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RESEARCH ARTICLE

Impact of Varicocelectomy on the Sperm Function Parameters in Relevance to Reproductive Hormonal Profile in Infertile Men

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Abstract

Background: Varicocele is associated with a decrease in fertility and testicular functions. If varicocele is a consequence of a congenital lack in the vein drainage of the testicular grove, its occurrence is caused by the presence of vein reflux. An abnormal spermiogram is more frequent in men with varicocele than in those with normal parameter findings .The presence of varicocele in the general male population is 15-20%. In addition, varicocele causes infertility in 30-50% of all marriages. Moreover, according to WHO (2000), the infertility of a great number of couples is caused by varicocele, which is the main cause of male infertility (30-50%).

Objectives: To identify the impact of varicocelectomy on the sperm function parameters and reproductive hormones.

Subjects and Methods: This randomized study was carried out on 35 infertile men with different varicocele grades, their age ranged between 19-42 years old through the period from December 2011 to Feburary 2013 in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad-IRAQ.

The cases were subjected to evaluate the varicocelectomy effect on the certain sperm function parameters in relevance to their reproductive hormones.

Results: The results of this study was revealed a highly significant ($p<0.001$) increase in the sperm function parameters of infertile men with all grades of varicocele after varicocelectomy state compared with before state for the same subjects. While there were decrease in reproductive hormones (FSH,LH,PRL) after varicocelectomy in comparison with before state except the testosterone level which were higher in different grades of varicocele infertile patients with a highly significant difference ($p<0.001$) in comparison with before state.

Conclusions: in conclusion the varicocelectomy have a good role in the improvement of the major sperm function parameters, as a result of the varicocelectomy there were an improvement in the testicular hormones as showed by testosterone level corrections.

Introduction

Varicoceles are found in 35% to 40% of men who have primary infertility and in75% to 80% of men who have secondary infertility but are found in only 15-20% of the general population [1,2]. The association with infertility

has been recognized for more than 50 years [3]. Varicocele causes a duration-dependent decline in semen parameters and testosterone production [4,5].

Venous dilation is thought to impair the counter-current heat exchange mechanism in the scrotum [6]. Pooling of venous blood is likely to cause the increased intratesticular temperature and the progressive, duration dependent decline in testis function observed in patients who have varicoceles [7,8]. Repair of a varicocele can prevent further testicular damage, improve sperm production, and improve testosterone production [4]. Ligation of a varicocele may help prevent infertility and low testosterone levels after repair [9,10]. Several investigators have sought to determine preoperative characteristics of a varicocele that would predict response to varicocelectomy[11,12]. Patients who had larger varicoceles were found to have greater improvements in semen analysis parameters after the procedure than men who had smaller varicoceles [13]. Semen analysis parameters improved in men who had clinically nonpalpable varicoceles detected by ultrasound, and a cutoff of a venous diameter of 3 mm or reversal of flow have been suggested as operating criteria [14–17]. It uses the microsurgical, subinguinal approach to repair varicoceles [18].

It believes that this approach with optical magnification minimizes complications and produces the best results by ligating all of the internal spermatic and cremasteric veins that contribute to the formation of varicoceles. The testicular artery is identified, preventing damage to this structure. Also, cremasteric arteries and lymphatic channels are preserved, which prevents the formation of hydroceles [19]. Use of the operating microscope results in a hydrocele rate of approximately 1% compared with up to a 30% rate of hydrocele formation 6 months postoperatively after conventional inguinal and laparoscopic approaches [20,21]. Recurrences are not uncommon and are seen in up to 15% to 25% of men using nonmicrosurgical approaches but in less than 1% of men using microsurgical approaches [21]. Loupes under 2.5 do not provide enough power to reliably identify the testicular artery or lymphatics. Other methods of varicocele surgery include the conventional inguinal, the retroperitoneal, and the laparoscopic approaches.

Radiologic embolization represents another option to correct varicoceles. In this procedure, the testicular veins are accessed percutaneously, and an alcohol-based sclerosant or coils are used to embolize the veins. Two large investigations demonstrated resolution of varicoceles in 83% of men. Significant improvement after embolization was noted for sperm density, motility,and morphology [22,23].

Surgical ligation of varicoceles reduces intratesticular temperature to the normal range [24] and improves semen parameters and Leydig cell function of the testis.

