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Research Article

The Effect of Varicocelectomy on Oxidative Stress and Sperm Dna Fragmentation in Infertile Males: Results from A Prospective and Controlled Study

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Abstract

Introduction: While male infertility is a cause of concern and treatment of varicocele is a clinical challenge, Oxidative Stress (OS) and related Sperm DNA Fragmentation (SDF) have been identified as significant causes of male infertility in men with varicocele. Although there is no direct evidence for improved pregnancy rates, varicocelectomy might reduce OS. Here, we aim to explore the role of varicocele repair as a means of reducing SDF and improving fertility.

Materials and Methods: Sixty infertile males (median age 35; IQR 27-45) with varicocele demonstrated by clinical and ultrasound examination were enrolled in this single centre study from November 2022 to January 2023. Forty patients were included in an intervention group and 20 in a control group of healthy young men. All patients in the intervention group underwent varicocelectomy. SDF levels were examined in semen from all patients before and 3 months after varicocele repair. The main outcome measures were reduction of SDF level after varicocele repair, improvement of sperm parameters, and pregnancy rate.

Results: The difference in SDF values in the intervention group before and after varicocele repair was 25% (p=0.01). Varicocele repair was associated with a higher pregnancy rate in the treatment group compared to controls (p=0.03).

Conclusions: Varicocelectomy reduced SDF levels in semen and increased pregnancy rate as compared with controls.

Introduction

Varicocele is found in approximately 15-20% of the general male population and is considered one of the major causes of male infertility, , with a frequency of about 40% in men with primary infertility and 80% in those with secondary infertility [1]. Varicocele appears as a dilatation of the pampiniform plexus that overlies and surrounds the testicle, and causes poor sperm production and alteration of seminal parameters [1-3]. However, the pathogenetic mechanisms through which varicocele induces disruption of spermatogenesis has not yet been fully clarified [4]. The suggested mechanisms are venous stasis with consequent testicular hypoxia, increasing testicular temperature and oxidative stress with the latter considered the central cause of infertility due to negative impact on the sperm quality varicocele [5]. Spermatozoa are extremely vulnerable to oxidative insult, which induces axonemal damage and increased midpiece sperm morphological defects, leading to reduced sperm motility. Furthermore, it has been demonstrated that varicocele alters the integrity of sperm DNA, which is considered essential for the successful process of fertilization and embryonic development in both spontaneous and assisted conceptions [6,7]. Although surgical correction of varicocele has been shown to reduces oxidative activity and restores fertility, the effect of varicocele surgery on reducing oxidative stress remains controversial [8-11]. The aim of our study was to extend the evidence base on the effect of surgical correction of varicocele on semen quality and sperm DNA fragmentation levels in adult males, as well as the pregnancy rate of infertile couples..

Materials and Methods

Study Design and Patients' Populations

This study included 60 male patients aged between 27 and 45 years. Forty presented with clinical diagnoses of varicocele and associated infertility in the urology department of an Italian teaching hospital. All underwent varicocelectomy as part of the treatment group. Twenty men, also with varicocele, but with no fertility problem served as controls and did not undergo surgery. The study was specifically designed to assess the causes of male infertility, and causes related to female partners were not included in the analysis. Varicocele was considered the primary cause of infertility in the 40 patients in the intervention group as all other causes were excluded. All underwent andrological examination revealing clinical signs of varicocele, confirmed by ultrasound and scrotal echo color-Doppler performed by two experienced urologists. The varicocele was classified into three grades as recommended by the European Association of Urology guidelines, All forty patients enrolled in the interventions group underwent varicocele surgery, while the remaining 20 only underwent control examinations after three months. Evaluation of seminal parameters was performed at the time point for the diagnosis of varicocele (first visit to the urology clinic), for all patients included in the study; three months after surgery for the 40 patients undergoing varicocelectomy; and three months after diagnosis for the 20 patients who did not undergo surgery (control group). The seminal parameters were correlated with the patient's age, the grade of varicocele, and study group (intervention vs control). Finally, the spontaneous pregnancy rate and the successful Medically Assisted Reproduction (MAR) rate were evaluated in the 1-year follow-up in both groups. Patients in the intervention group were seen by the urologist at 3 weeks pre-intervention and at 3 months after varicocelectomy.

