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Comparison of Nitric Oxide Concentration in Seminal Fluid between Infertile Patients with and without Varicocele and Normal Fertile Men

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ABSTRACT

Introduction: Elevated nitric oxide (NO) levels have been shown to have toxic effects on sperm function and motility. This study was conducted to compare NO levels in the seminal fluid of infertile men with varicocele with those of infertile and fertile men without varicocele.

Materials and Methods: Semen samples were obtained from 40 infertile men with varicocele (group 1), 40 infertile men without varicocele (group 2), and 40 fertile volunteers without varicocele (group 3). NO levels in the seminal plasma of patients in each group were measured and compared. In infertile men with varicocele, semen parameters, including sperm count and motility, and grade of varicocele were also determined.

Results: Mean NO concentrations were 52.34 ± 26.62 μmol/L, 37.06 ± 20.39 μmol/L, and 33.7 ± 18.99 μmol/L in groups 1, 2, 3, respectively. Concentrations in group 1 were significantly higher than those in groups 2 and 3 (P = 0.001). In group 1, no significant correlations were seen between NO concentrations and grades of varicocele, sperm count, sperm motility, or ages of the patients.

Conclusion: Data from the current study suggest a possible role of NO in damaging the sperm function in varicocele as demonstrated by an increased concentration of NO in the seminal fluid of infertile men with varicocele compared with the seminal fluid of fertile and infertile men without varicocele.

KEY WORDS: varicocele, male infertility, nitric oxide, seminal fluid

Introduction

Nitric oxide (NO) was first described in 1979 as a potent relaxant of peripheral vascular smooth muscles, with an action mediated by cyclic guanosine phosphate (cGMP).[1] Subsequently, endothelium-derived relaxing factor was identified as NO or a chemically unstable nitrous precursor.[2] NO is synthesized from endogenous L-arginine by nitric oxide synthase (NOS). It has several roles in biological processes, such as neurotransmission (in nonadrenergic, noncholinergic pathways), tumor cell killing, and inflammatory and immune responses.[3]

Recent studies have shown that NO levels increase in the spermatic veins and seminal plasma of patients with varicocele.[4,5] Some observations have indicated that NO could modulate sperm functions. Low concentrations of exogenous NO donors have been shown to enhance human sperm motility, viability, capacitation, and binding to the zona pellucida.[6-8] Conversely, at higher concentrations, they decrease human sperm motility and induce sperm toxicity.[9,10]

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Varicocele is the most common correctable cause of male infertility; however, the mechanism by which varicocele affects testicular function remains unclear.[11]

In the present study, we compared NO levels in the seminal fluid of infertile men with varicocele with those of infertile and fertile men without varicocele. We also evaluated the relationship between NO concentration and total sperm count, sperm motility, and grade of varicocele in infertile men with varicocele.

Materials and Methods

From October 2003 to November 2004, semen samples were obtained from 40 infertile men with varicocele (group 1), 40 infertile men without varicocele (group 2), and 40 fertile men without varicocele (group 3). The patients in groups 1 and 2 were recruited from outpatient infertility clinics of the Dr. Shariati Hospital and Royan Infertility Institute, Tehran, Iran, on a nonrandomized basis. Healthy age-matched volunteers with proven fertility were selected from among male employees of the Dr. Shariati Hospital. This case-control study was approved by ethical committee of Tehran University of Medical Sciences. Informed consent was obtained from all subjects, before entering the study.

Infertility was considered when no pregnancy had occurred despite couples having regular intercourse without contraception for at least 1 year. Inclusion criteria were clinically diagnosed varicocele, being the only risk factor of infertility in the couples of group 1; and proven infertility in men without clinical varicocele for group 2. In groups 1 and 2, the mean duration of infertility was 3.1 years (range, 1 to 8 years); partners had a mean age of 24.2 ± 4.36 years; and there was no female factor contributing to a couple's infertility, as reported by the consulting gynecologist. The third group consisted of men without clinical varicocele, proven recent fatherhood (less than 2 years), and normal results on genital examination.

Exclusion criteria were the presence of active genitourinary infection, leukocytospermia (> 1 million WBC/mL), and treatment with nitrate derivatives.

