RELATIONSHIP OF SPERM PARAMETERS WITH LIPID PEROXIDATION IN ASTHENOZOOSPERMIC AND NORMOZOOSPERMIC MALES

HEIDAR TAVILANI, MAHMOUD DOOSTI, HOJATOLLAH SAEIDI, SEYED MOHAMMAD HOSSEINIPANAH, AND HASAN HASANI BAFERANI

From the Department of Medical Biochemistry, Hamadan University of Medical Sciences, Hamadan, the Department of Medical Biochemistry, Tehran University of Medical Sciences, Tehran, the Department of Medical Pathology, Tehran University of Medical Sciences, Tehran, and the Department of Anatomy, Kashan University of Medical Sciences, Kashan, I.R. Iran.

ABSTRACT

Background: The lipid peroxides and their degradation products are highly toxic to spermatozoa and may play a major role in sperm dysfunction. The purpose of this study was to determine the level of lipid peroxidation as indicated by malondialdehyde (MDA) in the spermatozoa and seminal plasma of asthenozoospermic and normozoospermic males. In addition, correlation of lipid peroxidation with motility grades: a (rapid progressive), b (slow progressive), c (nonprogressive), d (immotile), percent of normal morphology, acrosome and tail defect, and concentration of spermatozoa was determined.

Methods: MDA of spermatozoa and seminal plasma was determined in 35 asthenozoospermic and 15 normozoospermic men by spectrofluorometry. Semen analysis was done according to the WHO standard.

Results: MDA in the spermatozoa of asthenozoospermic patients was significantly higher than normozoospermic males (0.14 ± 0.01 and 0.09 ± 0.01 nmol/$10*10^6$ spermatozoa respectively, mean$\pm$SE). A significant negative correlation was observed between MDA of spermatozoa with grade a motility and concentration of spermatozoa. In addition, a significant positive correlation was observed between MDA of spermatozoa with the acrosome and tail defect. The MDA value in the seminal plasma of asthenozoospermic and normozoospermic patients was 1.35 ± 0.07 and 1.2 ± 0.08 nmol/mL seminal plasma respectively (mean$\pm$SE). The MDA of seminal plasma exhibited a negative correlation with grade a+b motility and positive correlation with grade c+d motility and head defect.

Conclusion: Lipid peroxidation has a deleterious effect on semen quality and MDA is an index of lipid peroxidation which may be a diagnostic tool for the analysis of infertility in asthenozoospermic patients.
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Keywords: Asthenozoospermia, Lipid peroxidation, Semen Parameters, Human Spermatozoa, Semen Analysis.

Corresponding author: Prof. Mahmoud Doosti, Department of Medical Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Fax: 021 88953004
Email : doostimd@sina.tums.ac.ir

INTRODUCTION

Peroxidation damage to the plasma membrane of spermatozoa has been suggested as an important pathological mechanism of male infertility.[1,2,3] The human sperm cell plasma membrane is particularly susceptible to oxidation due to the existence of a high concentration of polyunsaturated fatty acids (PUFA) in these membranes.[1,4,5] The toxic lipid peroxides are known to cause various impairments of the sperm cell, such as membrane damage and decrease in motility.[6,7] The reactive oxygen species (ROS) derived from spermatozoa themselves[2,6,8] or from white blood cells (WBC) which represent an additional powerful source of ROS in semen[4] are responsible for the peroxidation damage that has been proposed as a major factor in male infertility.[9,10] A simple tool to evaluate the effect of lipid peroxidation on the spermatozoa is the assay of sperm and seminal plasma malondialdehyde (MDA) which is a stable lipid peroxidation product.[11] The aim of this study was: 1) to determine the lipid peroxidation level, as expressed by MDA in the spermatozoa and seminal plasma of asthenozoospermic and normozoospermic males, 2) its possible correlation with motility (i.e. rapid progressive motility, grade a; progressive motility, grade b; nonprogressive motility, grade c; immotility, grade d), acrosome and tail defect, percent of normal morphology and concentration of spermatozoa.

