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Evaluation of Serum Antimullerian Hormone in Varicocele patients before and after Varicocelectomy

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Abstract

Background: A varicocele is abnormal dilation and enlargement of the scrotal venous pampiniform plexus which drains blood from each testicle. While usually painless, varicoceles are clinically significant because they are the most commonly identified cause of infertility. The severe damage caused by varicocele is correlated with impaired function of sertoli cells. AMH has been proposed as a direct marker of Sertoli cell function and an indirect marker of spermatogenesis. The aim of this study was to evaluate the serum level of antimullerian hormone in varicocele patients before and after varicocelectomy and assessment of its relation to semen parameters and varicocele grade. Methods: This study was conducted on thirty varicocele patients and twenty age and sex matched healthy volunteers as controls. Results: There was non significant difference was noted between patients and control groups as regards age. Varicocele grade showed significant improvement post-operative. Before varicocelectomy, sperm count and motility was significantly lower in patients than control. In contrast, abnormal forms was significantly higher in patients than control. The mean sperm count and motility significantly increased after than before varicocelectomy. In contrast, abnormal forms significantly declined after than before varicocelectomy. AMH level in patients before varicocelectomy was significantly lower than in controls while after varicocelectomy AMH level increased. Before varicocelectomy, AMH level showed a significant negative correlation with varicocele grade and abnormal forms. After varicocelectomy, AMH level showed a significant positive correlation with sperm count. Conclusion: The study confirmed that AMH decrease in varicocele patients and increase after surgical repair compared to controls.

Key words: Serum Antimullerian Hormone - Varicocele patients – Varicocelectomy

1.Introduction

Antimullerian hormone [AMH], also known as Mullerian-inhibiting substance [MIS], is a Sertoli cell-secreted protein that plays a major role in the development of internal male genitalia [1]. High expression of AMH in male gonads at 7th weeks of gestation promotes regression of the Mullerian duct [2].

Varicocele is an abnormal enlargement and bending of the pampiniform venous plexus in the scrotum. The adverse effects of varicocele on spermatogenesis are progressive and therefore decrease male fertility with time [3]. The severe damage caused by varicocele is correlated with impaired function of Sertoli cells [4].

An analysis of serum AMH levels in varicocele bearing patients was inconclusive. A study done on subfertile men including those complaing of varicocele indicated that circulating AMH levels in subfertile men were 60% lower than those in corresponding controls, accompanied by a decreased level of inhibin B, indicating the decreased function of Sertoli cells in varicocele patients [5].

In prepubertal and pubertal boys with varicocele, AMH concentrations were elevated accompanied by an increase in inhibin B levels, suggesting a compensatory increase in Sertoli cell function in the early-onset varicocele [6].

The aim of this study was to evaluate the serum level of antimullerian hormone in varicocele patients before and after varicocelectomy and compare their levels in healthy controls.

2.Subjects and Methods

2.1Subjects

This case control study was conducted on thirty varicocele patients and twenty age and sex matched healthy volunteers as controls. They were recruited from the outpatient clinic of Dermatology, Venereology and Andrology Department of Benha University hospitals from Dec 2019 to sept 2020.

2.2Inclusion criteria

- Patients with primary varicocele with age ≥ 18 years and were williy to participate in the study.

2.3Exclusion criteria

Any patients with any of the following conditions were excluded from this study.

- Presence of associated chronic diseases as diabetes mellitus, cardiovascular diseases and hypertension.
- Patients with secondary varicocele.
- Previous abdominal or pelvic surgery.
- Previous varicocelectomy.
- Patients with bleeding tendency or any other risk factors to do varicocelectomy.
- Chemotherapy and drugs that affect testicular function e.g: Dapsone, Lamotrigine, Colchicine and Cyclophosphamide.

All participants will be divided into two groups

- **Group [A]:** Thirty varicocele patients were subjected to varicocelectomy.

- **Group [B]:** Twenty healthy volunteers with no varicocele by clinical examination

2.4Administrative design

This study was approved by the Research Ethical Committee of Benha Faculty of Medicine, and was carried out according to the guidelines of the Helsinki declaration principles.

2.5Methods

Every participant was subjected to the following:

Written informed consent

A written informed consent was taken before the start of the study. No risks were found and any unexpected risk appearing during the study was clarified to the patients and the committee on time. All the records were confidential. The results of this study were used only in scientific purpose. The participation was voluntary and the patients were able to discontinue participation at any time without penalty or loss of benefits.

