# The Activities of Catalase in the Spermatozoa and Seminal Plasma of Patients with Asethenospermia; and their Relationship with Oxidants and Antioxidants

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## Abstract

The exact oxidants and antioxidants status in the seminal and spermatozoal patients with asthenospermia is still not clear. To add a new insight to the question, changes in the seminal and spermatozoal lipid peroxidation products (MDA), levels of glutathione (GSH) and activities of antioxidant enzyme catalase were measured in patients with asthenospermia. This work was undertaken to assess oxidative stress and antioxidant status in patients with asthenospermia. The study was conducted on 30 patients and compared to 20 controls. Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the student's t - test. It was observed that there was a significant increase in seminal and spermatozoal catalase activity and MDA levels; and a significant decrease in seminal and spermatozoal GSH in patients with asthenospermia when compared to controls. The results of this study suggest higher oxygen-free radical production, evidenced by increased MDA levels and catalase activities and decreased GSH levels, support to the oxidative stress in asthenospermia. The increased activities of antioxidant enzyme may be a compensatory regulation in response to increased oxidative stress.

## الخلاصة

أن المحتوى المضبوط للمؤكسدات ومضادات الاكسدة في النطف والبلازما المنويـة مـازال غيـر واضح. ولاضافة المزيد من الوضوح لهذا الموضوع ,تم دراسة مجموعة من المتغيرات الكيميائية الحياتية في النطف والبلازما المنوية للمرضى مثل الانـزيم المضـاد للاكسـدة (الكـاتليز) ومسـتويات نـاتج اكسـدة الدهون(المالون ثنائي الالديهايد) ومضاد الاكسدة (الكلوتاثيون).تضـمنت الدراسـة 30 مريضـا و20 مـن الاصحاء . لوحظ وجود زيادة معنوية في مستويات انزيم الكاتليز ومستويات ناتج اكسدة الدهون(المالون ثنائي الالديهايد) في النطف والبلازما المنويةللمرضى المصابين بوهن النطف عند مقارنتها بقيمها لدى الاصـحاء, كما وجد نقصان معنوي في مستويات مضاد الاكسدة (الكلوتاثيون).نتائج هذه الدراسة تدل على حصول زيادة في انتاج جذور الاوكسجين الحرة ,أثبتت بتزايد مستويات ناتج اكسدة الدهون(المـالون ثنـائي الالديهايـد) و الانزيم المضاد للاكسدة (الكاتليز) ونقصان مستويات مضاد الاكسدة (الكلوتاثيون, قد تكون زيادة فعالية انـزيم الكاتليز عملية تعويضية لزيادة الاجهاد التأكسدي

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**Abbreviations: - DTNB (5, 5'- Dithio bis(2-nitro benzoic acid)); ROS (Reactive oxygen species); GSH (Glutathione); MDA (Malondialdehyde); CAT(Catalase); PUFA(Poly unsaturated fatty acid); GPx(Glutathione peroxidase); GST(GlutathioneS-transferase); ml*(ml letter of homogenous solution of spermatozoa); LPO(Lipid peroxidation).**

## Introduction

In recent years, the generation of reactive oxygen species (ROS) in the male reproductive tract has become a real concern because of their potential toxic effects at high levels on sperm quality and function. ROS are highly reactive oxidizing agents belonging to the class of free radicals [1]. Many studies have indicated that high levels of ROS are detected in semen samples of 25% to 40% of infertile men [2,3]. However, Aitken *et al.*, suggests that small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities[4]. Poor sperm quality is linked to increased ROS generation as a consequence of the presence of excess residual cytoplasm. Spermatozoa undergo a remarkable transformation during the final stage of sperm differentiation and lose their cytoplasm to become mature spermatids. Following spermiation, any residual cytoplasm associated with spermatozoa is retained in the mild-piece region as an irregular cytoplasmic mass (as shown in figure (1)) [1]. If this residual cytoplasm occupies more than one third of the sperm head, it is termed a cytoplasmic droplet. Under these circumstances, the spermatozoa that are released after spermiation are thought to be immature and functionally defective. They are capable of producing increased amount of ROS.

