

N-acetyl cysteine's effect on semen parameters in a sample of Iraqi men with oligoasthenoteratozoospermia

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Abstract

Background: Infertility is recognized as the incapability of infertile couples to become pregnant following one year of unsafe intercourse, with male factors accounting for roughly half of the documented instances. Several reasons of male infertility factors have been reported; however, the actual cause in the majority of cases remains unknown.

Objective: To study prospectively the outcome of N-acetylcysteine on semen parameters in males with oligoasthenoteratozoospermia.

Patient and methods: A total 45 patients with idiopathic oligoasthenoteratozoospermia have received N-acetyl cysteine (NAC) for 12 weeks, their seminal fluid parameters were measured at the baseline and after 12 weeks.

Results: The results showed that after 3 months of NAC treatment, the motility was statistically significantly higher than pre-NAC baseline, with no statistically significant differences in both count and morphology.

Conclusion: These results confirmed that NAC has a positive effect in improving motility in infertile men and thus resulting in better spermatogenesis and sperm function.

Keywords: N-acetyl cysteine, seminal fluid parameters, male infertility, oligoasthenoteratozoospermia.

Introduction:

Infertility is recognized as the incapability of infertile couples to become pregnant following one year of unsafe intercourse, with male factors accounting for roughly half of the documented instances (1). Testicular dysfunction or reproductive tract obstruction are considered secondary to infertility (2). Several reasons of male infertility factors have been reported; however, the actual cause in the majority of cases is unknown (3). The majority of male infertility causes are linked to changes in the spermiogram's traditional characteristics, and the oligoasthenoteratozoospermia syndrome (OAT) has a combination of these changes (4). Low sperm concentration ($< 15 \times 10^6$ ml) and decreased motility are both symptoms of idiopathic OAT (progressive motility 32% and overall motility 40%), and improperly shaped spermatozoa (normal morphology of 30% by WHO 2021 guidelines or 4% by Kruger stringent criteria) in men without any sickness or probable risk factors such as varicocele or mumps that could impair their fertility (5, 6). N-acetylcysteine (NAC) is a precursor of L-cysteine, an amino acid, and glutathione (GSH), an antioxidant. (7). It is a powerful antioxidant with properties that can aid both male and female infertility (8).

Accordingly, the current research was designed with the goal of evaluating seminal fluid parameters in infertile men before and after NAC.

Patients and Methods

This prospective study was conducted at Al-Kut governorate, from November 2021 to April 2022. A total 45 infertile men (20-45) years of age for at least one year diagnosed as idiopathic oligoasthenoteratozoospermia according to the World Health Organization (WHO) classification (2010) were referred to the Center of Infertility in Al-Kut hospital and were subjected to this study. The control group included 20 apparently healthy fertile volunteers with normal seminal parameters according to the WHO 2010 (5). The Patients underwent further tests for diagnosis and management. They were not alcoholic and did not suffer any other significant systemic disorder such as diabetes, heart disease, kidney disease, or hepatitis. They had no hormonal imbalances which may interfere with the study parameters and impact the conclusion. The semen sample collection was done with a dry, sterilized, and warm disposable Petridish that is labeled with the patient's name, after an abstinence period of at least Two days and a total of Seven days of sexual abstinence. The Semen then is promptly transported to the laboratory, where it is kept at 37°C in an incubator (HERAEUS, Germany) until it liquefies. The sample

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was gently mixed after liquefaction, and 20 micro ml of the seminal fluid was diluted with 380 micro ml of semen diluents. The diluent was made by adding 50 g of NaHCO₃ with 10 ml of saturated formaldehyde solution at 40% concentration. After that, the ingredients were dissolved in distilled water and diluted to 1000 ml (dilution 1:20). Ten micro cc of the diluted sample was applied to the surface of the Neubauer hemocytometer under the cover slip after it had been mixed. After three minutes, the spermatozoa in the middle of 16 squares were counted, and the spermatozoa concentration was calculated using the following equation and expressed as million per ml. Spermatozoa concentration ($10^6/\text{ml}$) =

Number* dilution factor* multiplication (Dilution=20, Multiplication=10000)

