Seminal Plasma Soluble forms of Fas, Oxidants and Antioxidants in Infertile Men with Varicocele

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ABSTRACT

Varicocele is a common cause of male infertility Recent studies at the molecular level have demonstrated that varicocele can cause testicular nuclear DNA damage, apoptosis, and raised levels of reactive oxygen species. The present study was carried out on 120 men: 60 infertile (30 with varicocele and 30 without) and 60 fertile men (30 with varicocele and 30 without). Varicocele was diagnosed clinically and by ultrasonography. Standard semen analysis (sperm concentration, motility, morphology; and seminal leucocytic counts) was performed according to the criteria presented by World Health Organization in 2010. Then, the seminal plasma was assessed for levels of oxidants [malondialdehyde (MDA)], antioxidants [ascorbic acid, glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD)] and seminal antiapoptotic factor; soluble fibroblast associated (sFas) which has been identified in the testis as a key physiological regulator of apoptosis. The results revealed that patients with varicocele had significantly higher seminal oxidants (MDA P< 0.001) and significantly lower seminal antioxidants (SOD, GPx, CAT and ascorbic acid; P < 0.001) and sFas; (P<0.001); as compared to men without varicocele whether fertile or infertile. Patients with grade3 varicocele had significantly higher seminal oxidants, and significantly lower antioxidants and sFas as compared to grade 1 varicoceles (P<0.001). Also, infertile men with or without varicocele had significantly higher seminal oxidants (MDA P<0.001) and significantly lower seminal antioxidants (SOD, GPx, CAT and ascorbic acid; P<0.001) and sFas; (P<0.001); compared to fertile men with or without varicocele. Moreover, sperm concentrations in men with varicocele (fertile and infertile showed significant positive correlations with the levels of ascorbic acid (r=0.882 & 0.626,respectively, P<0.001 for each); with the activity of SOD (r=0.901&0.711 respectively, P<0.001 for each), GPx (r=0.909 &0.703 respectively, P<0.001 for each), CAT (r=0.751&0.679 respectively, P<0.001 for each), and sFas (r=0.750 &0.542, P<0.001& <0.005 respectively), and significant negative correlations with the levels of MDA (r= -0.896 & - 0.732 respectively, P<0.001 for each), In addition, sperm motility showed similar correlations as the sperm concentrations.

Conclusions: *The present results indicated that varicocele in fertile and infertile men is associated with increased oxidative stress (indicated with significant increase of MDA), and significant decrease in seminal antiapoptotic factor (sFas) and antioxidants (SOD, GPx, CAT and ascorbic acid). These associations were strongly correlated with increased grade of varicocele.*

Key Words: *Male infertility, antioxidant, oxidant, varicocele, apoptosis, sFas*

INTRODUCTION

Varicocele is an abnormal engorgement and dilatation of the pampiniform plexus above the testes. It is the most common cause of male infertility [(1)] and occurs in 5–25% of the adult male population, 35% of men with primary infertility and up to 80% of men with secondary infertility[(2,3)]. Though, the association of varicocele with infertility is recognized since centuries, yet the exact cause(s) of that abnormality remain(s) to be determined. Several theories have been suggested to explain the mechanism by which varicocele impairs male fertility. These theories include an increase in apoptosis, increased sperm DNA damage[(4)], oxidative stress, tissue hypoxia[(5,6)], degenerative changes in the seminiferous tubule, and immunological infertility caused by decreased production of Fas protein [(7)], increased scrotal temperature ; reflux of the metabolites of the kidney and adrenal gland in the renal veins and testicular hypoxia due to venous stasis[(8)]. These changes are considered to occur gradually as the duration of the varicocele increases [(9)].

Reactive oxygen species (ROS) are metabolites of oxygen, including superoxide anions, hydrogen peroxide, hydroxyl radical, hydroperoxyl radical and nitric oxide (NO). When presenting in excess, they can initiate pathological damage by inducing oxidative changes to cellular lipids, proteins and DNA[(10)]. Most cells are equipped with either enzymatic antioxidant systems, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat), or non-enzymatic antioxidant systems, such as uric acid, vitamin C, vitamin E and albumin. When these defenses are overwhelmed, cell function is affected. Seminal plasma possesses antioxidant scavengers and enzymes which might be deficient in some patients. Significant increase of superoxide anion and free radical activity has been demonstrated in some andrological conditions, such as leukocytospermia and varicocele where great oxidative stress produce different unstable, potentially toxic products[(11)]. Scrotal varicocele is found to be associated with elevated spermatozoal ROS production and diminished seminal plasma antioxidant activity[(12)]. Also, it has been demonstrated that surgical varicocelectomy improved semen parameters associated with decreased ROS and increased antioxidant seminal plasma levels [(13)].

