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Research Paper

Altered Antioxidant Status and Increased Lipid Per-Oxidation in Seminal Plasma of Tunisian Infertile Men

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Abstract

Human seminal plasma is a natural reservoir of antioxidants that protect spermatozoa from oxidative damages. There is evidence in literature supports the fact that impairments in seminal antioxidant and lipid per-oxidation status play important roles in the physiopathology of male infertility. Our present study forms the first one which was carried out in Tunisia. We evaluated the antioxidant status in the seminal plasma of 120 infertile men programmed to In Vitro Fertilization (IVF) for the first tentative. Patients were characterized by an idiopathic infertility. They were divided into three groups: normozoospermics who were considered as controls (n=40), asthenozoospermics (Astheno; n=45) and oligoasthenoteratozoospermics (OAT; n=35). Seminal activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) and the levels of glutathione (GSH), zinc (Zn) and malondialdehyde (MDA) were measured. With the significant increase of the seminal activities of SOD and GPX in normozoospermics group, there were positive correlations observed between this enzymes and sperm quality. Also, significant elevated rates of seminal zinc and GSH were observed in control group, but there was contradictory associations reflecting the effects of these antioxidants on semen parameters. However, we noted significant increase of MDA levels in groups with abnormal seminogram. We showed negative associations between this per-oxidative marker and sperm parameters. These results obviously suggested that impairment on seminal antioxidants is an important risk factor for low sperm quality associated to idiopathic infertility and as a result can lead to poor IVF outcome.

Key words: Oxidative damage, Antioxidant enzymes, Semen quality, Male infertility, Sperm abnormalities, lipid per-oxidation.

Introduction

Infertility is defined as the failure to conceive after 1 year of regular, unprotected intercourse with the same partner. The physiopathology of male infertility could be explained by a cascade of molecular and biochemical events which represents itself in most of cases by abnormal semen parameters [1]. The single, most common, defined cause of human infertility is defective sperm function. Recent evidences suggest the imbalance between per-oxidative and anti-oxidative substances in semen leads to oxidative stress, resulting in metabolic and functional disorders of male germ cells in some types of infertility[2]. Human spermatozoa, through their high amounts of polyunsaturated fatty acids and the little cytoplasm seques-

tering defensive enzymes are extremely vulnerable to oxidative attack [3]. They are rendered dysfunctional by lipid per-oxidation which triggers the loss of membrane integrity, causing increasing cell permeability, enzyme inactivation, and structural damage of DNA and cell death[4, 5].

Human seminal plasma is considered as an important source of antioxidants which might be useful in the prediction of sperm fertilizing potentials. It contains high levels of non-enzymatic antioxidants such as ascorbate, trace elements and thiol groups, as well as less substantial amounts of GSH. In addition, the antioxidant enzymes, SOD, catalase and GPX have all been found in this micro-environment [6]. SOD is the first enzymatic line of antioxidant defense and is one of the most important anti-oxidative defense enzymes. SOD is generated as a byproduct of aerobic metabolism. It scavengers both intracellular and extracellular superoxide radical and prevents the lipid per-oxidation of plasma membrane. SOD should be conjugated with catalase or GPX in order to prevent the action of hydrogen peroxide [7]. The enzyme "GPX" then converts H_2O_2 to water and oxygen, eradicating the ROS. GPX could protect the sperm against per-oxidative damage. It plays an important role in sperm maturation from the early events up to the onset of fertilization [8]. With the absence of GPX may lead to reduce fertilizing capacity[9]. Alternatively, ROS can be neutralized by non-enzymatic antioxidants [10].

