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EFFECTS OF OXIDATIVE STRESS AND HEAVY METALS OF MALE FERTILITY

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Abstract

Defective sperm function is the most common cause of male infertility. The major causative factor of this process is the presence of oxidative stress which induce lipid peroxidative damage to the sperm membrane. This process was partially evaluated by the assay of superoxide dismutase (SOD) activity and measurement of reduced glutathione (GSH) and malondialdehyde (MDA) levels. Many metals are discharged as environmental pollutants and may affect semen profiles. So, iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and cadmium (Cd) as well as the proteins pattern of both seminal plasma and spermatozoal homogenate supernatant were also evaluated. The present study includes 70 normospermic individuals (control, 32.4%) and 146 infertile men. The latter group were further classified according to the defects in their semen parameters into asthenozoospermic (n= 48), oligo-asthenozoospermic (n= 18), oligo-astheno-teratozoospermic (n= 41) and azoospermic (n= 39) groups. Significance of difference among the groups and coefficient of correlation between the parameters were tested statistically. The protein contents of seminal plasma were nearly the same in all groups but that of the spermatozoa were highly significantly increased (P<0.01) when compared to control. The spermatozoal proteins were correlated with their abnormalities (r= 0.78), densities (r= -0.5), and motilities (r= -0.7). On the other hand, MDA levels were highly significantly increased and the GSH levels were highly significantly decreased in both seminal plasma and spermatozoal

homogenate supernatant of the infertile men comparing with control. Also, they were correlated with the quality of the spermatozoa. Concerning SOD activity, it was highly significantly decreased (P<0.01) in seminal plasma but was highly significantly increased (P<0.01) in spermatozoal homogenate supernatant of the oligo-astheno-teratozoospermic males when compared to control. In conclusion, the parameters of oxidative stress and the levels of the heavy metals were associated with human male infertility and may be useful tools in predicting semen quality.

Keywords:
Human male infertility, malondialdehyde, superoxide dismutase, reduced glutathione, divalent heavy metals.

Introduction

Infertility affects 15% of couples and in 30% of these couples, the cause of infertility is associated with aberrations found in the male partner, termed male infertility. Defective sperm function is the most common cause of male infertility [(36)] . One of the factors that potentially can cause spermatozoal dysfunction is the oxidative stress [(27, 38)] .

Oxidative stress (OS) is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants. These oxidative molecules commonly known as reactive oxygen species (ROS) which are produced primarily by the physiological metabolism of O_2 in cells under aerobic conditions [(2)] . Spermatozoa, like all cells living in aerobic conditions, constantly face the oxygen that is required to support life, but its metabolites such as ROS can modify cell functions. Hence, ROS must be continuously inactivated. So, it is not surprising that a battery of different antioxidants is available to protect spermatozoa against oxidants [(51)] . Normally a balance is maintained between the amount of ROS produced and that scavenged by antioxidant [(33)] . Seminal OS arises when this equilibrium is disturbed as a consequence of excessive production of ROS and/or impaired antioxidant defense mechanisms

[6] . In this case, the antioxidant systems cannot eliminate the increased ROS leading to sperm malfunction and infertility [33] .

Spermatozoa are susceptible to OS-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids as well as high density of mitochondria in the midpiece of spermatozoa which may leak oxygen radicals beside that their cytoplasm contains low concentrations of scavenging enzymes. In addition, the intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing spermatozoa to supplement their limited intrinsic antioxidant defenses by depending on the protection afforded by the seminal plasma [51] .

In fact, ROS have beneficial or detrimental effects on sperm functions depending on the nature and concentration of the ROS involved, as well as the moment and the location of exposure [19] . Low levels of ROS play a key-role in physiological processes such as capacitation, hyperactivation, the acrosome reaction, and fertilization. However, excessive production of ROS can inflict severe damage to spermatozoa [2] . Excessive generation of seminal ROS can be produced by immature spermatozoa, such as immotile or morphologically abnormal spermatozoa and morphologically normal but functionally abnormal spermatozoa, **Plante *et al.*,** [50] and also by leukocytes due to inflammation in the seminal tract or lower urinary tract [46] .