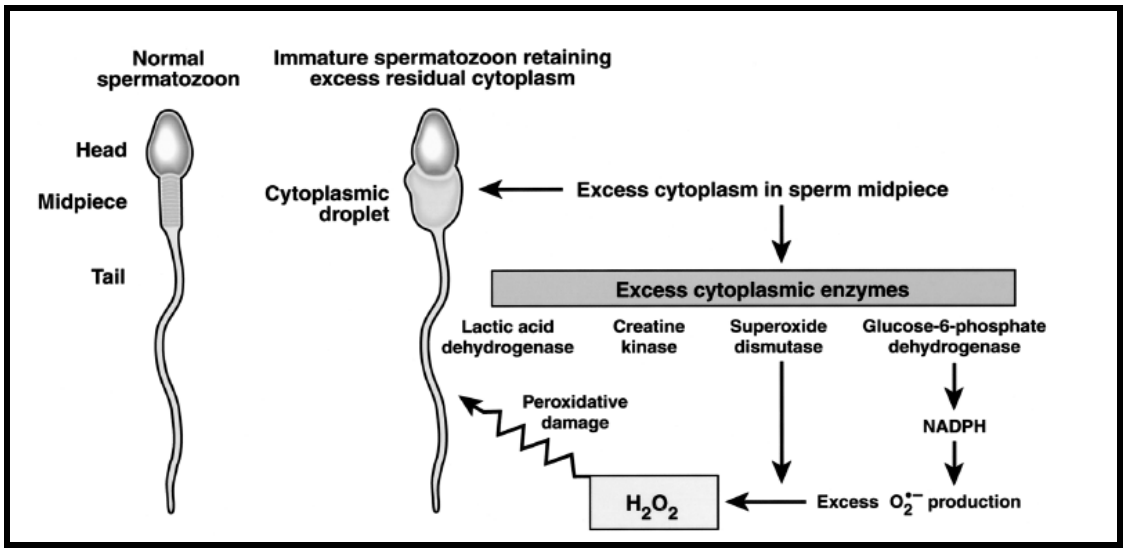


**Figure (1):- Mechanism of Increased Production of ROS by Abnormal Spermatozoa.** [1]

ROS may also initiate a chain mature of reactions that ultimately lead to apoptosis. Apoptosis is a natural process in which the body removes old and senescent cells; it is a process of programmed cell death. In human germ cells, apoptosis may help remove abnormal germ cells and prevent their overproduction. Multiple extrinsic and intrinsic cell factors control the process of apoptosis [5]. In a previous study, Sikka found that levels of ROS were positively associated with apoptosis in mature spermatozoa. Levels of caspases, which are proteases involved in apoptosis, correlated with levels of ROS. ROS can attack polyunsaturated fatty acids in the cell membrane leading to a chain of chemical reactions called lipid peroxidation. Fatty acids breakdown results in the

formation of various oxidatively modified products, which are toxic to cells and are finally converted into stable end products. The spermatozoal membrane contains large amounts of poly unsaturated fatty acids [7], which maintain its fluidity. Peroxidation of these fatty acids leads to the loss of membrane fluidity and a reduction in the activity of membrane enzymes and ion channels. As a result, the normal cellular mechanisms that are required for fertilization are inhibited. It is possible to measure the extent of peroxidative damage estimating the stable end products of lipid peroxidation such as malondialdehyde [8]. The aim of the present study is to assess spermatozoa and seminal plasma levels of glutathione and malondialdehyde and the activities of catalase in men with asthenospermia, compared to normospermic males.

## Materials and methods

Reagents unless otherwise stated, the reagents were purchased from BDH.

**Patients and Sample Preparations:-** Semen samples were obtained from 20 fertile men and 30 patients aged 30-38 years (mean age, 33 years) with asethenospermia infertility. All semen samples were collected in the infertility unit of the laboratory after a 3-5 day period of sexual abstinence, then incubated at 37°C and analyzed within 1 h.

