Anti-Mullerian Hormone Is a Significant Marker for Male Infertility

***Sajeda S. Al – Chalabi, **Yasir T.Al-Wattar, ***Islam M .Algalili**

***Department of physiology, College of Medicine, University of Mosul, Mosul, Iraq**
****Consultant Urologist, Al-Jumhory Teaching Hospital, Mosul, Iraq**
*****Department of Medical Microbiology, Al-Batool Teaching Hospital, Mosul, Iraq**

Received 8/12/2011 Accepted 5/1/2012

Abstract

To evaluate serum anti-Mullerian hormone (AMH) in men with normal, reduced sperm concentration and in azoospermic men as a possible clinical marker of male factor infertility. Prospective study Private clinic of Dr. Yasir Al-Wattar and Al-Batool Teaching Hospital in Mosul. This study was conducted on one hundred male subjects aged less than 50 years for the period from July to November 2011, infertile men were classified according to their sperm count into oligospermics (n=28) and azoospermics(n=47), twenty five men were normal (n=25).Serum concentrations of FSH and Testosterone were measured using ELISA, serum Anti-Mullerian hormone concentrations were measured using AMH/MIS enzyme linked immunosorbant assay kit. Significant differences in serum AMH, FSH and Testosterone concentrations were found between normal, oligospermic & azoospermic men. AMH negatively correlated with FSH in oligospermic and azoospermic men and positively with testosterone in azoospermics and negatively with testosterone in oligospermics. Anti-Mullerian hormone should be evaluated in patients with oligospermia and azoospermia, it may serve as a marker of male infertility.

ساجدة سعيد الجلبي ياسر الوتار إسلام الجليلي

الملخص

أجريت الدراسة على مئة رجل معدل أعمارهم اقل من خمسين سنة للفترة من تموز 2011-تشرين الثاني 2011 وذلك لتقييم تركيز هرمون أنتي مولي رين في الرجال الطبيعيين والرجال اللذين لديهم قلة في تركيز النطف واللذين لديهم انعدام النطف ولمعرفة إذا كان من الممكن استخدامه كعلامة في عقم الرجال . تم تصنيف الرجال العقيمين حسب عدد النطف الى مجموعة قلة النطف والى مجموعة انعدام النطف كذلك شملت الدراسة خمسة وعشرون رجل طبيعي . تم قياس مستوى هرمون أنتي مولي رين وهرمون المحفز للجريب والهرمون الذكري بواسطة جهاز أل ELISA. أظهرت النتائج اختلاف واضح في تركيز هرمون إلانتي مولي رين ، الهرمون المحفز للجريب والهرمون الذكري عندما تمت مقارنتهم بين الرجال الطبيعيين والرجال العقيمين . كذلك لوحظ وجود علاقة عكسية بين هرمون أنتي مولي رين والهرمون المحفز للجريب في الرجال العقيمين بينما كان هناك علاقة طردية بين هرمون أنتي مولي رين والهرمون الذكري في مجموعة انعدام النطف بينما كانت العلاقة عكسية مع الهرمون الذكري في مجموعة انعدام النطف.

Introduction

Anti-Mullerian hormone (AMH) is a glycoprotein that belongs to the transforming growth factor- B(TGF-B) a member of the super family of growth and differentiation factors. It was identified as a factor which is being synthesized by testicular Sertoli cells, induces regression of the Mullerian ducts during male fetal development. [1, 2]It continues to produce by the testes throughout life. It is thought to be involved in the inhibition of steroid hormone production in women of reproductive age. [3]. AMH not only induces regression of the Mullerian ducts during male sexual differentiation [4, 5, 6] but also plays a critical paracrine role in the regulation of Leydig cell development and testosterone biosynthesis [7, 8]. AMH has also been shown to inhibit proliferation of prepubertal progenitor Leydig cells and prevent regeneration of Leydig cells after chemical ablation. [9]. In the male, AMH level rises rapidly after birth, is highest during late infancy, then gradually declines until puberty. [10]. The decrease of AMH corresponds to the Sertoli cell maturation status with the onset of puberty and spermatogenesis, AMH is expressed only by immature Sertoli cells and by immunohistochemistry found only in tubules with spermatogenic arrest or Sertoli-cell-only syndrome. [11, 12]. The aim of the study is to verify in a large number of well- characterized men with normal or decreased sperm concentration, whether serum AMH determination would add any diagnostic advantage over current endocrine diagnostics.