MATERIAL AND METHODS

Study groups

Semen samples of infertile and fertile groups were obtained from men attending the Omid Fertility Clinic for infertility evaluation. All patients were evaluated with a complete history, physical examination (varicocele), drugs used, occupational exposure to heavy metals and cigarette smoking. Subjects were determined to be either normozoospermic (control) or asthenozoospermic after analysis of semen samples. Criteria for the asthenozoospermic (n=35) group were age 20-50 years, infertility for at least 12 months, sperm concentration of $>20*10^6$/mL and motility (grade a+b)<50% irrespective of morphology. In addition, 15 normal healthy men with semen characteristics that met the WHO (1999) criteria (sperm concentration>$20*10^6$/mL, motility grade a+b >50% and normal morphology> 14%) were enrolled as controls. Patients fulfilling the inclusion criteria were asked to participate in a research project (experimental study) which was duly explained to them. Written informed consents were obtained from all volunteers according to the criteria of the Ethical Committee of Tehran University of Medical Sciences.

Semen evaluation

All semen samples were collected by masturbation after 3 days of abstinence. After liquefaction of the samples, semen volume, its concentration (haemocytometer method), total count, morphology (Papanicolaou staining method), motility grades: a (rapid progressive), b (slow progressive), c (nonprogressive), d (immotile), acrosome and tail defect (Papanicolaou staining method) were determined using standard procedures (WHO 1999).[12] Semen samples with more than $1*10^6$/mL neutrophilic granulocytes (peroxidase staining, WHO, 1999) were excluded. After liquefaction of semen, spermatozoa were separated from seminal plasma by centrifugation (1000*g for 10 min at room temperature). Supernatant was removed immediately and pellets were resuspended in phosphate buffer saline (PBS; pH 7.2).[13]

Lipid peroxidation
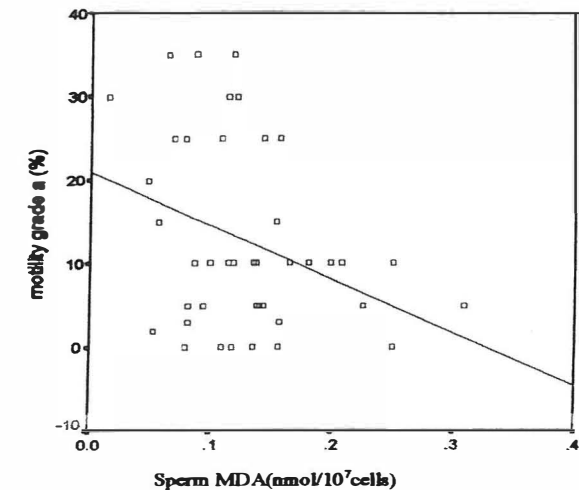
Mean concentration of spermatozoa in samples prepared for measurement was 10^7/0.2 mL. Lipid peroxidation in spermatozoa and seminal plasma was measured by reaction of thiobarbituric acid (TBA) with MDA according to Yagi.[14] Content of MDA was measured spectrofluorometrically using a Jasco (FP-6200) spectrofluorometer (excitation 515 nm, emission 553 nm). The MDA fluorescence intensity of spermatozoa and seminal plasma was determined using various concentrations of tetraethoxypropane as standards. The results are expressed as nmol MDA/10^7cells and nmole MDA/mL seminal plasma.

Statistical analysis

For comparing plasma MDA, total sperm count and acrosome defect, two independent sample t-tests were used. Since the distribution of sperm MDA and other semen parameters were not normally distributed, Mann-Whitney U test was applied to compare between asthenozoospermic and normozoospermic groups.

