

# EVIDENCE OF INCREASED OXIDATIVE STRESS AND DNA DAMAGES IN OLIGOSPERMIA

Arun William, Viji Krishnan, Abdul Gafoor C, Apoorva M, Heera Banu C and Dinesh Roy D

**Abstract**— Oligospermia is defined as a sperm density (count) less than 20 million/ml. Infertility affects approximately 15% of couples worldwide, among them 20–25% of reproductive problems being contributed to male factor. Male infertility is a relatively common condition affecting approximately 1 in 20 of the male population. Defective sperm function is held to be the largest, single and defined cause of human infertility. The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa has been defined as one of the important etiologies for male infertility. Generation and persistence of ROS in seminal fluid and sperm increase the rate of lipid peroxidation of sperm membrane which is manifested by a high MDA level. The present study was undertaken to evaluate the role of oxidative stress by measuring the level of oxidative stress marker, Malondialdehyde (MDA), in the sera of males suffering with oligospermia. The extent of somatic DNA damage in these subjects was quantified by using Cytokinesis Block Micronuclei Assay. These investigations were carried out in 56 subjects suffering with oligospermia and 15 healthy fertile men as control subjects. The MDA value and the micronuclei frequency was significantly elevated in study subjects as compared with that of control subjects. The finding of increased oxidative stress marker level may indicate that oxidative stress may be involved in the pathogenesis of sperm DNA damage leading to oligospermic condition as well as infertility in male subjects. These individuals can be better informed about the extent of somatic DNA damages, oxidative stress and genetic risks. This may help in preventing the sufferings of infertile subjects with oligospermia.

**Index Terms**— DNA damages, Double strand breaks (DSB), Infertility, Malondialdehyde (MDA), Oligospermia, Oxidative stress, Reactive oxygen species (ROS), Sperm DNA integrity

## 1 INTRODUCTION

Infertility is defined as the failure to achieve a pregnancy within one year of regular (at least three times per month) unprotected intercourse [1]. It affects approximately 15% of couples worldwide, among them 20–25% of reproductive problems being contributed to male factor [2]. Male infertility is a relatively common condition affecting approximately 1 in 20 of the male population. In a vast majority of infertile subjects sufficient numbers of spermatozoa are generated to initiate a pregnancy; however, the functionality of the spermatozoa has been compromised. As a result, defective sperm function is held to be the largest, single and defined cause of human infertility [3].

Oligospermia is defined as a sperm density (count) less than 20 million/ml [4] which may leads to male infertility [5]. 'Male factor' infertility is seen as an alteration in sperm concentration and/or motility and/or morphology [6].

Semen analysis remains the cornerstone in the evaluation of male infertility or oligospermia condition [5]. The primary causes of defective sperm function are undoubtedly multifactorial, involving a range of primary genetic, lifestyle and environmental factors, acting alone or, more frequently, in combination. At the level of the gamete, it is the oxidative stress that impairs the functional and structural integrity of these highly differentiated cells like sperm [7].

The oxidative stress not only disrupts the integrity of sperm DNA but also limits the fertilizing potential of these cells as a result of collateral damage to proteins and lipids in the sperm plasma membrane [7]. Oxidative stress is caused by an imbalance between pro-oxidants and anti-oxidants. This ratio can be altered by an increased level of reactive oxygen species or a decreased antioxidant defence mechanism [8]. Recombination is triggered by the generation of a DNA double strand break (DSB) within an amplicon. The occurrence of such lesions are particularly frequent in the male germ line, owing to the fact that spermatogenesis requires multiple cell divisions in an oxidative environment with depleted DNA repair enzymes [9]. Studies have also shown that elevated rate of DNA nicks and double strand breaks in sperm of infertile men could lead to infertility and 50% of miscarriages; this means that this individuals have a background of genetic instability that can be caused by their inability to repair DNA damage and are susceptible to mutagenic and clastogenic agents [10]. Sperm DNA damage is strongly associated with sperm function and infertility [11].

