid Disorder or other endocrine disorders
7. Patients having varicocele
8. Cytotoxic drug therapy/ radiation therapy.
9. Steroid / androgen therapy

Method

Only the male partners of primary infertile couples for more than one year were included for the study after taking a formal consent of the subject. Cases of azoospermia were selected from individuals reported at AFIP Clinical Pathology department for semen analysis. Relevant information of the subjects was collected and recorded in preformed data collection sheet. All selected study subjects were then subjected to evaluation for serum hormonal status of FSH, LH and Testosterone levels. Fifty (50) apparently healthy age matched normozoospermic controls of proven fertility were also evaluated in the same manner. To avoid the chance of secondary male infertility, normozoospermic fertile controls were selected only if the female partners had recent documented positive pregnancy test.

Collection of samples

In all cases semen analysis was done after sexual abstinence of at least 3 days and not more than 5 days. Before collection of the specimen individual was asked for bladder evacuation. Then semen specimen was collected in a sterile container by masturbation in a room with adequate privacy adjacent to the laboratory in AFIP. Semen analysis including sperm count was done by employing 'Improved Neubauer Counting Chamber' within one hour of collection by following WHO criteria (2010). The study subjects were classified as: normozoospermia (>15 million sperm /ml), Oligozoospermia (<15 million sperm/ml) and azoospermia (no spermatozoa).

Overnight fasting sera of the selected cases were collected. Serum FSH, LH, Testosterone levels were estimated by electrochemiluminescence immunoassay. The reference values used in the study were: FSH, 1.5-11.5 mIU/ml; LH, 1.1-8.2 mIU/ml; Testosterone, 2.7-17.1 ng/ml. The relationship of testosterone, LH and FSH were interpreted as per Table I.

Ethical Consent:
1. Informed written consent was taken from patient.
2. Patient's individual secrecy was maintained.

Statistical analysis:
Data was analyzed by standard statistical method (SPSS 20.0). Categorical variables were summarized with absolute values and proportions. Continuous variables were expressed as Mean ± 2SD and comparison between the two groups was done by t-test.

Table I: Interpretation of FSH, LH and Testosterone 7

Clinical condition	FSH	LH	Testosterone
Normal spermatogenesis	Normal	Normal	Normal
Primary Gonadal Failure or Hypergonadotrophic Hypogonadism	High	High	Normal/ Low
Secondary Gonadal Failure or Hypogonadotropic Hypogonadism	Low	Low	Low
Normogonadotrophic Hypogonadism	Normal	Normal	Low
Isolated LH elevation	Normal	High	Normal
Seminiferous tubule failure (Isolated FSH elevation)	High	Normal	Normal

Results

Table II: Distribution of study subjects according to age

Age range	Normozoospermic		Azoospermic	
	Number	%	Number	%
21-30	30	60%	26	52%
31-40	18	36%	22	44%
41-50	02	04%	02	04%
Total:	50		50	

Most of the study subjects belonged to 21 to 30 years age range in both the groups (table II).

SD: Standard Deviation & SE: Standard Error
Non-significant: ($p>0.05$) & significant: ($p\leq 0.05$)
Age distribution revealed no significant difference (i.e., $p>0.05$) between fertile normozoospermic & primary infertile azoospermic groups. So, the study was not biased due to age factor (Table III).