Varicocele was classified in 3 grades; grade 1, a pulse that can be palpated in the scrotum during a Valsalva maneuver; grade 2, a varicocele that is large enough for tortuous and dilated veins to be palpable without a Valsalva maneuver; and grade 3, a varicocele is visible through the scrotal skin.[12]

A sample size of 40 men in each group would be sufficient to detect a difference of 7 μmol/L in the mean of NO concentration in the seminal fluid, assuming a standard deviation of 9 μmol/L,[6] a power of 90%, and significance level of 5%.

Semen specimens were collected into sterile containers after 72 hours of abstinence in all participants. Specimens were allowed to liquefy for 30 minutes at room temperature, and a conventional semen analysis was performed under sterile conditions within 1 hour after collection. A fraction of semen was stored at -80°C for NO assay.

NO Analysis

Total nitrite and nitrate levels of seminal plasma were determined as a measure of NO radical production, using a Griess reagent. The Griess reagent consists of sulfanilamide and N-1-naphtyl ethylenediamine.[6,13] The frozen semen was allowed to thaw and reach a temperature of 25°C. The liquefied semen was then centrifuged. One hundred microliters of supernatant was mixed with 100 μL of Griess reagent. Enzyme linked immunosorbent assay (ELISA) was used to measure the photometric absorbance of the mixed solution at 540 nm. This would indirectly determine NO concentration in seminal plasma.

Statistical Analysis

Normality of distribution was checked as needed. The results of continuous variables were expressed as median (range) and mean (± SD). Owing to the abnormal distribution of some data, the nonparametric Mann-Whitney *U* and Kruskal-Wallis tests were used to compare the NO levels between groups. The relationship between NO levels in seminal plasma and semen parameters and grade of varicocele were investigated by correlation analysis. A level for *P* less than 0.05 was regarded as statistically significant.

Results

Mean ages of the patients in groups 1, 2, and 3 were 29.8 ± 5.36 years, 30.4 ± 5.17 years, and 27.9 ± 4.44 years, respectively (ANOVA, $P = 0.685$). The distribution of varicocele grades in group 1 was grade 1 in 11 patients (27.5%), grade 2 in 14 (35%), and grade 3 in 15 (37.5%).

The median NO concentrations in the seminal

plasma of patients in group 1 (infertile men with varicocele) were significantly higher than those of patients in group 2 (P = 0.006) and group 3 (P = 0.001); however, there was no significant difference between patients in groups 2 and 3 (P = 0.525). NO concentration and distribution in seminal fluids of the 3 groups are shown in Table 1 and Figure 1. There was no significant linear relationship between the mean NO concentration in the seminal plasma of patients in group 1 and total sperm count (r = 0.035, P = 0.831), sperm motility (r = 0.06, P = 0.713), and grade of varicocele (r = 0.06, P = 0.674). Also, there was no significant linear relationship between mean NO concentration in seminal plasma of patients in group 1 and age (r = 0.126, P = 0.44).

Discussion

In humans, NO is an important biologic substance and is found in a variety of tissues including those of the reproductive system. NO has been implicated as protecting against reactive oxygen species (ROS)-mediated damage; however, in situations of inappropriate NOS regulation, NO may exacerbate ROS-mediated pathology.[14] The relationship between ROS and varicocele has been the subject of several studies. It has been reported that varicocele is associated with elevated sperm ROS production and diminished seminal plasma antioxidant capacity.[15, 16]

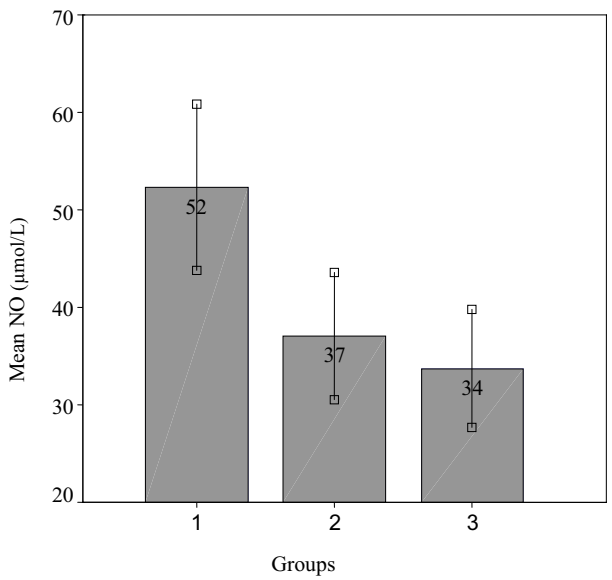

Fig. 1. NO distribution (µmol/L) in the 3 groups of infertile men with varicocele (group 1), infertile men without varicocele (group 2), and fertile men without varicocele (group 3)

