Research Article

The Association of Sperm DNA Fragmentation with Idiopathic Male Infertility

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ABSTRACT

Our objective is to detect DNA fragmentation index in Sperm in males suffering from an unknown reason infertility in case the sperm count was normal and with normal motility. A total of 100 Orient hospital patients with normal semen analysis and the selection of 30 samples in which the sperm count is normal and with normal motility. This study shows that the percentage of infertile cases who have an increased fragmentation rate of 30% is (10%) and the percentage of those who were infertile who have a fragmentation ratio have between 15-30% is (40%) of any Mediterranean person able to fertilize. The high percentage of sperm fragmentation in people who suffer from infertility refers to the need for a test to detect fragmentation with halosperm, finding of high DFI will in incurable cases point to direct referral to IVF or ICSI, instead of continuing attempts to achieve spontaneous pregnancy or using intrauterine insemination.

Keywords: DNA Fragmentation, DFI, Unexplained Male Infertility.

INTRODUCTION

Infertility is a very common health problem, affecting approximately 15-20% of couples who attempt pregnancy[1]. Male factor is assumed to be responsible in about 40-50 % of the infertile couples[2-3].

About 15% of the infertile men may carry a genetic abnormality, including numerical and structural chromosomal abnormalities[4].

Although the World Health Organization has estimated that up to 50% of the infertility cases are predominantly or partly caused by male factors[5], the incidence of infertile couples diagnosed as unexplained infertile is around 10-20%[6].

DNA fragmentation is now considered an important factor in the etiology of male infertility[7-9]. However, DNA fragmentation is not routinely assessed in semen analysis according to World Health Organization (WHO) guidelines[10], and approximately 15–30% of couples are 'diagnosed' with unexplained infertility after a routine analysis[11-12]. DNA damage may be present in men with both abnormal and normal semen parameters[7,13], and routine semen parameters are not robustly predictive of infertility or outcome of assisted reproduction treatment[14-15].

During the last decade the search for better predictors of male fertility has resulted in an increased focus on the sperm DNA integrity[16,17]. Now number of sperm chromatin integrity assays is available. Among the most frequently used are the Comet assay (single cell gel electrophoresis)[18], the TUNEL (terminal deoxynucleotidyl transferase-mediated dUDP nick end labelling) assay[19], the sperm chromatin dispersion (SCD)[20,21], and the sperm chromatin structure assay (SCSA)[22,23]. The clinical value of these different tests varies; however, SCSA, first described by Evenson[22] is shown to be an independent marker of fertility *in vivo* and may also help in selection of the most effective ART treatment in each individual couple[24].

In clinical practice, the traditional, manual-visual light microscopic methods for evaluating semen quality maintain their central role in assessment of male fertility potential. However, often a definitive diagnosis of male fertility cannot be made as a result of basic semen analysis. This consists of measuring seminal volume, pH, sperm concentration, motility, morphology and vitality[25].

Abnormalities in the male genome characterized by damaged sperm DNA may be indicative for male subfertility regardless of routine semen parameters[26,27], and these parameters do not reveal sperm DNA defects.

DNA fragmentation may be an important factor in unexplained infertility[28,29], however, ICSI with high DFI is not without its limitations. There is a significantly increased risk of miscarriage, implantation failure or failure to progress to delivery within this group[30-32] are being close to zero when DFI exceeds the level of 30%[33].

This Article will discuss how sperm DNA integrity assessment by help of halosperm can be used as a tool in diagnosis of infertility.

Using DNA fragmentation analysis routinely may allow couples to avoid costly assisted reproduction treatments, repeated failures or recurrent pregnancy losses by proceeding directly to ICSI. In addition, the source of DNA damage may be assessed and relevant treatment may increase the likelihood of spontaneous conception or

successful pregnancy using assisted reproduction technology and/or ICSI.

The halosperm distinguishes cells with intact DNA (large halo) from sperm cells with damage DNA (small or absent halo).