**Preparation of seminal plasma and spermatozoa for biochemical analysis:-**

For each sample, seminal plasma was separated from the spermatozoa 1 h after semen collection by centrifugation of 2 ml of seminal fluid at 1500 *g* for 10 min at 4°C and maintained at -30°C until analysis. For the analysis of catalase, glutathione, and malondialdehyde in spermatozoa, the pellet was resuspended in 10 volumes of medium NTPC (NaCl 113 mM, $NaH_2PO_4$ 2.5 mM, $Na_2HPO_4$ 2.5 mM, $CaCl_2$ 1.7 mM, D-glucose 1.5 mM, Tris 20 mM, EDTA 0.4 mM adjusted to pH 7.4 with HCl)[9] and centrifuged at 1500 *g* for 10 min at 4°C. This washing procedure was repeated three times. Triton X-100 (0.1%) was added to the pellets obtained and the samples were centrifuged again at 8000 rpm for half an hour in a refrigerated centrifuge. This concentration of Triton X-100 does not affect enzyme levels. The supernatant was used for enzymatic measurements in spermatozoa.

**Assay of catalase (CAT):-** CAT was assayed calorimetrically at 570 nm and expressed as katal/ml of samples as described by sinha [10], in brief, to 0.2 ml of sample was added 1.0 ml of 0.01 M pH 7.0 phosphate buffer solution, and 0.3 ml of 0.2 M $H_2O_2$. The reaction was stopped after three minute by 1.5 ml of dichromate-acid reagent.

**Determination of MDA concentration in spermatozoa and seminal plasma:-** MDA levels were analyzed according to method described by Buege [11].To 1 ml of the resulting supernatant was added 2 ml thiobarbituric acid reagent (15% W/V trichloroacetic (sigma) acid ,0.375% thiobarbituric acid and 0.25 N HCI) The solution was then heated at 100 C° for 15 min. After cooling, the precipitate was removed by centrifugation at 3000 g for 10 min, and the absorbance of the supernatant determined at 532 nm against a blank containing distilled water instead of biological sample. The concentration of MDA in the samples were calculated using the molar coefficient of molar extinction coefficient of MDA ($1.56*10^5$ $_M CM^{-1}$)

**Determination of the glutathione concentration in spermatozoa and seminal plasma** [12]:- To 0.2 ml of sample was added 0.8 ml of precipitating mixture (1.67 gm metaphosphoric acid, 0.2 gm EDTA and 30 gm NaCl to make 100 ml of solution). It was centrifuged at 5000 xg for 5 min and 0.5 ml of the filtrate was added to 1.0 ml of the 0.25 M tris buffer solution (pH:8,2), followed by the addition of 0.5 ml of DTNB reagent (20 mg of 5,5'-dithio bis(2- nitro benzoic acid (DTNB) (sigma) dissolved in 100 ml of 1% soduim nitrate). The absorbance of the supernatant determined at 412 nm against a blank containing distilled water instead of biological sample. The concentrations of glutathione in the samples were calculated using a standard curve constructed using reduced glutathione (sigma) dissolved in distilled water.

**Statistical analysis:-**

The results are expressed as number, range, convedance interval C.1 95%. The data were performed using Microsoft Excel version 6. The hypothesis testing was performed using student's (t) test taking $p < 0.05$ as the lowest limit of significance.

## Results

Catalase activity was investigated. Figure 2 shows catalase activity in spermatozoa and seminal plasma of patients with Asethenospermia infertility and control donors.

