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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 dilatation and expansion of the scrotal venous pampiniform plexus which drains blood from each testicle. While typically painless, varicoceles are clinically relevant since they are the most often recognized cause of infertility. The specific process by which varicocele causes infertility remains unclear. It is widely known that the pathophysiology of varicocele caused infertility is complicated and multifaceted. The severe damage produced by varicocele is connected with reduced function of sertoli cells. AMH has been considered as a direct marker of Sertoli cell activity and an indirect marker of spermatogenesis. So with prolonged varicocele , assessment of serum AMH be one of diagnostic studies in varicocele patients. Also, following varicocelectomy examination of percentage of success and reversal of normal tistecular function is an essential factor for raising of pregnancy rate. The purpose of this research was to analyze the serum level of antimullerian hormone in varicocele patients before and after varicocelectomy and evaluation of its connection to semen parameters and varicocele grade. Methods: This case control research was done 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. Results: There was non significant difference was noticed between patients and control groups as respects age. Varicocele grade showed substantial improvement post-operative. Before varicocelectomy, sperm count and motility was considerably lower in patients than control. In contrast, aberrant forms was much greater in patients than control. The mean sperm count and motility considerably higher after than before varicocelectomy. In contrast, aberrant forms dramatically diminished after than before varicocelectomy. AMH level in patients before varicocelectomy was substantially lower than in controls whereas after varicocelectomy AMH level rose. Before varicocelectomy, AMH level exhibited a substantial negative connection with varicocele grade and aberrant morphologies. After varicocelectomy, AMH level revealed a substantial positive connection with sperm count. AMH was a significant predictior of varicocele after adjusting for the influence of patients' age and smoking. Conclusion: The research indicated that AMH decrease in varicocele patients and rise after surgical repair compared to controls which linked with Sertoli cells alterations histopathologically and this the first study evaluated AMH after varicocelectomy. Thus, a finding that reinforces the importance of AMH as a trustworthy measure of Sertoli cell activity.

Key words: Serum Antimullerian Hormone, Varicocele patients, Varicocelectomy.

1. Introduction

MIS, or Mullerian-inhibiting substance, is a protein secreted by Sertoli cells that aids in the growth of male genitalia inside the body [1]. At the 7th week of gestation, high AMH expression in male gonads stimulates Mullerian duct regression [2].

Scrotal varicocele is caused by an abnormal bulging and curving of the pampiniform vein plexus. Male fertility declines over time as a result of varicocele's detrimental effects on spermatogenesis[3]. Sertoli cell dysfunction is linked to the severe damage produced by varicocele [4].

Serum AMH values in patients with varicocele were not conclusive. Subfertile men, including those with varicocele, had 60% lower levels of circulating AMH than control men, and this was accompanied by a 60% decrease in inhibin B, suggesting that the Sertoli cells in varicocele patients are less functional [5].

Prepubertal and pubescent boys with varicocele had elevated levels of AMH and inhibin B, suggesting that Sertoli cell function had increased in response to the elevated levels of AMH and inhibin B [6].

Varicocele patients' serum levels of antimullerian hormone before and after varicocele surgery were compared to those of healthy controls in this study.

2. Patients and Methods

2.1. Subjects

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.

2.2. Inclusion criteria

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

2.3. Exclusion 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

2.4. Administrative 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.

2.5. Methods

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

A normal control group of males and a group of men were used to collect the semen samples. Before and three months after varicocelectomy with varicocele. Abstain from ejaculating for at least 3-5 days before collecting samples. Masturbation was used to collect the semen, which was then placed in a pre-weighed sample container and left to liquefy for about 30 minutes at room temperature away from the lab. For 10 minutes, the samples were centrifuged at 900g. Finally, aliquots of seminal plasma [supernatant] were prepared, and stored at 80°C. The number of sperm was counted with a hemacytometer, the morphology of the slides was examined with phase-contrast optics, and the motility of the sperm was assessed on a heated [37°C] microscope stage for morphology assessment. Using World Health Organization [WHO] guidelines for normal semen characterization, liquefied samples were examined. Motility [percent] is 50, normal morphology [percent] is 14, and sperm concentrations [106/ml] are all at or above these standards.