MATERIALS AND METHODS

Materials

All chemicals were obtained from halosperm (halotech dna)

Semen donors

This retrospective study involved 100 male patients from infertile couples referred to the Orient hospital. Infertility was defined as the inability to conceive after at least 1 year.

Sperm quality measures

Standard semen parameter measurements

Samples were obtained by masturbation. Only one ejaculate from each patient was obtained. The patients were recommended 2-5 days of sexual abstinence, although in each case the actual abstinence period was noted. Samples were allowed to liquefy for 30 min. Standard semen parameters (volume, concentration and motility) were measured according to the World Health Organization (WHO) guidelines. Sperm concentration was assessed using positive displacement pipettes and an improved Neubauer hemocytometer. Sperm motility was graded into four groups: rapid progressive motility, slow progressive motility, non-progressive motility, or immotile sperm. Sperm morphology was assessed after Papanicolaou staining following WHO guidelines for the staining procedure.

A level of ≥ 5% was regarded as the threshold for normal morphology. Semen parameters were regarded as normal in men with sperm concentration ≥ 20 × 106/mL, progressive motility ≥ 50% and/or rapid progressive motility ≥ 25%, and a proportion of morphologically normal sperms ≥ 5%.

Sperm DNA damage assessment

Sperm DNA damage was evaluated by halosperm. The halosperm was applied following the procedure described earlier.

This test provides a reliable analysis of sperm DNA integrity that may help to identify men who are at risk of failing to initiate a healthy ongoing pregnancy.

Information about sperm DNA integrity may help in the clinical diagnosis, management and treatment of male infertility and may be of prognostic value in assessing outcome of assisted conception treatment.

Normal and healthy pregnancies do occur in couples where the male partner has a high percentage of sperm with fragmented DNA.

RESULTS

The results are reported showing 3 statistical categories of fertility potential:

DNA Fragmentation Index (%DFI; %sperm cells containing damaged DNA)

≤ 15% DFI = excellent to good sperm DNA integrity

15-30 % DFI = Mediterranean sperm DNA integrity

> 30 % DFI = very poor sperm DNA integrity

While the routine semen analysis can provide important information about male fertility such as sperm concentration and motility, it does not reveal any information about the integrity of the sperm's DNA.

The number of samples examined in a halosperm way in our study 30 sample and the number of samples studied in Orient Hospital in 2011 in this way 40 samples so calculated 70 sample and were discussed seventy cases where divided into three groups mentioned and in terms of relationship high fragmentation of DNA sperm with Smoking factor as shown in the Figure 1.

Sperm DNA can be damaged by exposure to chemicals, smoking, high temperature, and free radicals from normal metabolism. Sperm DNA fragmentation has been associated with decreased fertilization rate and miscarriages.

Research has shown that broken DNA in sperm does not disperse after acid denaturation. In contrast, normal sperm DNA disperses after special acid treatment to produce a halo of DNA chromatin around the sperm head.

The halosperm test makes use of this property to differentiate sperm with normal DNA from those with fragmented DNA. Sperm are first exposed to a colored agent that stains the DNA within the sperm capsule. Subsequent acid treatment makes the sperm membrane leaky. Like a coiled spring upon release, sperm DNA disperses quickly through the leaky membrane to form a halo around the head of the sperm. If the DNA coil is broken, it does not leak readily through the sperm membrane and thus remains confined within the sperm head.

Sperm that do not show the halo by the halosperm test are thought to contain broken DNA. When 30% or more sperm have fragmented DNA, the sample is considered to be abnormal.

Among 100 consecutive men under infertility investigation, 30 cases with the diagnosis 'unexplained infertility' were identified. In the group diagnosed with 'unexplained infertility' 43.3% of the men presented with 0 ≤ DFI ≤ 15 and 33.3% had 15% ≤ DFI ≤ 30% and 10% had DFI ≥ 30% as shown in Figure 2.