Apoptosis has been observed in testicular germ cells, and is thought to be one of the important factors in regulating the production of spermatozoa. Two major pathways (intrinsic and extrinsic) are known to induce apoptotic cell death; these pathways differ in how the death signal is transduced [(14)]. The intrinsic (or mitochondrial) pathway is induced by cellular stress; this involves Bcl-2, mitochondrial outer-membrane permeability, and caspase-9 [(14)]. The extrinsic (or death receptor) pathway is induced by specific ligands that engage death receptors; this involves Fas, the bindings and activation of caspase-8 [(14)].

The physiologic regulation of germ cell apoptosis was found to

involve signaling pathways triggered by Fas ligand (FasL). The Fas system has been implicated as a key regulator of germ cell apoptosis in the mammalian testis[15]. The Fas system in the testis has been identified as a paracrine signaling system by which Sertoli cells, expressing FasL, can initiate killing of Fas expressing germ cells. Fas or CD 95 is a type I membrane protein that mediates apoptosis. When Fas ligand (FasL) or anti-Fas antibody bind to Fas, apoptosis occurs [16]. **Moustafa et al.**[17] determined that infertile patients had high ROS levels in their seminal plasma and a higher percentage of apoptosis than normal healthy donors.

In addition, soluble forms of cell surface receptors such as soluble Fas (sFas) can be produced either by proteolytic cleavage of membrane bound receptors or by alternative splicing [18].

The aim of the present study is to assess the relationship of seminal oxidants, antioxidants and sFas to fertility in men with varicocele

SUBJECTS & METHODS

The current study included 120 men that were divided into four groups of 30 men each: Group I fertile men without varicocele, Group II fertile with varicocele, Group III infertile without varicocele, Group IV infertile with varicocele. All subjects were selected prospectively among the attendants of Andrology Outpatient Clinic of the Cairo University Hospital. Written informed consent was obtained from all participants. All men were evaluated clinically by physical examination in a warm room while the patient in standing position with and without Valsalva maneuver for clinical detection and grading of varicocele, if present [19]. The term clinical varicocele refers to those detectable by either visual inspection or palpation. The most widely used classification is the Dubin grading system [20]

Varicocele was graded into: grade I (palpable only during a Valsalva maneuver), grade II (palpable distension with the patient standing upright) or grade III (visible through the scrotal skin) [21]. Color Doppler Ultrasonographic (CDU) examination was conducted for assurance of varicocele and its grading. A radiologic diagnosis of varicocele was made when one or more veins had a maximal diameter of >3 mm [22] and retrograde flow was seen either at rest or under Valsalva maneuver. The fertile group included healthy men who had initiated at least one pregnancy and were therefore considered to have proven fertility. Infertile group included men from couples who had failed to conceive after at least one year of regular unprotected intercourse. The female partners of those men underwent gynecologic evaluations, and all results were normal. In all men, a detailed medical history, including men's occupation, the use of prescription medications, smoking habits and alcohol intake was obtained: all participants were non-smokers and alcohol non-users as sperm concentration and motility were reported to be negatively influenced by alcohol consumption [23] or tobacco smoking [24]. Patients were excluded from the study if there was history of

illicit drug use, exposure to any environmental or occupational toxicants, use of medication with proven toxicity on fertility, exposure to radiation or heat, mumps with orchitis, sexually transmitted or systemic diseases, cryptorchidism regardless of treatment, testicular torsion, hypogonadism (testicular volume <15 ml), genitourinary anomalies or infection, epididymal, or vas deferens alterations, and scrotal or inguinal surgery. To reduce the heterogeneity of the groups, the same exclusion criteria were applied to all of the male participants.

All subjects were assessed for semen analysis. Varicocele was on the left side in 55 men and bilateral on 5 men.