RESULTS

The mean values of the examined semen parameters in the asthenozoospermic and normozoospermic groups are illustrated in Table I. The mean ± SE of MDA value in spermatozoa of asthenozoospermic and normozoospermic groups were 0.14 ± 0.01 and 0.09 ± 0.01 nmol/10^7 spermatozoa respectively. There was a significant difference between MDA concentration in the two groups ($p< 0.01$). Spearman's correlation analysis revealed a significant negative correlation between MDA concentration in the

A

B

C
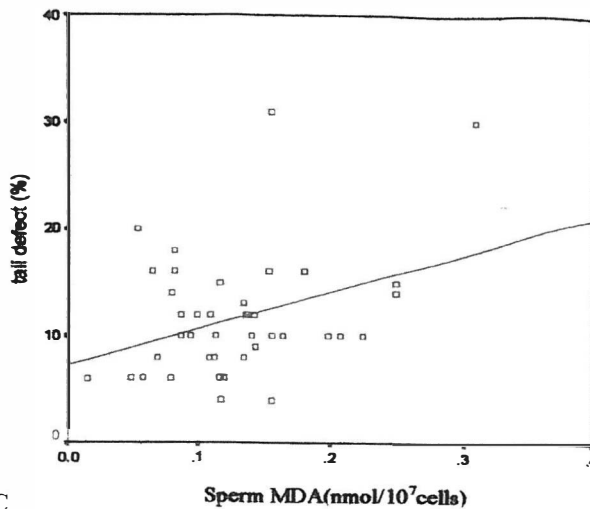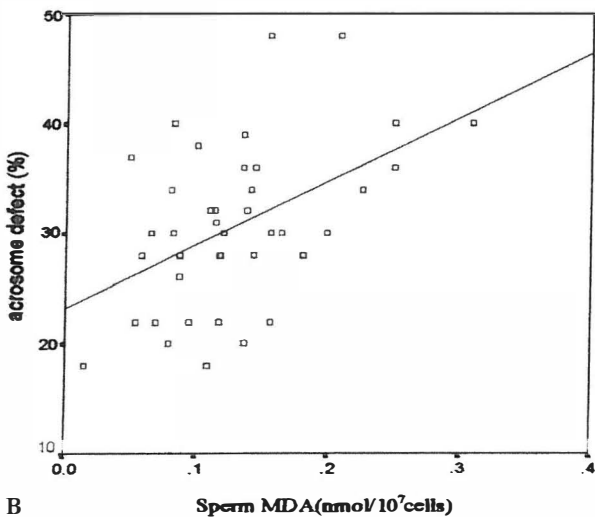
Fig. 1. Correlation between MDA concentration (nmol/10⁷cells) in all sperm samples (n = 50) with: (A) percent of motility grade a (a= rapid progressive, WHO, 1999) (r= -0.313, $p<0.05$), (B) percent of spermatozoa with acrosome defects (r= 0.407, $p<0.01$), and (C) percent of spermatozoa with tail defects (r=0.36, $p<0.05$).
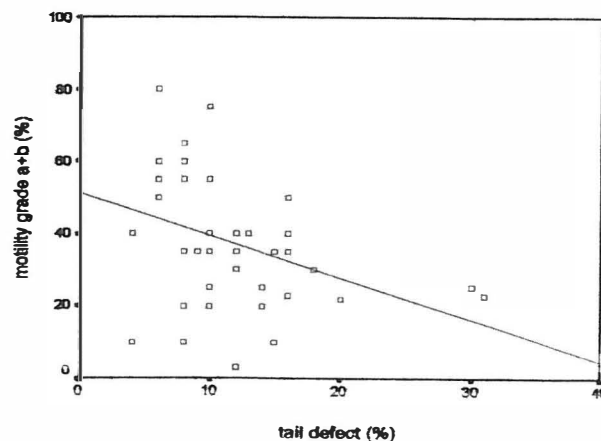

Fig. 2. Correlation between percent of motility grade a+b (a=rapid progressive, b=slow progressive, WHO, 1999) in all sperm samples (n=50) with percent of spermatozoa with tail defects (r=-0.43, $p<0.01$).

spermatozoa with grade a motility (Fig. 1A) and concentration of spermatozoa (r = -0.313, $p< 0.05$ and r=-0.36, $p<0.05$ respectively).

A significant positive correlation was observed between the MDA concentration of spermatozoa with acrosome and tail defects (Fig.1B and 1C) (r = 0.407, $p<0.01$ and r = 0.364, $p< 0.05$). In addition, a significant negative correlation was observed between motility grade a+b with tail defects (r= -0.43, $p<0.01$, Fig. 2).