II. Blood Sampling

A Five ml of fasting peripheral venous blood withdrawn under complete aseptic conditions into plain vaccutainer, allowed to clot for 10-20 minutes at room temperature then centrifuged for 20 minutes at the speed of 2000-3000 r.p.m, if precipitation appeared again.

Determination of serum AMH level

AMH ELISA kits was used to measure serum AMH level in sera of all participants. The kits was produced by Biokit for Research Service, Hagar Palace Building, Flat no. 5 2nd. floor - Victoria Alexandria Egypt E-mail: info@Biokit-eg.com, https: // www .bioki t-eg.com/, Tel: +201099405255

2.6Statistical Methods

In this study, SPSS version 25 was used for both data management and statistical analysis. The Shapiro-Wilk test and direct data visualisation approaches were used to examine quantitative data for normalcy. Numerical data were summarised using means and standard deviations, or medians and interquartile ranges, in accordance with normality tests. Numbers and percentages were used to summarise categorical data. Both pre- and post-operative statistical analyses were conducted using paired t-tests to compare quantitative data. The accuracy of AMH in detecting varicocele was evaluated by a ROC analysis. With a 95 percent confidence interval, the optimal cut-off point, and diagnostic indices were determined. The Pearson and Spearman correlations were used for the correlations. Varicocele was predicted using multivariate logistic regression analysis. Calculations included calculating the odds ratio and 95 percent confidence interval. There were no one-sided statistical tests. Statistical significance was defined as a value less than or equal to 0.05.

3. Results

As shown in Table (1), 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.

Table (1) Comparison of Varicocele grade before and after varicocelectomy in patients group.

Varicocele grade		n [%]	Z	*P*
Before operation	**Grade I**	7 [23.3]		
	Grade II	14 [46.7]		
	Grade III	9 [30.0]		
			-5.295	<0.001
After operation	**No varicocele**	26 [86.7]		
	Grade I	3 [10.0]		
	Grade II	1 [3.3]		

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] Table (2).

Table (2) Comparison between semen characteristics of patients before varicocelectomy and controls.

		Patients [n = 30]	Control [n = 20]	t	*P*
Count [million/mL]	**Mean ±SD**	27.3 ±13.2	60.7 ±14.5	8.332	<0.001
Progressive motility [%]	**Mean ±SD**	25 ±8	39 ±6	6.860	<0.001
Abnormal forms [%]	**Mean ±SD**	41 ±13	8 ±3	-12.810	<0.001

Independent t-test was used

AMH is measured in serum of patients before and after varicocelectomy and control group. As shown in table 3, 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] Table (3).

Table (3) Comparison between AMH level in the patients before varicocelectomy and control group.

		Patients before varicocelectomy [n = 30]	Control [n = 20]	*t*	*P*
AMH level [ng/ml]	Mean ±SD	1.601 ±0.661	3.894 ±1.394	6.861	<0.001

t: **Independent t-test was used**

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] Table (4).

Table (4) Comparison between semen characteristics of patients after varicocelectomy and control group.

	Patients [n = 30]	Control [n = 20]	*t*	*P*
Count [million/mL]	41.4 ±12.4	60.7 ±14.5	5.029	<0.001
Progressive motility [%]	38 ±12	39 ±6	0.071	0.944
Abnormal forms [%]	21 ±9	8 ±3	-7.852	<0.001

Independent t-test was used

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] Table (5).

Table (5) Correlation between AMH and other parameters before varicocelectomy.

	AMH level before varicocelectomy	
	r	***P***
Age [years]	0.077	0.685
Varicocele duration	0.19	0.316
Varicocele grade	**-0.365**	**0.047**
Count [million/mL]	0.188	0.328
PR [%]	-0.082	0.673
Abnormal forms [%]	**-.438**	**0.017**

Pearson's or Spearman's correlation was used r = Correlation coefficient

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 = 0.575] Table (6).

