

Article

Relationship between oxidative stress, varicocele and infertility: a meta-analysis



Dr Ashok Agarwal is the Director of Research at the Centre for Advanced Research in Human Reproduction, Infertility, and Sexual Function, and the Director of the Clinical Andrology Laboratory and Reproductive Tissue Bank. He holds these positions at The Cleveland Clinic Foundation, where he is a Professor at the Lerner College of Medicine of Case Western Reserve University and, since 1993, full staff in the Glickman Urological Institute, Departments of Obstetrics–Gynecology, Anatomic Pathology, and Immunology. Dr Agarwal has published extensively with over 235 original peer-reviewed articles, 24 book chapters, and over 550 presentations at scientific meetings. His research is focused on studies of the role of oxidative stress, DNA integrity, and apoptosis in the pathophysiology of male and female reproduction.

Dr Ashok Agarwal

Ashok Agarwal¹, Sushil Prabakaran, Shyam SSR Allamaneni
Centre for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics–Gynecology, Cleveland Clinic, 9500 Euclid Avenue, Desk A19.1, Cleveland, OH 44195, USA

¹Correspondence: Tel: +1 216 4449485, Fax: +1 216 4456049; e-mail: Agarwaa@ccf.org

Abstract

Varicocele is one of the leading causes of male infertility, and is present in almost 40% of infertile males. Recent understanding of the role of oxidative stress in male reproduction has led some researchers to postulate oxidative stress as the possible cause of sperm dysfunction in varicocele patients. The objective of the present study was to examine the published literature on the role of oxidative stress in patients with varicocele as the aetiology of their infertility. Twenty-three human studies were identified after an extensive search dealing with the role of oxidative stress in varicocele-associated infertility. Out of these studies, four were selected that measured similar types of reactive oxygen species (ROS) by a similar method of measurement. The data were then entered in the RevMan software for analysis. The overall estimate showed that patients have significantly higher concentrations of ROS than controls, with the mean difference being 0.73 (95% CI 0.40, 1.06, $P < 0.0001$). This translated to an ROS concentration of 4.37×10^4 cpm/ 20×10^6 spermatozoa/ml on the linear scale. Total antioxidant capacity levels were found to be significantly lower in the infertile varicocele patients, with 386 fewer trolox equivalents than the controls (95% CI –556.56–216.96, $P < 0.00001$). From the results, it can be concluded that there is increased oxidative stress in varicocele patients; however, more studies are needed to confirm this finding.

Keywords: antioxidants, infertility, oxidative stress, sperm dysfunction

Introduction

Varicocele is the abnormal tortuosity and dilatation of the veins of the pampiniform plexus within the spermatic cord. It is one of the leading causes of male infertility. About 15% of adult males are believed to have either clinical or subclinical varicocele, although the prevalence in infertile males is as high as 40% (Schoor *et al.*, 2001). Only one of every five varicocele patients seeks treatment for infertility. The aetiology of varicocele is multifactorial (Skoog *et al.*, 1997).

Varicocele-related pathology is suspected in infertility as it leads to elevated temperatures in the scrotum and testes, which has a deleterious effect on spermatogenesis (Saypol *et al.*, 1981; Goldstein and Eid, 1989). In addition to the disruption of spermatogenesis, there is also increased apoptosis of germ cells (Lue *et al.*, 1999).

Reactive oxygen species (ROS) are a group of free radicals, highly reactive compounds that have deleterious effects on many cellular organelles. Morphologically abnormal spermatozoa and leukocytes are the main sources of ROS in the seminal plasma (Esfandiari *et al.*, 2003; Saleh *et al.*, 2003). In varicocele patients, ROS production is enhanced irrespective of the fertility status. Several studies have found that the total antioxidant capacity (TAC) is reduced in varicocele patients. This imbalance between enhanced ROS production and diminished antioxidant capacity results in a condition called oxidative stress, which damages spermatozoa.

Several studies have reported oxidative stress in testicular tissue, spermatic vein blood and seminal plasma from infertile varicocele patients. The presence of oxidative stress could cause molecular and genetic defects leading to infertility (Benoff *et al.*, 2004).

Varicocele treatment can significantly improve sperm parameters (Madgar *et al.*, 1995; Kim *et al.*, 1999) and either decrease ROS concentrations or increase the antioxidant capacity (Mostafa *et al.*, 2001).