The mean ± SE MDA value in the seminal plasma of asthenozoospermic and normozoospermic groups were 1.35 ± 0.07 and 1.2 ± 0.08 nmol/mL seminal plasma respectively (Table II). There was no significant difference between MDA concentration of seminal plasma in the two groups. The MDA concentration in seminal plasma exhibited negative correlation with motility grade a+b (r= - 0.284, $p< 0.05$), positive correlation with sperm

head defect (r=0.38, $p<0.05$), grade c + d (r= 0.296, $p< 0.05$) and grade d motilities (r = 0.31, $p<0.05$).

DISCUSSION

The results presented in this study indicate two main points. First, the value of MDA spermatozoa was higher in asthenozoospermic sperm samples than in

Table I. Basic parameters of semen samples in normozoospermic (n=15) and asthenozoospermic (n=35) patients. Results are presented as mean ± SD.

Semen parameters	Normozoospermic (n=15)	Asthenozoospermic (n=35)
Volume (mL)	3.58 ± 1.16	3.35 ± 1.16
Sperm concentration (10^6/mL)	113 ± 60.53	84.29 ± 26.39*
Total sperm count (10^6 sperm /ejaculate)	384.8 ± 203.8	270.8 ± 131
Normal morphology (%)	22.7 ± 5.44	8.22 ± 3.19***
Acrosome defect (%)	27.91 ± 8.06	31.81 ± 7.66
White blood cell(10^6/mL)	0.66 ± 0.34	0.72 ± 0.47
Motility grade a[1] (%)	27 ± 5.9	6.5 ± 5.29***
Motility grade a+b[1] (%)	57.6 ± 9.80	28.7 ± 10.1***
Motility grade c+d[1] (%)	41.15 ± 10.02	69.8 ± 11.4***
Motility grade d[1] (%)	31 ± 9.87	55 ± 12***
Tail defect (%)	8.3± 3	13.1 ± 5.8**
Head defect (%)	28.3 ± 6	33.8 ± 7.2*

[1]Grade of sperm movement according to WHO criteria (World Health Organization, 1999).
a= rapid progressive, b=slow progressive, c=nonprogressive and d=immotile. *$p<0.05$,
**$p<0.01$, ***$p<0.001$

Table II. MDA concentration in sperm and seminal plasma of normozoospermic and asthenozoospermic patients. Results are presented as mean ± SE.

Type of seminal fluid	Sperm MDA (nmol/10^7 spermatozoa)	Seminal plasma MDA (nmol/mL seminal plasma)
Normozoospermic (n=15)	0.09 ± 0.01	1.2 ± 0.08
Asthenozoospermic (n=35)	0.14 ± 0.01*	1.35 ± 0.07

*$p<0.01$

normozoospermic sperm samples. Second, the MDA level in spermatozoa correlates significantly with semen parameters. It has been suggested that toxic membrane lipid peroxides may play a major role in sperm dysfunction.[6] The most widely used assay for lipid peroxidation involves the measurement of MDA, that can be measured readily by virtue of its capacity to form adducts with TBA[15] but sensitive detection of the TBA adducts generally requires a spectrofluorometer.[15] In this study MDA was measured spectrofluorometrically[14] without FeSO$_4$ to promote lipid peroxidation which is used by many researchers.[13,16] In our study MDA concentration in spermatozoa was almost similar to Soleiman et al.[17] who used specific absorbance coefficent by spectrophotometry but

was very different from that detected by Zabludovsky et al.[18] who used ferrous sulfate and sodium ascorbate as promoter and calculated MDA by molar absorbance coefficient. Since several components other than MDA might generate reaction products with similar spectral characteristics to the true TBA–adducts,[7] abnormal results could be obtained using specific absorbance coefficient method. In addition seminal plasma MDA concentration was much lower (almost 1/5th) than the concentration reported by Suleiman et al.,[17] due to interference with other compounds of seminal plasma that readily form TBA adducts, for example aldehyde functions. Therefore the spectrofluorometrical method for MDA measurement provides better results as compared

to spectrophotometery[15] and promoter induced lipid peroxidation.

