Zinc, Copper, and Superoxide Dismutase in Spermatozoa of Patients with Asthenospermia

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Abstract

Objective: To determine the level of Superoxide dismutase (SOD), Zinc and Copper in the seminal and spermatozoal of patients with asthenospermia.
Setting: All semen samples were collected in the infertility unit of the laboratory (in Amel clinic for detection and treatment of infertility/Iraq. Hilla city) after a 3-5 day period of sexual abstinence, then incubated at 37°C and analyzed within 1 h.
Methods: 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. Zinc and copper content was assayed by atomically spectrophotography,SOD enzyme was measured by biochemical assay. This work was undertaken to assess oxidative stress and antioxidant.
Results: It was observed that there was a significant increase in seminal and spermatozoal superoxide dismutase activity and copper content. While Zinc were significantly decreased.
Conclusions :The results of this study suggest higher oxygen-free radical production, evidenced by increased superoxide dismutase activities, support to the oxidative stress in asthenospermia. The increased activities of antioxidant enzyme may be a compensatory regulation in response to increased oxidative stress.
Keywords:Zinc**,**Copper,Superoxide dismutase(SOD),Human sperm

الخلاصة

لتحديد مستويات إنزيم السوبر اوكسيد دسميوتيز و عنصري الزنك و النحاس في النطف والبلازما المنوية للمرضى المصابين بوهن النطف. تم قياس مستويات الإنزيم بطريقة كيميوحيوية، أما الزنك والنحاس فتم قياسهما باستعمال جهاز الامتصاص الذري. أن المحتوى المضبوط للمؤكسدات ومضادات الاكسدة في النطف والبلازما المنوية مازال غير واضح. ولاضافة المزيد من الوضوح لهذا الموضوع ،تم دراسة مجموعة من المتغيرات الكيميائية الحياتية في النطف والبلازما المنوية للمرضى مثل الانزيم المضاد للاكسدة (السوبر اوكسيد دسميوتيز) ومستويات عنصري الزنك و النحاس .تضمنت الدراسة ٣٠ مريضا و٢٠ من الاصحاء . لوحظ وجود زيادة معنوية في مستويات انزيم السوبر اوكسيد دسميوتيز ومستويات عنصر النحاس في النطف والبلازما المنوية للمرضى المصابين بوهن النطف عند مقارنتها بقيمها لدى الاصحاء، كما وجد نقصان معنوي في مستويات عنصر الزنك.نتائج هذه الدراسة تدل على حصول زيادة في انتاج جذور الاوكسجين الحرة ،أثبتت بتزايد مستويات الإنزيم المضاد للأكسدة (السوبر اوكسيد دسميوتيز). زيادة مستويات الإنزيم المضاد للأكسدة قد تكون عملية تعويضية لزيادة الاجهاد ألتأكسدي.

Introduction

There is growing evidence that oxidative stress significantly impairs sperm functions. Due to their high content of polyunsaturated fatty acids spermatozoa are susceptible to damage induced by reactive oxygen species (ROS)[1-3]. To counteract the effects of ROS, spermatozoa and seminal plasma possess antioxidative systems to prevent cellular damage. However, due to the small cytoplasm in the mid piece of spermatozoa, the ability to scavenge oxidants is limited, 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)[4]

