ORIGINAL ARTICLE

SERUM INHIBIN B AS A DIAGNOSTIC MARKER OF MALE INFERTILITY

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Background: Infertility affects about 15% of all couples in the world. Approximately 40% of all infertility cases could be attributed entirely to male factors. Serum inhibin B has emerged as a sensitive marker of male fertility. Analysis of serum inhibin B reflects the relationship between inhibin B, Sertoli cell function and spermatogenesis. **Methods:** This validation study was conducted to calculate the sensitivity, specificity, positive and negative predictive value of serum inhibin B in diagnosis of male infertility, using semen analysis as the gold standard. One hundred and sixty men were included in the study, they reported for semen analysis for evaluation of male infertility. Sperm count was done per standard procedure. Serum inhibin B level was determined by ELISA. **Results:** Serum inhibin B level ≥80 ρg/ml was regarded as a normal response. The serum inhibin B test had 75% sensitivity, 93.1% specificity, 80.5% PPV and 90.7% NPV. **Conclusion:** Serum inhibin B has a positive correlation with sperm counts and could be used for evaluation of male infertility as a non-invasive predictor of spermatogenesis. The sensitivity, specificity and PPV are appropriate for clinical decision making and to avoid unnecessary testicular biopsies.

Keywords: Inhibin B, male infertility, sensitivity, specificity, predictive values

INTRODUCTION

Infertility is a health problem of multi-factorial aetiology with an estimated world-wide prevalence of 15%.[1] In about 50% of infertile couples, a male factor aetiology is demonstrable[2] (about 40% of all infertility cases may be attributed entirely to male partner, with another 20% to combined male and female factors of infertility)[3], based upon abnormal semen characteristics (i.e., sperm concentration, motility, morphology, etc.). Although sperm concentration between fertile and sub-fertile populations overlaps extensively,[4] oligozoospermia is the most common semen abnormality in infertile males[5].

Assessment of spermatogenesis plays a central role in evaluation of the infertile male. Semen analysis is a classical marker, while testicular biopsy is carried out to ascertain the cause of spermatic abnormalities, testicular impairment or an obstructive disorder.[6] Endocrine evaluation includes measurement of serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). These hormones have their diagnostic limitations. There is a need for an accurate additional marker of spermatogenesis. Recently serum inhibin B has emerged as a sensitive marker of male fertility.[7]

In 1932, Mc Cullagh postulated the existence of a specific hormone of the testis, i.e., inhibin B.[8] Inhibin B is a glycoprotein with a molecular mass of 32 KDa and consists of two sub units (α and β) connected with disulfide bonds.[9] It is produced by Sertoli's cells of seminiferous tubules in men[10] and is also present in spermatogonia, spermatocytes and early spermatids.[8] Inhibin B regulates FSH secretion in a negative feedback loop.[11] It acts as a 'messenger' from the testes, inhibiting FSH secretion from pituitary when sperm cells production has been stimulated enough, reflecting the testicular response to activity of the hypothalamic-pituitary-gonadal hormone (HPG) axis.[12] Inhibin B production depends both on FSH and spermatogenic status.[13]

Several studies[14–22] of serum inhibin B in relation to male reproductive health have been published internationally. The objective of this study was to calculate the sensitivity, specificity, positive and negative predictive value of serum inhibin B in diagnosis of male infertility, using semen analysis as the gold standard.

MATERIAL AND METHODS

One hundred and sixty men of age 18–60 years were included in the study after obtaining their informed consent. They reported for semen analysis as a part of their evaluation for male infertility. Serum of all subjects was obtained for inhibin B assay. Patients already on hormonal therapy for infertility were excluded from the study.

Semen specimen was obtained by masturbation, after abstinence for 3–7 days, in a clean, dry, sterile wide-mouth container; the same were kept at 37 °C and were analysed within two hours of collection. For inhibin B assay, 5 ml venous blood was collected in a plain bottle without anticoagulant and serum was separated by centrifugation. The serum aliquots were stored at -20 °C till analysis in batches.

Semen analysis for sperm count was done by using Neubaur chamber under light microscope. The serum inhibin B was assayed using the commercially

available ELISA kit from Diagnostic Systems Laboratories (DSL), Inc. (Texas, USA). The detection limit was 7 ρg/ml (linearity: 10–531 ρg/ml). The inter-assay coefficient variation (CV) was 6.7% and the within-assay CV was 4.5%.

