Introduction

The male factor infertility with defective sperm function is seen in about 24% of infertile couples. The common morphological features suggestive of abnormal sperm functions are decreased sperm count, i.e., oligospermia and decreased sperm motility. The cause of oligospermia and asthenozoospermia are multifactorial and not completely understood but the most commonly associated finding is increased levels of reactive oxygen species (ROS) in seminal fluid. The ROS are hydrogen peroxide, the superoxide anion (O$_2^-$), the hydroxyl radical (OH$^-$), and hypochlorite radical (OHCl$^-$). The normal sperm function (maturation, capacitation, acrosomal reactions, and fertilization) is associated with controlled release of ROS, but excessive levels cause sperm dysfunction. Immature, morphologically abnormal spermatozoa and seminal leukocytes are the main sources of ROS in human ejaculates. The membrane damage by lipid peroxidation caused by ROS is indicated by the high level of their by-product malondialdehyde (MDA) in semen. The antioxidant system in semen is mainly provided by the antioxidant system and reactive oxygen species with clinical semen parameters in infertile men.

Aims and Objectives: To determine the correlation of antioxidant system and reactive oxygen species with clinical parameters in infertile semen samples. Materials and Methods: Semen sample of fifty infertile men were divided into three groups: (1) Group I - Normospermic (count >15 million/ml), Group II - Asthenospermic (motility <32%), and Group III - Oligospermic (counts <15 million/ml) subjects based on the sperm count and sperm motility. The samples were also divided into two groups: (1) Group IV with semen pH >7.2 (25 samples) and Group V - Semen pH <7.2 (25 samples). The grouping was based on the WHO guideline for semen analysis. The semen antioxidant parameters like glucose-6-phosphate dehydrogenase (G-6-PDH) (spectrophotometric method Kornberg and Horecker, 1955), catalase (Maehly and Chance 1954), glutathione peroxidase (GPX) (Rotruck method), glutathione (GSH) (dithiobisnitrobenzoate method), superoxide dismutase (SOD) (direct method), and malondialdehyde (MDA) (thiobarbituric acid reactive substances assay kit method) were investigated. Mann-Whitney U-test was applied to compare the findings. Results: Of fifty semen samples there were 12 normospermic (sperm concentration ≥15 × 10$^6$/ml of ejaculates), 24 asthenospermic (sperm motility ≤32%), and 14 oligospermic (sperm concentration ≤15 × 10$^6$/ml of ejaculates) subjects. Results suggested that all asthenospermic males were found to have reduced motility and viability when compared with normospermic and oligospermic subjects. Activity of antioxidant parameters such as G-6-PDH, GPX, GSH, and SOD was decreased in case of asthenospermic subjects. The concentration of MDA was increased significantly (P < 0.001) in semen of asthenospermic subjects compared to normospermic and oligospermic subjects. Conclusion: The current study concludes that there is a significant relationship of ROS and semen parameters. Further studies will be needed in such subjects regarding role of effectiveness of dietary antioxidants in improving semen qualities.

Keywords: Antioxidant system, infertile, reactive oxygen species, semen parameters
by seminal fluid which comprises nonenzymatic antioxidants like Vitamin C and E, hypotaurine, taurine, L-carnitine, lycopene[18] and enzymes like superoxide dismutase (SOD), catalases, glutathione peroxidase (GPX) glucose-6-phosphate dehydrogenase (G-6-PDH), glutathione (GSH).[19] The antioxidant system scavenges the ROS and thus reduces abnormal spermatogenesis, DNA fragmentation, premature spermatogenesis, and cryodamage to spermatozoa during freezing. The dietary antioxidants improve semen quality in smokers and improve assisted reproductive technique results in oligoasthenospermic males.[19]

Aims and objectives

The primary objective of the present clinical investigation is to determine whether there exists a relationship between the antioxidant system and ROS with clinical parameters in seminal plasma of infertile men.

Materials and Methods

A case–control study was designed. Following Institutional Review Board approval, the semen samples were collected from the case and the control groups. Total fifty semen samples of infertile males attending the infertility clinic were collected into sterile plastic containers by masturbation after an abstinence period of 3–5 days and were analyzed within 1 h of collection. Semen analysis was carried out to measure sperm concentration, sperm motility, and sperm morphology. Samples with a leukocyte concentration >10⁶/ml of ejaculate and specimens with hyperviscosity were excluded from this study. Semen sample of fifty infertile men was divided into three groups: Group I - Normospermic (count >15 million/ml), Group II - Asthenospermic (motility <32%) and Group III - Oligospermic (counts <15 million/ml) subjects based on the sperm count and sperm motility. The samples were also divided into two groups: Group IV with semen pH >7.2 (25 samples) and Group V - Semen pH <7.2 (25 samples). The grouping was based on the WHO guideline for semen analysis.[10] The semen antioxidant parameters such as G-6-PDH (spectrophotometric method Kornberg and Horecker, 1955), catalase (Maehly and Chance 1954), GPX (Rotruck method), GSH (dithiobisnitro-benzoate method), SOD (direct method), and MDA (thiobarbituric acid reactive substances assay kit method) were investigated.

