Original Article

The Correlation between Varicocele, Sperm Parameters and Embryo Quality of ICSI Outcome in Infertile Men

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Abstract

Background & Aims: Varicocele is among the most common identifiable abnormality found in men evaluated for infertility. Despite the long history associated with varicoceles, there remains much controversy regarding their diagnosis and management. The aim of this study was to determine association between varicocele, sperm parameters, and embryo quality of ICSI outcome in infertile men.

***Materials & Methods*:** Fifty individuals with varicocele for Intracytoplasmic sperm injection (ICSI) treatment were included in this study. The control group included healthy men without varicoceles (n=50). Semen samples were analyzed according to the World Health Organization (WHO) criteria. Hormonal analysis of serum LH, FSH, and testosterone were measured. Sperm DNA fragmentation was assessed by SCD (Halo sperm), and then ICSI fertilization rate, percentage of high- quality embryos and chemical pregnancy rate were measured.

***Results*:** In semen analyses, the total sperm count, total motile sperm and normal sperm morphology were significantly lower in patients with varicocele. Also, the DNA fragmentation was significantly higher in patients with varicocele than patients in the control group ($p<0.001$). The level of reproductive hormones was different in varicocele and control groups ($p<0.05$). Fertilization rate and embryo quality were significantly lower in individuals with varicocele, when compared to men without varicocele ($p<0.05$). The significant negative correlation between DNA fragmentation, fertilization rate, and embryo quality were observed ($p<0.05$).

***Conclusion*:** The effect of varicocele on male infertility may be attributed to a decrease in sperm quality as well as an increase in DNA fragmentation, which leads to reduced embryo quality in patients with varicocele.

Keywords: Varicocele, DNA fragmentation, Fertilization, Embryo quality

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Introduction

Varicocele, an important factor in the male fertility, can impair sperm quality and fertility(1). Varicocele is the abnormal dilatation of the spermatic veins (2). It is commonly seen in nearly 40% of male population, affecting 15% of individuals at reproductive age, 35% of those with primary infertility, and up to 80% of men with secondary infertility (3-5).

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An increase in testicular temperature, scrotal hyperthermia, altered testicular blood flow, testicular hypo perfusion, hypoxia, and testicular hormonal dysfunction may result in varicocele-related testicular dysfunction (6). Recent studies have shown that infertile men with varicocele have abnormal semen parameters (count, motility, and morphology) (7). The correlation between varicocele and dysfunction of spermatogenesis has been well described (8). Perhaps one of the leading causes is high follicle - stimulating hormone (FSH) and low Serum testosterone among infertile men with varicoceles (9, 10).

Varicocele may affect the final stages of spermatogenesis and lead to changes at sperm condensation. During the final stages of spermatogenesis, histones are replaced by cysteine-rich protamines(11). According to one theory, increased thermal caused

damage to the DNA and proteins in the nucleus of spermatic tubules' cells and / or Leydig cells (12). Increased testicular temperature as oxidative stress factor is known to have adverse effects on sperm structure and function, such as DNA fragmentation (13). Sperm DNA integrity has been recognized as one of the important determinants of normal fertilization ,embryo development, implantation, and pregnancy rate (14, 15).

ICSI procedure is used to treat severe male factor infertility and to modify their diagnostic and therapeutic approach(16). Also, varicocelectomy have an essential role in the treatment of infertile patients with clinical varicocele and can significantly improve sperm quality and quantity for ART (17, 18). Therefore, we have evaluated the impact of varicoceles on semen quality, reproductive hormones, ICSI outcome and described the associations between DNA fragmentation, fertilization rate, and embryo quality in men with varicocele.

Method and Material

Study design and patients:

The ethics committee of Qom University approved the study (IR.QOM.REC.1399.011), and written consent was obtained from the participants. 50 patients who were diagnosed with varicoceles and were referred for infertility treatment at the fertility and infertility of Academic Center for Education, Culture and Research (ACECR), Qom, Iran from October 2018 to December 2019 participated in this study. 50 healthy men without varicoceles were included in the study. Inclusion criteria included male gender, age younger than 40 years, primary infertility, and left-sided varicocele (grades II and III) diagnosed by palpation and Doppler duplex ultrasound. We then extracted physical exam and demographic history from both groups. Semen samples were analyzed according to the World Health Organization (WHO) criteria. Hormonal analysis of serum LH, FSH, and testosterone were measured. Sperm DFI was assessed by Sperm chromatin disruption (SCD) (Halo sperm).

Sperm collection and semen analysis:

Semen samples were collected by masturbation after 2–5 days of sexual abstinence. After semen liquefaction, sperm parameters analysis was performed according to World Health Organization (19) guidelines, and strict criteria evaluated sperm Morphology after Diff-Quick staining.

