Original Article

Study of reduced glutathione in seminal plasma and spermatozoa nuclear chromatin decondensation test (NCDT) in human subjects with different fertility potential.

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Context: The nuclear chromatin decondensation test (NCDT) detects the ability of the sperm chromatin to undergo decondensation following fertilization. It is known that there is a positive relationship between concentrations of seminal plasma reduced glutathione and male fertility.

Aims: In this study, we try to evaluate correlation between seminal plasma glutathione and in-vitro sperm nuclear decondensation (NCDT).

Methods and Material: The present study was done on 80 male patients of 21-58 yrs of age which were divided into three groups i.e. Group I: Controls- fertile men (n=30) ; Group II: Normozoospermic infertile men (n=30) ; Group III: Oligozoospermic infertile men (n=20). Levels of the reduced glutathione were measured and spermatozoa nuclear chromatin decondensation test (NCDT) was done. These two parameters were then correlated.

Statistical analysis used: ANOVA test and Bonferroni's post test. Coefficient of correlation (r-value) was found to find relationship between different parameters.

Results: Reduced glutathione level (GSH) and NCDT score was significantly more in group 1 as compared to the other groups. Coefficient of correlation between reduced glutathione level (GSH) and NCDT score 0.62, 0.57 and 0.71 respectively in group 1, group 2 and group 3.

Conclusions: The ability of the sperm chromatin to undergo decondensation following fertilization is affected by reduced glutathione level in seminal plasma.

1. Introduction

Nuclear chromatin decondensation test (NCDT) is used to evaluate the ability of the spermatozoa to decondense following fertilization. During sperm nuclear condensation, in spermiogenesis, the disulfide bond between the cysteine molecules of sperm protamines is formed [1]. The ability of the sperm to decondense in vitro reflects the ability of the sperm to form male pronucleus in the wake of fertilization. NCDT has been recommended to detect the fertility status of human semen. Samples [2]. The male factor is considered a major contributory factor to infertility. Apart from the conventional causes for male infertility such as varicocele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma and tumors, a new and important cause has been identified and that is oxidative stress [3]. Oxidative stress is a condition associated with an increase rate of cellular damage. Oxidative stress arises as a consequence of excessive ROS production and/or impaired antioxidants defence mechanisms [3,4,5]. Owing to their deleterious effects on human spermatozoa, excessive ROS must be continuously inactivated. These reactive oxygen species (ROS) have been implicated as a major contributory factor in male infertility, as 40% of infertile men have detectable amounts of ROS in their semen, whereas no ROS activity in the semen of fertile men. Given that male factor problems make up the largest single cause of infertility, the role of antioxidants in male infertility has become very important [3,4].

So with this study, we had taken an attempt to find out how antioxidant affect the ability of the sperm to form male pronucleus in the wake of fertilization (assessed by NCDT test)? In the present
we had studied reduced glutathione level and NCDT score in males with different fertility potential. Our aim was evaluate relation between seminal plasma glutathione and in-vitro sperm nuclear decondensation (NCDT).

2. Materials and Methods

Semen samples were obtained from 80 male patients of 21-58 yrs of age with complaints of infertility (both primary and secondary infertility cases) referred to the infertility laboratory of Physiology department of Government medical college Nagpur and Control-healthy, adult, fertile, men in the age group of 21-58 yrs of age selected from the staff and their relatives of Government Medical College hospital, Nagpur. Detailed history of present and past illness as well as medical and surgical management was taken. Selected male partners were undergone thorough surgical examination of genito-urinary system to rule out the exclusion criteria. Subjects with normally developed genito-urinary organs were included in the study. An appointment for semen analysis was given to the patients who were referred from various departments of the hospital.

The subjects belonging to the group II and III were those having normal semen parameter, to which semen parameter of group I was used as control. Group II: Normozoospermic infertile men [2x10^6 spermatozoa/ml] (n=30) referred from various departments of Government Medical college hospital, Nagpur (n=30)

Group III: Oligozoospermic infertile men [<2x10^6 spermatozoa/ml] referred from various departments of Government Medical college hospital, Nagpur (n=20)

The subjects belonging to the group II and III were those having no issues in-spite of at-least one year of unprotected inter-course.

2.1. Exclusion criteria

1. Persons with occupation near hot furnace or in chemical industries using the substances like benzene or aniline dyes, which are known to produce alterations in spermatogenesis.
2. Patients with azoosperma as the nuclear chromatin decondensation test cannot be carried out.
3. Persons with history of drug addiction, smoking and alcohol intake.
4. Persons with previous history of hydrocele, varicocele, hernia and operations on genital tract.

The semen sample was centrifuged (3000 rpm for 10 minutes) to separate the plasma. The plasma is used to measure reduced glutathione and spermatozoa pellets were used to perform nuclear chromatin decondensation test (NCDT).