Seminal samples: The samples were collected by masturbation into sterile plastic screw-capped 50 ml containers, after 3–5 days of sexual abstinence. Seminal samples were allowed to liquefy for 30 minutes at room temperature and were then evaluated according to the recently adopted guidelines of World Health Organization criteria [25]. The variables taken into consideration for normal fertile semen were: ejaculate volume (1.5ml, 1.4 -1.7ml), sperm concentration (15 x 10^6/ml, 14 – 16 x 10^6/ml), and total (progressive and non-progressive) motility 40 % (38 – 42 %) .Seminal plasma was obtained after liquefaction by centrifuging at 1000 *g* for 10 minutes, followed by an additional centrifugation of the supernatant at 4000 *g* for 20 minutes to remove debris. Each sample was then divided into aliquots and stored frozen at − 20 °C till time of assay. Leukocyte concentrations in semen were quantified by a myeloperoxidase staining test [26]. The results were recorded as X 10^6 peroxidase-positive leukocytes/ml. of semen. Patients who had leukocytospermia (peroxidase-positive leukocytes was greater than 1X10^6 /ml. of semen) were excluded.

I-Assessment of oxidative stress indices in seminal plasma:

A-Oxidants: Determination of malondialdehyde (MDA): Proteins of the seminal plasma were precipitated by addition of trichloroacetic acid (TCA), then, thiobarbituric acid (TAB) reacts with malondialdehyde (MDA) to form thiobarbituric acid reactive product which is measured at 534nm [27].

B. Antioxidants:

1. Ascorbic acid: The ascorbic acid level of seminal plasma was measured by the spectrophotometric method described by **Kyaw** [28].
2. Spectrophotometric methods were used for estimating seminal plasma antioxidant enzyme activity i.e **Kraus and Ganther** [29] method for glutathione peroxidase (GPx); **Beers and Sizer** [30] method for catalase (CAT); and **Misra and Fridovich** [31] method for superoxide dismutase (SOD).

II- Human Fas/Apo-1 levels were determined in seminal plasma by an ELISA technique using an immunoassay kit, (Cat. No. KHS 9502, supplied by BioSource International Inc. California, USA) according to the method of **Cifone et al.** [32].

Statistical analysis:

The Kolmogorov-Smirnov test was used to view the data graphically and analyze how the data was distributed. Data were analyzed using

statistical package (SPSS Version 13). The results were presented as mean ± SD. For the comparison of statistical significance between two groups, unpaired student t test was used for the normally distributed continuous variables. For multiple comparisons, one-way analysis of variance (ONE-WAY-ANOVA) test followed by Least Significant Difference (LSD) were used. Correlations between variables were calculated using Spearman nonparametric method. A value of $p< 0.05$ was considered to be significant.

RESULTS

The age, semen characteristics and the investigated parameters of the studied 4 groups of subjects are presented in table 1. Infertile men with varicocele had significantly impaired semen quality (sperm concentration, motility and normal morphology), significantly higher seminal oxidants (MDA $P<0.001$) and significantly lower seminal antioxidants (SOD, GPx, CAT and ascorbic acid; $P <0.001$ for each) as well as sFas ($P<0.001$); as compared to their corresponding control infertile men without varicocele. Fertile men with varicocele compared to their corresponding control fertile men without varicocele showed the same abnormalities.

Varicocele in fertile men were 13 grade I, 9 grade II and 8 grade III, but in infertile men, varicocele were 7 grade I, 12 grade II and 11 grade III. The age, semen characteristics and the investigated parameters of the studied subjects with varicocele in fertile and infertile men according to the grade of varicocele are presented in tables 2 and 3.

Table (2) shows comparison of the age, semen characteristics and the investigated parameters of the studied 30 fertile subjects with varicocele according to the varicocele grade. Patients with grade 3 varicocele had significantly more impaired semen quality (sperm concentration, motility and normal morphology), significantly more higher seminal oxidants (MDA $P< 0.001$) and significantly more lower seminal antioxidants (SOD, GPx, CAT and ascorbic acid; $P < 0.001$ for each) as well as sFas ($P<0.001$) compared to fertile men with grade II varicocele. Also, varicocele grade II compared to varicocele grade I showed the same abnormalities.

Table (3) shows comparison of the age, semen characteristics and the investigated parameters of the studied 30 infertile subjects with varicocele according to the varicocele grade. Infertile patients with grade 3 varicocele had significantly more impaired semen quality (sperm concentration, motility and normal morphology), significantly more higher seminal oxidants (MDA $P< 0.001$) and significantly more lower seminal antioxidants (SOD, GPx, CAT and ascorbic acid; $P < 0.001$ for each) as well as sFas ($P<0.001$) compared to fertile men with grade II varicocele. Also, varicocele grade II compared to varicocele grade I showed the same abnormalities.

