Research Paper

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Role of Superoxide Dismutase and Glutathione Peroxidase in Infertility

Medical Science

KEYWORDS : Glutathione Peroxidase; Primary infertility; Reactive Oxygen Species; Superoxide Dismutase.

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ABSTRACT

A prospective study was planned to assess the role of Superoxide Dismutase(SOD) and Glutathione Peroxidase (GPx) in unexplained infertility of young Indian population. Blood SOD and GPx activity was assessed in 27 couples (27 males and 27 females) with unexplained infertility (mean age 29 ± 5.48years, group I) before antioxidant therapy and control (group II) having age related 14 couples (14 males and 14 females, 34 ± 8.26 years) using RANSOD & RANSEL kits(m/s Randox Pvt Ltd.). No significant difference was found in SOD and GPx activity in group I and group II (p< 0.191 and p< 0.279 respectively). After detailed evaluation of couples of group I, only 8 patients were found to have lower values of either SOD or GPx. Others had normal antioxidant activities. Studies in larger group are warranted.

INTRODUCTION:

Oxidative stress can cause potential harm to organs. When oxidative stress is over generated or the enzymatic and nonenzymatic antioxidant defense system is inefficient, it can stimulate some chain reactions causing cytotoxic effect [1]. SOD and GPx are the two major parts of oxidase defense system which quench the ROS and save peroxidation of the unsaturated fatty acids in cell membranes. Defective sperm function is the most common cause of infertility and until recently it was difficult to evaluate and treat. Mammalian spermatozoa membranes are rich in high unsaturated fatty acids and are sensitive to oxygen induced damage mediated by lipid peroxidation. The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and by contaminating leukocytes has been identified as one of the causes for male infertility [2]. Normally seminal plasma contains antioxidants, which quench ROS and protect spermatozoa from likely damage. In females, oxidative stress can cause low success rates of in vitro fertilization and embryo transfer[3].Oviductal and uterine environments where early embryos develop naturally, are characterized by an oxygen tension < 5 % [4]. Human embryos cultured in vitro are often exposed to 20% atmospheric oxygen; this may disturb the balance between prooxidants and antioxidants leading to oxygen stress [5]. In normal human subjects the oxidative stress is neutralized by antioxidants like superoxide dismutase and glutathione peroxidase which detoxify the superoxide anion and H_2O_2 produced by xanthine oxidase system and NADPH system respectively [6].Tarin and Trounson 1993 have suggested that culturing embryos in media with enhanced antioxidative capacity increased the blastulation rate in mice. Any reduction in antioxidant levels may lead to dysfunctional sperm or embryos [7]. Rodriguez et.al. 2001 have shown that genital infection is another cause of infertility worldwide, affecting not only fallopian tubes but all anatomic urogenital sites in both males and females [8]. In contrast to this, Polak et al 2000 have demonstrated that in female patients of unidentified infertility or idiopathic infertility the cellular superoxide dismutase in plasma and peritoneal fluid does not play a significant role [9]. The present study was planned to investigate whether there is any role of superoxide dismutase and glutathione peroxides in unexplained infertility.

MATERIALS AND METHODS

The study included group I with 27 couples of unexplained infertility (n= 54, 27 males and 27 females) with mean age of 29 ± 5.48 years. The informed consent was obtained from these individuals. 3 ml heparinised blood sample was collected and processed immediately for SOD and GPx. All the patients were advised to get routine biochemical, hormonal and microbiological examination done.

To obtain data from control (group II), 28 age related healthy fertile individuals (14 males and 14 females with mean age 34± 8.26 year) were selected for the study. The control population was randomly selected normal individuals.

METHODS

SOD and GPx were analysed photometrically using RANSOD & RANSEL commercial kits from M/s Randox Laboratories UK).

GPx

This method is based on Paglia and Valentine [18]. Glutathione Peroxidase catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured. GPx concentration was calculated and expressed as U/mL.

S O D

This method is based on method described in Ransod kit insert of Cat no. SD125 [19].The test employed xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyle tetrazolium chloride (INT) to form a red formazan dye. SOD present in the sample competes with the INT for superoxide radicals,inhibiting the production of formazan dye. SOD activity was defined as the amount of enzyme measured in terms of % inhibition of formation of dye. After obtaining % inhibition, units of SOD activity were calculated from standard curve.

STATISTICAL ANALYSIS

All values were reported as mean ± SD. Statistical significance between groups was computed using the Mann Whitney test and student's T test. P values lesser than 0.05 were considered significant

RESULTS

The mean SOD activity in group I was 137.2 ± 91 U/mL and in group II, it was 117 ± 32 U/mL (lab range 85-150 U/mL). The difference was not significant (p< 0.191).