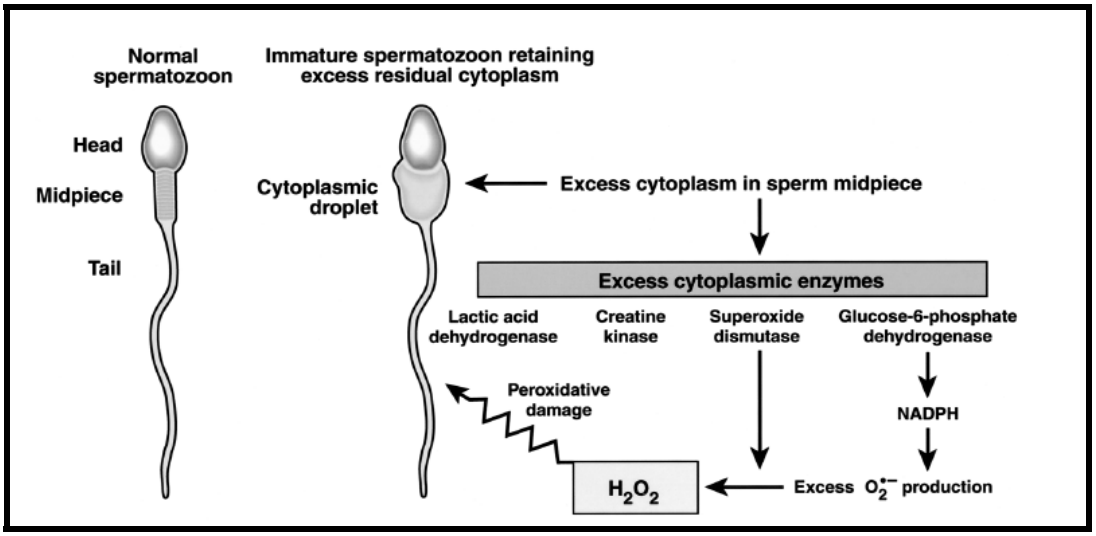

Fig 1. Mechanism of Increased Production of ROS by Abnormal Spermatozoa

Reactive oxygen species (ROS) play an important role in the human reproduction. Spermatozoa are rich in polyunsaturated fatty acids as well as susceptible to be attacked by ROS or membrane lipid peroxide ion[10]. There is a relationship been loss of motility and peroxidation of spermatozoal lipids[5,6]. Also, the superoxide radical has recently been implicated in the capacitation reaction[3].

There have been several studies on the existence of enzymatic defenses in spermatozoa and seminal plasma, such as superoxide dismutase (SOD) [7,8], catalase[9], and glutathione peroxidase (GPx)[10]. Besides these enzymes small molecules present in semen could also act as ROS scavengers such as vitamin C, urate[11], and glutathione[9]. Fig 2 show reduced glutathione (GSH), glutathione disulfide (GSSG), and enzyme which involved in the biosynthesis and breakdown of ROS in male reproductive tissues.

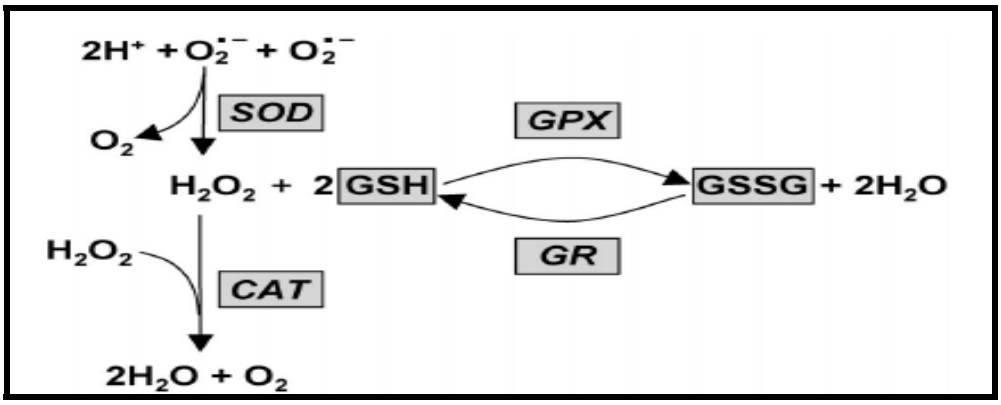

Fig 2. Diagrammatic Representation of The Relationship Between Antioxidant Enzymes, GSH and GSSG. SOD, Superoxide Dismutase; CAT, Catalase; GPX, Glutathione Peroxidase; GR, Glutathione Reductase; GSH, Reduced Glutathione; GSSG, Oxidized Glutathione