Lipid peroxidation (LPO) of sperm membrane is considered to be the key mechanism of this ROS-induced sperm damage leading to loss of the germinating ability or infertility [5] . Malondialdehyde (MDA) is an end product of LPO and considered as one of the important markers of OS. High levels of MDA represent high LPO rate [59] .

Protection against ROS and prevention of other damage are of critical importance, and can be provided by both enzymatic [superoxide dismutase (SOD)] and non-enzymatic [reduced glutathione (GSH)] antioxidants. GSH plays a central role in the defense against oxidative damage and toxins due to its ability to react directly with ROS by its free sulphydryl groups and GSH with GSH-related enzymes might play a role in sperm quality [23] . Also, SOD is

a very important antioxidant enzyme that involved in the inhibition of sperm LPO. It catalyzes the dismutation of the highly reactive superoxide anion radical ($O_2^{\cdot-}$) to form O_2 and H_2O_2 [13] .

The general population is exposed to metals at low concentrations either voluntarily through supplementation or involuntarily through intake of contaminated food and water or contact with contaminated soil, dust, or air [16, 9] . Human and animal evidence suggests that these metals may have adverse impacts on male reproductive health at relatively low levels leading to infertility. For example, Cd has been linked to poor human semen quality and DNA damage [57, 61] . While several other metals, such as zinc, copper and manganese are essential for good health but may be harmful above certain levels [32, 35, 8] . For example, Mn and Cu which act as cofactors for a variety of important enzymes, have been associated with reduced semen quality in humans [57, 39, 60] . On the other hand, low doses of metals such as Cu and Zn may have protective effects on male reproductive outcomes **Evenson *et al.*, [24] and Lyubimov *et al.*, [42]** and may assist in counteracting the effects of Cd, Pb, or other metals [57, 61] .

So, the aim of the present study was to assess the OS by measuring MDA levels and antioxidants by measuring GSH level and SOD activity, in addition to, investigation the influence of some heavy metals on male reproductive capacity such as the levels of Zn, Cu, Mn, Fe and Cd all in both the seminal plasma and spermatozoal homogenate supernatant of human subjects with different fertility potential.

SUBJECTS AND METHODS

Semen collection and analysis

All fresh semen samples were collected by masturbation after 3 days of sexual abstinence. Ejaculates parameters [sperm density, morphology, motility grades: a (rapid progressive), b (slow progressive), c (non-progressive), d (immotile)] were analyzed by a computer assisted sperm analyzer (CASA, Cell Soft 3000, Cryo Resources Co., U.S.A.). Samples with a leukocyte concentration more

than 1×10^6/ml were excluded. The liquefied semen samples were preserved under liquid nitrogen at -196 °C until assay. This method allowed a long-time sperm preservation without any risk of negative freezing impact on semen quality parameters [44] .

Semen classification

216 semen samples were classified into five groups based on WHO criteria **Group I** as control normozoospermics [70 cases] with sperm density of 20 millions/mL or more, sperm motility of 50% or more (a+b type motility), normal sperm morphology of 30% or more. **Group II:** asthenozoospermics [48 cases] have a problem in sperm motility. **Group III:** oligoasthenozoospermics [18 cases] have a problem in sperm density and sperm motility. **Group IV:** oligoasthenoteratozoospermics [41 cases] have a problem in sperm density, sperm motility and sperm morphology. **Group V:** azoospermics [39 cases] have no spermatozoa in semen.

Semen preperation

The frozen semen samples were leave to thaw at room temperature then centrifuged at 5000 r.p.m. for 15 minutes at +10 °C to separate spermatozoa from seminal plasma. The supernatant seminal plasma was then carefully removed and transferred to test tubes. The spermatozoal sediment was washed towice with physiological saline solution to remove the remained seminal plasma. After each procedure, the spermatozoa–saline mixture was centrifuged at 5000 r.p.m. for 10 minutes at +4°C [49,47] . Then, the separated spermatozoal pellet were treated with 2 ml homogenising buffer and homogenized mechanically for 5 minutes in ice bath and then subsequently centrifuged for 10 minutes at 5000 r.p.m. in a refrigerated centrifuge. The supernatant was then used for enzymatic measurements [18] .

