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Correlation of Sperm Count, Oxidative Stress, and Seminal Antioxidant Enzyme Activity

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Abstract Infertility is a reproductive health problem in the human population. The aim of the present study was to examine the relationship between sperm count, seminal antioxidant capacity, and oxidative stress markers in infertile men. Semen samples were obtained from 115 men and categorized on the basis of sperm count. Seminal oxidative and antioxidant markers are as follows: lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity were determined. Sperm count significantly correlated positively with sperm count. Sperm count showed significant negative correlation with LPO. Sperm count showed significant positive relationship with SOD and CAT activities. The SOD and CAT activities were positively whereas LPO level negatively associated with elevated sperm count. In conclusion insufficient antioxidant enzymes and increased oxidative stress may attribute to the risk of decreasing semen quality and fertility.

Introduction

Infertility is a common reproductive health problem affecting 13–15% of couples worldwide. The prevalence varies throughout developed and underdeveloped countries, being higher in the latter in which limited resources for diagnosis and treatment exist (Hamada *et al*., 2011). Infertility is either primary or secondary. In primary infertility no pregnancy has ever occurred. In secondary infertility however, there has been a pregnancy, regardless of the outcome. About 67.71% and 29.33% of patients have primary and secondary infertility, respectively (Muller and Daling 1989; Thonneau *et al*., 1991; Irvine., 1998).

Fraczek and Kurpisz., (2007) indicated that imbalance between peroxidative and antioxidative substances in semen leads to metabolic and functional disorders of male germ cells and may be a primary cause of some types of infertility. An increase in the seminal reactive oxygen species level has been reported in 40% of the infertile men. Although antioxidant defense system is active in the semen, its activity is limited as the amount of cytoplasm of the sperm cell is low (Lewis *et al*., 1997).

Keeping in view the oxidative damage to the sperm membranes that may result into the

impairment of cellular and subcellular functioning, permeability, integrity as well as morphological alterations, the aim of the present study is to investigate the role of seminal enzymatic antioxidant capacity and lipid peroxidation in the seminal plasma that exhibit sperm abnormalities which play an important role in the normal functioning of spermatozoa.

Material and Methods

Study population

The study was carried out in115 male partners from couples (ranged from 22 to 50 years) attending the Out Patient Department of the Andrology Unit of Mansura University Hospital, Mansoura , Egypt for the evaluation of infertility.

Subjects currently do not take any medication, tonics, or antioxidant supplementation and those suffering from any acute infection were excluded. The samples were categorized on the basis of sperm count into the following groups: idiopathic (unexplained infertility), oligozoospermic, severe oligozoospermic, and secondary infertility.

Another 18 normal fertile men (each has more than one child) were used as a control, ranged from 30 to 39 years old. The healthy donor specimens had the following criteria: sperm concentration >15 million/ml, >32% progressively motile (a+b) and >4% with a normal morphological shape (Cooper *et al*., 2010, WHO, 2010). The study was conducted according to the local ethical committee. Consent of each subject was taken after explaining the aims and objectives of the study and its benefit to individual and society.

Semen collection and sperm count

Semen sample was obtained from each case by masturbation after 3-4 days of sexual abstinence and collected in sterile plastic containers. Semen samples were allowed to liquefy for 20-30 min at room temperature. Sperm count was carried out in duplicate using hemocytometer and the mean was expressed in millions per milliliter.

Biochemical assays

Liquefied semen was centrifuged at 3000g for 15 min and seminal plasma was immediately processed for further analysis without freezing–thawing.

Superoxide dismutase

SOD was measured according to the method of Nishikimi *et al*., 1972.

Catalase

CAT activity was measured pectrophotometrically according to the method of Aebi (1984) by measuring the decrease in the concentration of hydrogen peroxide at 240 nm. The catalase activity was expressed as specific activity (unit/minute per milliliter of seminal plasma).

Total antioxidant capacity

Total antioxidant capacity was estimated by Colorimetric method following instruction provided with the kit (Bio-diagnostic,Giza,Egyot), (TA2513) according to (Koracevic, Koracevic *et al*., 2001).

