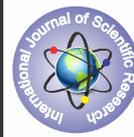


Effect of Superoxide dismutase(SOD) and Catalase in unexplained infertility.



Biological Science

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ABSTRACT

Sperm produce small amounts of free oxygen radicals (ROS) that are required for their normal function. It is important that these free radicals are removed as soon as they have performed their function so that their levels remain low if the balance of ROS production and antioxidant activity is disturbed, high levels of free oxygen radicals will build up, causing oxidative stress. This leads to sperm damage and consequently affects fertility. ROS can be simply measured in a fresh semen sample. The present study evaluates the semen analyses of unexplained infertility cases in two age group and is compared with normal fertile cases. ROS is measured in semen sample which shows decline in the activity of catalase enzymes and Superoxide dismutase(SOD)

Introduction

Infertility is an inability or reduced ability to produce a pregnancy within a reasonable period of trying, usually 12 months. Infertility is commonly defined as the failure of conception after at least 12 months of unprotected intercourse (Comhaire et al., 1993)[2]. Infertility is either primary, when no pregnancy has ever occurred, or secondary, where there has been a pregnancy, regardless of the outcome. About 67.71% and 29.33% of infertile couples have primary and secondary infertility, respectively (Mueller and Daling, 1989; Thonneu et al., 1991)[6,10]. Sterility is the total inability to produce a pregnancy. Relative infertility means fertility that is low in relation to what is expected but it is not absolute in as much as, the causes are often manageable. When several tests show no anomaly whatsoever, the condition is termed as unexplained infertility. The present study has been focused specifically on cases of unexplained infertility.

Material and Method

Semen samples were analyzed from selected cases referred with complaints of unexplained infertility and failure of sperm fertilizing ability, to evaluate for various structural and functional parameters of human spermatozoa, related to impaired fertilizing potential. The cases were divided into two age group ranges. Group II with age range from 20-30 years and Group III with age range 31-40 years. Normal volunteers of proven fertility, with no clinical history of infections or related disorders, were selected as controls for Group I, in an age matched manner viz., Group I of age range 20-30 years and Group IA of age range 31-40 years, as shown below:

- Group I Normal men of proven fertility. Age range 20-30 years (n=40).
- Group IA Normal men of proven fertility, Age range 31-40 years (n=40)
- Group IIA Men with history of unexplained infertility having sperm counts in the normozoospermic range, Age range 20-30 years (n=62).
- Group IIB Men with history of unexplained infertility, having sperm counts in the oligozoospermic range <40 million (n=46) Age range 20-30 years.
- Group IIIA Men with history of unexplained infertility having sperm counts in the Normozoospermic range, Age range 31-40 years (n=53).
- Group IIIB Men with history of unexplained infertility, having sperm counts in the oligozoospermic range <40 million (n=59) Age range 31-40 years

Semen samples were collected into sterile containers. After

liquefaction, samples were centrifuged to separate seminal plasma which was analyzed for volume and sperm count, sperm motility and morphology microscopically according to WHO guidelines [11]. Then the semen samples were analyzed for functional parameter i.e SOD and Catalase activities. The activity of super oxide dismutase (SOD) was assayed by modified spectrophotometric method of kakkar et al (1984) [4] and catalase activity was determined in human seminal plasma of normal and infertile groups by the modified method of Luck (1963) [5].

Results

The Superoxide dismutase and Catalase activities in semen samples of cases of unexplained infertility were found lower than those of normal subjects.

1. Superoxide dismutase (SOD) (E.C.1.15.1.1): Evaluation of SOD enzyme in samples of (Groups II A, II B and III B) cases of unexplained infertility (Table 1 and 2) as compared to the age-matched controls. Group III A showed a less significant decrease ($p < 0.01$) as compared to normal fertile groups IA (Table 20).
2. Catalase (E.C.1.15.1.1): A significant decrease ($p < 0.01$) was observed in the catalase activity in samples from individuals of the lower age bracket with normal sperm count (Group II A), whereas a highly significant decline ($p < 0.001$) was obtained in samples of Group II B, III A and III B, when compared to the age-matched normal control groups. (Tables 1 and 2)

TABLE I: SHOWING ACTIVITIES OF SUPER OXIDE DISMUTASE (SOD) AND CATALASE ACTIVITY IN SEMEN OF GROUP II MALES {CAUSES OF UNEXPLAINED INFERTILITY OF AGE RANGE 20-30 YEARS}

Group	Parameter	
	SOD (μ /mgpr)	Catalase (μ moles/min/mgpr)
Group I Normal n=40	8.25 \pm 1.4	18.9 \pm 2.3
Group II-A (Normospermia) (n=62)	3.25 \pm 0.2**	12.48 \pm 0.39*
Group II -B (moderate oligozoospermia) n=46	2.18 \pm 0.32**	7-6 \pm 0.95**