All data including demographical, clinical details and biochemical parameters were analysed using SPSS-11. Descriptive statistics were carried out to summarise the data. Mean and SD were calculated for numerical data. Frequency and percentage were calculated for qualitative data. Pearson's test was used to correlate the serum inhibin B levels with the total sperm count. Pearson's test was also used to correlate the age of subjects with the serum inhibin B levels and total sperm count. A *p*-value of <0.05 was considered as significant. Considering sperm count as gold standard, the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of serum inhibin B were calculated by 2×2 contingency table.

RESULTS

One hundred and eighty-four adult men were interviewed who reported for semen analysis. Twenty-four cases were excluded from the study on the basis of exclusion criteria, and 160 were enrolled for the study. Mean age of the patients was 31±6 years.

Amongst these 160 individuals, normospermic were 116 (72.5 %), oligospermic 19 (11.9%), and azoospermic were 25 (15.6%). Serum inhibin B level ≥80 ρg/ml was regarded as a normal response. The decrease in serum inhibin B concentration correlated well with reduction in spermatogenesis. In normospermic cases sperm count was 67±16 million/ml and serum inhibin B levels were 202.0±47.2 ρg/ml. In oligozoospermic cases sperm count was 8±6 million/ml and serum inhibin B levels were 44.7±24.5 ρg/ml, and in azoospermic cases serum inhibin B levels were 61±78 ρg/ml (Table-1).

Serum inhibin B levels positively correlated with sperm count (r= 0.792, p<0.0001). The age of men negatively correlated with sperm count (r= -0.163, p<0.039) and with serum inhibin B levels (r= -0.188, p<0.017) (Table-2). Serum Inhibin B had sensitivity 75%, specificity 93.1%, PPV 80.5%, and NPV 90.7%.

Table-1: Serum inhibin B levels in patients with normal and impaired spermatogenesis (n=160,Mean age= 31±6 years)

	Normospermic n=116	Oligospermic n=19	Azoospermic n=25
Sperm count million/ml	67±16	8±6	0±0.004
Serum inhibin B pg/ml (Mean±SD)	202±47.2	44.7±24.5	61±78*

*Note: The increased variation in serum inhibin B levels in azoospermic patients might be due to 'inclusion of Sertoli cell only syndrome (SCO) or obstructive causes for azoospermia'

Table-2: Correlations between serum inhibin B levels and sperm count

		Sperm count	Inhibin B levels
Sperm count	Pearson Correlation	1	0.792*
	Sig. (2-tailed)		p<0.0001
Inhibin B levels	Pearson Correlation	0.792*	1
	Sig. (2-tailed)	p<0.0001	

*Correlation is significant at 0.01 level

DISCUSSION

A significant positive correlation between sperm count and serum inhibin B levels was demonstrated. The results provide strong evidence that serum inhibin B is an important marker of competence of Sertoli cells and spermatogenesis, which is in accordance with studies carried out worldwide on serum inhibin B and spermatogenesis.

In this study mean age negatively correlated with sperm count and with serum inhibin B levels. Similar findings were observed in studies by Hu YA and Huang YF[23], and Mahmoud AM *et el*[24]. Kumanov P *et al*[25] reported a natural outcome of aging process.

Majority (72.5%) of males who reported for semen analysis/evaluation for male infertility had normal sperm count. The serum inhibin B estimation and its correlation with sperm count has been a subject of interest for the last so many years. There is no consensus on the limit of low sperm count that could be regarded as cause of infertility. However, it has been documented that conception rate decreases significantly with a sperm count of <20 million/ml.

