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Fertility Rate and Sperm DNA Fragmentation Index following Varicocelectomy in Primary Infertile Men with Clinical Varicocele: A Prospective Longitudinal Study

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Abstract

Background: Varicocele is one of the most common treatable causes of male infertility, and its treatment may be beneficial for fertility. This study aimed to evaluate fertility rate and DNA fragmentation index (DFI) following varicocelectomy in primary infertile men with clinical varicocele.

Materials and Methods: This prospective longitudinal study was conducted on primary infertility men, in a tertiary center from December 2018 to December 2019 with one-year follow-up. Data of the semen parameters, DFI (%), and fertility rate were gathered before, as well as 4 and 12 months after undergoing varicocelectomy. For data analysis, SPSS software and analytical test were used.

Results: Out of 76 patients who were analyzed, 22 (29%) became fertile and 54 (71%) remained infertile. Semen parameters and DFI (%) were improved significantly following varicocelectomy (P<0.001). Smoking history, occupational heated exposure, body mass index (BMI), and infertility duration were determined as predictors associated with fertility status (P<0.05).

Conclusion: Although varicocele repair improved the DFI, the fertility rate was achieved in less than one-third of patients; it seems that the other parameters, such as the history of smoking, occupational heated exposure, overweight, and duration of infertility should be considered as predictors of fertility status, in primary infertile men who are a candidate for varicocelectomy.

Keywords: DNA Fragmentation, Infertility, Semen Analysis, Varicocele, Varicocelecto

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Introduction

Infertility is defined as an incapability to conceive after one year of regular sexual intercourse without using contraceptive utilities (1). It has been estimated that more than 180 million people suffer from infertility worldwide, most of them (about 50%) due to the male factor (2). Although the cause of infertility in men remains unknown in about 50% of people on early assessment (3, 4), the previous studies demonstrated that sperm DNA fragmentation (SDF) has an important role in etiology of this abnormality (5, 6). On the other hand, male infertility is significantly associated with excess reactive oxygen species (ROS) in semen (5), and SDF is a type of ROS-related damage that is mostly observed in infertile men's sperm (7).

Varicocele is defined as an abnormal enlargement pampiniform plexus veins. It is associated in about 15% of all adult men, 35% of people with primary, and more than 80% of secondary infertilities (8). This disease is associated with infertility in men by several non-exclusive mechanisms, such as increased ROS production which can cause deoxyribonucleic acid damage to sperm DNA (9). Excessive amounts of ROS accompanied by antioxidant deficiency can cause oxidative stress (OS), which can lead to sperm nuclear and mitochondrial DNA damages (5). This mechanism causes point mutations, chromosome deletion, chromosomal rearrangements, and breaks in sperm DNA (10). Excessive sperm DNA break, which is called SDF, mostly appears in the semen

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of individuals with varicocele and other fertility problems (11). Previous studies also demonstrated a higher rate of SDF in the semen of patients with varicocele and varicocele correlation with ruined sperm DNA integrity. It was shown that varicocelectomy notably increased sperm DNA quality (12, 13). Another study demonstrated that varicocelectomy resulted in better sperm parameters, regardless of its techniques type (12). Sperm DNA fragmentation index (DFI) is a key parameter in fertility indicating both sperm DNA integrity and injury (14). Previous studies demonstrated DFI as an indication for the varicocele surgical treatment. Most of these studies focused on infertility without distinguishing between primary and secondary types of varicocele. So, this study aimed to evaluate fertility rate and DFI following varicocelectomy in primary infertile men with clinical varicocele.

Materials and Methods

Study design and participants

This prospective longitudinal study was conducted on primary infertility men in a tertiary center, from December 2018 to December 2019 with one-year follow-up. Primary infertile men with clinical varicocele and abnormal sperm parameters, in two semen samples with three weeks intervals undergoing varicocelectomy, were selected by consecutive sampling. The inclusion criteria were abnormal sperm parameters, any grade of clinical varicocele, younger than 35 years old, and lack of infertility factors in men's sexual partners. Exclusion criteria included serum high level of follicle-stimulating hormone (FSH; more than 12.3 mIU/ml) (15), testicular atrophy due to the other causes such as trauma or previous surgery, and secondary infertility. Additionally, patients with a history of epididymitis, cryptorchidism, orchitis, previous inguinal surgery, acute urinary tract infections, leukospermia, any disease or malignancy which may affect spermatogenesis, and history of underlying disease and medication affecting spermatogenesis were excluded.

