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## Reactive oxygen species and antioxidants relationship in the internal spermatic vein blood of infertile men with varicocele

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### Abstract

**Aim:** To assess the relation of reactive oxygen species (ROS) and antioxidants in the internal spermatic vein blood compared to the peripheral venous blood. **Methods:** Sixty-eight infertile oligoasthenozoospermic patients associated with varicocele were investigated. During inguinal varicocelectomy, blood samples of internal spermatic as well as median cubital veins were withdrawn. Three ROS factors (malondialdehyde [MDA], hydrogen peroxide H<sub>2</sub>O<sub>2</sub>, nitric oxide [NO]) and four antioxidants (superoxide dismutase [SOD], catalase [Cat], glutathione peroxidase [GPx] and vitamin C) were estimated in these blood samples. **Results:** Mean levels of tested ROS factors were significantly higher in the internal spermatic venous blood compared to those in the peripheral one (mean ± SD) (MDA 18.7 ± 1.4 nmol/mL vs. 15.4 ± 1.4 nmol/mL, H<sub>2</sub>O<sub>2</sub> 43.6 ± 8.0 μmol/mL vs. 30.8 ± 8.1 μmol/mL, NO 2.3 ± 0.5 nmol/L vs. 1.6 ± 0.4 nmol/L, *P* < 0.01). Mean levels of tested antioxidants were significantly lower in the internal spermatic venous blood compared to those in the peripheral one (superoxide dismutase 1 690.7 ± 130.0 U/mL vs. 1 818.5 ± 143.0 U/mL, catalase 38.9 ± 6.1 mol/L vs. 47.9 ± 10.2 mol/L, GPx 20.4 ± 8.1 U/mL vs. 23.0 ± 8.4 U/mL, vitamin C 0.3 ± 0.1 vs. 0.4 ± 0.1 mg/dL, *P* < 0.05). **Conclusion:** Internal spermatic venous blood of infertile male cases associated with varicocele demonstrated elevated levels of ROS and decreased levels of antioxidants compared to peripheral venous circulation. (*Asian J Androl* 2006 Jul; 8: 451–454)

**Keywords:** male infertility; varicocele; spermatic vein; reactive oxygen species; antioxidants

### 1 Introduction

Several theories have been advanced to explain the mechanisms by which scrotal varicocele impairs male fertility. These theories include scrotal hyperthermia [1], retrograde flow of adrenal or renal metabolites [2], Leydig

cell dysfunction [3] and hypoxia as a result of venous stasis or impairment of testicular artery perfusion [4].

Reactive oxygen species (ROS) are metabolites of oxygen, including superoxide anions, hydrogen peroxide, hydroxyl radical, hydroperoxyl radical and nitric oxide (NO). When presenting in excess, they can initiate pathological damage by inducing oxidative changes to cellular lipids, proteins and DNA [5]. Most cells are equipped with either enzymatic antioxidant systems, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat), or non-enzymatic antioxidant systems, such as uric acid, vitamin C, vitamin E and albumin.

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When these defenses are overwhelmed, cell function is affected.

Seminal plasma possesses antioxidant scavengers and enzymes, which might be deficient in some patients. Significant increase of superoxide anion and free radical activity has been demonstrated in some andrological conditions, such as leukocytospermia and varicocele where great oxidative stress produce different unstable, potentially toxic products [6]. Scrotal varicocele is found to be associated with elevated spermatozoal ROS production and diminished seminal plasma antioxidant activity [7]. Also, it has been demonstrated that surgical varicocelectomy improved semen parameters associated with decreased ROS and increased antioxidant seminal plasma levels [8].

The aim of the present study is to assess the relationship of ROS and the antioxidant levels in the internal spermatic vein of infertile patients associated with scrotal varicocele compared to those in the peripheral venous blood.

## 2 Materials and methods

The study group consisted of 68 infertile males, selected prospectively after consent from the oligoasthenozoospermic patients scheduled for inguinal varicocelectomy (grades I, II, III) at the Andrology Department, Faculty of Medicine Hospitals (mean age  $31.0 \pm 2.6$  years, ranging from 24 to 47 years), and all of them had been married for more than 2 years. Scrotal varicocele was diagnosed both clinically as well as by Duplex. Semen analysis was carried out according to World Health Organization guidelines. Associated conditions that might affect seminal ROS or antioxidant levels (leukocytospermia, smoking and diabetes) were excluded beforehand.

The levels of three ROS radicals (MDA,  $H_2O_2$  and NO) and four antioxidants (SOD, Cat, GPx and vitamin C) were estimated in the peripheral blood samples drawn from the median cubital vein as well as the internal spermatic vein(s) during varicocelectomy operation (inguinal approach; left or bilateral). MDA was determined using the photometric method described by Placer *et al.* [9].  $H_2O_2$  was measured spectrophotometrically. NO was determined using the photometric method of Green *et al.* [10]. SOD was estimated according to the method of Witerbourn *et al.* [11]. Cat was estimated using a colorimetric method as described by Sinha [12]. GPx level

was estimated using the method of Paglia and Valentine [13]. Vitamin C level was estimated calorimetrically.