Studies demonstrated that inhibin B concentration was higher in men with apparently normal fertility than in those with infertility and abnormal spermatogenesis[22] (except in those with obstructive azoospermia or spermatogenic arrest at some stages[16,26]). Men who underwent castration had undetectable inhibin B levels, confirming the fact that serum inhibin B reflect testicular function and, more precisely, Sertoli cell function.[22,27] The men with Sertoli-cell-only syndrome (SCO, i.e., absence of germ cells) have very low levels of inhibin B and there was a close correlation between the presence of SCO and the level of serum inhibin B.[23] There is an inverse relationship between serum inhibin B and FSH in adults.[28,29] Infertile males have low inhibin B and high FSH levels.[29,30] Serum inhibin B values <80 ρg/ml were reported as abnormal, suggesting male disorder or dysfunction.[22]

The present data showed significant differences in mean serum inhibin B levels between oligospermia/azoospermia and normospermia subgroups. Serum inhibin B levels were significantly reduced in infertile men with oligospermia/azoospermia, compared to males of normospermic group. In fact the serum inhibin B concentrations were usually not less

than 100 ρg/ml in men with intact spermatogenesis. All patients with a normal sperm concentration had mean serum inhibin B concentration 202±47.2 ρg/ml; all patients with reduced sperm concentration had a lower serum inhibin B concentration <80 ρg/ml, except in some azoospermic patients. The variation in serum inhibin B levels in azoospermic patients could be due to inclusion of 'SCO or obstructive causes for azoospermia' in this study. These results are in agreement with the findings of previous studies. Anderson RA[31] reported that serum inhibin B concentrations appeared more closely related to presence of germ cells. Inhibin B concentrations fell to undetectable levels following loss of all germ cells, e.g., by testicular irradiation. The direct positive correlation between serum inhibin B and sperm count in normal men indicates that serum inhibin B quantitatively reflects the number of spermatozoa being released. Similar results were observed by Pierik *et al*[8] who summarised the pros and cons of different markers of spermatogenesis, with specific focus on serum inhibin B and reported that the serum inhibin B levels were associated with classical markers of spermatogenesis, particularly testicular histology, and was the most accurate endocrine marker of spermatogenesis. A subnormal serum inhibin B level clearly reflected disturbed spermatogenesis. Anderson *et al*[32] reported that serum inhibin B concentrations correlated directly with testicular status based on concurrent biopsy specimens; serum inhibin B concentrations in adult men with normal spermatogenesis were higher than in subjects with maturation arrest (MA) or SCO syndrome. However, our study indicated that serum inhibin B was a useful marker of spermatogenesis and inhibin B production sufficient to maintain detectable serum concentrations in adults depends on spermatogenic activity.

In this study serum inhibin B showed 75% sensitivity and 93% specificity for spermatogenesis, keeping the cut-off levels of serum inhibin B >80 ρg/ml for normal spermatogenesis. Similar findings were observed in a study by Pierik *et al*[15], keeping the cut-off levels of inhibin B >139 ρg/ml for normal spermatogenesis (the difference between cut-off levels of serum inhibin B to discriminate oligospermia/ azoospermia from normospermia may be due to different methodology/kits for inhibin B assays), they reported that serum inhibin B had 83% sensitivity and 90% specificity (patients with obstructive azoospermia were excluded). The sensitivity of serum inhibin B could have been increased, had the cases of obstructed azoospermia and SCO syndrome been excluded by testicular biopsy or fine needle aspiration cytology (FNAC) of testes.

This study revealed 90.7% NPV and 85.5% PPV, which are in agreement with the results of a study by Jensen *et el*[29] that recorded 80% PPV of detecting sperm counts below 20 million/ml among men who's serum inhibin B levels were below 80 ρg/ml. In many studies, the sensitivity, specificity and PPV of serum inhibin B were calculated on the basis of sperm retrieval in men with non-obstructive azoospermia by TESE (testicular sperm extraction) procedure and after exclusion of obstructed azoospermic cases by testicular biopsies. In obstructed azoospermia[33] and SCO syndrome[19,23] the serum inhibin B levels were elevated or reached their lowest level respectively.

According to the above mentioned studies and this study, the serum inhibin B level has proven to be valuable in the evaluation of male infertility, and holds a promise for further research and application.

CONCLUSION

Serum inhibin B has a strong positive correlation with spermatogenesis. Inhibin B is the best known endocrine marker for spermatogenesis. The sensitivity, specificity and PPV are appropriate for clinical decision making and to avoid unnecessary biopsies. Estimation of serum inhibin B is a non-invasive predictor of spermatogenesis. Its estimation proves to be a non-invasive alternate for testicular biopsy and also for differentiating normal and impaired spermatogenesis in infertile males.

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