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The fertilizing potential of sperm depends not only on the functional competence of spermatozoa but also on sperm DNA integrity [12]. Classical semen analysis, which include sperm concentration, motility and morphology gives an approximate evaluation of the functional competence of spermatozoa, but does not always reflect the quality of sperm DNA. Men with normal sperm counts may still be infertile; the cause could be related to abnormal sperm DNA [13]. Sperm DNA integrity has an important role not only for fertilization but also for normal embryo and foetal development [14]. Sperm with compromised DNA integrity, regardless of the degree of DNA damage, appear to have the capacity to fertilize oocytes at the same rate as normal sperm [13]. However, the embryos produced by fertilization of an oocyte with DNA damaged sperm cannot develop normally [15].

The genetic integrity of the male gamete is essential for a successful and healthy pregnancy. Data in the literature suggest that the frequency of sperm cells with massive DNA fragmentation is a marker of sperm quality and a potential predictor of fertility [16]. Sperm DNA fragmentation (SDF) could be a result of unrepaired DNA breakage produced during the process of chromatin remodeling or could be a consequence of an apoptotic like DNA degradation process [17]. Oxidative stress may also induce SDF, when reactive oxygen species (ROS) generation overcomes the antioxidant scavenging activities of ROS [18].

The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa has been defined as one of the important etiologies for male infertility. Generation and persistence of ROS in seminal fluid and sperm increase the rate of lipid peroxidation of sperm membrane which is manifested by a high MDA level [19].

Strong evidence suggests that high levels of ROS mediate the occurrence of high frequencies of single and double strand DNA breaks commonly observed in the spermatozoa of infertile men [7]. Several studies reported that accumulation of oxidative DNA damages may lead to defective DNA repair capacity and spermatogenic failure. Hence the present study has become important to evaluate oxidative stress-induced nuclear DNA damage and its effects on sperm quality and to prevent this oligospermia condition.

## 2. MATERIALS AND METHODS

Fifty six males suffering from varying degrees of oligospermia were selected for this study. All these samples were referred from various infertility clinics for genetic testing to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala. Fifteen asymptomatic healthy fertile men formed the control group, and the detailed demographic, clinical history, lifestyle characteristics, and other relevant information were recorded using proforma.

8ml of venous blood was collected aseptically by venepuncture from all these study subjects and from that, 4ml of blood was set up for culture A and B. The culture A was for

detecting constitutional chromosome anomalies by peripheral blood lymphocyte culture method [20] and banded with GTG banding technique. The culture B was for quantitating the extent of somatic DNA damages by Cytokinesis block micronuclei (CBMN) assay [21]. The remaining 4ml of blood was transferred to plain tube and allowed to clot, serum separated immediately. The level of the malondialdehyde, the end product of lipid peroxidation, was determined using thiobarbituric acid as main reagent and measuring these values on photoelectric colorimeter at 540nm.

For detecting constitutional chromosome anomalies, cell division was arrested at metaphase and Giemsa stained as well as GTG banded. GTG banded slides were observed under microscope and good quality metaphases were photographed. From the prints each chromosome was cut down and pasted according to the size, position of centromere and the banding pattern. Karyotypes were prepared according to ISCN (1995) pattern.

The lymphocytes were cultured in sterile bottles using RPMI 1640 medium and were prepared for each subject. The media is supplemented with 100 units/ml penicillin, 100 units/ml streptomycin, 10% fetal bovine serum and 1% phytohemagglutinin. At 44 hr after initiation, cells were blocked in cytokinesis by adding Cytochalasin B (Sigma, final concentration, 4.5µg/ml). The total incubation time for all cultures was 72 hr. After incubation, the cells were fixed in 3:1 methanol/glacial acetic acid, dropped onto clean microscopic slides, air dried, and stained with Giemsa stain. For each sample, 1,000 binucleated cells were scored at 100X magnification. The number of micronuclei per 1,000 binucleated cells was recorded. The data was computed and analyzed using SPSS 11.3 for Windows.