Biochemical analysis

Protein content in the seminal plasma was determined by the biuret reaction according to the method of **Gornall *et al.*** [31] and in spermatozoal homogenates supernatant by method of **Lowry *et al.*** [41] . LPO was measured by the reaction of thiobarbituric acid with MDA

according to [54] . GSH level was determined by the method of [14] . SOD activity was assayed by the modified method according to. [20] .

Measurement of heavy metals levels

100 µl from seminal plasma or spermatozoal homogenate supernatant was added to 5 ml of nitric 69% (A.R.)/perchloric 70% (A.R.) acids mixture (4:1) and placed on a hotplate until complete digestion. The resulting residues was allowed to cool at room temperature then 4.9 ml of deionized bidistilled water and 100 µl of nitric acid 69% (A.R.) were added to the dried mixture. Determination of Zn, Cu, Mn, Fe and Cd was performed using the single-beam Perkin-Elmer atomic absorption spectrophotometer, with a single slit burner and an air acetylene flame [53] .

Statistical analysis

The statistical analysis was performed using Instate® program (version 2.03; from GraphPad software, U.S.A.). Differences among groups were compared using the Student t-test. A P-value <0.05 was considered statistically significant. Correlations between parameters were assessed using the Microcal ™ Origin® program`s coefficient (r-value) (version 0.6; from GraphPad software, U.S.A.). All parameters were expressed as mean ± standrad deviation.

RESULTS

The present work concerned with the evaluation of the factors affecting human fertility in males. Antioxidants (GSH, SOD) and LPO as represented by MDA in addition to heavy metals (Zn, Cu, Mn, Fe, Cd) were determined in seminal plasma and spermatozoal homogenate supernatant.

As can be seen from **table (1)**, there are no significant differences in seminal plasma protein between G_{II}, G_{IV} and control while, seminal plasma protein of G_{III} and G_V were significantly differ than control. G_{II}, G_{III}, G_{IV} and G_V showed highly significant decrease in level of GSH and activity of SOD when compared to control. On the other

hand, MDA levels in seminal plasma of G_{II}, G_{III}, G_{IV} and G_V were highly significantly increased than control.

Table 1: Contents of total protein (T.Protein, gm%), malondialdehyde (MDA, Mole10^{-7}/gm protein), reduced glutathione (GSH, µMole10^{-2}/mg protein) and superoxide dismutase (SOD, % inhibition) in seminal plasma of normozoospermic group (G_I, control), asthenozoospermic group (G_{II}), oligo-asthenozoospermic group (G_{III}), oligo-asthenoteratozoospermic group (G_{IV}) and azoospermic group (G_V).

Parameter / *Group*	T.Protein gm%	MDA Mole10^{-}	GSH µMole10^{-}	SOD % inhibition
Control (G_I)				
Range	3.19 – 7.86	0.3 – 0.95	5.64 – 14.03	59.26 – 96.87
M ± S.D.	5.71 ± 1.28	0.55 ± 0. 19	9.78 ± 2.23	74.5 ± 11.61
n.	70	36	48	47
G_{II}				
Range	3.06 – 7.78	1 – 4.3	3.44 – 8.66	58.18 – 75.93
M ± S.D.	5.51 ± 1.25	1.9 ± 0.84	6.81 ± 1.58	65.74 ± 4.39
n.	48	44	33	30
P	>0.05 ns	<0.0001**	<0.0001**	<0.0001**
G_{III}				
Range	2.86 – 7.28	1.41 – 3.34	2 – 4.9	54.66 – 67.27
M ± S.D.	5.05 ± 1.54	2.31 ± 0.72	3.24 ± 1.1	61.29 ± 4.16
n.	18	17	15	11
P	<0.05*	<0.0001**	<0.0001**	<0.001**
G_{IV}				
Range	3.33 – 7.86	2.4 – 5.06	1.52 – 4.32	34.25 – 50.68
M ± S.D.	5.44 ± 1.12	3.64 ± 0.77	3.1 ± 0.97	44.2 ± 4.97
n.	41	21	30	17
P	>0.05 ns	<0.0001**	<0.0001**	<0.0001**
G_V				
Range	3.81 – 9.22	1.26 – 3.43	0.98 – 3.25	35.62 – 85
M ± S.D.	6.23 ± 1.51	2.24 ± 0.66	2.43 ± 0.67	59.28 ± 13.61
n.	39	27	36	26
P	<0.05*	<0.0001**	<0.0001**	<0.0001**

- P>0.05 ns non significant; P<0.05* significant; P<0.01** highly significant.
- (n.) number of cases.

