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MALE INFERTILITY AND EJACULATE ENZYMES

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Abstract:

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. 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. 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. 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. Conclusions: It is recommended that such an analysis of DNA fragmentation determination in the sperm becomes part of the clinical trials of Andrology laboratories. The creatine kinase activity evaluation in the seminal plasma constitutes a useful biochemical marker in determining the potential for male fertility.

Key words: Creatine kinase, Sperm DNA fragmentation, Halotech/Halosperm.

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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 [1]. 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 [2]. Infertility is traditionally diagnosed based on a number of standard ejaculate parameters including volume, pH, morphology, mobility and number as recommended by the WHO Manual, which focuses on human ejaculatory examination [3]. Ejaculation routine analysis does not address the integrity of the DNA molecule, which plays a key role in diagnosing male infertility [4]. Research has shown that approximately 15% of infertile males tested have normal spermogram analysis parameters [5]. 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 [6].

Sperm DNA fragmentation:

DNA fragmentation is the separation or breaking of DNA yarns into parts. Increased levels of DNA damage have been associated with multiple infertility [7] which is the most common cause of the disease, increased abortion after assisted reproductive technology (ART) [8] Consequently, diagnostic tests that determine fragmented DNA in the ejaculate are a valid substitute for routine ejaculate analysis. The analysis for the determination of fragmented DNA in the sperm is the best determinant of male infertility [8].

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).

Figure 1. The working reagent for DNA fragmentation in the sperm

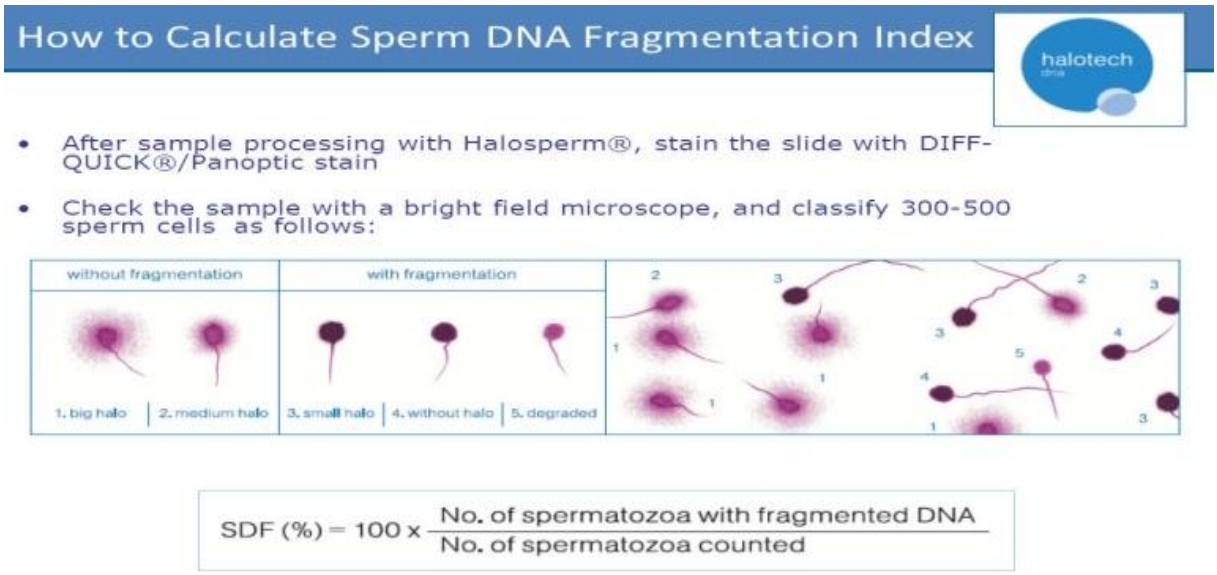

Figure 2. Formul for calculating percentage of fragmented DNA in sperm.

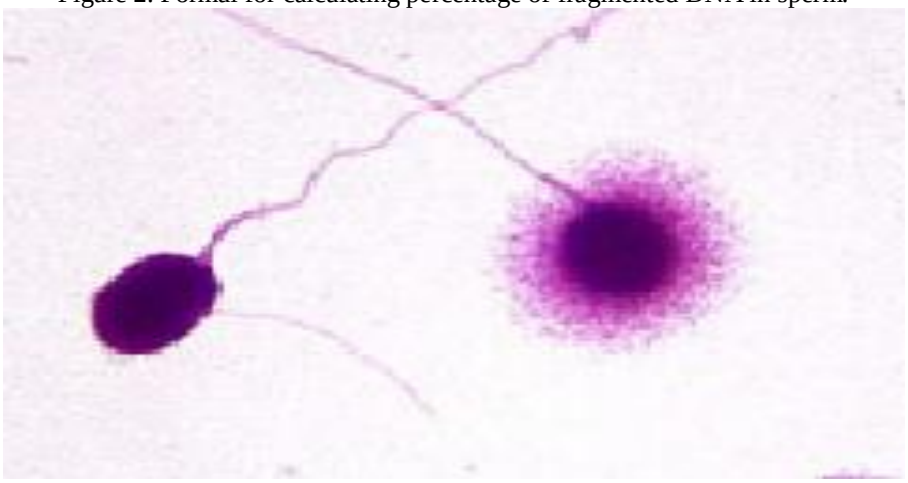

Figure 3. Photograph of a normal DNA sperm and a fragmented DNA sperm

Creatine kinase enzyme analysis in ejaculation:

Determination of the concentration of CK (Creatin kinase) enzyme is done by the photometric Reflotron analyzer (Roche, Germany). After preparing the ejaculate with the swim-up (PBS) ejaculate, 30 microliters of ejaculate were taken and put into the test strip and placed in Reflotron and determined the concentration of this enzyme.

