

SERUM TOTAL TESTOSTERONE AND INHIBIN B ARE THE BETTER MARKERS OF SPERMATOGENESIS THAN ANTI-MULLERIAN HORMONE IN OLIGOSPERMIC MEN

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ABSTRACT

A case-control study was carried out to assess the role of serum total testosterone, AMH and Inhibin B in evaluation of spermatogenesis in oligospermic men. The study was conducted in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Iraq and in Biochemistry Department, College of Medicine, University of Baghdad. Serum levels of total testosterone (ng/mL), Anti-Mullerian Hormone (AMH pmol/L), inhibin B (pg/mL), FSH (mIU/mL), LH (mIU/mL) and prolactin (ng/mL) were measured in oligospermic men (n = 30) and fertile controls (n = 20). Seminal plasma AMH and Inhibin B were also measured in oligospermic group. In addition, semen analysis was performed in oligospermic men according World Health Organization guidelines. Results revealed that the mean (\pm SD) values of serum total testosterone (4.91 ± 1.73 ng mL⁻¹, $P < 0.04$) and inhibin B (138.75 ± 55.34 pg/ml, $p < 0.003$) of oligospermic men were significantly lower than those in fertile men (8.0 ± 0.78 ng/mL, 243.19 ± 142.42 pg/mL; respectively). The mean of serum AMH was insignificantly reduced in oligospermic men when compared with that of fertile controls (1611.17 ± 777.04 pmol/L, 1854.32 ± 1549.4 pmol/L, respectively). Significant positive correlations were observed between serum total testosterone and semen concentrations ($r = 0.67$, $p < 0.0001$), motility ($r = 0.40$, $p < 0.03$) and morphology ($r = 0.43$, $p < 0.018$).

Keyword(s): Anti-Mullerian Hormone, Inhibin B, Total Testosterone, Semen Analysis, Oligospermic Men

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1. INTRODUCTION

Anti-Mullarian Hormone (AMH), also known as mullarian- inhibitory substance, is a dimeric glycoprotein hormone belonging to the transforming growth factor-beta family. It is produced by Sertoli cells of the testis in males and by ovarian granulose cells in females (LaMarca *et al.*, 2009). The regulation of AMH after birth is complex, basal levels of AMH are independent of gonadotropin regulation, for example, during childhood and in patients with hypogonadotropic hypogonadism. Throughout pubertal development, AMH correlates negatively with serum testosterone concentrations. This correlation persists if androgen levels are abnormally high (such as in activating mutation of the LH receptors) but gonadotropins are low (Young *et al.*, 1999). These findings suggest that AMH is down-regulated by androgens and not directly by gonadotropins (Tuttelmann *et al.*, 2009). However, in patients with androgen insensitivity or deficient androgen production, serum AMH levels are extremely elevated indicating that, without the inhibitory androgen effect, FSH stimulates AMH production (Wilhelm *et al.*, 2007). Two recent studies demonstrated the importance of seminal fluid AMH as a marker of spermatogenesis (Fenichel *et al.*, 1999; Fujisawa *et al.*, 2002), although this was not confirmed by a another study (Al-Qahtani *et al.*, 2005). The latter author also found significantly reduced serum AMH concentrations in oligospermic infertile men compared with control men and suggested its superiority to seminal plasma AMH in male factor infertility. However, this difference in serum AMH between men with normal and reduced sperm concentration was not confirmed by a second study performed by the same latter authors who measured serum and seminal plasma AMH in parallel in a small cohort of uncharacterized infertile men. Although, a correlation between serum AMH concentrations and sperm concentration, serum FSH and free testosterone was observed (Appasamy *et al.*, 2007).

With regard to Inhibin B, it is a glycoprotein hormone of gonadal origin, consisting of two dissimilar disulfide-linked subunits alpha and beta B, that has an inhibitory effect on gonadotropin production, definitely, the FSH production (Anderson *et al.*, 1997). Inhibin B has been shown to be a marker of spermatogenesis and sub fertile men generally have reduced serum concentrations of inhibin B (Klingmuller and Haidl, 1997) except in patients with obstructive azoospermia or spermatogenic arrest at some stages (Plerik *et al.*, 2001).

The aim of this study was to evaluate the role of serum total testosterone, AMH and Inhibin B measurements in assessment of spermatogenesis in oligospermic patients and to show their correlations with other hormones and seminal parameter values.