However **table (2)** revealed highly significant increase in spermatozoal protein content of G_{III} and G_{IV} and significant decrease in G_{II} when compared with control. Moreover, all abnormal groups (G_{II}, G_{III} and G_{IV}) showed highly significantly decreased in GSH levels of spermatozoal homogenate supernatant than control. While MDA levels of spermatozoal homogenate supernatant in all abnormal

groups (G_{II}, G_{III} and G_{IV}) were highly significantly increased comparing with control. Also, the mean activity of SOD in spermatozoal homogenate supernatant was highly significantly elevated in G_{IV} but significantly decreased in G_{II} when compared with control.

Table 2: Protein contents (µg protein/10^6sperm), malondialdehyde (MDA, Mole10^{-7}/gm protein), reduced glutathione (GSH, nMole/mg protein) and superoxide dismutase (SOD, % inhibition) in spermatozoal homogenate supernatant of normozoospermic group (G_I, control), asthenozoospermic group (G_{II}), oligo-asthenozoospermic group (G_{III}) and oligo-astheno-teratozoospermic group (G_{IV}).

Parameter / *Group*	**Protein** µg prtn/10^6	**MDA** Mole10^{-7}/gm	**GSH** nMole/mg	**SOD** % inhibition
Control (G_I) Range M ± S.D. n.	2.3 – 5.35 3.4 ± 0.77 39	0.6 – 1.9 1.19 ± 0.44 32	12.85 – 28.08 20.06 ± 4.79 20	27.78 – 57.53 41.56 ± 8.8 39
G_{II} Range M ± S.D. n. P	1.8 – 4.3 3.06 ± 0.80 30 <0.05*	1.55 – 3.95 2.51 ± 0.053 30 <0.0001**	11.67 – 20.67 16.51 ± 2.71 18 <0.01**	21.43 – 57.14 37.42 ± 10.15 25 <0.05*
G_{III} Range M ± S.D. n. P	5.12 – 13.9 9.3 ± 3.07 13 <0.0001**	2.82 – 5.2 3.64 ± 0.87 10 <0.0001**	3.5 – 6.8 4.5 ± 1.2 6 <0.0001**	26.63 – 53.01 40.63 ± 7.03 16 >0.05 ns
G_{IV} Range M ± S.D. n. P	13.7 – 33.4 23.5 ± 7.1 18 <0.0001**	4.4 – 9.9 5.72 ± 1.37 18 <0.0001**	0.45 – 1.1 0.85 ± 0.25 7 <0.0001**	28.59 – 69.7 50.23 ±13.09 32 <0.001**

- P>0.05 ns non significant; P<0.05* significant; P<0.01** highly significant.
- (n.) number of cases.

Zn and Cu concentrations of seminal plasma were highly significantly decreased in all abnormal groups (G_{II}, G_{III}, G_{IV} and G_V) when compared with control. However, Mn level was non-significantly decreased in G_{II} and G_{III} but was highly significantly

decreased in G_{IV} and G_V comparing with control. On the other hand, Fe and Cd concentrations were highly significantly increased in all abnormal groups (G_{II}, G_{III}, G_{IV} and G_V) than control **(Table 3)**. **Table (4)** illustrated that both Zn and Cu concentrations of spermatozoal homogenate supernatant decreased in G_{II} and G_{III} than control but they increased highly significant in G_{IV}. Also, Mn concentration increased highly significant in G_{IV} and significantly differ in both G_{II} and G_{III}. While in case of Fe and Cd concentrations, there were highly significantly increased in their concentrations in all abnormal groups (G_{II}, G_{III} and G_{IV}) and reached the highest value in G_{IV}.

Table 3: Concentrations of zinc (Zn, µg/mg protein), cupper (Cu, µg/mg protein), manganese (Mn, µg/mg protein), iron (Fe, µg/mg protein) and cadmium (Cd, µg/mg protein) in seminal plasma of normozoospermic group (G_I, control), asthenozoospermic group (G_{II}), oligo-asthenozoospermic group (G_{III}), oligo-astheno-teratozoospermic group (G_{IV}) and azoospermic group (G_V).

