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Original Article

Relationship between sperm progressive motility and DNA integrity in fertile and infertile men

Salah Elbashir [a], Yasmin Magdi [b,*], Ayman Rashed [c], Mohamed Ahmed Ibrahim [d], Yehia Edris [d], Ahmed Mostafa Abdelaziz [d]

[a] Department of Urology, Faculty of Medicine, Benha University, Egypt
[b] Al-Yasmeen Fertility and Gynecology Center, Benha, Egypt
[c] Department of Urology, Faculty of Medicine, 6th of October University, Egypt
[d] Department of Obstetrics and Gynecology, Faculty of Medicine, Benha University, Egypt

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ABSTRACT

Background: Progressive sperm motility was found to be predictive for in vitro and in vivo fertilization. There has been an increase in the literature of studies investigating whether DNA fragmentation could be associated with other semen parameters; however, few reports focused on the relationship between sperm DNA fragmentation and progressive sperm motility.
Purpose: We purposed here to determine the relationship between DNA fragmentation level and progressive sperm motility in different groups of infertile asthenozoospermic patients as well as in healthy men of proven fertility.
Study design: Retrospective study.
Setting: Andrology Laboratory of the Assisted Reproductive Technologies (ART) department of Elite fertility and gynecology center, Cairo, Egypt.
Patients and methods: Semen samples were collected and examined after liquefaction for 20 min at 37 °C from 182 patients. Patient were then classified as asthenozoospermic [(Mild asthenozoospermia; PR (progressive sperm motility) = 30–20%, n = 58), (Moderate asthenozoospermia; PR = 20–10%, n = 68) and (Severe asthenozoospermia; PR < 10%, n = 56)] and 32 fertile healthy men as a control.
Outcome results: Fertile healthy men showed lower sperm DNA fragmentation levels as compared with asthenozoospermic infertile men. There was a significant negative correlation of sperm DNA fragmentation using the modified sperm chromatin dispersion (SCD) test with motility ($r = -0.319$; $P < .001$) and progressive motility ($r = -0.474$; $P < .001$).
Conclusion: Overall, our data suggest that sperm DNA damage is strongly associated with both type and percentage of motility.

1. Introduction

Overall, infertility affects 15% of couples. A male factor is solely responsible in about 20–25% of infertile couples and contributory in another 30–40% [1]. A routine semen analysis according to World Health Organization (WHO) guidelines [2] has been recognized as the most important tool of male infertility diagnosis; *however*, none of semen parameters reflect the DNA integrity of spermatozoa or their fertilization capacity. Thus, sperm DNA fragmentation becomes a new essential parameter for estimating sperm quality [3].

Sperm motility is considered an important indicator of adequate metabolic activity of the sperm and strongly influences ICSI outcome [4]. Sperm motility has been categorized into three classes: progressive, non-progressive and immotile spermatozoa. Asthenozoospermia is one of the main causes of male seminal pathologies that affects about 19% of infertile men. It is characterized by decreasing in percentage of progressively motile (PR) spermatozoa below (32%). It affects approximately 19% of infertile men [5].

Sperm DNA fragmentation test provides approximate information about sperm DNA integrity that may help the clinicians to identify the cause of infertility [6], and therefore guide the couples

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* Corresponding author at: Al-Yasmeen Fertility and Gynecology Center, 5 Farid Nada Street, Benha 13511, Qalubyia, Egypt.
E-mail address: Yas.magdi@hotmail.com (Y. Magdi).

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to bespoke treatment for their particular needs. Various methods could be used to assess sperm chromatin abnormalities. These assays include the Sperm Chromatin Structure Assay (SCSA), the Sperm Chromatin Dispersion (SCD) test, the DNA Breakage Detection-Fluorescence in Situ Hybridization (DBD-FISH) assay, the In-situ nick translation (NT) assay, the Comet assay, and the TUNEL assay [7]. The Comet assay is simple and inexpensive; *however*, it is limited assay because it is time consuming and needs a highly skilled technician to interpret the results. The TUNEL and SCSA assays have been widely used in sperm DNA chromatin assessment, *however*, both of them are expensive. The clinical value of the DNA Breakage Detection-Fluorescence in Situ Hybridization (DBD-FISH) assay is limited because it is expensive, time consuming and less cumbersome. The NTL assay cannot be employed for routine clinical use because it lacks sensitivity and the results are not correlated with fertilization during in vivo studies [7,8]. Compared to other methods of assessing DNA fragmentation, the SCD test is a simple, fast and inexpensive technique. Data from several studies suggest that the sperm DNA fragmentation inversely correlate with the rates of fertilization and embryo development [9].

