

Relationship between Seminal Malondialdehyde Levels and Male Infertility

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ABSTRACT: Seminal oxidative stress (OS) results from an imbalance between reactive oxygen species (ROS) production and ROS scavenging by seminal antioxidants. Seminal OS is believed to be one of the main factors in the pathogenesis of sperm dysfunction and sperm DNA damage in male infertility. It is estimated that 25% of infertile men possess high levels of semen ROS, whereas fertile men do not have high levels of semen ROS. Although a controlled production of these ROS is required for sperm physiology (sperm hyperactivation, capacitation and acrosome reaction) and for natural fertilization, the excessive production of ROS by leukocytes causes sperm dysfunction. The aim of the current study was to investigate the possible relationship between seminal malondialdehyde levels (MDA) and male infertility. The present study included 80 semen samples that were obtained from men attending the andrology Outpatient Clinic, Mansoura University Hospital, Mansoura, Egypt and grouped into four groups: Normozoospermia (n=20), Asthenozoospermia (n=20), Astheno-Teratozoospermia (n=20), Oligo-Astheno-Teratozoospermia (n=20). In this study Seminal plasma concentration of malondialdehyde (MDA) per 10^8 sperm was moderately significantly increased in Asthenozoospermia and highly significantly increased in Astheno-Teratozoospermia and Oligo-Astheno-Teratozoospermia compared with the normal group. However, the concentration of alpha-glucosidase activity was highly significantly decreased in Asthenozoospermia, Astheno-Teratozoospermia and Oligo-Astheno-Teratozoospermia compared with the normal group. MDA/ 10^8 sperm had highly significant negative correlation with sperm concentration (million/ml), grade A motility (%) and normal morphology (%). Also, MDA/ 10^8 sperm had highly significant negative correlation with results of HOS test as well as α -glucosidase. In conclusion, the present study revealed a possible role of ROS production on male infertility.

I. INTRODUCTION

Defective sperm function is the most prevalent cause of male infertility and a difficult condition to treat. Many environmental, physiological, and genetic factors have been implicated in the poor sperm function and infertility. Evaluation of the potential causes of sperm damage leading to abnormal sperm function and infertility is an important area of investigation (Sikka, 1996). Free radical-induced oxidative damage to spermatozoa is one such condition which is recently gaining a considerable attention for its role in inducing poor sperm function and infertility. Controlled generation of reactive oxygen species (ROS) in spermatozoa is associated with normal physiological function. Uncontrolled and excessive production of ROS, however, seems to have a significant role as one of the major factors leading to an infertile status (Pasqualotto et al., 2000).

The aim of the current study was to investigate the possible relationship between seminal malondialdehyde levels (MDA) and male infertility.

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II. PATIENTS & METHODS

This prospective study was done during the period from August 2013 to January 2015; semen samples were obtained from men attending the andrology Outpatient Clinic, Mansoura University Hospital, Mansoura, Egypt. Semen samples were divided into four groups:

Group I: comprised 20 semen samples with Normozoospermia.

Group II: included 20 semen samples with Asthenozoospermia.

Group III: comprised 20 semen samples with Astheno- Teratozoospermia.

Group IV: included 20 semen samples with Oligo- Astheno-Teratozoospermia. All subjects were subjected to full history taking and physical examination. A general physical examination was performed to detect possible abnormality relevant to fertility in the following system: metabolic, endocrine, cardiovascular, respiratory, gastro-intestinal and neurological. Local examination of the testis, epididymis, vas deferens and inguinal region was done for all patient to detect possible abnormalities in order to be excluded.

