Original Article

Serum Prolactin Status in Primary Sub-Fertile Males with Azoospermia and Oligozoospermia

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Abstract

***Background**: Fertility is adversely affected by negative feedback of prolactin on hypothalamic secretion of gonadotropin-releasing hormone (GnRH). Hyperprolactinemia inhibits the pulsatility of GnRH secretion and may cause secondary hypogonadism and results in spermatogenic arrest and impaired sperm motility. Besides, prolactin is also directly related to spermatogenesis and steroidogenesis. **Objective**: This study was designed to assess the serum prolactin status in primary sub-fertile males with azoospermia and oligozoospermia. **Materials and Methods**: This study was carried out at Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment from January 2019 to December 2019 on 150 males. The study population included primary infertile males, 50 azoospermic and 50 oligozoospermic as cases and 50 age-matched normozoospermic males with proven fertility as control. Serum prolactin levels were estimated in fasting sera by electrochemiluminescence immunoassay in all the three groups. The reference value for serum prolactin was 4−18 ng/mL. **Results**: Mean age for normozoospermic fertile males, oligozoospermic and azoospermic infertile males were 30.73 ± 4.13 years, 31.46 ± 4.59 years and 32.34 ± 5.04 years respectively. Mean serum prolactin level in normozoospermic fertile males, oligozoospermic sub-fertile males and azoospermic sub-fertile males were 9.44 ± 3.46 ng/mL, 12.02 ± 11.78 ng/mL, and 10.48 ± 4.55 ng/mL respectively with no significant variation ($p>0.05$). Serum prolactin was within the normal range (4−18 ng/mL) in all (100%) normozoospermic fertile males, 84% oligozoospermic males and 90% azoospermic males. Eight (16%) oligozoospermic cases and five (10%) azoospermic cases had hyperprolactinemia. Among the oligozoospermic cases with hyperprolactinemia, four (50%) had elevated prolactin with hypergonadotrophic state and four (50%) cases showed isolated prolactin elevation. All five (100%) azoospermic hyperprolactinemia cases were associated with hypergonadotrophic state. **Conclusion**: Serum prolactin estimation should be evaluated in primary sub-fertile males with azoospermia and oligozoospermia. Cases of hyperprolactinemia should also be evaluated for gonadotroph status.*

***Key words**: Azoospermia; Oligozoospermia; Prolactin*

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Introduction

Conception normally is achieved within twelve months in 80−85% of couples not using contraception. Couples who present after this time should therefore be regarded as possibly sub-fertile and need evaluation. Male factor is at least partly responsible in about 50% of infertile couples, in 30% cases only due to male factor and in 20% cases due to factors related to both the partners.[1] This makes the evaluation and treatment of the male partners extremely important. At present the tools for evaluation of male infertility includes history, physical examination, semen analysis, biochemical examination, hormonal assay, immunological and radiological investigations, cytogenetic study and testicular biopsy. The successful and complete male germ cell development depends on the balanced endocrine interplay of hypothalamus, pituitary and the testes. Gonadotropins (FSH, LH) and testosterone are the prime regulators of germ cell development. Gonadotrophin releasing hormone (GnRh) is secreted from the hypothalamus in a pulsatile manner. This elicits episodic release of gonadotropins, i.e., follicle stimulating hormone (FSH) and luteinizing hormone (LH).[2] Prolactin has complex inter-relationship with the gonadotropins (LH and FSH). Hyperprolactinemia inhibits pulsatile secretion of gonadotropin-releasing hormone (GnRH), which produces secondary hypogonadism by decreasing the pulsatility of FSH, LH and testosterone. This in turn leads to spermatogenic arrest and impaired sperm motility.[3] Besides, prolactin plays a direct role in spermatogenesis and steroidogenesis as prolactin receptors have been detected in Sertoli cells and Leydig cells in testes causing primary hypogonadism.[4] Patients with hyperprolactinemia have decreased or normal serum gonadotropin levels and decreased testosterone levels.[5] Hence, fertility is adversely affected by negative feedback of prolactin on hypothalamic secretion of GnRH. Oligospermic patients with normal serum gonadotropins showing a relatively higher serum prolactin level proves its role in gametogenesis independent of gonadotropins.[4,6] Previous studies have also reported that, semen analysis of patients with hyperprolactinemia may even show azoospermia.[7,8] Hyperprolactinemia may result from either disinhibition (e.g., compression of the pituitary stalk or reduced dopamine levels) or excess production of prolactin from a prolactinoma. In addition to pituitary tumors (macroadenoma or microadenoma), hypothyroidism, liver disease and drugs such as phenothiazine, tricyclic antidepressants, and some antihypertensives cause hyperprolactinemia.[9] Excess production of prolactin has been implicated as a cause of both sub-fertility and erectile dysfunction. In one series of male sub-fertile patients, 4% were noted to have elevated serum prolactin level.[9] Only a very few studies have been carried out on serum prolactin level in different subgroups of infertile males. Hence, an attempt has been made to estimate serum prolactin in azoospermic and oligozoospermic infertile males.