A wet slide preparation or a fixed stained slide is required by the WHO method. Hematoxylin and eosin is generally the most often used stain, however the papanicolaou stain may also be used. On a slide, a 10 microliter drop of sperm was prepared. The wet slide preparation was used to determine the sperm morphology after placing a cover slip over the material. The average sperm head is oval in shape, with a 1.75 to 1.5 distance ratio (9). According to the 2010 WHO handbook, a morphologically "normal" sperm has a head (including acrosome), mid-piece, and tail. For example, a "normal" head is egg shaped with gentle contours. The acrosome is well-defined, clearly visible, stained uniformly bright blue and covering 30–60% of the sperm head's front portion. A "normal" mid-piece is 1 m wide and 1.5 times the length of the head, there are no cytoplasmic residues, and the head is axially connected to it without making a distinct angle. The tail which should be free of cytoplasmic residues is apically attached to the mid-post piece's acrosomal end, 45–50 m long, and without any acute bends (12). The motility of the spermatozoa was measured immediately after liquefaction, by placing a drop of the gently mixed seminal fluid on a clean slide and covering it with a cover slip, the slide was inspected microscopically (40x objectives) after two minutes to obtain the type of motility, and at least eight fields were examined.

The following are the categories of sperm motility according to the WHO definition (WHO, 2010):

- The term "progressive motility" refers to spermatozoa that are actively flowing, either in a straight line or in enormous circles, at any speed.
- Any form of mobility that is not progressive, referred to as "non-progressive motility", is moving in little

circles, the flagellar force hardly moving the head, or when only a flagellar can be observed.

- Immotility refers to a lack of movement.

Statistical analysis

SAS was used to do statistical analysis of the data (Statistical Analysis System - version 9.1). The significance of differences between means was determined using a one-way ANOVA and the Least Significant Differences (LSD) post hoc test. In addition, an independent t test was utilized to determine whether there was a significant difference between the two groups. Statistical significance is defined as a P value of less than 0.05. The validity of markers as disease indicators was determined using the receiver operation characteristic curve (ROC curve). The area under the curve was used to compare the markers. MedCalc Software was used to submit the analysis.

Results

Table 1 shows the mean of each group control, pre-NAC and post-treatment (600 mg per day) group (Asist plus, bilim®) for three consecutive months.

Table 1: Mean±SD for the studied parameters among the controls and the cases (Pre- and post-NAC)

Groups	Count	Motility	Morphology
Control	49.33±2.33a	37.00±1.07a	79.66±0.33a
Pre	35.87±3.44b	14.89±1.42c	63.22±3.37b
Post	44.18±3.12ab	25.60±1.80b	71.89±2.54ab
LSD	11.19	5.51	10.04

Means with a different letter in the same column are significantly different (P<0.05)

The mean sperm count for the control group was (49.3±2.33) compared to (35.9±3.44 and 44.2±3.12) for pre- and post-treatment values respectively; the results show that the difference between the mean count for the control group and pre-NAC values was significant (P<0.05). The mean count for the post-NAC value increased but did not differ significantly from the pre-NAC value. For motility, the mean for control group was (37.0±1.07), pre-NAC (14.9±1.42) and post-NAC (25.6±1.80) which is statistically significant from the pre-NAC value. As for the morphology, there is a statistically difference between the control group (79.7±0.33) and pre-NAC values (63.2±3.37), while there is no statistically significant difference with post-NAC value (71.9±2.54) as shown in figure 1.

