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Male Infertility and Sperm DNA Fragmentation

Afrim Zeqiraj[1*], Sheqibe Beadini[2], Nexhbedin Beadini[2], Hesat Aliu[2], Zafer Gashi[1], Shkelzen Elezaj[3], Sadi Bexheti[4], Agim Shabani[5]

[1]Department of Biochemistry, FAMA Colleague, Prishtina, Kosovo; [2]Department of Biology, University of Tetovo, Republic of Macedonia; [3]Clinic of Urology, Regional Hospital of Peja, Kosovo; [4]Faculty of Medicine, University of Tetovo, Republic of Macedonia; [5]Department of Chemistry, University of Tetovo, Republic of Macedonia

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***Correspondence:** Afrim Zeqiraj. Department of Biochemistry, FAMA Colleague, Prishtina, Kosovo. E-mail: afi_istog@hotmail.com

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Abstract

BACKGROUND: One of the main factors affecting male infertility is DNA fragmentation in sperm. Male infertility is a heterogeneous group of disorders, known causes account for only 30-50%, and unknown cause (idiopathic) constitute the rest. Infertility involves nearly 15% of couples in the reproductive age, and only the male problem involves about 40% of the problems.

AIM: We have studied our DNA damage to sperm cells of a group of infertile males (113 patients) with abnormal sperm parameters (oligoasthenospermia and oligospermia) and a group of male patients (80 patients) with normal semen parameters (normospermia) to document whether the Sperm Chromatin Dispersion (SCD) analysis could increase the information obtained from the sperm routine analysis to explain the causes of infertility.

MATERIALS: A group of 193 patients were analysed, 113 patients in the working group and 80 patients in the control group were screened. The ejaculate samples were taken by the patient to whom the reason for the analysis was explained. All patients were from the Republic of Kosovo. Samples are collected from 2014/2018. Sperm Chromatin Dispersion (SCD) analyses in the ejaculate were analysed by the Biolab Zafi laboratory in Peja.

RESULTS: Clinical data were compared between the two groups by one-way ANOVA, mean ± SD, student's t-test. A p-value of less than P < 0.05% was considered statistically significant. Outcomes: In our study, we have gained significant (P < 0.05) results in the workgroup and the control group across all hormonal parameters, sperm parameters, and fragmented DNA in the sperm.

CONCLUSION: Based on our obtained results we can conclude that DNA fragmentation in spermatozoa is useful in the selection of unsuitable DNA sperm for use in ART methods. We conclude that our DNA fragmentation analysis results are encouraging and can be used for diagnostic purposes in determining male infertility.

Introduction

Male-factor infertility reportedly accounts for 30–40% of cases of couple infertility. Infertility is defined as the inability to achieve a successful pregnancy after 12 months of unprotected intercourse or therapeutic donor insemination (Practice Committee of the American Society for Reproductive Medicine, 2013) [1]. The natural desire of human beings is to propagate their lineage and is part and parcel of human evolution. The inability to conceive and produce a pregnancy results in the depression in couples. This infertility or subfertility forces couples to seek a solution as the problem results in social stigma. One in eight couples encounters problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Three per cent of women remains involuntarily childless, while 6% of parous women are not able to have as many children as they would wish [2]. Male fertility can be reduced as a result of congenital or acquired urogenital abnormalities, malignancies, urogenital tract infections, increased scrotal temperature (e.g. as a consequence of varicocele), endocrine disturbances, genetic abnormalities and immunological factors [2]. In about 15% of infertile male subjects, genetic abnormalities may be present, including chromosome aberrations and single gene mutations [3]. The cause of infertility can be attributed to male, female or due to both the partners and in a significant number the cause of infertility is unexplained. Hence simultaneous evaluation of both male and female partners of the infertile couple should

be done. Appropriate investigative modalities and the proper assisted reproductive procedure adopted will bring a definitive change in partners affected by infertility. Affected male partner of infertile couples should undergo semen analysis as a primary procedure. When the male partner alone is involved in infertility, it can save time and procedures can be accelerated which is also less expensive. One of the main factors affecting male infertility is DNA fragmentation in the sperm.

Male infertility is a heterogeneous group of disorders, the known causes account for only 30-50%, and the unknown cause (idiopathic) is the rest of infertility. It is of great importance to consider the effects of DNA damage on the sperm because half of the DNA in the progeny comes from the father's unit [4]. As a consequence of the complex anatomical and functional integration of the reproductive system, spermatogenesis in the germinal epithelium and the regulatory role of the hypothalamohypophysial-testicular axis are very susceptible; their changes become apparent even in the deterioration of fertility [5]. The hormone responsible for spermatogenesis is LH [6]. This glycoprotein regulates the testosterone synthesis of the extra tubular Leydig cells. FSH controls spermatogenesis by affecting both the germinal epithelium and Sertoli cells [7].

