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HIGHER FREQUENCY OF TAIL DEFECTS IN HUMAN SPERMATOZOA CORRESPOND TO HIGHER LEVELS OF DNA FRAGMENTATION

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Abstract: Numerous studies have demonstrated the presence of increased levels of sperm DNA fragmentation in patients with poor sperm morphology. The majority of studies have shown that specific head abnormalities (long, amorphous and micro heads) are associated with the presence of spermatozoa with immature chromatin and higher DFI (DNA fragmentation index). During spermatogenesis the process of chromatin compaction *is considered to be tightly linked* to the head morphology. However, it could also be suggested that there is relationship between DNA integrity and other morphological defects.

A retrospective study was performed during the period 2015 - 2016. A native sperm from 98 patients undergoing ICSI procedure were used for analysis of DNA fragmentation and sperm morphology. Using the Sperm Chromatin Structure Assay (SCSA), the number of DNA fragmented sperm, expressed as DNA fragmentation index (DFI) was evaluated. Sperm morphology was assessed by Kruger strict criteria after staining procedure with Sperm Stain kit (Microptic S.L.). Spearman's rank correlation test was performed to evaluate the correlation between the frequency of the morphological defects and levels of DNA fragmentation.

Among all types of morphological defects, tail defects showed the strongest correlation with DNA fragmentation of human spermatozoa. A significant positive correlation was observed between DFI and percentage of cumulative tail defects ($r = 0.54$; $p < 0.001$), acephalic cell (pinhead) ($r = 0.46$; $p < 0.001$), short tail ($r = 0.37$; $p = 0.022$) and coiled tail ($r = 0.34$; $p = 0.015$).

In conclusion, the analysis of our data indicates that the frequency of occurrence of tail defects should be considered as a valuable biomarker of sperm DNA fragmentation.

INTRODUCTION

The origin and effect of sperm DNA fragmentation on male fertility and ongoing pregnancy has been intensively studied. It is already known that infertile man with poor sperm morphology have an increased number of spermatozoa with high levels of DNA fragmentation (Lopes et al, 1998; Irvine et al, 2000). Furthermore, fertilization by sperm with high DNA fragmentation results in low blastocyst formation rates, poor embryonic development, low implantation rate and increased probability of spontaneous abortion (Hammadeh et al, 1998; Lopes et al, 1998b; Larson et al, 2000; Virant-Klun et al, 2002; Benchaib et al, 2003; Seli et al, 2004; Virro et al, 2004; Loutradi K. Et al., 2006; Simon L. Et al. 2014). The main possible causes of DNA fragmentation in human spermatozoa could be an aberrant chromatin packaging during spermatogenesis (Shamsi M et al., 2008), defective apoptosis before ejaculation (Sakkas et al, 2002), excessive production of reactive oxygen species in the ejaculate (Moustafa et al, 2004; Saalu L., 2010), decreased levels of antioxidants (Shamsi M., 2010 et al.) and excessive exposure to xenobiotics (Bain et al., 2004).

The evaluation of morphological characteristics of human spermatozoa seems to be another useful tool for an accurate prediction of successful fertilisation, implantation and on-going pregnancy (Lundin K. et al., 1997; Kruger T., 1999; Van Waart J. Et al., 2001). Sperm morphology is already associated with sperm DNA damage since morphological abnormalities of sperm could be useful indicators of the measured levels of sperm DNA fragmentation (Shen H., 2002; Darin B., 2010). Studies attempting to determine the relationship between specific morphological abnormalities and high levels of DNA fragmentation are scarce. Different authors have revealed an association between DNA fragmentation and tail abnormalities (Muratori M., 2000), cytoplasmic droplets (Said T., 2005) and head defects (Zini, A. et al., 2009; Cassuto N. et al., 2012; Fortunato A. et al., 2013). Determination of the existing correlations between specific morphological abnormalities and elevated degrees of DNA fragmentation in human sperm could be of crucial importance for better prediction of successful in-vitro fertilization procedures and improved sperm selection strategies.

The purpose of the present study was to determine the relationship between specific types of morphological abnormalities in human spermatozoa evaluated according to Kruger strict criteria and the degree of DNA fragmentation, measured by the sperm chromatin structure assay (SCSA).

MATERIALS AND METHODS

Study design

A retrospective study was performed at the Nadezhda Women's Health Hospital, Sofia, Bulgaria during the period 2015 - 2016. A native sperm from 98 patients undergoing ICSI procedure were used for analysis of DNA fragmentation and sperm morphology.

