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ANALYSIS OF FRAGMENTED DNA IN INFERTILE MALE EJACULATE IN DUKAGJINI PLAIN IN THE REPUBLIC OF KOSOVO

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Abstract:

Aim: Infertility is defined as without the ability of a sexually active mate to reach a pregnancy despite regular and unprotected sexual intercourse for more than 12 months. Analysis of fragmented DNA in the sperm. The fragmented DNA analysis was performed according to the procedure mentioned in the instruction of the Halosperm / Halotech DNA reagent, S.L. Madrid, Spain. Materials and methods: The statistical processing of the data is done with the statistical package SPSS 22.0. Patients received for analysis were all from the Republic of Kosovo. The sampling period was 2014/2018. All analyses were performed at Biolab Zafi, Laboratory in Peja, Republic of Kosovo. The difference is significant if P <0.05. Results: The study included 257 males of different age groups, of whom 169 were in the study group and 88 in the control group. In the study group were men with asthenospermia, oligospermia and oligoasthenospermia, whereas in the control group were men with normospermia. Conclusions: We can conclude that the results of DNA fragmentation fragmented into the sperm are encouraging and can be used for diagnostic purposes in determining male infertility. Such an assessment of DNA damage to the sperm can serve clinicians to assist spousal couples with infertility in the selection of IVF / ICSI assisted medical support technique. It is recommended that such an analysis of DNA fragmentation determination in the sperm becomes part of the clinical trials of Andrology laboratories.

Key words: *Sperm DNA fragmentation, Kit Halotech/Halosperm, IVF / ICSI.*

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INTRODUCTION:

Infertility is defined as without the ability of a sexually active mate to reach a pregnancy despite regular and unprotected sexual intercourse for more than 12 months (Agarwal et al., 2011). Infertility is recognized by the World Health Organization (WHO) as public concern for health (Boivin et al., 2007). Of all couples trying to have children, 17% - 25% will be diagnosed as infertile, where about 50% of these cases are attributed to male infertility (Venkatesh et al., 2011). Of 25% of couples who do not get pregnant within a year, 15% of them seek medical treatment for infertility and less than 5% of them are left without children. Approximately 50% of the cases the basic infertility etiology lies only with males (Poongothai et al., 2009). In addition, in approximately 60-75% of cases, no cause is found in males and is called idiopathic (unknown) factor. Male infertility may be the result of genetic damage, oxidative stress, tumors, BMI, urogenital tract infections, temperature increase, varicocele, hormonal disorders, smoking, drug-related toxicity (WHO, 2000). In men in whom the cause of infertility is idiopathic, they have no previous history of fertility problems and findings are normal in physical and hormonal examinations. Diagnostic processing of idiopathic cases involves the analysis of ejaculate which usually reveals the reduction of the number of spermatozoa (oligozoospermia), the reduction of sperm interstitial mobility (asthenozoospermia) or the presence of morphologically abnormal spermatozoa (teratozoospermia). If these anomalies occur together, the entirety of all these abnormalities is described as oligoasthenoteratozoospermia (OAT). The presence of genetic abnormalities such as chromosomal and genetic levels is a major concern for couples choosing assisted reproduction techniques (ART), which offer the ultimate hope for these couples to have their descendants (WHO, 2000). However, no matter what reference values were used for the spermogram analysis, it still has its own deficiencies. Ejaculation routine analysis does not address the integrity of the DNA molecule, which plays a key role in diagnosing male infertility (De Jonge, 2012). Research has shown that approximately 15% of infertile males tested have normal spermogram analysis parameters (Omran, Bakhiet & Dashti, 2013). As a result, there was considerable care in the DNA fragmentation in the ejaculate and in its connection with reproductive results. High levels of fragmented DNA analysis have been associated with male infertility (Zini, 2011; Tamburrino et al., 2012). Although all sperm may look the same during an observation, human ejaculation has different biochemical and physiological characteristics that can be identified in each ejaculate specimen (Singh et al., 2011).

Analysis of fragmented DNA in the sperm:

The fragmented DNA analysis was performed according to the procedure mentioned in the instruction of the Halosperm / Halotech DNA reagent, S.L. Madrid, Spain. First, LS (lysis solution) is placed in the room temperature, the agarose ependorf tube is placed in water for 5 min at 90-100C until the agarose is fluidized, then the agarose ependorf is placed in the water bath at 37C and left for 5 min, is taken from ejaculation 5-10 mil / ml and mixed with agarose, 25 microliters of the mixture and placed in special glass for this procedure and covered with 22x22 mm cover glass. Care should be taken that the position of the glass is in horizontal position and placed on a glass or metal plate and placed in the refrigerator at 4C for 5 min. Then, denaturing solution (DA) is prepared: 80 microliters of DA and 10 mL distilled water are added and mixed. Then the cover glass is removed carefully and the working glass is incubated for 7 min in the DA prepared solution, then the glass is incubated for 25 min at 10 ml of lysis solution (LS), then incubated in distilled water for 5 min and continued from 2 min incubation at 70%, 90% and 100% ethanol concentrations, and then left to dry at room temperature after drying can be stored for months. Prior to microscopy, the preparation of the preparation with May-Grunwald-Giemsa is done. Results are obtained by counting 300 sperm counts for each patient. The fragmented DNA results will be in this form: (DNA Fragmentation Index, DFI), (DNA Fragmentation Index -DFI).

