Effects of *N*-acetylcysteine on Semen Parameters and Oxidative/Antioxidant Status

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OBJECTIVES To examine whether a beneficial effect of *N*-acetylcysteine (NAC) on semen parameters and oxidative/antioxidant status in idiopathic male infertility exists. The production of reactive oxygen species is a normal physiologic event in various organs. However, overproduction of reactive oxygen species can be detrimental to sperm and has been associated with male infertility.

METHODS Our study included 120 patients who had attended our clinic and were diagnosed with idiopathic infertility according to medical history and physical and seminal examination findings, as initial evaluations. The patients were divided randomly into 2 groups. Those in the study group (60 men) were given NAC (600 mg/d orally) for 3 months; the control group (60 men) received a placebo. The oxidative status was determined by measuring the total antioxidant capacity, total peroxide and oxidative stress index in plasma samples. The sperm parameters were evaluated after NAC treatment and were compared with those in the control group.

RESULTS NAC had significant improving effects on the volume, motility, and viscosity of semen. After NAC treatment, the serum total antioxidant capacity was greater and the total peroxide and oxidative stress index were lower in the NAC-treated group compared with the control group. These beneficial effects resulted from reduced reactive oxygen species in the serum and reduced viscosity of the semen. No significant differences were found in the number or morphology of the sperm between the 2 groups.

CONCLUSIONS We believe that NAC could improve some semen parameters and the oxidative/antioxidant status in patients with male infertility. UROLOGY 74: 73–76, 2009. © 2009 Elsevier Inc.

Reactive oxygen species (ROS) are products of normal cellular metabolism. Most of the body energy is produced by the enzymatically controlled reaction of oxygen with hydrogen in the oxidative phosphorylation occurring within the mitochondria during oxidative metabolism. Free radicals are formed during this enzymatic reduction of oxygen to produce energy. Under certain conditions, increases in oxidants and decreases in antioxidants cannot be prevented, and the oxidant/antioxidant balance shifts toward the oxidative state. This results in oxidative stress, which has been implicated in >100 disorders, including infertility.[1] Male factor infertility accounts for up to one half of all cases of infertility and affects 1 in 20 men in the general population.[2] Evidence has suggested that ROS-mediated damage to sperm is a significant contributing factor in 30%-80% of all cases.[3] ROS, including oxygen ions, free radicals, and peroxides, cause infertility by 2 principal mechanisms. First, ROS damage the sperm membrane, which in turn reduces the sperm's motility and its ability to fuse with the oocyte. Second, ROS directly damage sperm DNA, compromising the paternal genomic contribution to the embryo.[4]

Idiopathic male factor infertility has been linked to oxidative stress by several research groups. One of the principal causes of this association is the observation that morphologically abnormal sperm has an increased capacity to generate ROS but also a reduced antioxidant capacity.[5] It is not surprising that sperm oxidative stress is commonly identified in the idiopathic infertile male population. Even men with normozoospermic idiopathic infertility exhibit significantly greater seminal ROS production and lower antioxidant capacity compared with fertile men.[6] Therefore, the reduction of ROS activity in semen by the addition of scavenging agents might be a useful approach in treating male factor infertility. Some scavenging agents, such as *N*-acetylcysteine (NAC), have been tested in vitro or in vivo.[7-9] These substances have been shown to reduce the effects of ROS activity on sperm parameters, but their efficacy and clinical usefulness are still controversial.[10]

Owing to the enormous detrimental effects of ROS on sperm parameters, we used NAC in an attempt to lower ROS activity in the plasma. NAC has been used to treat or counteract various diseases and conditions, including a variety of respiratory illnesses, heart disease, smoking,

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heavy metal poisoning, and acetaminophen poisoning, as influenza prevention, epilepsy. It has also been shown to have immunologic functions.[11-14] In the present study, patients with idiopathic male infertility were given NAC (600 mg/d orally) for 3 months. The sperm parameters and oxidant/antioxidant status of the plasma were compared between these patients and a control group that did not receive NAC.

MATERIAL AND METHODS

The institutional review board of Infertility Central, Department of Urology, Harran University (Sanliurfa, Turkey) approved the present study. Institutional ethical committee approval was in accordance with the principles of the Declaration of Helsinki. All subjects provided informed consent.

Study Subjects

A total of 120 patients who attended our clinic were evaluated for idiopathic infertility. They had normal sperm parameters, as determined by medical history and physical and seminal examination findings, as initial evaluations. Infertile patients with well-known pathologic features such as varicocele, leukospermia, hormonal abnormalities, and/or obstruction were excluded from the study. The additional exclusion criteria included the presence of cryptorchidism, vasectomy, abnormal liver function, cigarette smoking, and alcohol consumption. The patients with idiopathic infertility with normal sperm parameters were included in the present study. Using sealed envelopes, these patients were randomly allocated to the study group (60 men) or control group (60 men). The patients in the study group were given NAC (600 mg/d orally for 3 months, and those in the control group received a placebo, with the pills containing sugar). The patients in the study and control groups were unaware of whether they were receiving the drug or placebo. After 3 months, the total antioxidant capacity (TAC), total peroxide (TP) level, and oxidative stress index (OSI) were determined to assess the oxidative status of the plasma. The sperm parameters were also evaluated. The results were compared between the study and control groups.