Materials and Methods

This study was carried out at Armed Forces Institute of Pathology (AFIP) in the Department of Biochemistry, Dhaka Cantonment from January 2019 to December 2019 on 150 males. The study population included primary infertile males, 50 azoospermic and 50 oligozoospermic as cases and 50 age matched normozoospermic males with proven fertility as control. Convenient purposive random sampling was carried out.

Inclusion criteria of sub-fertile cases were i) age: 24 years to 45 years, ii) male partner of primary sub-fertile couple for more than 1 year, iii) azoospermia evident by semen analysis and iv) oligozoospermia evident by semen analysis. Inclusion criteria of normozoospermic fertile males were i) age: 24 years to 45 years, ii) male partner of fertile couple, iii) normozoospermia evident by semen analysis.

Exclusion criteria were i) inadequate specimen, ii) coexisting genitourinary tract infection, iii) coexisting systemic illness, e.g., diabetes mellitus, chronic renal disease, heart disease, brain tumor etc, iv) thyroid disorder or other endocrine disorders, v) patients having varicocele, vi) cytotoxic drug therapy/radiation therapy, vii) steroid/androgen therapy.

Only the male partners of primary infertile couples for more than one year and normozoospermic fertile males were included in this study after taking a formal consent. Cases of azoospermia and oligozoospermia

were selected from individuals reported at AFIP clinical pathology department for semen analysis. Relevant information of the subjects were collected and recorded in preformed data collection sheet. All selected study subjects were then subjected to evaluation for serum prolactin levels. Fifty apparently healthy age-matched normozoospermic males 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 specimens

In all cases semen analysis was done after sexual abstinence for at least 3 days but 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 sperms/mL), oligozoospermia (<15 million sperms/mL) and azoospermia (no spermatozoa). Overnight fasting sera of the selected cases were collected. Serum prolactin levels were estimated by electrochemiluminescence immunoassay. The reference value for serum prolactin was 4−18 ng/mL.

Ethical consent

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

Statistical analysis

Data were analyzed by standard statistical method SPSS version 20.0. Data were expressed as mean ± 2SD and comparison was done for azoospermia and oligozoospermia with the control group. Comparison between the groups was done by t-test or one way ANOVA.

Results

The study was carried out on 50 azoospermic primary sub-fertile males, 50 oligozoospermic primary sub-fertile males and 50 normozoospermic fertile males. Table I shows distribution of study subjects according to age range and Table II shows the mean age of three groups. No significant variation (p>0.05) was observed in age of three groups. So, age-related variation did not affect the hormonal assay interpretation.

Age distribution revealed no significant difference (p>0.05) among the normozoospermic, oligozoospermic and azoospermic groups.

Table III shows the mean of serum prolactin levels in study subjects. No significant variation (p> 0.05) in serum prolactin levels was observed among these three groups. Table IV shows the distribution of study subjects by serum prolactin concentration.

Serum prolactin was within the normal range (4−18 ng/mL) in all (100%) normozoospermic fertile males, 84% oligozoospermic males and 90% azoospermic males. Remaining, 16% oligozoospermic cases and 10% azoospermic cases had hyperprolactinemia.

Table I: Distribution of study subjects according to age

Age range in years	Normozoospermic		Oligozoospermic		Azoospermic		p value
	Number	Percentage	Number	ercentage	Number	ercentage	
21−30	30	60%	25	50%	26	52%	>0.05
31−40	18	36%	23	46%	22	44%	
41−50	02	04%	02	04%	02	04%	
Total	50		50		50		

Non-significant: (p>0.05); Significant: (p≤ 0.05)

Table II: Mean and standard deviation of age in study subjects

Study subjects	Age	p value
	Mean ± 2SD	
Normozoospermia	30.73±4.13	>0.05
Oligozoospermia	31.46±4.59	
A***zoospermia***	32.34±5.04	

Non-significant: (p>0.05); Significant: (p≤ 0.05)

Table III: Mean ± SD of serum prolactin in study subjects

Study subjects	Serum prolactin (ng/mL) Mean ± 2SD	p values	
Normozoospermic	9.44 ± 3.46	Oligospermic	>0.05
		Azoospermic	>0.05
Oligospermic	12.02 ± 11.78	Control	>0.05
		Azoospermic	>0.05
Azoospermic	10.48 ± 4.55	Control	>0.05
		Oligospermic	>0.05

Non-significant: (p>0.05); Significant: (p≤0.05)