SD: Standard Deviation; SE: Standard Error
The Mean FSH level in azoospermic infertile males was 18.50 ± 2.89 mIU/mL with a range of .70 mIU/ml to 100 mIU/ml. In contrast, Mean FSH level in the age matched fertile normozoospermic group was 4.26 ± 0.34 with a range of 1.6 mIU/ml to 8.4 mIU/ml. and levels were within the reference limit (Table IV).
The Mean LH level in azoospermic infertile males was 10.01 ± 1.11 mIU/mL with a range of 0.6 mIU/ml to 42 mIU/ml. In contrast, the LH level in the age matched fertile normozoospermic group was 4.31 ± 0.20 with a range of 2.6 mIU/ml to 6.4 mIU/ml. and levels were within the reference limit (Table IV).
FSH and LH levels were found to have significant increment ($p<0.001$) in azoospermic infertile males in contrast to normozoospermic group (Table IV).
The Mean ± SE of the Testosterone level in azoospermic infertile males was 5.48 ± 0.35 ng/ml with a range of 0.9 ng/ml to 12,2 ng/ml. In contrast, the Testosterone level in the age matched fertile normozoospermic group was 6.12± 0.39 ng/ml with a range of 3.8 ng/ml to 13 ng/ml. and the levels were within the reference limit. But, in azoospermic group, 09 cases had Testosterone level below the lower normal limit. Data analysis by t-test revealed no significant ($p>0.05$) differences for testosterone level in between the normozoospermia and azoospermia cases (Table IV).

Reference range: FSH: 1.5-11.5 mIU/ml**; LH:** 1.1- 8.2 mIU/ml**; Testosterone:** 2.7-17.1 ng/ml
In case of azoospermia, elevated FSH was the predominant (48%) finding, in contrast all the normozoospermic fertile males (100%) had normal FSH level. Again, in case of azoospermia, elevated LH was the predominant

Table III: Mean, Standard Deviation and Standard Error of Age in Study subjects

	Study subjects	Mean	Std. Deviation	Std. Error	Significance
Age	Normozoospermia	30.73	4.13	0.75	>0.05 Put exact p value here
	Azoospermia	32.34	5.04	0.71	

Table IV: Comparison of Variables by t-Test between Normozoospermic group and Azoospermic group

Dependent Variable	Normozoospermic Group			Azoospermic Group			Significance
	Mean	SD	SE	Mean	SD	SE	
FSH level (mIU/ml)	4.26	1.84	0.34	18.50	20.44	2.89	Put exact p value here ≤ 0.001
LH level (mIU/ml)	4.31	1.11	0.20	10.01	7.84	1.11	≤ 0.001
Testosterone level (ng/ml)	6.12	2.17	0.39	5.48	2.47	0.35	>0.05

Table V: FSH, LH, Testosterone Comparison in Study Subjects.

	Azoospermia (n=50) versus Normozoospermia (n=50)		
	Normal Azoo Vs Normo	High Azoo Vs Normo	Low Azoo Vs Normo
FSH	23 (46%) vs 50 (100%)	24 (48%) vs 00 (0%)	03 (6%) vs 00 (0%)
LH	20 (40%) vs 50 (100%)	28 (56%) vs 00 (0%)	02 (04%) vs 00 (0%)
Testosterone	41 (82%) vs 50 (100%)	00 (0%) vs 00 (0%)	09 (18%) vs 00 (0%)

Table VI: Etiological categorization of 50 azoospermic patients.

Variants	Number	(%)
No abnormality/other endocrine abnormality	17	(34%)
Primary gonadal failure	24	(48%)
Hypogonadotrophic hypogonadism	02	(4%)
Normogonadotrophic hypogonadism	01	(2%)
Seminiferous tubule failure	02	(4%)
Isolated LH elevation	03	(6%)
Isolated FSH suppression	01	(2%)
Total	50	100%

(56%) finding, in contrast all the normozoospermic fertile males (100%) had normal LH level. Among 50 azoospermic infertile males, 41 had normal and 09 had low testosterone level. In azoospermia, normal testosterone was the predominant (82%) observation and all the normozoospermic fertile males (100%) had normal testosterone level. None of the study subjects had elevated testosterone level (Table V).

Majority (48%) of azoospermic patients were found to have primary gonadal failure and second common (34%) was due to non-specific abnormality or other endocrinopathy. None of the normozoospermic fertile males had abnormality in hypothalamo-pituitary-gonadal axis.

