

Pre-operative Serum Level of Inhibin B as a Predictor of Spermatogenesis Improvement After Varicocelectomy

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Purpose: Due to various reasons, spermatogenesis might not improve after varicocelectomy. Inhibin B, a sertoli cell glycoprotein, has proved itself as a marker of spermatogenesis. In this study, we measured serum level of inhibin B in patients with varicocele and through comparing pre and post operative semen analysis data, we tried to use serum level of inhibin B as a predictor of spermatogenetic improvement.

Materials and Methods: This prospective clinical trial was carried out between September 2007 and September 2008 on 36 infertile men with high grade unilateral or bilateral varicocele. Scrotal ultrasonography and measurement of seminal parameters and serum level of inhibin B were performed for the patients and after confirmation of impaired spermatogenesis, they underwent a subinguinal nonmicroscopic varicocelectomy by a single surgeon. Physical examination, scrotal ultrasonography, and semen analysis were repeated at postoperative months of 3 and 6. Statistical data analysis was done by independent and paired sample *t* test and Spearman's Rho test.

Results: Mean size of the testis remained the same ($P = .5$), but mean sperm density, normal morphology, and motility all increased statistically significant after the operation ($P < .05$, $P = .042$, $P = .023$). A significant relationship was found between serum levels of inhibin B and the testis sperm count and morphology ($P < .05$), but not sperm motility ($P > 0.05$).

Conclusion: It seems that serum level of inhibin B can be used as a reliable pre-operative marker of testicular potential activity and can also predict chance of spermatogenesis after varicocelectomy and save patients from useless surgical procedures.

Keywords: varicocele, spermatogenesis, sertoli cells, Inhibin B

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INTRODUCTION

Varicocele is identified by the presence of dilated and tortuous veins in the spermatic cord. About 15% of the normal men population and up to 40% of infertile men experience varicocele⁽¹⁾ and it seems to be the underling disease in 70% of men with secondary infertility.⁽²⁾ Varicocele is recognized as the most surgically correctable cause of male

infertility, and a varicocele repair is the most commonly performed surgical procedure in treatment of male infertility.⁽³⁾

Inhibin B is a 32-KD glycoprotein which consists of two chains (α , β).⁽⁴⁾ Based on the β chain, Inhibin B is classified to two subgroups (A, B) and is detectable by radioimmunoassay and enzyme-

linked immunosorbent assay.⁽⁵⁾ Inhibin B is secreted by the sertoli cells in response to Follicle-stimulating hormone (FSH)⁽⁵⁾ and regulates gametogenesis by negative feedback effect on FSH secretion.⁽⁶⁾ It selectively inhibits FSH synthesis and has local paracrine functions on the testis.⁽⁷⁾ Inhibin B has been reported as an endocrine marker to monitor normal gonadal function in both sexes⁽⁶⁾ and is identified as an effective predictor of improvement of spermatogenesis after varicocelectomy.⁽⁸⁾ Recently, Inhibin B has been reported to be a more sensitive factor in study of azoospermic men than the testis size, FSH, and even the testis biopsy.⁽⁹⁾ A correlation has been found between serum level of inhibin B, total sperm count, and the testis volume⁽¹⁰⁾ while in a study by Fugisawa and colleagues, no significant correlation was seen between serum level of inhibin B and post varicocelectomy FSH, testosterone, the testis volume, and sperm count, but it was concluded that differences in serum level of inhibin B after varicocelectomy may be a predictor of improvement in the testis function.⁽¹¹⁾ Turkyilmaz and associates in 2006 also confirmed that increase in serum level of inhibin B is correlated with improvement of testis function after varicocelectomy.⁽¹²⁾

In this study, we evaluated the efficacy of serum level of inhibin B as a predictor of improvement in the seminal indices after varicocelectomy.

MATERIALS AND METHODS

From September 2007 to September 2008, 65 infertile men with varicocele presented to the urology clinic. Our study inclusion criterion was diagnosis of grade II-III uni or bilateral varicocele on physical examination by an experienced urologist. Patients with a past history of cryptorchidism, scrotal surgery, and solitary testis or who had a history of medical therapy for infertility were excluded.

Of 65 patients, 35 subjects were enrolled in the present study. After a 3-day abstinent period, semen specimens were collected for computer aided seminal analysis, and serum specimens were obtained for measurement of inhibin B at 8 a.m. All these measurements were done in a single reference andrology laboratory. Ultrasonographic

evaluation was performed to assess the testis volume by empirical formula of Lambert ($\text{length} \times \text{width} \times \text{height} \times 0.71$) and to rule out testicular abnormalities. Blood specimens were centrifuged in the room temperature for 15 minutes by 2000 rounds per minute, and collected serum were kept in a refrigerator in -25°C . Serum level of inhibin B was measured by double antibody immunoenzyme metric assay, using solid phase sandwich enzyme-linked immunosorbent assay test (DSL-10-84100i, Diagnostic Systems Laboratories, Webster, Texas, USA) by specific monoclonal antibody against β_B chain for inhibin B, with detection limits between 7 and 1000 pg/mL.

