Analysis of Malondialdehyde and Reduced Glutathione in Spermatozoa of Infertile Men and Their Relation with Sperm Quality Parameters

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ABSTRACT:

BACKGROUND:

Male factor infertility is a multifactorial condition that is observed in the infertile couples. However, a proportion of infertile men have now been shown to have defective sperm functions related to oxidative stress (OS) which is a significant pathology,

AIM OF THE STUDY:

To estimate the indicators of OS by measuring malondialdehyde (MDA) and reduced glutathione (GSHr) levels in sperms of the infertile and fertile men and to evaluate the correlations of them with sperm quality parameters.

METHODS:

The levels of MDA and GSHr were measured spectrophotometrically at The Higher Institute of Infertility Diagnosis and Assisted Reproductive Technique /Al-Nahrain University, Baghdad, Iraq, between February till August 2019.

RESULT:

It was found that the mean of MDA levels was significantly higher in spermatozoa of infertile men ($P \leq 0.0001$) and mean of GSHr levels was significantly lower in spermatozoa of infertile men compared to spermatozoa of fertile controls ($P \leq 0.00 \cdot 1$). Furthermore, there were no statistical significant correlations between MDA and GSHr with sperm quality parameters.

CONCLUSION:

Evaluation of MDA and GSHr levels in spermatozoa of the infertile men provide valuable assays for estimation of OS which help in planning treatment for male infertility.

KEYWORDS: Glutathione, Male infertility, Malondialdehyde, Oxidative stress, Reactive oxygen species, Spermatozoa.

INTRODUCTION:

Infertility is inability of a sexually active, non-contracepting couple to achieve pregnancy in one year. Male reproductive function is found to be deficient in 50% of infertile couples [(1)]. Apart from the conventional causes of male infertility such as varicocoele, infections…etc; a new important cause has been identified: oxidative stress (OS) [(2)].

Oxidative stress occurs when the balance between the production of reactive oxygen species (ROS) and the inherent antioxidant capacity of any system is distorted [(3)].

Spermatozoa (especially, defective or immature) had been shown to be one of the main sources of ROS. The main target of OS in spermatozoa is the plasma membrane [(4)].

The plasma membrane contains polyunsaturated fatty acids (PUFAs) in abundance. Unfortunately, the presence of double bonds in these molecules makes them vulnerable to ROS attack, initiating a chain of chemical reactions called lipid peroxidation (LPO) which is grossly damaging to the spermatozoa. Malondialdehyde is the most common lipid peroxides [(5)].

Normally, spermatozoa are equipped with an elaborate defense system including scavenger molecules which protect against cell injury and death via detoxification of ROS. Glutathione (GSH) is the most abundant endogenous molecule in spermatozoa [(6)]. It is a tripeptide comprised of glutamate, cysteine and glycine. In healthy cells, more than 90% of the total GSH pool is in reduced

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form (GSHr) and less than 10% exists as oxidized form (GSSG). Disturbances in GSH homeostasis have been implicated in the etiology and/or progression of a number of human diseases including infertility [6].

The purpose of this study was to (a) estimate the LPO and the antioxidant in spermatozoa of infertile men and to (b) assess their impact on sperm quality parameters. In order to fulfill this goal, the study measured the levels of MDA and GSHr in the spermatozoa of 70 infertile men.

MATERIALS AND METHODS:

A case-control study was employed. Seventy infertile men (patients) and 30 fertile men (controls) were studied, their age range from 20-45 years.

The infertile men had unable to conceive despite more than 12 months of unprotected intercourse and had at least one defect in sperm parameters either in concentration, motility or normal morphology, and consulting for infertility diagnosis and treatment at The Higher Institute of Infertility Diagnosis and Assisted Reproductive Techniques /Al-Nahrain University, Baghdad, Iraq. The men included in the control group were in general good health with normal sperm parameters and had proven paternity in the last 12 months.