An important endogenous antioxidant in humans is the tri-peptide glutathione which plays a central role in the defense against oxidative damage and toxins [1]. GSH can exist in two forms; both as reduced monomer (GSHr) and as an oxidized dimer (GSSG). However, GSHt indicates total GSH which includes the monomeric and dimeric forms as tripeptide [11]. It has been detected intracellularly within the sperm and extracellularly in the seminal plasma[12]. In actual fact, it is essential to know that intracellular GSH levels are regulated by a complex series of mechanisms including rate of synthesis and regeneration by glutathione reductase, GSH utilization and GSH efflux to extracellular compartment. Effectively, GSH transported out of cells provides the source of plasma GSH and GSSG. GSH and GSSG are substrates of the extracellular enzyme δ-glutamyl transferase (GGT) which is the only enzyme which can break the δ-peptide linkage. GGT can either transfer the δ-glutamyl group of GSH, GSSG or GSH-conjugates to amino acid acceptors to give δ-glutamyl peptides and cysteinylglycine or directly hydrolyze GSH to glutamate and cysteinylglycine. Cysteinylglycine can then be cleaved by a dipeptidase. The free amino acids and δ glutamyl amino acids produced by both of these reactions can be transported back into cells and used to regenerate GSH [11]. When present in extracellular space, GSH is able to react directly with cytotoxic aldehydes produced during lipid per-oxidation and thus protect the sperm plasma membrane [8]. Fertility promoting role of GSH has been established through different studies where GSH has been found to be reduced in oligo and azoospermics then normozoospermics [12]. Moreover, in some clinical trial GSH when given exogenously to infertile men, was found to cause significant increase in sperm motility [13]. Nevertheless, studies have shown a higher concentration of GSH in the seminal plasma of azoospermics than the oligo or normozoospermics [14].

Among the several trace elements in human seminal plasma, there is zinc. The concentration of zinc in human seminal plasma is higher than in other tissues [15]. In seminal plasma it stabilizes the cell membrane and nuclear chromatin of sperm [16]. Zinc is also an important antioxidant which acts directly and as a cofactor of Cu/Zn SOD against ROS. There is extensive evidence that human seminal plasma Zn has an important role in physiologic functions of sperm and that reduced levels result in low quality of sperm and reduced chances of fertilization [16]. Total content of zinc in mammalian semen is high and has been found to be critical to spermatogenesis, but there have been conflicting reports on the effect of seminal Zn on sperm quality [17]. Wong WY et al [18] indicated that there is no significant difference between Zn content in fertile and infertile men, but Mankad M et al [19] found a significant difference between them. However, it is difficult to measure the effectiveness of one antioxidant in isolation of another because there appears to be cooperation between antioxidants [18].

Keeping in view the oxidative damage to the sperm membranes that may result into the impairment of sperm functioning and integrity, our present study attempt was made to measure the levels of enzymatic antioxidants (SOD and GPX) and non-enzymatic antioxidants (different forms of glutathione and zinc) and at the same time to assess the oxidative stress by measuring MDA levels in the seminal plasma of human subjects with idiopathic infertility. Correlation between the MDA levels and fertility potential as well as MDA level and different antioxidants were tested statistically.

Material and Methods

Study population: The study was carried out in 120 male partners from couples (range, 27–49 years) undergoing IVF or intracytoplasmic sperm injection (ICSI) as an infertility treatment. The study design included three groups based on the ejaculate param-

eters. Group I (n=40) consisted of males with normal ejaculate (Normozoospermia), group II (n=45) consisted of patients with only abnormal motility (Asthenozoospermia) and group III which consisted of associated abnormal motility, count and morphology (Oligoasthenoteratozoospermia = OAT). General details of the study groups and semen characteristics were described in **table 1 and table 2**.

Exclusion criteria: A detailed medical history and andrological examination was performed for all studied cases. Subjects currently on any medication or antioxidant supplementation were not included. Also, patients with varicocele, leucospermia, those suffering from any acute infection, smokers and alcoholic men were excluded from the study because of their well-known high seminal ROS levels.

Study consent: A written consent of each subject was taken after explaining the aims and objectives of the study and its benefit to individual and society.

Table 1: General characteristics of the study population.

parameters	Characteristics	Number(n)	Percentage (%)
Age (Years)	≤30	36	30
	>30	84	70
Duration of infertility (years)	≤10	99	82,5
	>10	21	17,5
Type of infertility	Primary	83	69,16
	Secondary	37	30,83
Area	Rural	34	28,33
	Urban	86	71,66
Diet	Vegertarian	72	60
	Mixed	48	40
Sperm criteria	Normozoospermics	40	36,36
	Asthenozoospermics	45	40,90
	OAT	35	31,81

Table 2: Seminal characteristics (Values± SD) with respect to sperm count.

