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Predictive Factors of Sperm Recovery in Azoospermic Patient Post Varicocele Ligation (December 2020 to December 2021)

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Abstract **Background:** Varicocele, an abnormal enlargement of the pampiniform venous plexus in the scrotum, affects around 15% of men and 40% of fertility-problem men. It is believed to impair sperm production. This study aims to evaluate the frequency and predictive factors of successful sperm recovery in azoospermic patients post varicocele ligation. **Methodology:** A cross-sectional hospital-based study, was conducted in Ibn Sina Specialized Hospital and Hawa Infertility Center, within the period from December 2020 to December 2021, included 70 infertile patients with NOA and palpable varicocele on their physical examination, data was collected through direct interview closed ended questionnaire, analyzed by computer using Statistical Package For social science (SPSS) version 25.0. **Results:** Seventy patients were included in this study with mean age of 36.4±2.5 years. Mean infertility duration was 4.15±2.04 years. Body mass index was normal in over half of patients (52.9%), 40 patient (57.1%) had varicocele Grade III, followed by 30 (42.9%) had varicocele Grade II, most of patients 44(62.9%) underwent unilateral varicocelectomy. histopathology showed sertoli only cell in 11(29.7%), maturation arrest 14(37.8%), and hypospermatogensis in 12(32.4%). Semen analysis /TESE in 3-6 months post varicocele ligation was positive in 27(38.6%), and negative in 43(61.4%). patients age (P value =0.017) duration of infertility (P value =0.012). BMI (P value=0.007) Grade of varicocele. (P value=0.006) were significant predictors of successful sperm recovery after varicocelectomy. **Conclusion:** Varicocelectomy is an acceptable modality of treatment for non-obstructive azoospermic patients with clinically palable varicocele, especially in young age , non obese, with hyopspermatogensis in histopathology, a higher grade of varicocele, and a shorter duration of infertility, which showed a high rate of sperm recovery.

Key Words Homocysteine, Hyperhomocysteinemia, Cardiovascular disease

1. Introduction

A varicocele is an abnormally dilated pampiniform plexus, which is the venous network that drains blood from the testicles. The varicocele prevalence in the general population is estimated to be 15%; however, the prevalence is 35% among men with primary infertility and 81% among men with secondary infertility. The detrimental effects of varicoceles on fertility and the benefit gained by their repair have been debated among andrologists for almost 60 years. Since Tulloch reported the first unassisted pregnancy following varicocele repair in an azoospermic man in 1952, the effect of varicocelectomy on male infertility has become a hotly debated topic [1].

Increased scrotal temperature caused by varicocele's impeded blood drainage in the pampiniform plexus of the spermatic cord is thought to cause spermatogenesis to deteriorate with time. Varicocele can cause generalized sperm dysfunction, defined by poor semen quality ranging from oligospermia to full azoospermia [2]. The prevalence of azoospermia or severe oligospermia with varicocele has been reported to be between 4.3 and 13.3% [3].

Varicocelectomy increases spermatogenesis as well as Leydig cell activity [4]. Although it has been extensively documented that varicocelectomy improves sperm parameters in oligoasthenoteratozoospermia (OAT) patients, its efficacy in men with azoospermia is yet unknown [5]. In injured testes,

clinical data suggests that localized or 'patches' of sperm production occur. It's thought that men with non-obstructive azoospermia (NOA) have homogeneously or randomly distributed spermatogenesis in their testes. A major portion of testicular tissue represents spermatogenesis in the first situation, whereas a large portion of testicular tissue may be devoid of focused advanced spermatogenesis in the second condition [6].

In about 4–14 percent of cases, azoospermia is linked to a varicocele [6]. In previous investigations, the effect of varicocelectomy in these men was proven to be less significant [7], but recent studies in men with azoospermia have revealed that varicocele repair results in a slight improvement in semen parameters [8].

The ability to obtain motile sperms in the ejaculate is the principal benefit of varicocelectomy in situations of NOA. Another advantage of varicocele repairs in azoospermic men is that they boost the success rates of assisted reproductive procedures (ART) like intracytoplasmic sperm injection (ICSI) or testicular sperm extraction (TESE) by creating motile sperms from the fresh ejaculate [6].

According to some previous studies, varicocele correction microsurgicaly improves semen parameters and the production of motile sperms in the ejaculate by around 40% in patients with azoospermia [9]. Varicocele repair may result in a moderate improvement in semen quality for men with azoospermia or severe oligoasthenospermia, which could have a substantial impact on a couple's fertility prospects [10].