Preparation of samples: Fresh semen samples were collected from cases by masturbation, after 2-7 days sexual abstinence and allowed to liquefy for 15-30 minutes. After complete liquefaction, the liquefied semen was divided into 2 parts: one part was used for performance of computer assisted semen analysis and acrosin activity test according to the recommendation of the World Health Organization (WHO, 2010). Sperm morphology was evaluated by phase contrast microscope and Sperm Mac stain (Fertipro, Belgium). WBCs were determined by peroxidase stain (Endtz, 1972). Assay of functional integrity of sperm membrane by hypo-osmotic swelling test was performed according to WHO (1999). The other part was centrifuged at 1000 rpm for 15 minutes to separate seminal plasma and sperm pellet. The supernatant (seminal plasma) was divided into two tubes and stored at -20°C until analysis: First tube was analyzed for Malondialdehyde (MDA) by colorimetric method (Draper et al., 1993), Second tube was analyzed for seminal alpha-glucosidase activity (Guerin et al., 1986).

Assay of alpha- glucosidase activity: Measurement of alpha-glucosidase activity in seminal plasma was done according to the colorimetric method described by Guerin et al. (1986), and Cooper et al. (1988) to evaluate the function of the epididymis as the bulk of the activity of alpha-glucosidase in semen has been shown to depend on secretion by the epididymis (Cooper et al., 1990). The test is based on demonstration of the yellow colored P-nitrophenol released from a chromogenic substance (P-nitrophenyl- α -D-glucopyranoside) by the activity of α glucosidase. Alpha glucosidase was measured using (Episcreen Kit; FertiPro, 32,8730 Lotenhulle, Belgium). Extinction of clear aspirated supernatant was assessed by spectrophotometer at wavelength 405nm against (blank). The concentration of α glucosidase was calculated from the standard curve as shown in figure (1).

Determination of Malondialdehyde (MDA) 'Thiobarbituric acid reactive substances': Proteins of the seminal plasma are 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, 1ml of supernatant (seminal plasma) was added to 1ml of 17.5% Trichloroacetic acid and 1ml of 0.6% thiobarbituric acid and mixed well. After mixing, the tubes were put in boiling water bath for 15 minutes and then allowed to cool slowly, 1ml of 70% Trichloroacetic acid was added to the mixture, mixed well and incubated at 37°C for 20 minutes and lastly, the mixture was centrifuged at 2000g for 15 minutes. The absorbance reading of the supernatant portion was taken at 534nm against reagent blank, using spectrophotometer (Genova, serial no. 1722, Jenway, UK) and the concentration of MDA in the seminal plasma was calculated using its molar extinction coefficient (1.56×10^5 mol/lcm) according to Beer-Lambert Law. MDA concentration = (Absorbance of the samples / 1.56) $\times 10 =$ nmol/ml.

Assay of the functional integrity of the sperm membrane "Hypo- Osmotic Swelling (HOS) Test": A property of the cell membrane is its ability to permit the transport of molecules selectively. When exposed to hypo-osmotic conditions, water will enter the spermatozoon in an attempt to reach osmotic equilibrium. This inflow of water will increase sperm volume and the plasma membrane will bulge (balloon) giving minimum surface to volume ratio. The

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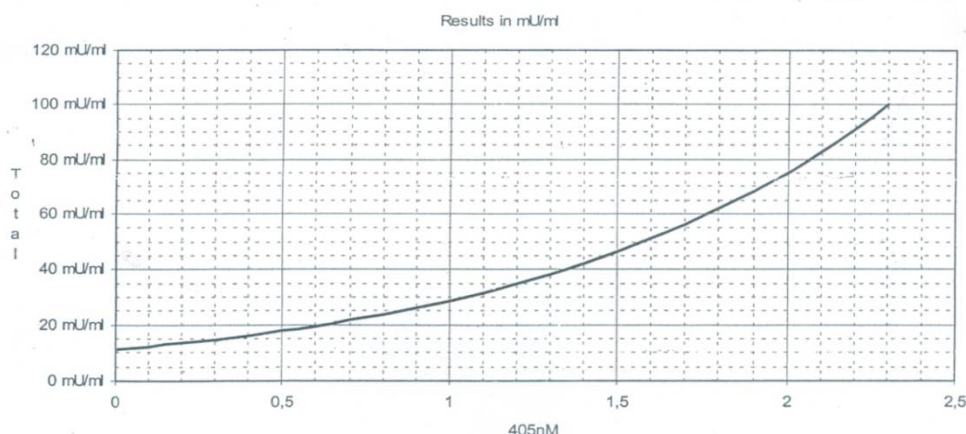
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sperm tail appears to be particularly susceptible to such hypo- osmotic conditions .These induced alterations in sperm morphology are visible with the phase – contrast microscope and could assess the functional integrity of the sperm membrane (**Jeyendran et al ., 1984 ; WHO, 1999**), 1.0 ml aliquot of freshly prepared and warmed working solution was added to 0.1ml of liquefied semen and mixed well, The mixture was incubated at 37 ° C for 30 minutes and the sperm were then examined under phase – contrast microscope.Swelling of sperm is identified as changes in the shape of the tail as shown in figure (2) .The number of swollen cells were counted in duplicate in a total of 200 spermatozoa and the mean percentage was calculated. The HOS test is considered normal for a semen sample if more than 60% of the spermatozoa undergo tail swelling . If less than 50% of the spermatozoa show tail swelling, the semen specimen is considered to be abnormal.

