

Relationship of antioxidant system and reactive oxygen species with clinical semen parameters in infertile men

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ABSTRACT

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 (12). 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) (dithiobisnitro-benzoate 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 $\geq 15 \times 10^6$ /ml of ejaculates), 24 asthenospermic (sperm motility $\leq 32\%$), and 14 oligospermic (sperm concentration $\leq 15 \times 10^6$ /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

Introduction

The male factor infertility with defective sperm function is seen in about 24% of infertile couples.^[1,2] The common morphological features suggestive of abnormal sperm functions are decreased sperm count, i.e., oligospermia and decreased sperm motility.^[3] 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.^[4,5] The ROS commonly produced by human spermatozoa (as well as leukocytes) are hydrogen peroxide, the superoxide anion ($\bullet\text{O}_2^-$), the hydroxyl radical ($\text{OH}\cdot$), and hypochlorite radical ($\text{OHCl}\cdot$).^[6,7] The normal sperm function (maturation, capacitation, acrosomal reactions, and fertilization) is associated with controlled release of ROS, but excessive levels cause sperm dysfunction.^[8] Immature, morphologically abnormal spermatozoa and seminal leukocytes are the main sources of ROS in human ejaculates.^[9] The membrane damage by lipid peroxidation caused by ROS is indicated by the high level of their by-product malondialdehyde (MDA) in semen.^[10,11] The antioxidant system in semen is mainly provided

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by seminal fluid which comprises nonenzymatic antioxidants like Vitamin C and E, hypotaurine, taurine, L-carnitine, lycopene^[5] and enzymes like superoxide dismutase (SOD), catalases, glutathione peroxidase (GPX) glucose-6-phosphate dehydrogenase (G-6-PDH), glutathione (GSH).^[12] 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.^[13]

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 $>10^6$ /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.^[14] 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 \pm standard deviation. Standard *t*-test was applied. The results were analyzed using SigmaStat

3.5 Version software (Systat Software Inc). Mann-Whitney U-statistic was used for comparison between different groups. All hypothesis tests were two-tailed with statistical significance assessed at the $P < 0.001$ level with 95% confidence intervals.

Results

Of fifty semen samples, there were 12 normospermic (sperm concentration $\geq 15 \times 10^6$ /ml of ejaculates), 24 asthenospermic (sperm motility $\geq 32\%$), and 14 oligospermic (sperm concentration $\geq 15 \times 10^6$ /ml of ejaculates) subjects. The sample was also divided into two groups with semen pH >7.2 (25 samples) and semen pH <7.2 (25 samples). 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

Table 1: Semen parameters in each group

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 \pm 0.8	2.4 \pm 0.7	1.8 \pm 0.5	2.7 \pm 1.1	2.2 \pm 0.9
Sperm count (million/ml)	56.55 \pm 17.7	42.67 \pm 10.2	12.11 \pm 3.2	49 \pm 14.5	34 \pm 18.2
Sperm motility (%)	54.55 \pm 6.2	18.09 \pm 8.1	38 \pm 12.7	48.76 \pm 4.4	34 \pm 5.8

Table 2: Antioxidant parameters

Sperm types	Antioxidant parameters					
	G-6-PDH (IU/L)	Catalase (U/mg of protein)	GPX (U/mg of protein)	GSH (mg/dl)	SOD (U/mg of protein)	MDA (nmoles/ml)
Normospermic (12)	24.62±0.73	2.07±1.45	72.07±0.67	9.01±3.63	6.57±0.33	1.61±0.41
Asthenospermic (24)	18.99±0.58	2.28±1.42	60.88±0.99	6.26±0.10	6.43±0.21	2.11±0.72
Oligospermic (14)	26.57±3.92	2.77±2.41	116.18±2.23	6.62±1.41	1.46±0.45	2.89±0.58

G-6-PDH: Glucose-6-phosphate dehydrogenase; GPX: Glutathione peroxidase; GSH: Glutathione; SOD: Superoxide dismutase; MDA: Malondialdehyde

Table 3: Comparison between different groups

Comparison between	Number of samples	Clinical and antioxidant parameters	Mann-Whitney U-statistic		
			U	T	P
I and II	36	Motility	8.00	358.00	<0.001
		Viable count	-	3.696	<0.001
		G-6-PDH	-	5.81	<0.001
		Catalase	288.00	78.00	<0.001
		GPX	0.00	366.00	<0.001
		GSH	18.00	228.00	<0.001
		SOD	-	11.03	<0.001
		MDA	288.00	78.00	<0.001
II and III	38	Motility	266.00	215.00	0.078
		Viable count	91.50	349.50	0.021
		G-6-PDH	171.00	270.00	0.939
		Catalase	141.00	300.00	0.416
		GPX	-	25.95	<0.001
		GSH	12.00	354.00	<0.001
		SOD	0.00	441.00	<0.001
		MDA	-	3.89	<0.001
I and III	26	Motility	12.00	234.00	<0.001
		Viable count	132.00	114.00	0.013
		G-6-PDH	60.00	186.00	0.225
		Catalase	-	7.06	<0.001
		GPX	168.00	78.00	<0.001
		GSH	249.00	192.00	0.014
		SOD	166.00	80.00	<0.001
		MDA	-	0.174	<0.001
IV and V	50	Motility	144.00	806.00	0.001
		Viable count	145.50	804.50	0.001
		G-6-PDH	208.00	742.00	0.043
		Catalase	478.50	471.50	0.001
		GPX	300.00	650.00	0.816
		GSH	207.00	743.00	0.041
		SOD	299.50	650.50	0.808
		MDA	-	2.805	0.007

G-6-PDH: Glucose-6-phosphate dehydrogenase; GPX: Glutathione peroxidase; GSH: Glutathione; SOD: Superoxide dismutase; MDA: Malondialdehyde

as compared to the semen of subjects with pH >7.2. In case of other antioxidants such as GPX, SOD, and GSH were declined in asthenospermic subjects compared to oligospermic and normospermic subjects. However, the activity of GPX and SOD were increased in oligospermic subjects as compared to normospermic subjects. Whereas the activity of catalase was not found increasing significantly in asthenospermic subjects and oligospermic subjects as compared to normospermic subjects. Moreover, the activity of catalase was increased in subjects with semen pH <7.2 as compared to subjects with semen 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 as compared with the control group.^[15] Similarly, Samant *et al.* in 2009^[16] 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 *et al.*^[17] 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 normospermic men were significantly lower than in asthenoteratospermic men ($P = 0.049$) and oligoasthenoteratospermic men ($P = 0.001$) and had a negative correlation with sperm count, motility, and morphology. A larger study was conducted by Shi *et al.*^[18] 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 ($r = 0.150, P < 0.05$).

Although significant correlation has been between low TAC levels and abnormal semen parameters, significant benefits of dietary antioxidants has not yet been established. *In vitro*, the sperm damage by exogenous oxidants has been prevented by antioxidant supplements, but their effect on endogenous ROS is still under debate.^[19,20]

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 asthenospermic and oligospermic subjects compared to normospermic 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 *et al.*^[16] and Hosseinzadeh Colagar *et al.*^[17] In a clinical review Sharma and Agarwal^[21] concluded that MDA concentration exhibits an excellent inverse relationship with

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 finding 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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