Table (6) Correlation between AMH and other parameters after varicocelectomy.

	AMH level after varicocelectomy	
	r	***P***
Varicocele grade	-0.293	0.117
Count [million/mL]	**0.431**	**0.017**
Progressive motility [%]	0.064	0.737
Abnormal forms [%]	-0.107	0.575

Pearson's or Spearman's correlation was usedr = Correlation coefficient

Multivariate logistic regression was done for the prediction of varicocele. It showed that AMH was a significant predictior of varicocele [OR = 0.068, 95% CI = 0.013 – 0.354, P = 0.001], after controlling for the effect of patients′ age and smoking Table (7).

Table (7) Multivariate logistic regression analysis for prediction of varicocele**.**

	OR [95% CI]	*P*
Age [years]	1.033 [0.912 - 1.171]	0.610
Smoking	3.264 [0.148 – 72.151]	0.454
AMH level	0.068 [0.013 - 0.354]	0.001

OR: Odds ratio 95% CI = 95% confidence interval

4. Discussion

Patients who had varicocelectomy had considerably lower blood AMH levels than the controls in the present research. Varicocele-induced Sertoli cell dysfunction may have contributed to a reduction in serum AMH. AMH concentrations in oligoozospermic infertile patients differed significantly from those of healthy controls [MSD; 140.3 254, 249 167 pmol/l, respectively, P= 0.0337] in this study. A substantial correlation (r=0.339, P=0.035) was found between AMH levels in the seminal fluid and sperm counts. The authors speculated that spermatogenic dysfunction and Sertoli cell immaturity may be linked to reduced AMH concentrations. Sertoli cell dysfunction may be linked to poor spermatogenesis if the AMH content in the seminal fluid is high enough.

According to Pierik et al. [10], a very low blood AMH level was found in people with testicular disease (e.g. partial testicular dysgenesis, extended cryptorchidism, prolonged varicocele) in the present investigation.

Men with male factor reproductive issues were found to have mean AMH levels that were not substantially different from those of the control group (P=0.9), according to Al-Qahtani and colleagues [11]. According to this research, AMH levels were considerably lower in male factor infertility patients compared to those in the control group [2.8–0.34ng/ml; 4–43–0•43ng/ml; P 0.01].

Infertile oligoasthenozoospermia had lower seminal AMH levels than controls, according to Mostafa et al. [12] [MSD; 30.5 10.3, 41.5 10.9 pmol/l respectively, P 0.05]. Seminal AMH was also linked to higher levels of sperm concentration and motility in the testes, as well as lower levels of aberrant sperm forms in the testes.

According to Goulis et al. [13], subfertile males with varicocele had substantially lower circulating AMH levels than matching controls [3.9 ng/ml; 11.6 ng/ml, P.05]. In addition, Tuttelmann et al. [14] discovered that AMH levels were lower in males with oligospermia who were unable to conceive [4.9 1.3 ng/ml vs 6.31.8 ng/ml, P 0.084]. The authors hypothesised that AMH might serve as a biomarker for Sertoli cell activity and maturation in adult men with poor spermatogenesis.

According to Goulis et al. [15], there was no significant difference in peripheral vein AMH concentrations between males with varicocele [n= 61] and fertile controls (10.1+0.5 pg/dL vs. 10.4+0.8 pg/dL, respectively, P 0.9) in the present investigation.

For AMH concentrations to fall in the peripheral vein, more substantial injury to the sertoli and/or germ cells must be sustained, according to the authors.

There was no statistically significant difference between the AMH values of patients with varicocele (n=20) and the controls (n=20), according to Turan et al. [16], who found that AMH levels were lower in the latter group (n=20).

This may be due to Sertoli cell insufficiency in AMH synthesis.

Varicocelectomy improved varicocele grade and semen parameters, according to the findings of this research. P0.001, sperm count and progressive motility rose, while aberrant forms reduced dramatically after varicocelectomy [P0.001]. P0.001, on the other hand, demonstrated considerable improvement in the varicocele grade.

