CLINICAL UTILITY OF INHIBIN B AS A BIOMARKER OF MALE INFERTILITY

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ABSTRACT

Background: Infertility is a health problem that involves about 15–20% of couples. Male factor is supposed to contribute in approximately 40–50% of the infertility cases. Inhibin B is considered one of biomarkers of spermatogenesis and testicular function.

Objective: To measure the potential role of serum FSH and Inhibin B in assessment of spermatogenesis among infertile male partner and to estimate sensitivity and specificity, positive and negative predictive value of serum inhibin B.

Methodology: In this cross sectional study, out of one hundred and seventy eight adult males, who visited pathology department of BVH Hospital, for semen analysis, one hundred and forty four were selected. Sperm count was performed per WHO 2010 guidelines and infertile males were compared for endocrine profile with age matched fertile group. Infertile subjects were further categorized into normospemic, oligospermic and azoospermic groups and serum FSH and inhibin B level were evaluated by applying ANOVA in each group. Data was analyzed by SPSS 20.

Results: Amongst these 146 individuals, normospermic were 69 (47 %), oligospermic 48 (33%), and azoospermic were 29 (20%). Serum FSH (12.2±9.6IU/L) was highest while inhibin B (83.48±66.7pg/ml) was lowest in azoospermic group. Serum inhibin B more than75 ρg/ml was regarded as a normal response. Serum Inhibin B had sensitivity 78%, specificity 90.3%, PPV 79.2%, and NPV 91% using sperm count as gold standard.

Conclusion: Serum inhibin B is significantly related to spermatogenesis. It can be diagnostic markers for male infertility work up and will provide encouraging and promising results in correct application of spermatogenesis.

Key words: Inhibin B, FSH, Semen, Infertile male

INTRODUCTION

Infertility is a health problem that involves about 15–20% of couples.[1] Male factor is supposed to contribute in approximately 40–50% of the infertility cases. [2,3] Semen analysis, endocrine evaluation and testicular biopsy are possible variables to be performed to investigate male factor. Semen analyses with detail about sperm quantity and quality is considered a reliable baseline infertility investigation. There are substantial biological and laboratory instability and variability being observed in a sample so semen analysis has to be performed multiple times to establish trend. Proper history about abstinence length, recent ailment or testicular heat exposure is important while interpreting semen analysis (SA).[4, 5] The regular iteration and overlapping of "normal" SA ranges in the World Health Organization (WHO) guidelines make it difficult for clinician to discriminate fertile from infertile men and manage male infertility cases.[6] Moreover comparison of semen reports of different lab may vary as interpretation of different pathologist will be different. Blood samples are easier to obtain than ejaculates or biopsy.[7]

Moreover, advancing research technology and techniques have made it possible to use better biomarkers to identify possible causes of male fertility.[8] Follicle-stimulating hormone is considered a principal endocrine marker in evaluation of male testicular function. It exerts its action on sertoli cell to initiate and facilitate the process of spermatogenesis but its levels are influenced by hypothalamus.[9] Sertoli cells in turn secrete inhibin B, a protein hormone which inhibit FSH secretion by negative feedback effect. So it can also be used to establish cause of infertility whether due to testicular damage or an obstructive lesion.[10,11]

In the literature, potential role of FSH and Inhibin B as reliable markers of infertility is debatable.Some studies attempted to prove Inhibin-B to be better[12]or equal[13,14] to FSH, while in other [15,16] combination would be better predictor of spermatogenesis and testicular function.

The purpose of this study was to measure the potential role of serum FSH and Inhibin B in assessment of spermatogenesis among infertile male partner and to estimate the sensitivity, specificity,

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positive and negative predictive value of serum inhibin B considering semen analysis as gold standard in work up of male infertility.

METHODOLOGY

This cross sectional study included 146 men who were referred for semen analysis from infertility clinic of Bahawal Victoria Hospital, from October 2017 to November 2018. Informed consent was taken after approval of study from ethical committee. The infertile men with previous surgeries for undescended testicles, varicocelectomy, orchiectomy and taking any hormonal preparation forinfertility treatment were excluded from the study.

The levels of endocrine markers, such as FSH, LH, testosterone, prolactin and inhibin B were measured in cases and control groups. Semen samples were taken in a clean dry sterile wide-mouth container after abstinence for 3 days. Sperm count was performed on semen by using Neubaur chamber under light microscope within two hours of collection. "Lower Reference Limit" (LRL) for sperm parameters were as follows: sperm concentration 15million/ mL; forward progressive motility 32%; vitality 58% and normal sperm morphology 4%, with leukocytes< 1 million/mL. Venous blood was taken in a clot activator tube and serum was separated by centrifugation. The serum FSH, LH and testosterone were performed on Architect i1000SR (Abbott) chemiluminescence based technology. Serum inhibin B measurement was performed byusing the commercially available Gen IIELISA kit from Beckman Coulter. The detection limit was 7 ρg/ml (linearity: 10–531 ρg/ml). The coefficient variation (CV) for intrassay was 8.1% and the intraassay CV was 6.5%.

