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Relationship between Lipid Peroxidation and Integrity of Sperm Plasma Membrane in a Sample of Iraqi Infertile Men

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Abstract: *The current study aims to investigate the relationship between lipid peroxidation, represented by seminal malondialdehyde (MDA) level, and integrity of sperm plasma membrane, represented by the hypo-osmotic swelling (HOS) test score, in infertile men. One hundred and twenty Iraqi men (20 fertile and 100 infertile) shared in this study during their attendance to the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al- Nahrain University. The mean age of the fertile men was (30.46±0.78) years while the mean age of infertile men was (32.58±0.64) years with the duration of infertility (5.36±0.33) years. Semen samples were collected and the parameters of seminal fluid analysis were assessed; also, the HOS test score and seminal MDA level were determined. The results showed a significant (P<0.05) decrease in sperm concentration, sperm motility, sperm grade activity (progressive sperm motility and non-progressive sperm motility), total progressive sperm and normal sperm morphology with a significant (P<0.05) increase in sperm agglutination and round cells count in the infertile group compared with the fertile group. The percentage of sperm HOS test score in the infertile men was significantly (P<0.05) lower than that in the fertile men while seminal MDA level in the infertile men was significantly (P<0.05) higher than in the fertile men. Also, the results revealed a significant negative correlation (r = -0.48, P<0.05) between HOS test score and seminal MDA level in the infertile men. In conclusion, increased seminal MDA levels together with poor response of spermatozoa to hypo-osmotic stress in infertile men could represent the pathological impact of lipid peroxidation on the spermatozoa membrane and consequently on sperm function and quality. Lipid peroxidation may alter sperm membrane functional intergrity adversely and contribute to abnormal sperm function.*

Keywords: *Hypo-osmotic swelling test, Infertile men, Lipid peroxidation, Malondialdehyde*

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I. Introduction

The general definition of infertility is a lesser capacity to conceive than the mean capacity of the general population [1]. Primary infertility is the term used in reproductive medicine for a couple who failed to achieve a pregnancy for one year of marriage and who was never pregnant before, while secondary infertility is the term applied to couple who meet criteria for primary infertility but at some time in the past have been pregnant [2]. Infertility affects 15% of the married couples and in about 50% of cases male factor is the predominant causative factor, where both qualitative and quantitative defects are seen in sperm production [3]. However, when dealing with infertile couples, it is important to recognize that the duration of infertility is a critical prognostic factor, and in couples with a history of primary infertility of longer than 3 years, the possibility of achieving an unassisted pregnancy is low [4]. Semen analysis is the first tool which a medical practitioner uses it to assess the male factor in an infertility workup [5]. Sperm concentration is basic parameter for assessing male fertility, and there have been many calls for global standardization of this test [6]. Sperm motility gives a measure of the integrity of the sperm axoneme and tail structures as well as the metabolic machinery of the mitochondria, while sperm morphology is a surrogate measure of the integrity of DNA packaging and the quality of spermatogenesis [7].

One of the simplest methods to evaluate the plasma lemma of sperm cells is the hypo-osmotic swelling (HOS) test. The traditional HOS test, originally presented by [8], enables reproduction specialists to determine the functional intactness of sperm membranes as spermatozoa "swell" under hypo-osmotic conditions due to the influx of water, and the expansion of the membranes causes the tails to coil [9]. Considering the importance of

this test, a large number of studies have evaluated and reported associations between HOS test and semen parameters, and also between HOS test and fertilization rates and pregnancy outcomes after both in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [10,11].

The oxidative stress is one of the causative factors for the male infertility, the action of reactive oxygen species (ROS) must equal the antioxidant of the body but in the infertile male there is disparity between them and because that there are problem effect on sperm plasma membrane and destruction the poly unsaturated fatty acid (PUFA) by the free radical production from ROS, and form the lipid peroxidation, also effect on the sperm motility and DNA structure [12]. Malondialdehyde (MDA) is one of the final products of lipid peroxidation in seminal plasma [13]. Toxic lipid peroxides are known to cause various impairments of sperm cells and may play a major role in the etiology of male infertility. Malondialdehyde is an index of lipid peroxidation which may be a diagnostic tool for the analysis of infertility [14,15].

The present study aims to investigate the relationship between lipid peroxidation, represented by measurement of seminal MDA level, and integrity of sperm plasma membrane, represented by the HOS test score, in a sample of Iraqi infertile men.