The objective of this study was to evaluate the alterations in the concentrations of ROS and antioxidants in infertile varicocele patients by combining several publications from the literature. The results may help determine if oxidative stress is increased in these patients. Furthermore, it will help to assess the role played by oxidative stress in the pathophysiology of infertility in varicocele patients.

Materials and methods

Primary articles from which data were taken were located by searching Ovid Medline using the exploding option, and Web of Knowledge, PubMed, BIOSIS and EMBASE. The following keywords were used: varicocele, oxidative stress, free radicals, reactive oxygen species, lipid peroxidation, antioxidants, total antioxidant capacity, DNA damage and male infertility. Articles were searched from 1988 to the present. Cross-references were checked in each of the studies, and relevant articles were retrieved.

Twenty-three human studies were identified that dealt with the role of oxidative stress in varicocele-associated infertility. Two authors of this article analysed all of these studies by going over the actual articles. Only those studies that were accepted by both of the reviewers were included. However, in the event of disagreement among the reviewers, the study was re-examined by both the reviewers. The study was included or excluded from the final analysis upon consensus. The inclusion criteria were based mainly on the oxidative stress markers measured and methodology. Only studies with patients having at least 12 months of infertility were included. Studies on fertile patients with varicocele or patients with leukocytospermia were excluded.

The selection of studies for antioxidant measurement in this meta-analysis was based on methodology. Multiple tests are available to measure the TAC, such as oxygen radical absorbance capacity, the ferric reducing ability of plasma and the trolox equivalent antioxidant capacity assays (Agarwal and Prabakaran, 2005). These assays used enhanced chemiluminescence or calorimetric techniques to measure the antioxidant status of the semen (Said *et al.*, 2003). Enhanced chemiluminescence technique for TAC measurement has been widely used and its results are validated by many researchers (Sharma *et al.*, 1999; Pasqualotto *et al.*, 2000). Therefore, it was decided to include only those studies which measured the total antioxidants by the enhanced chemiluminescence technique for the meta-analysis.

Out of the 23 studies that were identified, four measured ROS by chemiluminescence with luminol as the probe in samples prepared from semen (Hendin *et al.*, 1999; Sharma *et al.*, 1999; Pasqualotto *et al.*, 2000; Saleh *et al.*, 2003). All of these four studies had a control group comprising males with no history of infertility or varicocele and normal semen parameters. It was not possible to apply more strict criteria to the control populations due to the small number of studies. The ROS values were log transformed (ROS + 1) due to the wide range of ROS concentrations. Data were presented as the mean \pm SD of the log-transformed values

and expressed as 10^4 counted photons per minute per 20×10^6 spermatozoa. In these same studies, TAC was measured using the enhanced chemiluminescence method, and the results were presented as molar trolox equivalents.

The raw data obtained from individual studies were examined for completeness of observations and to avoid duplication of data. Average ROS concentrations and TAC were calculated from the original data set along with their corresponding standard deviations after excluding individuals with leukocytospermia and azoospermia. These data were entered into the RevMan software used by the Cochrane Collaborative (RevMan v 4.2.8, Cochrane IMS). A random effects model was applied for calculating weighted mean difference. This measure was chosen to enable clinically relevant interpretation of the results. The data were examined for heterogeneity using the Chi-squared statistic, and overall significance was assessed using a z-score. A significance level of 0.05 was chosen for all statistical tests.

The selected studies included varicocele patients attending an infertility clinic with a clinical diagnosis of infertility. Studies were performed with infertile varicocele patients and donors (fertile males with no clinical varicoceles and having normal semen parameters). Oligo- and azoospermic samples (defined as a sperm concentration below 20×10^6 /ml) as well as leukocytospermic samples (defined as more than 1×10^6 peroxidase-positive white blood cells/ml of semen) were excluded from the analysis in both the subjects and controls. All patients and controls were recruited at a tertiary care facility for the evaluation of infertility. Controls were recruited from a group of healthy normospermic donors.

Results

Of the 23 original articles obtained, seven studies measured either reactive oxygen species or lipid peroxidation concentrations in semen, serum or testicular tissue, five measured nitric oxide concentrations and two measured reactive oxygen species or lipid peroxidation and nitric oxide concentrations. Antioxidants were measured in 10 studies, of which eight demonstrated decreased antioxidant concentrations. Only four studies fulfilled the inclusion criteria for conducting the meta-analysis (Hendin *et al.*, 1999; Sharma *et al.*, 1999; Pasqualotto *et al.*, 2000; Saleh *et al.*, 2003).