None of sperm parameters should be considered alone in male infertility assessment as all directly impact upon initiation a healthy ongoing pregnancy [10]. A significant negative correlation between DNA fragmentation and sperm concentration, motility and morphology has been previously reported [11].

The purpose of our study was to (1) determine the relationship between DNA fragmentation levels and progressive sperm motility in normozoospermic fertile men and asthenozoospermic infertile patients, and (2) verify whether the sperm progressive motility is correlated with DNA fragmentation in ejaculated semen using the modified sperm chromatin dispersion (SCD) test or not.

2. Materials and methods

This retrospective study was performed from September 2015 to June 2016. Semen samples were obtained from 214 men attending the andrology Laboratory of Assisted Reproductive Technologies (ART) department of Elite fertility and gynecology center, Cairo, Egypt.

The subjects, 214 enrolled in the study, were classified into two groups. Fertile group (Control; they were able to conceive and bear children through normal sexual activity two years ago) was 32 healthy men with normal semen characteristics (Normozoospermia) according to the criteria of WHO 2010 [5]. The second group were 182 infertile asthenozoospermic men that had at least one year of regular unprotected intercourse with no pregnancy achieved. They were subdivided into three sub-groups on the basis of their progressive motility (PR) [(Mild asthenozoospermia; PR = 30–20%, n = 58), (Moderate asthenozoospermia; PR = 20–10% , n = 68) and (Severe asthenozoospermia; PR < 10%, n = 56)]. Gynecologic evaluation and fertility workup of all female partners failed to reveal any abnormality.

2.1. Semen analysis

Semen samples of patients were collected in sterile containers by masturbation after 5 days of sexual abstinence and examined after liquefaction for 20 min at 37 °C. Volume, pH, concentration and motility were evaluated according to WHO guidelines [2].

2.2. Sperm morphology assessment

Air-dried seminal smears were fixed and stained with Diff-Quik stain (Baxter Healthcare, McGaw Park, IL), according to the manufacturer's instructions. Normal sperm morphology was scored according to WHO criteria [2] and strict criteria by Kruger [12] using a x100 oil-immersion brightfield objective. At least 200 sperm were assessed.

2.3. Measurement of leukocytes

Leukocyte staining method (LeucoScreen; Ferti Pro M.V., Aalter, Belgium) was used to indicate the presence and number of leukocytes. A drop of 10μ of semen mixed with 10 μl of working solution (30 μl of reagent 2–1 ml of reagent 1 of LeucoScreen). Yellow to brown stained cells were considered as peroxidase positive cells.

2.4. DNA fragmentation analysis

The SCD test processed using commercial kit (Halosperm®, Laboratorios INDAS, Madrid, Spain). The spermatozoa were immersed in a melted agarose matrix at 37 °C. On a slide, a drop of 8 μl of mixed sperm agarose initially treated with an acid solution (solution 1) for 7 min to denature the DNA with DNA breaks, and directly treated with lysing solution (solution 2) for 20 min to deproteinize the nucleoids. After removal of nuclear proteins, fixation was done using ethanol and then slide was stained using solution 3 for 6 min and solution 4 for 7 min. Non-fragmented sperm DNA appeared with a core and with a peripheral halo of dispersion of DNA loops. Fragmented sperm DNA appeared with very small or no halo of DNA dispersion.

2.5. Statistical analysis

The data were tabulated and analyzed using the computer program SPSS (Statistical package for social science) version 16. Data are presented as mean ± standard deviation.

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:

1. Student's *t*-test and Mann-Whitney test (z test):- Used to compare mean of two groups of quantitative data of parametric and non-parametric respectively.
2. ANOVA test (F value) and kruskal-wallis test (x2): Used to compare mean of more than two groups of quantitative data of parametric and non-parametric respectively.
3. Correlation coefficient: to find relationships between variables.

A P value < .05 was considered statistically significant (S), while > .05 was considered statistically insignificant. P value < .01 was considered highly significant (HS) in all analyses.

3. Results

Table 1 lists the comparison between the basic semen parameters, of the 214 men enrolled in the study. There were no significant differences between the two groups in terms of their age, semen volume, and BMI (Table 1). However, BMI was significantly higher of severe asthenozoospermic sub-group compared with control healthy men (BMI: 25.56 ± 2.06 and 24.69 ± 0.60 years, respectively, P = .015) (Table 2).

Differences between fertile and infertile sub-groups were non-significant for sperm count and morphology, as shown in Table 2. The mean leukocytes concentration comparable between the fertile and infertile sub-groups men was (0.156 ± 0.37 versus 0.076 ± 0.26, 0.097 ± 0.27 and 0.35 ± 0.57 × 106/ml, not significant).

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