ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 09 | September - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EFFECT OF OXIDATIVE STRESS ON INFERTILE MALE

Anatomy

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ABSTRACT

Reactive oxygen species (ROS) levels in semen are believed to play physiological as well as pathological roles in male fertility (Venkatesh et al, 2009). Through the present study we try to find out the clinical significance of ROS levels in infertile men of Indian origin. The study was conducted on 25 idiopathic infertile men who were collectively termed as infertile group (IG) and 25 fertile men who were termed as control group (CG). Both the groups were analysed for semen parameters and ROS using W.H.O. criteria and chemiluminescence assay. The experimental results indicated significant difference in two groups in terms of semen parameters and ROS level. Compared to control group, infertile group showed significant difference in the sperm count (CG/IG: 66.2±18.1/11.7±3.9), percent sperm motility (CG/IG : 68±19.5/16±4.4) and percent normal morphology (CG/IG : 77.5±15.1 /8.1±1.5). Infertile group showed significantly ($p<0.001$) higher ROS levels (183.60±50.93cpm)/ 106 spermatozoa compared to fertile controls (5.84±0.91cpm)/ 106 spermatozoa. Catalase activity in the seminal plasma of the infertile group were also found to be significantly lower ($p<0.001$) as compared to the control group. No significant correlation was found between ROS levels and semen parameters. Results indicates that clevated ROS levels in the idiopathic Indian infertile men can be one of the underlying reasons for impaired fertility hence the present methodology can prove useful in better understanding of the actiology and improved selection of antioxidant regimen in the treatment of male infertility.

KEYWORDS

INTRODUCTION

Oxidative Stress (OS) is a condition established due to an imbalance between antioxidant levels and reactive oxygen species(ROS) (Ahmed, 2005). OS has been reported to be a causative factor in variety of diseases like diabetes, cancer, aging, atherosclerosis, rheumatoid arthritis, etc (http://www.dojindo.com/newsletter/review_vol2.html), but recently their role in male infertility has been emphasized (Garg and Garg, 2011). Chromosomal abnormalities, (Dada et al, 2006) which include Y chromosome deletion (Dada et al, 2002) and high testicular temperature (Dada et al, 2002) are important aetiological factors in idiopathic cases. Apart from spermatozoa and leukocytes, which are the sources of ROS in semen, external factors such as smoking environment exposure to high temperature, pollution, toxic chemicals, environment endocrine disruptors and genitourinary infections (Tremellen, 2008) have also been reported to be associated with oxidative stress. These factors impair the physiological function of spermatozoa (Tunc, 2010). ROS may cause damage to different parts of the spermatozoa, including nuclear and mitochondrial DNA (Agarwal, 2008). However, unlike somatic cells mature spermatozoa lack cytoplasm. Since cytoplasm is the major source of anti-oxidants, lack of cytoplasm in the mature spermatozoa causes deficiency in both antioxidant defense and endogenous repair mechanism (Venkatesh el al, 2009). Under natural conditions the deficiency of antioxidant system is compensated by the enzymatic and non-enzymatic antioxidants in seminal fluid of different origin (Agarwal et al, 2004). The decreased antioxidant enzymes or increased ROS level disrupts the physiological function of spermatozoa and impairs sperm motility and the process of fertilization. Under physiological conditions, spermatozoa produce small amounts of ROS, which are needed for capacitation and acrosomal reaction. Superoxide anion also appears to play a role in this process. Though, ROS at low level facilitates capacitation, acrosome reaction and hyper activation (Rivlin et al. 2004: Griveau and Lannou. 1997 : Agarwal et al, 2003).

Normally, cells are able to defend themselves against ROS damage through the use of enzymes such as superoxide dismutasos, catalases, lactoperoxidases, glutathione peroxidases and peroxiredoxins. Small molecule antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E), uric acid, and glutathione also play important roles as cellular antioxidants. Similarly, polyphenol antioxidants assist in preventing damage by ROS by scavenging free radicals. In living cells the main sources of ROS are mitochondrial respiratory chain and lymphocytes and external factors such as smoking, air pollution, exposure to xenobiotics, and electromagnetic radiation (Ahmed, 2005 ; Venkatesh et al 2009).

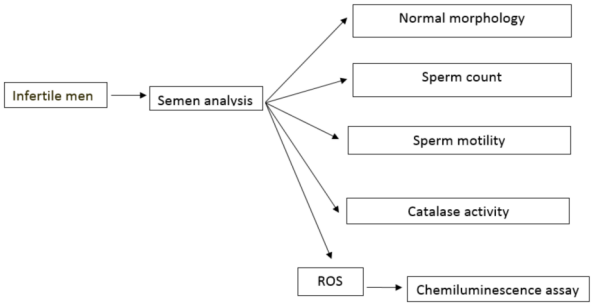
The unique structure of sperm is well defined with compact packing of the nuclear DNA that passes genetic information to the offspring. However, the plasma membrane of spermatozoa possesses high content of polyunsaturated fatty acids (PUFA), which are highly susceptible to ROS (Duru et al, 2000). The excess ROS production can cause damage to the normal spermatozoa by inducing lipid peroxidation resulting in alteration of sperm function and fertilizing capacity (Moustafa et al. 2004). Studies also reported that excess ROS are known to cause sperm nuclear DNA damage (Moustafa, et al, 2004) and a high degree of sperm mitochondrial mutation (Yakes and Houten, 1997). As sperm motility is most important indicator of fertility potential mt mutation have profound adverse affect on sperm function. However, it is very important to distinguish and characterize the cause of impaired sperm function whether it is in nuclear genome (cylogenetic abnormality or Y chromosome deletion) or is it a mtDNA defect (Kumar et al, 2007, Dada et al, 2008) due to increased ROS production. So that ROS production may be the good diagnostic and prognostic marker in men opting for assisted reproduction techniquc (ART). It is very important to understand the actiology of ROS production in infertile men and thus this study has been conducted with aim to asses OS and relate increased ROS levels in infertile men with oligozoospermia and severely impaired sperm motility.