A study by Thakur et al. reviewed 104 patients with infertility and NOA with palpable varicocele who underwent microsurgical varicocelectomy. Out of them, 18.26% had sperm analysis post-operatively, and 29 (34.11%) had sperms in their testis. The fertilization rate was 89.65%, and the sperm retrieval rate was 34.11% higher in NOA men with varicocele. This effect was more significant after successful intracytoplasmic sperm injection [10].

A study involving 83 men with NOA and left varicocele underwent microsurgical VR with simultaneous testicular biopsy. Sperm recovery was confirmed in 24% of patients within 12 months. Among 23,003 genes, up-regulated cell cycle-related genes and down-regulated antioxidant genes were found in men with sperm recovery [11].

A study by Abdel-Meguid et al. assessed sperm recovery and relapse of azoospermia in 31 men. Results showed that sperm recovery was evident in 32.3% of patients, with hypospermatogenesis and late maturation arrest being the most common. Histological patterns showed a significant correlation with recovery, while no predictors of relapse were identified. Varicocelectomy could recover motile sperm in men with nonobstructive azoospermia, palpable varicoceles, hypospermatogenesis, or late maturation arrest [12].

The pampiniform plexus of the spermatic cord, it is linked to infertility and gradual testicular injury. In about 4–14% of instances, azoospermia is linked to a varicocele. Varicocele repair may result in a moderate improvement in semen quality for men with azoospermia or severe oligoasthenospermia, which could have a substantial impact on a couple's fertility prospects. Therefore, this study aimed to evaluate the role of microsurgical subinguinal varicocelectomy in men with varicocele, to determine the predictive parameters of post-operative improvement and to assess the result of varicocelectomy on sperm retrieval rate.

2. Materials and Methods

A. Study design

A cross-sectional hospital-based study.

B. Study area

This study was conducted in Ibn Sena Specialized Hospital and Hawa Infertility Center.

C. Study duration

The study was conducted within the period from December 2020 – December 2021.

D. Study Population

1) Inclusion Criteria

- All infertile patients with NOA and palpable varicocele on their physical examination were included.

2) Exclusion Criteria

1) Non-palpable varicocele.
2) Female factor infertility.
3) Obstructive azoospermia.
4) Genetic abnormalities like Y-chromosome microdeletion and Klinefelter syndrome.
5) unwilling to participate.

E. Sample size and sampling technique

Total coverage of 70 cases during study period, due to rare cases.

F. Data collection tools

Predesigned structured questionnaire was used to collect data, included sociodemographic information's, patient's history and clinical examinations will be collected from patients file, Data will be collected by researcher.

G. Data entry, analysis and presentation

Data entered, cleaned, and analyzed using Statistical Package of Social Science SPSS software version 26.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics in term of frequency tables with percentages and graphs. Means and standard deviations, presented with relevant graphical representation for quantitative data. Program. Chi squire test was used to test the relationship between the variables, P value will be considered significant if </= 0.05 (CI 95%).

H. Ethical considerations

Written ethical clearance and approval for conducting this research obtained from Sudan Medical Specialization Board

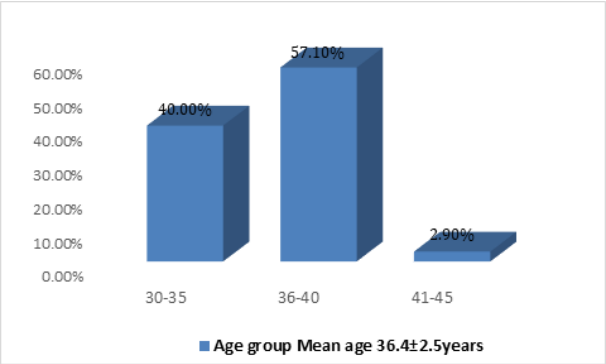

Figure 1: Age groups of patients underwent varicocele ligation

Ethical Committee and by (EDC). Written permission was obtained from the administrative authority of Ibn Sina Specialized Hospital and Hawa Infertility Center. Written consent was obtained from all participants. Confidentiality was considered intentionally, data will be used anonymously by using code numbers instead of names in order to protect participants' identity and keep securely, and information was used for research purpose only.