II. Materials and Methods

Subjects

One hundred and twenty Iraqi men (20 fertile and 100 infertile) have been involved in this study during their attendance to the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University. The studied subjects have been divided into two groups as follow:

Fertile group: This group consists of 20 fertile men. The mean ages were (30.46 ± 0.78) years with a range from 19 to 45 years old.

Infertile group: This group consists of 100 infertile men. The mean ages were (32.58 ± 0.64) years with a range from 19 to 49 years old. The duration of infertility was (5.36 ± 0.33) years with a range from 2 to 10 years.

The two groups were handed questionnaire asking for some descriptive data such as age; also, the other characteristic was reported for the infertile group which involved (duration and type of infertility).

Seminal fluid analysis (SFA)

Semen samples were collected by masturbation after 3-5 days of sexually abstinence and then they were incubated at 37ºC to allow liquefaction. Seminal standard parameters following World Health Organization guidelines [16] were determined on all samples which including physical parameters (volume, liquefaction time, and pH) as well as using light microscopy to determine sperm concentration, sperm motility, sperm grade activity (progressive sperm motility, non progressive sperm motility, and immotile sperm), total progressive sperm, normal sperm morphology, sperm agglutination, and round cells count.

Hypo-osmotic swelling test (HOS-test)

The HOS test was performed by mixing 0.1 ml of semen with 1.0 ml of a 150 mOsm/ L NaCl as a hypo-osmotic solution. The mixture was incubated for 30 minute at 37ºC in 5% CO_2. Then, 10 µl of the mixture was placed on a slide and mounted with a cover and examined at a magnification of 40X objective under a light microscope. Swellings of the sperm were identified by coiling of spermatozoa tails. At least 100 spermatozoa were counted in at least ten different fields, and the percentage of swollen sperms was calculated [8].

Seminal malondialdehyde (MDA) level determination

Semen sample was centrifuged at 3000 rpm for 10 minutes after liquefaction to get the seminal plasma. Seminal MDA level was determined according to manufacture recommended procedure by using Bioassay kit through an enzyme linked immune sorbent assay (ELISA) technique. This assay employs the competitive inhibition enzyme immune assays technique [15].

Statistical analysis

Statistical analysis was performed with statistical package for social sciences (SPSS), version18, and computer software. All results were expressed as mean $\pm$ SE. Statistical comparisons between groups were made using student's-test., and $P<0.05$ was considered statistically significant. Pearson correlation coefficients were calculated to check the relationship between variables [17].

III. Results

Descriptive characteristics of the infertile men

Distribution of the infertile men according to their descriptive characteristics is shown in (Table 1). The highest percentage of the infertile men was in the age group of ≤ 29 years comprising (48%) of them, while the lowest percentage of the infertile men was in the age group of 40 to 49 years comprising (14%) of them. The highest percentage of the infertile men had duration of infertility between 3 to 5 years comprising 44% of them, while the lowest percentage of the infertile men had duration of infertility ≥9 years comprising (12%) of them. The majority of the infertile men (70%) complained of primary infertility while the minority of them (30%) complained of secondary infertility.

Table (1): Descriptive characteristics of the infertile men

Variables	No.	(%)
Age (years)		
≤ 29	48	(48)
30-39	38	(38)
40-49	14	(14)
Duration of infertility		
≤2	28	(28)
3-5	44	(44)
6-8	16	(16)
≥9	12	(12)
Type of infertility		
Primary	70	(70)
Secondary	30	(30)

Semen analysis

The results of semen analysis for the fertile and the infertile men (Table 2) revealed non-significant (P>0.05) differences in liquefaction time, semen volume, and semen pH between the two groups. A significant (P<0.05) decrease was seen in the sperm concentration, sperm motility, sperm grade activity (progressive sperm motility and non-progressive sperm motility), total progressive sperm and normal sperm morphology in the infertile group compared with the fertile group; while a significant (P<0.05) increase was seen in immotile sperm, sperm agglutination and round cells count in the infertile group compared with the fertile group.

Table (2): Semen parameters of the fertile and infertile groups.