Patient management

According to the routine protocol, before the surgery, all patients were examined by a single urologist who conducted the varicocelectomy (Inguinal technique by conventional approach). Varicocele grading was determined according to the WHO criteria and Sarteschi criteria by urologist examination and color Doppler ultrasonography (CDU) (16). The semen and SDF level tests were done, and semen parameters and percentage of DFI were determined. The patient partners were also visited by a gynecologist, and they had normal fertility potential. All data were recorded in patients' clinical documents. Only fertile men with normal fertility potential spouses were included in the study. Patients were monitored during the 4th and 12th months after surgery. During this time, their semen was tested, and their DFI (%) and their partner's pregnancy testing were checked and recorded in checklists.

Demographic data

Demographic characteristics, including patient's age, partner's age, duration of the marriage and duration of primary infertility, as well as histories of smoking and occupational exposure were recorded into checklists using clinical patients' documents.

Hormone analysis

Serum FSH levels were evaluated in the all patients by blood sample early morning (before 10 a.m), and those with high FSH levels (>12.3 mIU/ml) were excluded (15).

Semen analysis

The semen analysis test was conducted within two to five days of abstinence before the varicocelectomy and again within four to 12 months after the surgery. The test was performed to evaluate semen parameters of the patients, including sperm count, motility, morphology, and DFI. Standard semen analysis was conducted within one hour after sample collection and semen parameters including semen volume, density, sperm morphology, total motility and progressive motility were compared to the normal levels according to World Health Organization (WHO) 2010 classification. Next, they were recorded in the checklists. According to this protocol, semen parameters must be in 1.5 ml volume, 15 million concentrations, 58% vitality, 32% progressive, 40% total motility, and 4% normal morphology (17).

DNA fragmentation index measurements

A semen sample was taken for the SDF measurement, like semen analysis and it was analyzed by sperm chromatin structure assay (SCSA) method. Abnormal sperm percentage (abnormal chromatin structure) was expressed as DFI (%), indicating ratio of single-stranded (denatured) DNA to the total DNA (18).

Fertility outcome

The occurrence of pregnancy during the 12 months follow-up was assessed by taking a detailed history of the patients and monitoring onset of pregnancy in the patients with delayed menstruation. The onset of pregnancy was monitored by measuring the beta-human chorionic gonadotropin (beta-hCG) levels in blood.

Data analysis

The data were analyzed using version 24 of SPSS (IBM Corp., USA) software. The results for quantitative data are reported as mean ± standard deviation (SD) for data with normally distribution or median (IQR) or as frequency (%) for not normally and qualitative data. The Shipro-wilk, Skewness and Kurtosis tests were used to verify the normality distribution. Chi-square, independent samples t test, and Mann-Whitney test were used to compare the

variable between fertile and infertile patients. Analytical tests, such as repeated measure ANOVA, Friedman, and LSD post hoc tests, were used to compare DFI (%) and semen parameters at times before, four months, and one year after surgery. Univariate and multivariate regression tests were used to explore correlation between fertility outcomes and related factors. The significance level was considered 0.05, and the two-tailed assay examined the significance.

Ethical considerations

The research followed the tenets of the Declaration of Helsinki. This study was carried out under the authority of the Guilan University of Medical Sciences, Ethical Research Committee (IR.GUMS.REC.1398.486). Accordingly, written informed consent was taken from all participants before any intervention. Besides, the authors ultimately observed ethical issues (including plagiarism, data fabrication, and double publication).

Results

Of the 83 cases, seven patients (8.5%) were excluded due to not completing the follow-up duration, and 76 (91.5%) were included in the final analysis. Out of 76 patients who were analyzed, 22 (29%) became fertile and 54 (71%) remained infertile. Demographic and clinical characteristics of the patients by fertility status (fertile/infertile) are summarized in Table 1.

Table 1: Demographic and clinical characteristics of the studied patients with fertility status

Variable	Sub-variable (n)	Fertility status		P value
		Fertile	Infertile	
Smoking history	No (33)	16 (48.5)	17 (51.5)	0.001*
	Yes (43)	6 (14)	37 (86)	
Occupational heated exposure	No (40)	17 (42.5)	23 (57.5)	0.006*
	Yes (36)	5 (13.9)	31 (86.1)	
Varicocele grade	Grade 1 (6)	1 (16.7)	5 (83.3)	0.426*
	Grade 2 (37)	9 (24.3)	28 (75.7)	
	Grade 1 (33)	12 (36.4)	21 (63.6)	
Patient age (Y)		34.63 ± 5.23	33.9 ± 4.83	0.582**
Spouse age (Y)		30.95 ± 5.84	30.33 ± 4.88	0.641**
BMI (kg/m^2)		23.18 ± 1.75	25.12 ± 2.17	<0.001**
Infertility duration (Y)		2.43 ± 1.10	3.90 ± 2.49	0.003***

Data are presented as n (%) and mean ± SD. BMI; Body mass index, n; Frequency, SD; Standard deviation, *; Chi-square, **; Independent samples t test, and *; Mann-Whitney**