	Normozoospermics (n=40)	Asthenozoospermics (n=45)	OAT (n=35)
Age (years)			
Mean ± SD	38,5±56	38,04±5,37	37,37±4,87
Min-Max	27-49	29-47	31-49
Volume (ml)			
Mean ± SD	3,15±1,18	3,34±1,45	3,21±1,44
Min-Max	2-5,5	2-7,5	2-7,5
Sperm motility (%)			
Mean ± SD	42,72± 13,22	26±9,67	18,40±9,62
Min-Max	40-100	5-40	1-30
Sperm Count (Million/ml)			
Mean ± SD	75,86± 23,83	45±25,00	10±6,51
Min-Max	40-100	20-100	1-15
Abnormal morphology (%)			
Mean ± SD	45± 13,74	59±8,63	81,78±12
Min-Max	39-67	41-72	77-98

Note: Max= maximum, Min = minimum, ml= milliliter, SD= Standard Deviation, OAT= Oligoasthenoteratozoospermics.

Sample collection: Semen was obtained by masturbation technique after at least 3 days of sexual abstinence. Samples were collected into sterile containers for immediate transportation to the laboratory. They were examined immediately after 30 minutes of liquefaction according to WHO guidelines (World Health Organization). Spermiograms included semen volume (ml), sperm count (%), sperm motility (%) and abnormal morphologic features (%). Sperm motility was classified into four categories: rapid progressive motile (Type a), slow progressive motile (Type b), non-progressive motile and immotile spermatozoa, and was assayed at exactly 0.5 and 2 h after liquefaction. Total progressive motility was defined as the combination of type a rapid motility and type b slow progressive (At least 30% of sperm should have normal motility (categories a + b)). Morphology was measured by recording the percentage of abnormal forms in the sample. We are based on the classification of "David" and at least 30% of sperm should have normal morphology, which was characterized by normal heads, mid-piece and tails. A fraction of each semen sample included in our study was designated for determination of antioxidants and lipid per-oxidation levels. Seminal plasma was obtained after centrifugation at 3500 rpm for 15minutes and it was loaded in the Eppendorf tubes (3ml) and stored at -80°C awaiting antioxidant analysis.

Chemicals: All reagents and chemicals were of analytical grade or higher purity and obtained from standard commercial suppliers. Ultra-pure water was received from water purification Milli-Q system (Millipore Corporation, USA).

Antioxidant enzyme essay: Total SOD activity was determined using pyrogallol as a substrate by the method of Marklund and Marklund (1974) [20]. This method is based on pyrogallol oxidation by the superoxide anion ($O_2^{\circ-}$) and its dismutation by SOD. One Unit (U) of total SOD and CuZnSOD is defined as the amount of enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%. GPX activity was assayed by the subsequent oxidation of NADPH at 240 nm with t-butyl-hydro-peroxide as a substrate [21]. GPX units (U/g protein) were defined as µmol NADPH oxidized/g protein.

Analytical methods for determination Zinc levels: For all the experiment, flame atomic absorption spectrophotometry (AAS) was adopted for Zn determination. The measures were implemented using a Zeenit 700-Analytik-Jena, Germany (Flame and Graphite-Furnace AAS), equipped with deuterium and Zeeman background correction, respectively, as recommended by the manufacturer. Detection limits for Zn (Flame AAS) were 0.47 µg /l.

Determination of GSH and GSSG contents: The total glutathione (GSH), reduced glutathione and oxidized glutathione (GSSG) were measured spectrophotometrically in deproteinized supernatant fractions from the semen by the method of Akerboom and Sies (1981) [22] using 5,5′-dithiobis (2-nitrobenzoic acid). Absorbance values were compared with standard curves from known amounts of GSH standards.

Determination of LPO level: LPO was estimated by measuring thiobarbituric-acid reactive substances (TBARSs) and was expressed in terms of malondialdehyde content according to the method of Yagi (1976) [23].

Determination of Protein amounts: The protein content in supernatant was estimated by the biuret method [24] using Serum Albumin Bovine as standard (BSA).

Statistical analysis: The mean levels of seminal plasma oxidative parameters were expressed as means ± SE. Differences among different studied groups were assessed by SPSS 11.0 followed by protected least significant difference student's test (T-test for independent samples). Pearson correlation analysis was conducted to investigate the relationship between variables. Values were considered statistically significant when $P<0.05$ ($P<0.05$= significant; $P<0.001$= highly significant).

Results

The mean levels of seminal activities of antioxidant enzymes ($\times 10^{-3}$ U/g of proteins), GSH (µmol/l), zinc (mg/l) and seminal plasma MDA (µmol/l) of all studied groups were shown in tables 3 and 4.