Several studies have demonstrated improvements in semen parameters, testosterone production, and pregnancy outcomes. Semen parameters improve in 60% to 80% of men after repair [2,17,25]. Bilateral repair in men who have a large unilateral and small contralateral varicocele and repair in younger men may have a greater beneficial effect on sperm parameters and androgen production than repair in older men [26-29].

Subjects and Methods

The study was conducted in the High Institute of Infertility Diagnosis and ART's at Al- Nahrain University through the period from December 2011 to February 2013.Thirty five infertile men with varicocele Grade I,II,III were involved in this study who examined by a urologist.

Physical examination: patients were examined in a warmed room in supine and standing position (for 5 minutes).The scrotal contents were palpated between thumb and forefingers to assess volume, position and consistency of testis and epididymis on both sides. Each spermatic cord was palpated in the standing position and during the Valsalva maneuver. The patients were divided according to the grade of varicocele. The severity of the varicoceles is graded I ,II and III using the system of Dubin and Ameral [30] as the followings: grade I(small) varicoceles palpable only during Valsalva maneuver and men with subclinical varicocele were included in this group(n=15) , grade II (medium) varicocele palpable in standing position(n=10) and grade III (large) varicocele detectable by visual scrutiny alone(n=10).

Ultrasound Doppler: The Ultrasound study was performed to optimize the detection of blood flow. The examination done with patient in supine and standing position too. Each side of the scrotum was scanned and vascular channels in the spermatic cord were noted, and the vessel diameters were measured by electronic clippers .Any testicular abnormalities , spermatocele , epididymal lesions and hydrocele were recorded. The Valsalva maneuver was used in the supine and erect positions. The maximal diameter of the scrotal veins was recorded and the presence of the venous plexus was noted. The flow velocity of the main vein was measured before and after the Valsalva maneuver. The sonographic diagnosis of varicocele was based on the detection of two to three venous channels one of which measured >3mm in diameter and reflux during the Valsalva maneuver. Patients with 2.7mm or less in diameter were regarded as subclinical varicoceles as they were not detected by palpation [31]. Freshly ejaculated samples of semen were collected by masturbation from infertile men with different grades of varicocele directly into a clean, dry and sterile disposable plastic Petri–dish in an especially allocated room for this purpose in the Institute. For each subject with acquaintance in the abstinence period from 3-5 day, the sample was

transported to the semen analysis laboratory immediately. After liquefaction time each sample was divided into two equal portion (each 1ml) for two lines first line: 1ml for before activation and second: 1ml for after activation. After 30-60 minutes each semen sample was allowed to liquefy according to methods described previously [32,33].After complete liquefaction, the semen was analyzed by a macroscopic and microscopic examination using the standardization of WHO, (1999) [34].

- Microscopic examination

1- Sperm concentration:

Assessment of sperm concentration is done by the estimation of the number of sperm per milliliter, the number was multiplied by a factor of one million [34-36]. A drop of 10µl spermatozoa suspension was placed on a microscopic slide and covered with a cover slip [37,38]. Certain sperm function parameters were recorded according to guidelines of WHO (1999) too [34].

2- Sperm motility:

The microscopic field was scanned systematically and the motility of each spermatozoon encountered was graded:-

- A -Rapid and linear progressive motility.
- B -Rapid non linear or linear non rapid progressive motility.
- C-Non progressive motility.
- D- Immotile.

3- Sperm morphology:

The percentage of morphologically normal sperms was performed by using the same prepared slides for sperm motility. At least 100 spermatozoa were calculated by dividing the mean number of normal spermatozoa in four high power microscopic fields under magnification of (40x) [36-38] on the number of sperm concentration [39].

- Sperm media preparation

The recommended sperm media in study is Ham's F-10 according to studies of [32, 33, 40].

- As a method of sperm *in vitro* activation the wash and spin method was done for all samples as described by [41].Then the microscopic examination were done after the *in vitro* sperm activation (wash and spin method).All these procedures had been repeated for each subject and the final readings were after *in vitro* activation in two lines:

1-Before Varicocelectomy

2-After Varicocelectomy within period ranged from (6 months-9 months) [42] after varicocelectomy , therapy and according to the patient attendance.

Reproductive Hormonal Assay:

Measurements of reproductive hormones concentrations (FSH, LH, Prolactin and Testosterone) were performed by fluorescence assay (mini VIDAS, bioMerieux, France).

Data of sperm parameters and hormones concentrations were statistically analyzed by using appropriate statistical tests.

Results

Fifteen out of the 35 (43%) patients had varicocele grade I as the main infertility cause.

