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ORIGINAL ARTICLE

The impact of coexisting sperm DNA fragmentation and seminal oxidative stress on the outcome of varicocelectomy in infertile patients: A prospective controlled study



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KEYWORDS

Sperm DNA damage;
Varicocele;
Oxidative stress;
Infertility

ABBREVIATIONS

AO, acridine orange;
DFI%, DNA fragmentation index, percentage of sperm with denatured nuclei;
LH, luteinising hormone;
NO, nitric oxide;

Abstract Objectives: To study the relationship between sperm DNA fragmentation (SDF) and reactive oxygen species (ROS) levels in infertile patients with varicocele, and to examine the beneficial effect of varicocelectomy and elucidate predictors of improvement after repair.

Patients, subjects and methods: We prospectively studied 60 patients with varicocele and abnormal semen variables who attended the outpatient clinic complaining of infertility for ≥ 12 months. In all, 25 patients (41.7%) had bilateral varicoceles and 35 (58.3%) had left varicoceles. The DNA fragmentation index (DFI%, percentage of sperm with denatured nuclei), ROS and total non-enzymatic antioxidant capacity (TAC) were measured. Inguinal varicocelectomy was performed in all patients. At 3–6 months postoperatively, all measurements were repeated. A control group, comprised of 20 normozoospermic fertile men, was included. Regression analysis was used to examine predictors of improvement.

Results: The mean (SD) DFI% in the 60 infertile patients with varicocele was 29.9 (8.3) and 7.56 (2.84)% in the controls; ROS levels were 4.49 (0.9) in patients and 2.62 (0.8) photons/min in controls; and the TAC was 0.97 (0.4) in patients

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PBS, phosphate-buffered saline;
 ROS, reactive oxygen species;
 SCSA, sperm chromatin structure assay;
 SDF, sperm DNA fragmentation;
 TAC, total antioxidant capacity;
 TMSC, total motile sperm count

and 1.5 (0.5) mM in controls; with highly significant differences between the patients and controls. The DFI% showed a positive correlation with ROS levels, whilst the total motile sperm count (TMSC) had a significant negative correlation with DFI%, ROS levels and grade of varicocele, whilst there was significant positive correlation with TAC. The grade of varicocele and duration of infertility were related to the presence of higher levels of ROS and increased of DFI%. Postoperatively, improvement (measured as a > 50% increase in TMSC) occurred in 40 of 55 (73%) patients available at follow-up, with a significant reduction in the mean (SD) DFI% from 29.49 (8.58) to 18.78 (7.23)%, ROS levels from 4.49 (0.88) to 3.27 (1.3) photons/min (both $P < 0.001$), and a significant increase in the mean (SD) TAC from 1.01 (0.44) to 2.05 (0.51) mM ($P < 0.001$). Responders had a shorter infertility duration and lower preoperative DFI% and ROS levels. Regression analysis showed that DFI% is a predictor of improvement after varicocelectomy.

Conclusion: SDF was shown to have a negative impact on improvement after varicocelectomy. Hence, DFI% could be recommended as a prognostic test in infertile patients with varicocele to help decision-making as regards the necessity and the anticipated outcome of varicocelectomy in patients with infertility.

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Introduction

Varicocele is one of the major causes of infertility in men, occurring in 15–20% of the general male population [1]. In men with a normal semen analysis varicocele is found in 11.7%, whilst in those with abnormal seminal variables varicocele is found in 25.4% [2]. The pathogenesis of testicular dysfunction and infertility in association with varicocele is probably due to multiple factors, e.g. venous stasis leading to testicular hypoxia, elevation of testicular temperature, reflux of toxic metabolites from adrenal or renal origin, and impairment of the hypothalamic–pituitary–gonadal axis [3,4]. Reactive oxygen species (ROS) are reactive molecules or free radicals generated as a by-product of normal aerobic metabolism by the reduction of oxygen [5]. Their expression at physiological levels has roles in sperm capacitation and cellular differentiation, whilst oxygen-free radicals at concentrations beyond physiological limits result in oxidative stress [6]. Previous reports have shown that infertile men with clinically diagnosed varicocele have high levels of seminal oxidative stress, as evidenced by increased levels of ROS and reduced total antioxidant capacity (TAC), suggesting that sperm dysfunction in patients with varicocele may be in part related to oxidative stress [7–9]. DNA integrity with the presence of single- and double-strand DNA breaks was found in the ejaculates of infertile men as consequence of oxidative stress [10]. Sperm DNA integrity is one of the essential determinants of normal fertilisation and embryo growth in natural and assisted conception [11–13]. Currently, the sperm chromatin structure assay (SCSA) is a clinically applicable method for calculating the sperm DNA fragmentation index (DFI%,

percentage of sperm with denatured nuclei) to determine the susceptibility of sperm DNA to denaturation [14]. There is no conclusive evidence that a varicocelectomy improves spontaneous pregnancy rates; however, its beneficial effect on various sperm variables [15], improvement of sperm DNA damage [16], and improvement of the testicular microcirculation has been evaluated [17]. Varicocele repair also has a beneficial effect in reducing seminal oxidative stress [18]. Recently, Esteves et al. [19] hypothesised that a decrease in the DFI% after varicocele repair could serve as an indicator of oxidative stress alleviation, and recommended further evaluation of varicocele repair on DFI%. Considering the above-mentioned findings, the aim of the present study was to investigate the relationship between sperm nuclear DNA damage and ROS levels in ejaculated spermatozoa of infertile patients with varicocele and to examine the beneficial effect, if any, of varicocelectomy and to elucidate predictors of improvement after repair.

Patients, subjects and methods

Study design and patient population

A prospective open-label study conducted at the Department of Urology, Department Dermatology Venereology and Andrology and Department of Clinical and Chemical Pathology at Benha University between November 2013 and May 2015, included 60 infertile men complaining of inability to conceive for ≥ 12 months of unprotected intercourse and abnormal seminal variables (reduced sperm concentration, motility and morphology on two or more semen samples) associated with unilateral or bilateral clinical varicocele

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