Table 4 shows the correlations between sperm concentrations and sperm motility with the investigated parameters in fertile and infertile men with varicocele. Sperm concentrations

in fertile men with varicocele (n=30) showed significant positive correlations with the levels of seminal ascorbic acid (r=0.882); activity of SOD (r=0.901), activity of GPx (r=0.909), activity of CAT (r=0.751); and with the levels of sFas (r=0.750); and significant negative correlations with MDA levels (r=- 0.896), P values were <0.001 for each). In addition, sperm motility showed similar correlations as the sperm concentrations: with MDA (r=- 0.882), with sFas (r= 0.754,), with ascorbic acid (r= 0.893,), with SOD activity (r= 0.827), with CAT activity (r= 0.758), and GPx activity (r=0.917,) P values were <0.001 for each). In infertile men with varicoceles (n=30) sperm concentrations showed significant positive correlations with the levels of seminal ascorbic acid (r=0.626); activity of SOD (r=0.711), activity of GPx (r=0.703), activity of CAT (r=0.679); and levels of sFas (r= 0.542) and significant negative correlations with MDA levels (r=- 0.732), P values were <0.001 for each). In addition, sperm motility showed similar correlations as the sperm concentrations: with MDA (r=- 0.766, P<0.001), with sFas (r= 0.585, P<0.005), with ascorbic acid (r= 0.699, P<0.001), with SOD activity (r= 0.778, P<0.001), with CAT activity (r= 0.657, P<0.001), and GPx activity (r=0.813, P<0.001).

Table (1): Comparison of the age, seminal characteristics and the investigated parameters of the studied 4 groups of subjects (mean ± SD)

Parameters	Fertile controls		Infertile men		F	P and significance
	Fertile without varicocele n =30	Fertile with varicocele n =30	Infertile without varicocele n =30	Infertile with Varicocele n = 30		
Age, years	36.20± 2.66	36.17±4.96	36.47±3.67	36.93±5.25	0.207	>0.9
Volume , (ml)	3.50 ± 0.63	3.16±0.77	2.73±083[a*]	2.69±0.86[a*]	7.304	<0.001*
Sperm Concentration ($X10^6$/ml.)	78.47 ± 9.32	56.07±6.37[a]	18.5±3.01[a*]	14.0±1.66[a*†]	822.08	<0.001*
Sperm Motility (%)	65.27 ± 7.5	52.5±6.66 [a]	30.6±5.63[a*]	21.07±3.05 [a*†]	344.25	<0.001*
Normal Sperm Forms (%)	61.77±10.39	51.17±8.78[a]	40.63±5.87[a*]	31.27±7.06[a*†]	77.4	<0.001*
Seminal Leukocytes ($X10^6$/ml.).	0.43 ± 0.14	0.48±0.102	0.56±0.089[a*]	0.61±0.09[a*]	16.79	<0.001*
MDA (Pmol/mg protein)	7.22 ± 1.06	8.73±1.06 [a]	10.0±1.17[a*]	12.67±1.58[a*†]	104.53	<0.001*
Ascorbic Acid (mg/dl.)	1.37 ± 0.39	1.08±1.32[a]	0.86±0.14[a*]	0.67±0.20[a*†]	54.829	<0.001*
GPx (mU/ml. protein)	69.73 ± 8.94	55.6±4.59[a]	45.6±4.99[a*]	31.5±7.01[a*†]	178.39	<0.001*
CAT (U/ml.)	134.0± 25.41	106.0±15.90[a]	85.1±8.92[a*]	68.27±16.01[a*†]	78.09	<0.001*
SOD (U/ml.)	35.93 ± 7.51	27.53±3.85[a]	22.5±3.80[a*]	16.57±3.18 [a*†]	84.23	<0.001*
s-Fas (ng/ml)	6.57 ± 0.57	6.18±0.80[a]	5.8 ±0.62[a*]	4.75±0.58 [a*†]	43.221	<0.001*

[a] Significant difference vs with fertile controls

* Significantly different vs fertile control with varicocele.

† Significantly different vs infertile without varicocele

Table (2): Comparison of the investigated semen parameters in fertile men with varicocele according to grades of varicocele (mean ± SD.).