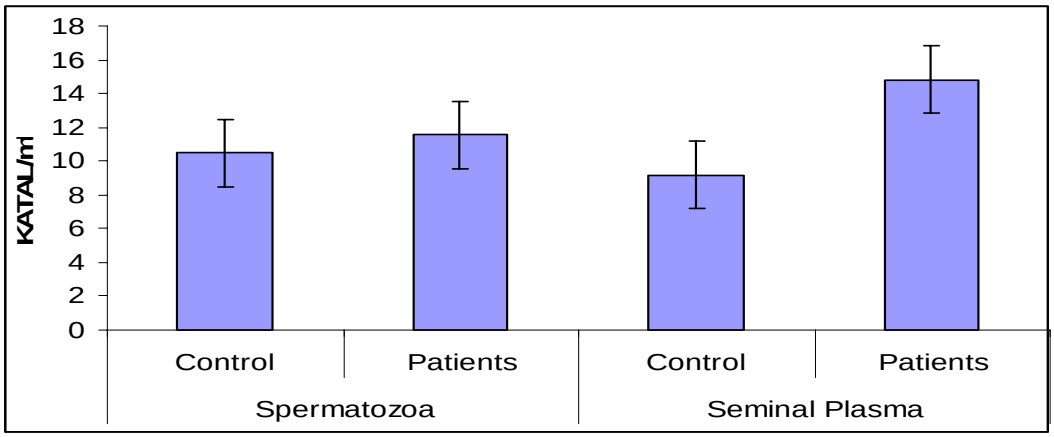


**Fig (2):- CAT Activity in Spermatozoa Kat/ml* and Seminal Plasma Kat/ml of Patients with Asethenospermia Infertility and Control Donors.**

Malondialdehyde; the end product of lipid peroxidation was estimated. Fig 3 shows the levels of malondialdehyde in spermatozoa and seminal plasma of patients with Asethenospermia infertility and control donors.

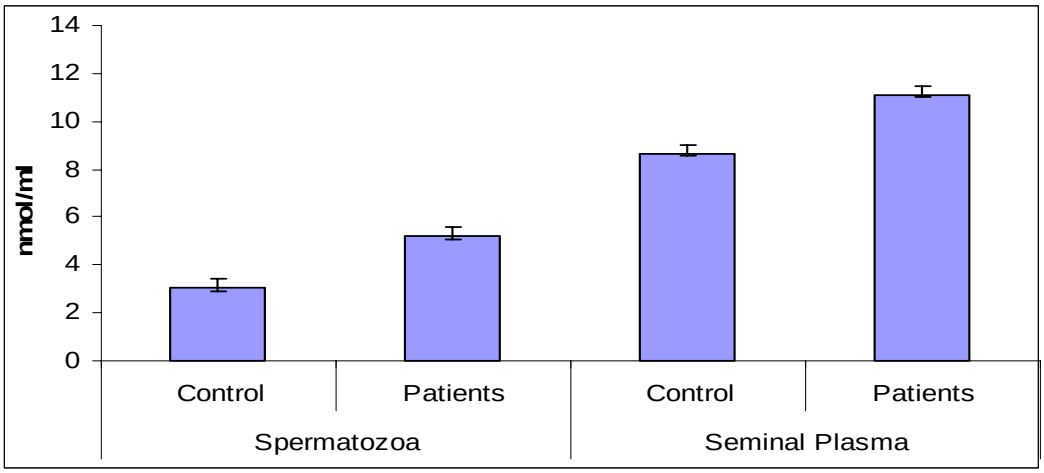


**Fig (3):- MDA Levels in Spermatozoa nmol/ml* and Seminal Plasma nmol/ml of Patients with Asethenospermia Infertility and Control Donors.**

Finally, the levels of non protein thiol compound were investigated. Fig 4 shows the levels of glutathione in spermatozoa and seminal plasma of patients with Asethenospermia infertility and control donors.