Biochemical analysis

Comparison of results between control group and asthenozoospermic patients: Student test showed significant decline SOD ($P=0.008$) and GPX ($P=0.02$) activities in the seminal plasma of asthenozoospermic men in comparison with controls **(Table 3)**. A highly significant increase ($P<0.001$) in zinc levels occurred in normospermic group compared to asthenospermic patients. Besides, Total GSH and reduced GSH showed significant elevation in seminal plasma of controls ($P=0.03$ and $P=0.008$; respectively). However, GSSG was lightly increased in asthenospermic men than normospermics (**Table 4**).

Table 3: The activities of the seminal antioxidant enzymes in the study groups.

Parameters	Control (n=40)	Astheno (n=45)	OAT (n=35)	P-Value	
				Control Vs. Astheno	Control Vs. OAT
SOD ($\times 10^{-3}$ U/g)					
Mean ± SD	2,58± 2,03	1,07±0,5	0,87±0,38	0,008	0,003
Min-Max	1,07-7,41	0,14-2,41	0,51-6,51		
GPX ($\times 10^{-3}$ U/g)					
Mean ± SD	9,56± 4,87	6,35±2,69	3,90±2,01	0.02	<0.001
Min-Max	2,14-19,44	2,27-12,19	0,71-6,63		

Note: SOD= superoxide dismutase, GPX= glutathione peroxidase, Max= maximum, Min= minimum, SD= standard deviation, Astheno= asthenozoospermics, OAT= Oligoasthenoteratozoospermics. Data are expressed as means ± SD. P≤0.05=significant; P≤.001=highly significant.

Table 4: Glutathione, Zinc and MDA levels in the study groups.

Parameters	Control (n=40)	Astheno (n=45)	OAT (n=35)	P-Value	
				Control Vs. Astheno	Control Vs. OAT
GSHt (µmol/l)					
Mean ± SD	53,6± 20	38,69±24	45,42±17,7	0.03	NS
Min-Max	25,62-99,5	7,13-96,80	14,25-68,4		
GSSG (µmol/l)					
Mean ± SD	22 ± 12,3	22,89±9,8	9,85±4,8	NS	0.02
Min-Max	2,61-99,5	2,60-79,08	2,26-35,5		
GSHr (µmol/l)					
Mean ± SD	31,5 ± 19,2	15,7±13	35,5±16,1	0.008	NS
Min-Max	4,09-55	0,99-44,9	11,64-63,7		
Zn (mg/l)					
Mean ± SD	97,6 ± 34,9	43,4±24,9	32,4±7,9	<0.001	<0.001
Min-Max	56,23-169,2	10,7-102,3	0-201,8		
MDA(µmol/l)					
Mean ± SD	18,89±7,61	29,60±4,83	32,48±7,98	<0.001	<0.001
Min-Max	5,73-33,44	21-37,53	22,37-45		

Note: GSHt= total glutathione, GSSG= oxidized glutathione, GSHr= reduced glutathione, MDA= malondialdehyde acid, Astheno= asthenozoospermics, OAT= Oligoasthenoteratozoospermics. Data are expressed as means ± SD. P≤0.05=significant; P≤.001=highly significant.

Antioxidant levels among normozoospermics and OAT group: The data in **table 3** showed that seminal SOD and GPX activities in seminal plasma of controls were more and significantly important than seminal plasma of OAT patients (P=0.003 and p<0.001; respectively).Concentrations of different GSH forms in seminal plasma of the two groups of patients were in order GSHt>GSHr>GSSG. It was noted a significant increase in seminal GSSG (P<0.001) of control group. But, there are no significant differences were seen in mean concentrations of GSHt and GSHr (**Table 4**).

Seminal malondiadehyde (MDA) content: As regards MDA, means of seminal concentrations were significantly different between the group of patients and controls (P<0. 001), but conversely to antioxidants we found an increased concentration in patient group compared to control group (28,70 vs. 18,89 µmol/l; respectively). Effectively, seminal MDA amounts in both groups of patients, asthenozoospermic and OAT men, showed also highly significant increase (P≤0.0001) in comparison with control group (**Table 4**). In actual fact, we noted an elevated rate of seminal MDA in OAT (32,48±7,98)cases compared to asthenozoospermic patients (29,60±4,83) but the difference was not significant. Additionally, elevated rates of seminal MDA were strongly and negatively associated to sperm motility (P<0.001; r=-0.478**). Meanwhile, there is no correlation between seminal lipid-per-oxidation and the percentage of abnormal morphology, but a negative and not significant relationship was found among MDA and sperm count

(**Table 5**). On the other hand, we estimated correlations that can be exist between MDA and antioxidants studied and we found high negative correlations with SOD activity (P<0. 001, r=-0.490**) and GSSG levels (P<0.004, r=-0.369**). Though, there are no correlations noted between MDA and GPX, Zn GSHt and GSHr (**Table 6**).