STATISTICAL ANALYSIS: was done by using SPSS (Statistical Package for the Social Sciences) program version 16 on windos 7.The quantitative data were expressed as mean , standard deviation Mann-Whitney test was used as a test of significance for comparison of two group. The qualitative data were presented in the form of number and percentage . Spearman rank correlation coefficient was done to study the relation between variables.The result is considered significant if p-values less than or equal 0.05 ,if P less than or equal 0.01 is considered moderately significant and if P less than or equal 0.001 is considered highly significant.

III. RESULTS

In the present study, table (1) and figure (3) show halo diameter, halo percent(%), acrosin activity index and the results of HOS test (%) in all studied groups, Table (2) and figure (4) show the malondialdehyde (MDA) (nmol / 10⁸ sperm), alpha-glucosidase(mU/ ml) in all studied groups, Table (3) show the correlation of MDA/sperm with semen parameters of all studied groups and Table (4) show the correlation of MDA/sperm and α - glucosidase (mU/ml) in different groups.



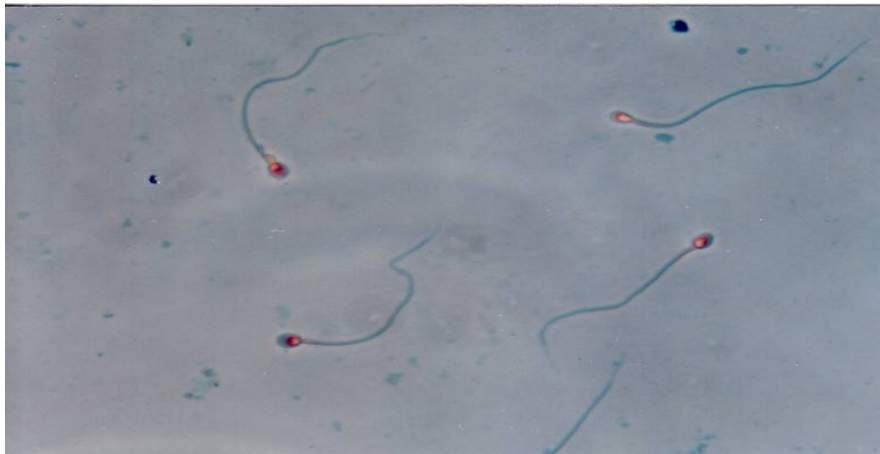
Figure(1): Standard curve of alpha glucosidase

Measurement of alpha-glucosidase activity in seminal plasma based on demonstration of the yellow colored P-nitrophenol released from a chromogenic substance by the activity of α glucosidase. α glucosidase is secreted by epididymis and used for evaluation of epididymal function. The concentration of α glucosidase was calculated from standard curve.