Semen samples from all 100 men were collected from that institute from February till August 2019.Subjects with chronic diseases, varicocele, genital infections, those currently on any medication or antioxidant supplementation, smokers, leukocytospermia and azoospermia were excluded from this study.

Sample collection and preparation

All semen samples were produced by masturbation into sterile cups after 3 days of sexual abstinence. Semen samples were allowed to liquefy at least for 30 min at room temperature before analysis. Semen analysis was performed according to WHO guidelines (2010) to obtain volume, sperm concentration, motility and morphology.

After routine semen analysis, the remaining portion of semen was divided into two portions to analyze for research parameters. One portion of liquefied semen (1000µl) was centrifuged at 4000 xg for 10 min then the plasma supernatant was discarded. After washing twice in (1000µl) of a solution of NaCl (0.9%), the sperm pellet was stored at −20°C until assayed for MDA.

The second portion (500µl) of the semen was centrifuged at 2500 xg and 4°C for 5 min then the plasma supernatant was discarded. After washing twice in phosphate buffer solution (PBS), the sperm pellet was stored at −20°C until assayed for GSHr.

Biochemical Procedures

a. Determination of malondialdehyde

Lipid peroxidation was measured by determining the MDA production, using Thiobarbituric Acid (TBA) method [7]. The sperm pellet was suspended in 1 ml of distilled water. Another 1 ml of distilled water was transferred to another glass tube served as a blank. To each of the tubes (blank and sample), a 500µl of TBA reagent was added (0.67g of 2-TBA dissolved in 100 ml of distilled water with 0.5g of solid NaOH and 100 ml glacial acetic acid added).This mixture was heated for 1 hour in a boiling water bath. After cooling, the tube was centrifuged for 10 min at 4000 xg and the supernatant's absorbance was read on a spectrophotometer at 534 nm.

b. Determination of reduced glutathione

The sperm pellet was suspended in 1000 µl of distilled water, mixed well and centrifuged at 3000 xg and 4℃ for 10 min. By the method of Anderson [8], using metaphosphoric acid (6%), the GSHr levels were measured spectrophotometrically at 400 nm in deproteinized sperms lysate samples.

Statistical Analysis

Data were analyzed by statistical packages of SPSS 18.0 (statistical packages for social sciences-version 18.0, LED Technology, USA). Data of both groups (fertile and infertile) were expressed as mean ± standard error of mean (SEM). Student-t-test was used to compare values from both groups. Spearman correlation was used to correlate the chemical variables with sperm parameters and the r (correlation coefficient) was calculated with its p value. P values < 0.05 considered statistically significant.

RESULTS:

Table 1 depicts sperm quality parameters for both infertile and fertile men. As only men with male factor infertility were recruited into the study, it was expected that the infertile group would have

lower sperm quality than the fertile controls Statistical analysis revealed that there were significant differences observed in terms of sperm concentration, total motility and normal morphology between infertile and fertile study groups. In addition, the levels of MDA and GSHr of studied men are summarized in Table 1.

The mean and standard error of mean (Mean ± SEM) of MDA levels in spermatozoa of patients group was 0.497 ± 0.027 µM / 10^7 spermatozoa versus 0.218 ± 0.018 µM / 10^7 spermatozoa of fertile men group. Statistical analysis showed a significant elevation in the mean of MDA levels in spermatozoa of infertile compared to fertile men ($P \leq 0.0001$), indicating a dramatic increased OS in spermatozoa of these patients Figure 1.

The mean ± SEM of GSHr levels in spermatozoa of infertile subjects was 0.255 ± 0.009 while in controls was 0.438 ± 0.039 µM / 10^6 spermatozoa.

A statistical significant decline was found in mean of GSHr levels in spermatozoa of infertile patients in comparison to controls ($P \leq 0.0001$). The results revealed a significant decrease in the availability of antioxidant reserve in spermatozoa of these patients to respond to the resulting damage Figure 2.