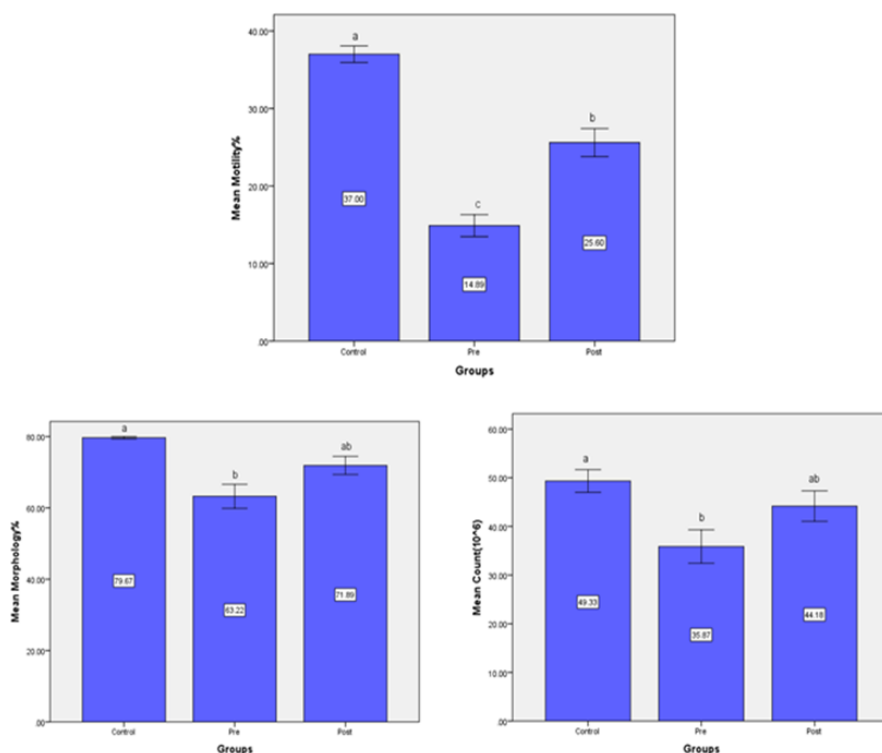


Figure 1: Seminal Fluid Analysis means in control, pre- and post-NAC groups

The result of seminal fluid analysis found that the motility was the best marker for diagnosis (figure 2, table 2) as it has the highest sensitivity (91.1%) and specificity (100%).

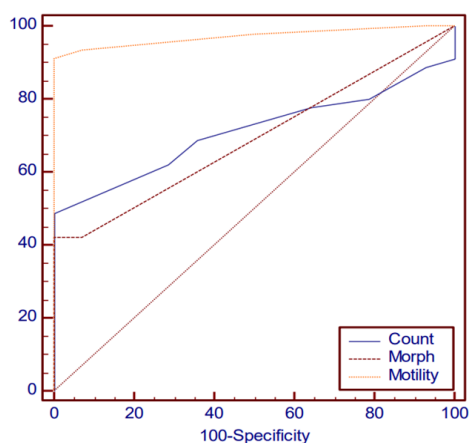


Figure 2: Seminal fluid analysis ROC curve

Table 2: ROC curve values of sensitivity, specificity and cut off point for Seminal Fluid Analysis

Variable	AUC	SE ^a	95% CI	Sensitivity	Specificity	Cut off
Count	0.713	0.0643	0.580 to 0.823	48.9	100	<= 30
Motility	0.690	0.00695	0.557 to 0.804	91.1	100	<= 25
Morphology	0.971	0.0199	0.890 to 0.997	42.2	100	<= 70

Discussion:

Many couples throughout the world are still concerned about infertility. Anti-sperm antibodies, sperm DNA damage, high levels of reactive oxygen species (ROS), and sperm dysfunction are all possible reasons of infertility in males with no obvious cause (10). Idiopathic male infertility, is characterized by an unexplained decline in semen quality; in other word infertility of unknown origin (11). High levels of free radicals have a deleterious impact on sperm parameters, leading to a reduction in the sperm count, motility, and typical appearance of sperm (12). The present study indicates that the administration of NAC (600mg once daily) over three months to patients with idiopathic oligoasthenoteratozoospermia can improve the parameters of seminal fluid analysis. Researchers looked at a variety of features in a three-month in vivo study using 600 mg of NAC per day, including chromatin unfavorable changes, all of the sperm parameters improved considerably after this therapy (13). The impact of reactive oxygen species (ROS) is fundamental in numerous activities of human spermatozoa under physiological settings. Semen, in fact, necessitates the involvement of ROS at various stages of maturation (14). However, ROS overproduction in the sperm of infertile males is well-documented, it has the ability to induce long-term oxidative damage to a wide range of biological molecules (proteins, nucleic acids, and polyunsaturated fatty acids in biological membrane lipids), affecting spermatozoa's activity and lifespan. (15). In the current study, NAC is used as an antioxidant to modulate the ROS levels and hence improve seminal fluid parameters. The results shown that the mean count of post-treatment group increased but did not differ significantly from the pre-NAC group. The mean of the post-NAC treatment group was significantly different from the pre-NAC group in terms of motility. The mean morphological results did not differ statistically from the post-treatment group. A study on 60 men who were given NAC (600 mg/d orally) for three months and 60 men as a control group who received a placebo showed that the NAC group had a significantly improved semen concentration, mobility, and texture. There were no significant differences in the quantity or shape of the sperm between the two groups (16). It is reasonable to target oxidative stress in the treatment of idiopathic infertility, whether through anti-oxidant supplements or otherwise. It has long been recognized as a potent antioxidant. NAC's antioxidant action in vivo can be attributed to at least three separate pathways, including: On particular oxidant species, it exerts a direct antioxidant effect which is due to its free thiol group's capacity to react with nitrogen species and reactive oxygen (17). This reducing agent can directly scavenge a variety of non-enzymatic processes by the formation of reactive oxygen and nitrogen species (ROS/RNS),

