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A comparative investigation of anti-Müllerian hormone (AMH) and various biochemical parameters in patients with cryptorchidism, oligospermia, or varicocele*

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Aim: To investigate the serum AMH and various biochemical parameter levels in patients with cryptorchidism, oligospermia, or varicocele.

Materials and methods: This study included 100 participants, divided into 5 groups: cryptorchidism (n = 20), varicocele (n = 20), oligospermia (n = 20), and 2 control groups [control 1, 8-12 years old (n = 20) and control 2, 18-24 years old (n= 20)]. Using the blood samples drawn from both patient and control groups, AMH, testosterone, FSH, LH, and prolactin parameters were investigated.

Results: AMH values measured were found to be 73.04 ± 44.5 ng/mL and 84.81 ± 59.1 ng/mL in 20 children with cryptorchidism and 20 healthy children (both groups aged between 8 and 12 years), respectively (P = 0.56). In the present study, AMH values were found to be 5.19 ± 4.9 ng/mL and 7.66 ± 5.6 ng/mL in 20 patients with oligospermia and 20 healthy individuals (both groups aged between 18 and 24 years), respectively (P = 0.10). AMH values in 20 patients with varicocele and 20 healthy controls aged between 18 and 24 years were determined to be 7.15 ± 5.8 ng/mL and 7.66 ± 5.6 ng/mL, respectively (P = 0.82). No significant P values were determined, but the decrease in values was remarkable.

Conclusion: New studies with larger number of participants are needed in order to determinate AMH levels in patients with varicocele, cryptorchidism, or varicocele for diagnostic criteria.

Key words: Anti-Müllerian hormone, varicocele, infertility, cryptorchidism

Kriptorşit, oligospermi ve varikoselli hastalarda anti-müllerian hormon (AMH) ve farklı biyokimyasal parametrelerin karşılaştırılması

Amaç: Kriptorşit, oligospermi ve varikosel tanılı hastalarda AMH ve farklı biyokimyasal parametrelerin kıyaslanması yoluyla değişimin saptanması.

Yöntem ve gereç: Çalışmaya 18-24 yaş arası varikosel (n = 20), oligospermi (n = 20) ile 8-12 yaş arası kriptorşit (n = 20) hasta grupları; 8-12 yaş arası (n = 20) ve 18-24 yaş arası (n = 20) sağlıklı bireyler kontrol gruplar olarak alındı. Hasta ve kontrol gruplarından kan örnekleri alınarak AMH, testosteron, FSH, LH, ve prolaktin parametreleri çalışıldı.

Bulgular: Çalışmada pubertal dönemde (8-12 yaş arası) 20 kriptorşitli çocuk ile 20 normal çocuk AMH değerleri sırasıyla 73,04 ± 44,5 ng/mL ve 84,81 ± 59,1 ng/mL olarak bulunurken (P = 0,56) postpubertal dönemde (18-24 yaşarası) 20 oligospermili hasta ile 20 normal birey AMH değerleri sırasıyla 5,19 ± 4,9 ng/mL ve 7,66 ± 5,6 ng/mL (P = 0,10) ve 20 varikoselli hasta ile 20 normal birey AMH değerleri ise sırasıyla 7,15 ± 5,8 ng/mL ve 7,66 ± 5,6 ng/mL (P = 0,82) olarak saptandı.

Sonuç: AMH düzeylerinin belirlenmesine yönelik daha geniş katılımcı ile yapılacak yeni çalışmalarla varikosel, oligospermi ve kriptorşidik vakalarda serum AMH ölçümünün tanı kriteri olabileceği düşünülmektedir.

Anahtar sözcükler: Anti-Müllerian hormon, varikosel, infertilite, kriptorşidizm

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Introduction

Hormonal factors are important diagnostic criteria in the evaluation of varicocele, cryptorchidism, and oligospermia because these disorders can lead to infertility. It is very difficult to determine the underlying mechanisms of disorders occurring during fetal, prepubertal, and pubertal periods. The effects of newly discovered hormones, such as AMH and inhibin, have been proven in this process. Therefore, it can be said that recent studies have concentrated on the newly discovered hormones—AMH and inhibin—due to their effects on patients with varicocele, cryptorchidism, or oligospermia.

