Sperm Chromatin Condensation Assay in Varicocele Patients Before and After Varicocelectomy

Thesis

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Ahmed Atef El-Beltagy

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List of Abbreviations

ROS	Reactive oxygen species
DFI	DNA fragmentation index
DSBs	Double Stranded breaks
ELV	Experimental lab. varicocele
FISH	Fluorescence in situ hybridization
ICSI	Intracytoplasmic sperm injection
PCNA	Proliferating Cell Nuclear Antigen
PR	Pregnancy rate
RCTs	Randomized controlled trials
RNS	Reactive nitrogen species
SCD	Sperm chromatin dispersion
SCSA	Sperm chromatin structure assay
SDF	Sperm DNA fragmentation
SSBs	Single stranded breaks
TAC	Total antioxidant capacity
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick-end labeling
VEGF	Vascular endothelial growth factors

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INTRODUCTION

A varicocele is an abnormally dilated pampiniform plexus, which is the venous network that drains blood from the testicles. The varicocele prevalence in the general population is estimated to be 15%; however, the prevalence is 35% among men with primary infertility and 81% among men with secondary infertility. The detrimental effects of varicoceles on fertility and the benefit gained by their repair have been debated among andrologists for almost 60 years ***(Gorelick et al., 1993).***

Many theories have been postulated to explain the mechanism through which varicocele can affect fertility e.g. elevated temperature, adrenal hormone reflux, gonadotoxic metabolite reflux, altered testicular blood flow, antisperm antibody formation, alterations in the hypothalamic-pituitary gonadal axis, and oxidative stress ***(Naughton et al., 2001).***

It used to be thought that a varicocele would result in the so-called stress pattern as seen in the traditional semen analysis in the form of decreased percentage of moving sperms and sperms with abnormally shaped heads ***(Andrade-Rocha et al., 2007).***

However, varicocele would not only affect traditionally tested semen parameters but can also affect other sperm

functions and /or properties that cannot be assessed using routine semen analysis. Among these are sperm chromatin condensation and sperm DNA fragmentation ***(Bertolla et al., 2006).***

Several techniques are employed to assess DNA fragmentation and/or sperm chromatin condensation including cytochemical assays, flow cytometric-based sperm chromatin structure assay, comet assay, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay. Among these, cytochemical assays (e.g. aniline blue staining and toluidine blue staining) have been reported to be sensitive, simple, and inexpensive since they do not require special instruments as -for example-in flow cytometry ***(Talebi et al., 2012).***

The chromosomes of sperm cells are tightly packaged into a complex of DNA and protamines, as somatic histones are replaced during spermiogenesis. Aniline blue (AB) staining is used for visualization of sperm chromatin condensation based on the detection of lysine residues with AB as a measure of an excess of histones remaining bound to the sperm DNA ***(Marcon et al., 2004).***

AIM OF THE WORK

The aim of this study is to assess the degree of DNA fragmentation of sperms in varicocele patients before and after varicocelectomy compared to control

1. VARICOCELE

1.1. Definition and Incidence

Varicocele is defined as an abnormal dilatation of the testicular veins in the pampiniform plexus and retrograde blood flow in the internal spermatic veins as a result of incompetent or absent valves. Normally, reverse blood flow is prevented by small, one-way valves. Defects in these valves or compression of the veins by adjacent structures can cause vessel dilatation ***(Gat et al., 2004).***

It is considered one of the main causes of male infertility; affecting 15-25% of the adult male population, 35% of men with primary infertility and up to 80% of men with secondary infertility. The increased prevalence of varicoceles among men with secondary infertility suggests that this vascular lesion has a progressive rather than static effect on male infertility ***(Gorelick et al., 1993)*** and highlights the acquired and progressive nature of infertility due to varicocele ***(Cozzolino and Lipshultz, 2001).***

The observed increase in the prevalence of varicocele may be due to either an absolute increase in the number of men with varicocele who have secondary infertility or a relative increase due to reduction in the incidence of other etiologies that cause male infertility ***(Jarow, 2001).***

1.2. Diagnosis

1.2.1. Clinical Diagnosis of Varicocele

Varicocele may be symptomatic with pain and swelling. At the level of examination, the clinical suspicion of varicocele depends upon the expertise of the evaluating physician. Careful palpation of the spermatic cord on both sides will reveal the presence of the varicocele that feels like a bag of worms ***(Dubin and Amelar, 1977)***. Valsalva's maneuver should be done while performing gentle traction on the cord to prevent cremasteric muscle contraction with resultant shortening and thickening of the spermatic cord that may give a false impression of cord impulse due to varicocele ***(Nagler et al., 1997).***

Palpable varicoceles have been classified clinically in three grades. Grade 1 varicoceles persistent venous reflux that end before Valsalva's; grade 2 varicoceles persistent venous reflux throughout the maneuver; and grade 3 varicoceles are visible and palpable even without Valsalva's maneuver ***(Dubin and Amelar, 1970).***

However, the limitation of physical examination was demonstrated in a multicenter study sponsored by the World Health Organization ***(WHO, 1985).***

1.2.2. Scrotal Ultrasonography

The diagnosis of varicocele by sonograghy is considered if the veins in the pampiniform plexus are more than 3mm in diameter and is confirmed if they change in size with the patient in the upright position or during Valsalva maneuver ***(McClure and Hricak, 1986).***

1.2.3. Color Doppler US

This technique is now available on most models. Flow detection is far simpler than in continuous Doppler mode, but grading is subjective if pulse or TM Doppler mode is not used. The best recording site is probably the inguinal canal, because the flow is faster than in the scrotum, making reflux more readily detectable during the Valsalva's maneuver. The examination can be done sagittally or cross-sectionally. In the latter case the scrotal skin should be moderately invaginated in the external orifice of the inguinal canal to obtain a good acoustic window. It has been graded according to degree of reflux grade I (brief) reflux lasts less than 1 second and is considered physiologic, grade II (intermediate) reflux lasts 1-2 seconds and decreases during the Valsalva's maneuver then disappears prior to the end of the maneuver, and grade III (permanent) reflux lasts more than 2 seconds and has a plateau aspect throughout the Valsalva's maneuver ***(Cornud et al., 1999).***

1.3. Pathophysiology of Varicocele in Infertility

1.3.1. Introduction

According to the comprehensive reviews published in the past 10 years regarding the pathophysiology of varicocele, several hypotheses on the impairment of spermatogenesis have been proposed, including endocrine and testicular paracrine disturbances, heat stress, hypoxia, oxidative stress, accumulation of toxic substances, genetic factors, and autoimmunity, all leading to decreased proliferation of germ cells and/or apoptosis ***(Marmar, 2001; Schoor et al., 2001; Agrawal et al., 2009).***

1.3.2. Heat Stress

Heat stress is the most plausible cause of the impairment of spermatogenesis in men with varicocele, and has been investigated since 1941 ***(MacLeod et al., 1941).***

Spermatogenesis is temperature-sensitive and proceeds optimally at approximately 36°C in men. The internal spermatic artery (surrounded by the pampiniform plexus) maintains the testes at 35-36°C, 1-2°C lower than the core temperature, by the countercurrent heat-exchange system ***(Zorgniotti and Saelfon, 1998).***

Varicocele causes an average scrotal temperature increase of 2.6°C by the dilatation of the venous plexus ***(Naughton et al., 2001).***