MATERIALS AND METHODS:

The statistical processing of the data is done with the statistical package SPSS 22.0. From the statistical parameters are calculated the arithmetic average, the standard deviation, the minimum and the maximum value. Qualitative data testing was done with the X2-test of quantitative data that had normal T-test distribution, while those with non-normal distribution with the Mann-Whitney test or Kruskal Wallis test. The difference is significant if P <0.05. Patients received for analysis were all from the Republic of Kosovo. The sampling period was 2014/2018. All analyses were performed at Biolab Zafi, Laboratory in Peja, Republic of Kosovo.

RESULTS:

The study included 257 males of different age groups, of whom 169 were in the study group and 88 in the control group. In the study group were men with asthenospermia, oligospermia and oligoasthenospermia, whereas in the control group

were men with normospermia. With the distribution of the surveyed by age group and X2-test groups we have distinguished significant statistical significance (X2 = 10.28, P = 0.006). In the study group compared to that less control was under the age of 30 and older aged 40+ years.

Table 1. Average Age of Researched by Groups

Age-group (Years)	Study group		Control group			
	N	%	N	%	N	%
<30	32	18.9	30	34.1	62	24.1
30-39	73	43.2	39	44.3	112	43.6
40+	64	37.9	19	21.6	83	32.3
Total	**169**	**100.0**	**88**	**100.0**	**257**	**100.0**
X^2-test, P-value	X^2=10.28, P=0.006					

Table 2. Average Age of Researched by Groups

Age-group (Years)	Study group	Control group	Total
N	169	88	257
Mean	36.7	34.3	35.9
SD	7.1	8.1	7.5
Min	16	18	16
Max	51	56	56
T-test, P-value	T= 2.508, P=0.0128		

Table 3. Comparison of parameters between study group and control group

Parameters	Study group	Control group	t-Testi	P-value
N	169	88		
Nr. of sperm in 1 mil	19.25 ± 19.45	61.43 ± 34.48	U'=13215	P < 0.0001
General Mobility of spermatozoa (%)	28.37 ± 17.86	57.15 ± 10.83	U'=13761	P < 0.0001
Progressive Movement e (a), (%)	13.37 ± 11.85	25.51 ± 7.47	U'=12210	P < 0.0001
Medium Movement (b+c) (%)	15.39 ± 11.37	31.74 ± 10.10	U'=12630	P < 0.0001
No movement (%)	70.98 ± 17.58	42.67 ± 10.88	U'=13753	P < 0.0001
Normal morphology (%)	15.85 ± 13.48	42.58 ± 15.21	U'=13426	P < 0.0001
Abnormal morphology (%)	84.09 ± 13.51	57.67 ± 15.24	U'=13365	P < 0.0001
Sperm DNA fragmentation (%)	29.96 ± 7.56	14.91 ± 3.88	U'=14543	P < 0.0001

DISCUSSION:

The analysis of the fragmentation of DNA fragmentation has been developed and improved by (Fernández et al., 2005). This method is simple and easy to perform in Andrology laboratories and is available in the market as Halosperm / Halotech kit, DNA, Spain. Our results of fragmented DNA analysis in infertile males studied ($p < .0001$) are in complete harmony with the results obtained from (Fernández et al., 2005) ($p < .001$). Conventional ejaculate analysis only evaluates sperm concentration, mobility, and morphology, so it is an incomplete study, as it does not contain the analysis of one of the most important parameters, the integrity of the DNA molecule. The results from this PhD study show that fragmented DNA analysis in infertile male sperm in percent is significantly higher than in male fertile. Spermatozoids with abnormal morphology and the lower percentage of mobility have more DNA damage to sperm than normal spermatozoa. Our results have provided evidence of a significant relationship between several parameters of ejaculate and fragmented DNA in the sperm. The sperm-fragmented DNA analysis, estimated by Sperm Chromatin Dispersion (SCD), showed a very clear negative relationship between sperm motility and morphology. The control group had a lower percentage of fragmented DNA in the sperm compared with the working group. (Irvine et al., 2000) evaluated the relationship between the parameters of ejaculation and integrity of the fragmented DNA molecule of a group of infertile patients and a group of patients. (Sun et al., 1997) have shown a significant negative correlation between ejaculate parameters and DNA damage to the sperm. However, some studies have shown prognostic and diagnostic limitations of ejaculate parameters for infertile couples, where male infertility diagnosis is still based on the conventional ejaculatory analysis in routine clinical practice (Simon et al., 2010). Our results are consistent with the studies (Sakkas, et al., 1999) that gained high DNA fragmentation values in infertile male sperm groups compared to the male control group. The sperm DNA (Sperm DNA Fragmentation Index) was significantly higher in the group of infertility patients compared to those of the control group. From these results, it can be concluded that the determination of sperm DFI can be used to distinguish infertile males from male fertile (Diallo et al., 2015). Each laboratory technique to analyze fragmented sperm DNA in each androgenic or assisted reproductive technology (ART) should be simple, low cost, and without complex equipment (De Jonge C., 2012).

CONCLUSIONS:

We can conclude that the results of DNA fragmentation fragmented into the sperm are encouraging and can be used for diagnostic purposes in determining male infertility.

Such an assessment of DNA damage to the sperm can serve clinicians to assist spousal couples with infertility in the selection of IVF / ICSI assisted medical support technique.

It is recommended that such an analysis of DNA fragmentation determination in the sperm becomes part of the clinical trials of Andrology laboratories.

Conflicts of interest:

The Authors declare that there are no conflicts of interest.

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