Semen parameters (Mean ± SE)		Fertile group (N=20)	Infertile group (N=100)	(WHO, 2010) criteria
Semen volume (ml)		$2.85^{a} \pm 0.07$	$2.64^{a} \pm 0.08$	1.5
Liquefaction time (minute)		$38.13^{a} \pm 0.21$	$40.61^{a} \pm 1.28$	≤60
Semen pH		$7.74^{a} \pm 0.21$	$7.62^{a} \pm 0.19$	≥7.2
Sperm concentration (million/ml)		$66.46^{a} \pm 5.84$	$48.71^{a} \pm 3.42$	≥15
Sperm motility (%)		$62.87^{a} \pm 2.38$	$53.12^{b} \pm 1.80$	≥40
Sperm grade activity(%)	Progressive sperm motility (%)	$48.62^{a} \pm 2.63$	$34.56^{b} \pm 1.45$	
	Non progressive sperm motility (%)	$27.25^{a} \pm 0.84$	$18.58^{b} \pm 0.96$	
	Immotile sperm (%)	$24.13^{b} \pm 0.38$	$46.86^{a} \pm 1.79$	
Total progressive sperm (million/ml)		$92.02^{a} \pm 6.34$	$44.47^{b} \pm 5.32$	
Normal sperm morphology (%)		$55.35^{a} \pm 2.85$	$40.39^{b} \pm 1.49$	≥30
Sperm agglutination (%)		$8.27^{b} \pm 1.74$	$22.74^{a} \pm 2.20$	<10
Round cells count (HPF)		$3.68^{b} \pm 0.63$	$8.86^{a} \pm 0.52$	<5

- Means with different superscripts within each row are significantly different (P<0.05).
- Means with similar superscripts within each row are non-significantly different (P>0.05).

Hypo-osmotic swelling (HOS- test)

The percentage of sperm HOS test for the infertile men (57.21 ± 1.12) % was significantly (P<0.05) lower than those of the fertile men (73.66 ± 1.66) % as shown in (Fig. 1).

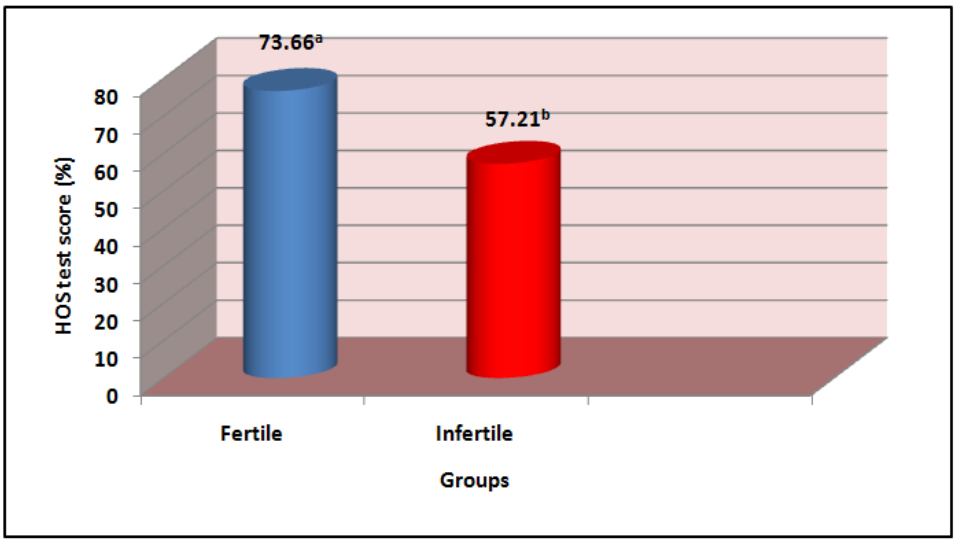

Figure (1): Hypo-osmotic swelling test score in the fertile and the infertile groups.

- Means with different superscripts within each column are significantly different (P<0.05).
- Means with similar superscripts within each column are non-significantly different (P>0.05).

Seminal MDA level

Seminal MDA levels were found to be significantly (P<0.05) higher in the infertile men (1380.82±29.29) ng/ml than in the fertile men (954.74±28.23) ng/ml as shown in (Fig. 2).