However, LH secretion is regulated by the negative feedback of the testosterone in the vascular system. The serum LH concentration reflects the function of Leydig cells; it is an important factor in the differential diagnosis between primary sociopathy and hypothalamo-hypophyseal hormone deficient [8]. Despite some deficiencies, spermogram analysis is generally acceptable and is considered reliable in evaluating male fertility [9]. Spermogram analysis does not always indicate the quality and sperm health [10]. For the accurate disclosure of genetic information, the integrity of the DNA molecule is essential [11].

The main causes of early pregnancy loss are genetic abnormalities in the sperm genome (EPL) [12]. Independent fertility indicators in couples subject to ART can be used for DFI [13]. The diagnostic test used today (SCD) is a lightweight and fast test based on the sperm chromatin dispersion [14]. Normal sperm creates DNA halo zones [15]. It has been established that if sperm DNA fragmentation exceeds 30%, sperm quality is significantly reduced [16]. Based on the percentage of DNA fragmentation, it is possible to choose the right technique in assisted medical care clinics. Patient selection based on sperm parameters to perform DNA fragmentation analysis is advisable.

We have studied our DNA damage to sperm cells of a group of infertile males (113 patients) with abnormal sperm parameters (oligoasthenospermia and oligospermia) and a group of male patients (80 patients) with normal semen parameters (normospermia) to document whether the Sperm Chromatin Dispersion (SCD) analysis could increase the information obtained from the sperm routine analysis to explain the causes of infertility.

Materials and Methods

There were analysed 193 patients, 113 patients in the working group and 80 patients in the control group. 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.

Sperm analysis was done according to World Health Organization guidelines (WHO, 2010) [17].

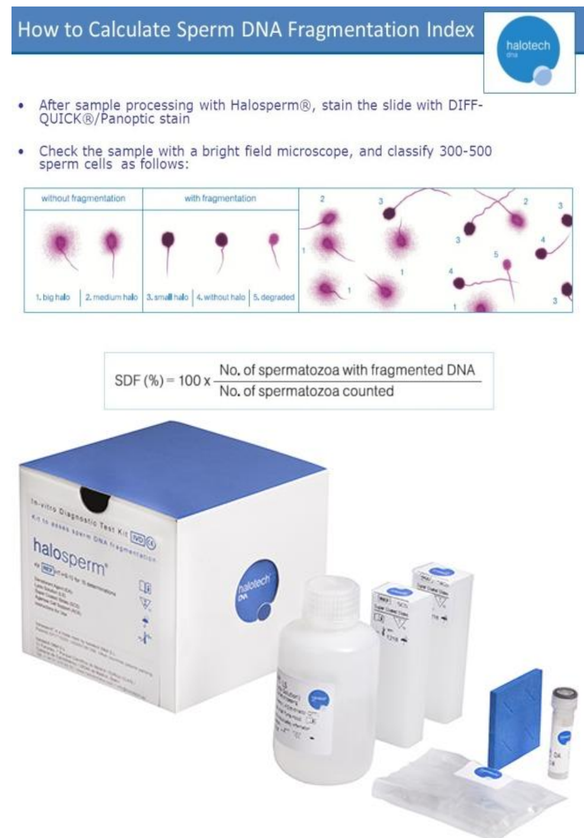

Figure 1: Sperm DNA fragmentation kit

Analysis of hormone levels of FSH, LH, prolactin, testosterone, will be made by ECIA (Eng. electrochemiluminescence immunoassay) in a mini VIDAS ® (bioMérieux, Marcy l'Etoile, France), in the laboratory clinic "BIOLAB-Zafi" Peja, R. of Kosovo.

Clinical data were compared between the two groups by one-way ANOVA, mean ± SD, student's t-test. A p-value of less than $p < 0.05\%$ was considered statistically significant.

Results

From the results obtained after analysing and comparing hormonal parameters in both groups of patients to take analysis (working group and group control) received the following results.

Table 1: Table presentation of the two groups of patients taken for analysis

	Infertility group (113 patients) Average/Std	Fertile group (80 patients) Average/Std	t-test	Significant P < 0.05
FSH	10.35 ± 8.17	6.42 ± 4.12	4.748	p<0.00001
LH	8.14 ± 4.96	4.98 ± 3.15	2.759	p<0.01
Prolactin	16.56 ± 4.61	10.29 ± 8.97	3.543	p<0.00001
Testosterone	2.85 ± 1.78	5.46 ± 3.76	2.754	p<0.001
Number in 1 million sperm	19.67 ± 19.79	61.43 ± 34.47	-8.247	p<0.00001
The general moving	28.29 ± 18.37	57.15 ± 10.83	-10.151	p<0.00001
Movement A	14.09 ± 10.86	25.21 ± 7.46	-7.359	p<0.00001
Movement B+C	14.03 ± 10.88	31.73 ± 10.09	-9.928	p<0.00001
Without moving	71.59 ± 18.34	42.7 ± 10.9	11.554	p<0.00001
Normal morphology	16.90 ± 14.47	42.6 ± 15.2	-9.696	p<0.00001
Abnormal morphology	83.09 ± 14.47	57.2 ± 16.2	8.991	p<0.00001
DNA- fragmentation (SDF%)	34.53 ± 4.68	14.91 ± 4.02	11.476	p<0.00001

The Table 1, shows that all hormones (FSH, LH, prolactin, and testosterone) defined in patients with infertility are at the significantly higher ($p < 0.05$) degree compared with hormones designated the control group.