Collecting and Analyzing Samples

Semen Analyses. The median of 2 pre- and post-treatment semen (with ≥2-week intervals) analyses was evaluated. The semen samples were obtained by masturbation after 3-5 days of sexual abstinence. After liquefaction, the samples were analyzed according to the World Health Organization (1999) guidelines to determine the volume, pH, sperm concentration, motility, morphology, and viscosity. Semen viscosity was estimated by introducing a glass rod into the sample and measuring the length of the thread on withdrawal of the rod. Ejaculates with normal viscosity had a thread length of <2 cm, and those classified as hyperviscous had a thread length >2 cm.

Biochemical Analyses. For the biochemical analysis, the patients fasted for 10-12 hours before approximately 5 mL of blood was withdrawn from the cubital vein into heparinized tubes. The blood samples were centrifuged immediately at 3000 rpm for 10 minutes. The plasma was separated and stored at −80°C until analysis. TAC and TP were measured at the same time for all samples.

TAC Determination. The TAC of the plasma was measured using a novel automated colorimetric measurement method developed by Erel.[15] In this method, hydroxyl radicals, the most potent of the biologic radicals, are produced by the Fenton reaction, followed by their reaction with the colorless substrate *O*-dianisidine to produce the bright yellow-brown dianisyl radical. On addition of a plasma sample, the oxidative reaction initiated by the hydroxyl radicals present in the reaction mix is suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of the TAC of the plasma. This assay has excellent precision (coefficient of variation <3%),[16] and the results are expressed as mmol Trolox Eq/L.

TP Determination. The plasma TP concentration was determined using the FOX2 method,[17] with minor modifications.[18] The FOX2 method is based on the oxidation of ferrous ions to ferric ions by the various types of peroxides in the plasma samples. The ferric ion complexes with xylenol orange, and the absorbance of the colored product is measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulfate (9.8 mg) in 250 mM H_2SO_4 (10 mL) for a final concentration of 250 mM ferrous ion in acid. This solution was then added to 90 mL of high-performance liquid chromatography-grade methanol containing 79.2 mg of butylated hydroxytoluene. Finally, 7.6 mg of xylenol orange was added by stirring for 100 mL of the working reagent (250 mM ammonium ferrous sulfate, 100 mM xylenol orange, 25 mM H_2SO_4, and 4 nM butylated hydroxytoluene, in 90%% [vol/vol] methanol). The blank reagent was prepared as described for the working reagent, except that the ferrous sulfate was omitted. Plasma (200 mL) was mixed with 1.8 mL of FOX2 reagent. After incubation at room temperature for 30 minutes, the sample was centrifuged at 12 000*g* for 10 minutes, and the absorbance of the supernatant was measured at 560 nm. The TP content of the plasma was determined as the difference in absorbance between the test and blank samples, using a solution of hydrogen peroxide as the standard. The coefficient of variation for the individual plasma samples was <5%.

OSI Calculation. The percent ratio of the TP level to TAC level was accepted as the OSI. For calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated according to the following formula[18]: OSI (arbitrary unit) = TOS (μmol hydrogen peroxide Eq/L)/TAC (mmol Trolox Eq/L).

Statistical Analysis

Student's *t* test and correlation analyses were performed using the Statistical Package for Social Sciences for Windows, release 11.5 (SPSS, Chicago, IL), and $P < .05$ was considered statistically significant. The results are given as the mean ± standard deviation of the mean.

RESULTS

The study population included 120 patients, and the results are summarized in Table 1. No statistically significant differences were found between the study and control groups with regard to age (33.1 ± 4.5 vs 32.8 ± 3.7 years, respectively) or the duration of infertility (4.1 ± 1.7 vs 5.2 ± 2.8 years, respectively) and the initial semen parameters. NAC showed significant improving effects

Table 1. Oxidative status of plasma and sperm parameters stratified by group

Variable	Study Group (n = 60)	Control Group (n = 60)	*P* Value
Oxidative status			
TAC	1.34 ± 0.4	0.816 ± 0.21	<.001
TP	21.9 ± 3	28.5 ± 4	<.001
OSI	38.07 ± 0.4	72.04 ± 0.56	<.001
Sperm parameters			
Volume (mL)	2.48 ± 75	1.44 ± 51	<.05
Viscosity (thread length, cm)	1.99 ± 19	2.59 ± 31	<.001
Liquefaction time (min)	15.6 ± 4	20.4 ± 5	<.001
Concentration ($\times 10^6$/mL)	21.85 ± 1108	22.32 ± 22.00	NS
Motility (%)	31.29 ± 8.62	20.33 ± 8.43	<.05
Morphology (%)	26.37 ± 0.03	25.35 ± 0.2	NS

TAC, total antioxidant capacity; TP, total peroxide; OSI, oxidative stress index; NS, not significant.

on the semen parameters (volume, $P < .05$; motility, $P < .05$; and viscosity, $P < .001$) compared with the control group. These benefits of NAC were thought to be attributable to the reduction of ROS in the plasma and the reduction of semen viscosity. The number and morphology of the sperm did not differ significantly between the 2 groups. The TAC (1.34 ± 0.4 vs 0.816 ± 0.21, respectively; $P < .001$) was greater and the TP and OSI (21.9 ± 3 vs 28.5 ± 4, respectively, $P < .001$; and 38.07 ± 0.4 vs 72.04 ± 0.56, respectively; $P < .001$) were lower in the plasma of the study subjects than in the plasma of the control subjects.