Table IV: Distribution of study subjects by serum prolactin concentration

Serum prolactin status	Number (%) of study subjects		
	Normozoospermia	Oligozoospermia	Azoospermia
Normal	50 (100)	42 (84)	45 (90)
High	00 (00)	08 (16)	05 (10)
Low	00 (00)	00 (00)	00 (00)
Total	50 (100)	50 (100)	50 (100)

Table V: Gonadotrophs and testosterone status in hyperprolactinemia with oligozoospermia and azoospermia

Gonadotrophs and testosterone status in hyperprolactinemia	Oligozoospermia	Azoospermia	Total cases
Hypergonadotrophic state (high FSH, high LH, normal testosterone)	04/13	05/13	09/13 (69.2%)
Isolated hyperprolactinemia (normal FSH, normal LH, normal testosterone)	04/13	-	04/13 (30.8%)
Total	08/13	05/13	

Table V shows gonadotrophs and testosterone status in hyperprolactinemia with oligozoospermia and azoospermia. In oligozoospermia group total 08 subjects had hyperprolactinemia; among them 04 (50%) cases had elevated prolactin with hypergonadotrophic state and 04 (50%) cases showed isolated prolactin elevation. In all of the eight cases serum testosterone was within normal range. All azoospermic five cases (100%) with hyperprolactinemia had hypergonadotrophic state.

Discussion

This study revealed that normal serum prolactin was the predominant finding in azoospermic (90%), oligozoospermic (84%), and in the normozoospermic fertile males (100%). Mean prolactin level in primary sub-fertile males with azoospermia and oligozoospermia were 10.48 ng/mL and 12.02 ng/mL respectively. Both were within normal range but slightly higher than mean prolactin level, 9.44 ng/mL found in normozoospermic fertile males. Variations among these three groups were statistically insignificant ($p>0.05$). This study is also consistent with the findings of Al-Daghistani & Abdel-Dayem[10] where the serum prolactin level was found relatively elevated in azoospermia and in oligozoospermia in contrast to that of fertile control group but in all groups mean prolactin values were within the range. Mean serum prolactin level in the study of Shahroona et al[11] on 100 infertile males having oligozoospermia or azoospermia was 18.07 ± 6.94 ng/mL. It was significantly higher ($p<0.001$) in contrast to 20 apparently healthy control with 5.45 ± 1.13 ng/mL. It was different from us and may be due to inclusion of more number of apparently healthy males in our study.

In our study, among the infertile sub-groups total 13 cases (13%) were found to have elevated prolactin in contrast to normal prolactin level in all normozoospermic males. Hyperprolactinemia was present in 08/50 (16%) primary infertile oligozoospermic males and 05/50 (10%) primary infertile azoospermic males. Our study is also supported by Carter et al[12], reporting hyperprolactinemia causing infertility in approximately 11% oligozoospermic males.[13] So, serum prolactin estimation is necessary in evaluation of primary infertile male presenting with azoospermia or oligozoospermia.

In our study on 150 study subjects (50 apparently healthy and 100 sub-fertile males having either oligospermia or azoospermia), 13 sub-fertile males had elevated prolactin. Among them, four had elevated prolactin with normogonadotrophic state, and nine cases had elevated prolactin with hypergonadotrophic state, of whom 5 were azoospermic and 4 were oligozoospermic. A similar study by Shahroona et al[11] in Multan on 120 subjects (20 apparently healthy and 100 infertile males having either oligospermia or azoospermia) reported 25 cases (25%) to have hyperprolactinemia. Among them, 12/25 (48%) cases had normogonadotrophic state and 13/25 (52%) had hypergonadotrophic hypogonadism, i.e., primary hypogonadism. Among them 9 had azoospermia and 4 had oligospermia. Both our study and Shahroona et al[11] had similar findings, i.e., that relatively more cases of hyperprolactinemia were associated with hypergonadotrophic state than normogonadotrophic state. High serum FSH levels of hypergonadotrophic state attempt better spermatogenesis in hyperprolactinemia. This has been shown by Yazawa et al[14] who has experimentally demonstrated that prolactin induces apoptosis in spermatogonial stage of the testes in Japanese newt and addition of FSH to these tissue cultures could reverse this state. Our study found three-fourths, Shahroona et al[11] found three-fourths and Merino et al[15] found two-thirds of the patients with oligozoospermia and azoospermia had normal prolactin levels. All these findings make it difficult to find a relevant role for serum prolactin on sperm concentration.

All normozoospermic fertile males had normal prolactin level but 16% oligozoospermic and 10% azoospermic cases had prolactin level beyond upper limit. But statistically there was no significant difference in mean prolactin levels among the three groups. Majority cases of hyperprolactinemia with azoospermia or oligozoospermia were associated with hypergonadotrophic state. Hence, serum

prolactin should be estimated for evaluation of primary infertile males with azoospermia and oligozoospermia.

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