Correlations between different seminal antioxidants studied and semen parameters: As showed in **table 5**, correlation studies showed high positive associations of seminal SOD and activity to motility (P<0.001; r= 0,494**) and sperm count (P<0,001; r= 0,424**). Positive and strong relationships were also demonstrated between GPX and sperm motility (P=0,04 ; r= 0,263*) and sperm concentration (P<0,001 ; r= 470**). Negative, but not significant correlation was found between the two enzymes and percentage of abnormal morphology. At the same time, seminal concentration of zinc was positively associated to sperm motility (P=0.003; r=0.378), sperm count (P<0.001; r=0.486) and abnormal morphology (P<0.001; r=0.413). For different forms of glutathione only oxidized form showed a negative and significant correlation with abnormal morphology (P=0.002, r=0.400).

Correlations between studied oxidative parameters: Correlation study showed high negative associations among MDA content and both SOD activity (r=-0.490**; P<0. 001) and GSSG (r=-0.369**; P=0.004) concentration in seminal plasma of infertile patients. In contrast, strongly positive relationships were found between seminal GSHt amounts and GSSG (r=0.516**; P<0.001) and GSHr (r=0.725**; P<0.001). Furthermore, we found positive correlations between seminal GPX activity and SOD (r=0.272*; P=0.03) and zinc concentration (r=0.305*; P=0.02). On the other hand, we noted negative but not significant correlations between seminal MDA and GPX and Zn and GSHt. Another negative and not significant correlation was found among GSHr content and GSSG level and SOD and GPX activities (**Table 6**).

Table 5: The value of Pearson's correlation coefficients calculated between the oxidative parameters and the criteria of semen quality.

Parameters	Motility	Sperm concentration	Abnormal morphology
SOD	**0,49****	**0,42****	Negative NS
GPX	**0,26***	**47****	Negative NS
Zn	**0,37****	**0.48****	**0.41****
GSHt	NS	NS	NS
GSSG	NS	NS	**-0.40**
GSHr	NS	NS	NS
MDA	**-0.47****	Négative NS	NS

Note: SOD= superoxide dismutase, GPX= glutathione peroxidase, Zn= zinc, GSHt= total glutathione, GSSG= oxidized glutathione, GSHr= reduced glutathione, MDA= malondialdehyde acid.

** Correlation strongly significant P≤0.001; * Significant correlation P<0.05.

Table 6: Correlations between studied oxidative stress variables.

Parameters	SOD	GPX	Zn	GSHt	GSSG	GSHr	MDA
MDA	**-0.49****	Negative NS	Negative NS	Negative NS	Negative NS	NS	-
GSHt	NS	NS	NS	-	**0.51****	**0.72****	Negative NS
GSSG	NS	NS	NS	**0.51****	-	Negative NS	Negative NS
GSHr	NS	NS	NS	**0.72****	NS	-	NS
SOD	-	**0.27***	NS	NS	NS	NS	**-0.49****
Zn	NS	**0.30***	NS	NS	NS	NS	Negative NS

Note: SOD= superoxide dismutase, GPX= glutathione peroxidase, Zn= zinc, GSHt= total glutathione, GSSG= oxidized glutathione, GSHr= reduced glutathione, MDA= malondialdehyde acid. Pearson correlation analysis was conducted to investigate the relationship between variables. ** Correlation strongly significant P≤ 0.001; * Significant correlation P≤ 0.05.

Discussion

It is well-known that Spermatozoa themselves contain negligible levels of antioxidants, and thus render the cells particularly reliant to their immediate environment for protection [25]. Human seminal plasma contains several antioxidants compromising enzymatic and non-enzymatic systems that play an important role in the normal function of sperm. Many studies suggested that decreased levels of antioxidants in seminal plasma might be a potential cause of infertility but there were always contradictory between reports [25]. It is important to make a note of contradictions and controversial outcomes found and cited in articles. In point of fact, these differences can be due to several variables like the criteria included and excluded for patient selection. In actual fact, effects of abstinence time on the results of semen analysis were eliminated in this study by the abstinence time of subjects. The abstinence time was not different at either of the collection times.