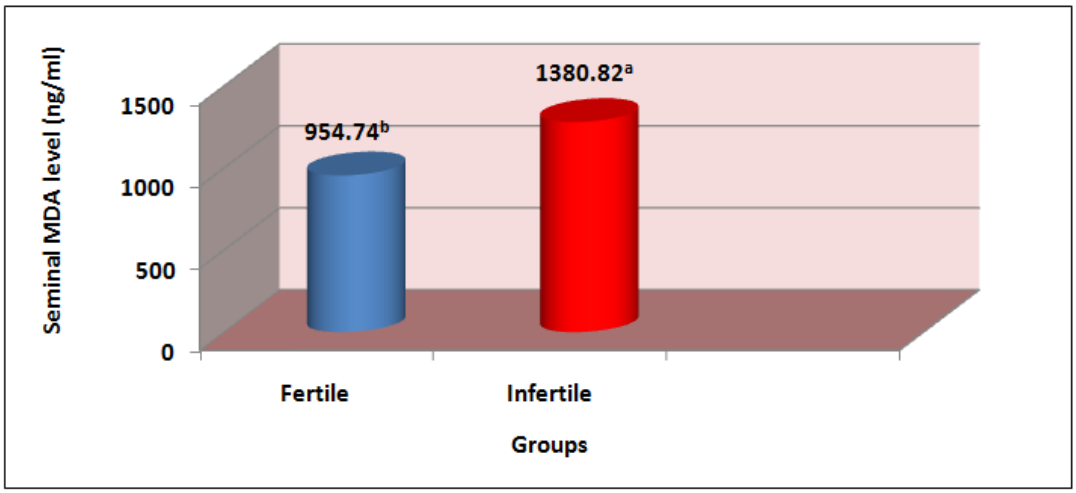

Figure (2): Seminal MDA levels in the fertile and the infertile groups.

- Means with different superscripts within each column are significantly different (P<0.05).
- Means with similar superscripts within each column are non-significantly different (P>0.05).

Correlation coefficient between HOS test score and sperm parameters in infertile men

The correlation was studied between HOS test score and sperm parameters in the infertile men. The results (Table 3) showed that HOS test score revealed a highly significant positive correlation with sperm concentration (r = 0.64, P<0.01), sperm motility (r = 0.71, P<0.01), progressive sperm motility (r = 0.69, P<0.01), and non-progressive sperm motility (r = 0.70, P<0.01); while a highly significant negative correlation was found with immotile sperm (r = - 0.71, P<0.01). Also, a significant positive correlation was found with total progressive sperm (r = 0.42, P<0.05) and normal sperm morphology (r = 0.49, P<0.05).

Table (3): Correlation coefficient between HOS test score and sperm parameters in the infertile men.

Sperm parameters	HOS test score	
	Correlation coefficient (r)	Level of significant (P)
Sperm concentration	0.64	P<0.01**
Sperm motility	0.91	P<0.01**
Progressive sperm motility	0.69	P<0.01**
Non-progressive sperm motility	0.80	P<0.01**
Immotile sperm	-0.91	P<0.01**
Total progressive sperm	0.42	P<0.05*
Normal sperm morphology	0.49	P<0.05*

- * Correlation is significant at the 0.05 level.
- ** Correlation is significant at the 0.01 level.

Correlation coefficient between seminal MDA level and sperm parameters in the infertile patients

The correlation was studied between seminal MDA level and sperm parameters in the infertile men. The results (Table 4) showed that non-significant correlation was found between seminal MDA level and sperm concentration; while a highly significant negative correlation was found with sperm motility (r = -0.86, P<0.01), progressive sperm motility (r = -0.67, P<0.01), and non-progressive sperm motility (r = -0.58, P<0.01); and a highly significant positive correlation was found with immotile sperm (r = 0.62, P<0.01). Also, a significant negative correlation was found with total progressive sperm (r = -0.45, P<0.05) and normal sperm morphology (r = -0.50, P<0.05).

Table (4): Correlation coefficient between seminal MDA level and sperm parameters in the infertile men.

Sperm parameters	Seminal MDA level	
	Correlation coefficient (r)	Level of significant (P)
Sperm concentration	-0.20	NS
Sperm motility	-0.86	P<0.01**
Progressive sperm motility	-0.67	P<0.01**
Non-progressive sperm motility	-0.58	P<0.01**
Immotile sperm	0.62	P<0.01**
Total progressive sperm	-0.45	P<0.05*
Normal sperm morphology	-0.50	P<0.05*

- NS: Non-significant.
- * Correlation is significant at the 0.05 level.
- ** Correlation is significant at the 0.01 level.

Correlation coefficient between HOS test score and seminal MDA level in the infertile men

The correlation was studied between HOS test score and seminal MDA level in the infertile men. As shown in (Fig. 3), a significant negative correlation was found between HOS test score and seminal MDA level in the infertile men (r = -0.38, P<0.01).