Subjects and Methods

This prospective study was conducted on one hundred men aged less than 50 years who attended an infertility clinic of Dr. Yasir Al-Wattar and infertility clinic at Al- Batool Teaching Hospital between July2011-November2011. Infertile men were grouped into azoospermic men (n=47) their mean age 35.87±7.32 years and oligospermic men (n=28) their mean age 38.25±8.78years. Twenty five normal men their mean age 29.96±4.34 years were used as control. All subjects were questioned about IVF trials, testicular biopsy , mumps , venereal disease , varicocele, drugs which may interfere with fertility and all underwent complete clinical and physical examination. The subjects considered infertile according to WHO criteria. [13]. Serum concentrations of FSH and testosterone were measured using ELISA. Serum AMH concentrations were measured using AMH/MIS enzyme linked immunosorbant assay kit (immunotest material USA). Data analysis was done using Minitab program. Pearson correlation and unpaired T-test was used. P-Value $\leq$ 0.05 was considered significant throughout the study.

Results

The laboratory values of hormones were significantly different in infertile men as compared to normal men as shown in table (1). That serum testosterone concentrations and AMH concentrations were significantly lower in oligospermic and azoospermic men as compared to normal men. While, serum FSH concentrations were found to be significantly higher than controls. Table 2 shows that serum testosterone and serum AMH concentrations were significantly lower in azoospermic men as compared to normal men. While serum FSH was significantly higher in azoospermic men as compared to normal men. Table 3 shows that oligospermic men had higher testosterone and AMH and lower serum FSH concentrations as compared to azoospermics. AMH correlated negatively with FSH and testosterone in oligospermic men (table4) while in azoospermic men there was a positive correlation between serum AMH and testosterone and a negative correlation between serum AMH and FSH concentrations were found.

Table (1):- Mean±SD of serum hormones in infertile men and controls

Hormones	Infertile men(n=75)	Control(n=25)	t- value	p- value
Testosterone(ng/ml)	4.24±2.12	6.48±1.62	5.52	0.001
FSH mIU/L	17.5±14.9	5.31±1.56	-6.94	0.001
AMH(ng/ml)	1.30±0.16	3.03±0.48	9.24	0.001

Unpaired t- test was used to determine the presence of significant differences between infertile and normal men.

Table(2):- Comparison of serum hormones in azoospermic and control men using unpaired t-test.

Hormones	Azoospermics(n=47)	Control(n=25)	t- value	p-value
Testosterone(ng/ml)	3.33±1.44	6.48±1.62	-8.15	0.001
FSH mIU/L	24.4±14.9	5.31±1.56	8.70	0.001
AMH(ng/ml)	0.544±0.80	3.03±0.48	-16.35	0.001

Table(3):- Comparison of serum hormones in azoospermic and oligospermic men using unpaired t-test.

Hormones	Azoospermics (n=47)	Oligospermics (n=28)	t- value	p-value
Testosterone(ng/ml)	3.33±1.44	5.76±2.22	-5.18	0.001
FSH mIU/L	24.4±14.9	5.05±1.73	8.80	0.001
AMH (ng/ml)	0.544±0.80	2.58±1.23	-7.79	0.001

Table(4):- Correlation analysis between serum AMH , testosterone and FSH in men grouped according to study protocol.

Group	FSH (mIU/L)	Testosterone(ng/ml)
Normal men(n=25)	0.282 (N.S)	0.089 (N.S)
Oligospermic men (n=28)	-0.232*	-0.161*
Azoospermic men (n=47)	-0.42*	0.376*

Discussion

Several studies with questionable results have focused on the value of serum FSH and AMH to predict the status of spermatogenesis in the testes of men. The present study compares some serum biomarkers in normospermic, oligospermic and azoospermic men. The results showed significant difference in FSH, testosterone and AMH concentrations in azoospermic men as compared to normospermic men. this is an indication of defective spermatogenesis and as a result of feedback control probably by inhibin B or may be a direct involvement of AMH.[14]. Serum AMH was found to be significantly lower in men with oligospermia and azoospermia as compared with controls. This is in accordance with results of a previous study. [15]. 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. [16]. AMH was found to be negatively correlated with serum

testosterone this in agreement with other studies. [17]. The negative correlation between AMH and FSH this in lines with previous studies. ([18, 19] either might reflect an involvement in the signaling and regulation of FSH or most probably to be a symptom of impaired or immature Sertoli cells [20]. In conclusion, AMH should be carefully evaluated in oligospermic and azoospermic men. As anti-Mullerian hormone is a marker of both Sertoli cell proliferation and protein synthesis activity in response to FSH before puberty and also a useful marker of FSH action in the assessment of testicular function in the prepubertal boys. [14]