Andrological Laboratory Examinations

All semen samples underwent comprehensive analysis of the seminal fluid (WHO 2010), and an assessment of Sperm DNA Fragmentation using the Halosperm test for Sperm Chromatin Dispersion (SCD method) expressed as % DFI (DNA Fragmentation Index) [12]. This analysis reports spermatozoa with abnormal chromatin structure. For patients in the intervention group samples were obtained after 4 days of sexual abstinence starting 3 weeks before varicocelectomy and standard semen analysis was performed within 1h of collection according to the WHO guidelines 2021. An abstinence period of 4 days was also applied before the follow-up visit for patients in the intervention group and in both visits for patients in the control group. Sperm parameters were considered normal when levels were equal to or exceeded the 5th percentile as accepted lower limit reference. Sperm DNA damage was measured using the Sperm Chromatin Structure Assay (SCSA). All seminal fluid examinations were carried out by the same specialist operator.

Surgical Procedure

All patients included in the intervention group underwent microsurgical inguinal varicocelectomy slightly modifyed from the technique described by Mirilas [13]. In brief, we perform an inguinal incision with opening of the external oblique aponeurosis in the direction of its fibers. The spermatic cord s grasped with a Babcock clamp, placed over a penrose drain and delivered through the incision. The operating microscope is then brought into the field. Veins are stripped free of associated lymphatics, doubly ligated with hemoclips or 4-0 silk ties and divided. Small veins are controlled with bipolar electrocautery. Lymphatics, cremasteric fibers, the vas deferens and associated vasal vessels are preserved. The external oblique aponeurosis is closed with a continuous absorbable suture. Scarpa's and Camper's fascia are similarly reapproximated with interrupted monofilament absorbable suture. The skin is then closed with a 5-0 monofilament absorbable running subcuticular suture.

Statistical Analysis and Ethical Considerations

The study was been planned as retrospective cohort study. Linear regression was used for identification of predictors that could affect the outcome of varicocelectomy. The Students' t-Test was used for continuous values. Statistical analysis was performed as follows: continuous variables were presented as median and IQR and categorical variables were presented as absolute (n) and relative (%) frequency distributions. T tests were used to compare average performance between the first and second visits. The threshold of statistical significance was set at $p<0.05$. All reported p-values are two-sided. All statistical analyses were performed using SPSS 23.0 (IBM Corporation, Armonk, NY, USA). All anamnestic, clinical and laboratory data containing sensitive information about patients were de-identified to ensure analysis of anonymous data only. The de-identification process was performed by non-medical staff by means of dedicated software. According to Italian law, this study did not require written informed consent from patients. The study was conducted in line with the Good Clinical Practice guidelines and the ethical principles laid down in the latest version of the Declaration of Helsinki.

Results

Baseline data

At the enrolment the median SDF levels were 23.0% (18.0-27.0) in the study group and 23.5% (19.0-26.0) in the control group. Table 1 shows all demographic, clinical and laboratory characteristics of enrolled patients (Table 1).

Characteristic	Overall N = 60	Intervention group N = 40	Control group N = 20	p-value
Age (years)	37.0 (34.0-40.5)	37.0 (33.5-42.0)	36.0 (34.5-38.0)	0.326
Sperm concentration (millions/mL)	47.5 (24.5-77.5)	49.0 (18.5-75.0)	47.5 (34.0-83.5)	0.51
Progressive motility (%)	20.0 (15.0-30.0)	20.0 (15.0-30.0)	22.5 (20.0-30.0)	0.404
Total motility (%)	50.0 (40.0-65.0)	45.0 (40.0-65.0)	55.0 (45.0-65.0)	0.258
Normal morphology (%)	12.0 (10.0-13.0)	12.0 (10.0-13.0)	12.0 (10.0-13.0)	0.632
SDF (%)	23.0 (18.0-26.5)	23.0 (18.0-27.0)	23.5 (19.0-26.0)	0.689

Figures in parentheses refer to range. P-values refer to comparisons between groups. The null hypothesis was that there was no difference between the groups.

Table 1: Demographic and laboratory data at enrollment of the entire study cohort and by treatment. SDF: Sperm DNA Fragmentation.

Semen Parameters at Follow-Up

Patients with mildly altered seminal parameters experienced greater benefits from varicocele correction surgery than patients with significantly altered parameters, as it led to the restoration of normal values (reverting to the 5th percentile according to WHO 2010) within three months post-surgery (data not shown). The procedure led to an improvement in total motility and even more in progressive motility, which went from an average of 10% to an average of 28%, while remaining slightly below the parameters at the 5th percentile of the WHO 2010 (Data not shown). Table 2 shows the values of all semen parameters at the follow-up evaluation compared with the enrolment in the two groups (Table 2).