3. Results

In Figure 1, 70 participants were involved, with an average age of 36.4±2.5 years. The largest proportion, 40 individuals (57.1%), fell within the 36-40 age bracket.

Table 1 shows that average duration of infertility was 4.15±2.04 years, with most patients (33, 47.1%) experiencing infertility for 1-3 years, followed by 27 patients (38.6%) experiencing infertility for 4-6 years. In terms of body mass index (BMI), more than half of the patients (52.9%) had a normal BMI, while 25 patients (35.7%) were classified as overweight. The mean volume of the right testicle was 17.8±5.2 (ranging from 5 to 30), and the mean volume of the left testicle was 17.4±5.5 (ranging from 6.5 to 35). Hormonal tests revealed that the mean follicle-stimulating hormone (FSH) level was 12.7±4.1 ng/ml (range: 3-20 ng/ml), the mean luteinizing hormone (LH) level was 9.54±2.4 ng/ml (range: 4-14 ng/ml), and the mean testosterone level was 3.42±1.8 ng/ml (range: 1-8 ng/ml). Varicocele Grade III was most common among patients (40, 57.1%), followed by Varicocele Grade II (30, 42.9%).

Among the patients who underwent varicocelectomy, most (44, 62.9%) had undergone unilateral surgery, with 25 patients (56.8%) having the procedure on the left side. Testicular sperm extraction (TESE) during varicocelectomy yielded positive results for 42 patients (45.7%) and negative results for 38 patients (54.3%), while 28 patients (40%) did not undergo TESE. Among patients with negative TESE results, histopathology revealed sertoli cell-only syndrome in 11 patients (29.7%), maturation arrest in 14 patients (37.8%), and hypospermatogenesis in 12 patients (32.4%). Post-varicocele ligation semen analysis conducted within 3-6 months showed

Feature	N (%)
Infertility Duration (years)	
* 1-3 years	33 (47.1%)
* 4-6 years	27 (38.6%)
* 7-9 years	8 (11.4%)
* 10+ years	2 (2.9%)
Body Mass Index (BMI)	
* Normal (18-25)	37 (52.9%)
* Overweight (26-30)	25 (35.7%)
* Obese (31-35)	8 (11.4%)
Testicular Volume (mL)	
* Right	17.8 ± 5.2
* Left	17.4 ± 5.5
* Total	17.6 ± 5.3
Hormones (Mean±SD)	
* FSH (ng/mL)	12.7 ± 4.1
* LH (ng/mL)	9.54 ± 2.4
* Testosterone (ng/mL)	3.42 ± 1.8
Varicocele Grade	
* Grade I	0 (0%)
* Grade II	30 (42.9%)
* Grade III	40 (57.1%)
Varicocelectomy	
* Unilateral	44 (62.9%)
* Bilateral	26 (37.1%)
* Side of Unilateral (n=44)	
* Left	25 (56.8%)
* Right	19 (43.2%)
Testicular Sperm Extraction (TESE)	
* Positive	42 (5.7%)
* Negative	66 (94.2%)
Histopathology of Negative TESE	
* Sertoli-cell only	11 (15.7%)
* Maturation arrest	14 (20%)
* Hypospermatogenesis	12 (17.1%)
Sperm Recovery (3-6 months post-surgery)	
* Positive	27 (38.6%)
* Negative	43 (61.4%)

Table 1: Characteristics and outcomes of 70 patients underwent varicocele repair surgery

positive results in 27 patients (38.6%) and negative results in 43 patients (61.4%).

Table 2 shows significant association between the duration of infertility and successful sperm recovery, as indicated by a P value of 0.012. A statistically significant relationship between BMI and successful sperm recovery, with a P value of 0.007. A significant association was found between testicular volume and successful sperm recovery, with a P value of 0.013. A significant association was detected with a P value of 0.006, suggesting a relationship between varicocele grade and successful sperm recovery. The table also indicated a potential association between the type of varicocelectomy and successful sperm recovery, with a P value of 0.07. A significant association was observed between histopathological findings and successful sperm recovery, with a P value of 0.000.

Table 3 shows that younger age groups (30-35 and 36-40) had higher percentages of positive sperm recovery compared to the older age group (41-45). BMI in the range of 18-25 had higher percentages of positive sperm recovery compared to those in the higher BMI categories of 26-30 and 31-35.