Complete history taking

- Personal history: Name, age, occupation, residence and smoking or special habit of medical importance.
- History of the present condition including: onset, course and duration of varicocele.
- Past history: History of medications [type and duration], associated systemic diseases and previous surgery.

General and local examination

General examination

- It was done to exclude systemic disease.

Local examination

It was done to diagnose clinical varicocele which is detectable by both visual inspection and palpation. Scrotal examination was done from two different planes using valsalva maneuver and the patient was examined in two positions first in supine then in standing position. Then inspection and palpation was done for swelling to evaluate testis and spermatic cord.

The diagnosis of varicocele was made by the precence of dilated or tortuous segment. The varicocele grade was according to Dubin and Amelar grading system:

- **Grade I**: Dilated veins are palpable only during valsalva.
- **Grade II** : Dilated veins are easily palpable at rest but not visible during valsalva.
- **Grade III**: When the distended venous plexus bulges Visibly through the scrotal skin and is easily palpable at rest [7].

Scrotal Doppler

Doppler was done to confirm the presence of varicocele in query cases.

Laboratory investigations

I. Semen evaluation

The semen samples were measured from normal control men and men With varicocele before and three months after varicocelectomy. Subjects were asked to abstain from ejaculating for 3-5 days prior to sample collection. Semen sample was obtained by masturbation and collected in a pre-weighed sample container in a room close to the laboratory and were allowed to liquefy for about 30 min at room temperature. The samples were centrifuged at 900g for 10 min. Seminal plasma [supernatant] was then separated and aliquots were prepared and stored at − 80°C . Sperm concentration was determined by counting using a hemacytometer, slides for morphology assessment were stained with the modified Papanicolaou method and examined with phase-contrast optics and motility was assessed on a heated [37°C] microscope stage. Liquefied samples were analyzed according to World Health Organization [WHO] guidelines used for normal semen characterization. The reference values for total sperm number (39 million/ml), sperm concentration (15 million spermatozoa per milliliter of ejaculated volume), vitality (58% live), progressive motility must exceed (32%), total (progressive + non-progressive) motility (40%) and morphologically normal forms (>4.0% typical forms). [8].

Determination of serum AMH level

- A five ml of fasting peripheral venous blood were withdrawn under complete aseptic conditions into plain vaccutainer.
- The samples were allowed to clot for 10-20 minutes at room temperature.
- The samples were centrifuged for 20 minutes at the speed of 2000-3000 r.p.m.
- If precipitated appeared, the samples were centrifuged again.
- The superadded serum was stored at -20°C until time of assay.
- AMH ELISA kits was used to measure serum AMH level in sera of all participants. The kits was produced by Sunred biokit company for Research Service, Shanghai, Catalogue No: 201-12-1053,

Evaluation of semen analysis

was repeated with same previous steps three months after the operation.

Evaluation of serum AMH

was repeated with same previous steps three months after the operation.

2.6Statistical Methods

Data management and statistical analysis were done using SPSS version 25. [IBM, Armonk, New York, United States]. Quantitative data were assessed for normality using the Shapiro-Wilk test and direct data visualization methods. According to normality testing, numerical data were summarized as means and standard deviations or medians and ranges. Categorical data were summarized as numbers and percentages. Quantitative data were compared between study groups using independent t-test, and were compared pre and post-operative using paired t-test. ROC analysis was done for using AMH in diagnosing varicocele. Area Under Curve [AUC] with 95% confidence interval, best cut-off point, and diagnostic indices were calculated. Correlations were done using Pearson's or Spearman's correlation. Multivariate logistic regression analysis was done for the prediction of varicocele. The odds ratio and the 95% confidence interval were calculated. All statistical tests were two-sided. P values less than 0.05 were considered significant.

3.Results:

Varicocele showed significant improvement post-operative **[P < 0.001].** About one quarter were grade I [23.3%], and about half of the patients were grade II [46.7%], while about one-third were grade III [30.0%] before varicocelectomy. Most patients showed no varicocele [86.7%] and Only 10% and 3.3% were grades I and II, respectively after varicocelectomy.

Before varicocelectomy, sperm count was significantly lower in patients than control [Mean ±SD: 27±13.2, 60.7±14.5 respectively; P<0.001]. Also, progressive motility was significantly lower in patients than control [Mean ±SD: 25±8, 39±6 respectively; P<0.001]. In contrast, abnormal forms was significantly higher in patients [41%] than control [8%] [P < 0.001] .