Assessment of the correlation between demographic and clinical characteristics with fertility status showed that there was no statistical significance between varicocele grade, patient's age, and spouse's age with fertility status, while regarding the smoking history, occupational exposure history, body mass index (BMI), and infertility duration was significant (Table 1). When we adjusted variables, the results showed that all of the studied variables in multivariate regression analyses, smoking history, occupational heated exposure history, BMI, and infertility duration were determined as predictors associated with fertility status. Infertility duration with an odds ratio of 2.3 per one year increasing age and BMI with an odds ratio of 1.62 kg/m^2 increased the risk of infertility. Occupational exposure with an odds ratio of 4.7 compared to the patients without occupational exposure, and smoking compared to non-smoking patients with an odds ratio of 4.41 fold increased risk of infertility (Table 2).

Table 2: Determination of predictors associated with fertility status by using multivariate regression models

Variable	OR	Standard error	P value	95% CI	
				Lower	Upper
Smoking history	4.41	0.719	0.038	1.086	18.160
Occupational heated exposure	4.70	0.740	0.036	1.103	20.052
Infertility duration	2.30	0.307	0.006	1.265	4.214
BMI	1.62	0.179	0.007	1.145	2.312

OR; Odds ratio, CI; Confidence interval, and BMI; Body mass index.

In this study, we used DFI (%) to determine sperm DFI, following varicocelectomy at different times, such as before, four, and 12 months (s) after varicocelectomy. Results demonstrated that the percentage of DFI during 12 months of follow-up was significantly reduced. After varicocelectomy, there was a statistically significant reduction in the DFI at 12 months compared to the 4 months after the procedure as well as before it. Moreover, the reduction of DFI at 4 months after compared to the before varicocelectomy was significant (Table 3).

As indicated in Figure 1, the percentage of DFI during the 12 months follow-up after varicocelectomy was reduced.

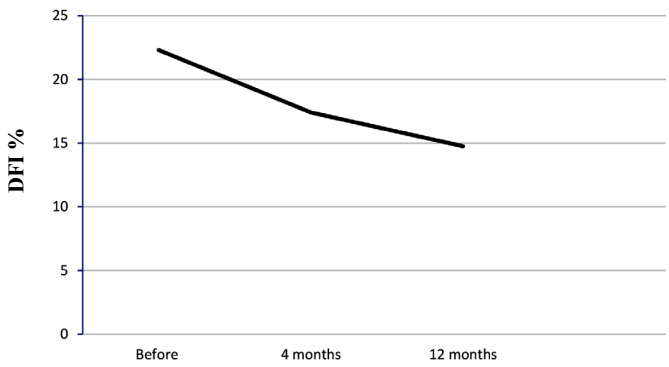

Fig.1: Comparison of DFI percentage during 12 months' follow-up after varicocelectomy. DFI; DNA fragmentation index.

The results of semen analysis, following the varicocelectomy, showed that the difference in normal morphology and total motility percentage during 12 months follow-up (before, four and 12 months after varicocelectomy) was not significant; while in terms of progressive motility percentage, sperm concentration and semen volume, it was significant. As time passed, progressive motility and sperm concentration were significantly increased. However, the semen volume was decreased after four months and then increased after 12 months (Table 4).

Table 3: Comparison of DFI (%), before, four and 12 months after varicocelectomy

Time	Before (mean ± SD)		4 months (mean ± SD)		12 months (mean ± SD)	P value
DFI (%)	22.31 ± 0.99		17.41 ± 0.79		14.76 ± 0.70	<0.001*
First time	Second time	Mean difference	Std. Error	P value	95% CI (lower)	95% CI (upper)
4 months	Before	- 4.91	0.831	<0.001**	- 6.565	- 3.256
12 months	Before	- 7.58	0.895	<0.001**	- 9.372	- 5.805
	4 months	- 2.67	0.609	<0.001**	- 3.891	- 1.464

DFI; DNA fragmentation index, CI; Confidence interval, *; Repeated measurement ANOVA, **; LSD post hoc test, and Std. Error; Standard error.

Table 4: Semen analysis, following varicocelectomy, during 12 months follow-up

Semen parameter	Before	4 months	12 months	P value
Semen volume (ml)	3.27 ± 1.48	2.88 ± 1.04	3.20 ± 1.35	0.025*
Sperm concentration (15×10^6 sperm/ml)	38.05 ± 22.42	45.82 ± 25.62	56.72 ± 27.28	<0.001**
Sperm morphology (%)	33.15 ± 34.87	38.97 ± 35.88	41.77 ± 33.94	0.096*
Total sperm motility (%)	53.48 ± 19.73	53.78 ± 20.79	55.95 ± 17.23	0.473*
Progressive sperm motility (%)	31.78 ± 14.54	39.29 ± 16.45	42.62 ± 17.42	<0.001*

Data are presents as mean ± SD. *; Friedman and **; Repeated measurement ANOVA.