Subinguinal varicocelectomy was performed for all of the patients by a single urologist. Four weeks after the operation, a Doppler ultrasonography examination was performed to confirm the improvement of varicocele by demonstrating lack of venous back flow in all of the subjects. We followed up the patients for 3 and 6 months, and seminal analysis and scrotal ultrasonography were performed to evaluate changes in the seminal indices and the testis volume.

Eventually, data were analyzed using SPSS (Statistical Package for the Social Science, version 16.0, SSPS Inc, Chicago, Illinois, USA) software. For independent variables we used independent sample *t* test and for dependent variables, paired sample *t* test was utilized. *P* values less than .05 were considered statistically significant.

RESULTS

Three subjects refused to continue the follow-up after 3 months and were excluded from our study. The mean age of the patients was 25.32 ± 4.13 years. All the patients had left side varicocele with a 15% prevalence of bilateral varicocele. Mean serum level of inhibin B was 110 ± 38 pg/mL. Patients were classified into 2 groups based on serum inhibin level: Group A (inhibin B < 110 pg/mL, 16 subjects) and Group B (inhibin B > 110 pg/mL, 17 subjects). The mean serum levels of inhibin B for groups A and B were 70.78 (SD = 10.32) and 211.47 (SD = 16), respectively. The mean age for groups A and B were 26.26 ± 1.3 and 24.71 ± 2.2 years, respectively, which showed no significant difference between two groups (*P* = .36)

The mean testis volume was 16.58 ± 23 mL, 16.86 ± 14 mL, and 16.94 ± 22 mL before the varicocelectomy and at 3 and 6 months postoperation, respectively, which showed no significant changes in the testis volume before the operation and during the follow-up periods ($P = .5$). The mean sperm count was $11.62 \pm 0.22 \times 10^6$ /mL before the operation which increased to $13.36 \pm 0.15 \times 10^6$ /mL and $19.5 \pm 0.33 \times 10^6$ /mL at 3 and 6 months after the operation, respectively, which showed a significant correlation between varicocelectomy and improvement in sperm count at 3 and 6 months ($P = .032$). There was also a significant improvement in sperm count from 3 to 6 months ($P = .036$).

The mean sperm motility was $14.27 \pm 9\%$ which increased to $16.97 \pm 11\%$ at 3 months and $23.82 \pm 6\%$ at 6 months, which showed a significant improvement in sperm motility after varicocelectomy ($P = .023$). The mean normal sperm morphology was $8.317 \pm 0.54\%$ which increased to $11.09 \pm 0.87\%$ and $16.36 \pm 0.22\%$ at 3 and 6 months after the operation, respectively, and showed a significant improvement in sperm morphology ($P = .042$).

The mean volume of the testis was 15.3 ± 2.5 mL, 15 ± 1.22 mL, and 34 ± 1.1 mL for group A and 17.8 ± 2.32 mL, 17.97 ± 2.11 mL, and

18.26 ± 3.21 mL for group B before and at 3 and 6 months after varicocelectomy, respectively, which showed a significant difference between two groups, and we detected a significant correlation between the testis size and serum level of inhibin B. The mean sperm count for group A was 11.6 ± 3.32 , 13.21 ± 4.32 , and $14.76 \pm 2.22 \times 10^6$ sperm/mL and for group B was 11.64 ± 4.41 , 13.5 ± 2.22 , and $23.96 \pm 3.44 \times 10^6$ sperm/mL before the operation, and at 3 and 6 months after the procedure, which showed no significant difference at 3 month, while it was significant at month 6 for each group and there was also a significant correlation between serum inhibin B level and improvement in sperm count at month 6 (spearman's rho = 0.567) (Figure 1). Analysis showed that we may be able to calculate postoperative sperm count by having pre-operative serum inhibin B level using the following formula: $Y = 13.452 + 0.042 X$ ($Y =$ sperm count at 6 months after varicocelectomy, $X =$ pre-operative serum inhibin B level).

The mean sperm motility and density before the operation and at 3 and 6 months after varicocelectomy was $14.188 \pm 3.3\%$, $16.25 \pm 4.32\%$, and $21.81 \pm 3.12\%$ for group A and $14.353 \pm 2.11\%$, $17.647 \pm 3.11\%$, and $25.71 \pm 4.32\%$ for group B. *T* test showed no significant difference between the two groups, but we detected moderate improvement in sperm motility for each group after varicocelectomy.