Semen analysis

In all cases, after 3-5 days of sexual abstinence, semen samples were collected. All samples for evaluation were allowed to liquefy for at least 20 minutes at 37°C and then evaluated for sperm concentration, motility, and morphology according to the guidelines of the World Health Organization (WHO, 2010). The SCSA

was done following the established protocol of Evenson and Jost (1994). More than 10000 sperm were evaluated for each semen sample and the results were expressed as percent DNA fragmentation index (%DFI). For the morphological evaluation by Kruger strict criteria, a smear was made from 5 ml semen, and stained by the Sperm Stain kit (Microptic S.L.). A x1000 magnification was used, and the morphology of 100 sperm cells was determined for each sample.

Statistical analysis

Statistical data analysis was performed using SPSS v.21. Data were reported as mean ± SD. Spearman's rank correlation test was performed to evaluate the correlation between the frequency of the morphological defects and levels of DNA fragmentation. The *P* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 illustrates baseline characteristics of the studied population, selected morphological variables and DNA fragmentation index (DFI). The mean semen parameters were in the normal range. Patients with a relatively high variety of morphological defects and DNA fragmentation index were included.

Table 1. Characteristic of the study population, standard semen analysis parameters, selected morphological defects and DFI value.

Variable		Mean ± SD	Range (Min-Max)
Age (years)		38 ± 6	30 (24-54)
Standard semen variables	Sperm Volume (ml)	3.14 ±1.40	7 (1-7)
	Sperm concentration (cells x 10^6)	79.11 ± 34.45	350 (1-350)
	Sperm motility (A %)	19.27 ± 10.03	46 (0-46)
	Sperm motility (B %)	20.17 ± 7.04	40 (0-40)
	Sperm morphology (Kruger) (%)	4.25 ± 2.04	11 (1-12)
Sperm morphological defects (%)	Cumulative Tail defects	12.85 ± 9.23	61 (1-62)
	Cumulative Midpiece defects	17.45 ± 9.04	52 (2-54)
	Cumulative Head defects	83.96 ± 19.20	82 (49 - 131)
	Macrocephalic heads	15.85 ±10.56	46 (2-48)
	Microcephalic heads	17.24 ± 7.04	60 (1-61)
	Pyriform heads	9.65 ± 4.05	37 (1-38)
	Amorphous heads	17.40 ± 9.51	58 (1-59)
	Pinheads	6.34 ± 3.73	26 (1-27)
	Short tails	4.73 ± 2.62	21 (1-22)
	Coiled tails	5.39 ± 2.44	30 (1-31)
	Double tails	2.47 ± 1.07	3 (1-4)
DNA fragmentation	DFI (%)	21.54 ± 15.83	75 (4-79)

Table 2. Significant correlation (P<0.05) between sperm morphological defects (%) and DNA fragmentation index (DFI) (%). *r – Spearman Correlation coefficient.

Morphological defects	r	DNA fragmentation
Cumulative Tail defects	0.54	DFI
Pinhead	0.46	
Short tail	0.37	
Coiled tail	0.34	
Cumulative Head defects	0.29	
Pyriform heads	0.29	
Microcephalic heads	0.26	
Amorphous heads	0.25	

Correlations between human sperm morphological abnormalities and DNA fragmentation are listed in Table 2. Among all types of morphological defects, tail defects showed the strongest correlation with DNA fragmentation. A significant positive correlation was observed between DFI and percentage of cumulative tail defects ($r = 0.54$; $p < 0.001$), acephalic cell (pinhead) ($r = 0.46$; $p<0.001$), short tail ($r = 0.37$; $p = 0.022$) and coiled tail ($r = 0.34$; $p = 0.015$) (Fig.1).

In our study DNA fragmentation index was also related with head abnormalities such as the frequency of occurrence of microcephalic (r=0.26), amorphous (r=0.29) and pyriform heads (r=0.29) (Table 2). However, the obtained values of correlation coefficients were relatively smaller compared to those with tailed abnormalities.