Parameter / *Group*	**Zn** µg/mg prtn	**Cu** µg/mg prtn	**Mn** µg/mg prtn	**Fe** µg/mg prtn	**Cd** µg/mg prtn
Control (G_I)					
Range	2.69 – 5.89	0.05 – 0.13	0.051 – 0.119	0.42 – 0.9	0.01 – 0.02
M ± S.D.	3.93 ± 0.76	0.09 ± 0.03	0.087 ± 0.026	0.68 ± 0.13	0.015 ± 0.005
n.	15	17	10	17	16
G_{II}					
Range	1 – 2.5	0.03 – 0.07	0.05 – 0.11	0.56 – 1.15	0.04 – 0.08
M ± S.D.	1.91 ± 0.56	0.04 ± 0.02	0.079 ± 0.022	0.95 ± 0.19	0.065 ± 0.017
n.	11	10	11	11	11
P	<0.0001**	<0.0001**	>0.05 ns	<0.0001**	<0.0001**
G_{III}					
Range	0.67 – 1.41	0.03 – 0.07	0.04 – 0.1	0.72 – 1.18	0.09 – 0.13
M ± S.D.	1.1 ± 0.29	0.04 ± 0.02	0.069 ± 0.02	1 ± 0.17	0.114 ± 0.018
n.	5	6	6	6	6
P	<0.0001**	<0.0001**	>0.05 ns	<0.0001**	<0.0001**
G_{IV}					
Range	1.18 – 2.91	0.02 – 0.04	0.04 – 0.08	0.92 – 1.86	0.06 – 0.1
M ± S.D.	2.17 ± 0.56	0.03 ± 0.01	0.055 ± 0.015	1.36 ± 0.29	0.072 ± 0.013
n.	15	18	15	15	17
P	<0.0001**	<0.0001**	<0.001**	<0.0001**	<0.0001**
G_V					
Range	1.5 – 3.41	0.03 – 0.1	0.02 – 0.06	0.99 – 2.11	0.06 – 0.13
M ± S.D.	2.13 ± 0.61	0.06 ± 0.02	0.045 ± 0.017	1.498 ± 0.39	0.083 ± 0.028
n.	15	16	10	17	16
P	<0.0001**	<0.001**	<0.001**	<0.0001**	<0.0001**

Table 4: Concentrations of zinc (Zn, µg10^2/mg portein), cupper (Cu, µg10^2/mg portein), manganese (Mn, µg10^2/mg portein), iron (Fe, µg10^2/mg portein) and cadmium (Cd, µg10^2/mg portein) in spermatozoal homogenate supernatant of normozoospermic group (G_I, control), asthenozoospermic group (G_{II}), oligo-asthenozoospermic group (G_{III}) and oligo-astheno-teratozoospermic group (G_{IV}).

Parameter / *Group*	**Zn** µg10^2/mg prtn	**Cu** µg10^2/mg prtn	**Mn** µg10^2/mg prtn	**Fe** µg10^2/mg prtn	**Cd** µg10^2/mg prtn
Control (G_I)					
Range	0.83 – 1.97	0.04 – 0.1	0.03 – 0.09	1.1 – 2.7	0.01 – 0.029
M ± S.D.	1.28 ± 0.38	0.07 ± 0.02	0.049 ± 0.019	1.7 ± 0.54	0.021 ± 0.005
n.	18	15	10	18	23
G_{II}					
Range	0.47 – 0.95	0.02 – 0.05	0.04 – 0.09	1.62 – 3.85	0.04 – 0.09
M ± S.D.	0.72 ± 0.18	0.03 ± 0.01	0.07 ± 0.02	2.6 ± 0.76	0.061 ± 0.02
n.	10	10	9	9	12
P	<0.0001**	<0.0001**	<0.05*	<0.001**	<0.0001**
G_{III}					
Range	0.53 – 0.98	0.03 – 0.06	0.023 – 0.048	1.89 – 3.76	0.1 – 0.16
M ± S.D.	0.79 ± 0.18	0.05 ± 0.01	0.03 ± 0.01	2.56 ± 0.72	0.14 ± 0.03
n.	6	5	6	5	5
P	<0.01**	<0.05*	<0.05*	<0.01**	<0.0001**
G_{IV}					
Range	1.34 – 3.43	0.04 – 0.16	0.1 – 0.24	2.03 – 5.31	0.17 – 0.47
M ± S.D.	2.29 ± 0.68	0.16 ± 0.04	0.16 ± 0.05	3.78 ± 1.08	0.29 ± 0.1
n.	17	16	14	18	16
P	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**

- P>0.05 ^{ns} non significant; P<0.05* significant; P<0.01** highly significant.
- (n.) number of cases.