	Grade 1 Varicocele n = 13	**Grade II Varicocele n= 9**	**Grade III Varicocele n= 8**	**F**	**Significant**
Age, years	36.08±3.34	36.11±6.39	35.88±4.97	0.006	>0.9
Volume , (ml)	3.11±0.89	3.2 ±0.73	3.170 ±0.89	0.035	>0.9
Sperm Concentration ($X10^6$/ml.)	61.92±3.20	55.22±1.09[a]	47.75±3.15 [ab]	67.893	<0.001*
Sperm Motility (%)	58.33±3.26	51.67±2.50 [a]	43.75±2.31 [ab]	70.577	<0.001*
Normal Sperm Forms (%)	58.33±5.36	47.78±4.41[a]	42.5±2.67 [ab]	35.255	<0.001*
Seminal Leukocytes ($X10^6$/ml.).	0.39±0.05	0.50±0.05[a]	0.61±0.064 [ab]	41.187	<0.001*
MDA (Pmol/mg protein)	7.75 ± 0.45	8.94 ± 0.17 [a]	10.06 ± 0.68 [ab]	62.509	<0.001*
Ascorbic Acid (mg/dl.)	1.01 ± 0.11	0.83 ±0.07 [a]	0.56 ±0.07 [ab]	61.430	<0.001*
GPx (mU/ml. protein)	59.83 ± 2.89	54.44± 1.67[a]	50.0 ±1.2 [ab]	54.177	<0.001*
CAT (U/ml.)	117.50±9.65	100.56 ±3.91[a]	89.38±.5.15 [ab]	29.396	<0.001*
SOD (U/ml.)	30.83 ±1.95	26.89 ±2.20[a]	23.0 ±2.51 [ab]	32.810	<0.001*
s-Fas (ng/ml)	6.88 ± 0.46	6.11 ± 0.22 [a]	5.06 ±0.18 [ab]	69.871	<0.001*

[a]: Compare Grade II varicocele and Grade III with Grade I varicocele.
[b]: Compare Grade II varicocele with Grade III heavy varicocele

Table(3): Comparison of the investigated semen parameters in infertile men with varicocele according to grades of varicocele (mean ± SD.).

	Grade 1 Varicocele n = 7	**Grade II Varicocele n= 12**	**Grade III Varicocele n= 11**	**F**	**Significant**
Age, years	36.14±5.67	36.58±5.07	36.72±6.75	0.022	>0.9
Volume , (ml)	2.94 ±0.99	2.71 ±0.79	2.52 ±0.89	0.507	>0.6
Sperm Concentration ($X10^6$/ml.)	15.86±1.46	14.42±1.38[a]	12.36±1.12 [ab]	16.228	<0.001*
Sperm Motility (%)	25.71±1.89	20.33±0.78 [a]	18.91±1.81 [ab]	46.220	<0.001*
Normal Sperm Forms (%)	37.14±3.93	32.92±4.98[a]	29.0±2.83 [ab]	8.713	<0.001*
Seminal Leukocytes ($X10^6$/ml.).	0.51±0.04	0.59±0.05[a]	0.69±0.07 [ab]	21.794	<0.001*
MDA (Pmol/mg protein)	10.71 ± 0.49	12.33 ± 0.98 [a]	14.27 ± 0.65 [ab]	46.769	<0.001*
Ascorbic Acid (mg/dl.)	0.92 ± 0.04	0.71 ±0.15 [a]	0.47 ±0.07 [ab]	39.474	<0.001*
GPx (mU/ml. protein)	41.71 ± 2.36	31.58± 3.65[a]	24.91 ±1.87 [ab]	75.777	<0.001*
CAT (U/ml.)	86.14±5.40	73.42 ±8.33[a]	51.27±.8.76 [ab]	45.359	<0.001*
SOD (U/ml.)	20.71 ±1.25	17.0 ±1.28[a]	13.45 ±1.92 [ab]	48.232	<0.001*
s-Fas (ng/ml)	5.64 ± 0.48	4.75 ± 0.45 [a]	4.18 ±0 .64 [ab]	15.92	<0.001*

[a]: Compare Grade II varicocele and Grade III with Grade I varicocele.
[b]: Compare Grade II varicocele with Grade III heavy varicocele

Table (4): Correlation study of the investigated semen parameters with sperm motility and sperm concentration i**n** fertile and infertile men with varicoceles (mean ± SD.).