The statistics are presented as mean  $\pm$  SD. ROS and antioxidant levels of the two sources of venous blood were compared using unpaired *t*-test. Significance was based on a type I error of 0.05.

## 3 Results

Mean levels of the investigated ROS factors (MDA,  $H_2O_2$  and NO) were significantly higher in the internal spermatic vein blood compared to those in the peripheral venous blood (Table 1). Also, the levels of the four tested antioxidants (SOD, Cat, GPx and vitamin C) were significantly lower in the internal spermatic vein blood compared to those in the peripheral venous blood (Table 2).

Table 1. Comparison of tested reactive oxygen species (ROS) levels in median cubital and internal spermatic venous blood. Values are mean  $\pm$  SD.

|                                   | Median cubital<br>vein blood | Spermatic<br>vein blood | <i>P</i> |
|-----------------------------------|------------------------------|-------------------------|----------|
| Malondialdehyde (nmol/mL)         | 15.4 $\pm$ 1.4               | 18.7 $\pm$ 1.4          | < 0.01   |
| Hydrogen peroxide ( $\mu$ mol/mL) | 36.8 $\pm$ 8.1               | 43.6 $\pm$ 8.0          | < 0.01   |
| Nitric oxide (nmol/L)             | 1.6 $\pm$ 0.4                | 2.3 $\pm$ 0.5           | < 0.01   |

Table 2. Comparison of tested antioxidants levels in median cubital and internal spermatic venous blood. Values are mean  $\pm$  SD.

|                               | Median cubital<br>vein blood | Spermatic<br>vein blood | <i>P</i> |
|-------------------------------|------------------------------|-------------------------|----------|
| Superoxide dismutase (U/mL)   | 1818.5 $\pm$ 143.0           | 1690.0 $\pm$ 130.0      | < 0.05   |
| Catalase (mol/L)              | 47.9 $\pm$ 10.2              | 38.9 $\pm$ 6.1          | < 0.05   |
| Glutathione peroxidase (U/mL) | 23.0 $\pm$ 8.4               | 20.4 $\pm$ 8.1          | < 0.05   |
| Vitamin C (mg/dL)             | 0.4 $\pm$ 0.1                | 0.4 $\pm$ 0.1           | < 0.05   |

## 4 Discussion

Controlled generation of ROS was shown to have a role in many physiological sperm functions, such as hyperactivation, capacitation and acrosome reaction [14]. Pasqualotto *et al.* [15] investigate the relation between oxidative stress, semen characteristics and clinical diagnosis in investigated infertile patients. They report that the total antioxidant levels in patients with idiopathic infertility, varicocele, vasectomy reversal and infection

with varicocele were significantly less than those in the fertile controls [15]. They conclude that the total antioxidant capacity might contribute strongly to the pathophysiology of male infertility, irrespective of the clinical diagnosis.

Many findings support the hypothesis that male infertility associated with scrotal varicocele is at least in part related to oxidative stress. Varicocele patients were found to have decreased antioxidant defenses both at the local (seminal plasma) and systemic (blood plasma) levels [16]. Lewis *et al.* [17] report increased ROS generation in 40% of the general infertile population, compared to 80% in the infertile varicocele population. Ozbek *et al.* [18] demonstrate increased NO production in the spermatic vein in men with varicocele, pointing to its responsibility for spermatozoa dysfunction in these cases.

In the present study, the mean levels of the investigated ROS factors (MDA, H<sub>2</sub>O<sub>2</sub> and NO) in the internal spermatic vein blood show significant increase compared to those in the peripheral venous circulation. However, that of investigated antioxidants, SOD, Cat, GPx and vitamin C, showed significant decrease. We could propose a relationship between these observations and the pathophysiology of varicocele associated with male infertility. This goes with the local hypoxic condition as an adverse effect of varicocele [4, 19] and is also supported by improved ROS as well as antioxidant levels after varicocelectomy operation [8, 20].

Actually, scientific articles discussing this point were few. Barbieri *et al.* [16] measure the total antioxidant potential defenses, not individually, of varicocele patients at both seminal plasma and the systemic circulation. They report that the antioxidant defenses decrease in both of them. They conclude that varicocele-associated oxidative stress is evidenced not only at the local site but also at the systemic levels. Our results also supported the statement of Ozbek *et al.* [18] who find that NO level had almost a double level in the internal spermatic vein than in the peripheral circulation in a group of 14 infertile patients with varicocele. They conclude that increased NO levels in the internal spermatic vein of varicocele patients might be responsible for sperm dysfunction and varicocele-associated male infertility.

However, our study lacks control groups due to ethical demands. If the differences observed between local and systemic blood are responsible, at least in part, for reduced fertility in varicocele patients, it would be nec-

essary to investigate whether similar changes took place in fertile men with varicocele and/or in infertile men without varicocele.

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