A significant part of men diagnosed as unexplained infertile according to traditional diagnostic methods has

remarkably high degrees of fragmented sperm DNA. Apart from adding to our understanding of biology of infertility our finding has clinical implications.

DISCUSSION

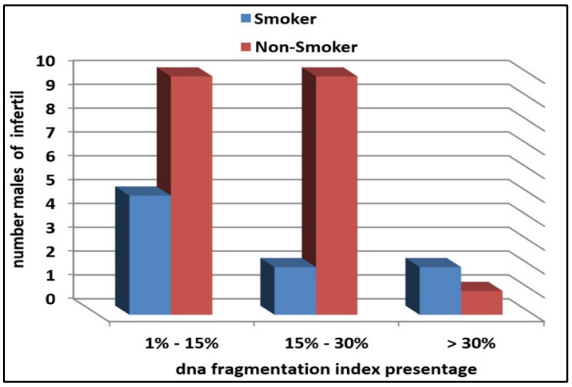

Figure 1: The relation between of DNA Fragmentation Index and Smoking.

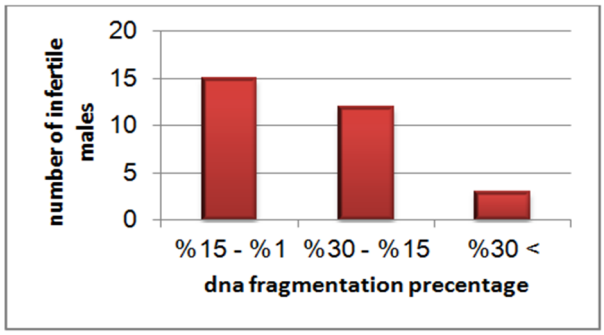

Figure 2: Distribution of infertile men according to DNA fragmentation index.

Most fertility clinics evaluate semen samples simply by conventional analysis, which does not ensure the absence of a male factor problem[34,35].

The level of DNA fragmentation correlates negatively with pregnancy and delivery in both natural and assisted conceptions, although not after intracytoplasmic sperm injection (ICSI), as will be discussed[36-38].

The basic requirement in our research was the normal sperm count and normal motility when measuring the parameters in the semen analysis and this is what was also adopted in Evangelini, 2014.

The SCD (halosperm) test utilizes the ability of intact DNA to loop around the nucleus once embedded in agarose, giving a characteristic 'halo' appearance[39].

We arrived in this research to the conclusion that the percentage of infertile cases who have an elevated to about 30% DFI is the ratio of 10% of people and this result has been approached largely as a result of Oleszczuk and Giwercman, 2013.

Tobacco smoke contains high concentrations of ROS including O2 and OH-, shown to participate in Fenton reactions to produce H2O2 and cause DNA damage[40] and in our study this factor where it had a ratio of DFI > 30% in our study were smokers 100% but nevertheless we came up in our study that cases with lowered percentage of DFI from the 15% who are capable of fertilization were smokers 62.5% refers to the significant increase for people and their natural link smoking factor Saleh confirmed by high-ups in his study in 2002.

Varicocele is the leading cause of male factor infertility, with an incidence of 15% in the whole male population and 40% among infertile men[41] and that as many researchers such as Osadchuk Kovich, 2014 concluded high link-ups for pre-exposure process of varicose veins and this agrees with our study as the infertile individuals who had the DFI rise to about 30% were previously subjected to the process of varicose veins.

That halosperm can make a valuable contribution to semen analysis and consequently be an effective predictive tool for assessing male-factor infertility.

our results suggest that sperm DNA integrity assessment may help to differentiate men with fertility problems and can therefore be of help in counseling of infertile couples.

Furthermore, the finding of high DFI will in incurable cases point to direct referral to IVF or ICSI, instead of continuing attempts to achieve spontaneous pregnancy or using intrauterine insemination.

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