Values are Mean \pm S.E. * $p < 0.01$ ** $p < 0.001$

TABLE II: SHOWING ACTIVITIES OF SUPER OXIDE DISMUTASE (SOD) AND CATALASE ACTIVITY IN SEMEN OF GROUP III MALES (CAUSES OF UNEXPLAINED INFERTILITY OF AGE RANGE 31-40 YEARS)

Group	Parameter	
	SOD (μ /mgpr)	Catalase(μ moles /min/mgpr)
Group I-A Normal n=40	8.9 \pm 0.9	1.29 \pm 1.8
Group III-A (Normospermia) (n=53)	5.6 \pm 0.1*	4.8 \pm 0.5**
Group III -B (moderate oligozoospermia) n=59	3.3 \pm 0.7**	3.2 \pm 0.9**

Values are Mean \pm S.E. *p<0.01 **p<0.00

DISCUSSION

The spermatozoa unlike other cells are unique in structure and functions and susceptible damage by lipid peroxidation Alvarez et al 1987[1] it has been reported that increase in reactive oxygen species (ROS) can cause the destruction of all cellular structure, including membrane lipids, hence in the present study LPO has been used as a marker of oxidative damage to the sperm membrane lipids. ROS may cause defect in sperm function through lipid peroxidation (Sharma and Agarwal 1996)[9]. Therefore, lipid peroxidation and levels of anti oxidants have been analysed in present study since they are implicated in disturbances of sperm function, ROS are produced by variety of semen components including immotile and morphologically abnormal spermatozoa, leucocytes, and morphologically normal but functionally abnormal spermatozoa (Plante et al., 1994)[7]. Antioxidants in general are compounds which dispose, scavenge and suppress the formation of ROS or oppose the actions of the antioxidant studied, the enzymes, superoxide dismutase and catalase play significant role.

The most abundant free radical are superoxide and anion (O_2^-) and its derivatives. The superoxide is highly reactive damaging free radical which appears to act via peroxidation of membrane lipids. The superoxide is inactivated by superoxide dismutase (SOD), the only enzyme known is effective only when it followed by increase in catalase or glutathione peroxidase. SOD is related to scavenging of reactive oxygen species (ROS). Sanocka et al 1996[8] demonstrated the decrease in SOD activity in infertile male when compared to normal. This data is comparable to our present data, which also showed low level of activity in semen sample in groups of unexplained fertility. Alvarez et al. (1987) showed that SOD level in spermatozoa is positively correlated in sperm mobility. Hsieh et al. (2002)[3] studied the effect of SOD activity in spermatozoa and seminal plasma on sperm quality and concluded that SOD activity of sperm and seminal plasma were not significantly correlated with seminal quality. However, our data revealed a significant decrease in SOD activity and increase in LPO level. The decrease SOD activity therefore leads to increased effect of free radical damage on spermatozoa, thus having a detrimental effect, and lowering sperm fertilizing capacity. Our finding therefore corroborated with those of Alvarez et al. (1987) who suggested that SOD provides major component of antioxidant defense of human spermatozoa against the damaging effect of free radical. Powerful antioxidant Enzyme catalase enhance the activity of SOD. In present investigation the catalase activity was lower in sample of males in all groups of unexplained infertility as compared to the normal control men of proven fertility. Along with SOD, this decline in activity of Catalase thus indicated an inadequate protective enzymatic machinery to the action of free radicals. This leads to increased effect of free radicals and ROS attack on sperm cell, which subsequently shows decline in structure and function thereby accounts for poor sperm fertilizing ability.

SUMMARY AND CONCLUSION

The present study has provided evidence that the enzyme system in human seminal plasma protects spermatozoa from peroxidative damage. The contribution of these antioxidant enzymes Superoxide dismutase and Catalase is imperative in order to understand the alteration in sperm function. The Superoxide dismutase and Catalase activities in semen samples of cases of unexplained infertility were found lower than those of normal subjects. This decline in activity in SOD and Catalase indicated an inadequate

protective enzymatic system against the action of the free radical.

Our investigation shows that catalase activity was lower in samples of males in all age groups of unexplained fertility as compared to the normal men with proven fertility. This leads to effect of free radical and ROS attack on the sperm cell which subsequently shows decline in structure and function and thereby accounts for poor sperm fertilizing ability. This led to increased free radical attack on the sperm cell, which subsequently showed a decline in sperm function and alteration in sperm metabolism of sperm. Hence we conclude 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.

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