References

1. Razich DF, Naeimeh T and Maryam A. Serum level of Anti-Mullerian hormone in early follicular phase as a predictor of ovarian reserve and pregnancy outcome in assisted reproductive technology cycles. Arch of Iranian Med 2008; 11(4): 371-376.
2. Rodina AV, Gukasova NV, Makarov VA, Kondrasheva IG, Khomgakov GA,Posypanova, Popova ON, YumoskalevaYU and Severa SE. Localization of Mullerian inhibiting substance receptors in various human cancer cell lines. Biochemistry (Moscow).2008; 75(7): 797-805.
3. Jang H Kim.Mullerian inhibiting substance, physiology and clinical applications . J of Women's Med 2009;2(2): 45-47.
4. Arango NA, Lovell-Badge R, Behringer RR. Targeted mutagenesis of the endogenous mouse MIS gene promoter. In vivo definition of genetic pathways of vertebrate sexual development. Cell 1999; 99:409-419.
5. Behringer RR, Cate RL, Froelick GJ, Palmiter RD, Brinster RL. Abnormal sexual development in transgenic mice chronically expressing Mullerian inhibiting substance. Nature 1990; 345: 167-170
6. Lee MM, Donahoe PK, Mullerian inhibiting substance: a gonadal hormone with multiple functions. Endocr Rev 1993; 14:152-164
7. Lee MM.MIS actions in the developing testes in: Goldberg E. ed. The testis: from stem cell to sperm function. New York: Springer- verlag: 30-42.
8. Srivaman V, Niu E, Matias JR, Donahoe PK, Maclaughlin DT, Hardy MP, Lee MM. Mullerian inhibiting substance inhibits testosterone synthesis in adult rats. J Androl 2001; 22: 750-758.
9. Salva A , Hardy MP, Wu XF, Sottas CM, Maclaughlin DT, Donahoe PK, Lee MM. Mullerian inhibiting substance inhibits rat Leydig cell regeneration after ethylene dimethane sulphonate (EDS) ablation. Boil Reprod 2004; 70:600-607.
10. Lee MM, Donahoe PK, Hasegawa T, Silverman, B., Crist GB, Best S. *et al.* Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab 1996; 82: 571-60
11. Maymon BB, Toger L, Paz G, Kleiman SE, Schreiber L,Botchan A. sertoli cell maturation in men with azoospermia of different etiologies. Ferti Steril 2002;77:904-9
12. Steger K, Rey R, Louis F, KlieschS, Behre HM, Nieschlag, E. Revertion of the differentiated phenotype and maturation block in Sertoli cells in pathological human testis. Hum REPROD 1999; 14: 136-43
13. World Health Organization. laboratory manual for the examination of human semen and semen- cervical mucus interaction.4th ed. New York: Cambridge University Press , 1999; Appendix IA, 60-1
14. Tuttelmann F, Nina D, Axel PN Jenny AV, Eberhard N , Manuela S. anti-Mullerian hormone in men with normal and reduced sperm concentration and men with maldescended testes.Fertil Steril 2009; 91(5):1812-1818
15. Al- Qahtani A, Muttukvishna S, Appasamy M, Johous J, Granfield M,

Visser JA. Development of a sensitive enzyme immunoassay for anti-Mullerian hormone and the evaluation of potential clinical applications in males and females. Clin Endocrinol 2005; 63: 267-73

16. Young J, Rey R, Couzine B, Chanson P, Josso N, Schaison G. anti-Mullerian hormone in patients with hypogonadotropic hypogonadism. J Clin Endocrinol Metab 1999; 84:2696-9
17. Rey R, Lordereun RI , Carl JC, Barbet P, Cate RL, Roger M. Antimullerian hormone and testosterone serum levels are inversely related during normal and precious pubertal development. J Clin Endocrinol Metab 1993; 77: 1220-6
18. Fuji SM, Yamasaki T, Okada H, Kamidonos S. the significance of anti-Mullerian hormone concentration in seminal plasma for spermatogenesis. Hum Reprod 2002; 17(4): 968-70
19. EI Garem YF, EI Arini AF, EI Beheiry AH, Zeid SA, Comhaire FH. Possible relationship between seminal plasma inhibin B and spermatogenesis in patients with azoospermia J Androl. 2002; 23(6) : 825-9
20. Appasamy M, Muttukrishna S, Pizzey AR, Ozturk O, Groome NP, Serhal P. Relationship between male reproductive sperm DNA damage and markers of oxidative stress in infertility. Reprod Biomed Online 2007; 14: 159-65.