Lipid peroxidation

The lipid peroxide level in the seminal plasma was measured using a thiobarbituric acid reactive substances assay, which monitors MDA (malonaldialdehyde) production, based on the method of Ohkawa *et al*., (1979). The amount of MDA was calculated using an extinction coefficient ($1.56\times105\ M^{-1}\ cm^{-1}$). The MDA intensity was measured at 535 nm and expressed as nanomoles per milliliter of seminal plasma.

Statistical analysis

Data was computed with the statistical package for the social science, windows 7 versions, USA (SPSS17 software).Variable with normal distribution were expressed as mean ± SD. In these variables, the T test was applied for group differences. Non parametric data were expressed as median. The Kalmogorove-Smirnov test was check normal distribution of data. For correlation analysis, spearman's

correlation coefficients were calculated with two- tailed *P* value. Value of $P<0.05$ was considered significant (Levesque, 2007).

Results

One hundred and fifteen cases of men with infertility of different etiologies and eighteen cases of fertile men were included in our work. The general characteristics of the study population are given in Table 1. The mean age of the study population was 31.5±6.2 years and most of the subjects were residing in the urban areas. The diagnosis was made on the basis of sperm count. Among the study subjects fifteen were oligozoospermic, fifteen sever oligozoospermic, seventeen secondary infertility (there was a pregnancy regardless to the outcome), and the remaining sixty eight idiopathic (had normal sperm count with unexplained infertility).

Table 1:- General characteristics of study population

No	Parameter	Characteristics	N (%)
1	Age	≤ 30 years	76 (54.36%)
		>30 years	57 (45.64%)
2	Area	Rural	54 (42.72%)
		Urban	79 (57.28%)
3	Sperm count millions per milliliter)	Control	18 (22.3%)
		Idiopathic	68 (66.5%)
		Oligozoospermic	15 (11.06%)
		Severe oligozoospermic	15 (11.06%)
		Secondary infertility	17(12.7%)
4	Motility grade a%	Control	18 (23.7%)
		Idiopathic	68 (33.89%)
		Oligozoospermic	15 (34.0%)
		Severe oligozoospermic	15 (34.0%)
		Secondary infertility	17 (23.3%)
5	Motility grade ab%	Control	18 (47.0%)
		Idiopathic	68 (24.05%)
		Oligozoospermic	15 (20.4%)
		Severe oligozoospermic	15 (20.4%)
		Secondary infertility	17 (31.8%)
6	Occupation	literal profession	93 (70.87%)
		non literal profession	40 (29.13%)

Where:
N: Total count of subjects
%: Per cent of total count of subjects

The results illustrated in Fig. 1 showed a very highly significant decrease in superoxide dismutase activity in seminal plasma and sperm in the different infertile groups as compared with that in control group.

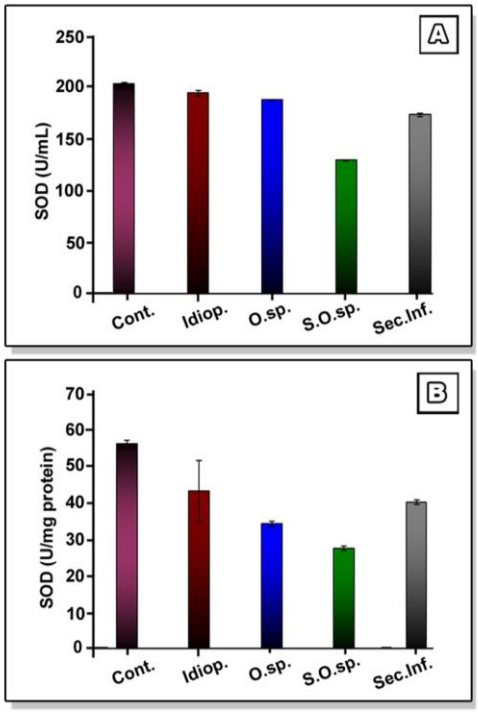

Fig.1:- Superoxide dismutase (SOD) activity in seminal plasma (A) and sperm (B) of the infertile and control groups