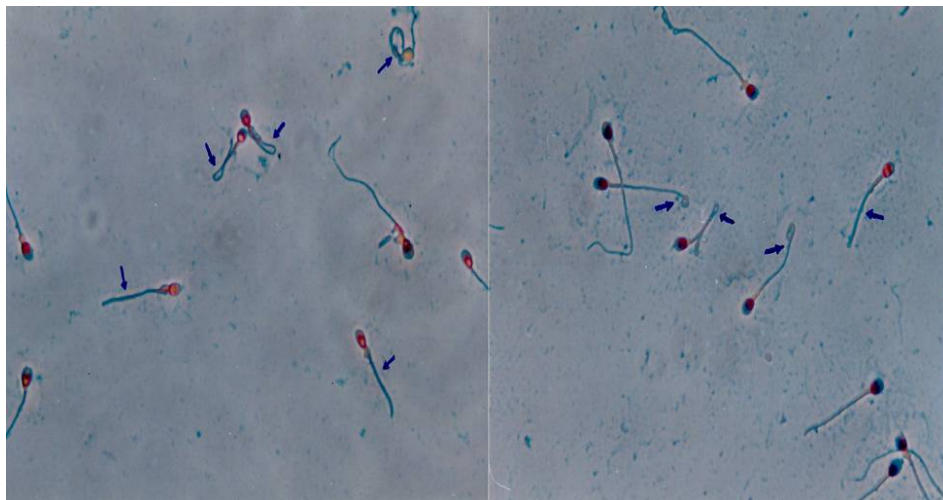
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A. Spermatozoa before HOS test (no tail swelling).



B. Spermatozoa positive HOS test (arrows indicate different types of tail swelling).

Figure (2): Photomicrograph of (500X) sperm MAC stain spermatozoa Acrosome ,midpiece , and tail are green in color , and the nucleus is red .

Swelling of sperm is identified as changes in the shape of the tail as shown in fig2(A,B) .The number of swollen cells were counted in duplicate in a total of 200 spermatozoa and the mean percentage was calculated .The HOS test is considered normal for a semen sample if more than 60% of the spermatozoa undergo tail swelling . If less than 50% of the spermatozoa show tail swelling ,the semen specimen is considered to be abnormal .

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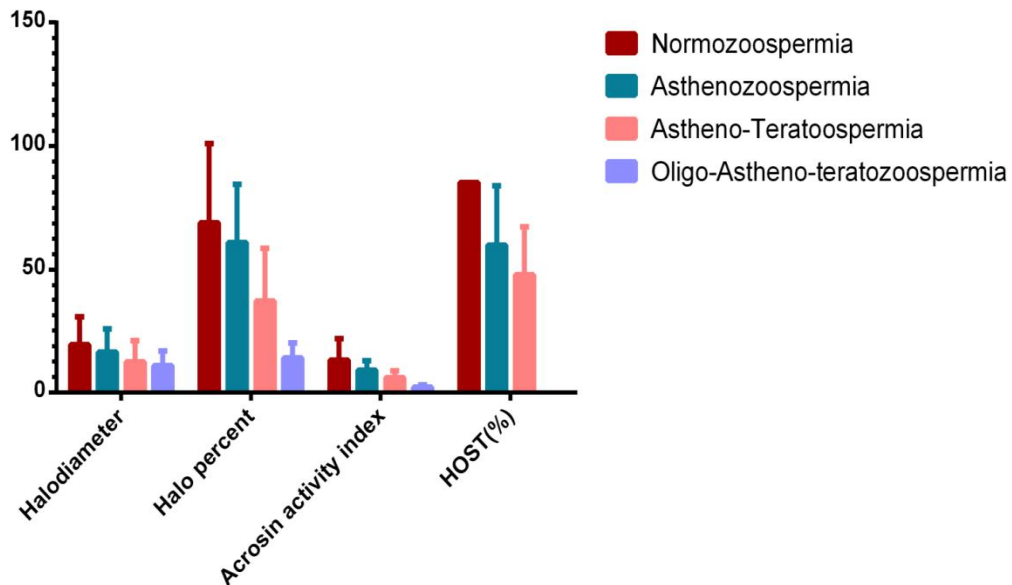


Figure (3): halo diameter of all studied group.

figure (3) show halo diameter, halo percent(%), acrosin activity index and the results of HOS test (%) in all studied groups. They were highly significantly decreased in asthenozoospermia, astheno-teratozoospermia and oligo-astheno-teratozoospermia compared with the normal group.