such as hypochlorous acid (HOCl), peroxyxynitrous acid (ONOOH), peroxy radical (RO₂•), hydroxyl radical (OH•), and hydrogen peroxide (H₂O₂), and could also affect the nitric oxide pathway (18). NAC's ability to function as a precursor of Cysteine (which has a lower intracellular concentration), NAC has an indirect antioxidant impact as a key component and rate-limiting step in glutathione formation (GSH) which is a well-known direct antioxidant and a substrate of various antioxidant enzymes. NAC's role as a cysteine and intracellular GSH prodrug is explained by this characteristic (19). A disulphide-breaking effect and the potential to replenish thiol pools, that modulate the redox state as another mechanism that contributes to NAC's indirect antioxidant activity in reduction ability.

Conclusion:

The results of the current study proved the positive effect of NAC in decreasing the ROS and restoring the oxidant balance level by its antioxidant effect resulting in better spermatogenesis and sperm function.

Authors' Contributions:

Fatima Sinan Salman designed, performed the experiments, and wrote the manuscript. Huda Ibrahim AlQadhi supervised the research. Baraa Abd Al Kareem collected the data.

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تأثير ن-أسيتيل سستين على معاملات السائل المنوي لدى عينة من الرجال العراقيين المصابين بوهن، إمساخ وقلة النطاف

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الخلاصة

المقدمة: يُعرّف العقم بأنه عدم قدرة الأزواج المصابين بالعقم على الحمل بعد عام واحد على الأقل من الجماع غير المحمي، حيث تمثل العوامل الذكورية نصف الحالات الموثقة تقريباً. تم تحديد عدة أسباب لعقم الذكور ولكن السبب الحقيقي في غالبية الحالات غير معروف.

الأهداف: دراسة تأثير ن-أسيتيل سستين على معاملات السائل المنوي في المرضى الذين يعانون من قلة، وهن وإمساخ الحيوانات المنوية.

المرضى والمنهجية: تلقى مجموع 45 مريضاً مصاباً بقلة، وهن وإمساخ الحيوانات المنوية مجهول السبب ن-أسيتيل سستين لمدة 3 أشهر وتم قياس معاملات السائل المنوي قبل وبعد 12 أسبوع من العلاج.

النتائج: أظهرت النتائج بعد 3 أشهر من استخدام علاج ن-أسيتيل سستين وجود فرق معنوي في حركة النطف مقارنة بالنتائج قبل استخدام العلاج مع عدم وجود فرق معنوي في عدد وشكل الحيوانات المنوية.

الاستنتاج: أكدت النتائج التأثير الايجابي ل ن-أسيتيل سستين في تحسين حركة الحيوانات المنوية في المرضى المصابين بالعقم مما يؤدي الى تحسين تكوين الحيوانات المنوية ووظيفتها.

الكلمات الرئيسية: ن-أسيتيل سستين، معاملات السائل المنوي، عقم الرجال، وهن، إمساخ وقلة النطاف.