

Research article

Evaluation of oxidative stress in idiopathic male infertility in the Iraqi populationMayasah Muthanna Khalid¹, Mohammed Ibrahim Nader², Rasha Abdulla Hussein Mahood³¹Department of Anesthesia Technique, The University of Mashreq, College of Medical Sciences Techniques, Baghdad, Iraq^{2,3}Institute of Genetic Engineering and Biotechnology for Postgraduate studies, University of Baghdad, Iraq

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ABSTRACT

Introduction and Aim: Idiopathic infertility accounts for at least 30% of all cases of infertility, and oxidative stress has been identified as a novel developing factor in idiopathic male infertility. Oxidative stress occurs when antioxidant defense mechanisms are outmatched by the creation of reactive oxygen species (ROS). The purpose of this research was to examine the impact of enzymatic antioxidants (catalase and superoxide dismutase; CAT and SOD) on sperm DNA fragmentation and associated sperm alteration in Iraqi males with idiopathic infertility.

Materials and Methods: One hundred infertile guys (50 oligozoospermia and 50 asthenozoospermia) and 50 normospermia) had their superoxide dismutase, catalase, and malondialdehyde levels tested.

Results: Our study showed that the sperm DNA fragmentation index (DFI) of patients who suffered from oligozoospermia and asthenozoospermia was higher than that of individuals who suffered from normozoospermia. Seminal plasma SOD and MDA levels were higher in infertile participants compared to controls ($P < 0.001$). The concentration of CAT in the seminal plasma of oligozoospermic and asthenozoospermic men was not significantly different from that of normospermic men. DFI correlated negatively with seminal antioxidant status (SOD, CAT) and MDA, while CAT correlated positively with SOD and MDA.

Conclusion: The impacts of oxidative stress include the breakage of DNA in sperm, with lipid peroxidation being the primary cause of idiopathic male infertility. This is associated with less successful fertility outcomes in couples. According to the findings that we obtained, the sperm DNA fragmentation index as well as malondialdehyde are substantially associated with the quality of the sperm.

Key words: Idiopathic infertility; CAT; SOD; MDA; Sperm DNA fragmentation.

INTRODUCTION

Infertility is a disorder that can be caused by a number of different factors and affects 15% of couples. The inability to achieve a spontaneous pregnancy after one year of regular, unprotected sexual interaction is the definition of this condition (1). Nearly half of all cases of infertility can be attributed to male factors, with female variables also playing an important role. The study of basic sperm parameters is typically used as the foundation for diagnosing male infertility (2). According to the recommendations of the WHO (3), a large number of men who have infertility are incorrectly identified as having an unknown reason. Recent studies have brought up the possibility that oxidative stress may play a part in idiopathic male infertility. Oxidative stress, also known as OS, refers to a situation in which there is an imbalance between the formations of reactive oxygen species (ROS) and antioxidant defense systems (4). Numerous reproductive functions, such as sperm maturation and hyperactivation, the acrosome response, and fertilization, are dependent on physiological levels of reactive oxygen species (ROS). However, high ROS concentrations are detrimental to numerous cellular processes. Several major contributions to the downstream effects of oxidative stress on sperm maturation, motility, and function have been discovered, including lipid peroxidation, mitochondrial

dysfunction, DNA damage, and apoptosis, these effects are all caused by free radicals (5).

Antioxidants are found in high concentrations in human seminal plasma, which helps to preserve spermatozoa from being damaged by oxidation. Researchers have found that 30-80 percent of infertile males had elevated levels of reactive oxygen species (ROS). The antioxidant capacity of enzymatic defense molecules (catalase (CAT) and superoxide dismutase (SOD) present in seminal plasma, as well as the level of the lipid peroxidation marker malondialdehyde (MDA), and the percentage of sperm DNA fragmentation index (DFI), can all be used to estimate the presence of reactive oxygen species. This allows one to deduce that reactive oxygen species are present (6).