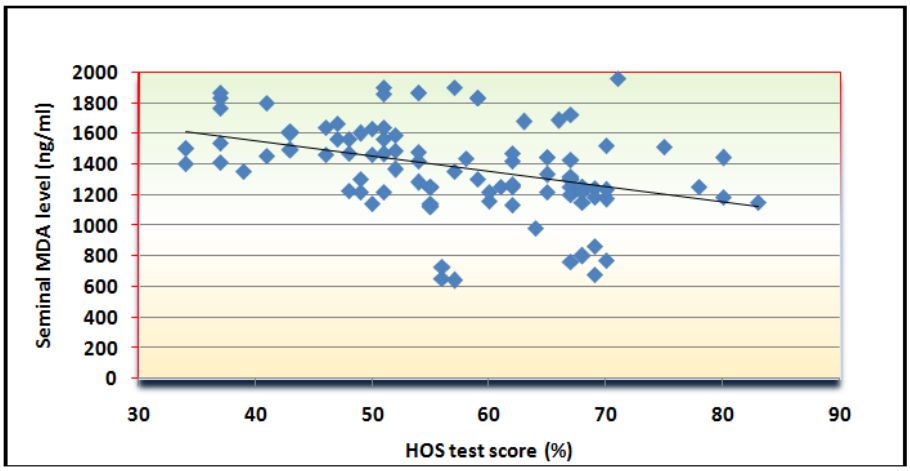

Figure (3): Correlation coefficient between HOS test score and seminal MDA level in the infertile men (r = -0.38, P<0.001).

III. Discussion

In the current study, distribution of the infertile men according to their descriptive characteristics is in broad general agreement with previous studies [18,19] in which a sample of Iraqi infertile men was included. The reason of increased rate of infertility in low age category may be related to exposure to the pollution, a previous study has provided evidence of an association between exposure to high levels of air pollution and increased DNA damage in human sperm [20]; while the lowest percentage of the infertile men in the progressed age categories may be related to the relatively a decease number of men who are choosing to have children at older age. An increase in the percentage of infertile men in the duration of infertility between 3 and 5 years may be due to an increase awareness of men as being the cause of infertility and the earlier interest in seeking medical advice, while a decrease in the percentage of infertile men with increasing duration of infertility which indicates reduced attendance to infertility clinic because the hopeless feelings of being a father that make them discontinue treatment. The high percentage of primary infertility in the infertile men could be attributed to hidden genetic defects because many of idiopathic infertility have a genetic basis [21]. Also, this finding may be explained on the ground that the patients with primary infertility are stronger desire to seek medical advice particularly when the male factor is the cause of infertility in the couples, while the infertile men with secondary infertility may not seek medical consultation since they are already have children.

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 [22]. Semen analysis remains an essential tool for the identification of infertility and the diagnosis of its severity. Among the parameters reported in a semen analysis, it is not yet known which one would be the most associated with fertility. While multiple reports point to sperm morphology as the parameter with the most discriminatory power, others indicate that sperm concentration and/or motility are the most valuable [23].

The results of semen analysis for the studied cases (20 fertile men and 100 infertile men) are similar to that reported by many authors in Iraq [19,24]. Regarding the results of the infertile men, the decline in sperm concentration may be due to the lifestyle risk factors such as: cigarette smoking, alcohol consumption, chronic stress, and nutritional deficiencies [25]. Also, there are several reasons that interfere with spermatogenesis such as environmental factors including exposure to heat, radiation, and pollutions [26]. The low percentage of sperm motility or sperm grade activity may be due to the presence of toxic agents in the environment and occupational exposure to toxic chemicals. A previous study reported a reduction in sperm motility in men exposed to environmental toxic substances and the authors suggested that these substances may cause changes in the physicochemical characteristics of the semen, resulting in increased viscosity and inhibiting the rapid forward progression of the sperm [25]. It has been reported that hyperviscous seminal plasma has an adverse strict effect on sperm motility [27]. On the other hand, other reasons were stated such as spermatozoal structural defects, genital tract infection, antisperm antibodies (ASA), and partial ductal obstruction [28]. The low percentage of normal sperm morphology can be due to a variety reasons such as infection, testicular stress (e.g. varicocele, poor sperm production, environmental toxins), and hormonal imbalance [29]. Also, altered sperm morphology might reflect disturbances during spermiogenesis, spermiation, and sperm passage during epididymis [30].

Increase percentage of agglutinated sperm may attribute to infections of the male genital tract or to the presence of ASA in the seminal plasma. It has been reported that infections of the male genital tract are associated with ASA formation and sperm agglutination [31]. Increased round cells count indicates that most of the semen samples showed infections by elevated leukocytes and phagocytes concentrations. Excessive presence of leukocytes in the semen can interfere with the quality of the semen and more importantly with the functional status of sperm fertilizing ability [32].