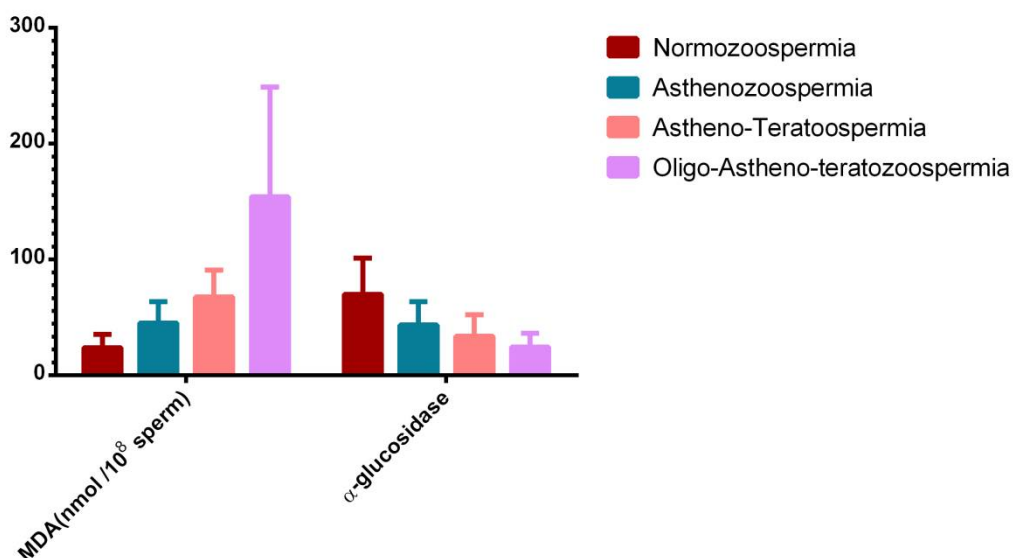


Figure (4): MDA(nmol)/10⁸ sperm and α-glucosidase(mU/ ml) in all studied groups.

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Seminal plasma concentration of malondialdehyde (MDA) per 10^8 sperm was moderately significantly increased in asthenozoospermia and highly significantly increased in asthenoteratozoospermia and oligo-asthenoteratozoospermia compared with the normal group. However, the concentration of alpha-glucosidase activity was highly significantly decreased in asthenozoospermia, asthenoteratozoospermia and oligo-asthenoteratozoospermia compared with the normal group.

	Normo.(n=20)	A (n=20)	AT (n=20)	OAT (n=21)
Halo diameter	19.3±11.3	16.3±9.4 P= 0.002	12.4±8.5 P < 0.0001	10.9±5.9 P < 0.0001
Halo percent	69±32.1	61±23.5 P < 0.0001	37±21.7 P < 0.0001	14±6.1 P < 0.0001
Acrosin activity index	13.1±8.7	9±3.8 P = 0.0003	6±2.8 P < 0.0001	2.12±0.9 P < 0.0001
HOST(%)	85.3	59.9±24.1 P < 0.0001	48±19.4 P < 0.0001	39.7±14.5 P < 0.0001

Table (1): Halo diameter, halo percent, acrosin activity index and HOS test (%) in all studied groups.

Halo diameter was highly significantly decreased in asthenozoospermia ($P < 0.001$), asthenoteratozoospermia ($P < 0.001$) and oligo-asthenoteratozoospermia ($P < 0.001$) compared with the normal group. Also, halo percent was highly significantly decreased ($P < 0.001$) in asthenozoospermia, asthenoteratozoospermia and oligo-asthenoteratozoospermia compared with the normal group. As regard to acrosin activity index, it was highly significantly decreased ($P < 0.001$) in asthenozoospermia, asthenoteratozoospermia and oligo-asthenoteratozoospermia compared with the normal group. The results of HOS test were highly significantly decreased ($P < 0.001$) in asthenozoospermia, asthenoteratozoospermia and oligo-asthenoteratozoospermia compared with the normal group.