The major ROS in sperm cells is ($O_2^{\bullet-}$), which is converted by SOD to hydrogen peroxide and then by CAT enzymes to the harmless products H_2O and O_2 . Catalase is the only antioxidant enzyme with a higher turnover rate; one molecule of this enzyme can break down more than 1 million molecules of H_2O_2 in a second (7). Specific biomarkers are frequently used to evaluate the incidence of OS. Malondialdehyde (MDA), a damage marker for OS and a byproduct of lipid peroxidation, is however one biomarker. The other OS marker is SDF, which is used to evaluate the quality

of sperm and represents the consistency of the gamete's genetic content (8).

MATERIALS AND METHODS

Study subjects

One hundred and fifty men participated in the study as volunteers and provided semen to the Kamal Al-Samurai Specialized Hospital for Infertility and IVF, Baghdad, Iraq for analysis. The study conducted from April 2021 to February 2022, was approved by the Hospital local Ethics Committee (December, 2020). An experienced andrologist conducted an interview with each of the male participants in this study. Age, height, weight, body mass index (BMI), whether or not the patient used alcohol, medication, reproductive history, and occupation were some of the patient data that were recorded and examined.

Inclusion criteria

Participants in this study were couples who had been living together for at least a year and engaging in frequent, unprotected coitus but were unable to conceive. The men in these couples either had an abnormal sperm count or abnormal sperm motility.

Exclusion criteria

Men who have azoospermia, taking antioxidant supplements or other medications, those with a history of medication use, those with major systemic diseases and chronic illnesses, previous health conditions, testicular hydrocele or varicocele, mumps following

puberty, who have received radiation therapy or chemotherapy, as well as alcoholic drinkers were excluded from the study.

Sample collection

Seminal fluid samples were obtained by stimulation from both fertile and infertile men after 3-7 days of abstinence. A portion of the fluid collected was subjected to liquefaction for 30 minutes for macroscopic and microscopic evaluation. The remaining was distributed into two Eppendorf tubes and the plasma separated by centrifugation. One was used for studying the biochemical parameters catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) using specific ELISA kits (Human ELISA Kit (Enzyme Level and Sunlong Biotech Co., LTD, China) as well as determining the protein concentration. The other tube was used in studying sperm DNA fragmentation (SDF) by Acridine orange assay (9).

Statistical analysis

The International Business Machines Statistical Package for the Social Sciences (IBM SPSS®) Statistics Version 26 (SPSS Inc., USA) was utilized in order to do the analysis on all of the data that was gathered from this study. The values obtained are expressed as Mean \pm SD. The statistical significance of the difference in means of results was assessed by using the student's *t*-test. The $P < 0.05$, $P < 0.001$ was considered to be statistically significant (10).

Table 1: Comparison of the seminal parameters between patients and control

Parameters	Mean \pm SE			P-value
	Oligospermia group (n=50)	Asthenospermia group (n=50)	Control group (n=50)	
Age (year)	31.52 \pm 6.1 ^a	31.96 \pm 5.3 ^a	30.52 \pm 4.175 ^a	NS 0.612
Volume (1.4-1.7)	1.7 \pm 1.116 ^b	1.58 \pm 0.746 ^b	2.668 \pm 0.563 ^a	0.00041**
PH (>7.2)	7.484 \pm 0.3579 ^a	7.56 \pm 0.2872 ^a	7.149 \pm 0.571 ^b	0.002*
Count (> 15 \times 10 ⁶ /ml)	0.00984 \pm 0.00422 ^b	0.02476 \pm 0.00823 ^b	50.24 \pm 6.06 ^a	0.00021**
Grade A (%)	0.4 \pm 2.0 ^b	0 \pm 0 ^b	65.6 \pm 11.02 ^a	0.0005**
Grade B (%)	14.2 \pm 6.24 ^b	11 \pm 4.787 ^b	25 \pm 6.45 ^a	0.00011**
Grade C (%)	29 \pm 7.5 ^a	20.6 \pm 5.27 ^b	6.8 \pm 4.975 ^c	0.0003**
Grade D (%)	56.4 \pm 12.12 ^b	68.4 \pm 8.0 ^a	2.6 \pm 2.93 ^c	0.0006**
Nor. Sperm (> 4%)	29.8 \pm 6.69 ^b	29.6 \pm 8.77 ^b	79.4 \pm 6.34 ^a	0.0002**
Abo.Sperm (< 85%)	70.2 \pm 6.69 ^a	70.4 \pm 8.77 ^a	20.6 \pm 6.34 ^b	0.006*