Table 1. *NO levels (µmol/L) in the 3 groups of infertile men with varicocele (group 1), infertile men without varicocele (group 2), and fertile men without varicocele (group3)*

Group	Mean ± SD	Median (Range)
1	52.34 ± 26.62	46.80 (17.23 to 100)
2	37.06 ± 20.39	30.91 (7.40 to 93.75)
3	33.70 ± 18.99	28.90 (2.90 to 100)

Kruskal-Wallis, P = 0.001

Some studies show that NO may modulate sperm functions. Low levels of NO, generated under physiological conditions, might be beneficial for sperm functions, but excessive levels of NO under pathological situations (eg, infections or endometriosis) might be toxic for sperm. Infertile patients, especially those with pyospermia, have higher NO levels in the seminal plasma than do fertile controls, and a positive correlation has been found between the level of NO and number of immotile sperm.[17]

NOS is selectively inhibited by NG-nitro-L-arginine-methyl ester (L-NAME). One study has indicated that endothelial NOS (eNOS) plays a role in human sperm's capacity to fuse with an oocyte but not in zona pellucida binding.[18] This study showed that L-NAME, added from the onset of capacitation, strongly inhibits sperm-oocyte fusion.

The relationship between infertility, varicocele, and NO concentration has not been clearly identified. Some animal and human investigations have shown that NO concentration increases in seminal plasma, spermatic veins, and Leydig cells of patients with varicocele.[4,5,19] In a study by Romeo and coworkers, it was found that elevation of NO in adolescents with varicocele creates an oxidative stress status and as such, should be an indication for varicocele treatment.[15]

Aksoy and colleagues compared semen samples from 55 infertile patients with varicocele and 48 normal controls. The median NO concentration in the seminal plasma of patients with varicocele was significantly higher than it was in controls (P < 0.001). A significant negative correlation was noted between NO and sperm motility (r = -0.29, P = 0.003) and also sperm count (r = -0.26, P = 0.008).[5] In another study, the same authors showed increased levels of NO in the seminal plasma of patients with varicocele and oligo-

and/or asthenozoospermia, compared with oligo- and/or asthenozoospermia in subjects without varicocele and a control group. In that study, 19 men with varicocele and oligo- and/or asthenozoospermia (group 1), 30 patients without varicocele and oligo- and/or asthenozoospermic (group 2), and 20 healthy subjects (control group) were recruited. The authors also showed an inverse correlation between NO concentration in the seminal plasma and sperm motility and concentration.[20]

In the present study, regardless of semen analyses, we found that NO concentrations in the seminal plasma of infertile men with varicocele were significantly higher than those in both infertile and fertile men without varicocele. However, we could not show any correlation between seminal plasma NO levels and sperm count, sperm motility, grade of varicocele, and age of infertile men with varicocele. In view of some supportive studies,[5,20] our negative result needs further consideration. One possible explanation could be the effect of the laboratory technician's performance.

Whether or not the increased seminal plasma NO in varicocele translates into a detrimental effect on spermatogenesis and fertility should be clarified by further studies with larger sample sizes. In addition, there remain other unanswered questions that should be considered by further research: Could the increased seminal plasma NO levels be used to decide which adolescent with varicocele should be followed and which should undergo varicocelectomy? Could this and other similar studies contribute to the introduction of new medical therapies for varicocele using NOS inhibitors such as L-NAME?

Conclusion

This study demonstrates a statistically significant increase in NO concentration in the seminal fluid of infertile men with varicocele as compared with that in fertile and infertile men without varicocele. However, age, semen parameters, and grades of varicocele did not show a significant correlation with NO concentration. This study provides an opportunity for further studies to examine NO as a possible factor that is detrimental to sperm function in varicocele.

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