Characteristic	Overall N = 60	Intervention group (Varicocelectomy) N = 40	Control group N = 20	p-value
Sperm concentration (millions/mL)	43.50 (26.00 - 75.00)	46.00 (27.00 - 75.00)	35.00 (23.50 - 72.50)	0.35
Progressive motility (%)	25.00 (15.00 - 30.00)	25.00 (20.00 - 30.00)	17.50 (15.00 - 25.00)	0.037
Total motility (%)	55.00 (45.00 - 65.00)	55.00 (50.00 - 70.00)	50.00 (35.00 - 57.50)	0.111
Normal morphology (%)	12.00 (10.00 - 13.00)	12.00 (10.00 - 13.00)	10.00 (8.50 - 12.50)	0.159
SDF (%)	18.00 (15.00 - 22.00)	15.00 (12.50 - 20.00)	22.00 (17.00 - 25.00)	0.004
Sperm concentration difference (millions/mL)	8.00 (-11.00 - 31.00)	13.50 (7.00 - 42.50)	12.50 (3.00 - 40.50)	<0.001
Progressive motility difference (%)	0.00 (0.00 - 5.00)	5.00 (0.00 - 5.00)	5.00 (0.00 - 5.00)	<0.001
Normal morphology difference (%)	0.00 (0.00 - 0.50)	0.00 (0.00 - 2.00)	0.00 (-1.50 - 0.00)	<0.001
SDF difference (%)	-6.00 (-8.00 - -2.00)	-7.00 (-8.50 - -6.00)	-1.00 (-2.00 - 0.00)	<0.001

Figures in parentheses refer to range. P-values refer to comparisons between groups. The null hypothesis was that there was no difference between the groups.

Table 2: Laboratory data at follow-up and difference between follow-up and baseline values of the entire study cohort and by treatment. SDF: Sperm DNA Fragmentation.

SDF Levels at Follow-Up

The Sperm DNA Fragmentation index significantly decreased for the majority of patients after undergoing surgery, decreasing from an average value of 25% to a post-operative average value of 16% (Figure 1). In the most severely affected patients (those with all seminal parameters altered), the surgery was only effective in reducing sperm DNA fragmentation (% DFI was reduced by 15 percentage points) (Figure 1). The patients who did not undergo surgery (control group) did not show significant changes in seminal parameters after three months and the DFI was also unchanged, passing from a value of 20% to 19% (Figure 1). Table 2 shows the SDF levels at the follow-up evaluation compared with the enrolment in the two groups.

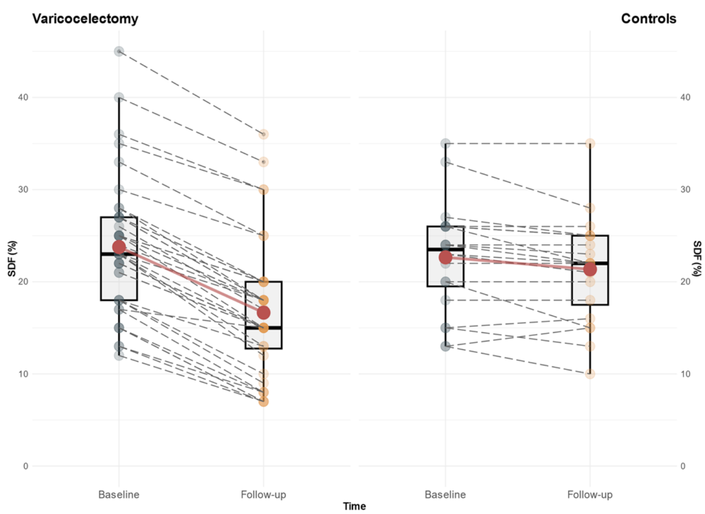

Figure 1: Levels of SDF at baseline and follow-up in patients undergoing varicocelectomy and in controls. The color intensity of points is proportional to the number of patients presenting the specific SDF value. Red points indicate mean levels. SDF: Sperm DNA Fragmentation.