* ($P \leq 0.05$), ** ($P \leq 0.001$), NS: Not significant. Different letters indicate significant differences ($p \leq 0.05$ or $p \leq 0.001$) between columns

RESULTS

Based on the semen analysis, the participants in this study were grouped as men with oligozoospermia, men with asthenozoospermia and a normal healthy control group. The seminal parameters checked and comparison of values between patients and control is presented in Table 1. We observed no statistically significant difference between the groups in terms of

age and this result allowed for the exclusion of age as a potential confounding factor for the semen parameters.

Enzymatic antioxidant levels in seminal plasma

The examination of seminal plasma samples taken from fertile and infertile males has the potential to yield a number of significant pieces of information. Firstly, there were statistically significant increase ($p \leq 0.05$) in the levels of Superoxide dismutase (SOD) in control group samples when compared to infertile

(oligozoospermia and asthenozoospermia) groups (69.4 ± 69.9 , 228.8 ± 55.4 , 230 ± 59 , respectively). Catalase enzyme levels in the serum of seminal plasma samples did not show any statistically significant

differences between the viable and infertile (oligozoospermia and asthenozoospermia) groups (0.89 ± 0.09 , 0.89 ± 0.13 , 0.89 ± 0.13 , respectively) as shown in Table 2.

Table 2: The semen plasma superoxide dismutase (SOD) and catalase (CAT) enzyme levels in the studied groups

Parameters Groups	SOD ng/ml (mean \pm SD)	CAT ng/ml (mean \pm SD)
OligoZoospermia	228.8 ± 55.4^b	0.8935 ± 0.135^a
Asthenozoospermia	230 ± 59.0^b	0.8928 ± 0.13^a
Control	690.4 ± 6.0^a	0.8947 ± 0.0938^a
P-value	0.0021*	0.998 NS

*($P \leq 0.05$), NS: Non-Significant; Different letters show significant differences ($p \leq 0.05$ or $P \leq 0.001$) between columns.

Evaluation of oxidative stress parameters

Malondialdehyde (MDA)

The result of the biochemical test showed a significant increase ($p \leq 0.001$) of malondialdehyde (MDA) in seminal plasma of idiopathic infertile groups oligozoospermia and asthenozoospermia compared to fertile (control) group (106.01 ± 11.25 , 106.7 ± 12.82 , 64.68 ± 9.09 , respectively) as shown in Table 3.

Sperm DNA fragmentation index (DFI)

Staining with acridine orange was used to evaluate the level of DNA damage in sperm. Those spermatozoa

that displayed a spectrum of fluorescence ranging from green to yellow had undamaged DNA, whereas those that displayed a spectrum of fluorescence ranging from orange to red had damaged DNA (Fig. 1). Table 4 presents the results of a comparison of the DNA fragmentation analysis of sperm in the three groups that were investigated. Based on these results, DFI of the sperms in the oligozoospermia ($38.41 \pm 7.97\%$) and asthenospermia ($36.02 \pm 4.93\%$) groups significantly increased ($P \leq 0.001$) compared with the control ($7.911 \pm 2.49\%$) group.