As indicated in Fig. 2, there was a significant decrease in the catalase activity in the seminal plasma of the idiopathic group as compared with that in control group and a very highly significant decrease in catalase activity in oligozoospermic, severe oligozoospermic and secondary infertility groups as compared with that in control group in seminal plasma. Also, there was a very highly significant decrease in catalase activity in sperm of the idiopathic, oligozoospermic, severe oligozoospermic and secondary infertility groups as compared to the control group. As Fig.3, there was a very highly significant decrease in total antioxidant capacity levels in seminal plasma in the different infertile groups compared to the control group.

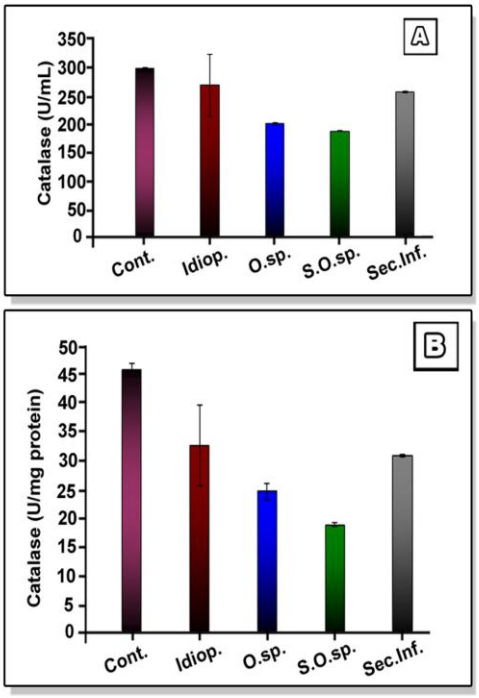

Fig.2:- Catalase activity in seminal plasma (A) and sperm (B) in the infertile and control groups

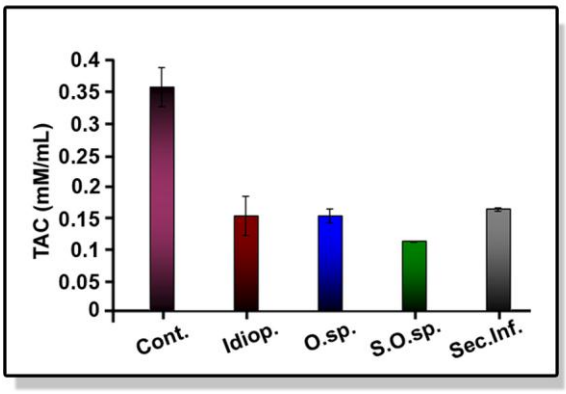

Fig.3:- Total antioxidant capacity level in the seminal plasma in the infertile and control groups

The results shown in Fig. 4 shows a very highly significant increase in MDA levels in seminal fluid in all infertile groups compared with that in control group. Also, there was a very highly significant increase in oligozoospermic, severe oligozoospermic and secondary infertility groups in MDA level in sperm compared to control group. On the other hand, there was insignificant change in the spermatozoal MDA level in the idiopathic group.

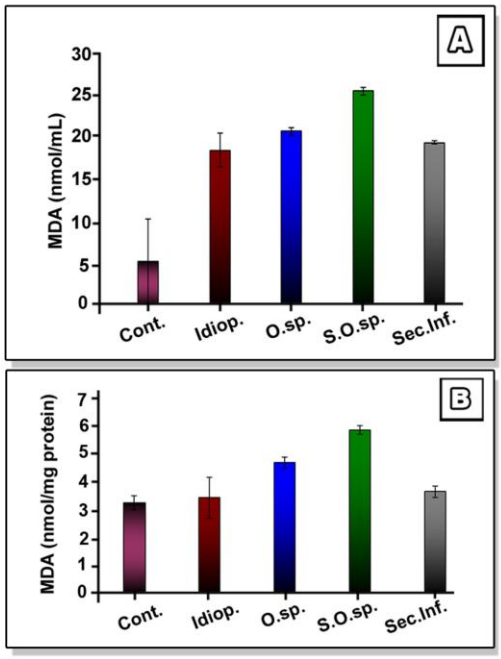

Fig .4:- Malondialdehyde (MDA) level in seminal plasma (A) and sperm (B) in the infertile and control groups

