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Sperm Chromatin Structure Assay in Oligospermic Infertile Men

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Abstract

Impairment of spermatogenesis and defective sperm function is the most prevalent cause of male infertility. Recent reports indicate that increasing male infertility could be due to genomic abnormalities. Therefore the present study was aimed to evaluate sperm DNA fragmentation in oligospermic infertile as well as fertile men and to study correlation of sperm DNA fragmentation with sperm parameters. The study is prospective, comprises 30 oligospermic infertile men and 30 fertile men. Sperm DNA integrity in terms of DNA fragmentation Index (DFI) was assessed by flow cytometer. Sperm DNA fragmentation was found to be significantly increased in oligospermic infertile men as compared to fertile men. It was negatively correlated with sperm count and sperm motility. Therefore assessment of sperm DNA damage can be used to identify cause of infertility in patients with poor semen quality. This needs to be considered while treating patients with sperm quality anomalies for better outcomes of infertile patients.

Keywords: Sperm Chromatin Structure Assay (SCSA); DNA Fragmentation Index (DFI), Oligospermia.

Introduction

It has been established that sperm DNA integrity plays a significant role in sperm function and fertilizing capacity[1]. In this perspective, sperm chromatin defects have been studied comprehensively in the past decade as a cause for male infertility [2]. The etiology of sperm DNA damage is most likely multi-factorial but compromised due to condensation or nuclear maturity defects, DNA breaks or DNA integrity defects, protamine deficiency, apoptosis and oxidative stress [3].

Numerous of methods have been developed to assess sperm DNA or chromatin integrity. Some of them detect single or double strands breaks of sperm DNA. Other methods are based on the fact that defects in the sperm chromatin structure have been linked with increase DNA instability and sensitivity to denaturing stress [4].

Evenson and et al [5,6]. described the sperm chromatin structure assay (SCSA) and used for both animal and human semen [7]. The SCSA utilizes the metachromatic properties of the fluorescent stain acridine orange (AO), and the extent of DNA denaturation after an acidic treatment is determined by measuring the shift from green fluorescence (double-stranded, native DNA) to red fluorescence (single-stranded, denatured DNA) using flow cytometry. At present, the SCSA is widely used in the human fertility clinic [7,8,9]. SCSA has shown to be an independent marker of fertility in vivo and may also have a potential to contribute for successful use of ART in future [10].

The purpose of this work was to determine sperm DNA fragmentation Index (DFI) by sperm chromatin structure assay in oligospermic infertile men and to assess its correlation with sperm quality parameters.

Material and Methods

Study design

The present study was carried out in Department of Biochemistry, Department of Obstetrics and

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Gynecology, MGM Medical College and Group of Hospitals, Navi Mumbai and National Institute for Research in Reproductive Health (NIRRH). The Institutional Ethical Committee clearance was obtained for the present study.

Thirty male subjects aged 21-45 years, whose partners had conceived within a year and having sperm count ≥ 20 million / ml with motility ≥ 50% in forward progression were selected from general population and considered as fertile (Control Group). Thirty infertile men with oligospermia (Sperm count < $20x10^6$ sperm cells / ml) referred from Department of Obstetrics and Gynecology, aged 21-45 years, without any treatment, whose wives had not conceived after one year of regular, unprotected intercourse. The wives of infertile subjects included had no apparent causes of infertility like tubal blockage or ovulation disorders.

Subjects currently on medication or antioxidant supplementation and azoospermia due to obstructive causes were excluded from the study. The written consent was taken from fertile and oligospermic infertile men. Samples were collected by masturbation in wide mouth sterile plastic container after minimum of three days of abstinence. After liquefaction, samples were processed by conventional analysis to determine sperm count, sperm motility and sperm morphology according to WHO criteria.

Sperm DNA integrity Assessment

Sperm DNA integrity was assessed by the Sperm Chromatin Structure Assay (SCSA) by flow cytometry by Evenson et al. [11] method. (FACSDiva Version 6.1.3 flow cytometer (BD Biosciences, USA). The assay is based on the metachromic properties of DNA binding fluorescent dye, acridine orange (AO). AO intercalates with DNA and emits green fluorescence when bound to intact, double strand DNA and red fluorescence when bound to single strand, fragmented DNA. The percentage of red sperm is called DNA fragmentation index (DFI).

Results

Results were expressed as mean ± SD for each parameter. Statistically significant differences among infertile oligospermic men and fertile groups are indicated in Table 1 along with their significant values. Percent sperm DNA fragmentation was significantly increased in oligospermic infertile men (40.27 ± 8.87) as compared to fertile men (11.65 ± 3.38). Sperm count and sperm motility were significantly decreased whereas sperm abnormal morphology was significantly increased in oligospermic infertile men as compared to fertile men (p<0.001). Sperm DNA fragmentation was negatively correlated with sperm count, motility and positively correlated with sperm abnormal morphology. (Table No.2)

Table 1: The mean values of sperm count, sperm motility, sperm abnormal morphology and sperm DNA fragmentation in fertile and oligospermic infertile men.