AMH, previously known as Müllerian inhibiting substance, belongs to a superfamily of dimeric glycoproteins, structurally similar to transforming growth factor-b, activins, and inhibins (1). In the male fetus, AMH is secreted exclusively by the immature Sertoli cells in the testes. The main biological function of AMH is to cause the involution of Müllerian ducts in male embryos (2). During male sexual differentiation, AMH inhibits differentiation of the Müllerian ducts into the uterus, fallopian tubes, and upper vagina (3). In addition to this principal role of AMH, its continued postnatal expression supports the hypotheses that AMH may have other roles, such as the regulation of gonadal function, testicular descent, lung development, and suppression of tumor growth (4). AMH is synthesized as a large precursor with a short signal sequence followed by the preprohormone that forms homodimers. Prior to secretion, the mature hormone undergoes glycosylation and dimerization to produce a 140 kDa dimer of identical disulfide-linked subunits (5,6).

Circulating levels of AMH remain high until the onset of puberty, and then progressively decrease due to the inhibitory effects of intratesticular testosterone (7). During puberty, AMH levels decline with testosterone production. AMH inhibits the differentiation of mesenchymal cells into Leydig cells and decreases the expression of several steroidogenic enzymes (8).

Recent studies indicate that male differentiation requires the secretion of 3 testicular hormones: AMH, produced by fetal Sertoli cells, induces the regression of the Müllerian ducts (9); testosterone, produced by Leydig cells, promotes the development of Wolffian duct derivatives and the masculinization of the external male genitalia (10); and finally, insulin-like 3 (Insl3) mediates transabdominal testicular descent into the scrotum (11). In this respect, clinical applications of serum AMH assay include isolated abnormalities of external genitalia, such as microphallus or cryptorchidism.

AMH is secreted at high levels during the early period of fetal life. However, the serum AMH concentration declined after 12 months and stays within normal limits after pubertal stage (12). If AMH production in Sertoli cells is insufficient, then some defects, such as gene mutation and AMH receptor gene defect, will be encountered (13). AMH plays critical regulatory roles in the gonadal development and reproductive fertility (14). Therefore serum AMH levels may be useful as diagnostic criteria to determine urological dysfunctions, such as varicocele, cryptorchidism, and varicocele.

The aim of the study was to determine serum AMH levels in men with different causes of urological dysfunctions. We undertook this study to determine whether the levels of AMH would help in the identification and evaluation of cryptorchidism, oligospermia, or varicocele.

Materials and methods

This study was performed in 100 participants, divided into 5 groups; 20 with cryptorchidism, 20 with varicocele, 20 with oligospermia, and 20 in control group 1 (8-12 years old) and 20 in control group 2 (18-24 years old).

None of the patients had exhibited a metabolic disease before admission. The subjects were selected from the patients that presented to the urology department and were diagnosed with oligospermia, varicocele, or cryptorchidism. The oligospermia group consisted of subfertile men and were evaluated, including history and spermiogram, according to World Health Organization criteria (15). Color Doppler ultrasonography and pelvic computed tomography were performed in bilateral varicocele (grade III) and cryptorchidism groups as clinically indicated. Varicocele and control groups were selected from among fertile men. The study was approved by the ethics committee of Selçuk University Medicine Faculty, Turkey.

Blood samples (10 mL) were drawn after a 12-14 h-fasting in the morning. The blood samples were separated after coagulation. Serum testosterone, FSH, LH, and prolactin levels were analyzed using the chemiluminescence method. Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) using the AMH/MIS ELISA kit (Immunotech-Beckman, Marseilles, France).

Statistical analysis

Statistical analyses were carried out with SPSS (Version 10.0). The Kruskal Wallis one-way analysis of variance was used, followed by the Mann-Whitney U test to evaluate the differences between control groups (control 1 and 2) and patient groups (cryptorchidism, oligospermia, and varicocele) (16).

Data were expressed as mean ± standard error ($\bar{X}$ ± SE). Results were considered to be statistically significant at $P < 0.05$.

Results

The findings are presented in Tables 1-3. Serum AMH values were found to be 73.04 ± 44.5 ng/mL and 84.81 ± 59.1 ng/mL in 20 children with cryptorchidism and 20 healthy children (both groups aged between 8 and 12 years), respectively.

AMH values were found to be 5.19 ± 4.9 ng/mL and 7.66 ± 5.6 ng/mL in 20 patients with oligospermia and 20 healthy individuals (both groups aged between 18 and 24 years), respectively. AMH values in 20 patients with varicocele and 20 healthy controls aged between 18 and 24 years were determined to be 7.15 ± 5.8 ng/mL and 7.66 ± 5.6 ng/mL, respectively.

This study demonstrated that serum AMH levels of the patient groups were lower than those of the control groups but the difference was not significant ($P > 0.05$).

Table 1. Levels of parameters in cryptorchidism group and control group (1).

