

The Effects of Varicocele on DNA Morphology

Keywords: Varicocele; Infertility; Sperm DNA damage

Abstract

Varicocele is one of the major causes of infertility in men. Although there are different theories related to the pathophysiology of the varicocele, the exact pathophysiologic mechanism remains unknown. Sperm DNA damage may be the cause of undiagnosed infertility problems. The standard semen examination may not be sufficient to determine the etiology of infertility. For this reason, extra tests are needed to identify sperm DNA damage. Despite conflicting reports, many studies have shown that varicocele repair improves the quality of sperm DNA.

Varicocele and Infertility

Infertility is defined as a failure to achieve pregnancy in spite of regular unprotected sex. The male infertility by 50% contributes to this important health problem affecting about 15-20% of couples in society [1,2]. A study carried out by the World Health Organization (WHO) in 1992, which included 9,034 infertile couples, indicated varicocele in 25.4% of men with disordered sperm parameters and in 11.7% of men with normal sperm parameters [3]. Varicocele is the dilatation of the pampiniform plexus veins within the spermatic cord [4]. Varicocele occurs in roughly 15% of the adult male population. The rate of occurrence increases to 21-41% in men with primary infertility and to approximately 80% in those with secondary infertility [5,6]. Varicocele is found on the left side of the scrotum in 75-95% of cases. While its bilateral likelihood is 10%, the likelihood of varicocele occurring on the right side of the scrotum is 2% [7,8].

Etiology and Pathophysiology

Although there are different theories related to the disease pathophysiology, the effects of varicocele on testicular dysfunction and infertility are not completely understood. However, many pathophysiological mechanisms related to this status have been described. These mechanisms include increased scrotal and intra testicular temperature [9,10], hypoxia [11], low back of adrenal and renal toxic metabolites [12], increased venous pressure [13], immunologic factors [14], apoptosis [15], and impairment of enzymatic functions [16]. Many factors related to varicocele and their risk statuses are shown in Table 1.

Among these mechanisms is the most extensively studied and accepted mechanism of increased testicular heat [17]. In previous studies, the increased heat was shown to decrease the levels of heat-shock proteins by affecting androgen synthesis [18]. Researchers demonstrated that increased heat caused by varicocele resulted in disrupted semen parameters and reduced testis tissue-specific heat-shock protein (HSPA2) expression when compared to the fertile group. Removal of this issue by varicocelectomy has been shown to ameliorate semen parameters and enhance HSPA2 expression [19]. In addition, the heat increase caused by varicocele affects spermatogenesis by reducing DNA polymerase activity [20].



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Submission: 10 July 2014

Accepted: 01 October 2014

Published: 03 October 2014

Table 1: Risk factors related to varicocele.

Risk factors	Risk status
Testicular temperature	increased heat
Hypoxia	increased
Oxidative stress	increased
Tissue-specific heat- shock proteins	decreased levels of expression
DNA damage	increased
Ychromosome microdeletion	increased
Venous pressure	increased
Antioxidant capacity	decreased

Another mechanism related to the association between varicoceles and male infertility is increased oxidative stress (OS) [21,22]. Varicoceles may increase reactive oxygen species (ROS) production and decrease antioxidant capacity. Thus, varicoceles can cause OS [23-25]. A number of studies have demonstrated an increase of OS in serum, semen, and testicular tissues of patients with a clinical diagnosis of varicocele. This suggests that the impairment of sperm parameters found in men with varicocele may be related to oxidative stress [26-31]. Furthermore, OS may cause mtDNA deletions in human spermatozoa and mtDNA mutations can cause impairment of sperm functions [32]. In addition, an abnormal amount of ROS and antioxidants is connected to sperm motility defects and sperm quality [33,34].

Other pathologies that could affect the spermatogenesis in patients with varicocele are Y chromosome micro deletions, acrosome reaction disorders, protamine deficiency, and endocrinological changes. Whereas Y chromosome micro deletions are found in 0.3-7% of infertile men, the rate goes up to approximately 18% in varicocele cases with poor semen parameters and testicular hypotrophy [35,36]. Previous studies have suggested that the varicocele may disrupt the acrosome reaction occurring between sperm and the zona pellucida

[37]. Throughout standard spermiogenesis, 85% of histones are supplanted with protamines, which causes the sperm chromatin condensation that is necessary for conserving paternal genes and facilitating fertilization [38,39]. Several studies have shown a defect in the histone/protamine exchange in patients with varicocele [38,40]. Studies investigating the relationship between varicocele and hormonal dysfunction have reported that varicocele decreases the levels of total testosterone and significantly increases hormone levels after varicocelectomy [41].