Normo. = Normozoospermia, A = Asthenozoospermia, AT = Asthenoteratozoospermia, OAT = Oligo-Asthenoteratozoospermia, n= number of cases. Significant ($P \leq 0.05$).

Table (2): malondialdehyde (MDA) (nmol / 10^8 sperm) and alpha glucosidase (mU/ ml) in all studied groups.

Seminal plasma concentration of malondialdehyde (MDA) per 10^8 sperm was moderately significantly increased ($P < 0.01$) in asthenozoospermia and highly significantly increased ($P < 0.001$) in asthenoteratozoospermia and oligo-asthenoteratozoospermia compared with the normal group. However, the concentration of alpha-glucosidase activity was highly significantly decreased ($P < 0.001$) in asthenozoospermia, asthenoteratozoospermia and oligo-asthenoteratozoospermia compared with the normal group.

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	Normo. (n=20)	A (n=20)	AT (n=20)	OAT (n=21)
MDA(nmol /10 ⁸ sperm)	23.69±11.7	45.17±18.4 P = 0.001	67.69±23.14 P < 0.0001	153.97±94.81 P < 0.0001
α -glucosidase (mU/ ml)	69.8±31.5	43.6±19.8 P < 0.0001	33.7±18.5 P < 0.0001	24.6±11.8 P < 0.0001

Normo. = Normozoospermia, A = Asthenozoospermia, AT = Asthenoteratozoospermia, OAT = Oligo-Asthenoteratozoospermia, n= number of cases. Significant(P≤0.05).

Table (3): show Correlation of MDA/sperm with semen parameters of all studied groups.

In this table, MDA/sperm had highly significant (P<0.001) negative correlation with sperm concentration (million/ml) ($\rho = -0.86$), grade A motility (%) ($\rho = -0.78$) and normal morphology (%) ($\rho = -0.83$). Also, MDA/sperm had highly significant (P<0.001) negative correlation with results of HOS test ($\rho = -0.7$)/

Variable	MDA/sperm (nmol /10 ⁸ sperm)	95% CI for r	P value
Concentration (million/ml)	$\rho = -0.86$	-0.91, -0.79	P<0.0001 Negative
Normal morphology (%)	$\rho = -0.83$	-0.89, -0.75	P<0.0001 Negative
Grade A motility (%)	$\rho = -0.78$	-0.85, -0.68	P<0.0001 Negative
HOST (%)	$\rho = -0.7$	-0.8, -0.57	P<0.0001 Negative

ρ =correlation coefficient

* Significant(P≤0.05).

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Table (4): shows the correlation of MDA/sperm and α - glucosidase in different groups:

This table shows highly significant ($P < 0.001$) negative correlation between MDA/sperm and α - glucosidase ($\rho = -0.81$).

Variable	MDA/sperm (nmol /10 ⁸ sperm)	95% CI for r	P value
α - glucosidase (mU/ml)	$\rho = -0.81$	-0.87, -0.72	$P < 0.0001$ Negative

ρ =correlation coefficient

* Significant ($P \leq 0.05$).

IV. DISCUSSION

Male infertility is considered to be responsible for about 30-50% of all cases of infertility (Abarikwu, 2013). A number of factors have been claimed for male infertility as; congenital or acquired urogenital abnormalities, urogenital tract infections, varicocele, endocrine disturbances, genetic abnormalities, immunological factors and idiopathic in 40-50% of cases (Jungwirth et al., 2004). The pathophysiology 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, but it still not enough for complete diagnosis as nothing is sure about the etiology, and how that it disturbs the male fertility potential. Many of these events are very characteristic and seem to be specific for certain etiology. Thus, these changes may be used as markers if they could identify a certain mode of pathogenesis (WHO, 2000). Oxidative stress is one of the most frequent causes of male infertility. Probability of infertility is high in men with high levels of oxidative stress and DNA damage which can be determined by sperm motility which is reduced by ROS, so role of the oxidative stress in male infertility is more pronounced (Saalu, 2010). Low level of ROS is necessary for normal function of spermatozoa, which is mainly produced by immature spermatozoa and leukocytes. Abnormal spermatozoa can increase the levels of ROS which inhibits maturation of spermatozoa. H₂O₂ and anion superoxides are well known for main cause of impaired function of spermatozoa. High levels of ROS in semen may cause abnormal and dysmorphic spermatozoa, thus, effecting their capability for fertilization (Zare et al., 2013). In this study, MDA per sperm is significantly increased in cases of oligo-astheno-spermia. This is in agreement with (Badade et al., 2011) who demonstrated that lipid peroxidation of the membrane lipid may disturb the functions carried out by the sperm membrane.