Table 3: The semen plasma MDA level in the study groups

Parameter Groups	MDA ng/ml (mean \pm SD)
OligoZospermia	106.01 ± 11.25^a
Asthenozoospermia	106.7 ± 12.82^a
Control	64.68 ± 9.09^b
P-value	0.00031**
LSD	9.073

*($P \leq 0.05$), NS: Non-Significant; Different letters show significant differences ($p \leq 0.05$ or $P \leq 0.001$) between columns.

Table 4: The semen DFI level of patients in the study groups

Parameter Groups	DFI % (mean \pm SD)
Oligozoospermia	38.41 ± 7.97^a
Asthenozoospermia	36.027 ± 4.939^a
Control	7.911 ± 2.491^b
p-value	0.00041**
LSD	8.684

*($P \leq 0.05$), NS: Non-Significant; Different letters show significant differences ($p \leq 0.05$ or $P \leq 0.001$) between columns.

Correlation between SOD, CAT, MDA and sperm DFI

In the current study, we used the Spearman's correlation coefficient test to examine many relationships between the researched parameters (superoxide dismutase, catalase, malondialdehyde levels, and the percentage of sperm DNA fragmentation index) for infertile men (Table 5). Analysis of the correlations among enzymatic antioxidative mechanisms, MDA and DFI

(Fig. 2) indicated that there was a negative correlation between CAT and DFI in the infertile group (-0.231). DFI also correlated negatively with MDA and SOD levels ($r = -0.162$ and -0.166 respectively). Furthermore, CAT showed statistically significant ($P > 0.05$) positive correlation with MDA ($r = 0.908$) and SOD ($r = 0.882$). Also, SOD was observed to be positively correlated with MDA and this interaction was statistically significant ($P > 0.05$).

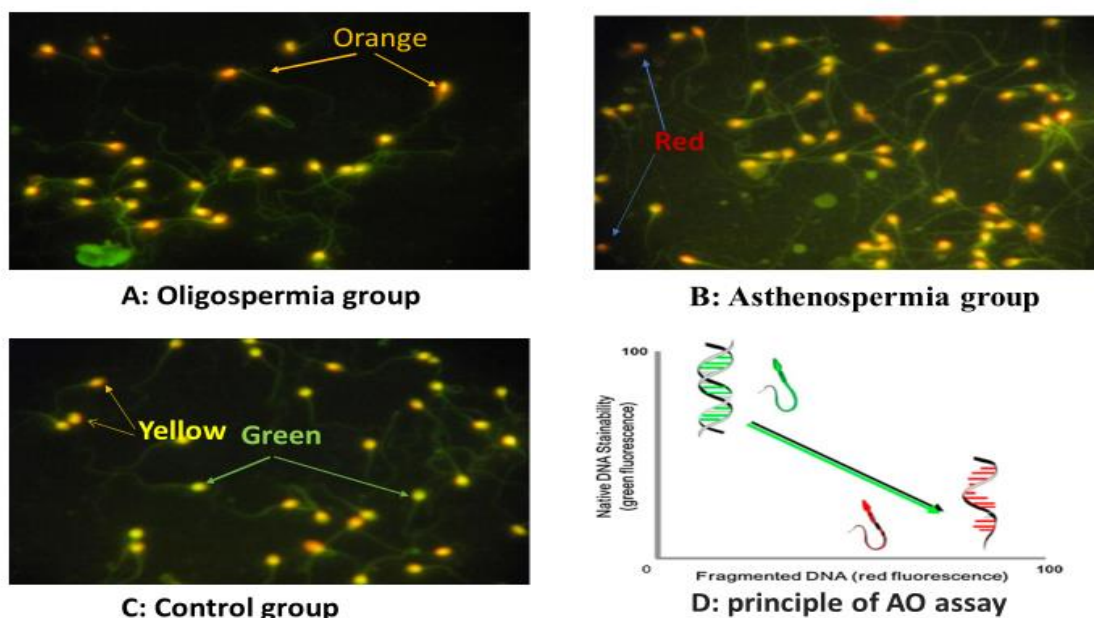


Fig. 1: A, B and C: A wide variety of different patterns may be seen in colored photos of DNA fragmentation in sperm that were acquired using a fluorescence microscope with a magnification setting of 20x (native DNA) to red (denatured DNA). D: Principle of Acridine orange (AO) assay.