Antioxidants in semen serve to protect ejaculated spermatozoa from oxidative stress such as that which occurs in the female reproductive tract. The lipid peroxidation of unsaturated fatty acids in sperm membranes is one of the most important effects from ROS-induced cell damage what may lead to persistent infertility[3]. Antioxidant enzymes, such as SOD and GPx, could dispose hydroperoxide and other ROS. A balance is maintained between the amount of ROS and that scavenged by antioxidant. Cellular damage arises when this equilibrium is disturbed, especially when the cellular scavenging systems cannot eliminate the increased ROS. Lipid peroxidation could damage the cell plasma membrane, which leads to loss of cytosolic components and cell death .In general, a balance is maintained between the amount of ROS and that scavenged by antioxidant. Cellular damage arises when this equilibrium is disturbed, especially when the cellular scavenging systems cannot eliminate the increased ROS. The aim of the present work was to investigate the seminal enzymatic and nonenzymatic antioxidant capacity in semen samples that demonstrate asthenozoospermia. SOD activity and the levels of zinc and copper were determined in asthenozoospermic samples.

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 (in Amel clinic for detection and treatment of infertility/Iraq. Hilla city) 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 4ºC and maintained at -30°C until analysis. For the analysis of Superoxide Dismutase (SOD), Zinc and Copper in spermatozoa, the pellet was resuspended in 10 volumes of medium NTPC (NaCl 113 mM, NaH2PO4 2.5 mM, Na2HPO4 2.5 mM, CaCl2 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.

Determination of Superoxide Dismutase (SOD) activity :-

SOD was measured according to the method of Marklund and Marklund,(1974)[32].

Determination the level of zinc and copper in spermatozoa and seminal plasma: -Zinc levels were analyzed according to method described by Vaubourdolle *et.al*. (1985)[12], by using an atomic absorption spectrometer (Shimadzu AA) after diluting the total homogenized 200-fold in distilled water. Copper also analyzed by an atomic absorption spectrometer according to method described by Yuyan et.al (2008)[33].

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

Superoxide Dismutase activity was investigated. Table 1 shows Superoxide Dismutase activity in spermatozoa and seminal plasma of patients with Asethenospermia infertility and control donors.

Table 1. SOD Activity in Spermatozoa mU/ml* and Seminal Plasma mU /ml of Patients with Asethenospermia and that of Healthy Controls.

		Mean	SD	SE	P -Value	Sign.
Seminal plasma	**Control**	0.288	0.1	0.042	------	------
	Patient	0.83	0.14	0.02	<0.01	Sign.
Spermatozoa	**Control**	0.52	0.06	0.013	------	------
	Patient	0.68	0.18	0.03	<0.01	Sign.

Zinc; an essential trace element required for the action of more than 200 metallo enzymes[13] was estimated. Table (2) shows the levels of Zinc in spermatozoa and seminal plasma of patients with Asethenospermia infertility and control donors

Table 2. Zinc levels in Spermatozoa mmole/L* and Seminal Plasma mmole/Lof Patients with Asethenospermia and that of Healthy Controls

		Mean	**SD**	**SE**	**P -Value**	**Sign.**
Seminal plasma	**Control**	0.13	0.033	0.007	------	------
	Patient	0.108	0.012	0.002	<0.05	Sign.
Spermatozoa	**Control**	0.132	0.031	0.006	------	------
	Patient	0.096	0.011	0.001	<0.05	Sign.

Finally, the levels of Copper were investigated. Table (3) shows the levels of Copper in spermatozoa and seminal plasma of patients with Asethenospermia infertility and control donors.

