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Department of Reproduction and Andrology, Medical University of Lublin
Department of Biochemistry, Faculty of Veterinary Medicine
Agricultural University of Lublin

JOANNA TKACZUK-WŁACH, MARTA KANKOFER,
GRZEGORZ JAKIEL

Activity of superoxide dismutase and glutathione peroxidase in human semen in normozoospermia and spermatopathy

Sensitivity of the human spermatozoa to oxidative stress was first described by McLeod in 1943. The author demonstrated negative effects of high oxygen levels on sperm motility (7).

Later studies were focused on examining reactive oxygen species (ROS) in the human spermatozoa and on their possible role in pathogenesis of male infertility. The studies showed that infertile men had elevated levels of ROS, which suggested that production of ROS by the human spermatozoa was likely to be one of the reasons of male infertility (12). Moreover, it was revealed that both normal and pathological spermatozoa are involved in ROS production. It is also noteworthy that reversed correlation was observed between ROS levels and some seminal parameters, e.g. motility, particularly in the A movement, or volume (6, 12).

Reactive oxygen species not only damage the cytoplasmatic membrane of spermatozoa but also unfavourably affect their motility. Moreover, their destructive effects on DNA of sperms or on the process of male sex cell and female egg cell coupling were observed. There are several studies confirming that even a spermatozoon with ROS-induced genetic damage is still capable of oocyte fertilization. This last finding is particularly relevant to provide safety of increasingly common procedures of artificial fertilization during which the sex cells are exposed to substantially higher oxidative stress (2). The human spermatozoa have their own measures to protect themselves against harmful effects of reactive oxygen species, i.e. superoxide dismutase SOD and glutathione peroxidase GSH-Px. A number of studies performed so far revealed that these two enzymes were present in the human semen. This fact inspired us to undertake the study concerning the activity of both enzymes in the human sperm in fertility disorders.

MATERIAL AND METHODS

The analysis involved the semen samples collected from 35 men aged 26-39. All the patients were diagnosed and treated for infertility in the Department of Reproduction and Andrology, Medical University of Lublin in 2001 and 2002. The semen was obtained by masturbation after 3-5 days of sexual abstinence and placed in sterile containers (11). 0.5 ml of each sample were collected and analysed according to the WHO standards (11). None of the patients selected for the study showed leukocytospermia (the leukocyte level lower than $1x10_6$/ml). On the basis of their results the patients were divided into 4 groups according to the WHO criteria (11). First, the patients were classified according to the number of spermatozoa in 1 ml of semen and thus the first group with normozoospermia (≥ 20 mln of spermatozoa/ml) and the second group with oligozoospermia (less than 20 mln/ml) were formed. Next, the patients were divided according to sperm motility – the third group – with normal motility according to the WHO standards (11), i.e. higher than or 50% and the fourth group – with motility lower than 50%. After liquefaction, the semen samples were frozen at –29° C. Before the determinations, the samples were defrosted at 22° C.

Determination of superoxide dismutase activity (DOS) (10). Each portion of semen was mixed with 0.4 ml of the chloroform: ethanol mixture (3:5), supplemented with 0.1 ml of 0.9% NaCl and centrifuged for 10 min. at 3000 x g. The supernatant was used in the enzyme determinations. The incubation mixture contained: 1.8 ml of carbonate buffer (0.05 mol/l, pH 10.2) and 0.1 ml of supernatant mixed with 0.1 ml of adrenaline (18 mg/10ml 0.1 mol/l HCl) – as a reaction substrate. Absorbance was measured in the spectrophotometer at 340 nm after 10-minute incubation. The enzyme activity was expressed as % of inhibition of adrenaline autooxidation. 50% inhibition was accepted as the enzymatic unit (U). This unit was converted into the number of spermatozoa (no/0.1 ml of semen). The method is based on the measurements of adrenaline autooxidation inhibition by dismutase in the alkaline medium.

Determination of glutathione peroxidase activity (GSH-Px) (8). The whole human semen was used. The incubation mixture contained: phosphatic buffer (0.05 mol/l, pH 7.0) – 2.58 ml, NADPH (0.0084mol/l) – 0.1 ml, glutathione reductase (100 units/mg protein) – 0.01 ml, sodium azide (1.125 mol/l) – 0.01 ml and glutathione (0.02 mol/l) – 0.1 ml. The mixture was supplemented with 0.1 ml of semen and 0.1 ml of substrate, i.e. hydrogen peroxide solution (0.022 mol/l). Absorbance was measured in the spectrophotometer after adding substrate between the 2^{nd} and 4^{th} minute at 340 nm. The enzyme activity was expressed in nkat and converted into the number of spermatozoa (no/0.1 ml of semen). The method was based on the measurements of a decrease in absorbance between the 2^{nd} and 4^{th} min. of incubation which resulted from the change of NADPH into NADP.