Parameters	n	Control Group (1) (aged 8-12 years) $\bar{X}$ ± SE	Cryptorchidism Group $\bar{X}$ ± SE	P
AMH (ng/mL)	20	84.81 ± 59.1	73.04 ± 44.5	0.56
Testosterone (nmol/L)	20	0.92 ± 1.5	0.91 ± 1.3	0.31
Prolactin (mIU/L)	20	10.26 ± 5.3	13.32 ± 8.4	0.25
FSH (mIU/mL)	20	1.25 ± 1.1	1.79 ± 2.4	0.88
LH (mIU/mL)	20	0.54 ± 0.8	0.74 ± 1.4	0.86

Table 2. Levels of parameters in varicocele group and control group (2).

Parameters	n	Control Group (2) (aged 18-24 years) $\bar{X}$ ± SE	Varicocele Group $\bar{X}$ ± SE	P
AMH (ng/mL)	20	7.66 ± 5.6	7.15 ± 5.8	0.82
Testosterone (nmol/L)	20	5.21 ± 2.3	5.24 ± 1.8	0.92
Prolactin (mIU/L)	20	15.88 ± 8.8	16.14 ± 13.3	0.38
FSH (mIU/mL)	20	2.95 ± 1.8	5.20 ± 5.5	0.15
LH (mIU/mL)	20	3.79 ± 2.1	4.38 ± 2.2	0.41

Table 3. Levels of parameters in oligospermia group and control group (2).

Parameters	n	Control Group (2) (aged 18-24 years) $\bar{X}$ ± SE	Oligospermia Group $\bar{X}$ ± SE	P
AMH (ng/mL)	20	7.66 ± 5.6	5.19 ± 4.9	0.10
Testosterone (nmol/L)	20	5.21 ± 2.3	4.49 ± 2.4	0.34
Prolactin (mIU/L)	20	15.88 ± 8.8	18.44 ± 19.3	0.49
FSH (mIU/mL)	20	2.95 ± 1.8	10.85 ± 12.2	0.05*
LH (mIU/mL)	20	3.79 ± 2.1	5.04 ± 3.8	0.38

Discussion

Male infertility is characterized by particular multifactorial clinical problems, especially during the early period of sexual development. It is quite significant to determine possible defects during this period. In addition to mediating the crucial aspect of fetal reproductive tract development, AMH plays regulatory roles in postnatal development and gonadal maturation (17). It was concluded that a disproportionate correlation exists between different age groups and AMH levels.

Prepubertal period serum AMH levels were observed to be higher than postpubertal period in our study (Tables 1 and 2). In a previous study, the serum AMH level of the cryptorchidism group was found to be lower (40.04 ± 4.9 ng/mL) compared with controls (53.46 ± 7.51 ng/mL) (18). These results are similar with those observed in our study (Table 1), although the difference was not statistically significant (P = 0.56).

In terms of serum testosterone levels, the scores were found to be lowest in children with cryptorchidism, which is consistent with a previous study (19).

As shown in Tables 1-3, the levels of testosterone, prolactin, FSH, and LH were found to be lower in subjects at the age between 8 and 12 years in both patient and control groups compared with those at the age between 18 and 24 years in both patient and control groups, whereas serum AMH levels were found to be higher in the former. These results are compatible with those reported in the literature (20,21).

In the present study, a decreased serum AMH level was observed in the oligospermia group compared with controls. Although no serum AMH levels have been reported to be determined in oligospermia group in previous studies (22), low serum AMH levels were determined in this group in our study (Table 3), which was not statistically significant (P = 0.10). In another study, serum AMH levels in varicocele and control groups were reported as 750 pmol/L and 378 pmol/L, respectively (23). The values in this study are different from those found in our study. AMH values in patients with varicocele were determined to be lower compared to controls but the difference was not to significant (P = 0.82). Pierik reported very low serum AMH levels in individuals with abnormal testes (24). In an experimental study conducted on adult rats by Sriraman et al., serum AMH levels were observed to reach high local concentrations in a 4-h period after the administration of intratesticular injection of serum AMH. In that study, serum AMH levels and testosterone concentration rates were 574 ± 60 ng/mL and 0.7 ± 0.1 ng/mL, respectively (25). It can be concluded from these results that an injection of AMH could be beneficial within the prepubertal period.

The presence of AMH type II receptor in the prostate and the initiation of growth-regulatory pathways by AMH in prostate cancer cells suggest that the prostate may be a target tissue for AMH action (26). Further experimental studies will also help to determine whether AMH would be of potential therapeutic benefit in the treatment of prostate cancer. However, the presence of AMH type II receptor in the breast and the ability of AMH

ligand to block proliferation of breast cancer cells suggest that AMH may have a physiological role in regulating the proliferation of mammary epithelial cells (27,28). These points remain unclear and need to be investigated further.

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