Table 3. Copper levels in Spermatozoa mmole/L* and Seminal Plasma mmole/Lof Patients with Asethenospermia and that of Healthy Controls

		Mean	SD	SE	P -Value	Sign.
Seminal plasma	Control	0.151	0.095	0.02	------	------
	Patient	0.174	0.02	0.003	<0.05	Sign.
Spermatozoa	Control	0.148	0.07	0.015	------	------
	Patient	0.162	0.014	0.002	<0.05	Sign.

Discussion

The normal functioning of mammalian cells is dependent upon maintaining an intricate balance between the production and elimination of reactive oxygen species[14,15]. The increase in oxidative modification of proteins with aging has been associated with oxygen-free radical generating systems. This increase can be inhibited by superoxide dismutase[15].

As shown in Fig (3) , SOD activity was found to be increased in the present study.

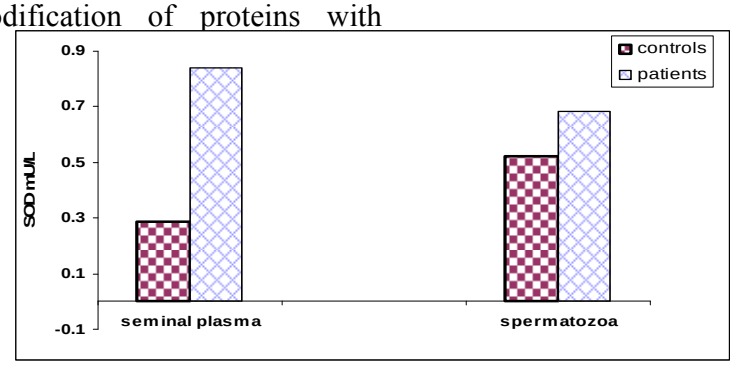

Fig 3. SOD Activity in Spermatozoa mU/ml* and Seminal Plasma mU /ml of Patients with Asethenospermia and that of Healthy Controls.

The increment of SOD could beyond to high levels of $O_2^{\cdot-}$, which act to induce the activity of SOD;(Fridovich (1983) has been demonstrated that cells capable of increasing synthesis of SOD in response to hyperoxidant stress[16]. SOD and its two isozymes have a significant role to dispose, scavenge, and suppress the formation of ROS via spontaneously dismutates ($O_2^{\cdot-}$) anion to form O_2 and H_2O_2, while catalase converts $H_2O_2$to O_2 and H_2O.

$$2\,(O_2^{\cdot-}) + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

$$H_2O_2 \xrightarrow{Catalase} H_2O + 1/2\,O_2$$

These data recommended that high production of H_2O_2 as a result of high SOD activity, H_2O_2 has been shown to be the most operative toxic agent on the spermatozoa[17].

Zinc is an essential trace element required for the action of SOD enzyme[18]. As shown in Fig (4) , Zinc levels was found to be decreased in the present study .

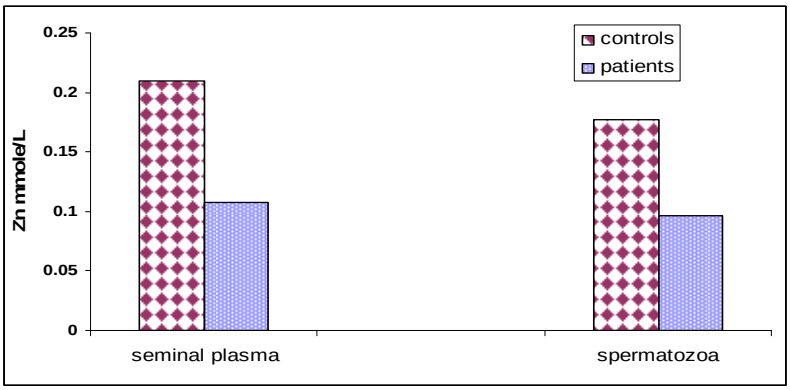

Fig 4. Zinc levels in Spermatozoa mU/ml* and Seminal Plasma mU /ml of Patients with Asethenospermia and that of Healthy Controls.