Correlations

All possible relationships between the studied parameters and semen profiles were examined in overall groups to investigate the influence of these parameters on sperm quality and thence male fertility as shown in **table (5)**.

Correlations with Sperm density

Sperm density was highly significantly and inversely correlated with MDA, Fe and Cd concentrations of both seminal plasma and spermatozoal homogenate supernatant. Also, it was highly significantly and inversely correlated with the concentrations of protein, Zn and Mn in spermatozoal homogenate supernatant. While, it was significantly and inversely correlated with SOD activity and Cu

concentration of spermatozoal homogenate supernatant. On the other hand, sperm density had highly significant and direct correlation with GSH levels of both seminal plasma and spermatozoal homogenate supernatant, SOD activity and Zn concentration of seminal plasma. While, the sperm density was directly and significantly correlated with seminal plasma protein, Cu and Mn levels.

Correlations with total progressive sperm motility

The total progressive sperm motility was inversly and highly significantly correlated with the concentrations of MDA, Fe and Cd in both seminal plasma and spermatozoal homogenate supernatant. Also, inverse and highly significant correlations were found between the total progressive sperm motility and the levels of spermatozoal protein, Zn, Cu, Mn and SOD activity in spermatozoal homogenate supernatant. Furthermore, seminal plasma protein concentration correlated inversely and significantly with the total progressive sperm motility. On contrary, direct and high significant correlations were found between the total progressive sperm motility with the concentrations of GSH, Zn, Cu, Mn and SOD activity in seminal plasma. Also, the total progressive sperm motility was directly and highly significantly correlated with the levels of GSH in spermatozoal homogenate supernatant.

Correlations with sperm abnormality

There were inverse and highly significant correlations between sperm abnormality with the levels of GSH, Zn, Cu, Mn and SOD activity in seminal plasma. Inverse and highly significant correlations were also observed between sperm abnormality and GSH level in spermatozoal homogenate supernatant. On the other hand, sperm abnormality was directly and highly significantly correlated with protein, MDA, Fe and Cd concentrations in both seminal plasma and spermatozoal homogenate supernatant. The same findings were observed between sperm abnormality and SOD activity, Zn, Cu and Mn concentrations in spermatozoal homogenate supernatant.

Table 5: **The correlations between the studied parameters with semen profiles.**

Parameter	Semen parameters								
	Sperm density			Sperm motility			Sperm abnormality		
Seminal plasma	r	p	n	r	p	n	r	p	n
Prtn	0.14	0.03	217	-0.14	0.03	178	0.03	0.0002	178
MDA	-0.45	<0.0001	145	-0.7	<0.0001	118	0.76	<0.0001	118
GSH	0.63	<0.0001	162	0.77	<0.0001	126	-0.67	<0.0001	126
SOD	0.39	<0.0001	120	0.69	<0.0001	94	-0.65	<0.0001	94
Zn	0.5	<0.0001	61	0.58	<0.0001	46	-0.49	0.0005	46
Cu	0.3	0.03	67	0.63	<0.0001	51	-0.6	<0.0001	51
Mn	0.3	0.04	52	0.5	0.0006	42	-0.5	0.001	42
Fe	-0.5	<0.0001	66	-0.73	<0.0001	49	0.69	<0.0001	49
Cd	-0.5	<0.0001	66	-0.5	<0.0001	50	0.47	0.0006	50
Spermatozoal homogenate	r	p	n	r	p	n	r	p	n
Prtn	-0.5	<0.0001	99	-0.7	<0.0001	100	0.78	<0.0001	100
MDA	-0.54	<0.0001	89	-0.81	<0.0001	90	0.83	<0.0001	90
GSH	0.59	<0.0001	50	0.49	0.0002	51	-0.64	<0.0001	51
SOD	-0.21	0.03	112	-0.37	<0.0001	112	0.48	<0.0001	112
Zn	-0.39	0.005	51	-0.54	<0.0001	51	0.67	<0.0001	51
Cu	-0.35	0.02	46	-0.55	<0.0001	46	0.67	<0.0001	46
Mn	-0.44	0.007	37	-0.76	<0.0001	37	0.76	<0.0001	37
Fe	-0.52	0.0001	50	-0.84	<0.0001	50	0.7	<0.0001	50
Cd	-0.54	<0.0001	55	-0.8	<0.0001	55	0.86	<0.0001	55