SOD, GPX, GSH and trace elements are important indicators of antioxidant status. Since it is difficult to measure the effectiveness of SOD in isolation of another antioxidant, we chose to determine SOD activity in seminal plasma of infertile men associated to GPX activity, zinc level and GSH amount [26]. The analysis of our results indicated a significantly lower seminal SOD activity detected in infertile groups compared to normozoospermic men. This outcome confirms previously published observations of the other authors [27, 6]. In contrast, Zini A et al. [10] showed highly seminal SOD activities in azoospermic and non-azoospermic men compared to fertile group. Our study like several studies Marzec-Wróblewska U et al [6] and Murawski M [27] showed also highly significant and positive correlations between seminal SOD activity and semen parameters, sperm concentration and overall motility, which are regarded as the most important criteria for normal fertilizing ability of the spermatozoa. We noted also a negative but not significant association of seminal SOD to abnormal morphology. However, some investigators suggested that the beneficial impact of SOD activity concerns only sperm movement, whereas no influence on sperm count has been noticed [28, 29]. The important activity of seminal SOD in control group and the noted associations between this enzyme and sperm quality proved the ability of this enzymatic antioxidant to remove $O_2^{\circ-}$ and its important biological role in controlling the fertilizing potential of this highly specialized cell. On the other hand, decrease of the capacity can result in accumulation of $O_2^{\circ-}$and indirectly in excessive membrane lipid per-oxidation which is responsible of abnormal sperm motility determined as sperm hyper-activation which was confirmed by the important amounts of seminal MDA found in seminal plasma of infertile patients and the high negative correlation ($P<0.0001$; $r=-0.490^{**}$) noted between this enzyme and MDA in our study. SOD is also considered as a pro-oxidant by the conversion of the superoxide anion into a quite stable and invasive free radical, H_2O_2. To efficiently recycle H_2O_2, two enzymatic activities are available: catalase and GPX. In our research we tried to explore the seminal activity of GPX in seminal plasma of our collected samples[30].

Conversely to Tamer et al., [31], we noted that the activity of seminal GPX was lower in abnormal groups than normozoospermic patients. Giannattasio et al. [32] showed also that the seminal GPX activity from healthy subjects was 10 times greater than that from infertile males. Efficiently, the increased activity of GPX in seminal plasma of normozoospermic men suggested that higher activity of this enzyme catalyzes the ROS which might protect sperm against per-oxidative damage [33] and also plays role in sperm maturation from the early events up to the onset of fertilization [9]. Reduction of GPX in seminal plasma may lead to reduce fertilizing capacity and defective sperm quality [10]. Recently, Dandekar et al. [34] and Hsieh et al. [35] demonstrated that GPX activities in seminal plasma correlate positively with Sperm motility which is in agreement with our findings. Successfully, we established strongly positive relationships between seminal GPX activity, sperm motility ($P=0,04$; $r= 0,263^{*}$) and sperm concentration ($P<0,001$; $r= 470^{**}$). However, we observed a negative but not significant correlation among this enzyme and percentage of abnormal sperm morphology. These associations reinforce the benefic effects of GPX in scavenging of ROS and consequently in the safeguard of good sperm quality. Positive and strongly relationship which was noted between seminal SOD and GPX activities confirms that it is difficult to measure the effectiveness of one antioxidant in isolation of another because there appear to be in cooperation [26]. This association supported the concomitant effect of the two enzymes against deleterious effects of lipid per-oxidation and oxidative stress affecting sperm quality [36]. But, the absence of significant correlation among GPX activity and MDA content in seminal plasma suggested that the seminal GPX is still not a useful tool in determining sperm fertilizing potential and this may be due to the case limitation or the complex interactions between the ROS and numerous antioxidants. In fact, the regulation of sperm function

and the mechanisms involved may be complex and multi-factorial.