## 3. RESULTS

TABLE 1:

COMPARISON OF MEAN CBMN FREQUENCY AND MDA VALUE AMONG THE STUDY AND CONTROL SUBJECTS

Category	Number	Mean CBMN frequency	MDA Value
Study Subjects	56	14.08	2.13
Control Subjects	15	11.16	1.08

Fifty six subjects with oligospermia, suffering from varying degrees of infertility and 15 healthy fertile subjects were selected and analyzed; the results were recorded. Detailed demographic, lifestyle and clinical characteristics were studied. The age of the study subjects ranged from 21 to 50 with a mean age of 34.83. The CBMN assay was performed to

quantify the extent of DNA damage and the malondialdehyde (MDA) was estimated to evaluate the level of oxidative stress among these study subjects. The mean CBMN frequency of the study subjects was higher (14.08) than the control subjects (11.16). The MDA value was also higher (2.13) in study subjects, when compared to that of the control subjects (1.08) [Table 1]. The mean CBMN frequency and the MDA values were statistically increased among the study subjects than the control subjects.

The demographic characteristics among the study subjects were studied. The age range of the study subjects ranged from 21-50 and the highest mean CBMN frequency was observed among the age group 41-50 (14.20). The birth order of the study subjects ranged from 1 to 9 and the highest mean CBMN frequency was shown by the birth order less than or equal to 3 (14.12). This study demonstrated that the mean CBMN frequency decreases with increased birth order. Majority of study subjects belonged to the religion, Hindu (n=37, 66.07%), Muslim (n=10, 17.85%) followed by Christian (n=9, 16.07%). The highest mean CBMN frequency was recorded in Christian (14.12) and Muslim (14.12). Majority of the study subjects (64.28%) belonged to rural area followed by urban (28.57%) and coastal (7.14%) and an increased mean CBMN frequency was shown by those residing at rural area.

The life style characteristics were also considered in this study. Only 3 among the 56 study subjects had the family history of infertility/sub fertility with the highest mean CBMN frequency of 14.3. Only 4 had the family history of cancer among first or second degree relatives with high mean CBMN frequency of 14.08 and only 2 showed the history of chronic illness with highest mean CBMN frequency of 14.75. History of smoking was reported in 21.42% subjects and 3.57% of study subjects had habit of alcoholism with high mean CBMN frequency of 14.12 and 14.55 respectively. Parental consanguinity was reported in 14.28% study subjects with a highest mean CBMN frequency of 14.13. This clearly indicates a significant relationship between the mean CBMN frequency and the history of infertility, cancer, chronic illness, smoking, alcoholism and parental consanguinity, etc. Simultaneously, a higher MDA value was observed among those subjects with higher mean CBMN frequency which suggests that lifestyle modifications can minimize the extent of oxidative stress.

Among 56 study subjects 87.5% showed normal karyotype and 12.5% showed abnormal karyotype. The mean CBMN frequency was found to be higher (14.41) in those subjects who had an abnormal karyotype (Table 2). This study revealed that the abnormal karyotype shows an increased mean CBMN frequency. An abnormal karyotype was found in 12.5% of the men with oligospermia. Thus the chromosomal abnormalities are more frequently observed in population of oligospermic males than the general population.

TABLE 2:

DISTRIBUTION OF MDA VALUE AND MEAN CBMN FREQUENCY ACCORDING TO KARYOTYPE OF THE STUDY SUBJECTS

	Variable	Number	Percentage	MDA Value	Mean CBMN Frequency
Karyotype	Normal	49	87.50%	2.09	14.03
	Abnormal	7	12.50%	2.36	14.41

#### 4. DISCUSSION

Oligospermia (less than 20 million spermatozoa m/L) is the condition with reduced number of sperms in males [22] and may lead to infertility condition. Infertility is a problem for many couples wishing to conceive. According to World Health Organization, it affects approximately 15% of couples worldwide, among them 20-25% of reproductive problems being contributed to male factor. Male infertility is a global health problem and affects one man in 20 in the general population [2].