The mean Glutathione Peroxidase activity in patients of group I was 6888 ± 4248 U/Land that of group II was 7428 ± 2319 U/l (biological reference range 5109-9747 U/L) (Table 1).The p value was <0.279 (Table 1).

Out of 27 couples, only 8 unrelated patients had low antioxidants , 2 had low SOD (25 %,), 4 patients (50 %) had low GPx activity and another 2 had (25 %) both low GPx and SOD activity when compared with control (p<0.00)(Table II).

DISCUSSION

In present patient series, the mean superoxide dismutase and glutathione peroxidase activity was comparable in group I and II. However, from group I, only 8 unrelated patients showed low antioxidants and clinical signs of infertility. It was difficult to understand the role of antioxidants in infertility from this patient series. Findings of our study corroborates with the findings of Polak et al 2000 , who has demonstrated no significant role of antioxidants in female patients of unidentified infertility or idiopathic infertility [9].

Positive correlation between antioxidants and infertility in various sample types like serum, peritoneal fluid etc has been reported. Intracellular glutathione levels of spermatozoa are known to be decreased in certain populations of infertile men. Compared with a control group, the infertile men in all groups had significantly higher levels of ROS and lower levels of total antioxidants. There is strong clinical evidence to show that men diagnosed with infertility have high levels of oxidative stress that may impair the quality of their sperm [10].

Infertility in males could be attributed to either impaired sperm function or damaged sperm morphology. Impaired sperm function is an obvious and general cause of unidentified infertility in males [11]. Human spermatozoa are rich in polyunsaturated fatty acids and therefore are susceptible to free radicals attack mediated by membrane lipid peroxidation. These free radicals damage the sperm's structure and DNA material. The free radicals are quenched by antioxidants present in seminal plasma. If antioxidant levels are reduced in seminal plasma it may lead to infertility in males [2]. In 1977 Jones and Mann have reported the free radical induced lipid peroxidation as one of the causes for an immediate arrest of motility and the irreversible loss of respiratory and fructolytic functions of spermatozoa [12]. Recently, it has been reported that reactive oxygen species affect sperm morphology and functions [13].Very few reports are available in literature on correlation of seminal plasma or peritoneal fluid SOD and GPx with male and female infertility respectively [13,14,15]. There is scarcity of literature showing correlation of blood superoxide dismutase and glutathione peroxidase with male and female unexplained infertility.

The lower total antioxidant capacity of seminal plasma in infertile men compared to fertile men has been reported [14] .Polak et al, 2001 has reported low total antioxidant status in peritoneal fluid of women with unidentified infertility. The peritoneal fluid diffuses into the tubal environment and its components may affect sperm transport and survival, because spermatozoa are very sensitive to oxidative damage. The reactive oxygen species in the fluid may be detrimental to sperm function [15]. In another study by same investigators low GPx levels were found in peritoneal fluid of women with unidentified infertility [16]. Mohanty et al 2001 showed supplementation of Lycopene (an antioxidant preparation) has a definite role in management of idiopathic oligoasthenospermia. The improvement in seminalogram with supplementation of Lycopene indicates that low antioxidant concentration leading to abnormal sperm count and motility causes infertility. [17]

CONCLUSIONS

We could not establish any correlation between SOD, GPx and infertility. The varied findings of different investigators on the role of SOD and GPx as an antioxidant in reproduction are still a subject of debate. Hence, a study in a larger number of patients is warranted to ascertain their potential advantages in infertility.

Table 1: Mean blood Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) concentration in Group I and Group II.

Parameters	**Group I (Mean ± S.D.)**	**Group II (Mean ± S.D.)**	p value
SOD(U/mL)	137.2 ± 91	117±32	p<0.191
GPx (U/L)	6888±4248	7428±2319	p<0.279

Table 2: Correlation of GPx and SOD levels with clinical findings of some of the infertile individuals

S.NO.	CLINICAL FINDINGS	SOD(U/ml)	GPx (U/l)
1. Male	Low sperms count (15,400,000/ml)	**82**	7686
2. Female	Tubal blockage	**71↓**	**5643↓**
3. Male	Low sperm motility(<10%), count normal(50,800,000/ml)	**75↓**	**5644↓**
4. Female	Tubal blockage	**80↓**	11289
5.female	Repeated abortions	220	**4618↓**
6. Male	20,000,000/ml(low sperm count),G.U.T infection	300	**3900↓**
7. Male	85 million / ml	660	**4516↓**
8. Female	Tubal blockage	660	**1026↓**

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