GPX activity is related with the balance between the GSHr (reduced form of glutathione) and GSSG (oxidized form of glutathione) [37]. But, little information about the effects of seminal glutathione on male fertility potential is adequate till date. Moreover, there are lot of controversy and variability in the findings. In fact, as regards our investigation, we confirmed the presence in human seminal plasma of detectable amounts of different forms of GSH, While other studies found GSH levels below the limit of detection (<2.5µM) in seminal plasma of infertile men[14, 38]. We however found higher levels of Total GSH in seminal plasma of normozoospermics compared to any other abnormal groups, but the difference was statistically significant only after comparison with asthezoospermic group (P=0.03). The important amounts of seminal GSH in control group confirmed the positive contribution of this free thiol as an antioxidant to maintain the good quality and motility of sperm. While stating that, other investigations could not observe any difference in GSH concentration between fertile and sub-fertile men [18]. Ochsendorf et al. [14] found out moderate reduction of GSH in oligozoospermics compared to normozoospermics. Others have noted that GSH levels must be significantly reduced in seminal plasma of infertile males compared to fertile ones [39] and this notes corroborated with our findings. Hesham et al [1], were also in accord with us, they noted a highly significant decrease of mean GSH level in both azoospermic and oligozoospermic groups compared to normospermic one [1]. In effect, as we indicated GSH exists into two forms, reduced (GSHr) and oxidized (GSSG) forms. It is well known that GSHr is the most abundant form and this evidence was confirmed by our results. Effectively, we noted that the most important form was the GSHr in seminal plasma of all groups included in this study. In actual effect, we found significant decrease of seminal GSHr content in asthenozoospermics compared to control men (P=0.008). These data suggested that a lower level of seminal GSHr in asthenozoospermc subjects can be associated to a decline of sperm motility, however higher levels of this element in seminal plasma can guide to an enhanced sperm movement. Effectively, the presence of low amounts of GSHr in samples with abnormal sperm motility remains to be explained. While asthenozoospermic patients showed decreased levels of seminal GSHr, oliasthenoteratozoospermic (OAT) group in comparison with controls, showed an elevated content of seminal GSHr sustained with a significant decline in GSSG levels (P=0.02). This elevation of GSHr in OAT group may be due to the contribution of excessive ROS produced by the abnormal spermatozoa leading to up-regulation of thiol synthesis in order to protect sperm from oxidative damage. Even glutathione therapy was found to improve the semen quality [27]. For this reason we tried to estimate correlations that can be exist between the seminal GSH levels and the sperm criteria. We noted a significant and negative correlation among seminal GSSG and the percentage of abnormal morphology. This result provides evidence that GSSG in seminal plasma seem to protect the quality of sperm cell membrane and morphology [40]. This result was compatible with those observed by Bhardwaj A et al [12] and Chaudhari A.R et al [41] who also observed positive correlation between seminal content of GSH and normal sperm morphology. We also found a significant negative correlation between GSSG and MDA levels of seminal plasma which was in agreement with Chaudhari A.R et al [41]. Therefore, GSH might have some fertility enhancing role by reducing lipid per-oxidation. On the other hand, there were no correlations between seminal GSHt and GSHr with the sperm parameters. This outcome which was in agreement with Garrido et al.[40] not negotiate the beneficial role of GSH to minimize oxidative damage to the sperms but more extended series of clinical trial will be needed.

The activity of total SOD was yet measured in seminal plasma of our subjects. Since zinc forms an essential part of this antioxidant enzyme (Copper/zinc SOD), we decided to determine its seminal levels and to estimate its influence on semen quality. Our outcomes showed significant elevation (P<0.001) of seminal zinc content in control group compared to the two abnormal groups. Several studies support this result [19, 27, 42, 43]. They mentioned that decrease in Zn concentration guides to an increase in oxidation of DNA, proteins, and lipids which can lead to the loss of sperm integrity [44, 45, 46, 47]. Increased ROS in the seminal plasma of infertile men may explain the decrease of the effective concentration of Zn, increasing the harmful effects of ROS to sperm cells that are associated with abnormal sperm parameters [45]. In this actual fact, there were many controversy reports indicating that there is no significant difference between Zn content in fertile and infertile men [45, 48, 49]. In addition, positive associations were observed between this trace element and sperm motility and sperm count. Our outcomes were in agreement with many investigations reporting that high concentration of seminal Zn was associated with enhanced sperm parameters including sperm count [38,45,46,16] and sperm motility [16,44,45,50]. Zhao et al [42] also observed a positive relationship between poor production of sperm and poor