Several studies reported that oxidative stress is a major factor in the etiology of male infertility [23]. Oxidative stress induces un-repairable DNA damages. It is suggested that normal persons are more resistant to mutagens compared to patients with various chromosome instability syndrome and cancer. Through cytogenetic analysis, variation in susceptibility to mutagen induced genomic damage can be quantitated.

The purpose of the study was to evaluate the role of oxidative stress and DNA damage in the development of oligospermia. 56 subjects with varying degree of oligospermia risk markers were selected for this study and MDA test and CBMN assay was performed. In this study, the demographic, lifestyle factors and clinical characteristics were concerned. The study demonstrated that the mean CBMN frequency of oligospermia subjects shows a positive correlation with advancing age, family history of infertility, family history of cancer, history of chronic illness, smoking, drinking, parental consanguinity and karyotype, and the mean CBMN frequency was varied according to birth order, religion, residence, education and occupation.

The older men seem to produce more sperm with DNA damage, which derives from three potential sources: oxidative stress, abortive Fas-mediated apoptosis or deficiencies in natural processes such as recombination and chromatin packaging that induce DNA strand breaks [7]. In this study a positive correlation exists between the increase in CBMN frequency and advancing age among the study subjects. The highest mean CBMN frequency was reported in the age group 41 to 50.

Occupational chemical exposure has also led to oligospermia and non-motility and those occupational exposures include heavy metals, solvents, and fumes (notably welding

fumes). More refined analysis of each semen parameter confirmed the higher risk of asthenospermia in subjects exposed to heavy metals [24]. The present study showed increased mean CBMN frequency in non sedentary occupation.

In cases of azoospermia or severe OAT (oligo-asthenoteratospermia), there may be deletions in the azoospermic factor (AZF) region of the Y chromosome. The presence of a Y deletion means that the defect will be passed to sons, who will then also be affected by spermatogenic disturbances or failure [25] present study also observed that the subjects suffering from oligospermia with family history of infertility shows high mean CBMN frequency.

Alcohol has been shown to have a deleterious effect on all levels of male reproductive system. Alcohol induced reduction in levels of testosterone, LH and FSH not only hampers their normal morphological development and maturation of spermatozoa (producing significant teratozoospermia), but also slows down the sperm production by testicular germ cells, especially in heavy alcoholics [26]. In the present study it has been found that the subjects with alcohol consumption reported to have highest CBMN frequency. Thus the study suggests alcohol abuse significant risk factor for oligospermia. Previous studies conducted on fertile male smokers showed reduction in semen volume in comparison to nonsmokers and this reduction in semen volume was in proportion to the number of cigarettes smoked [27]. The present study also observed cigarette smoking as a risk factor for oligospermia.

If the parents were first cousins, both sperm counts and motility parameters were significantly reduced when compared with the others [28]. In the present study the study subjects with parental consanguinity showed high mean CBMN frequency. The reduction in the number of sperm or function may be caused by either a chromosomal or a single gene disorder. Higher frequencies of chromosomal abnormalities ranging from 5% to 27% are found in infertile males than in general male population. Various chromosomal abnormalities reported are numerical or structural abnormalities of sex chromosomes, Robertsonian translocation, paracentric inversions of autosomes and marker chromosomes 9. With decreasing sperm counts, there is a progressive increase in the frequency of chromosomal abnormalities which are more common with severe oligospermia [29]. In this study the abnormal karyotype showed a high mean CBMN frequency suggesting relatively high range of DNA damage than others.

## 5. CONCLUSION

The subjects who had reported for the risk factors such as smoking, alcohol consumption showed higher values of both mean CBMN frequency and MDA. The level of mean CBMN frequency was higher among subjects who have the family history of cancer, chronic illness and infertility. Overall men population seems to be more predisposed to infertility. This is mainly due to lifestyle changes and sometimes occurs as hereditary problems. Increasing awareness of the role of genetics in the etiology of diseases and its overall impact on

the burden imposed on individuals, families and society has led to the emergence of modern clinical cytogenetics. These individuals can be better informed about extent of somatic DNA damages, oxidative stress and genetic risks. This may aid in preventing the sufferings of infertile subjects with oligospermia.

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