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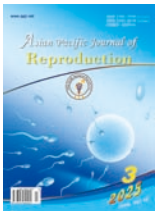

Letter to Editor

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Sperm DNA fragmentation and sperm chromatin decondensation status in patients with extreme and total isolated teratospermia: A prospective study

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Total or severe teratospermia affects the prognosis of fertility and causes serious problems for patients undergoing assisted reproduction[1]. The pathophysiological mechanism of teratospermia is unclear. It has been shown that patients with sperm parameters abnormalities and abnormal morphology have a high rate of fragmentation and sperm DNA decondensation[2,3], and that sperm DNA fragmentation analysis could be used as a predictor factor of fertility potential[4]. Therefore, functional tests such as sperm nuclear DNA integrity and chromatin condensation quality are required to make an appropriate clinical diagnosis[5]. Consequently, the source of DNA damage can be determined and can be appropriately treated thus increasing the likelihood of spontaneous conception or successful pregnancy using assisted reproductive technology[6].

A prospective comparative study was conducted involving infertile patients referred to our Reproductive Biology Department for sperm exploration from January 2021 to December 2021. Two groups of patients were recruited, one group of patients with isolated total or extreme polymorphic teratospermia (typical forms 3% according to David classification)[7] (group 1, n=32), and a control group including men with normal semen parameters and female factor of infertility (group 2, n=15). Patients with clinical varicocele, leucocytospermia and oligospermia or asthenospermia were excluded from the study. For each patient, standard semen parameters analysis, DNA fragmentation test (TUNEL assay) and sperm chromatin decondensation (SCD) assay were assessed according to the World Health Organization (WHO) 2021 recommendations[8]. This protocol was approved by the local ethics committee of Military Hospital of Tunis and all patients had previously given informed consent for the study.

This study aimed to answer the following question: did patients with isolated teratospermia have higher sperm DNA damage and defective chromatin condensation than normal men and even more is there a link between sperm DNA compaction defects, DNA fragmentation and poor morphology sperm?

Semen specimens were collected after three to five days of sexual abstinence. After liquefaction of semen at room temperature, standard semen parameters and sperm morphology were analyzed according to WHO 2021 criteria[8]. Then, sperm samples were prepared for immediate aniline blue staining assay, and for subsequent DNA TUNEL fragmentation test, and conserved at –20 ℃ until the assay.

SCD was evaluated by aniline blue coloration which detects the presence of histones thus indirectly inferring the existence of lower amounts of protamine in the sperm nucleus[9]. The aniline blue staining was carried out according to WHO recommendations[8]. To this staining, the samples were washed twice in 5 mL phosphate buffered saline (PBS; pH 7.4) and centrifuged at 600 g for 10 min. The obtained pellet was dissolved in 1 mL of PBS and two smears were carried out for each patient. Then, smears were fixed in 0.75% buffered glutaraldehyde for 30 min. Each slide was washed and marked with 5% aniline blue solution and mixed with 4% acetic acid (pH 2.5-3.0) for 5 min. Spermatozoa with residual histone showed blue colored nuclei while spermatozoa with normal chromatin condensation were unstained. A total of 300 sperm cells of each sample were analyzed under oil immersion at magnification ×1 000. Heads with high blue coloration corresponded to abnormal SCD with abundant histones. An ejaculate with a rate of blue stained spermatozoa less than 20% was accepted[9].

For TUNEL assay, the fresh semen was washed twice in 5 mL PBS (pH 7.2) and centrifuged at 1 500 g for 5 min. The sediment was then twicely fixed in methanol/acetic (3:1) acid and centrifuged.

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Table 1. Characteristics of patients with total and extreme teratozoospermia and controls.

Parameters	Teratozoospermia group (n=32)	Control group (n=15)	P value
Age, years	37.0±4.6	34.5±6.3	0.616
Coventional semen parameters			
Volume, mL	3.59±1.54	3.13±1.03	0.200
Vitality, %	77.09±13.08	84.20±7.57	0.051
Concentration, $\times 10^6$/mL	52.09±39.92	71.80±32.55	0.899
Progressive mobility, %	30.78±11.30	38.93±7.33	0.066
Total mobility, %	36.41±11.09	44.33±7.04	0.062
Advanced sperm morphology assessment			
Typical form, %	1.53±0.11	16.73±5.59	<0.001
Multiple anomalies index	2.02±0.38	1.38±0.15	0.002
Tapered head, %	21.72±8.61	13.60±4.78	0.058
Abnormal base, %	57.56±14.89	17.27±6.31	0.006
Acrosome abnormalities, %	23.90±7.92	12.97±3.49	0.007
Cytoplasmic droplets, %	3.66±0.45	3.13±0.21	0.040
Bent neck, %	27.00±13.49	15.27±4.57	<0.001
Missing flagellum, %	0.060±0.001	2.000±0.250	<0.001
Two tailed, %	0.220±0.049	0.47±0.07	0.017

Data are expressed as mean±SD.