24+2+1= 27 should have testosterone below normal, but you showed only 9 cases had low testosterone

Discussion

The incidence of primary endocrine defects in sub-fertile men is less than 3% and is rare in men with sperm concentrations greater than 5 million per ml.[8] In this study, the endocrine milieu, related to spermatogenesis and sperm maturation was evaluated. We measured the serum FSH, LH and Testosterone levels in infertile azoospermic males and age matched normozoospermic fertile cases. Our study showed, 100% normozoospermic fertile and 34% azoospermic males had no abnormality in their hypothalamo-pituitary-gonadal axis. Commonest disorder detected was hypergonadotrophic hypogonadism in cases of azoospermia.

Mean FSH and LH levels had significant rise in azoospermic infertile males in contrast to normozoospermic fertile males. In all normozoospermic cases testosterone levels were normal but 18% azoospermic cases had decreased testosterone level. In normozoospermic fertile males, mean testosterone level was slightly higher than azoospermic infertile males but statistically no significant difference between the groups was noted.

Our study findings of significantly elevated gonadotropins (FSH and LH) levels in infertile azoospermic males than that of fertile normozoospermic males are in accordance with the studies Put only reference [9-14] found mean LH and FSH levels, 19.85 mIU/mL and 24.8 mIU/mL in azoospermic infertile males are also very similar to our findings. How is it going give up it didn't and that's it that's Change the language of highlighted ASH.

The present study also showed isolated cases of LH or FSH hypersecretion, a very similar finding to a study by Aisha Bano et al.[15] In this study isolated FSH elevation with normal LH and normal testosterone levels were found in two (4%) cases of azoospermia. High FSH have been reported to be associated with human subfertility by different studies.[16-18] Various studies have also showed, elevated serum FSH levels are associated with azoospermia.[18-20] Abnormal spermatogenesis may have a normal serum FSH, but a marked elevation in serum FSH is clearly indicative of abnormality in spermatogenesis.[7] It has been reported that, in addition to FSH secreting pituitary adenoma or testicular failure, hyperactivity of the FSH axis, could also be due to mutations of FSH receptor.[21]

In our study, isolated LH elevation with normal FSH and normal Testosterone level were found in in three (6%) cases of azoospermia. It has also been demonstrated that hyperactivity of the LH axis, leading to male infertility could be due to mutation of LH receptor gene. [21]

FSH has role in the maturation of spermatozoa.[22] Isolated FSH suppression with normal LH and normal testosterone level was found only in one (2%) case in azoospermia. This finding of our study is also supported by the study of Aisha Bano et al. reveals a relationship between male infertility and low levels of serum FSH.[15]

None of the sub-fertile case had isolated LH suppression. All normozoospermic proven fertile males had normal FSH, LH, as well as normal Testosterone level. The changes of LH, FSH may be one of reasons that cause the dysfunction of spermatogenesis and sperm maturation in patients with azoospermia and oligospermia.[23]

Testosterone is essential for normal sperm development. Low testosterone concentration i.e. hypogonadism results in infertility. [24] In this study, mean serum testosterone level was slightly higher in fertile normozoospermic males than azoospermic sub-fertile groups but the difference was statistically insignificant in between the two groups; similar observation was reported by other researchers [13,25,26]

The study limitation was that, the testicular histopathological study was not carried out and so the hormonal findings of the individuals could not be matched with and confirmed by the findings of testicular histology, like primary gonadal failure, hypospermatogenesis, spermatid arrest and sertoli cell only syndrome. Another limitation was other endocrinopathy was not considered in our study.

Conclusion

Measurement of hormones of hypothalamo-pituitary-gonadal axis is an essential aspect in evaluation male infertility. Abnormalities in hormone production e.g. FSH, LH and testosterone in patients of male infertility with azoospermia is not only a diagnostic tool in finding the etiology but also an indication for appropriate therapeutic considerations in azoospermia. It is not necessary to measure FSH, LH and testosterone in normozoospermic males.

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