Feature	Total	Sperm recovery		P value
		Positive	Negative	
Infertility Duration (years)				0.012
* 1-3 years	33 (47.1%)	18 (25.7%)	15 (21.4%)	
* 4-6 years	27 (38.6%)	9 (12.9%)	18 (25.7%)	
* 7-9 years	8 (11.4%)	0 (0.0%)	8 (11.4%)	
* 10+ years	2 (2.9%)	0 (0.0%)	2 (2.9%)	
Body Mass Index (BMI)				0.007
* Normal (18-25)	37 (52.9%)	20	17	
* Overweight (26-30)	25 (35.7%)	28.6%	24.3%	
* Obese (31-35)	8 (11.4%)	7	18	
Testicular Volume				0.013
* Right mean volume ±SD	NA	18.7±4.1	16.3±6.5	
* Left mean volume ±SD	NA	18.9±4.1	15.06±6.6	
Hormones				
* FSH (ng/mL)	NA	13.5±4.9	12.1±3.4	0.176
* LH (ng/mL)	NA	8.17±2.2	10.39±2.2	0.000
* Testosterone (ng/mL)	NA	3.85±2.10	3.14±1.5	0.112
Varicocele Grade				0.006
* Grade I	0 (0%)	NA		
* Grade II	30 (42.9%)	6 (8.6%)	24 (34.3%)	
* Grade III	40 (57.1%)	21 (30.0%)	19 (27.1%)	
Varicocelectomy				0.07
* Unilateral	44 (62.9%)	17 (24.3%)	44 (62.9%)	
* Bilateral	26 (37.1%)	10 (14.3%)	16 (22.9%)	
Histopathology of Negative TESE				0.000
* Sertoli-cell only	11 (15.7%)	0 (0.0%)	11 (100%)	
* Maturation arrest	14 (20%)	0 (0.0%)	14 (100%)	
* Hypospermatogenesis	12 (17.1%)	10 (27.0%)	2 (5.4%)	

Table 2: Characteristics and outcomes of 70 patients underwent varicocele repair surgery and associations between these factors and successful sperm recovery

Age group	Total	Follow up		p value
		positive	negative	
30-35	15	13	28	0.017
	21.4%	18.6%	40.0%	
36-40	12	28	40	
	17.1%	40.0%	57.1%	
41-45	0	2	2	
	0.0%	2.9%	2.9%	
Total	27	43	70	
	38.6%	61.4%	100.0%	
BMI	Total	positive	negative	0.007
18-25	20	17	37	
	28.6%	24.3%	52.9%	
26-30	7	18	25	
	10.0%	25.7%	35.7%	
31-35	0	8	8	
	0.0%	11.4%	11.4%	
Total	27	43	70	
	38.6%	61.4%	100.0%	

Table 3: Association between participants' follow up with age group and BMI of participants

4. Discussion

In this study seventy patients underwent varicocele ligation were included the rate of successful sperm recovery was 27(38.6%), Several studies on NOA have shown improvement benefits in semen parameters of 20–50% cases, Abdel-Meguid et al. [12] study found motile sperm recovery was evident in 10 of 31 (32.3%) patients, including 9 of 31 (29%) who demonstrated sperm recovery in the initial follow up semen analysis. and Shiraishi et al. [11] stated sperm recovery was confirmed in 20 patients (24%) also Pavan-Jukic et al. [13] TESE success rate of testicular sperm retrieval rate was 50.0%, while lower rate recorded by Spermatogenez et al. [14] sperm was detected in semen in 15.6% (5/32) of the patients and Thakur et al. [10] study which documented after varicocele repair of 104 patients, motile sperms in the ejaculate detected in 19 patients (18.26%). on other hand Matthews et al. [15] reported 54.5% (12 out of 22) patients had motile sperm in the ejaculate.

Testicular histology is considered to be a significant predictive factor of outcome [16], [17]. Patients with maturation arrest at the spermatocyte stage and germ cell aplasia had not shown improved semen quality; on the other side, azoospermic men with hypospermatogenesis or maturation arrest at the spermatid stage had shown improved semen quality post-operatively [18]. in this study TESE during varicocelectomy was done to 42 patients 4(5.7%) were positive, and 38(54.3%) were negative. Among patients with negative TESE, histopathology showed sertoli only cell in 11(29.7%), maturation arrest 14(37.8%), and hypospermatogensis in 12(32.4%). testicular biopsy of majority of patients who had achieved motile sperm post-varicocelectomy we found hypospermatogenesis. this is consistent with Thakur et al. [10] study that among the 19 patients who had achieved motile sperm post-varicocelectomy found hypospermatogenesis in 14 patients. also Shiraishi et al. [11]study stated the histological patterns of hypospermatogenesis late but not Sertoli cell only (SCO) and early MA, have been reported to be reliable for predicting the presence of sperm in the ejaculate. Therefore, later stage of spermatogenesis are necessary for men to benefit from varicocelectomy . On the other hand, an improvement in spermatogenesis was not observed in patients with SCO and MA, [18], [19]. So, this result again supports that the most important histopathological predictor of post-operative sperm in the ejaculate was hypospermatogenesis.