Determination of hormonal analysis:

The hormonal levels in serum of FSH (Human Follicle Stimulating Hormone ELISA Kit (ab108678)(U/ml), LH (Human Follicle Stimulating Hormone (ab108678)(IU/L), and testosterone (Testosterone ELISA Kit (ab108666)(ng/ml) were measured using ELISA (Biotek -ELx 800-enzyme-linked immunosorbent assay).

Determination of DNA fragmentation (SCD):

For examination of sperm DNA fragmentation, Sperm chromatin dispersion (SCD) test (the Halo sperm

kit, INDAS laboratories, Spain) was used. For each sample, 200 sperms were evaluated under the ×1000 objective of an optical microscope. In this method, the normal sperm (without fragmented DNA) produce halos (large or medium halos) and abnormal sperm (with fragmented DNA) produce either small halos or no halos (20).

ICSI technique and embryo culture:

Oocytes that had the first polar body (MÌÌ oocytes) were used for

Intracytoplasmic sperm injection (ICSI). Four hours after oocyte retrieval, a single sperm with normal morphology was injected to inseminate oocyte. At 16–18 hours after ICSI, fertilization was confirmed by the presence of two pronuclei (2PN) under an Olympus inverted microscope (IX71) with a Hoffmann modulation contrast system at × 400 magnification.

Three-days post- ICSI, embryo quality was assessed based on a three-point scoring system (21): I) symmetric blastomeres and no fragmentation II) unequal of blastomere's size and shape and <30 %fragmentation and III) unequal of blastomere's size and shape and >30 %fragmentation. Chemical pregnancy was defined as serum βhCG ≥20 IU/L (VIDAS kit) measured 14 days after embryo transfer.

Statistical Analysis:

Statistical analysis was performed using SPSS version 20.0 (Chicago, IL, USA). The difference between two independent groups was analyzed using an independent Student's t - test and mean difference (MD). Significance level was set at $p<0.05$

Results

Clinical and demographic characteristics:

The study population consisted of 50 individuals with grades II and III varicocele. The mean ages of male participants were 30.1 ± 4.4 (range: 22-45) years. There was no statistically significant difference in weight, and Body Mass Index (BMI) between two groups ($p>0.05$) (Table 1).

Table 1: Comparison of the Clinical and demographic characteristics in the varicocele and control groups. ($p>0.05$).

Clinical and demographic characteristics	Control (n=50)	Varicocele (n=50)	P-value
Age, years	31 (22-35)	32 (22-38)	P= 0.07
Weight, kg	73.0 (59.4–95.0)	73.1 (59.0–93.8)	P=0.11
BMI, kg/m2	22.3 (18.7–28.4)	21.4 (18.2–25.8)	P= 0.8
Cigarettes daily	0 (0–20)	0 (0–15)	P= 0.2
Alcohol consumption	0(0–35)	0(0–38)	P=0.10

Data are shown as mean ± SE. No difference was observed between the mean of variables in the varicocele group compared with control group. BMI: Body Mass Index.

Effects of varicocele on seminal parameters:

Semen analysis revealed that the sperm concentration, total sperm count, total motile sperm were significantly lower in varicocele group than the control group ($p<0.001$). According to Diff-Quick staining, sperm with normal morphology were significantly higher in the control group, whereas sperm head anomalies were significantly higher in the varicocele group ($P<0.001$). When the samples were evaluated with SCD stanning, the percentage of DNA fragmentation was 33±2.12 and15.1±1.0 for the varicocele and control groups, respectively ($p<0.001$). There was no statistically significant difference in the volume of semen between the groups ($p>0.05$) (Table 2).

Table 2: Comparison of the conventional semen parameters and DNA fragmentation analyses between two groups ($p<0.05$).

Sperm Parameters	Control (n=50)	Varicocele (n=50)	P-value
Volume (ml)	2.6±0.3	2.01±0.1	0.18
Concentration (million/m)	50.5±1.23	28.6±2.13	0.0001
Total sperm count (million/m)	153±12.05	88.4±9.23	0.002
Normal morphology (%)	7.1±0.13	3.5±0.5	0.001
Total Motility (%)	63.23±2.01	39.12±3.2	0.002
DFI (Halo sperm) (%)	15.1±1.03	33±2.12	0.001

Data are shown as mean ± SE. Significant differences for the comparison between two groups are bold type. DFI; DNA Fragmentation Index.

Effects of varicocele on fertilization outcome:

The embryo quality was assessed based on a three-point scoring system. I) Cells are of equal size; no fragmentation, II) Cells are of equal size; minor fragmentation only, and III). Cells are of equal or unequal size; fragmentation is moderate to heavy.