The final pellet was dissolved in 1 mL methanol/acetic acid (3:1) and stored at –20 ℃ until further processing.

The presence of DNA strand breaks was evaluated by the TUNEL assay using the TACS® TdT-DAB kit, in situ apoptosis detection kit. The frozen tubes were centrifuged and washed 3 times, and then 2 slides were carried out for each patient. The assay was performed according to the manufacturer's instructions.

A total of 500 sperm cells of each semen sample were analyzed using optic microscope at magnification ×1 000. Normal cells considered as having intact DNA were colored in green and sperm cells with fragmented DNA were colored in brown. When sperm DNA fragmentation index (DFI) is less than 15%, DNA is qualified as excellent, if 15%<DFI≤25%, DNA is considered heterogeneous, and, if it is higher than 25%, the chances of success in *in vitro* fertilization (IVF) are compromised[10]. As shown in Table 1, both groups have comparable average age [(37.0±4.6) years *vs.* (34.5±6.3) years; P>0.05]. The mean of typical forms and multiple anomalies index were significantly different compared to the control group [(1.53±0.11)% *vs.* (16.73±5.59)%, P<0.001; (2.02±0.38) *vs.* (1.38±0.15), P=0.002; respectively].

By analyzing the morphological abnormalities in both groups, significant differences were reported in the category of head abnormalities, in term of absent or malformed acrosome in the teratospermia group compared to the control one [(23.90±7.92)% *vs.* (12.97±3.49)%, P=0.007], and in term of abnormal base in the teratospermia group compared to the control one [(57.56±14.89)% *vs.* (17.27±6.31)%, P=0.006] (Table 1). The rate of SCD was significantly higher in the teratospermia group compared to the control group [(44.34±13.04)% *vs.* (11.97±4.12)%, P=0.002]. No significant association was found between SCD and conventional semen parameters. However, further analysis of morphological abnormalities showed a high correlation between abnormally condensed chromatin and head abnormalities (thinned head, acrosome abnormalities, abnormal base) and mid piece abnormalities. These results showed a link between spermatozoon head abnormalities and SCD. The sperm DFI was (6.13±2.58)% in the control group *vs.* (27.0±7.1)% in the teratospermia group (P=0.003). Using Spearman's correlation test, a significant association was noted between sperm DNA fragmentation and progressive motility, total motility, sperm total count and typical forms (r=-0.439, P=0.003; r=-0.429, P=0.003; r=-0.378, P=0.009; r=-0.787, P=0.0001; respectively).

However, there is no evident correlation detected between sperm DFI and paternal age or, other semen parameters as volume or vitality. Sperm motility and concentration for our 47 patients were negatively correlated with DFI (r=-0.428, P=0.003; r=-0.379, P=0.009; respectively). A slightly positive but not significant correlation was observed between DNA fragmentation and SCD (r=0.625, P=0.09).

In conclusion, our study found that isolated total or extreme teratospermia is associated with a higher rate of SCD and DNA damage which seems to be even more present in head defect class. This suggests that semen head defects could be partly due to reduced nuclear compaction. Consequently, this parameter could be directly an indication of antioxidant treatment to correct DNA damage and achieve each attempted assisted reproduction. Only sperm DNA fragmentation was significantly and negatively correlated to sperm concentration and motility, and this suggests that DFI is more associated with sperm parameters than SCD especially that we did not notice a significant correlation between the two technologies. Further studies are needed to confirm these encouraging preliminary findings.

Conflict of interest statement

Authors declare that they have no conflicts of interest.

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Authors' contributions

Sonia Jellad, Sarra Maatallah, and Molka Bouricha contributed to the study design, analyzed and interpreted the data. Sonia Jelladand and Molka Bouricha drafted and revised the manuscript. Sonia Jella and Molka Bouricha revised the manuscript and final approval. Sonia Jellad, Molka Bouricha, and Sarra Maatallah read and approved the final manuscript.

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