Varicocelectomy versus antioxidants in infertile men with isolated teratozoospermia: A retrospective analysis

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Cite this article as: Fathi A, Castiglione F, Mohamed O, Alsagheer GA, Mahmoud O, Saber-Khalaf M. Varicocelectomy versus antioxidants in infertile men with isolated teratozoospermia: A retrospective analysis. Turk J U. 2021; 47(4): 279-284.

ABSTRACT

Objective: To compare the outcome of microscopic subinguinal varicocelectomy versus oral antioxidants for the management of male infertility due to isolated teratozoospermia.

Material and methods: Clinical patients' database of a tertiary infertility referral center was retrospectively reviewed to collect data on male infertility due to isolated teratozoospermia from July 2014 to January 2019. Seventy-two patients who underwent microscopic subinguinal varicocelectomy for their clinically palpable varicoceles were considered as a study group, while 60 patients who were treated with antioxidants (L-carnitine, vitamin C, and vitamin E) were included as controls. The improvement in the sperm morphology, sperm DNA fragmentation (SDF), and the achievement of natural pregnancy were compared between the two groups.

Results: In the varicocelectomy group, all sperm parameters and SDF statistically improved 6 months after the operation (*P*-value <.001). In the control group, the percentage of sperm morphology and SDF statistically improved 6 months after the treatment (*P*-value <.05). The improvement in sperm parameters, SDF, and the pregnancy rate was statistically higher in the varicocelectomy group compared to the control group. Within the range of follow-up (12-18 months), 30.5% of patients' partners achieved natural pregnancy in the varicocelectomy groups compared to 16.7% in the control group.

Conclusion: We believe that varicocelectomy has a positive effect on the sperm morphology, SDF, and pregnancy rate in male infertility caused by isolated teratozoospermia.

Keywords: Antioxidants; infertility; male; teratozoospermia; varicocele.

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Submitted: 11.01.2021

Accepted: 28.04.2021

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Available online at www.turkishjournalofurology.com

Introduction

Varicoceles represent one of the main causes of male infertility. The prevalence of varicoceles is estimated to be 15% in the normal population, 45% of primary male infertility patients, and up to 80% among males with secondary infertility.[1] Varicoceles usually cause concomitant defects in sperm motility, count, and morphology; however, isolated defects can occur.[2] Recent studies showed that varicocelectomy leads to an improvement in sperm motility and count.[3,4] According to the recent EAU guidelines, varicoceles repair should be recommended in cases of clinically palpable varicoceles, abnormal semen parameters, and normal or potentially correctable female infertility.[5]

Teratozoospermia is defined as the percentage of spermatozoa with normal form under the lower reference limit, which is 4%.[6] Recent studies have documented that oxidative stress is associated with sperm DNA fragmentation and sperm chromatin condensations, which are characteristics of men with isolated teratozoospermia. Oxidative stress is due to an imbalance between reactive oxygen species (ROS) overproduction and antioxidant scavenging activities in semen. Other studies suggest an association between teratozoospermia and apoptosis.[7,8] The negative effects of

Turk J Urol 2021; 47(4): 279–284
DOI: 10.5152/tju.2021.21013

oxidative stress on sperm parameters, fertilization rate, and pregnancy rate introduced the use of antioxidants in male subfertility.[9]

Isolated teratozoospermia has detrimental effects on natural pregnancy and the success rate of intrauterine insemination.[10] However, some studies have shown that men with abnormal morphology can have a natural pregnancy.[11,12] Few studies in the literature have evaluated the effect of varicocelectomy on the improvement of isolated teratozoospermia; some reports have shown no improvement while others have documented improvement of the sperm morphology.[13–17]

To date, none of the above-mentioned studies evaluated the effect of varicocelectomy on sperm DNA fragmentation (SDF) in patients with isolated teratozoospermia. Our study aims to assess the effect of microsurgical varicocelectomy compared to oral antioxidants on the improvement of sperm morphology, the percentage SDF, and the pregnancy rate in patients with isolated teratozoospermia.

Material and Methods

Study Design

The medical records of patients with isolated teratozoospermia who have been treated at our tertiary Andrology Center between July 2014 and January 2019 were retrospectively reviewed. Inclusion criteria were as follows: at least a 1-year history of male factor subfertility and a clinically palpable varicocele associated with isolated teratozoospermia according to the 2010 WHO criteria.[18] Patients with subclinical varicoceles, associated female factor fertility, recurrent varicoceles, cryptorchidism, genital infection, pyospermia, obesity, alcohol or drug abuse, patients exposed to gonadotoxins, or patients with cancer were excluded from the analysis.