The mean normal sperm morphology was 9.5 ± 2.11 , 11.31 ± 3.22 , and 13.38 ± 4.11 for group A and 7.133 ± 7.11 , 10.2 ± 3.21 , and 18.52 ± 3.43 for group B before the procedure and at 3 and 6 months after varicocelectomy, which showed no significant difference in normal sperm morphology for two groups, but showed significant improvement at months 6 ($P = .021$). It seems that with increase in serum level of inhibin B before varicocelectomy, there will be more improvement in sperm morphology at 6 months after the operation (Figure 2). Following formula was designed to predict changes in sperm morphology after the operation; $Y = 12.039 + 0.03 X$ ($Y =$ normal sperm morphology after varicocelectomy, $X =$ serum level of inhibin B before procedure).

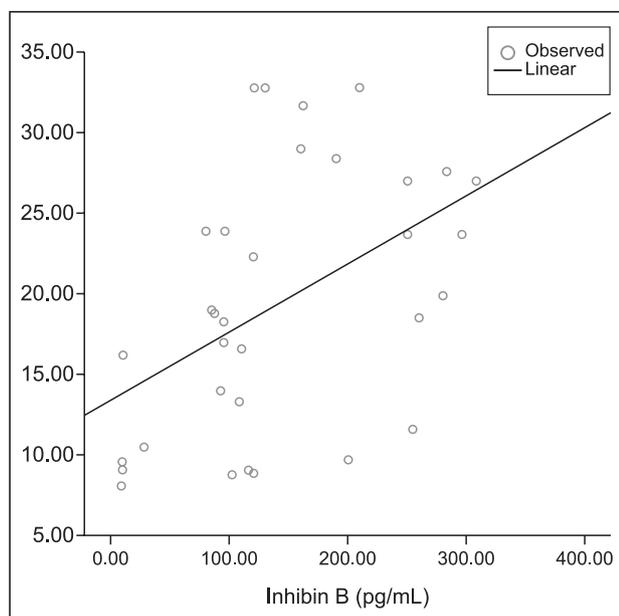


Figure 1. Regression analysis of pre-operative serum level of inhibin B against 6 months postoperative sperm counts.

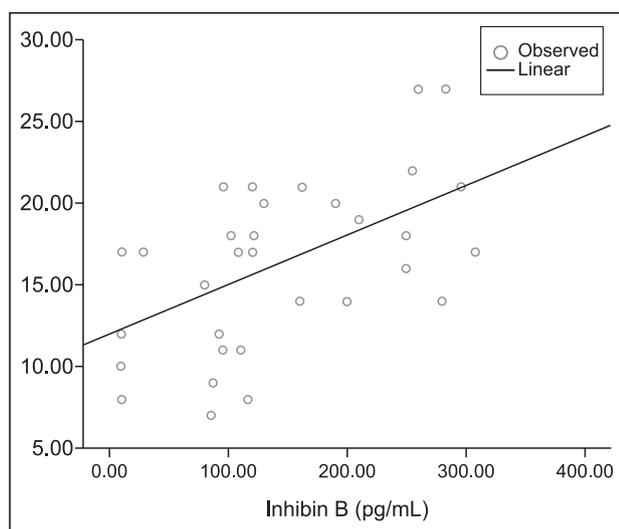


Figure 2. Regression analysis of pre-operative serum level of inhibin B against 6 months postoperative normal sperm morphology.

DISCUSSION

Deleterious effects of varicocele can cause reduction in function and number of the testicular cells,⁽¹³⁾ and subsequently leads to decreased testis volume, spermatogenesis, inhibin B production,⁽²⁾ and lowered serum level of inhibin B.⁽¹⁴⁾ In patients with a higher pre-operative serum level of inhibin B, it can be assumed that varicocele has had less destroying effects on the testicular parenchyma.⁽¹⁵⁾ Thus, more improvement in spermatogenesis may be anticipated after varicocelectomy. In this study, a significant correlation was found between serum level of inhibin B and the testis volume as well as improvement in spermatogenesis indices (sperm count and morphology) post varicocelectomy.

In accordance with our findings, Nowroozi and coworkers concluded that higher pre-operative serum inhibin B level was correlated with a better testicular histology and more chance of positive testicular biopsy in azoospermic patients.⁽¹⁶⁾ Bohring and Krause also reported that inhibin B can be used as a serum marker of sertoli cells function and patients with normal serum level of this marker will have better spermatogenesis and testicular functions.⁽¹⁷⁾ Romeo and colleagues reported decreased serum level of inhibin B in adolescents with varicocele and a significant statistical correlation between serum level of inhibin B and the testis volume.⁽¹⁸⁾ In another

study, Fujisawa and associates reported that pre-operative serum inhibin B concentration could not reliably predict a response to varicocelectomy. However, a change in serum inhibin B concentration after varicocelectomy might be helpful to evaluate the improvement of testicular function after varicocelectomy.⁽¹¹⁾

CONCLUSION

It may be concluded that pre-operative serum level of inhibin B can be used to predict postoperative improvement in spermatogenesis indices. However, further detailed and comprehensive studies are required in this regard. Evaluation of predictive efficacy of serum inhibin B level on smaller subclinical varicoceles also may be a good research area for further studies.

CONFLICT OF INTEREST

None declared.

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