2.2. Method of Estimation of reduced Glutathione (GSH)

Reduced glutathione (GSH) is estimated by methods based on principles of methods of Moron et al [6]. 0.5 ml of seminal plasma was taken in a test tube and 2ml of distilled water was added, mixed well. Then centrifuged for 5 min. at 5000rpm. 0.5 ml of supernatant was taken, to which 0.5ml of TCA (5%) was added and then centrifuged for 10 min. at 10,000 rpm.

0.5 ml of supernatant was taken to which 2.5ml of phosphate buffer (pH 8) was added. To this 1ml DTNB was added. This solution was inverted for 3 times to mix. The absorbance was read on spectrophotometer at 412nm within 4 min. of preparing the mixtures. Standard graph of reduced glutathione GSH concentrations was plotted. Determination of reduced glutathione GSH concentration in seminal plasma were done from the graph.

2.3. Nuclear chomatid decondensation test (NCDT):[7,8]

The test detects the ability of the spermatozoal nuclear material to decondense in vitro. The semen sample is centrifuged (400 g for 15 minutes) to separate the plasma. The pellets were washed in 0.05 M borate buffer twice. Nine volumes of 6 mM EDTA and 1% SDS mixture are added to one volume of sample. The equal volume of 2.5% glutaraldehyde in 0.05 M borate buffer is added. It is incubated at 37°C for 60 minutes. A drop of this mixture is transferred to pre-cleaned glass slide and covered with coverslip to observe under phase optics (400X). The numbers of condensed and decondensed heads are counted and their percentage is calculated.

Statistical analysis was done using ANOVA test and Bonferroni's post test and P values < 0.05 were taken as significant. (p < 0.05 was considered statistically significant). The relationship between different parameters was tested by calculating coefficient of correlation (r value). All the calculations were done by using Graph pad prism 5 software. Approval for the above study was taken from institutional ethics committee.

3. Results

The present study was done on 80 male subjects including controls in the age group 21-58 yrs. Significant decrease was seen in reduced glutathione level in group 2 and group 3. When intergroup comparison was done between group 2 and 3, Group 3 showed significant decrease. In the NCDT score, significant decrease was noted only in group 3. Group three also showed significant decrease. In the NCDT score, significant decrease was noted only in group 3. Group three also showed significant decrease when compared with group 2. When the values of reduced glutathione were correlated with NCDT test, results showed significant positive correlation. The r value was found to be 0.62, 0.57 and 0.71 respectively in group 1, group 2 and group 3. The correlation was found to be very highly significant in the group 1, group 2 and group 3 (p<0.001).
The male factor is considered a major contributory factor to infertility. Apart from the conventional causes for male infertility such as varicocele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma and tumors, a new and important cause has been identified: oxidative stress [3]. Antioxidant like reduced glutathione help to reduce the effect of oxidative stress. These antioxidants can be defined as Substances that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate. Our hypothesis behind the study was that decreasing seminal plasma antioxidants levels could have significant role in etiology of impaired sperm function. Present findings clearly demonstrate the importance of reduced glutathione (GSH) in the maintenance of sperm DNA integrity (NCDT test). Antioxidants like reduced glutathione prevent the structure of DNA of spermatozoa from damage.

Our result are in accordance with the various studies [9,10,11,12,13]. Spermatozoa utilizes the reduced glutathione present in seminal plasma to maintain its motility, viability, mitochondrial status, oocyte binding capacity and fertilizing capability [9,11]. Decreased reduced glutathione concentration in seminal plasma causes reduced antioxidant activity resulting in disruption in the membrane integrity of spermatozoa as consequence of increased oxidative stress [14,15]. Chen et al [16] found that decreased concentration of seminal plasma reduced glutathione positively correlated with sperm mitochondrial DNA. Thus NCDT test is definitely affected by the levels of the reduced glutathione and decreasing seminal plasma glutathione levels could have significant role in etiology of impaired sperm function however in one study there was no significant relationship between sperm DNA damage and total antioxidant capacity, suggesting other mechanisms for sperm dysfunction [17]. Also Donnelly et al[18] found no significant effect of addition of reduced glutathione to sperm preparation medium on sperm progressive motility or baseline DNA integrity.

Similarly other antioxidant may also play the role in maintain the sperm function [19,20]. Sierens [19] et al found significant positive effects of ascorbic acid levels in seminal plasma on DNA integrity of spermatozoa. So in the future we recommend to find out correlation with other antioxidant level so as to find role of other antioxidant. Present findings clearly demonstrate the importance of reduced glutathione (GSH) in the maintenance of sperm DNA integrity (NCDT test), thus antioxidants could have an important therapeutic role to play in the clinical management of male infertility [3,21].

5. References


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