The age of patients and partners, smoking history, duration and type of infertility, basic sperm parameters, and SDF were collected. A detailed urogenital examination was performed. Varicoceles examination was done in a sitting and standing position. The study was approved by the local Institutional Review Board of South Valley University (REC 50/3/19) and conforms to the provisions of the Declaration of Helsinki.

Main Points

- Oxidative stress and SDF are associated with decreasing the percentage of normal sperm forms.
- Varicocelectomy and antioxidants improved the percentage of normal forms, SDF, and pregnancy rate.
- The improvement after varicocelectomy was higher than oral antioxidants.

Semen Analysis and Sperm DNA Fragmentation Test

Two semen samples were provided by the patients after 3-5 days of sexual abstinence, and a standard semen analysis was performed within 1-hour after collection according to the WHO 2010 guidelines. Another two semen samples were obtained 6 months after varicocelectomy or medical treatment. Semen analysis with better results was taken into consideration for the baseline and the follow-up. Part of the sample was used for the measurement of SDF using the sperm chromatin dispersion, which is known as the Halo test.[19] The Halo test is based on the principle that sperms with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops, which is observed in sperm with nonfragmented DNA, following acid denaturation and removal of nuclear proteins. This was confirmed by the analysis of DNA fragmentation using the specific DNA Breakage Detection-Fluorescence In Situ Hybridization. The percentage of spermatozoa with abnormal chromatin structure is represented by the SDF, which is the ratio of single-stranded (denatured) DNA to the total DNA. SDF above 25% was considered abnormal.[20] The semen samples were all analyzed by the same embryologists.

Varicocelectomy

Microsurgical subinguinal varicocelectomy was successfully performed under spinal anesthesia. All procedures were performed using a Karl Zeiss operating microscope with a magnification from 10× to 20×, using the standard surgical technique.[21]

A total of 72 patients had a microscopic subinguinal varicocelectomy after obtaining a written informed consent. The control group included 60 patients who refused surgery and were prescribed antioxidants (L-carnitine 1 g, two tablets per day and vitamin C 1 g/day and vitamin E 400 mg/day in a single tablet) for 6 months.

The study outcome measures were:

A. Change in the percentage of normal forms and SDF which was measured before and at the 6th month of follow-up for the two groups.
B. The percentage of improvement in sperm morphology (>4% normal forms) for the two groups.
C. Spontaneous pregnancy rates at 6th and 12th months.

Statistical Analysis

The statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 26 (IBM SPSS

Corp.; Armonk, NY, USA). Frequency tables with percentages were used for categorical variables and descriptive statistics. Median (range) was used for numerical variables. The Mann–Whitney U test was used for the analysis of the changes in the sperm parameters before and after the intervention, while the Wilcoxon t-test was used to compare the changes in sperm parameters and the pregnancy rate across the two groups. A Chi-square test was used to compare the nominal data between the two groups. A *P*-value of less than .05 was considered statistically significant.

Results

This study included 132 male patients with a 1-year history of male subfertility and isolated teratozoospermia. Seventy-two patients underwent microsurgical subinguinal varicocelectomy (study group), while 60 patients were on the medical treatment (control group). All patients completed the 1-year minimum follow-up period. The median (range) age of the patients and their wives in the study group was 33 (24-41) and 24 (19-34) years, respectively, and in the control group, it was 34.5 (24-43) and 26 (19-34) years, respectively. There were no statistically significant differences between the two groups in terms of their smoking history, infertility duration, varicoceles laterality, and type of infertility (Table 1). Also, sperm count, percentage of progressive motility, percentage of normal forms, and the SDF% were statistically comparable between the two groups (Table 2). The majority of the patients presented with primary infertility which was $\geq$85% in both groups.

All sperm parameters improved after 6 months of the varicocelectomy or medical treatment. Also, the percentage of SDF statistically decreased in both groups (*P* values $<$.001 and $<$.05, respectively). On the other hand, the difference between the preoperative and the post-operative sperm concentration and motility was statistically insignificant. The improvement in sperm parameters and SDF was statistically higher in the varicocelectomy group compared to the control group (Table 2). When the percentage of normal morphology was evaluated in detail, 50 (69.4%) patients had an increase in the percentage of normal morphology, and 35 (48.6%) patients became normal morphology after the varicocele repair. On the other hand, 25 (41.6%) patients had an increase in the percentage of normal morphology, and 15 (25%) became normal sperm morphology in the medical treatment group (*P*-value = .008).