Figure1 shows the percent distribution of infertile men with different varicocele grades as the main cause of infertility in these cases.

Twenty out of 35 (57%) patients were infertile with varicocele grade II,III ,each grade can be represented by 10 patients.

In this study it has been carrying out a comparison for each grade of varicocele infertile patients between the two lines (before and after varicocelectomy). In infertile men with varicocele grade I, after varicocelectomy they exhibited higher sperm function parameters with a high significant ($p<0.001$) difference compared with before varicocelectomy (table 1). Regarding the hormonal profile it showed that there are a decrease in the hormones(FSH,LH and Prolactin) levels after varicocelectomy compared with before state, the decrease were with no significant difference except the difference in Prolactin level which were with a high significant ($p<0.001$) value, while the testosterone level were higher than before varicocelectomy state with a high significant ($p<0.001$)

difference which could be ascribed to the role of varicocelectomy within improvement of spermatogenesis process then the testicular hormones roles correction (table 1).

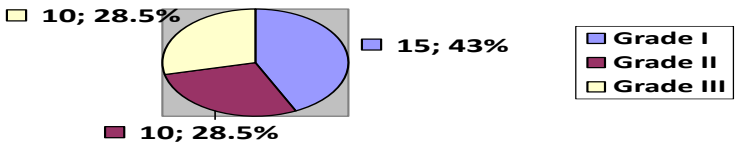

Figure (1): Percent distribution of Infertile patients with different varicocele grades (n=35)

Table (1): Comparison of Sperm Function, Reproductive Hormones parameters before and after varicocelectomy in infertile with varicocele grade I.

Parameter	Before Varicocelectomy	After Varicocelectomy	Significances
	Mean±SE	Mean±SE	
Sperm Concentration (Million/ml)	33.66±4.57	46.00±3.99	HS
Morphologically Normal Sperm (%)	26.47±2.78	31.93±1.47	HS
Sperm Motility Grade (a+b) (%)	30.20±4.54	34.87±2.91	HS
Sperm Motility Grade (a+b+c) (%)	51.33±4.51	58.67±3.22	HS
FSH (miu/ml)	5.15±1.18	4.57±0.44	NS
LH (miu/ml)	4.25±0.35	4.03±0.33	NS
PRL (ng/ml)	9.52±0.71	8.06±1.51	HS
Testosterone (ng/ml)	5.99±0.23	8.28±0.36	HS

n=15, M±SE=mean± standard error, HS=P<0.001, NS=Not significant

Regarding the infertile patients with varicocele grade II, after varicocelectomy they exhibited higher levels of all sperm function parameters than before state with a highly significant ($p<0.001$) difference(table 2).After varicocelectomy they exhibited also lower levels of hormones (FSH,LH and Prolactin) than before state with no significant difference, while they exhibited highly significant ($p<0.001$) increased testosterone in comparison with before state (table 2).

Table (2): Comparison of Sperm Function, Reproductive Hormones parameters before and after varicocelectomy in infertile with varicocele grade II.

Parameter	Before Varicocelectomy	After Varicocelectomy	Significances
	Mean±SE	Mean±SE	
Sperm Concentration (Million/ml)	29.90±5.03	37.60±1.95	HS
Morphologically Normal Sperm (%)	25.50 ±3.00	28.20 ±3.46	NS
Sperm Motility Grade (a+b) (%)	28.00±2.55	29.20±3.31	NS
Sperm Motility Grade (a+b+c) (%)	49.70±3.68	55.50±4.22	HS
FSH (miu/ml)	5.88±1.80	5.32±1.63	NS
LH (miu/ml)	4.16 ±0.93	3.88 ±0.90	NS
PRL (ng/ml)	11.06±2.08	9.69±1.91	NS
Testosterone (ng/ml)	5.38±0.22	6.90±0.34	HS

n=10, M±SE=mean± standard error, HS=P<0.001, NS=Not significant

In infertile patients with varicocele grade III, after varicocelectomy they also exhibited a highly significant ($p<0.001$) increase in sperm function parameters in comparison with before varicocelectomy (table 3).
Regarding the hormones, after varicocelctomy they were a highly significant ($p<0.001$) decrease in all hormones except in FSH the decrease were with no significant difference, while the testosterone level were highly significant increased in comparison with before state as same as the previous varicocele grades (table1,2,3).

Table (3): Comparison of Sperm Function, Reproductive Hormones parameters before and after varicocelectomy in infertile with varicocele grade III.