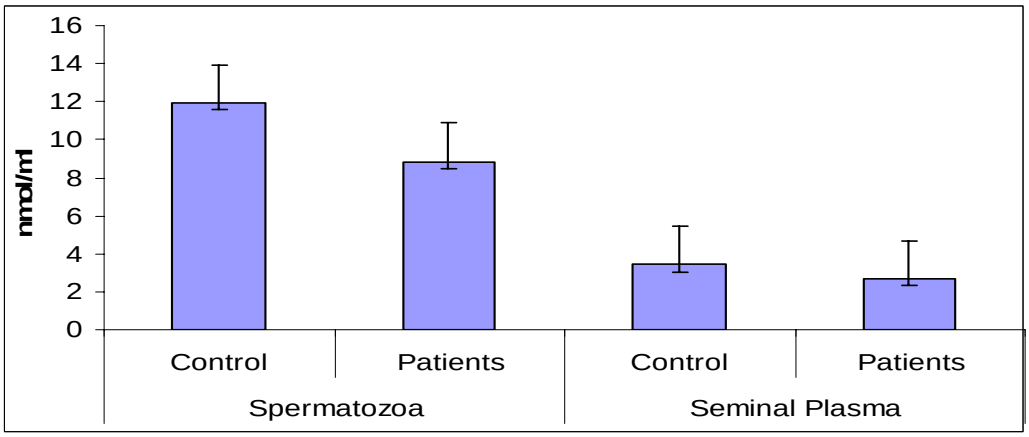


**Fig (4):- GSH Levels in Spermatozoa nmol/ml* and Seminal Plasma nmol/ml of Patients with Asethenospermia Infertility and Control Donors.**

## Discussion

The metabolism of human sperm produces different reactive oxygen species (ROS) such as $H_20_2$, $0_2^-$, and $^-0H$, which are potentially harmful to the sperm plasma membrane with its high content of polyunsaturated fatty acids [13, 14] Semen plasma contains many factors that protect spermatozoa against free radical toxicity[15];they include enzymatic ROS scavengers such as catalase. Catalase is an enzyme which protects the cells from accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant, in which it works as a peroxidase [16] Catalase activity is increased in spermatozoa and seminal plasma of patients with asethenospermia when it is compared with that of healthy controls as shown in-table- I -.

**Table 1:- Catalase Activity in Spermatozoa Kat/ml* and Seminal Plasma Kat/ml of Patients with Asethenospermia and that of Healthy Controls.**

| | | Mean | SD | SE | 95%C.I. | | P Value | Sign. |
|---|---|---|---|---|---|---|---|---|
| | | | | | Ll | L2 | | |
| Seminal plasma | Control | 9.16 | 3.066 | 0.685 | 10.7142 | 7.6117 | ---- | ---- |
| | Patient | 14.80 | 6.717 | 1.226 | 12.0326 | 17.581 | <0.01 | Sign. |
| Sperms-tozoa | Control | 10.54 | 2.387 | 0.533 | 11.747 | 9.3321 | ---- | ---- |
| | Patient | 11.56 | 1.916 | 0.349 | 12.351 | 10.766 | <0.05 | Sign. |

The increment of catalase could be beyond to the high levels of leukocytes in seminal plasma of patients with asethenospermia which acts to increase the generation of $0_2^-$ (Anurada et al., [17] found that super oxide radical induces the activity or catalase); also, the increment of levels of H202 may be induce the activity of catalase. Lipid peroxidation is broadly defined as "oxidative deterioration of PUFA" (i.e., fatty acids that contain more than two carbon-carbon double bonds [18]).

The LPO cascade occurs in two fundamental stages: initiation and propagation; propagation of LPO depends on the antioxidant strategies employed by spermatozoa. One of the byproducts of lipid peroxide decomposition is malondialdehyde. This byproduct has been used in biochemical assays to monitor the degree of peroxidative damage in spermatozoa[19,20].

Malondialdehyde levels increase in spermatozoa and seminal plasma of patients with asethenospermia when compared with that of healthy controls as shown in table 2.