Figure 4. Reflotron

Ejaculation analysis:

The ejaculate samples are collected in a separate room after the abstention of 3-7 days, masturbating in a sterile bottle containing patient records, then immediately settling into the incubator at 37ºC, and after 30-60 minutes semen analysis. Semen analysis was done according to 2010 WHO recommendations (V manual).

Cut-off reference values for semen characteristics as published in consecutive WHO manuals

Semen characteristics	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010
Volume (mL)	ND	≥ 2	≥ 2	≥ 2	≥ 1.5
Sperm count (10^6/mL)	20-200	≥ 20	≥ 20	≥ 20	≥ 15
Total sperm count (10^6)	ND	≥ 40	≥ 40	≥ 40	≥ 39
Total motility (%)	≥ 60	≥ 50	≥ 50	≥ 50	≥ 40
Progressive motility	≥ 2	≥ 25%	≥ 25% (a)	≥ 25% (a)	≥ 32% (a+b)
Vitality (%)	ND	≥ 50	≥ 75	≥ 75	≥ 58
Morphology (%)	80.5	≥ 50	≥ 30	(14)*	≥ 4*
Leukocyte count (10^6/mL)	< 4.7	< 1.0	< 1.0	< 1.0	< 1.0

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

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. Comparison of parameters between the study group and the control group

Parameters	Study group (Mean ± SD)	Control group (Mean ± SD)	Testi	P-value
N	169	88		
Sperm DNA fragmentation (%)	29.96 ± 7.56	14.91 ± 3.88	U'=14543	P < 0.0001
CK (IU/10^8sperm cells)	270.76 ± 92.87	160.39 ± 31.20	U'=13271	P < 0.0001

Study group males had higher Sperm DNA fragmentation values than male control group groups with significant statistical (Mann-Whitney test, U '= 14543, P <0.0001).
Study group males had higher creatine kinase (CK) values compared to men in the control group with significant statistical (Mann-Whitney test, U '= 13271, P <0.0001).

DISCUSSION:

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 [9]. Moreover, in the procedure used with SCD, sperm tails remain intact, allowing sperm cells to be distinguished from other types of cells (eg leukocytes). 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 [10]. Also [11] have found high frequency of fragmented DNA in infertile males (p <.001), the results that match the results obtained in our study. Our results of fragmented DNA analysis in the sperm (p <.001) are close to the results obtained from [12] which have found high frequency of fragmented DNA on a significant scale in infertile male sperm (p <.001). Our results are consistent with the results obtained by [13], which have found high frequency in significant (p <.001), fragmented DNA in ejaculates between the infertile group and fertility. Our results obtained by DNA fragmentation in the sperm (p <.001) are consistent with the results obtained by [14], which have gained value of fragmented DNA analysis on a significant scale (p <.001). The results of our analysis of DNA fragmentation in the sperm (p <.001) are consistent with the results obtained by [15], which have obtained significant (p <.001). The results of our study are consistent with the results obtained from [16], which have gained significant frequency at significant levels of fragmented DNA analysis (p=.001), in sperm of male infertile compared to the control group.

Creatine kinase:

Various studies show how sperm cells work, often associated with increased activity of key enzymes, one of these enzymes is CK (creatine kinase) [17]. Enzymes in the seminal fluid are not directly responsible for the loss of sperm cells but act as biochemical markers for normal differentiation of sperm cells. Our study shows that CK enzyme activity in the seminal fluid represents a valuable biochemical marker in reducing the number of sperm cells, reducing sperm mobility and reducing the fertilising potential of the sperm. High CK enzyme activity in the human seminal fluid is an indicator of cytoplasmic residues in immature spermatozoa cells. Concentration of CK enzyme in the seminal fluid is several times higher than that of serum, indicating that the CK enzyme is locally generated by gonads [18]. Our results are in full compliance with WHO 2010 criteria that describe the normal parameters for the spermogram analysis. The results of this study regarding CK activity in the seminal fluid show that there is a negative correlation between reduced spermogram parameters and CK enzyme activity in the seminal fluid of infertile and fertile patients. Our results show statistically significant differences (p <, 00001) between infertile and fertile male groups. Our results are similar to those published by [19]. The biochemical method used in this study measures the enzymatic activity of CK in the seminal human fluid and is made using the principles of dry chemistry. The biochemical method used in this paper to determine the activity of the CK enzyme gives results in a short, cost-effective and very reliable period. Therefore, the evaluation of CK enzyme activity in the seminal fluid constitutes a useful biochemical marker in determining the fertilising potential of males.

CONCLUSIONS:

Sperm Chromatin Dispersion (SCD) serves to evaluate sperm quality by supplementing the information provided by the conventional ejaculate analysis. 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 sperm DNA (Sperm DNA Fragmentation Index) was significantly significant in the infertility patient group compared to the fertility patient group. 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. The creatine kinase activity evaluation in the seminal plasma constitutes a useful biochemical marker in determining the potential for male fertility. In our study we have gained a high degree of creatine kinase enzyme on a significant scale among the infertile group and the fertile patient group.

Conflicts of interest:
The Authors declare that there are no conflicts of interest.

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