2. MATERIALS AND METHODS

This infertility case-control study was carried out in High Institute of Infertility Diagnosis & Assisted Reproductive Technologies, Al-Nahrain University, Iraq and in Biochemistry Department, College of Medicine, University of Baghdad during the period from June 2011 to December 2011. The study consisted of 30 oligospermic men, ages ranged from 22-44 years (mean±SD, 33.40±6.21 year). Patients were included in this study depending on confirmed seminal fluid analyses results according to the World Health Organization Laboratory Manual (World Health Organization, 2000). Semen specimens were collected by masturbation after a period of 3-5 days of sexual abstinence. After liquefaction, manual semen analysis was performed to determine sperm concentration and motility. Normal value for sperm concentration was $\geq 20 \times 10^6$ /mL, patients who have had value of more than zero and less than 20×10^6 /mL were considered as oligospermic patients and included in this study. Twenty healthy men with proven fertility were served as control group. These fertile men ages ranged from 23-45 years (mean±SD, 33.10±7.17 year) and the pregnancy of the female partner had been achieved by normal sexual relations and also should have had a sperm concentration 20×10^6 /mL or greater.

Five milliliters of peripheral venous blood was aspirated from each patient and control subject. Blood samples were collected in plain tubes, allow to clot and then centrifuged at 2500 rpm for 10

minutes. The serum was separated and stored in aliquots at -20°C until the day of assay. The hormonal and biochemical studies included in the present study were the measurements of serum concentrations of Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Total Testosterone (TT), Anti-Mullerian Hormone (AMH) and inhibin B (InH B). The serum concentrations of FSH, LH and TT were measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique according to methods reported by Haymond and Gronowski (2006), kits were provided by HUMAN GmbH, Wiesbaden, Germany. Serum levels of AMH was estimated by ELISA technique using the method of Kricka (2000) and Inhibin B by procedure of (Groome and Evans, 2000), kits were supplied from Diagnostic Systems Laboratory, USA. The ELISA study was performed using Biotek Instrument, USA.

We used the Statistical Package for Social Sciences (SPSS Inc., Chicago IL, USA) version 15 and Minitab analysis programs statistical studies. We used ANOVA and Student's t-tests to test for statistical significance. Linear regression was utilized to test for correlation between different studied parameters and the significance of the r-value was assessed by related t-test. *P*-values of less than 0.05 were considered significant.

3. RESULTS

This study included 30 oligospermic men and 20 healthy fertile men. The mean(\pm SD) values of age, Body Mass Index (BMI) and sperm concentrations are found in the **Table 1**, which showed that there was no significant difference in mean age between the patients (33.40 ± 6.21 year) and the control men (33.10 ± 7.17 year). However, the mean value of BMI of oligospermic men (33.06 ± 5.11 Kg/m^2) was significantly higher than that of fertile controls (28.94 ± 4.38 Kg/m^2 ; $p < 0.049$). The mean (\pm SD) value of sperm concentrations was investigated only in oligospermic men and was found to be ($8.67\pm 5.1\times 10^6$ sperms/ml). **Table 2** showed the mean (\pm SD) values of serum concentrations of FSH, LH and TT of patient and control groups. The mean values of serum FSH and LH concentrations of oligospermic men (5.81 ± 2.87 mIU/mL, 4.52 ± 2.35 mIU/ml) were significantly higher than those of fertile controls (4.80 ± 2.07 mIU/mL, 3.67 ± 1.34 mIU/ml; $p < 0.045$, $p < 0.044$; respectively). The mean value of serum TT concentrations of oligospermic men (4.91 ± 1.73 ng mL^{-1}) was significantly lower than that of fertile men (8.0 ± 0.78 ng mL^{-1} ; $P < 0.040$).

Table 1. Mean (\pm SD) values of age, body mass index (BMI), and sperm

Parameter	Oligospermic men (n = 30)	Fertile controls (n = 20)	P-value
Age (Year)	(33.40 ± 6.21)	(33.10 ± 7.17)	NS
BMI (Kg/m^2)	(33.06 ± 5.11)	(28.94 ± 4.38)	0.049
Sperm concentration (million/ml)	($8.67\pm 5.1\times 10^6$)		

Table 2. Mean (\pm SD) values of serum concentrations of FSH, LH, and total

Parameter	Oligospermic men (n = 30)	Fertile men (n = 20)	P-value
FSH mIU/ml	5.81 ± 2.87	4.80 ± 2.07	0.045
LH mIU/ml	4.52 ± 2.35	3.67 ± 1.34	0.044
Total testosterone (ng/ml)	4.91 ± 1.73	8.0 ± 0.78	0.040

Table 3. Mean (\pm SD) values of Serum and Seminal Plasma Concentrations

Parameter	Oligospermic Men (n = 30)	Fertile Controls (n = 20)	P-value
SAMH (pmol/L)	1611.17 \pm 777.04	1854.32 \pm 1549.4	NS
SPAMH (pmol/L)	3199.98 \pm 2197.7		
SINH B (pg/ml)	138.75 \pm 55.34	243.19 \pm 142.42	0.003
SPINH B (pg/ml)	336.78 \pm 175.84		