A total of 118 patients and 76 normal healthy donors (controls) were included in the meta-analysis. TAC results were missing for two patients, and ROS data were missing for one of the control subjects. No heterogeneity was found in either outcome. The overall estimate indicated that patients have significantly higher concentrations of ROS than controls, with the mean difference being 0.73 (95% CI: 0.40, 1.06, $P < 0.0001$). This translated to a ROS concentration of 4.37×10^4 cpm/ 20×10^6 spermatozoa/ml on the linear scale (**Figure 1**) [formula: $\log(\text{ROS} + 1) = 0.73$; $\text{ROS} = 10^{0.73} - 1$].

TAC concentrations were found to be significantly lower in the infertile varicocele patients when compared with the controls. Overall, varicocele patients had 386 fewer trolox equivalents than the controls (95% CI: -556.56, -216.96, $P < 0.00001$) (**Figure 2**).

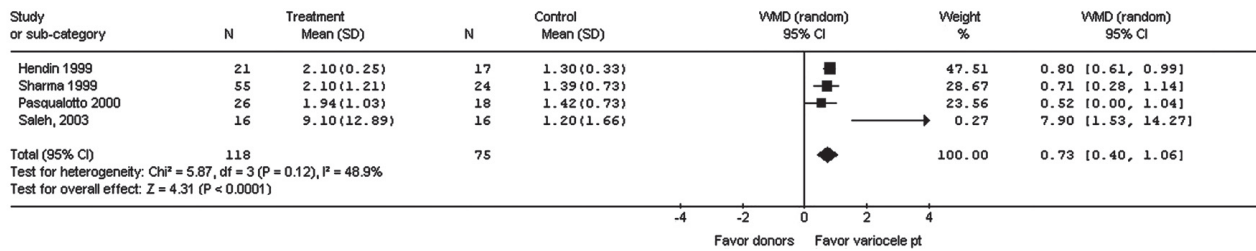


Figure 1. Forrest plot of meta-analysis on the concentrations of reactive oxygen species in varicocele patients compared with donors (WMD = weighted mean difference; CI = confidence interval; N = number of subjects).

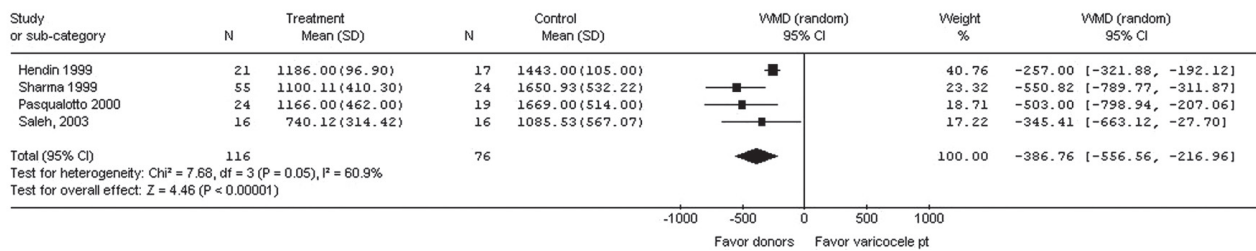


Figure 2. Forrest plot of meta-analysis on the concentrations of total antioxidant capacity in varicocele patients compared with donors (WMD = weighted mean difference; CI = confidence interval; N = number of subjects).

Discussion

The results indicate that there is an increase in oxidative stress (high ROS, $P = 0.0001$; and low TAC concentrations, $P < 0.00001$) in infertile patients diagnosed with varicocele. The increased production of ROS could result in greater utilization of antioxidants and therefore lower concentrations (Mostafa *et al.*, 2001). This may distort the delicate balance between ROS and antioxidants and lead to oxidative stress (Allamaneni *et al.*, 2004; Agarwal *et al.*, 2005). Oxidative stress might hinder the process of spermatogenesis and lead to the poor semen parameters seen in these patients, which are generally restored after varicocele repair (Zini *et al.*, 2005). Studies included in the meta-analysis demonstrated a strong correlation between semen parameters and ROS concentrations.

The test of heterogeneity was not significant for ROS ($P = 0.12$) or TAC ($P = 0.05$) in the present meta-analysis. This allowed us to use either a fixed or random effects model. It was decided to use the random effect model to generalize and extrapolate the findings to other situations similar to that of the included studies.