Within the range of follow-up (12-18 months), 22 (30.5%) patients' partners achieved natural pregnancy in the varicocelectomy groups compared to 10 (16.7%) in the control group (*P*-value = .009). Apart from the post-operative pain and scrotal swelling that resolve spontaneously, no post-operative complications were reported after varicoceles ligations. All patients were discharged the same day of surgery.

Discussion

This study demonstrated the superior positive effect of microsurgical subinguinal varicocelectomy on the improvement of the percentage of sperm parameters, SDF, and the natural pregnancy compared to the medical treatment in subfertile men with clinically palpable varicoceles and isolated teratozoospermia.

Isolated teratozoospermia is rarely encountered in patients with clinically palpable varicocele. Sperm morphology is an

Table 1. Patients Demographic and Clinical Data

Variable	Control group (60 patients)	Varicocelectomy group (72 patients)	*P*
Age, median (range) year	34.5 (24-43)	33 (24-41)	.06
Wife age, median (range) year	26 (19-34)	24 (19-34)	.16
Infertility duration (months), mean (SD)	25.5 (15-37)	26 (17-39)	.86
Smoking, n (%)			.73
Yes	30 (50%)	33 (45.8)	
No	30 (50%)	39 (54.2)	
Varicocele, n (%)	15 (25)	16 (22.2)	.84
Unilateral	45 (75)	56 (77.8)	
Bilateral			
Infertility, n (%)			.89
Primary	42 (86.7)	63 (87.5)	
Secondary	8 (13.3)	9 (12.5)	

Turk J Urol 2021; 47(4): 279-284
DOI: 10.5152/tju.2021.21013

important parameter of the fertilizing ability of the sperm. A recent study suggested that men with isolated teratozoospermia showed a significantly increased level of single sperm DNA breaks, oxidative stress, and sperm apoptosis when compared to controls.[8] Conflicting results exist about the effect of teratozoospermia on the pregnancy rate. Spiessens et al.[10] indicated that isolated teratozoospermia decreases not only the rate of spontaneous pregnancy but also the success rate of intrauterine insemination and in vitro fertilization (IVF).[10] Thus, more sophisticated techniques like intracytoplasmic morphology-selected sperm injection have been developed to chose sperms with better morphology to magnify the success rate of IVF and/or intracytoplasmic sperm injection (ICSI), with added cost and effort.[22] In contrast, Kovac et al.[12] found that 25% of his patients achieved a natural pregnancy with 0% normal morphology with a median follow-up of 2.5 years.

Few studies evaluated the effect of varicocelectomy on sperm morphology. In 1999, Vazquez et al.[13] documented a significant improvement in the sperm morphology after varicocelectomy using Krugar morphology. However, the number of patients was 33 with mixed oligozoospermia, asthenozoospermia, and teratozoospermia.[13] Okeke et al.[14] reviewed the records of 167 subfertile men, of whom 58 patients had isolated teratozoospermia. All sperm parameters statistically improved after varicocelectomy except sperm morphology. In 2008, Cakan et al.[15] compared 29 patients with isolated teratozoospermia who had varicocelectomy to 23 patients with no treatment. They demonstrated that varicocelectomy statistically improved the sperm morphology compared to the control group who did not receive any treatment. At 1-year, 17.5% of their patients had a normal pregnancy.[15] In our study, 30.5% achieved normal pregnancy after microsurgical varicocelectomy at 1-year. All these studies were based on the 1999 WHO classification of semen analysis, using Kruger's strict criteria, and teratozoospermia was defined as normal sperm morphology of less than 14%. In our study, we used a cutoff value of 4%.

In 2013, Cakiroglu studied 49 patients with isolated teratozoospermia who had varicocelectomy. Teratozoospermia was seen in 40 patients after the operation, and this indicated non-statistically significant improvement in the sperm morphology after varicocelectomy.[16] Choe and Seo[17] evaluated 80 patients with isolated teratozoospermia who underwent varicocelectomy.