- (r) correlation coefficient. The correlation is weak at r = 0.5, moderate at r = 0.5 - 0.75 and strong at r = 0.8 - 1.0.
- P>0.05 ^{ns} non significant; P<0.05* significant; P<0.01 highly significant.**
- (n.) number of cases.

Discussion

The Pathophysiology of male infertility could be explained by a cascade of molecular and biochemical events which represent in most cases by abnormal semen parameters. Growing evidence indicates that imbalance between peroxidative and antioxidative substances in semen leads to metabolic and functional disorders of male germ cells and may be a primary cause of some types of infertility [(25)] .

Extensive study on the peroxidation of phospholipids in mammalian sperm had demonstrated that peroxidation reaction causes

membrane damage which leads to loss of sperm quality and membrane integrity [22, 52] . In the present study, there is a highly significant increase in the mean levels of MDA in both seminal plasma and spermatozoal homogenate supernatant of subfertile males compared to control as well as a highly significant and inverse associations between MDA and sperm quality either in seminal plasma or spermatozoal homogenate supernatant indicating that LPO is a probable cause of idiopathic male infertility. These results are in agreement with [33,17,56] .

In case of azoospermic males, it was found that seminal plasma MDA mean level was significantly higher than that of control but still lower than its level in G_{IV} which has the highest percentage of abnormal sperm. This result suggests that MDA level in seminal plasma is not entirely derived from spermatozoa but may, at least in part, originate from the existence of LPO in the accessory glands as seminal vesicles, prostate and epididymis as was reported by [34] .

It is worth mentioning that MDA levels of spermatozoal homogenate supernatant were significantly higher than that of its seminal plasma in all groups. This may lead to suggest that spermatozoa represent the main source of MDA in semen as was reported by **Gil-Guzman *et al.*** [30] . Also, the higher susceptibility of spermatozoa to peroxidative damage may be due to the presence of high density of mitochondria in their midpiece which may leak excessive oxygen radicals in cytoplasm with a simultaneous lackage in its cytoplasmic antioxidant enzymes. These imbalance make the ability of spermatozoa to scavenge the oxidants become limited as was reported by [29,1]

Ebisch *et al.* [21] reported no significant difference in seminal plasma GSH level between fertile and subfertile men. While **Nabil *et al.*** [45] **and Chaudhari *et al.*** [17] found significant decrease in seminal plasma GSH levels of subfertile males compared to fertile ones. Even GSH therapy was found to improve the semen quality [40] . However, **Bhardwaj *et al.*** [15] showed that in the spermatozoa of patients with oligozoospermia, the GSH level of sperm was significantly lower than normospermic subjects.

In the present work, a highly significant decrease in the mean levels of GSH were found in both seminal plasma and spermatozoal homogenate supernatant of subfertile males compared to control. Also, GSH levels of seminal plasma and spermatozoal homogenate supernatant were linked directly with sperm quality parameters. These results provide evidence that the levels of GSH seem to play a role in male fertility and sperm quality.

Furthermore, it was found that the mean levels of GSH in seminal plasma were highly significantly increased than its level in spermatozoal homogenate supernatant for all groups. This may lead to suggest that seminal plasma may be the main source of GSH in semen. Because spermatozoa are such specialized cells, most cytoplasmic enzymes are extruded during the final stages of the sperm maturation process, which enables sperm to attain their characteristic morphology [(3)]. As a result spermatozoa lack the common cytoplasmic antioxidants found in other cell types due to they have very low amount of cytoplasm and are therefore particularly sensitive to oxidative damage [(58)] . So, the natural compensation for this deficiency is by providing an array of antioxidants in the seminal plasma to protect the spermatozoa [(37)] **.**