As indicated in Table 3, there was a statistically significant difference between the two groups in this study with regard to the overall fertilization rate and embryo quality ($p<0.05$). Embryo quality includes the number of embryos Grade I ($p<0.05$), number of embryos Grade II ($p<0.05$), number of embryos Grade III ($p<0.05$). There were no statistically significant differences in the clinical pregnancy in varicocele groups compared to control groups ($p>0.05$).

Table 3: clinical outcome – comparison between control and varicocele groups ($p<0.05$)

Parameters	Control (n=50)	Varicocele (n=50)	P-value
FR (%)	56.9±3.5	34.7±2.2	0.03
No. of embryos Grade I	3.33±0.9	1.53±0.83	0.02
No. of embryos Grade II	3.4±1.2	1.87±0.9	0.02
No. of embryos Grade III	3.8±1.1	1.73±0.8	0.01
No. of clinical pregnancy (%)	33%	28%	0.07

Data are shown as mean ± SE. Significant differences for the comparison between two groups are bold type. FR; Fertilization rate.

Effect of varicocele on the level of basal reproductive:

The results of this study showed that testosterone level was significantly lower in the varicocele group than the control group ($p<0.05$). As well as in this study was showed statistically significantly higher at the levels of FSH, LH hormones in varicocele group than the control group ($p<0.05$).

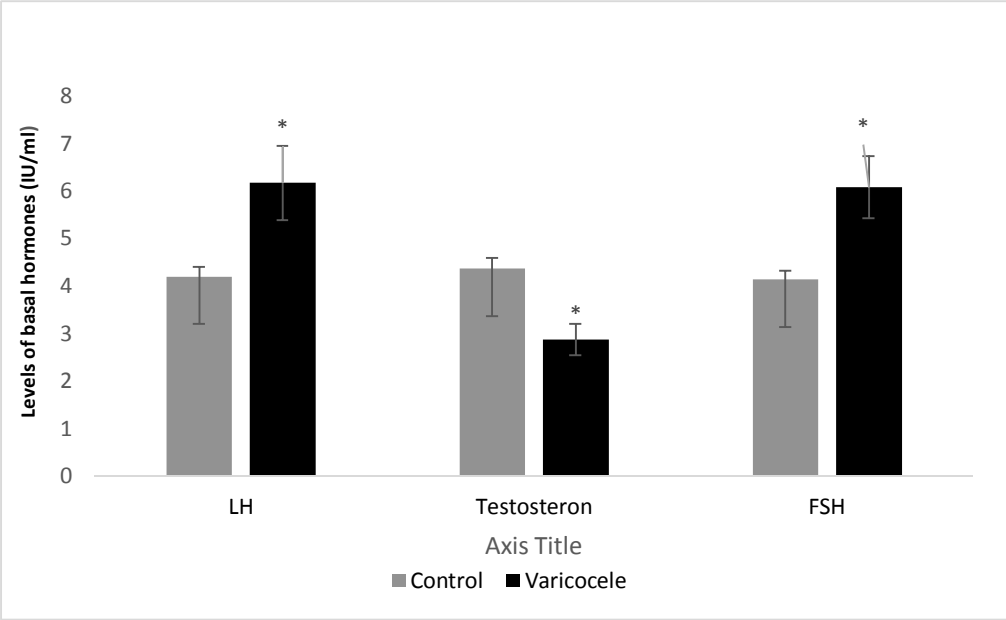
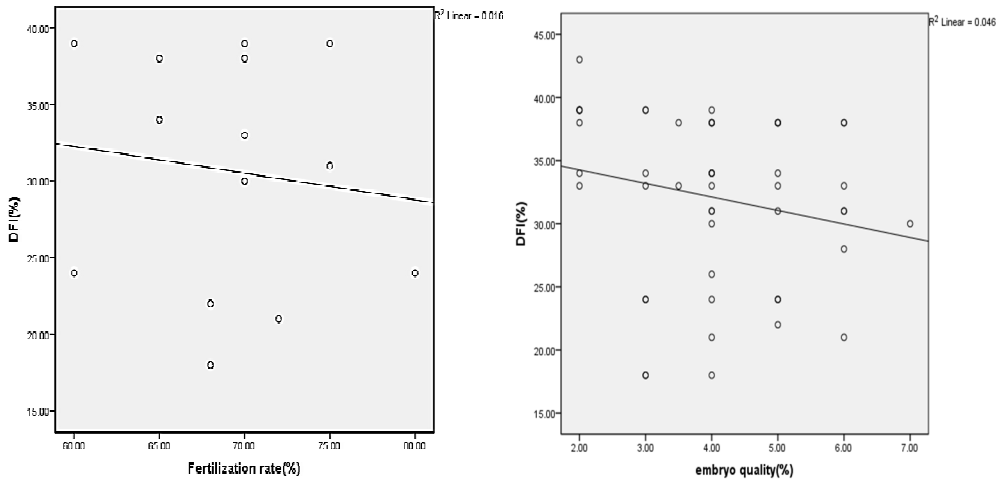


Figure 1: Comparison of (FSH, LH, and testosterone levels) between two groups ($p<0.05$), *; Indicate significant difference in varicocele and control groups.