This study showed that testicular volume was normal among patients with successful sperm recovery (P value=0.013), moreover LH was found significantly reduced in patients with sperm recovery (P value =0.000), and no significant differences reported in FSH, and Testosterone. (P value =0.176) (P value =0.112) respectively, in line Shiraishi et al. [11] stated testicular size has been reported to be significantly larger in men with complete spermatogenesis than in men with SCO or MA. In addition, FSH level was a valuable predictor for the presence of sperm in the ejaculate after varicocelectomy , although no statistically significant difference was observed in the FSH level and testicular size [11], [20] in contrast Shiraishi et al. [11] and Aboutaleb [21] studies showed lower FSH levels were found in patients who had viable sperms in their ejaculates after the surgery and other hormonal parameters did not show a significance to be used as predictive factors in future studies. found no correlation between serum LH and TT levels and re-spermatogenesis after varicocelectomy.

In many studies examining the relationship between NOA and varicocelectomy, certain predictive factors have been thoroughly researched. In the present study, we found that patients age (P value =0.017) duration of infertility (P value =0.017). Grade of varicocele (P value=0.006) were significant predictors of successful sperm recovery after varicocele

repair. Different finding reported by Shiraishi et al. [11] that Patient age, varicocele grade, were not correlate with TESE-positive, in addition Pavan-Jukic, et al [13] stated there was no statistical difference in age, grde of varicocele and hormonal levels in the TESE-positive and negative groups.

This study was limited by the small sample size, short-term follow up and noncontrolled design. Randomized controlled trials to examine the recoverability of sperm in ejaculate following varicocele repair vs no treatment should provide better evidence regarding the predictors. However, it seems that randomized controlled trials examining relapse of azoospermia are impractical.

5. Conclusion

Our study showed there was statistically significant association between sperm recovery in NOA post varicocele ligation and certain situations such as younger age, non-obese males, shorter duration of infertility, higher Grade of varicocele, and hypospermatogensis in histological examination. In addition, normal Testicular volume and low LH play successful role in sperm recovery. .

6. Recommendations

Further prospective studies with randomization and large sample numbers are required in future. Relapse rate of and ability to achieve pregnancy should be addressed in further studies.

7. List of abbreviation

- ART Assisted Reproductive Techniques
- ICSI Intracytoplasmic Sperm Injection
- CI Confidence Interval
- CT Computed Tomography
- FSH Follicle-Stimulating Hormone
- LH Luteinizing Hormone
- OR Odds Ratio
- AOR Adjusted Odds Ration
- CFR Case Fatality Rate
- MA Maturation Arrest
- NOA Non-Obstructive Azoospermia
- OAT Oligoasthenoteratozoospermia
- PCNA Proliferating Cell Nuclear Antigen
- SCO Sertoli Cell Only
- SPSS Statistical Package For Social Science
- TESE Testicular Sperm Extraction

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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Questionnaire

Predictive factors of successful sperm recovery in azoospermic patient post varicocele ligation

Serial no.

1) Age: ..... years
2) Infertility duration...../years
3) BMI 18-25 ( ) 26-30 ( ) 31-35 ( )
4) Testicular Volume Rt testicle ..... Lt testicle .....
5) Hormonal test: FSH: ..... LH: ..... Testosterone: .....
6) Varicocele Grade I ( ) Grade II ( ) Grade III ( )
7) Varicocelectomy: Unilateral ( ) Bilateral ( )
8) If unilateral: Left ( ) Right ( ) Not applicable ( )
9) TESE Positive ( ) Negative ( )
10) If negative, the Testicular histopathology show Sertoli only cell ( ) Maturation arrest ( ) Hypospermatogensis ( )
11) Follow up after: 3 – 6 months (semen analysis) Positive ( ) Negative ( )