Fig.5 shows a significant negative correlation between sperm count and malondaldehyde level in seminal plasma (r =- 0.839, p=0.000) and sperm (r =- 0.45 p=0.0000,). Fig.6 shows that superoxide dismutase level was increased with the increase in sperm count, Statistical analysis showed a positive correlation between sperm count and superoxide dismutase activity in plasma (r = 0.543, p=0.000) and sperm (r = 0.648, p=0.000). Fig. 7 shows that the activity of catalase in plasma & sperm was elevated with the increase in sperm count indicating a positive correlation between sperm count and catalase activity in plasma (r = 0.582, p=0.000) and sperm (r=0.767, p=0.000).

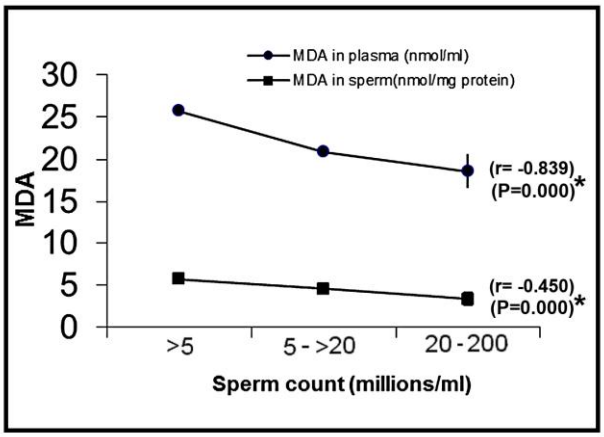

Fig .5: A correlation between sperm count and malondaldehyde level in plasma & sperm

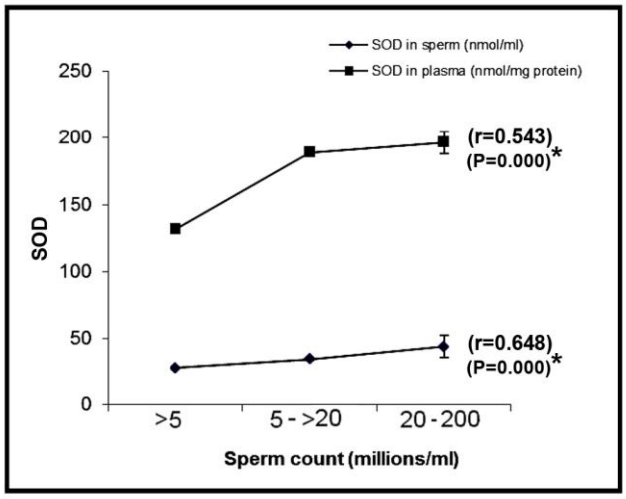

Fig .6: A correlation between sperm count and superoxide dismutase activity in seminal plasma and sperm

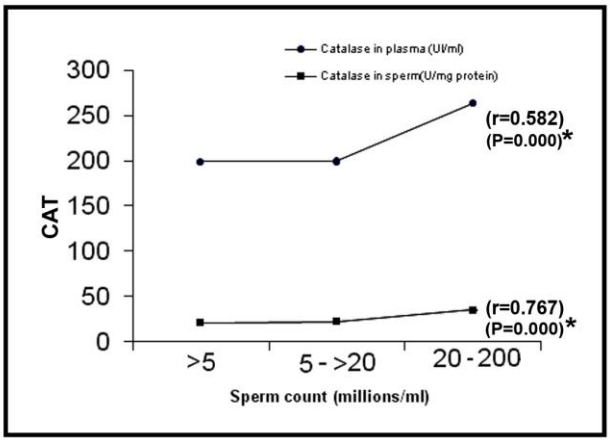

Fig .7: A correlation between sperm count and catalase activity in seminal plasma and sperm

Discussion

It is known that declining semen quality affects fertility as the adequate level of sperm number, motility, and morphology are necessary for the reproductive success. Oxidative stress has been hypothesized as a cause of peroxidative injury to the sperm membrane and a consequent impairment of the related functional properties, such as sperm motility (Sharma and Agarwal 1996). Increased oxidative stress in semen may be associated with reduced sperm fertilizing potentials, impaired metabolism, morphology, and motility (Cummins *et al*., 1994).