The hypo-osmotic swelling test was originally used as a sperm function test to evaluate the integrity of the sperm membrane. The present results revealed that the percentage of sperm HOS test score was significantly lower in the infertile men compared to the fertile men. This finding is in agreement with a previous study which stated that sperm samples from fertile subjects have normal HOS test scores and that those from infertile subjects have low HOS test scores [33]. The spermatozoal membrane contains large amounts of PUFA, which maintain its fluidity and integrity; peroxidation of these fatty acids leads to the loss of membrane flexibility and a reduction in the ability to swell and expand covering the tail when exposed to hypoosmotic solutions [34]. It has been hypothesized that defects in the functional integrity of the sperm membrane, which can be detected by the HOST test, may reduce the potential for fertility by causing implantation failure or increasing the rate of spontaneous miscarriage rather than simple fertilization failure [35]. The human sperm membrane is under constant threat of oxidative damage due to large quantities of PUFA in their plasma membrane and relative lack of scavenging antioxidant enzyme in their cytoplasm. Decrease in swollen coiled sperm percentage may be due to direct interaction of ROS with PUFA in the cell membrane leading to a chain of chemical reactions results in the formation of various oxidative modified products, which are toxic to cells. Hypo-osmotic stress test is the simplest preliminary test which could be used as a preliminary marker test for sperm tail damage by ROS [34].

Regarding the correlation between HOS test score and sperm parameters in the infertile men, the results revealed a highly significant positive correlation with sperm concentration, sperm motility, progressive sperm motility, and non-progressive sperm motility; while a highly significant negative correlation was found with immotile sperm. Also, a significant positive correlation was found with total progressive sperm and normal sperm morphology. These findings are in accordance with those reported by [33]. The sperm swelling is induced when exposed to hypo-osmotic and the sperm plasma membrane can be considered functionally active. The current results could be explained on the ground that a motile and normal sperm has a physically intact plasma membrane, thus suggesting the normal functionality of the plasma membrane of these swollen sperm. While an immotile and morphologically abnormal sperm has a functionally inactive plasma membrane so that it does not swell when exposed to hypo-osmotic solution.

The current results showed that seminal MDA levels were significantly higher in the infertile men than in the fertile men. This is in agreement with the findings of [36,33]. Malondialdehyde can be used as a marker of oxidative stress; rise in seminal MDA levels could be due to increased generation of ROS due to the excessive oxidative damage generated in these infertile men; these oxygen species in turn can oxidize many other important biomolecules including membrane lipids [37,38]. Seminal plasma MDA is stable peroxidation product and its estimation helps to evaluate the effect of peroxidation on sperm. High levels of seminal MDA in infertile men that indicate excessive ROS is responsible for peroxidation of membrane PUFAs and disturbance of the functions carried out by the sperm membrane and impairs the fertilizing capacity of spermatozoa [37].

Concerning the correlation between seminal MDA level and sperm parameters in the infertile men, the results showed that higher seminal MDA levels were negatively associated with sperm motility, progressive sperm motility, non-progressive sperm motility, total progressive sperm and normal sperm morphology; while they were positively associated with immotile sperm. These findings are in accordance with several studies which stated that lipid peroxidative degradation of sperm membrane may be responsible for abnormal sperm concentration, motility, and morphology. Also, oxidative damage to spermatozoal membrane resulted in impairment of these sperm parameters [37,38]. Thus evaluation of seminal MDA could help in distinction and treatment of male infertility especially in idiopathic cases. Also, it could be beneficial diagnostic tool for defining sperm fertilization potential [39].

In accordance with those reported by previous study [33], the results obtained in the present study revealed a significant negative correlation between HOS test score and seminal MDA level in the infertile men. High levels of seminal MDA represent increased lipid peroxidation rates and oxidative damage to the sperm plasma membranes, and it became clear that lipid peroxidation may alter sperm membrane functional intergrity adversely [33]. Lipid peroxidation of spermatozoa membrane is

responsible for causing perturbation of membrane structure and function (transport processes, maintenance of ion and metabolite gradient, and receptor mediated signal transduction) [40].

V. Conclusion

Increased seminal MDA levels together with poor response of spermatozoa to hypo-osmotic stress in infertile men could represent the pathological impact of lipid peroxidation on the spermatozoa membrane and consequently on sperm function and quality. It became clear that lipid peroxidation may alter sperm membrane functional intergrity adversely and contribute to abnormal sperm function.

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