The demographic data and biochemical parameters were analyzed using SPSS-20. The numerical data analysis was done by calculating, Mean, SD and qualitative data were analyzed by frequency and percentage. Data were analyzed using one-way analysis of variance (ANOVA).The correlation of serum FSH and inhibin B levels with total sperm count were established by Pearson's test. A p-value of <0.05 was considered as significant. The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of serum inhibin B were measured against sperm count (gold standard) by 2×2 contingency table.

RESULTS

Out of one hundred and seventy eight adult males who visited pathology department for semen analysis, one hundred and forty four were selected. A total of 146 patients who were enrolled for study, 23 had history of cryptorchidism and 34 had varicocele, 15 infertile males had previous history of genital tract infections. The infertility of 29 patients was associated with chronic illnesses i.e TB, Chronic renal diseases, diabetes, obesity while torsion, testicular hematoma and inguinal hernia was present in 9 cases and. 40 patients were labeled to have idiopathic infertility. Mean age of the patients was 32±6 years. The endocrine profile of infertile men was compared with age match fertile group of 30 males.

Table I: Mean (±SD) values of Endocrine Profile between infertile and fertile subjects.

Parameters	Infertile group N=144	Fertile group n=30	P value
Serum FSH(IU/L)	10.7±7.1	5.4±1.8	0.003
Serum LH (IU/L)	9.6±5.6	3.2±2.1	0.001
Serum Prolactin(µg/L)	6.5±1.9	5.9±1.3	0.38
Serum testosterone (nmol/L)	5.6±10.5	8.7±4.2	0.001
Serum inhibin B (pg/ml)	127.2± 67.8	207.9±53.72	0.0001

Table II: ANOVA Post Hoc Multiple Comparison Analysis

Groups	Number of subject	FSH IU/L (mean± SD)	Inhibin B pg/ml (mean± SD)
Normospermic	69	7.9±3.2‡	165.4±50.4**
Oligospermic	48	8.6±5.1	98.3±3.6
Azoospermic	29	12.4±9.6	83.5±66.7
Total subject	146	10.7±7.1	127.9±67.8

‡Group I vs. group II, P=.14; group II vs. group III, P+.04; group I vs. group III, P=.0001.
**Group I vs. group II, P=.0001; group II vs. group III, P=.001; group I vs. group III, P=.0001.

The inhibin B and total testosterone values were significantly showing decrease trends in the patients than in the controls (p<0.05). The levels of serum FSH (10.71±7.1 IU/L vs 5.4±1.8 IU/L) and LH (9.6±5.6 IU/L vs 3.2±2.1 IU/L) concentrations were

significantly higher than those of fertile controls. Although, difference observed in serum prolactin concentrations (6.5±1.9 IU/L vs 5.7±1.3 IU/L) was present but they were not statistically important. (p>0.05)(Table I). The infertile group was divided into three categories on basis of semen report; 69 were included in normospermic (47%), 48 were in oligospermic (33%) and azoospermia was observed in 29 cases (20%) with sperm count of 61±19, 9±4, 0±0.0003 million/ml respectively. The serum FSH and inhibin B concentrations were compared among three groups. Azospermic patients showed highest concentration of FSH and lowest levels of Inhibin B as seen in table II. Post Hoc multiple comparisons through LSD (Lit significant difference) analysis of Serum inhibin B and FSH was performed and results showed that for inhibin B values difference among three groups is statistically significant while difference between group I vs group III and group II vs group III were significant for serum FSH levels. Pearson correlation demonstrated positive association (r= 0.794, p<0.0001) between serum Inhibin B and sperm count. Serum Inhibin B had sensitivity 78%, specificity 90.3%, PPV 79.2%, and NPV 91% using sperm count as gold standard.