Zinc has several advantages in reproduction system; the first ,It has been reported to influence the process of spermatogenesis[19], controls sperm motility[20], stabilizes sperm membrane[21] and it plays an important role in prostate, epididymal and testicular functions[22]. The decrement of Zinc might be to high levels of $O_2^{\cdot-}$. (Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa in human semen after ejaculation[23]. That's means; seminal plasma and spermatozoa, exerts protective, antioxidant-like activity sufficient to cope with the excessive amount of superoxide anions because of its high content of zinc[24]. More than one study have demonstrated that zinc therapy results in significant enhancement in sperm quality with increases in sperm density, progressive motility, and enhanced conception and pregnancy outcome in human[25-27] and in animals[18,28].

Copper is found in a number of enzymes and is involved in connective tissue and structural protein formation[34]. It status may directly or indirectly affect nerve physiology and hormonal regulation[29]. As shown in Fig (5) , Copper levels was found to be decreased in the present study.

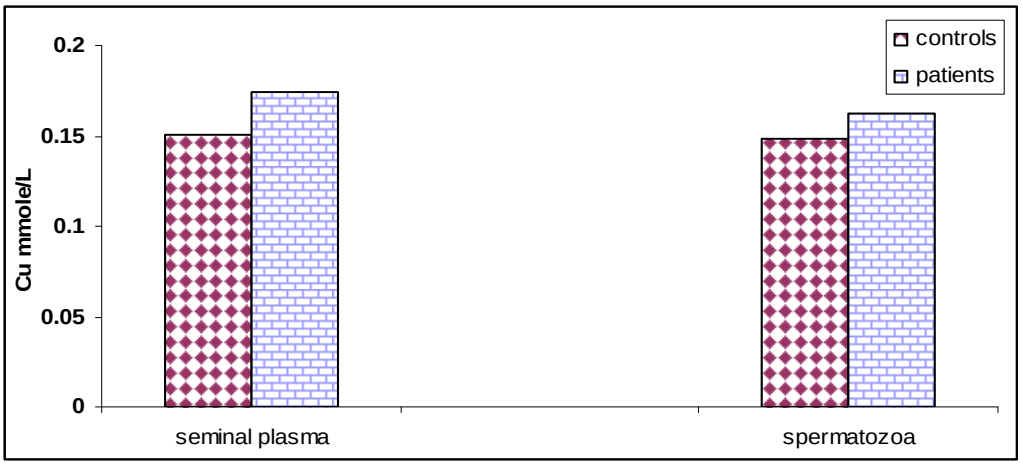

Fig 5. Copper levels in Spermatozoa mU/ml* and Seminal Plasma mU /ml of Patients with Asethenospermia and that of Healthy Controls.

The increment of Copper might be high levels of oxidants in semen of patients because oxidants due to releasing copper from tissues. Excess copper can cause damage tissues[30].

There are two mechanism to induce oxidative stress via copper ;the first was initiated by antioxidants (e.g., tocopherols) present in the LDL. The tocopherol was first converted to free radical by donating an electron to Cu(II) [generating Cu(I)]. The tocopherol radical then slowly induces the autoxidation of LDL(eq 1-5)[31]. The second mechanism include the generated Cu(I), which can decompose peroxides (LOOH) by a Fenton-type reaction (eq 5) and initiate more radical chain reactions.

$$Cu(II) + AOH \rightarrow Cu(I) + AO^{\bullet} + H^{+} \quad (1)$$
$$AO^{\bullet} + L\text{-}H \rightarrow AOH + L^{\bullet} \quad (2)$$
$$L^{\bullet} + O2 \rightarrow LOO^{\bullet} \quad (3)$$
$$LOO^{\bullet} + L\text{-}H \rightarrow LOOH + L^{\bullet} \quad (4)$$
$$Cu(I) + LOOH \rightarrow Cu(II) + LO^{\bullet} + HO^{-} \quad (5)$$

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