Varicocele and DNA damage

In recent years, another mechanism that has been the focus of studies investigating the relationship between varicocele and spermatogenesis is sperm DNA damage. Many studies have demonstrated that sperm DNA damage is detected in a high percentage of infertile males and that treatment provided significant improvement in levels [42-44].

The nucleus of the sperm gets condensed throughout the last phases of spermatogenesis. Thus, sperm DNA becomes more resistant to agents that are damaged. Varicocele may influence the last phases of spermatogenesis and lead to impairments in sperm parameters [45-47]. It is likely that this situation is one of the reasons for unexplained infertility. Sperm DNA damage in particular may be the cause of undiagnosed infertility problems [48]. The standard semen examination may not be sufficient to determine the etiology of infertility. For this reason, extra tests are needed to identify sperm DNA damage.

A recently published meta-analysis showed that patients with varicoceles had significantly higher sperm DNA damage than controls [49]. The sperm DNA fragmentation index (DFI: percentage of sperm with denatured nuclei) contains single and/or double-stranded DNA breaks, which may affect the infertility [50,51]. Researchers proposed that sperm nuclear DNA fragmentation happens even when there are no alterations in sperm quality (as evaluated by routine seminal analysis). Therefore, finding classic sperm parameters does not ensure the absence of DNA damage in the sperm of varicocele patients [52,53]. DNA damage can be caused by intra- or extra-testicular factors. The main intra-testicular factors are decreased levels of DNA polymerases, abnormalities in chromatin packing, abortive apoptosis, micro deletions of chromosomal rearrangements, and increased oxidative stress. External factors that cause DNA damage are age, hormonal component, drugs, cigarette smoking, and testicular hyperthermia [39,54-57]. Among these factors, apoptosis and oxidative stress are the most researched.

The authors showed that OS influences the integrity of the sperm chromatin and induces both single-strand and double-strand DNA breaks [58,47]. Moreover, abnormal amounts of ROS cause oxidative stress and impair spermatozoa DNA at both the nuclear and mitochondrial levels [59]. Polyunsaturated fatty acids in the plasma membrane of sperm cells are highly susceptible to damage by ROS. In this process, ROS causes lipid peroxidation and biomembrane damage and can impair DNA by causing deletions and mutations [60,61]. In addition, authors have been reported in relationship with an increased ROS and total antioxidant capacity both in infertile patients and those with varicocele [62].

Animal studies have stated that apoptosis is an important regulator of spermatogenesis in normal and pathological situations [63,64]. Miura et al. showed that the expression of Fas proteins plays an important role in the apoptosis process increase during an acute heat situation of murine testes [65]. Researchers inferred that the FasL mRNA levels of patients with varicocele were higher than in the control group. They also introduce an adverse connection between sperm concentration and FasL mRNA levels [66]. In addition, Wu et al. assessed apoptosis markers in varicocele patients and they found that nuclear DNA damage and externalization of phosphatidylserine (which plays a key role in the apoptosis process) are increased in varicocele patients [67].

Although many studies to be conducted, the adverse effect of DNA fragmentation on fertilization and pregnancy rates is still a controversial issue. There are various reasons for this, such as the application of several techniques for identifying DNA fragmentation and being of individuals with different sperm characteristics [68-70].

Varicocele and Treatment

Varicoceles can be corrected with surgical methods such as microscopic varicocelectomy [71]. Many studies have shown that multiple semen parameters, sperm DNA quality, and pregnancy rates improve after a varicocelectomy [72-74]. Moreover, Chen et al. have reported that varicocele repair decreases ROS levels. Therefore, varicocelectomies may repair spermatogenesis and decrease DNA damage due to reduced ROS levels [63]. In addition, Esteves et al. reported that varicocelectomies improve clinical pregnancy and live birth rates by intra cytoplasmic sperm injection. Researchers have shown that varicocelectomies increase the total number of motile sperm and decrease the sperm defect score [75]. Jiang et al. reported that DNA fragmentation index values were significantly correlated to the outcome of intracytoplasmic sperm injection [76]. These facts demonstrate that a varicocelectomy can improve not only the quantity but also the quality of sperm. However, several studies have shown no improvement in semen quality after varicocele repair [77]. These differences in results may be due to the use of different approaches for semen testing and the heterogeneity of patient groups, which includes clinical diversity, individual genetic background, duration of varicocele, and the socioeconomic status of patients [5]. In conclusion, varicocele is an important problem affecting infertility in men. According to the literature, varicocele repair can correct the many pathologies of varicocele.

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