COMMENT

In the present study, NAC had beneficial effects on some semen parameters (ie, volume, motility, and viscosity). These benefits of NAC were thought to be attributable to the reduction of ROS in the plasma and a reduction of semen viscosity. Free radical species cause oxidative damage to numerous different molecules in the cells, including membrane lipids, proteins, carbohydrates, and DNA, and have been implicated in the pathogenesis of many diseases. The physiologic generation of ROS in semen affects the functional properties of sperm.[3] In addition, ROS overproduction as a result of an imbalance between pro-oxidant and antioxidant levels is highly toxic to the sperm plasma membrane; thus, ROS may have a role in the etiology of defective sperm function. Experimentally induced high levels of ROS can damage sperm motility.[6] Because of the enormous detrimental effects of ROS on the sperm parameters, the present study was performed to determine the beneficial effects of NAC on the semen parameters and the oxidative/antioxidant status in idiopathic male infertility.

NAC, developed in the 1960s, is the *N*-acetyl derivative of the naturally occurring amino acid L-cysteine.[19] NAC is virtually nontoxic and is widely used to reduce the viscosity and elasticity of mucus because of its ability to reduce disulfide bonds. NAC has the potential to interact directly with oxidants and, as with many thiols (such as glutathione), is an excellent scavenger of hydroxyl radicals.[20] In addition to its scavenger function, a great deal of evidence has shown that NAC also promotes cellular glutathione production; thus, NAC might reduce or even prevent oxidant-mediated damage in cell cultures or animals.[21,22] In the present study, we administered NAC (600 mg/d orally) for 3 months, because we believe it to be a safe drug and its median lethal dose is very high. The results from animal studies have confirmed that the drug is neither teratogenic nor mutagenic.[23] An overdose of the drug causes a minimal allergic reaction, especially after intravenous administration, and no contraindications for its use have been demonstrated, other than known hypersensitivity to NAC.[24] None of the patients in the present study reported any side effects with use of the drug. A few reports have been published on the effects of NAC on human semen parameters in vitro and in vivo. Oeda et al.[7] incubated human semen samples with and without NAC (1.0 mg/mL) at room temperature and found that NAC improved total sperm motility and significantly reduced ROS, suggesting that NAC might improve the function of impaired sperm. Our findings were compatible with those of Oeda et al.[7] In a study examining the in vivo scavenging of ROS in human semen, Akiyama[8] assessed ROS generation and sperm function before and after NAC administration, concluding that although sperm density and sperm motility did not improve, sperm function tended to improve and the ROS level decreased significantly in human semen after NAC administration. In contrast to the study by Akiyama,[8] we found that sperm motility improved after NAC treatment. In addition, the sperm volume, viscosity, and liquefaction time were significantly improved in the NAC-treated group compared with the control group in the present study. In the study by Elzanaty et al.,[25] seminal viscopathy was associated with male infertility. Semen viscosity is important because spermatozoa can become tangled in the fibrous or mucoid mass of the semen, preventing their migration from the seminal plasma into the cervical track fluids and the site of fertilization. Siciliano et al.[26] reported severe impairment of both high- and low-molecular-weight antioxidative systems in hyperviscous semen. Owing to its ability to reduce disulfide bonds, NAC is widely used to reduce the viscosity and elasticity of mucus.[21] The proper viscosity

of both the semen in men and the cervical mucus in women is important for fertility. Badawy et al.[27] reported that NAC showed mucolytic activity and usually improved the character of cervical mucus without the need for an estrogen supply. In agreement with these findings, the results of the present study have indicated that the viscosity of sperm improved with NAC treatment.

NAC is virtually nontoxic and is widely used to reduce the viscosity and elasticity of mucus because of its ability to reduce disulfide bonds.[20] NAC also have been shown to reduce the effects of ROS activity in semen, which is a factor to reduce viscosity. Thus, we believe that the decreased viscosity might be responsible for the semen volume increase. However, to understand the real mechanism, additional studies should be performed. We also investigated the oxidative/antioxidant status in plasma after NAC administration. The TAC was greater and the TP and OSI were lower in the plasma after NAC treatment. Taken together, the results of the present study suggest that antioxidants such as NAC protect sperm from ROS damage while also reducing the production of oxygen radicals. However, the limitation of the present study was that the TAC was measured indirectly in the plasma rather than from the seminal plasma, which might be more accurate. However, we think the evaluation of plasma would be simpler and more practical in clinical use, especially during follow-up.

CONCLUSIONS

NAC might be useful in infertile patients through its beneficial effect on some semen parameters to protect sperm cells from peroxidative damage and improve their functional properties.

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