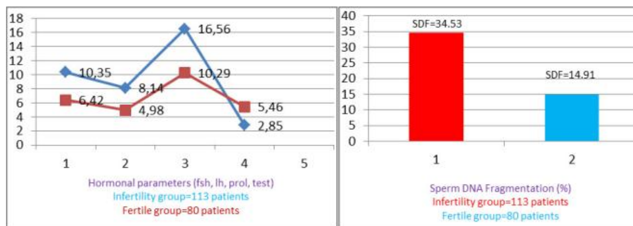

Figure 2: Graphical presentation of Sperm DNA Fragmentation (%)

In our work, we have gained significant results between the working group and control group on all spermogram parameters ($p < 0.001001$), and in DNA fragmentation in the sperm ($p < 0.001001$).

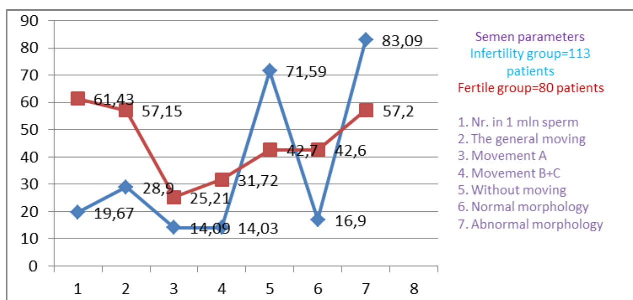

Figure 3: Graphical presentation of sperm parameters between two groups of patients taken for analysis

Discussion

The FSH, LH and testosterone evaluation is useful in the management of male infertility. FSH is necessary for initiation of spermatogenesis and maturation of spermatozoa [18]. In infertile men, a higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage, and was shown to be associated with severe oligozoospermia [19] [20] and reported that elevated levels of serum FSH with increasing severity of seminiferous epithelial destruction.

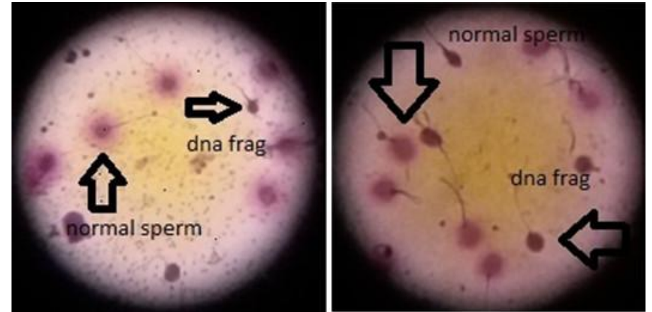

Figure 4: Photo by microscope during Sperm DNA Fragmentation analysis

However, FSH acts directly on the seminiferous tubules whereas LH stimulates spermatogenesis indirectly via testosterone. FSH plays a key role in stimulating mitotic and meiotic DNA synthesis in spermatogonia [21]. The increase in serum levels of gonadotropins might have disrupted the spermatogenic process leading to the decline in the sperm count and infertility [22]. In the present study, elevated serum levels of FSH and LH were observed in oligozoospermia and asthenozoospermia males when compared with normozoospermic men [23]. Also found that the high values of FSH, LH, Testosterone affect the reduction of sperm parameters [24].

From this paper, we have gained higher percentages of DNA than fragmented into infertile male sperm that had abnormal morphology and decreased mobility. Our results are consistent with the studies (Sergerie et al., 2005) [25] that found a DFI in the infertility group was significantly higher than in the fertile group (40.9 ± 14.3% compared to 13.1 ± 7.3%) and the mean sperm concentration in the infertile group was also significantly lower compared to the fertile group ($62.9 \pm 33.2 \times 10^6$/ml compared to $102.4 \pm 66.4 \times 10^6$/ml). DFI can be used to distinguish infertile men from fertile men [26]. A man with DFI sperm ≥ 26.1% has 2.84 times higher risk for infertility than the male with DFI sperm of < 26.1%.

In conclusion, determining Hormones (FSH, LH, Prolactin, Testosterone) of infertile men is a major step towards predicting infertility. Results of hormonal analysis and sperm parameters serve as an indicator

for medical personnel to determine male infertility. From our original work, we can conclude that with the increase of hormone parameters there is a reduction of sperm parameters (total number, total movement, movement A, movement B, normal form, abnormal form) and reduction of male reproductive capacity. We have concluded in our paper that there is a negative correlation between DNA fragmentation, mobility and morphology of sperm in male infertile. Based on our obtained results we can conclude that DNA fragmentation in spermatozoa is useful in the selection of unsuitable DNA sperm for use in ART methods. We conclude that our DNA fragmentation analysis results are encouraging and can be used for diagnostic purposes in determining male infertility.

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