Correlation between DNA fragmentations, fertilization rate and embryo quality in men with varicocele:

Data analysis has shown that there is a negative correlation between DNA fragmentation percentage and fertilization (Fig2A). Sperm DNA fragmentation also was negatively associated with embryo quality in men with varicocele (Fig 2B).



A B

Figure 2: Correlation between Fertilization rate (A) and embryo quality (B) with DNA fragmentation index (DFI) in men with varicocele.

Discussion

Our results demonstrated that patients with the varicocele had lower sperm concentration, total sperm count, and sperm motility compared to the control group, which is consistent with previously reported results (3, 22, 23). The results of this study showed that the number of sperm with abnormal morphology was significantly higher in the varicocele group. The adverse effect of varicocele on sperm quality can be attributed to many factors such as an increased testicular temperature, testicular damage, and hormonal profile abnormalities, and reflux of toxic metabolites from the adrenal glands(24).

One of the critical events in the pathology of varicocele is the excessive production of ROS (25). In terms of pathological conditions, two roles have been envisaged for the overproduction of ROS: ROS-induced damage to the sperm membrane reduces sperm motility and the ability of the sperm to fuse with the oocyte, and ROS directly damages sperm DNA and subsequently affects the genomic integrity of the embryo (26). Oxidative DNA damage caused by the extent of oxidative stress (OS) led to a reduction in sperm quality in patients with varicoceles (27).

According to the results, the data has demonstrated that there is an increase in DNA fragmentation in the sperm of the varicocele group compared to the control group. In this regard, several pieces of evidence have demonstrated the relationship between varicocele and sperm DNA damage (28, 29). The final steps of spermatogenesis have an important role in sperm function, and fertility. Disorders affecting varicocele on sperm DNA denaturation in the later stages of spermatogenesis may lead to abortive apoptosis or altered fertility potential (30).

There are many aspects still unknown today about the effects of varicocele on hormonal levels (31). Higher levels of FSH and LH indicate that a subtle Leydig cell dysfunction was associated with varicocele and may cause changes in Sertoli cellular function, and decrease testosterone production by Leydig cells (32, 33). Following other authors (10, 34), our study shows that men with varicocelehad a higher LH and FSH concentration, although testosterone level was significantly lower than the control group. These differences in male reproductive hormones seen in infertile patients with varicoceles could be related to the lower sperm concentration and lower sperm motility (35). In our study, we found that in patients with varicocele ICSI resulted (fertilization rate and embryo development) significantly lower compared to fertile men. Therefore, varicocele might have a negative impact on fertilization rate and embryo quality following ICSI. Also, a negative correlation with sperm DNA fragmentation, fertilization rate, and embryo quality is observed in varicocele patients. The negative effect of sperm DNA damage on embryo development might have been modulated by the ability of the oocyte to repair sperm DNA damage before the first cleavage (36, 37). Recent studies evaluated that abnormal sperm chromatin packaging is correlated with the reduced ability of spermatozoa to fertilize oocytes in standard, conception or during ART procedures (38-40). A high percentage of DNA fragmentation index values in varicocele patients may be associated with a lower fertilization and embryo quality in assisted reproductive techniques.

The conclusions outlined above mainly support the varicocele repair (Varicocelectomy), and antioxidant therapy may benefit sperm quality and success for couples with varicocele-related infertility who require ART to initiate a pregnancy (41). Barekat et al. (42) suggested the use of N-acetyl-L-cysteine, as an antioxidant, after varicocelectomy, and reported that post-operation antioxidant treatment reduced ROS levels and improved chromatin integrity and pregnancy

rates. Therefore, varicocele repair should be offered as part of treatment for infertile couples with palpable varicoceles.

Conclusion

In conclusion, Varicocele remains a topic with many controversies for the couples seeking fertility. Our clinical study of infertile men showed that semen quality was significantly impaired in men with varicocele. Despite a decrease in fertilization rate and embryo quality transfer in varicocele patients, no significant effect on clinical pregnancy was observed.

Conflict of interest statement

We declare that we have no conflict of interest.

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