MATERIAL AND METHODS

Semen analysis and ROS level tests were conducted on idiopathic infertile group and control group (each comprising of 25 men) using chemiluminescence assay. All the men were recruited from the Departments of Urology and Obstetrics and Gynecology after obtaining their informed consent and Institute Ethics Committee approval. Semen samples were obtained by masturbation in a sterile plastic container after 3- 4 days of sexual abstinence. After liquefaction at 37°C, semen analysis was performed manually as per WHO (1999) guidelines. For morphology, 10 il of semen smear was prepared in a clean slide and fixed with 90 percent.

Atleast 200 sperms per sample were evaluated for morphological defects .400 il of raw semen was taken in duplicate for ROS estimation. To 400 il of liquefied neat semen, 10 il of luminol (5-amino-2.3,-dihydro1,4-phthalazinedione: Sigma, USA), prepared as 5 mM stock in dimethyl sulphoxide (DMSO), was added. 10 il of 5 mM luminol in DMSO served as blank. 25 il H_2O_2 with 10 il luminol was used as a positive control. All the samples were measured in duplicate and the average of the readings was taken. Levels of ROS were assessed by measuring the luminol-dependant chemiluminescence with the single

detector luminometer (Sirius, Berthold Detection Systems GmbH, Pforzheim, Germany) in the integrated mode for 15 min. The values were expressed as x 10^4 relative light unit per minute (RLU/ min) per 20 x 10^6 spermatozoa. Figure 1 depicts the study design adopted in the present study



RESULTS

Compared to control group, infertile group showed significant difference (Table 1) in the sperm count (CG / IG: 66.2±18.1 / 11.7±3.9), percent sperm motility (CG / IG: 68±19.5 /16±4.4) and percent normal morphology (CG/IG : 77.5±15.1 / 8.1±1.5). Infertile group showed significantly ($p<0.001$) higher ROS levels (183.60±50.93cpm) /10^5 spermatozoa compared to fertile controls (5.84±0.91cpm) / 10^6 spermatozoa. Catalase activity in the seminal plasma of the infertile group were found to be significantly lower ($p<0.001$) compared to the control group.

Table 1: Sperm Characteristics In Infertile And Control Group

Characteristics	Infertile group	Control Group
Sperm count	11.743.9	66.2+18.1
Percent sperm motility	2 6+6.5	68+19.5
Percent normal sperm morphology	8.1+1.5	77.5+15.1

DISCUSSION

OS is a well established condition in variety of diseases including male infertility. The reason behind the sperm dysfunction in idiopathic infertility is still under debate. When semen analysis fails to detect the underlying pathology of sperm dysfunction, molecular analysis like cytogenetics, Y chromosome screening has gained much importance as they are considered important tools for couples opting for ART (Dada et al, 2006; Dada et al, 2002 ; Dada et al, 2003 ; Kumar et al, 2007; Dada et al, 2008 ; Dada et al, 2007). Generally antioxidants are present in every cell to scavenge the free radicals generated in the environment and thus prevent the damage caused to the cell. But, in spermatozoa, due to shedding of cytoplasm during spermiogenesis, the absences of enzymatic antioxidants are usually compensated by the seminal constituents of different origin (Yeung et al, 1998). In our study the antioxidants enzymes (Catalase anu Gpx) were found to be significantly lower and ROS level were significantly higher in infertile group as compared to controls. But there was no significant difference in the Superoxide dismutases (SOD) levels between two groups. This may be due to over expression of SOD as a result of OS to compensate the decreased level. A similar result has been reported in other studies (Zini et al, 1993). Many studies have also reported the elevated level of malondialdhyde (MDA) and ROS levels in infertile patients (Tavilani et al, 2005; Aydemir, 2008). Decreased antioxidant enzyme level and total antioxidant capacity (Mahfouz et al. 2008; Khosrowbeygi and Zarghami, 2007; Mahfouz, 2008) in the seminal plasma of infertile has also been stated in earlier studies. Decreased sperm function may be due to low antioxidant level or increase lipid peroxidation due to OS (Koca et al. 2003).

A very high population of sperm with decreased motility in our cases can be explained due to additive effect of membrane peroxidation and mt mutation induced decreased ATP production. However, it has also been studied that the activity of oxidative phosphorylation (OXPHOS) depends on the genetic back ground of mitochondria that affects the sperm motility the genetic back ground of mitochondria that affects the sperm motility (Spiropoulos et al, 2002). As mtDNA are source of production of ATP through OXPHOS, they also are site of ROS production and first site of ROS induced damage. Moreover, increased ROS may cause damage or alter DNA through oxidation or covalent binding (Ruiz et al, 2000). OS may also damage or alters the DNA sequence of the mitochondrial genome thus decreasing the Production of ATP and further increasing the leakage of free radicals through electron transport chain. Since increased mtDNA copy per cell has been reported during spermatogenesis (Diez et al, 2003), defect in the mitochondrial genome may increase the free radical production in sperms as compared to other somatic cells. ROS may play another pathological role in ultrastructural changes of spermatozoa leading to additive effect on impaired fertility. Ejaculates with high ROS can lead to high nuclcar DNA fragmentation, which is a promutagenic change that could generate mutation in the offspring (Aitken, 2001). Thus the screening of ROS and enzymes in infertile cascs can help to understand the aetiology of motility disorders in infertile men and better selection of antioxidant regimen in the treatment of male infertility. Still extensive study is required to ascertain the role of antioxidants in ROS elevated in male infertility through randomized and controlled clinical study.

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