AMH is measured in serum of patients before and after varicocelectomy and control group. AMH level in patients before varicocelectomy was significantly lower than in controls [Mean ±SD: 1.601 ±0.661ng/ml, 3.894 ±1.394ng/ml respectively; P<0.001].

After varicocelectomy, sperm count was significantly lower in patients than control [41.4 ±12.4, 60.7 ±14.5 respectively; P<0.001]. In contrast, abnormal forms was significantly higher in patients than control [21 ±9, 8 ±3 respectively; P<0.001]. No significant difference was reported regarding progressive motility [38 ±12, 39 ±6 respectively; P=0.944].

Before varicocelectomy, AMH level showed a significant negative correlation with varicocele grade [r = -0.365 & P = 0.047] and abnormal forms [r = -0.438 & P = 0.017]. However it showed non significant correlation between AMH level and patients' age [P = 0.685], varicocele duration [P = 0.316], sperm count [P = 0.328], and progressive motility [P = 0.673].

After varicocelectomy, AMH level showed a significant positive correlation with sperm count [r = 0.431 , P = 0.017]. However it showed no significant correlations between post-operative AMH and varicocele grade [r = -0.293, P = 0.117], progressive motility [r = 0.064, P = 0.737], and abnormal forms [r = -0.107, P = 0.57].

4.Discussion

In the current study, serum AMH level in patients before varicocelectomy was significantly lower than in controls. The decreased serum AMH could be explained by varicocele effects on Sertoli cells functions. This result was consistent with **Fujisawa et al.** [9] who showed a significant difference between seminal AMH concentrations of oligoozospermic infertile patients and controls **[M±SD**; 140.3 ± 254, 249 ± 167 pmol/l respectively, **P=** 0.0337]. The seminal concentration of AMH was correlated significantly with sperm concentrations [r=0.339, P=0.035]. The authers suggested that lower concentrations of AMH may be related to spermatogenic dysfunction and immaturity of Sertoli cells. Seminal AMH concentration was helpful for determining the extent of Sertoli cells dysfunction which may be associated with defective spermatogenesis.

The result of the current study is consistent with **Pierik et al.** [10] who reported a very low serum AMH levels in individuals with testicular pathology [e.g. partial testicular dysgenesis, prolonged cryptorchidism, prolonged varicocele].

Al-Qahtani et al. [11] showed that mean AMH levels in semen samples of male factor infertility group which assested for ART and control group weren't significantly different [17.54 ± 5 ng / ml; 10.2 ± 4 ng / ml respectively, P=0.9]. However, serum

levels of AMH in the male factor infertility group were significantly lower than those in the control group [2.8 ± 0.34 ng / ml; 4·43 ± 0·43 ng / ml respectively; **P** < 0.01] which agree with the result of the current study.

Mostafa et al. [12] showed lower seminal level of AMH in infertile oligoasthenozoospermia compared to controls [M±SD; 30.5 ± 10.3, 41.5 ± 10.9 pmol/l

respectively, P < 0.05]. In addition seminal AMH was positively correlated with testicular sperm concentration, sperm motility percent and negatively with sperm abnormal forms percent.

Goulis et al. [13] reported that circulating AMH levels in subfertile men with varicocele were significantly [60%] lower than those in corresponding controls [3.9±0.345ng/ml; 11.6± 7.7 ng/ml respectively, P<.05]. Also Tuttelmann et al. [14] reported that AMH in the infertile men with oligospermia was found to be slightly lower than in the men with normal sperm concentration [4.9 ± 1.3 ng/ml; 6.3±1.8 ng/ml respectively, P < 0.084]. The authers suggested that AMH might play a role as a marker of Sertoli cell function and maturation status in adult males when spermatogenesis is impaired.

The result of the current study was inconsistent with **Goulis et al.** [15] who reported a non significant difference between men with varicocele [n= 61] and fertile controls concerning peripheral vein AMH concentrations [10.2+0.5 pg/dL; 10.4+0.8 pg/dL, respectively, **P**< 0.9] .The authers suggested that AMH is a less sensitive marker of testicular function; thus, more extensive damage in sertoli and/or germ cells has to be suffered for the AMH concentrations to be decreased in the peripheral vein.

Turan et al. [16] showed AMH values in patients with varicocele [n=20] were determined to be lower compared to controls but the difference was non significant [7.15 ± 5.8 ng/mL; 7.66 ± 5.6 ng/mL, respectively, **P** = 0.82].This can be explained by insufficient production of AMH in Sertoli cells.