RESULTS

The findings were statistically analysed using a STATISTICA 5.5 programme. The distribution of the studied variables was checked by the W. Shapiro-Wilk test. Due to inconsistency with normal distribution, the relations were detected using the Spearman nonparametric rank correlation. The accepted error of estimation was 5% and $p<0.05$ was statistically significant.

The results were presented graphically in correlation scatterplots. In the group with oligozoospermia a statistically significant increase in SOD was observed ($p<0.05$) accompanied by a decrease in the number of spermatozoa in 1 ml of semen ($p=0.0009$, $R=-0.654$) (Fig. 1). Similarly, the level of GSH-Px was elevated in men with oligozoospermia

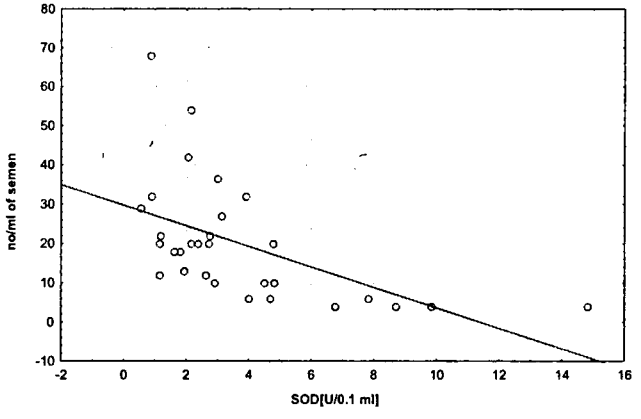

Fig. 1. Superoxide dismutase (SOD) in whole semen in relation to the number of spermatozoa

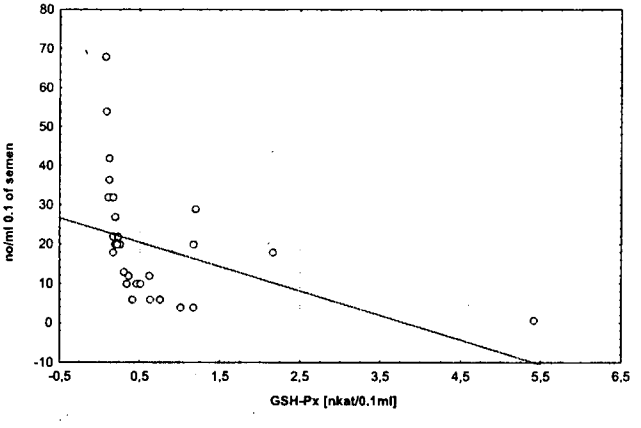

Fig. 2. Glutathione peroxidase (GSH-Px) in whole semen in relation to the number of spermatozoa

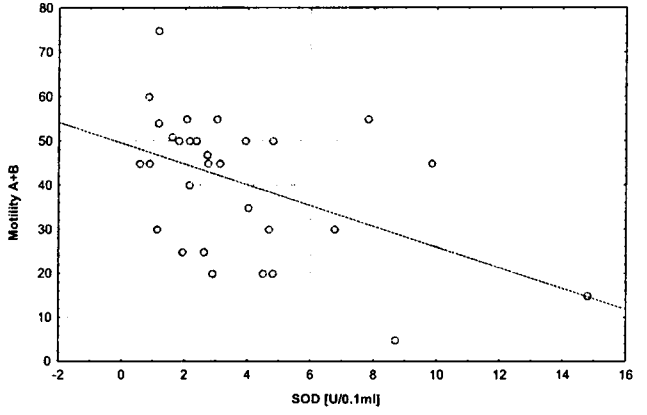

Fig. 3. Superoxide dismutase (SOD) in whole semen in relation to sperm motility

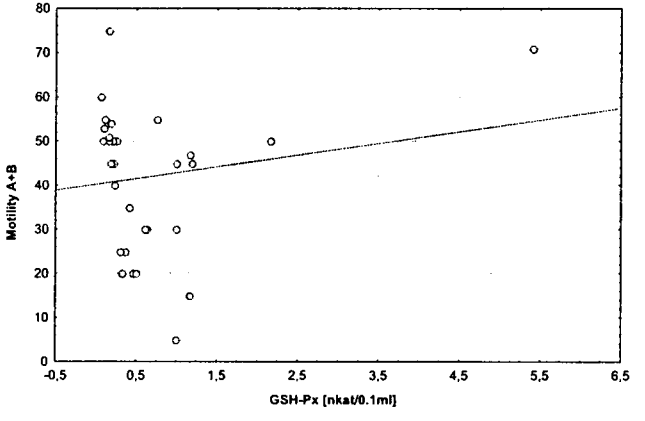

Fig. 4. Glutathione peroxidase (GSH-Px) in whole semen in relation to sperm motility

($p<0.05$) (Fig. 2). Correlation was found to be negative ($p=0.000001$, $R= -0.803$). The activities of SOD and GSH-Px were statistically significantly higher in men with low (<50%) motility than in those with high motility ($p=0.019$, $p=0.011$; $R= -0.425$, $R= -0.449$, respectively) (Figs 3, 4).