fertile men with varicoceles n= 30				
	Sperm concentration		**Sperm motility**	
	r	**P**	**r**	**P**
MDA (Pmol/mg protein)	- 0.896	<0.001*	- 0.882	<0.001*
Ascorbic Acid (mg/dl.)	0.882	<0.001*	0.893	<0.001*
GPx (mU/ml. protein)	0.909	<0.001*	0.917	<0.001*
CAT (U/ml.)	0.751	<0.001*	0.758	<0.001*
SOD (U/ml.)	0.901	<0.001*	0.827	<0.001*
s-Fas (ng/ml)	0.750	<0.001*	0.754	<0.001*
infertile men with varicoceles n= 30				
	Sperm concentration		**Sperm motility**	
	r	**P**	**r**	**P**
MDA (Pmol/mg protein)	- 0.732	<0.001*	- 0.766	<0.001*
Ascorbic Acid (mg/dl.)	0.626	<0.001*	0.699	<0.001*
GPx (mU/ml. protein)	0.703	<0.001*	0.813	<0.001*
CAT (U/ml.)	0.679	<0.001*	0.657	<0.001*
SOD (U/ml.)	0.711	<0.001*	0.778	<0.001*
s-Fas (ng/ml)	0.542	<0.005*	0.585	<0.005*

DISCUSSION

The present study included 60 men with varicocele: 30 fertile and 30 infertile. Fifty five of the 60 varicoceles (91.7%) were on the left side and five varicoceles (8.3%) were bilateral. Variation in the incidence of varicocele on both sides may be related to difference in the configuration of the right and left spermatic veins. The right spermatic vein drains into the inferior vena cava obliquely, while the left spermatic vein drains into the left renal vein at a right angle. In addition, the insertion of the left spermatic vein is 8-10 cm higher than that of the right spermatic vein which results in 8-10 cm greater pressure on the blood flow from the left spermatic vein [(33)]**.** The spermatic veins contain valves which help to prevent retrograde blood flow. Therefore, absent or defective valves can lead to an increased pressure within the spermatic veins and consequently varicocele formation. Venous valves are more often absent on left side than right, and the left vein may also suffer compression under the superior mesenteric artery and the aorta [(34)]. These factors have been proposed to be responsible for the preponderance of left sided varicocele (90%) compared to bilateral varicocele (10%)[(35)]. However, **Yigitler et al.**[(36)] have reported that

varicocele is on left side in 98% of cases

Varicocele has been implicated as a major cause of male infertility[37], but the pathophysiology remains unclear. Clinical varicocele is found in about 15% of the general population including adolescents and adults (in 35% of men with primary infertility and in up to 80% of men with secondary infertility [38]. Although the varicocele is clinically evident, not all men with varicocele are infertile. Several studies suggest that an individual with varicocele even with a normal semen analysis or documentation of previous fertility is at risk of subsequent loss of testicular function and fertility [39,40]. At present, despite numerous reports, the pathogenetic mechanisms by which varicocele induces testicular dysfunction and infertility are not completely understood. **Smith et al.**[41] have mentioned that the proposed mechanisms include reflux of toxic metabolites from adrenal or renal origin, impairment of the hypothalamic–gonadal axis, venous stasis leading to testicular hypoxia and elevation of testicular temperature. Recent studies have shown that infertile men with varicocele have elevated levels of sperm-derived reactive oxygen species [42].

Despite the several different theories that aim to explain the impact of varicocele on testicular function, none can fully clarify the variable effect of varicocele on human spermatogenesis and male fertility [43]**.** Proposed mechanisms include hypoxia and stasis, testicular venous hypertension, autoimmunity, elevated testicular temperature, reflux of adrenal catecholamines, and increased oxidative stress [40]. Also, clearly, the factors contributing to abnormal sperm function caused by varicocele that lead to infertility are ambiguous. Research conducted during the last decade has provided growing support to the concept that oxidative stress (OS) is one of the main causes of male infertility. Oxidative stress occurs either as a result of increased levels of reactive oxygen species or insufficient antioxidant capacity [44].

A number of studies have demonstrated high levels of seminal oxidative stress, as evidenced by increased levels of ROS and reduced total antioxidant capacity (TAC) in men with a clinical diagnosis of varicocele, suggesting that sperm dysfunction in varicocele patients may be, at least in part, related to oxidative stress [45]. In addition, oxidative stress has been shown to affect the integrity of the sperm genome by causing high frequencies of single- and double-strand DNA breaks which are often detected in the ejaculates of infertile men [17,41,46] and by altering membrane fluidity, permeability and impairment of sperm functional competence [41].