DISCUSSION

The evaluation for cause of fertility is typically initiated for couples who have been failing to conceive naturally for some period of time. The initial laboratory investigation of male infertility includes at least two semen analyses to establish a trend in reproductive potential but substantial variability in seminal parameters between and within male patients have been observed.[6,7]

Men should be properly guided about abstinence period and delivery protocols that should be followed to produce consistent and accurate results.[6] When sample is received in laboratory, analysis and interpretation of sample showed inter and intra individual variability. Moreover, the biopsy of testicular tissue is taken from different areas which may not be representative of the functional status of entire organ. The discovery of highly sensitive and specific assay of inhibin B made it possible to employ it as possible marker of male infertility. The functional capability of whole testis can be established by single measurement of easily collected blood sample. [17]

The result of this study are in agreement with that of Saleh et al which postulated that in infertile group serum FSH and LH were significantly increased while serum total testosterone and inhibin B were significantly lower in comparison to fertile men.[18] Majority of infertile male (47%) were categorized in normospermic group in our study as that of Jequier et al.[19] but sperm count did not coordinate and compatible to fertility potential of male. Even the results of some studies revealed that in almost half of non-obstructive azoospermic patients, it is possible to retrieve sperm despite variation in testicular size, serum FSH levels and clinical presentations concentration[20-23] but there is consensus that conception rate decreases with sperm count <15 million/ml.[6,7] It is essential to find reliable marker that anticipate and correlate well with sperm count. Many researches were conducted on measurement of serum inhibin B and its association with sperm count in the past. However, discovery of sensitive inhibin B assay makes it possible to predict and correlate with the presence of spermatozoa and provides high diagnostic priority in azospermicnon obstructive infertility cases.[23,24] It is possible to retrieve spermthrough testicular sperm extraction technique and use fresh and cryopreserved sperm to achieve successful treatment of infertility before ovarian stimulation. It is also helpful in decreasing emotional and financial loss of failed treatment.[23-25]

Similar results were observed in subgroup analysis, oligospermic, azoospermic and normospermic males showed different values of serum inhibin B. The levels were significantly decreased in oligospermic and azoospermic subjects in contrast to normospermic group. The mean serum inhibin B concentration of 165.4±50.4ρg/ml was observed in normal sperm count while mean concentration below 98.3ρg/ml was noted in other two groups except in some azoospermic cases. They showed wide variation in serum inhibin B levels because 'Sertoli cell only syndrome and obstructive azoospermic cases were also included in this study. The possible explanation of these findings is that alteration in testicular function can be predicted by serum inhibin B estimation efficiently rather than FSH, an indirect biomarker of spermatogenesis. Although, FSH is important hallmark for evaluation and differentiation between peripheral and central disorders of male infertility.

In our study, sperm count of male appears to be significantly and positively correlated with inhibin

B. The other results also revealed that it is a valuable indicator of integrity and functional capacity of Sertoli cells and its spermatogenesis activity even when serum inhibin B levels were compared to that of testicular biopsy.[26-28]

The positive relationship between sperm count and serum inhibin was in accordance with Pierik et al,[26] study with suggestion that serum inhibin B concentration could be indicator of spermatozoa being produced in testis. In this study, keeping the serum inhibin B cut off values at 59ρg/ ml demonstrated sensitivity of 78%, specificity 90.3%, PPV 79.2%, and NPV 91% for spermatogenesis. The results of some other studies showed that serum inhibin B had 83% and 75% sensitivity and 90% and 93% specificity (obstructive azoospermic cases were excluded) at levels of>139 ρg/ml and more than80pg/ml.[26, 28] The difference between cut-off levels of serum inhibin B to differentiate among three groups may be due to variation in instruments, techniques and reagent kits for inhibin B measurement. The sensitivity of assay could be increased if testicular biopsy or fine needle aspiration cytology (FNAC) of testes could be performed to exclude obstructed azoospermia and SCO syndrome cases. The results of NPV and PPV of 91%and 79.2% of this study todetect sperm counts below 15 million/ml were comparable to two other studies showing negative and positive values of 90.7%, 89%, 85.5% and 80% respectively.[28,29] The limitations of our study are that we included obstructed azoospermia and SCO syndrome in azospermic group that produced variation in serum inhibin results. Different methodologies were used for analysis of serum FSH and inhibin B concentration.

CONCLUSION

Serum inhibin B is significantly related to spermatogenesis. It is one of optimum markers for male infertility work up and will provide encouraging and promising results in application of spermatogenesis. The suitable clinical decision for spermatogenesis is possible at appropriate cut off and harmful effects of unnecessary biopsies can be avoided. Its estimation along with FSH prove to be a noninvasive substitute for testicular biopsy and differentiation among different subgroups of infertile subjects can be made.

Authors Contribution: FA: Idea generation and study design. **SSH:** Drafting, critically reviewed the paper. **SS:** Data Collection. **FAZ**: Bibliography. **MTG:** Supervised the study and Literature review. Data analysis and interpretation. All authors critically revised and approved its final version.

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