In the present study, LPO was found to have significantly negative correlation with sperm count. All infertile subjects showed higher seminal MDA content as compared to normal men. However, Suleiman et al., (1996) showed

no significant negative correlation between seminal plasma level of MDA and sperm count as well as motility but they observed that spermatozoal MDA concentration was higher with decreased sperm motility. The results of the present study are in confirmation with the earlier findings (Shiva *et al*., 2011).

It is known that free radicals can react with lipids. MDA formation has been known to be an early marker for lipid peroxidation. Sperm count was observed to have significant negative correlation with lipid peroxidation. An inverse relationship was found between MDA level in seminal plasma and sperm concentration. Oxidative stress frequently represented as an MDA formation has been recognized in accordance with male fertility (Aitken *et al*., 1989; Agarwal *et al*., 2003). The source of cytotoxic oxygen radicals is frequently intracellular, as in the case of oligozoospermic males whose spermatozoa generate particularly high levels of ROS (Gomez *et al*., 1996). Seminal plasma is well endowed with an array of antioxidant defense mechanism to protect spermatozoa against oxidants that compensates for the deficiency in cytoplasmic enzymes in the spermatozoa (Aydemir *et al*., 2007).

Sperm count was significantly correlated with the seminal SOD and CAT activities and it was found to be higher with increasing ranges of sperm count, which indicated that decline in SOD might be involved in the abnormal semen quality. Some infertility cases reveal intensive aberrations in chromosomes 13, 20, and 21, which contain sequences coding for superoxide dismutase (Brown., 1995). It has also been reported that SOD activity survey in seminal plasma could be a useful tool for determining sperm fertilization potential and could improve the diagnosis of male infertility (Murawski *et al*., 2007). Khosrowbeygi et al., (2004) observed that both catalase activity and total antioxidant capacity (TAC) were significantly correlated with sperm motility and morphology. Similarly, in the present study, a significant positive correlation of CAT was found with sperm count. These results indicate that, CAT activity was significantly associated with the increasing ranges of sperm count.

In conclusion, the present results suggest that the semen samples consisting of adequate sperm count was observed to be associated with improved antioxidant activity, which might reveal enhanced activity against oxidative stress.

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العلاقه بين عدد الحيوانات المنويه و الضغط التأكسدى و الانزيمات المضاده للاكسده الموجوده فى السائل المنوى

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يعتبر العقم مشكله تكاثريه فى مجتمعات الانسان. الهدف من الدراسه الحاليه هو ايجاد العلاقه بين عدد الحيوانات المنويه و السعه التأكسديه و العوامل المسببه للضغط التأكسدى للسائل المنوى فى الرجال المصابين بالعقم. تم اخذ عينات السائل من مائه و خمسة عشر رجل و تم تصنيفهم على اساس عدد الحيوانات المنويه. تم قياس الاكسده الفوقيه للدهون كمثال لعوامل الضغط التأكسدى و كذلك تم قياس سوبر اكسيد دسميوتيز و الكاتاليز و السعه الكليه لمضادات الاكسده كمثال للعوامل المضاده للاكسده. ثبت ان هناك ارتباط عكسى بين عدد الحيوانات المنويه و الاكسده الفوقيه للدهون و على النقيض فهناك ارتباط طردى بين عدد الحيوانات المنويه و السوبر اكسيد دسميوتيز و الكاتاليز و السعه الكليه لمضادات الاكسده. مما سبق يتضح ان وجود كميات غير كافيه من الانزيمات المضاده للاكسده و زياده الضغط التأكسدى قد يتسبب فى تقليل جودة السائل المنوى و من ثم تقليل الخصوبه.