Statistical analysis

Data are presented as mean ± standard deviation. All hypothesis tests were two-tailed with statistical significance assessed at the P < 0.001 level with 95% confidence intervals.

| Parameter               | Normospermic (n=12) | Asthenospermic (n=24) | Oligospermic (n=14) | With pH >7.2 (n=25) | With pH <7.2 (n=25) |
|-------------------------|----------------------|-----------------------|---------------------|---------------------|---------------------|
| Volume (ml)             | 3.2±0.8              | 2.4±0.7               | 1.8±0.5             | 2.7±1.1             | 2.2±0.9             |
| Sperm count (million/ml)| 56.55±17.7           | 42.67±10.2            | 12.11±3.2           | 49±14.5             | 34±18.2             |
| Sperm motility (%)      | 54.55±6.2            | 18.09±8.1             | 38±12.7             | 48.76±4.4           | 34±5.8              |

Results

Of fifty semen samples, there were 12 normospermic (sperm concentration ≥15 × 10⁶/ml of ejaculates), 24 asthenospermic (sperm motility ≥32%), and 14 oligospermic (sperm concentration ≥15 × 10⁶/ml of ejaculates) subjects. The mean volume in normospermic males was 3.2 ± 0.8 ml whereas in asthenospermic and oligospermic samples the volume was 2.4 ± 0.7 ml (P = 0.05) and 1.8 ± 0.5 ml (P < 0.001), respectively. Due to distribution bias, significant difference in sperm count and motility was noted between normospermic and oligospermic group and normospermic and asthenospermic groups respectively. A significant difference was noted in motility between Group IV and Group V (P < 0.001) [Table 1].

The levels of G-6-PDH were significantly reduced in asthenospermic subjects and significantly raised in oligospermic samples (P < 0.001). No significant change was observed in catalase levels in all three groups. The other antioxidants such as GPX, SOD, and GSH were declined in asthenospermic subjects compared to oligospermic and normospermic subjects (P < 0.001). However, the activity of GPX and SOD were increased in oligospermic subjects as compared to normospermic subjects. The variations observed were significant (P < 0.001) [Table 2].

On application of Mann–Whitney U-test between different groups, significant differences were noted in all seminal parameters antioxidant system and MDA levels in Groups I and II. Between Group II and Group III only GPX, GSH, and SOD were significantly altered. While Groups I and III showed significant differences in motility, catalases, GPX, SOD, and MDA levels, no significant differences were noted on comparing Group IV and Group V [Table 3].

Discussion

From the outcome of this study, it is evident that there is a strong underlying correlation between the antioxidant system and the clinical semen parameters. The activity of G-6-PDH was decreased in asthenospermic subjects and increased insignificantly in oligospermic subjects and semen of subjects with PH <7.2.
In 2007 Khosrowbeygi and Zarghami compared the values of total antioxidant capacity (TAC), free 8-isoprostane and activities of catalase and SOD of 46 abnormal seminal parameter samples (asthenozoospermia, asthenoteratozoospermia, and oligoasthenoteratozoospermia) with normozoospermic males. In their results, there was no change in SOD levels, but level of TAC and catalase activity was significantly lower in cases compared with the control group.\(^{16}\) Similarly, Samant \textit{et al}. in 2009\(^{17}\) compared MDA concentration, SOD and catalase activity between thirty normozoospermic and thirty oligoasthenozoospermic samples. The MDA levels were increased with a significant reduction in catalases and SOD activity was noted in oligoasthenozoospermic group. Hosseinzadeh Colagar \textit{et al}.\(^{21}\) correlated the levels of TAC and MDA concentration with semen parameters in 46 samples. The seminal plasma TAC level had a significantly positive correlation with sperm count, motility, and morphology. In contrast, MDA levels in normozoospermic men were significantly lower than in asthenoteratozoospermic men (\(P = 0.049\)) and oligoasthenoteratozoospermic men (\(P = 0.001\)) and had a negative correlation with sperm count, motility, and morphology. A larger study was conducted by Shi \textit{et al}.\(^{22}\) over 225 infertile men. Compared with fertile men, seminal plasma TAC in other infertile groups was significantly lower (\(P < 0.01\)). There were significantly made positive correlation between seminal plasma TAC and sperm density (\(r = 0.182, P < 0.05\), as well as sperm with grade a (\(r = 0.150, P < 0.05\)).

MDA is the end product of polyunsaturated lipids peroxidation by ROS. Hence, the high level of MDA concentration reflects the lipid peroxidation in sperm cell member and also inhibits the sperm motility and viability. This reactive aldehyde causes toxic stress in sperm cells by generating aldehyde ion.\(^{10,11}\) In the current study, the concentration of MDA was increased in asthenozoospermic and oligozoospermic subjects compared to normozoospermic subjects and increased in subjects with semen pH <7.2 as compared to subjects with semen pH >7.2. Similar results were observed by Samant \textit{et al}.\(^{16}\) and Hosseinzadeh Colagar \textit{et al}.\(^{17}\) In a clinical review Sharma and Agarwal\(^{21}\) concluded that MDA concentration exhibits an excellent inverse relationship with the seminal plasma TAC concentration exhibits an excellent inverse relationship with sperm concentration.
the sperm-oocyte fusion and a direct relationship with sperm morphology.

**Conclusion**

The present study mainly pointed the fact that a strong correlation exists between the antioxidant system in the seminal plasma of asthenospermic subjects and clinical semen parameters. However, it could not find such a relationship in oligospermic and normospermic subjects in clinical infertility.

**Limitation**

The study population was based on a clinic-based cohort of infertile males thereby excluding any comparisons with fertile males. Although the study clearly demonstrates the altered antioxidant system and raised MDA levels in asthenozoospermic and oligospermic males, it lacks in implementing these findings in clinical scenarios to improve seminal parameters in such cases.

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**Conflicts of interest**

There are no conflicts of interest.

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