Fertility markers assessed *in vitro*, such as fertilization rate, embryo cleavage, implantation, pregnancy, and live birth rates are all negatively affected by abnormal high levels of sperm DNA damage [47].

In the present study, men with varicocele (fertile and infertile) compared to men without varicocele (fertile and infertile) show significantly impaired semen quality presented by lower concentration, motility and normal morphology; significantly higher MDA (marker of

lipid peroxidation and indicator of oxidative stress) and significantly lower antioxidants presented by activities of SOD, CAT, GPx and the ascorbic acid levels (table 1). It is already known that testicular development is compromised in adolescents with varicocele; this fact can adversely affect sperm quality[48,49]. **Yeşilli et al.**[50] have demonstrated increased seminal plasma lipid peroxidation indicated by elevated MDA, and lowered sperm count in men with varicocele compared to controls.

The present findings are comparable to those reported by **Mostafa et al.** [51] who demonstrated that MDA, hydrogen peroxide and 5 antioxidants (catalase, glutathione peroxidase and superoxide dismutase, vitamin C and E) were impaired in fertile and infertile men with varicocele compared to those without varicocele. The authors concluded that varicocele is associated with oxidative stress (OS) even in fertile normozoospermic men. Therefore, it may be speculated that men with varicocele may have a threshold value of OS over which male fertility may be impaired. Also, **Abd-Elmoaty et al.**[52] reported significantly higher levels of oxidants (MDA and nitric oxide) and reduced levels of antioxidants (SOD, GPx, CAT, and ascorbic acid) in semen of infertile men with varicocele and that seminal oxidative stress seen in men with varicocele is associated with sperm motility and grade of varicocele.

In the current study, the demonstrated abnormalities (sperm quality, oxidants and antioxidants, the levels of sFas) in fertile men with varicocele (table 2) and in infertile men with varicocele (table 3) are more marked in varicocele grade III compared to grade II and the latter were more marked than grade I.

Allamaneni et al.[53] have reported that seminal ROS levels showed significant correlation with left varicocele grade and significantly elevated seminal ROS levels were seen in men with left varicocele grade 2 and 3 compared to grade 1. **Moein et al.**[54] have reported impaired semen quality in infertile men with varicocele, and the impairment was correlated with grades I and II varicocele, while in grade III, the impairment was not visible probably due to the small number of cases in that grade (20 men in grade I, 20 men in grade II, 2 cases only in grade III).

Moreover, the present findings are in agreement with the results of previous studies using the chemiluminescence technique that allowed collective measurement of seminal ROS and total antioxidant capacity [12] and, later, by the use of ROS/TAC score as an index for OS [55]. Further, the present results also indicated significantly higher levels of seminal oxidants and lower levels of seminal antioxidants in infertile men with grade 3 varicocele as compared to grade 1. Such observation agreed with the findings of **Allamaneni et al.**[53].

The present results indicated a significantly lower percentage of normal sperm forms in fertile as well as infertile men with varicoceles compared to their controls without varicoceles. It has been reported that spermatozoa with mid-piece defects and excess residual cytoplasm is a

major source of ROS production in infertile men [56]. Retention of residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanisms that may be mediated by the cytosolic enzyme glucose 6-phosphate dehydrogenase[33]. Other studies [57,58] have shown that levels of ROS production in semen were negatively correlated with the percent of normal sperm forms as determined by the WHO guidelines [59] and by Kruger's strict criteria [60]. Recently, **Shamsi et al.**[61] have demonstrated a similar correlation.

In the present study, levels of seminal plasma antioxidants were significantly lower in fertile as well as infertile men with varicocele than their controls without varicocele. Furthermore, reduced sperm motility was correlated with low levels of seminal antioxidants [61]. A previous study has indicated that varicocelectomy reduces ROS levels and increases antioxidant activity of seminal plasma from infertile men with varicocele [13].