Table 2. Laboratory Data and the Pregnancy Rate at Baseline and after Treatment in the Two Studied Groups

	Control group (n = 60)	Varicocelectomy group (n = 72)	*P*-value**
Sperm concentration, median (range) million			
Baseline	25 (19-45)	24 (18-40)	.11
After treatment	26 (20-47)	28 (20-46)	.04
P-value*	.21	<.001	
% of progressive motility, median (range)			
Baseline	36 (34-40)	36.5 (34-41)	.34
After treatment	37.5 (35-41)	40 (37-48)	<.001
P-value*	.05	<.001	
% of normal forms, median (range)			
Baseline	0.9 (0-3)	1 (0-3)	.46
After treatment	1.5 (0-5)	3.5 (0-9)	<.001
P-value*	.04	<.001	
% of SDF, median (range)			
Baseline	33 (27-55)	32 (26-51)	.38
After treatment	29 (24-48)	25 (15-36)	<.001
P-value*	.03	<.001	
Patients with normal forms, n (%)	15 (25)	35 (48.6)	.008
Pregnancy at 6 months, n (%)	0 (0)	6 (8.3)	.03
Pregnancy at 12 months, n (%)	10 (16.7)	22 (30.5)	.009

*Change after the treatment compared to the baseline reading.

**Difference between the two groups.

Although there was a statistically significant improvement in sperm morphology after varicocelectomy, only 20% had normal sperm morphology after varicocelectomy. The natural pregnancy rate was not studied.[17] In a recent study that included 62 patients with isolated teratozoospermia, sperm morphology was statistically significantly improved after varicocelectomy. The sperms with normal sperm morphology and lower head abnormalities were statistically significantly higher in men who had children through natural pregnancy compared to men who had not.[23] In our study, the improvement in sperm parameters and SDF was statistically significant in the varicocelectomy group 6 months after the operation and was statistically higher compared to the control group.

There are some findings associated with varicoceles-induced male infertility. First, SDF is significantly higher in patients with varicoceles, and varicoceles repair has been associated with a reduction in the percentage of SDF.[24] Also, oxidative stress and ROS are important factors associated with varicoceles and male infertility. Varicoceles repair reduces oxidative stress and increases seminal antioxidants.[25] Antioxidants are naturally occurring or synthetic biomolecules that prevent free radical-induced damage by averting the formation of radicals, scavenging them, or promoting their decomposition in the body. Thus, they decrease the deleterious effects of the highly reactive radicals by converting ROS into different, less harmful molecules.[26] The European Academy of Andrology guidelines does not recommend for or against the use of antioxidants in oligoasthenoteratospermia.[27] Moslemi and Tavanbakhsh[28] proved that daily administration of vitamin E and selenium produced improvement in all sperm parameters in 52% of the 690 male infertile patients. Another randomized controlled trial done on 56 infertile men has shown a significant relationship between the administration of L-carnitine and the improvement of all sperm parameters especially sperm motility.[29] In a recent meta-analysis of the use of antioxidants in male infertility, vitamin E, selenium, zinc, and carnitine were suggested for the treatment of teratozoospermia.[30] In our study, both varicocelectomy and oral antioxidants reduced SDF and improved sperm morphology. However, the improvement was statistically higher in the varicocelectomy group.

Up to our knowledge, this is the first study that investigated the reduction in SDF after varicocelectomy and antioxidants in patients with isolated teratozoospermia. We acknowledge several limitations to our study. First, it is a retrospective study. Second, it has a small sample size. Larger sample size may statistically reveal the higher beneficial effect of varicocelectomy on the improvement of sperm morphology, SDF, and natural pregnancy compared to the medical antioxidants.

In conclusion, we believe that varicocelectomy is more effective in achieving natural pregnancy when compared to antioxidants. Varicocelectomy should be offered as a treatment option for infertile men with isolated teratozoospermia and clinically palpable varicoceles. Randomized control trials with a larger sample size and longer follow-up are recommended to prove or disprove these results.

Ethics Committee Approval: The study was approved by the local Institutional Review Board of South Valley University (REC 50/3/19) and conforms to the provisions of the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.S.K., O.M., A.F.; Design - M.S.K., O.M., O.M., G.A., F.C., A.F.; Supervision - M.S.K., O.M., A.F.; Materials - M.S.K.; Analysis and/or Interpretation - M.S.K., O.M., O.M., A.F.; Literature Search - M.S.K., O.M., G.A., F.C.; Writing Manuscript - M.S.K., O.M., G.A., F.C.; Critical Review - M.S.K., O.M., O.M., G.A., F.C., A.F.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Acknowledgments: Fabio Castiglione is supported by the Margaret Spittle Research Fellowship, University College of London Hospital, London, UK.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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