Pertaining to the results of this study in infertile men; no statistical significant correlations were observed between the levels of MDA and GSHr respectively with sperm parameters (concentration, total motility and normal morphology) Table 2.

Table 1: Comparison of sperm quality parameters between the infertile and fertile subjects.

Sperm quality parameters	Infertile n=70 Mean ± SEM (Range)	Fertile n=30 Mean ± SEM (Range)	P value
Sperm concentration (10^6 /ml)	40.12 ± 3.45 (1.0 - 120.0)	56.20 ± 5.79 (25.0 - 151.0)	0.015
Total sperm motility (%)	28.73 ± 2.25 (1.0 - 100.0)	70.07 ± 1.13 (55.0 - 85.0)	0.0001
Normal sperm morphology (%)	23.21 ± 1.25 (00.0 - 40.0)	37.53 ± 1.19 (31.0 - 66.0)	0.0001
MDA (µM/ 10^6 spermatozoa)	0.497 ± 0.027 (0.21-1.00)	0.218 ± 0.018 (0.10-0.57)	0.0001
GSHr (µM/ 10^6 spermatozoa)	0.255 ± 0.009 (0.11-0.57)	0.438 ± 0.039 (0.25-0.86)	0.0001

Note: Total sperm motility= progressive and non-progressive motile sperms, MDA= malondialdehyde, GSHr= reduced glutathione, SEM= standard error of mean. Data analysed using the student-t-test. $P < 0.05$= significant.

Table 2: The value of Pearson's correlation coefficients (r) calculated between levels of MDA and GSHr with sperm parameters.

Sperm parameter	MDA (µM/10^6 spermatozoa)		GSHr (µM/10^6 spermatozoa)	
	r	p	r	p
Sperm concentration (10^6/ml)	0.175	0.147	0.163	0.177
Total sperm motility (%)	0.100	0.412	0.89	0.462
Normal sperm morphology (%)	0.158	0.191	0.190	0.115

Note: Total sperm motility= progressive and non-progressive motile sperms, MDA= malondialdehyde, GSHr= reduced glutathione. Correlations between different variables at 0.05 level.

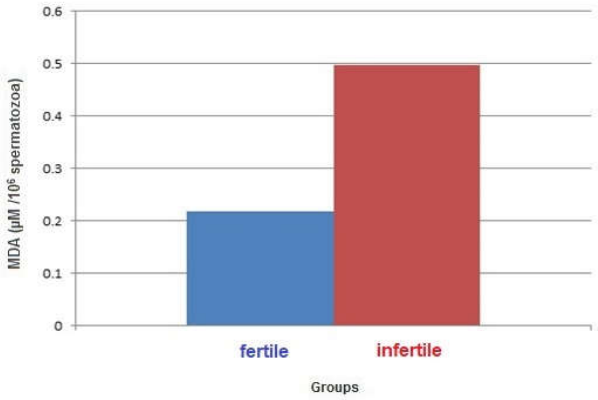

Figure 1: Levels of MDA in spermatozoa of infertile and fertile subjects.

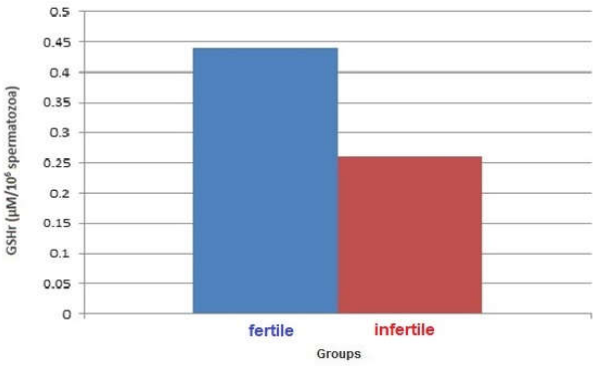

Figure 2: Levels of GSHr in spermatozoa of infertile and fertile subjects.