Apoptosis is a non-inflammatory response to tissue damage characterized by a series of morphological and biochemical changes [62]. In the context of male reproductive tissue, apoptosis helps in elimination of abnormal spermatozoa, thus maintaining the nursing capacity of the Sertoli cells. High levels of ROS disrupt the inner and outer mitochondrial membranes, inducing the release of the cytochrome C protein and activating the caspases and apoptosis. Apoptosis in sperm also may be initiated by ROS-independent pathways involving the cell surface protein Fas[63]. Fas is a type I membrane protein that belongs to the tumor necrosis factor-nerve growth factor receptor family and mediates apoptosis[64]. When Fas ligand or agonistic anti-Fas antibody binds to Fas, apoptosis occurs [65]. On the other hand, bcl-2, the inhibitor gene of apoptosis, protects the cell, most likely by mechanisms that reduce ROS production [66].

Although the Fas protein often leads to apoptosis, some of the Fas-labeled cells may escape apoptosis through abortive apoptosis. This result in a failure to clear all of the spermatozoa destined for elimination and thus, leads to a large population of abnormal spermatozoa in the semen. This failure to clear Fas-positive spermatozoa may be due to a dysfunction at one or more levels. First, the production of spermatozoa may not be enough to trigger apoptosis in men with hypospermatogenesis. In that case, Fas-positive spermatogonia may escape the signal to undergo apoptosis. Second, Fas-positive spermatozoa also may exist because of problems in activating Fas-mediated apoptosis.

Mitochondrial exposure to ROS results in the release of apoptosis inducing factor (AIF), which directly interacts with the DNA and leads to DNA fragmentation [67,68]. Seminal sFas levels were shown to be correlated with sperm concentration, sperm motility in varicocele-associated cases. Subnormal levels of sFas may be responsible for increased apoptosis induced by the Fas system as sFas could block Fas-dependent apoptosis, resulting in impaired

spermatogenesis in patients with varicocele; that lower sFAS levels were reversed by varicocelectomy [(69)]. High temperature and the increased production of heat shock proteins (HSP) and heat shock factors [(70)] could contribute, at least partly, to the lowered sFAS production

In conclusion, the finding of high seminal OS in varicocele patients may indicate that OS plays a role in the pathogenesis of varicocele-mediated male infertility. However, the exact etiology of OS elevation in association with varicocele is unclear. We also found a significant negative relationship between seminal OS and sperm parameters, which indicates that seminal OS plays an important role in the pathogenesis of varicocele-mediated male infertility. Finally, seminal OS levels were higher in the patients with high grades of varicocele, which may have important clinical implications and sFas could play a role in the germ cell apoptosis process in varicocele associated cases

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الفاس الذائب في بلازما السائل المنوي ، المؤكسدات ، ومضادات الأكسدة للرجال العقيمين مع دوالي الخصية

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تمثل دوالي الخصية نسبة كبيرة من اسباب العقم الثانوي وحدوثها يسبب نقصا في خصوبة الرجال. ان مضادات الاكسدة بالسائل المنوي لها دور مهم في التخلص من المؤكسدات التي لها تأثير سلبي في تكوين و حركة الحيوانات المنوية.

لذا فقد تم اختيار ٦٠ رجلا عقيما ، ٣٠ منهم مصاحب لدوالي الخصية و٣٠ غير مصاحب لدوالي الخصية، بالأضافة الي ٦٠ رجلا كامل الخصوبة ، ٣٠ منهم مصاحب لدوالي الخصية و٣٠ غير مصاحب لدوالي الخصية. تم جمع السائل المنوي وقياس معدلات المؤكسدات [ثنائي الدهيد المالون]، ومضاداتها [فيتامين ج ، كاتالاز ، جلوتاثايون بيراوكسيداز ،وسوبراكسيد دسميوتاز] ، والفاس الذائب وهو منظم لموت الخلايا المبرمج من المجموعات.الأربعة.

وقد أظهرت النتائج ارتفاعا في معدلات المؤكسدات ، ونقصا في معدلات مضادات الاكسدة ، والفاس الذائب في حالات العقم المصاحب لدوالي الخصية مقارنة بحالات العقم غير المصاحب لدوالي الخصية ، و في حالات ذات الخصوبة الكاملة. المصاحب لدوالي الخصية مقارنة بحالات ذات الخصوبة الكاملة. غير المصاحب لدوالي الخصية .كذلك لوحظ ايضا ان هذة التغيرات مرتبطة بدرجة دوالي الخصية. مما يرجح ان حدوث هذه التغيرات [ارتفاع المؤكسدات أو نقص معدلات مضادات الاكسدة المصاحب لدوالي الخصية] من المسببات لدوالي الخصية.