**Table 2:- Malondialdehyde levels in Spermatozoa nmol/ml* and Seminal Plasma nmol/ml of Patients with Asethenospermia and that of Healthy Controls.**

| | | Mean | SD | SE | 95%C.I. | | P Value | Sign. |
|---|---|---|---|---|---|---|---|---|
| | | | | | Ll | L2 | | |
| Seminal plasma | Control | 8.63 | 1.2583 | 0.2813 | 9.26 | 7.99 | ---- | ---- |
| | Patient | 11.1 | 2.2715 | 0.4147 | 12.03 | 10.16 | 0.00 | Sign. |
| Spermatozoa | Control | 3.02 | 1.1209 | 0.2506 | 3.59 | 2.45 | ----- | ---- |
| | Patient | 5.19 | 0.5597 | 0.1021 | 5.42 | 4.95 | 0.00 | Sign. |

Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules, including membrane lipids. Because most cytoplasmic enzymes are extruded during the final stages of the sperm maturation process [21], that's might make spermatozoa highly susceptible to peroxidative damage. Spermatozoa are particularly susceptible to ROS induced damage because their plasma membrane contains large quantities of poly unsaturated fatty acids and their cytoplasm contains low levels of scavenging enzymes [1]; also sperm preparation by centrifugation may be associated with generation of ROS. It has been reported that seminal plasma is rich in antioxidants and protects the spermatozoa from DNA damage and

lipid peroxidation [22].In humans, the glutathione (GSH) (Ly-glutamyl-Lcysteinyl-glycine) functions as the most important endogenous antioxidant involved in maintaining the pro-oxidant antioxidant balance in human tissues [23]. GSH is also involved in the metabolism and detoxification of cytotoxic and carcinogenic compounds, and in the elimination of reactive oxygen species (ROS) [24]. GSH levels decrease in spermatozoa and seminal plasma of patients with asethenospermia when compared with that of healthy controls as shown in table 3.

**Table** 3:- **GSH levels in Spermatozoa nmol/ml* and Seminal Plasma nmol/ml of Patients with Asethenospermia and that of Healthy Controls.**

| | | Mean | SD | SE | 95%C.I. | | P Value | Sign. |
|---|---|---|---|---|---|---|---|---|
| | | | | | Ll | L2 | | |
| **Seminal plasma** | **Control** | 3.44 | 0.2116 | 0.0473 | 3.54 | 3.33 | ---- | ---- |
| | **Patient** | 2.7 | 0.4582 | 0.0836 | 2.88 | 2.51 | <0.01 | Sign. |
| **Spermatozoa** | **Control** | 11.96333 | 2.2419 | 0.5013 | 13.09 | 10.82 | ----- | ---- |
| | **Patient** | 8.866667 | 0.2309 | 0.0421 | 8.96 | 8.77 | <0.01 | Sign. |

GSH and ROS are implicated in human reproduction. In humans, spermatozoa generate ROS which are known to affect hyper activation of spermatozoa, the acrosome reaction and the attachment of spermatozoa to oocytes thereby contributing to the fertilization of oocytes [25]. The decrease of GSH in spermatozoa and seminal plasma beyond to increase the levels of ROS, GSH undergo oxidation process to deplete this levels, such as the reaction between free radicals $0_2^{\bullet-}$, or -OH and glutathione 26

$$GSH + {}^{\cdot}OH \longrightarrow GS^{\cdot} + H_20$$

In the second type of reaction; GSH detoxifies ROS directly or via a GPx catalyzed reactions [27]

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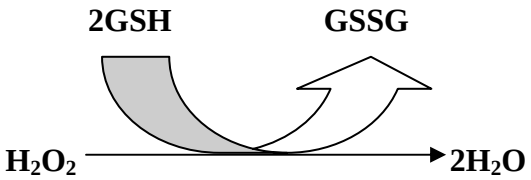


Also; GSH may be utilized by GST to formation thioether bond by conjugated hydrophobic compounds (hydrophobic compounds levels increased at high levels of oxidative stress [28]) with GSH. The decrease of the levels of GSH may be produced to regenerate ascorbic acid (vitamin C) and α -tocopherol (vitamin A) in seminal plasma and spermatozoa.

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