Pasqualotto *et al.*[(48)] and Murawski *et al.*[(44)] showed that seminal plasma SOD activity was significantly lowered in males with abnormal semen parameters comparing to the activity found in normospermic men. They also found that all semen parameters - concentration, percentage motility and morphology of sperm, were significantly and directly correlated with activities of seminal plasma SOD. Our results were in agreement with these results, suggesting that decreased seminal plasma scavenger antioxidant capacity, particularly in form of low SOD activity, may be responsible for male infertility and low sperm quality. However, **Tavilani *et al.*[(56)]** reported that there was no significant difference in the SOD activity in seminal plasma from normospermia and subfertile men.

The obtained results indicate a highly significant elevation in SOD activity only in spermatozoal homogenate supernatant of G_{IV} compared to control. Additionally, there was a highly significant and direct correlation between SOD activity and sperm morphological

abnormality. Since G_{IV} are characterized by high percentage of abnormal morphological spermatozoa which can generate high amount of ROS, the males of this group may be at greater risk of developing pathogenic levels of ROS a phenomenon which causes stimulation of SOD enzyme [55] . This may lead to expect higher SOD activity in spermatozoal homogenate supernatant of G_{IV} to help in overcome the LPO [18] . So SOD in spermatozoa seemed to be the most relevant enzyme in the protection of spermatozoa from LPO as was reported by [28] .

Generally, our finding showed that the activity of seminal plasma SOD was higher than that of spermatozoa. This confirm that seminal plasma is the potent source of SOD activity in semen which play an important role in preservation spermatozoa against free radical toxicity. In addition, the presence of a high SOD activity in seminal plasma than that in its spermatozoa may serve as an additional function. Superoxide radicals have been shown to induce hyperactivation and capacitation of spermatozoa. This induction would be suppressed in both male genitourinary system and vagina by the high SOD activity of seminal plasma. After movement to the upper female genital tract, the spermatozoa will encounter a much lower external SOD activity allowing a timely hyperactivation and capacitation [43] . Therefore, if spermatozoa SOD activity was higher than its seminal plasma it may be lead to acute male infertility like G_{IV}.

In the present work, seminal plasma Zn, Cu and Mn levels of subfertile groups were decreased compared to control and their deficiency associated with low sperm quality. On the contrary, Mn levels in spermatozoal homogenate supernatant of subfertile groups especially in G_{IV} were significantly increased than control and its increasing associated with low sperm quality. Additionally, spermatozoal homogenate supernatant levels of Zn and Cu in subfertile groups were lower than that of the control except G_{IV} in which there was a highly significant increase in Zn and Cu levels than control and inversely correlated with sperm quality. Furthermore, we found a highly significant increase in spermatozoal homogenate supernatant levels of Zn, Cu and Mn compared to their corresponding

levels in seminal plasma of all groups. This may be attributed to their role in sperm physiology. These results suggest that low doses of the essential metals such as Zn, Cu and Mn may improve sperm quality as their levels in seminal plasma [24, 42], may also have antioxidative properties and assist in counteracting the effects other toxic metals [57,61]. But excess levels of these metals as in spermatozoal homogenate supernatant have opposite effects on sperm quality leading to male infertility [39,60]. The direct correlation between Zn and Cu levels in spermatozoal homogenate supernatant with SOD activity in G_{IV} may be attributed to their stimulatory effect on SOD in presence of high ROS levels.

The obtained data revealed increase of Fe and Cd levels in spermatozoal homogenate supernatant of subfertile groups when compared to control with inverse correlations with sperm quality. The same behavior was observed in seminal plasma. In addition, their levels were higher in spermatozoal homogenate supernatant than the corresponding levels in seminal plasma. These suggest that Fe and Cd have a strong toxic effect on spermatogenesis as was reported by **Aydemir *et al.* [10] and Akinloye *et al.* [4]** and may induce apoptosis which deplete the sperm concentration leading to male infertility [12].

CONCLUSION

It is now well-accepted that lipid peroxidation and heavy metals induce a significant sperm membrane damage and markedly influences sperm quality, it is quite probable that such deleterious effect may account for some cases of male infertility and evaluation of metal ions and peroxidation parameters may be a part of infertile male workup in the near future.

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