Discussion

Varicocele is one of the known causes of infertility and the most common treatable disorder in infertile men (19). This treatable disease is found in about 40% and 80% of primary and secondary infertile men, respectively (20). Recent studies proposed association of elevated SDF levels with infertility in patients with varicocele (21). Additionally, the beneficial effect of varicocelectomy was found in patients with impaired sperm and clinical varicocele in the previous studies (20). In this study, during 12 months follow-up, fertility was achieved in 29% of 76 patients who underwent varicocelectomy. Previous studies reported different fertility rate, due to varicocelectomy. In a study performed by Sajadi et al. (22), 12.5% fertility rate was reported after varicocelectomy. Schlegel and Kaufmann (23), in a study on men with non-obstructive azoospermia, indicated that varicocelectomy resulted in 22% fertility. A rate of 31% fertility, following varicocelectomy, was achieved in a retrospective study on primary infertile men, by Ilktac et al. (24). Another study performed on primary infertile men by Mohamed al. (25) reported a fertility rate of 42.1%. It seems that, one of the most important mechanisms through which varicocelectomy can improve the fertility rate, is to increase perfusion of the testicular vessels and decrease temperature of the vessels, to remove the vessels that are the place of accumulation of venous blood with high temperature.

In this study, the fertility rate after varicocele treatment was achieved in less than one-third of the patients. Results showed that smoking, occupational exposure, BMI and infertility duration were the most predictors associated with infertility; however, the patient's age, spouses' age, and varicocele grade were not predictors. Consistent with our study Ren et al. observed a relationship between duration of infertility and incidence of pregnancy after varicocelectomy (26), and Bolat et al. (17) found no correlation between age and fertility rate. Studies also showed that smoking and presence of varicocele can cause more significant effect on semen analysis parameters and ROS via a synergistic effect (27). In contrast with our study, Choe and Seo (28) indicated that smoking was not associate with fertility status. Although in this study smoking had a negative effect on fertility, if duration of smoking and number of cigarettes smoked per day is evaluated, it may obtain more reliable results. On the other hand, low success rate in this study can be due to a shorter follow-up period, occupational exposure, high BMI or the surgical technique as with most of the previous studies.

Our results demonstrated that DFI during 12 months follow-up after varicocelectomy was significantly reduced. Furthermore, semen parameters were improved, following varicocelectomy, in some parameters, such as semen volume, progressive sperm motility and sperm concentration. This result was in line with the meta-analysis study performed by Birowo et al. (18), reporting that varicocele treatment reduced DFI, and this index can be an indication for varicocelectomy. In addition, they indicated that varicocele treatment can be beneficial in improving of sperm concentration, sperm progressive motility, and sperm morphology. Mohamed et al. (25) in their study found that semen parameters were improved after varicocele treatment in the both primary and secondary infertile men. Zampieri et al. (29) deduced that varicocelectomy improved rate of semen parameters. The other previous studies also showed similar outcomes, following varicocelectomy, on improving semen parameters (30). Alhathal et al. (31) demonstrated improvement of DFI and sperm progressive motility after varicocelectomy. Improvement of semen parameters and DFI, following varicocelectomy, in some other previous studies were approved (32-35).

The strengths of this study were the prospective, referral

center, and large sample size. Furthermore, a 12 months follow-up of semen parameters and SDF levels as well as a maximum follow-up of 12 months of pregnancy were performed, which were higher than similar studies. Precise registration of demographic information, history of smoking, BMI and occupational exposure history of the all participants were the other strengths of this study compared to the previous studies. However, there are some limitations in this study which should be noted. Firstly, Doppler Sonography was not performed after surgery to ensure the success of the procedure. Secondly, semen analysis was not performed in a common laboratory for the all patients, despite performing SDF assessment in a single laboratory. Thirdly, failure to use microscopic inguinal varicocelectomy as a gold standard surgical procedure for varicocele, due to limited equipment and facilities. Fourthly, lack of semen sample analysis with proper time in some patients. Finally, failure to study alcohol use, long-term use of mobile phones, as well as opium and drugs as factors affecting pregnancy and semen analysis parameters.

Conclusion

Although varicocele treatment improved DFI, the fertility rate was achieved in less than one-third of patients. It seems that the other parameters, such as the history of smoking, occupational exposure, overweight, and duration of infertility should be considered as predictors of fertility status, in primary infertile men who are a candidate for varicocelectomy.

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Authors' Contributions

A.H.M., Gh.M.; M.H.M., A.F.; Designed and directed the project, Data and statistical analysis, and Interpretation of data. E.K., N.R.H., M.P., R.R.; Contributed to sample preparation, Performed the experiments, and Wrote the paper. All authors read and approved the final manuscript.

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