Parameters	Fertile men (n=30)	Oligospermic Infertile men (n=30)
Sperm DNA fragmentation (%)	11.65 ± 3.38	40.27 ± 8.87**
Sperm Count (10^6millions/ml)	69.72 ± 13.47	9.35 ± 5.27**
Sperm Motility (%)	72.21 ± 13.11	63.57 ± 12.77*
Sperm Abnormal Morphology (%)	18.76 ± 4.74	35.92 ± 13.94**

*Significant (p<0.05), **Highly significant (p<0.001)

Table 2: Correlation coefficient of various parameters studied in oligospermic infertile men.

Parameters		DFI	Sperm count	Sperm motility	Morphology
DFI	r- value	-	-0.372*	-0.355*	0.332*
Sperm Count	r -value	-	1	0.311*	-0.278*
Sperm Motility	r -value	-	-	-	-0.253*

r = Pearson's correlation co-efficient.
*Significant (p<0.05) **Highly significant (p<0.001)

Discussion

The results of our study provided significant relationship between semen quality and sperm DNA fragmentation. DNA fragmentation was significantly increased in oligospermic infertile men and negatively correlated with sperm count and sperm motility. Our results are supported by Sahel et al [12]

and Irvine et al [13]. Saleh et al [12] reported that sperm DNA damage was significantly increased in men with idiopathic and male factor infertility and in men who failed to initiate a pregnancy after assisted reproductive techniques. Irvine et al [13] also demonstrated a significant proportion of infertile men have elevated levels of DNA damage in their ejaculated spermatozoa and noted highly significant negative correlations between DNA fragmentation and semen quality, particularly sperm concentration and motility. Over the last decade, several studies have confirmed that sperm DNA damage testing has strong associations with every early fertility check point. These comprised impaired fertilization, reduced implantation, slow early embryo development and miscarriage. Childhood cancers have also been associated with oxidative damage to sperm DNA as a consequence of paternal smoking [14].

Sun et al [15] have recently demonstrated a significant negative correlation between semen quality (motility, morphology, and concentration) and the presence of DNA strand breaks in spermatozoa from 285 men attending an assisted-conception clinic.

Several different levels of sperm chromatin abnormalities important to consider are: a) Damage of physical integrity of DNA b) Nuclear protein defects that might affect DNA compaction, and c) chromatin structural anomalies causing altered tertiary chromatin configuration. Environmental stress, gene mutations, and chromosomal abnormalities can disturb biochemical events that occur during spermatogenesis, which can ultimately lead to abnormal chromatin structure incompatible with fertility. Ova are able to repair sperm DNA damage to a certain extent. However, when sperm DNA damage is extensive, ovum may not have repair capacities to allow normal development [3,16]. External factors have also been involved sperm DNA damage. Lifestyle behaviors, radiation, heat exposure, medications and substance abuse are examples of such factors [16].

In our previous study, we reported that oligospermic semen samples with high levels of reactive oxygen species (ROS) which showed negative correlation with sperm count and motility [17]. ROS have been associated to development of male infertility. Oxidative stress in spermatozoa and damage DNA as well as ATP production could cause sperm dysfunction [18]. Oocyte fertilization and pregnancy can be affected when sperm DNA is damaged by oxidative stress [19].

Effects of severe oligozoospermia on intracytoplasmic sperm injection (ICSI) cycle outcomes studied by Hashimoto et al. [20] and reported that severely oligozoospermic patients had a significantly lower percentage of two pronuclei oocytes, indicating that good quality sperm is important for oocyte. A retrospective study by Arikan et al [21] concluded that the fertilization rate was significantly lower in groups with semen concentration < 107 sperm / mL.

Sperm DNA fragmentation was significantly increased in oligospermic men that could be the cause of infertility in oligospermic men. Therefore assessment of sperm DNA damage can be used to identify cause of infertility in patients with poor semen quality. This needs to be considered while treating patients with sperm quality anomalies for better outcomes of infertile patients.

Conclusion

In present study we have revealed that there was a negative correlation between sperm motility and sperm count with sperm DNA damage in oligospermic infertile men. Also there was positive correlation between sperm abnormal morphology and DNA damage in oligospermic infertile males.

Thus according to these results, we conclude that sperm DNA fragmentation was significantly increased in oligospermic men that could be the cause of infertility in oligospermic men. Therefore assessment of sperm DNA damage can be used to identify cause of infertility in patients with poor semen quality. This needs to be considered while treating patients with sperm quality anomalies for better outcomes of infertile patients.

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