The present study showed improvement of varicocele grade and semen parameters following varicocelectomy. After varicocelectomy, varicocele grade showed significant improvement [**P** < 0.001], sperm count and progressive motility significantly increased [**P**<0.001], but the abnormal forms significantly decreased [**P**<0.001].

Several previous studies showed improvement in semen quality following varicocelectomy in infertile men [17]. **Kibar et al.** [18] found a significant or even highly significant improvement of sperm count [**P** = 0.002] and motility [**P** = 0.001] after varicocelectomy. **Abdel-Meguid et al.** [19] reported a significant improvement in sperm count [**P** = 0.0001] and to an increase in sperm motility [**P**= 0.001]. Schauer et al. [20] highlighting a significant improvement in sperm count and motility after surgical varicocelectomy. Similarly, **Kim et al.** [21] detecting a significant gain of progressive sperm motility of 9.6% [**P**= 0.004], without any effect on sperm count and morphology after varicocelectomy. This limitations in the study were: absent of random allocation, different diagnostic methods such as ultrasound with Doppler, Doppler velocimetry and infrared imaging were used and various surgical approaches for varicocele ligation [Palomo or laparoscopic ligation of spermatic vien].

A study evaluated 18 men showed that varicocele repair resulted in an improvement of semen quality. Total motile sperm count increased from 6.4 [1.1–24.5] million at baseline to 11.1 [2.4–38.4] million at approximately three months and to12.5 [1.6–31.5] million at ≥12 months [P= 0.143], concentration increased from 10.7 [3.5–21.3] million sperm/cc semen at baseline to 14.5 [4.0–22.6] million sperm/cc semen at three months, and 16 [1.4–20.0] million sperm/cc semen at ≥12 months [P= 0.563] [22]. This significant data were consistant with the results of the current study.

Daria et al. [23] showed a significant improvement in morphology, sperm concentration [**P**= 0.015] and percentage of progressive and total motility [**P**= 0.022 and **P**= 0.039, respectively], with a significant decrease in the percentage of immotile sperms [**P**=0.013] after surgical varicocele repair. Other conventional semen parameters didn't significantly change after surgery.

The AMH may reflect the impaired Sertoli cells functions, so we correlate AMH level with semen parameters. In current study before varicocelectomy, AMH level showed a significant negative correlation with abnormal forms [r = -0.438 & **P** = 0.017] but no significant correlation with sperm count and progressive motility. After varicocelectomy, AMH level showed a significant positive correlation with sperm count [r = 0.431 & **P** = 0.017] but non-significant correlations with progressive motility and abnormal forms. The limitations of results may due to short duration [3 months] after the surgical repair to take the second semen samples, thus not sufficient time for recovery of abnormal forms and motility.

Appasamy et al. [24] reported that serum AMH in infertile men [oligozoospermic men] showed a positive correlation with sperm concentration [**r** = 0.46, **P** < 0.02] and semen volume **[r** = 0.30, **P** < 0.05]. This was explained as AMH, produced from testicular Sertoli cells may reflect Sertoli cell function and spermatogenesis. AMH is inhibited by testosterone secreted by Leydig cells under the influence of LH. Therefore, alteration in testicular androgens could interfere with

Sertoli cell function, thereby contributing to male infertility.

The relationship between sperm concentration and AMH serum or seminal plasma levels has been discussed, but the results are not consistent. The study of **Duvilla et al.** [25] observed seminal plasma AMH reported a positive correlation between AMH levels and sperm concentration [**P**<0.001].This can be explained by the levels of AMH in the seminal plasma of fertile donors are wide-ranged [3–340 pmol/L]. Levels are diminished in the case of NOA, and undetectable in the case of obstructive azoospermia.

Goulis et al. [5] described significantly decreased AMH levels in men with non-obstructive azoospermia compared to healthy men [4.6 [3.6] vs. 11.6 [7.7] ng/ml, **P**<0.001]. According to Radek et al., [26] sperm concentration seems to be a main pathological parameter in semen. The lowest levels of seminal plasma AMH were observed [3.29 ng/ml, **P**=0.0001] when pathological sperm count [less than 15 million] was present.

5.Conclusion

The study confirmed that AMH decrease in varicocele patients and increase after surgical repair compared to controls. Thus, a fact that strengthens the role of AMH as a reliable index of Sertoli cell function.

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