DISCUSSION :

The metabolism of spermatozoa produces different ROS which are potentially harmful in high levels to plasma membrane of high PUFAs content. Lipid peroxidation is an oxidative deterioration of PUFAs. One of the by-products of lipid peroxide decomposition is MDA [4,9]. It was found to be significantly higher in spermatozoa of infertile than that of fertile men. The results were concurrent with results of many researchers [9,10,11,12]. But contrary to results of Palani who found that no significant difference in MDA levels for infertile and fertile men respectively [13].

Increased MDA is due to increased ROS production by a variety of semen components including immotile or morphologically abnormal spermatozoa, low level of leukocytes, and morphologically normal but functionally abnormal spermatozoa [10].

The most significant effect of LPO is the perturbation of membrane (cellular and organellar) structure and function (transport processes, maintenance of ion and metabolite gradients, receptor-mediated signal transduction, etc.). High levels of ROS disrupt the inner and outer mitochondrial membranes and induce DNA damage which could accelerate germ cell apoptosis with subsequent decline in sperm concentration [10].Reduced motility may be due to a cascade of events that results in a decrease of axonemal protein and phosphorylation, with sperm immobilization; both of which are associated with a reduction in membrane fluidity that is necessary for sperm-oocyte fusion. Furthermore, LPO has a deleterious effect on ultramorphological status of spermatozoa and thereby on the male fertilizing potential [9,10,12,14].

Several studies had been shown that MDA associated with poor sperm quality, but this association was not statistically significant in this study. Similarly, Aleksandra et al. did not obtain any statistical association between MDA levels and sperm parameters [15,] but Colagar et al. had showed a significant negative

Normally, a balance is maintained between the amount of ROS produced (pro-oxidants) and that scavenged by a cell (antioxidants). Cellular damage arises when this equilibrium is disturbed especially when the cellular scavenging systems cannot eliminate the increased ROS [16].

Glutathione molecule is an effective antioxidant, can restore the physiological constitution of PUFAs in the cell membrane and prevent DNA damage [17].

In this study, we explored GSHr level in the spermatozoa of infertile and fertile men to estimate its contribution in a good sperm quality. The observations showed a highly significant decline in GSHr levels in spermatozoa of infertile men compared to the fertile counterparts. These results were in agreement with Bhardwaj et al. who found that GSH levels were significantly lowered in spermatozoa of infertile men in comparison to the fertile controls [18], and with Atig *et al* who found that GSHr levels were significantly higher in normozoospermics than in abnormal groups [19]. But in disagreement with Palani et al. and Ebisch et al. who reported that GSH levels in the spermatozoa did not differ significantly between infertile and fertile men [13,20].The decline of GSHr levels may be due to contribution of ROS in up-regulation of thiol synthesis (GSSG) to protect the spermatozoa from the oxidative damage [21].

The altered GSH status observed in the spermatozoa of infertile men might cause more oxidative damage with subsequent impaired sperm functions and process of fertilization. Hence, GSH might have some fertility enhancing role [22].

On the other hand, the findings of this study showed that there was no statistical significant correlation between GSHr levels and sperm parameters. The results were corroborative with oschendorf et al. who found no association between the GSH levels of men having different fertility potential with parameters of the spermiogram [23]. While Chaudhari et al. showed a significant positive correlation between them and Atig et al. showed a significant association of GSHr level with sperm motility and concentration [10,19] .

CONCLUSION:

It was shown that increased MDA levels in spermatozoa of infertile men group could represent the pathological effects of LPO on sperm function. In addition, the decrease of GSHr antioxidant can be a risk factor for sperm abnormality and associated male infertility. These data suggested that routine determination of MDA and GSHr levels in spermatozoa of infertile men provide valuable assays for estimation of OS which help in planning treatment for male infertility.

Recommendiation

Further studies are needed to determine the role of OS in male infertility. Recommonded uses of GSH level as additional parameter to assess male fertility, and therapeutic usage of GSH in treatment of male infertility should be studied extensively.

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