The lipid peroxides and their degradation products are highly toxic to spermatozoa and may play a major role in sperm dysfunction.[6] The extensive study on the peroxidation of phospholipids in mammalian sperm demonstrated that the peroxidation reaction caused membranc damage,which would inevitably lead to loss of motility and that the products of lipid peroxidation were also deleterious to membrane integrity.[4] Griveau et al.[19] have also shown that reactive oxygen species (ROS) cause a decrease in sperm motility, an increase in lipid peroxidation and a loss of membrane polyunsaturated fatty acids. In this study MDA concentration of spermatozoa is positively correlated with tail defect ($r=0.36$, $p<0.05$, Fig.1C), as well as a negative correlation between motility grade a+b and tail defect ($r=-0.43$, $p<0.01$, Fig. 2) was observed. Taking into account that docosahexaenoic acid (DHA) is the main and most widely polyunsaturated fatty acid found in the sperm tail and may contribute to membrane fluidity necessary for binding and flexibility of the tail required for motility,[20] thus oxidation of DHA could be the cause of decreased motility. In this study MDA concentration in spermatozoa and seminal plasma was found to have a significant negative correlation with grade a (Fig.1A) and grade a+b motility (respectively). In addition, a positive correlation was observed between seminal plasma MDA with grade c+d and grade d motilities that was similarly reported by Gomez et al.[16] which suggested that the immotile and nonprogressive motile spermatozoa are influenced by lipid peroxidation and loss of motility.

Morphology is one of the semen parameters reported to have an association with lipid peroxidation.[15] Ollero et al.[21] and Gil–Guzmat et al.[22] reported that levels of ROS production in semen were negatively correlated with the percentage of normal spermatozoa forms. It seems that products of lipid peroxidation are deleterious to membrane integrity[4] and an increase of abnormal formed features accompanies the increase of MDA in the spermatozoa and seminal plasma. Our finding that the MDA concentration of spermatozoa and seminal plasma is positively correlated with acrosomal (Fig.1B) and head defects (respectively) was supported by authors[18,23] who also reported that spermatozoa with high lipid peroxidation exhibit selective destabilization and loss of the acrosomal cap.

Seminal plasma is especially relevant for protecting spermatozoa from ROS and lipid peroxidation, since the cytoplasmic volume of spermatozoa is low in comparison to cell types and surrounding media.[24] The diminished cytoplasm of the spermatozoa reduces its capacity to retain an adequate load of protective molecules

inside the cell, which in turn protect the cell from extracellular ROS attack. Thus protection is provided by the extracellular environment.[24] Jones et al.[4] reported that the presence of seminal plasma has a preventive effect on the peroxidation reaction and that the enhanced rate of peroxidation caused by repeated washing of spermatozoa is due to a progressively more thorough removal of seminal plasma. In a normal situation, the seminal plasma contains antioxidant mechanisms, which are likely to quench ROS and protect against any likely damage to spermatozoa.[25]

The MDA concentration of spermatozoa was found to have a negative significant correlation with the concentration of spermatozoa. Many studies have demonstrated the association of lipid peroxidation with decreased sperm count.[26,27] Nonogaki et al.[28] suggested that lipid peroxidation occurs variably in different parts of human male genital organs which can explain a decrease in sperm concentration in asthenozoospermic patients. Furthermore, it has been proposed that oxidative damage is a possible cause of idiopathic male infertility involving disruption of spermatogenesis.[29]

In conclusion, the spermatozoa is a regionalized cell with specific functions localized in distinct regions: acrosome reaction take place in the anterior region of the head, sperm – oocyte plasma membrane fusion occurs in the equatorial region of the head and motion energy from mitochondria occurs in the midpiece, while motility is exhibited in the tail. This regionalization affects the distribution of both plasma membrane lipids and proteins,[30] and alteration of lipid composition by lipid peroxidation process can have deleterious effects on function and structure of spermatozoa. In this respect MDA as an index of lipid peroxidation provides a sensitive assay for diagnostic dysfunction of spermatozoa and may be a good tool for analysis of infertility in asthenozoospermic patients.

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