Pregnancy Rate

After one-year follow-up the pregnancy rate in the intervention group was approximately 17%: Spontaneous" in 7.5%; after Medical Assisted Reproduction (MAR II level) in 6.5%; and after Intra Uterine Insemination in 2.5% of cases. In the control group, spontaneous pregnancies were observed only in 5% of patients during one-year follow-up (Table 3).

	β	SE	p-value
Controls (vs. Varicocelectomy)	5.89	0.52	<0.0001
Age	0.03	0.05	0.538
Baseline progressive motility < 30%	-0.84	0.87	0.332
Varicocele grade > II (or bilateral)	-0.14	0.56	0.808

SE: Standard Error

Table 3: Coefficients of the linear regression of the Sperm DNA Fragmentation difference by treatment, age, impaired baseline motility and varicocele grade

Discussion

In this study we extended the evidence base on the effect of surgical correction of varicocele on semen quality. As compared with controls, varicocelectomy reduced sperm DNA fragmentation levels in semen from adult males and increased pregnancy rates of infertile couples within one year follow up. After the surgical intervention, there was a significant improvement in seminal parameters, especially in patients with mild forms of varicocele as has been shown by others [13]. Oxidative stress plays a key role in varicocele-related pathophysiology, leading to sperm DNA damage and compromised function. Our findings underline that varicocelectomy is a therapeutic avenue to mitigate the negative effects of oxidative stress on sperm quality and fertility, and agrees with observations by other authors on the efficacy of varicocele correction in restoring natural fertility and improving outcomes in MAR [10,11,13]. Varicocele is a prevalent condition affecting approximately 15% of men, and is associated with detrimental effects on male reproductive health, including inflammation, oxidative stress, and compromised seminal parameters such as sperm count, motility, and morphology [14]. The link between varicocele and inflammation is well-established, with chronic dilation of scrotal veins contributing to an elevated local inflammatory response [15]. It rectifies venous reflux and improves blood flow, resulting in a significant reduction in inflammatory markers post-surgery [16]. Studies report a substantial decrease in cytokine levels, such as interleukin-6 (IL-6) and Tumor Necrosis Factor-Alpha (TNF-α) [16,17]. Varicocele surgery restores normal venous circulation and ameliorate hypoxic conditions, leading to decreased Reactive Oxygen Species (ROS) levels and improved antioxidant defence [18]. A significant reduction in Malondialdehyde (MDA) levels and an increase in antioxidant enzyme activities have been demonstrated [19]. Varicocelectomy improves these parameters, and in patients es with mildly reduced values . a return to normal has been demonstrated within a relatively short timeframe post-surgery. Significant improvements in total motile sperm count and morphology have been reported after varicocelectomy interventions [20]. In severe cases with compromised sperm DNA integrity, varicocelectomy demonstrates a notable impact, with a documented 7%reduction in sperm DNA fragmentation post-surgery [21]. These findings suggests that varicocelectomy can exert a resolutive effect on different degrees male infertility, by addressing the intricate interplay between, inflammation, oxidative stress, and seminal parameters. However , further research is warranted to assess the long-term effects of varicocelectomy and refine patient selection criteria and the importance of personalized approaches in this complex reproductive disorder. Highly relevant data arse from the significant reduction of sperm DNA fragmentation which occurred after varicocelectomy also in patients with severely altered semen parameters [22-25]. A remarkable effect of reducing sperm DNA damage and improving the fertilizing capacity of the sperm has been shown only in natural fertilization but also in MAR. This situation where the quality of the sperm is crucial and depends fundamentally on the integrity of its DNA, deserves closer scrutiny in future studies [10,11]. The present study shows several limitations with the retrospective feature and the small number of enrolled patients being the most important. However, the accurate analysis of SDF in all enrolled patients and the use of pregnancy rate as an outcome, should be considered as strengths of the study.

Conclusions

Our study confirmed the effect of varicocelectomy on reducing SDF, improving semen parameters and increasing and pregnancy rate.. Integrating varicocelectomy into infertility treatment protocols will impact male reproductive health. Through a tailored approach the procedure can be recommended both for patients struggling with oxidative stress-related infertility, and as a preventive measure for men at risk of infertility due to varicocele. Our findings highlight the potential of varicocelectomy not only to alleviate oxidative stress but also to restore sperm integrity, thereby facilitating natural conception and improving outcomes in assisted reproductive technologies. Future research should focus on long-term benefits and optimizing patient selection for this surgical intervention in the era of increased negative effects of the environment on male fertility [26-28].

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