Table 5: Correlation coefficient between SOD, CAT, and MDA levels with DFI sperm percentage in infertile men

Correlations	DFI%	CAT	MDA
CAT	-0.231	-----	-----
P-value	0.107	-----	-----
MDA	-0.162	0.908	-----
P-value	0.26	**0.004	-----
SOD	-0.166	0.882	0.987
P-value	0.249	**0.0005	**0.0003

** ($P \leq 0.05$)

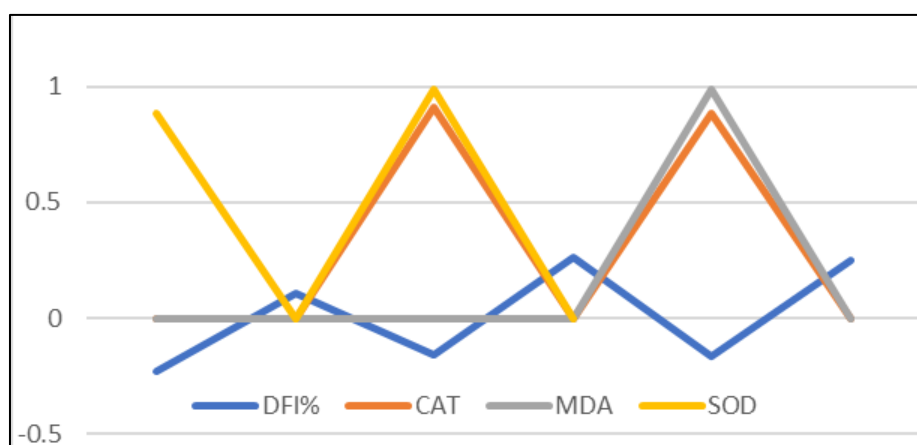


Fig. 2: Graph showing the correlation between SOD, CAT, and MDA levels with DFI sperm percentage in infertile men

DISCUSSION

The oxidative stress plays a role in male reproductive difficulties. This belief is based on the fact that oxidative stress is a prevalent mechanism underpinning the pathophysiology of male infertility. According to Esteves (11), oxidative stress can be discovered in the sperm of more than eighty percent of individuals who are fertile. Seminal plasma, which is released by accessory glands, is an extensive antioxidant system that contains both enzymatic and nonenzymatic components. These components work together to protect spermatozoa from the damaging effects of

reactive oxygen species (ROS). Spermatozoa are distinguished by an unusually high susceptibility to oxidative damage as a result of their structure. In this regard, sperm cells are particularly dependent on the availability of potent antioxidant qualities in the surroundings in which they are located (12).

In our investigation, we observed that SOD enzyme levels were considerably ($p \leq 0.05$) higher in the fertile group than in the infertile group. This is contrary to the findings of Rehman *et al.*, (13) who reported SOD levels to be significantly higher in the infertile group. SOD is an important part of the antioxidant scavenger