The mean (\pm SD) values of serum and seminal plasma concentrations of AMH and Inhibin B of studied groups are shown in **Table 3**. The mean value of serum AMH (SAMH) levels of oligospermic men (1611.17 \pm 777.04 pmol/L) was decreased compared with that of fertile men (1854.32 \pm 1549.40 pmol/L), but did not reach the significant level. While, the mean value of serum Inhibin B (SINH B) of patients group (138.75 \pm 55.34 pg mL⁻¹) was significantly reduced in comparison with that of controls (243.19 \pm 142.42 pg mL⁻¹; p<0.003). With regard to seminal fluid, the mean(\pm SD) value of AMH (SFAMH) and Inhibin B (SFINH B) of oligospermic men were found to be (3199.98 \pm 2197.75 pmol/L) and (336.78 \pm 175.84 pg mL⁻¹), respectively. The results of this study also revealed that there was significant positive correlation between the concentrations of serum AMH and each of serum Inhibin B (r = 0.64, p<0.0001) and SFAMH (r = 0.36, P<0.048) in oligospermic men. There was also significant negative correlation between SAMH concentrations and serum levels of each of FSH (r = -0.59, p<0.0008) and LH (r = -0.58, p<0.001) in patients group. Furthermore, borderline significant negative correlation was observed between serum Inhibin B and serum FSH levels in oligospermic men (r = -0.35, p<0.059). Moreover, the oligospermic men showed significant positive correlation between serum TT concentrations and seminal fluid observations, with sperm concentrations (r = 0.67, p<0.0001), sperm motility (r = 0.40, p<0.03) and sperm morphology (r = 0.43, p<0.018). However, the study did not reveal any significant correlation between the serum and seminal fluid plasma levels of AMH and Inhibin B with seminal fluid observations.

4. DISCUSSION

This study revealed that both of serum FSH and LH were significantly increased, while serum total testosterone was significantly lower in oligospermic men than in fertile men. Serum levels of AMH was insignificantly reduced and serum Inhibin B was significantly decreased in oligospermic men in comparison with those of fertile controls. The study also revealed the significant increased of seminal AMH and Inhibin B in oligospermic men compared with those of blood serum of the same group. These results are in agreement with that reported by (Tuttelmann *et al.*, 2009; Appasamy *et al.*, 2007) who observed that serum AMH in oligospermic infertile men was found to be slightly lower than in the men with normal sperm concentration. The latter authors concluded that serum AMH levels are not significantly affected by impaired spermatogenesis in general populations but are correlated with spermatogenic parameters in men with maldescended tests (Tuttelmann *et al.*, 2009). Goulis *et al.*, 2008; American Society for Reproductive Medicine, 2007) showed that serum AMH levels was significantly decreased in sub fertile men and can differentiate these patients from control men. However, the majority of sub fertile men in these latter studies were azoospermic, obstructive and non obstructive men. In humans, large amounts of AMH are produced during fetal and post-natal testicular development (Sweeney *et al.*, 1997). The expression and production of AMH is principally reduced at onset of puberty and this may reflect terminal differentiation of Sertoli cells. While some degree of AMH production in adults, after puberty is mainly in seminal plasma than in serum and this suggests that AMH is preferentially secreted at the apical pole of the Sertoli cell, toward the seminiferous tubular lumen (Meys *et al.*, 1999; Fenichel *et al.*, 1999). This may interpret the significantly higher concentrations of seminal plasma AMH

compared with serum AMH in oligospermic men of the present study. The negative correlations between serum levels of AMH and serum FSH and LH concentrations might reflect an important involvement of AMH in signaling and regulation of gonadotropin hormones and so the reproductive male system.

The present study found significant decrease of serum Inhibin B levels in oligospermic men than in fertile group along with borderline significant negative correlation between serum Inhibin B concentrations and serum FSH levels. These results confirmed that observed by Kumanov *et al.* (2006) who concluded that serum inhibin B measurement is a better marker of male fertility status and concentration of inhibin B in patients with infertility may provide useful information on spermatogenesis and possibly serve as a more direct marker of spermatogenesis than other classic routine hormones like FSH and LH. The negative correlation between serum levels of inhibin B and FSH support the fact that inhibin B is the testicular feedback signal for FSH (Uhler *et al.*, 2003). FSH is a gonadotropin glycoprotein synthesized and secreted by anterior pituitary gland, acts on Sertoli cells in the seminiferous tubules to initiate spermatogenesis. Sertoli cells produce and secrete inhibin B, a protein hormone which exerts a feedback effects on the anterior pituitary to inhibit FSH secretion (Meeker *et al.*, 2007). However, in the present study, the significant reduction of serum TT in oligospermic men compared with fertile men and its significant positive correlations with sperm concentrations, motility and morphology may pointed to the fact that measurement of this hormone TT and/or its free fraction is the better marker than AMH and Inhibin B in assessment of spermatogenesis in oligospermic men. Combination of serum TT and Inhibin B may improve such evaluation.

In conclusion, serum evaluation of both total testosterone and inhibin B might be the better indicators than serum AMH for fertility factor study in oligospermic men. Measurements of seminal plasma AMH and inhibin B, because of their large amounts compared to those in serum, may be useful in assessment of spermatogenesis in such patients and need for further studies.

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