Varicocelectomy has been shown to increase the quality of infertile men's semens [17]. A considerable or perhaps a very significant increase in sperm count and motility was discovered after varicocelectomy, according to Kibar et al. There was a considerable increase in sperm count and sperm motility, according to Abdel-Meguid and colleagues [19]. A considerable increase in sperm count and motility after surgical varicocelectomy was reported by Schauer et al. [20]. Similarly, following varicocelectomy, Kim et al. [21] found a 9.6% increase in progressive sperm motility (P= 0.004) but no change in sperm count or morphology. Some of the study's shortcomings include the absence of random allocation, the use of multiple diagnostic procedures such as ultrasonography with Doppler (Doppler velocimetry), as well as varied surgical approaches for varicocele closure (Paloma or laparoscopic).

A study of 18 males found that repairing a varicocele improved the quality of the semen. In only three months, the total number of sperm grew from 6.4 million [1.1–24.5], the concentration increased from 10.7 million sperm/cc semen at baseline to 14.5 million sperm/cc semen in three months, and 16 million sperm/cc semen in 12 months [P= 0.563] [22]. They were in agreement with the present study's findings.

Following varicocele surgery, Daria et al. [23] found that the proportion of immotile sperms decreased significantly [P=0.013], with a substantial improvement in the morphology, sperm concentration, and percentage of progressive and total motility [P=0.022 and P=0.039]. After surgery, there were no notable changes in any traditional semen characteristics.

The AMH level may be an indicator of Sertoli cell dysfunction, hence we use semen characteristics to correlate AMH levels. Prior to varicocelectomy, the AMH level was shown to be significantly correlated with aberrant forms (r = -0.438 & P = 0.017) but not with sperm count or progressive motility in the present investigation. [r] Sperm count increased significantly after varicocelectomy (r = 0.431, P = 0.017) but not significantly (r = 0.017, P = 0.017) with AMH levels. Semen samples taken three months after surgery may have had insufficient recuperation time to recover aberrant shapes and motility, which may have hampered findings.

Appasamy et al. [24] found a link between blood AMH levels and sperm and semen volume in infertile men [oligozoospermic males] (r = 0.46, P 0.02) and semen volume (r = 0.30, P 0.05). Sertoli cell activity and spermatogenesis may be reflected in the production of AMH by testicular Sertoli cells. Testosterone, which is released by Leydig cells under the influence of LH, blocks AMH activity. Male infertility may be exacerbated by changes in testicular androgens, which might affect Sertoli cell activity.

When it comes to sperm concentration and AMH serum or seminal plasma levels, the findings are not consistent. A research by Duvilla et al. [25] found an association between AMH levels and sperm concentration [P0.001]. Fertile donors' seminal plasma AMH levels vary from 3–340 pmol/L, which may account for this. NOA results in lower levels, whereas obstructive azoospermia results in none at all.

Males with non-obstructive azoospermia had considerably lower AMH levels than healthy men, according to Goulis et al. [5] [4.6 [3.6] vs. 11.6 [7.7] ng/ml, P0.001]. sperm concentration seems to be a major pathogenic characteristic in semen, according to Radek et al. [26]. When a pathological sperm count (less than 15 million) was present, the lowest seminal plasma AMH levels (3.3 ng/ml, P=0.0001) were found.

5. Conclusion

AMH decreased in varicocele patients and increased after surgical repair compared to controls, which coincided with Sertoli cell alterations histopathologically and this was the first research to evaluate AMH following varicocelectomy.. As a result, AMH is a more accurate indicator of Sertoli cell activity. For the examination of infertility, the amount of serum AMH in adult males was shown to be a reliable indicator of testicular tissue. After surgical varicocele correction, an increase in AMH was directly linked to an increase in sperm concentrations, which suggested an improvement in testicular tissue function. In order to provide the best care, individuals with varicocele should be monitored and treated promptly.

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