system and the polyunsaturated fatty acid in sperm is known to be protected by this system from oxidative stress and DNA fragmentation, ROS has been shown to cause apoptosis in germ cells, which can result in a lower sperm count (14). In contrast to the findings of Alhafadhi and Alkhafaji (15), which revealed that the levels of catalase (CAT) reduced significantly ($P < 0.001$) in the infertile males when compared to the fertile control group, the findings of the current investigation showed that infertile men had higher levels of the enzyme. Our research demonstrates that there is a strong and positive link between the concentration of CAT in seminal plasma and the percentage of both total and increasingly motile spermatozoa. This correlation was demonstrated in prior studies, which our data support. On the other hand, when measured against our findings, Ayad *et al.*, (16) found no correlation between the levels of seminal plasma SOD and the parameters measuring sperm count and motility. The importance of this enzyme in the prevention of ROS-induced oxidative damage and, as a result, the reduction of cytotoxicity to spermatozoa is indicated by the positive correlations that were found between sperm motility and normal count and SOD levels. Superoxide dismutase (SOD) and catalase (CAT) levels in seminal plasma were shown to be considerably lower in infertile individuals compared to those in control groups in a previous research, which clearly suggests their direct relationship with male fertility (17). Malondialdehyde (MDA), on the other hand showed significant increase ($p \leq 0.001$) in seminal plasma of idiopathic infertile groups compared to fertile groups. Our result is in agreement with the study by Al-Fleafil *et al.*, (18) who showed a significant increase in serum malondialdehyde (MDA) levels in the infertile groups in comparison to the fertile group. However, this observation contradicts the findings of Baszynsk *et al* (19) who found unexpected results of MDA concentrations and showed that lipid peroxidation was more intensive serum of the healthy controls than in the infertile group. Several studies have suggested that MDA concentrations in seminal plasma can be utilized to diagnose male infertility caused by an excess of reactive oxygen species in the male reproductive system. The lipid peroxidation of the sperm membrane is the primary source of sperm damage produced by ROS that results in infertility. MDA, a lipid peroxide byproduct, indicates the level of lipid peroxidation (20). The findings of this study corresponded with those of Gill *et al.*, (21), who observed that both fundamental sperm parameters and the integrity of sperm nuclear DNA are dramatically impaired in infertile men, as opposed to fertile men, these findings were based on the results of the acridine orange assay.

In comparison to the controls, the oligoasthenozoospermic and asthenozoospermic groups both showed a highly significant rise in their sperm DFI. This is similar with the findings of a previous Iraqi study that was carried out in Karbala,

Iraq (22) wherein the authors stated that the sperm DFI in men with asthenozoospermia and oligoasthenozoospermia was higher than in men who are normozoospermia. Normozoospermia men had a sperm DFI lower than that of men with asthenozoospermia.

It is considered that the sperm DNA fragmentation index is a more reliable biological indication of sperm quality than the sperm motility index, this is since intact DNA is necessary for the effective transmission of genetic material to the egg cell (23).

During the late stages of spermatogenesis, DNA damage in the form of single- or double-stranded breaks in DNA can be caused in spermatozoa by either endogenous mechanisms found in the testis or epididymis or by foreign substances that are met by sperm after they have been ejaculated (24). Our findings are also in accordance with a reported Baszynski *et al.*, (23), who show that sperm DNA fragmentation may not be correlated with MDA levels, suggesting that some fundamental sperm quality parameters may remain independent of MDA. Previous research has focused on the relationship between severe lipid peroxidation, elevated MDA level, declining sperm quality, and overall reproductive potential (20). However, there are many causes lead to male infertility, one of the antinuclear antibodies (25)

In Iraqi males, a correlation study undertaken by Fadhil *et al.*, (22) in Karbala displays that the concentration of catalase and superoxide dismutase in the seminal plasma of infertile men (oligozoospermia and asthenozoospermia) is negatively related to sperm DFI which was like the results seen in this study.

The process of sperm differentiation during spermatogenesis is associated with a decrease in cytoplasm content. As a result, sperms have a low level of intracellular antioxidant activity. At this level, SOD and CAT play an important role, in protecting the sperms against oxidative stress. Therefore, the enzymatic antioxidants found in seminal plasma, SOD and CAT play an even more significant role in ROS counteraction (26).

CONCLUSION

Patients with oligozoospermia and asthenozoospermia have significantly higher levels of sperm DNA fragmentation index and malondialdehyde, which suggests that sperm DFI and lipid peroxidation contribute to the reduction in semen parameters that contributes to male infertility. Patients with asthenozoospermia also have significantly higher levels of sperm DNA fragmentation index. We are hopeful that subsequent research will offer statistically valid explanations for this pattern, as it will have better controls.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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