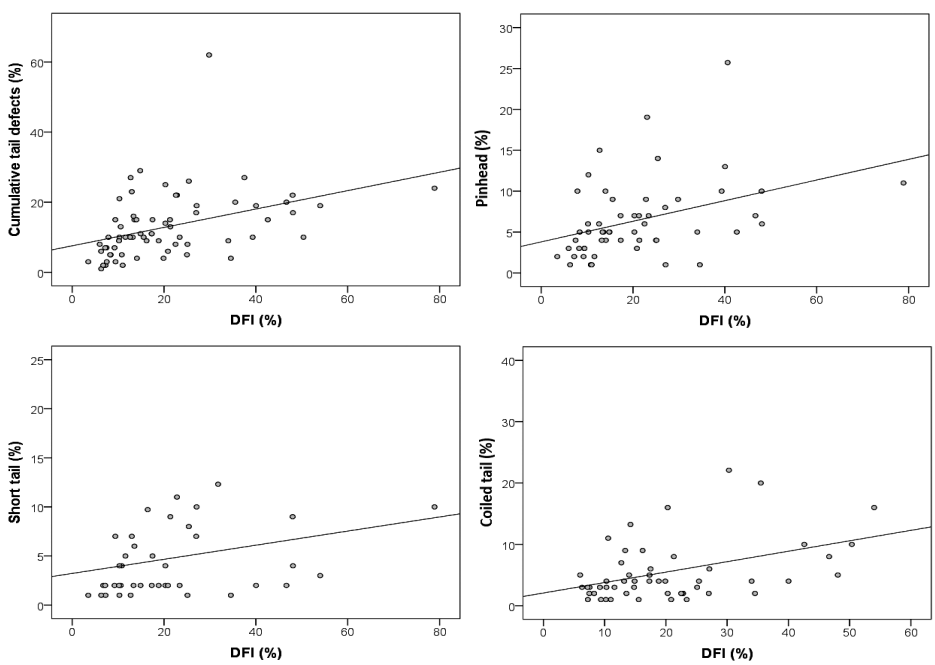

Fig. 1. Scatter graph illustrating correlations between specific types of morphological defects and DNA fragmentation index (DFI).

Sperm morphology and DNA fragmentation assessment are important components of the semen analysis protocols in cases of repeated IVF failures and both are tightly linked to male infertility. Although the causal relationship between these sperm parameters is not absolutely clear, the literature has demonstrated a positive link between them (Muratori M., 2000, Zini, A. et al., 2009; Daris, 2010; Cassuto N. et al., 2012; Fortunato A. et al., 2013). It is considered that DNA-fragmented spermatozoa retain specific abnormalities that are compatible with a lower degree of maturation (Muratori M. et al., 2000). DNA-fragmented sperm are less motile, more immature, with high frequency of morphological defects and with a lower functional integrity of the sperm membrane (Jeyendran et al, 1984).

In our study, higher percentage of human spermatozoa with specific tail defects indicated increased degree of DNA damage. A positive correlation between DNA fragmentation and cumulative tail defects were also obtained by Muratori (Muratori M., 2000) and Zini (Zini, A. et al., 2009). It should be noticed that an alternative method for assessment of DNA fragmentation (terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end labelling (TUNEL) assay) and swim-up selected spermatozoa was applied by Muratori while Zini assessed DNA damage by SCSA method. Despite the application of various techniques of detection, the similar final results obtained from both authors also support the existence of a relationship between sperm tail defects and the degree of DNA fragmentation.

Our results showed also relatively small but significant correlation between DNA fragmentation and the frequency of occurrence of sperm head defects. This type of relation was previously reported by other research groups (Zini, A. et al., 2009; Daris, 2010; Cassuto N. et al., 2012; Fortunato A. et al., 2013). They have found a positive relationship between DNA fragmentation and head abnormalities (cumulative head defects, the percentages of amorphous heads etc.). This correlation suggests that sperm head defects may in part be due to reduced nuclear compaction (Zini, A. et al., 2009). An association between these defects and aneuploidy could be regarded as another explanation of the observed tendency (Vicari E. et al., 2003; Calogero A. et al., 2003).

CONCLUSIONS

In conclusion, the analysis of our data indicates that the frequency of occurrence of tail defects should be considered as a valuable biomarker of sperm DNA fragmentation. High percentage of spermatozoa with specific tail abnormalities in human sperm samples suggest elevated degree of DNA fragmentation. There is a reasonable possibility for unsuccessful IVF/ICSI with such samples, since the potential risk of using spermatozoa with adverse DNA damage exists. Therefore, identification and selection of sperm cells without specific tail defects could improve the success in assisted reproductive technologies (ART).

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