sperm motility with a lower seminal content of Zn. These findings supported the extensive evidence defending the central contribution of seminal Zn to maintain the physiologic functions of sperm. Additionally, we noted a negative but not significant correlation between seminal Zn and MDA confirming that decreased levels of this antioxidant can lead to increased lipid per-oxidation and low quality of sperm [44, 45, 49]. At the same time, we noted conflicting results on the effect of seminal Zn on sperm quality. Effectively, we found a negative significant correlation between seminal Zn amounts and the percentage of abnormal spermatozoa. This was in accord with some studies stating associations between high concentrations of Zn and poor quality of sperm [51]. Consequently to these contradictory results, we demonstrated that physiologic levels of seminal Zn enhanced sperm motility and concentration, but the up-regulation leading to the large levels can be harmful to sperm morphology.

Impaired antioxidant statue was observed in seminal plasma of infertile men collected in our study and this may be an important risk factor of oxidant damage and make the sperm highly susceptible to lipid per-oxidation. For this reason, we determined MDA levels in order to clarify and interpret our results. Among the various methods for detection lipid per-oxidation, we chose to measure spontaneous MDA production, which reflects the per-oxidation of polyunsaturated phospholipids, the major components of sperm membrane [52]. The assay for sperm lipid per-oxidation used in this study involves the measurement of MDA, which forms an adduct with TBA [52] that can be detected with high sensitivity spectro-photometrically. MDA measurements are relevant because major loss of sperm function may occur with minimal damage to the membranes that envelop the sperm and/or divide key intracellular sperm compartments. Our results established highly significant increase ($P<0.001$) of mean level of MDA in seminal plasma was found in abnormal groups compared to normoozoospermics. Accordingly to these notes, lipid per-oxidative degradation of sperm membrane integrity may be held responsible for abnormal sperm motility, concentration and form. Thus, this oxidative damage is a probable cause of idiopathic male infertility involving disruption of spermatogenesis [53]. Our results were in agreement with Hesham et al. [1], Hsieh et al. [35] and Dandekar et al. [34]. Like us Fraczek et al. [54] noted significant elevated seminal MDA level in patients with oligoasthenoteratozoospermia. We were also corroborative with Tavilani et al. [55] and Ben Abdallah F et al. [56] who reported that MDA content was elevated in seminal of asthenozoospermic men. However, there is controversy about seminal MDA activity and the sperm quality. Kobayashi et al. [57] and Ben Abdallah F et al. [56] established significant correlations between MDA concentration in spermatozoa and the number of immotile spermatozoa which was in accord with our result. In fact, we revealed a significant negative correlation between MDA and sperm motility. In contrast, others demonstrated that the MDA concentration in the seminal plasma was not correlated with the sperm count and motility [58]. Besides, we observed a negative but not significant correlation between the MDA concentration and sperm count, which was compatible with the findings of Suleiman et al. [58] and not compatible with those of Hesham et al [1], Geva et al [59], Fraczek et al [54] and Kobayashi et al [57]. Increased MDA levels in seminal plasma of abnormal groups could represent the pathologic lipid per-oxidation effects on spermatozoa membrane and consequently on sperm motility and viability. Impaired antioxidant defense in seminal plasma of our infertile men help to clarify the important amounts of seminal MDA in these patients. Consequently, it seems that elevated lipid per-oxidation of human spermatozoa may explain loss of fertility in patients collected for our investigation.

Conclusion

To summarize, in this study we investigated whether the antioxidant status and the extent lipid per-oxidation in seminal plasma would be the best predictor of sperm function. Successfully, it was found that the MDA level was more important in infertile men than normozoospermics and it was negatively correlated with sperm motility and concentration. In contrast, antioxidant status was significantly increased in seminal plasma of control group and positively associated to sperm motility and count. This could provide the database about the effects of MDA and antioxidants upon sperm. While the elevated rates of GSHr in seminal plasma of OAT patients and the absence of correlations between GSHt and GSHr with sperm quality showed that the regulation of sperm function and the mechanisms involved may be complex and multi-factorial. Therefore, the positive correlation of seminal zinc with abnormal morphology not negotiate the beneficial role of this trace element but the up-regulation leading to the large levels can be harmful to sperm morphology. Finally, future research may include the studies using oxidative markers and antioxidant system on the large scale; the genetic susceptibility and their repercussions on IVF outcomes might be explored with respect to semen quality.

Conflict of Interests

The authors have declared that no conflict of interest exists.

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