The inverse correlation between MDA conc. on one hand and sperm concentration, motility, and morphology on the other hand corresponds with the well established deleterious impact of oxidative stress on spermatogenesis and sperm function. Other studies reported seminal oxidative stress to be one of the causes of disturbance of spermatogenesis and testicular damage manifested by marked decline of sperm concentrations and poor motility in infertile patients (Chen et al., 2002 and Nallela et al., 2004).

The increased levels of MDA was inversely correlated with a reduction of sperm motility. This is in agreement with Armstrong et al. (1999) who demonstrated a correlation between the seminal oxidative stress caused by ROS overproduction and the reduction of sperm motility. The link between ROS and reduced motility may be due to a cascade of events that result in a decrease in axonemal protein phosphorylation and sperm immobilization (Saraniya, et al., 2008) found a significant correlation between MDA concentrations and sperm motility and concentration. Also, increased levels of MDA in seminal plasma of infertile patients were associated with diminished percentage of normal sperm morphology. This is in agreement with (Palini et al., 2011) who demonstrated that levels of MDA correlate

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negatively with morphology. Studies have shown that the level of seminal oxidative stress was negatively correlated with the percentage of normal sperm forms (*Ollero et al., 2001*).

High levels of ROS are toxic to sperm quality and function. There was a significant negative correlation between MDA and sperm concentration, motility, and percentage of normal forms (*Elsaeid et al. 2006 and Hamad, 2014*). The relation between seminal oxidative stress and high sperm morphological deformity may be causal and related to the greater capacity of the morphologically abnormal spermatozoa to produce ROS (*Saleh and Agarwal, 2002*). It has been hypothesised that oxidative damage of mature sperm by ROS-producing immature sperm during their comigration from seminiferous tubules to the epididymis may be an important cause of male infertility (*Agarwal et al., 2003*). Other authors have found that sperm morphological irregularities are significantly correlated with creatine kinase (CK) activity in human spermatozoa. In this study, MDA concentration in seminal plasma was inversely correlated to the sperm membrane integrity assessed by HOS test. The excessive ROS exerts major destructive effects on the sperm membrane which is rich in polyunsaturated fatty acids (PUFA) making it highly sensitive to ROS. This high content of PUFA is not only necessary for optimal membrane fluidity required for tail movement, but also for the acrosome reaction and membrane fusion (*Zalata et al., 1998a and Bansal et al., 2014*). High levels of MDA in seminal plasma was associated with impaired epididymal function indicated by reduced alpha-glucosidase activity. During passage through the epididymis, spermatozoa undergo many dramatic changes to reach full maturation and gain the ability to move and to fertilize oocytes. Epididymal pathologic conditions could lead to abnormal events and production of morphologically and functionally immature spermatozoa with greater capacity to produce ROS. Also, epididymitis is associated, in most of the cases, with leukocytospermia which is the major source of high ROS production in semen.

V.CONCLUSION

It is concluded that the present study revealed a possible role of ROS production on male infertility.

It might be a marker for discrimination between fertile and infertile patients. Assessment of such oxidative stress status (OSS) may help in the medical treatment of male infertility by suitable antioxidants. Basic and clinical research on the involvement of ROS and antioxidants in maintaining normal sperm function is very much warranted.

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