Parameter	Before Varicocelectomy	After Varicocelectomy	Significances
	Mean±SE	Mean±SE	
Sperm Concentration (Million/ml)	15.70±4.24	30.50±4.21	HS
Morphologically Normal Sperm (%)	20.80±1.70	27.00±2.26	HS
Sperm Motility Grade (a+b) (%)	21.60±2.72	26.80±2.63	HS
Sperm Motility Grade (a+b+c) (%)	47.20±2.93	53.70±3.76	HS
FSH (miu/ml)	15.70±2.15	14.52±1.54	NS
LH (miu/ml)	11.35±0.75	10.54±0.73	S
PRL (ng/ml)	11.97±0.55	9.89±1.07	HS
Testosterone (ng/ml)	2.41±0.59	4.24±0.74	HS

n=10, M±SE=mean± standard error, HS=P<0.001, NS=Not significant, S= P<0.05

The pregnancy outcomes were shown by the wives of infertile patients with varicocele grades (I,II) who undergoing intra-uterine insemination (IUI).The pregnancy outcomes can be represented as: Three out of the 15 (20%) patients with varicocele grade I. One out of 10(10%) patients with varicocele grade II.

Discussion

The association between a clinical varicocele and impaired spermatogenesis is well described [43-45]. Most studies have reported varying degrees of hypospermatogenesis, as well as Sertoli cell changes and premature sloughing of germ cells into the seminiferous tubule lumen. In this study, it has been trying to study the impact of varicocelectomy with sperm function parameters and reproductive hormones of infertile men that complaining from varicocele as the main cause of infertility. The findings of the present study are in agreement with findings of Zini *et al*[46] and Schlesinger *et al*[47] and Ibrahim *et al*[48] demonstrating that overall, varicocele repair results in improved semen quality in 60–80% of infertile men. However, the true effect of adult varicocelectomy on male fertility remains controversial largely because of the paucity of randomized and controlled trials. Also, using the improvement in conventional semen parameters as an outcome measure after varicocelectomy is limited by virtue of the high degree of biological variability of these parameters. The present results show that the swim-up method for recovery of motile sperm is reliable. The washing procedure is necessary to remove prostaglandins, infectious agents and leukocytes [49].One advantage of the method is the limited number of technical steps that besides being more practical & important in the study aim avoids damage to the spermatozoal cytoplasmic membrane [50]. Furthermore, it was reported that common laboratory factors like centrifugation, washing, temperature fluctuation, and processing delay harmfully affect semen quality both positively and negatively due to direct influence of laboratory interventions on the cytoskeletal assemblies of sperm [51].Regarding after varicocelectomy selected period which is ranged from 6-9months this could be the best due to the optimum improvement of the disposal of residual sperm cytoplasm by the testis and/or epididymis in infertile men with different varicocele grades, in addition to the reduction process of the potential for ROS generation by human spermatozoa in these patients within this period [52].

The most important point in this study, our patients were selected based on the diagnosis of varicocele only and the impact of the surgical varicocelectomy method regardless the type of oral remedy after varicocelectomy and without paying attention to the other factors status like the affected side. This may explain the significant difference of the results with before varicocelectomy. These findings were so close to the findings of the study of Ketabchi *et al*[53].

In this study the effect of varicocelectomy on the reproductive hormonal profile was not so clear which could be ascribed to the absence of specific role of antioxidant therapy program and other administration of other remedy systems that must be taken regularly after varicocelectomy, these findings were reported by Agarwal *et al*[54].
The high significant difference in the comparison of testosterone level in different varicocele grades (before and after varicocelectomy) could be explained by the mechanism of impaired testicular function in patients with varicocele, also the elevated testicular and scrotal temperature, venous stasis and the resultant hypoxia, reflux of adrenal breakdown products in the testicular vein, and the most important one the lower intratesticular testosterone, and androgen receptor defects are possible causes[53].
The results of the pregnancy outcome were achieved due to the role of varicocelectomy of improved sperm function parameters after varicocele repair as the results reported by [55,56].

Conclusions

It have shown that in the infertile patients with different varicocele grades the varicocelectomy can improve the sperm function parameters , reproductive hormones levels, especially in patients with varicocele grade I and II, but it need more studies to provide an additional mechanisms like the most update therapy system and ART's as ICSI for the reported improvement in pregnancy rates after varicocele repair especially in infertile patients with varicocele grade III.

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