DISCUSSION

The negative impact of reactive oxygen species on the human spermatozoa and their function has been fully demonstrated (2). The first-line protectants are SOD and GHS-Px, whose activities in whole semen were examined in our study.

Alvarez et al. (4) reported that SOD was the main enzyme protectant of spermatozoa against ROS toxicity. It was showed that SOD was responsible for the removal of superoxide aninoradical O_2- which initiated lipooxidation. This reaction results in H_2O_2 formation. As the studies revealed the total amount of H_2O_2 present in the semen was formed due to the reaction catalyzed by SOD (4). HSH-Px with reductase, on the other hand, was responsible for several reactions removing H_2O_2 (9). The activities of SOD determined in various semen samples, even those from the same donor, vary while the activities of GSH-Px are usually constant (4). The activity of SOD in the spermatozoa collected from different Percol concentrations is three times higher in the population of morphologically abnormal spermatozoa. Moreover, positive correlation was found between the SOD level and induction of oxidative stress while negative correlation was observed between the enzyme and sperm motility as well as its ability to capacitate (1). Our findings led to similar conclusions – substantially increased activity of superoxide dismutase was found in group IV – with low motility (less than 50% of A+B motion).

Alkan et al. (3) demonstrated lower antioxidant activities (SOD, GSH-Px) in the seminal plasma of men with idiopathic infertility. According to the authors, this decrease may be responsible for increased production of ROS and may weaken "the quality" of semen, which is likely to result in infertility.

Similarly to Alkan, Durak et al. (5) studied the antioxidant level in the seminal plasma of 4 groups of men: fertile, with azoospermia, oligo- and normozoospermia. Increased antioxidative potential (SOD+GSH-Px) was detected in the seminal plasma of patients with azoospermia. The authors suggested that this increase was the mechanism compensating the lack of spermatozoa and protecting against oxidative stress. No significant differences in enzyme levels were found comparing the fertile, oligo- and normozoospermia groups. So, antioxidative potential in the seminal plasma of infertile men is not decreased. Furthermore, in the azoospermia group the enzyme activity is found to be the highest one. The results of the latest study oppose the findings reported by Alkan (3). Additionally, they question the usefulness and effectiveness of antioxidant therapy in patients with infertility unless damage to their antioxidative system was detected (5).

Our studies determine the antioxidative enzyme activities in whole human semen in the cases of normo- and oligozoospermia. The results show higher semen activity of superoxide dismutase and glutathione peroxidase in men with oligozoospermia. This seems to confirm the theory presented by Durak (5).

CONCLUSIONS

1. The activity of SOD and GSH-Px is related to the semen evaluation carried out according to the WHO standards.

2. Since the available literature data are full of discrepancies, further detailed studies on these issues should be performed.

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SUMMARY

The aim of the study was to assess the levels of antioxidative enzymes – superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the whole semen of men treated for infertility. The first group consisted of the patients with normozoospermia; the second – included the men with oligozoospermia. According to sperm motility, the patients were divided into the third group – motility higher than 50% and the fourth group – motility lower than 50%. The study showed that the levels of both enzymes were higher in men with oligozoospermia than those with normozoospermia. It was also found that the activities of SOD and GSH-Px were higher in men with low motility(< 50%) compared to high motility patients. The results confirm that the activity of superoxide dismutase and glutathione peroxidase is related to the parameters of human semen assessed according to the WHO standards.

Aktywność dysmutazy ponadtlenkowej SOD i peroksydazy glutationowej GSH-Px w pełnym nasieniu męskim w warunkach normozoospermii i patologii nasienia

Celem pracy była ocena poziomu enzymów antyoksydacyjnych - dysmutazy ponadtlenkowej (SOD) oraz peroksydazy glutationowej (GSH-Px) – w całym nasieniu u mężczyzn leczonych z powodu niepłodności. Pierwszą grupę stanowili mężczyźni z normozoospermią, drugą – mężczyźni z oligozoospermią. Następnie podzielono pacjentów według kryterium ruchliwości. Powstała grupa trzecia – ruchliwość wyższa niż 50 % oraz grupa czwarta – ruchliwość mniejsza niż 50 %. Przeprowadzone badania wykazały, że poziomy obu enzymów, SOD oraz GSH-Px, były wyższe u mężczyzn z oligozoospermią. Również po podzieleniu mężczyzn według kryterium ruchliwości okazało się, że aktywność SOD oraz GSH-Px jest wyższa w nasieniu mężczyzn z niską ruchliwością plemników (< 50%). Uzyskane wyniki potwierdzają, że aktywność dysmutazy ponadtlenkowej oraz peroksydazy glutationowej pozostaje w zależności z parametrami nasienia męskiego, ocenianymi standardowo według norm WHO.