Large-scale randomized controlled trials are still needed in support of the results. Studies should determine the aetiological role of oxidative stress in varicocele by demonstrating a decrease in oxidative stress measures and an improvement in clinically relevant fertility outcomes (pregnancy and assisted

reproduction success) after therapeutic intervention such as antioxidants supplementation and varicocelectomy. The mechanism by which oxidative stress affects spermatogenesis requires further investigation.

Oxidative stress parameters (such as ROS and lipid peroxidation) are significantly increased in infertile patients with varicocele compared with normal sperm donors. Antioxidant concentrations were significantly lower in infertile varicocele patients compared with controls. The presence of oxidative stress in the varicocele patients may play a role in the aetiology of infertility.

Acknowledgements

The authors thank the Cleveland Clinic's Glickman Urological Institute for its support of this study.

References

- Agarwal A, Prabakaran SA 2005 Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian Journal of Experimental Biology* **43**, 963–974.
- Agarwal A, Gupta S, Sharma RK 2005 Role of oxidative stress in female reproduction. *Reproductive Biology and Endocrinology* **3**, 28.
- Allamaneni SS, Naughton CK, Sharma RK *et al.* 2004 Increased seminal reactive oxygen species levels in patients with varicoceles

- correlate with varicocele grade but not with testis size. *Fertility and Sterility* **82**, 1684–1686.
- Benoff SH, Millan C, Hurley IR *et al.* 2004 Bilateral increased apoptosis and bilateral accumulation of cadmium in infertile men with left varicocele. *Human Reproduction* **19**, 616–627.
- Esfandiari N, Sharma RK, Saleh RA *et al.* 2003 Utility of the nitroblue tetrazolium reduction test for assessment of reactive oxygen species production by seminal leukocytes and spermatozoa. *Journal of Andrology* **24**, 862–870.
- Goldstein M, Eid JF 1989 Elevation of intratesticular and scrotal skin surface temperature in men with varicocele. *Journal of Urology* **142**, 743–745.
- Hendin BN, Kolettis PN, Sharma RK *et al.* 1999 Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *Journal of Urology* **161**, 1831–1834.
- Kim ED, Leibman BB, Grinblat DM *et al.* 1999 Varicocele repair improves semen parameters in azoospermic men with spermatogenic failure. *Journal of Urology* **162**, 737–740.
- Lue YH, Hikim AP, Swerdloff RS *et al.* 1999 Single exposure to heat induces stage-specific germ cell apoptosis in rats: role of intratesticular testosterone on stage specificity. *Endocrinology* **140**, 1709–1717.
- Madgar I, Weissenberg R, Lunenfeld B *et al.* 1995 Controlled trial of high spermatic vein ligation for varicocele in infertile men. *Fertility and Sterility* **63**, 120–124.
- Mostafa T, Anis TH, El-Nashar A *et al.* 2001 Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *International Journal of Andrology* **24**, 261–265.
- Pasqualotto FF, Sharma RK, Nelson DR *et al.* 2000 Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertility and Sterility* **73**, 459–464.
- Said, TM, Kattal, N, Sharma, RK *et al.* 2003 Enhanced chemiluminescence assay versus colorimetric assay for measurement of the total antioxidant capacity of human seminal plasma. *Journal of Andrology* **24**, 676–680.
- Saleh RA, Agarwal A, Sharma RK *et al.* 2003 Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. *Fertility and Sterility* **80**, 1431–1436.
- Saypol DC, Howards SS, Turner TT *et al.* 1981 Influence of surgically induced varicocele on testicular blood flow, temperature, and histology in adult rats and dogs. *Journal of Clinical Investigation* **68**, 39–45.
- Schoor RA, Elhanbly SM, Niederberger C 2001 The pathophysiology of varicocele-associated male infertility. *Current Urology Reports* **2**, 432–436.
- Sharma RK, Pasqualotto FF, Nelson DR *et al.* 1999 The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. *Human Reproduction* **14**, 2801–2807.
- Skooog SJ, Roberts KP, Goldstein M *et al.* 1997 The adolescent varicocele: what's new with an old problem in young patients? *Pediatrics* **100**, 112–121.
- Zini A, Blumenfeld A, Libman J *et al.* 2005 Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. *Human Reproduction* **20**, 1018–1021.

Received 13 December 2005; refereed 6 January 2006; accepted 6 February 2006.