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Frequency of Sperm DNA Fragmentation Index in Infertile Couples With Male Factor Infertility

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Abstract

Objectives: Today, sperm DNA fragmentation is considered as one of the causes of male infertility. The aim of this study was to investigate the frequency of sperm DNA fragmentation index (DFI) in male factor infertility.

Materials and Methods: This cross-sectional study was performed on 100 infertile men referred to Infertility Clinic of Zahedan (Iran) in 2017. Patients were selected using simple random sampling. All sperm parameters were analyzed for the patients. The sperm DFI was determined using Halosperm test and patients were divided into two groups with low sperm DFI (≤30%) and high sperm DFI (>30%).

Results: The mean age and sperm DFI of patients were 34.29 ± 4.18 years and %34.69 ± 19.13, respectively. From these patients, 55% had low DFI (less than 30%) and 45% had a high DFI (greater than 30%). The normal sperm morphology in the group with sperm DFI greater than 30% significantly was lower than that in the group with sperm DFI less than or equal to 30%.

Conclusions: The findings of this study indicated an inverse relationship between sperm DFI and sperm morphology. Conducting DFI tests to examine the anomalies and sperm DNA damage in infertile men is highly recommended.

Keywords: Sperm DNA fragmentation index, DFI, Infertility, Semen analysis, Male factor

Introduction

Infertility is a common disorder defined by the failure to achieve pregnancy after one year, despite unprotected sexual intercourse and without the use of contraception methods. According to the World Health Organization (WHO), failure to achieve pregnancy has involved more than 80 million people worldwide (1), and in Iran, a quarter of couples experience primary infertility during their life, which is higher than the global average. Over the past 50 years, scientists have found that all or part of the 40%-50% of infertility cases are related to male factors (1), however, for more than a century, semen analysis (S/A) has been used as a routine and simple test to assess the fertility of men. In many cases, the sperm parameters (concentration, motility, and morphology) cannot be considered as factors for predicting the male fertility (2), whereas, 15% of infertile men have normal spermograms (3).

Semen analysis is the key laboratory assessment of the partner of an infertile couple. The standard semen analysis measures the following factors (3):

Semen volume and pH, microscopic examination for: 1) Sperm concentration, sperm count, motility, and morphology, 2) Debris and agglutination, 3) Leukocyte count, 4) Immature germ cells.

The causes of male infertility can be divided into four main areas (4):

- Endocrine and systemic disorders, usually related to hypogonadotropic hypogonadism: 2% to 5%
- Primary testicular defects in spermatogenesis: 65% to 80% (of which the majority have idiopathic dysspermatogenesis, an isolated defect in spermatogenesis without an identifiable cause)
- Sperm transport disorders: 5%
- Idiopathic male infertility: 10% to 20%

Therefore, in several studies, the use of sperm function tests has been suggested. Several tests have been proposed based on the physiological and molecular performance of sperm in the fertilization process including the ability for sperm to connect to a transparent layer surrounding the oocyte during the first fertilization stage, the ability of the sperm to penetrate, and in other words, the ability to perform an acrosome reaction, the sperm DNA and sperm chromatin.

The quality of sperm chromatin is an important factor in maintaining the male fertility potential. Sperm DNA provides half of the genetic material to the embryo produced by fertilization; so half of the fertility success rate depends on the transmission of sperm DNA without defect along its pathway from the testes to the fallopian tubes in women, oocyte fertilization, and zygote formation (5).

The chromatin density facilitates the sperm transfer and protects the genome against destructive factors. The compression of DNA and the presence of antioxidants

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in seminal fluid protects the sperm DNA from oxidative damage (6). Approximately 10% of spermatozoa from fertile men and 20% of spermatozoa from infertile men have some degrees of sperm DNA fragmentation.

Environmental and occupational factors, infections of the urinary tract, age, inappropriate diet, the use of some drugs, smoking, alcohol, and so on are considered as effective factors in reducing the integrity of sperm DNA (6).

Fertilization by damaged sperm may reduce the fertility of sperm, increase failure of in vitro fertilization (IVF) cycles, decrease the quality of the embryos in IVF cycles and the fetal growth, and increase the abortion in the early stages of pregnancy (7). There is also a reverse and significant relationship between sperm DNA damage and morula and blastocyst formation, embryonic development, and pregnancy rate (7,8). Reduced DNA integrity also reduces the success rate of artificial reproductive technology including intracytoplasmic sperm injection, IVF, and intrauterine insemination (IUI) (9).

The results of a study on determining the threshold of sperm DNA fragmentation index (DFI) in infertility showed that DFI greater than 30% was significantly associated with infertility (10). A meta-analysis performed on four studies investigated the association between DFI and miscarriage after spontaneous pregnancy or IUI, and showed that the probability of successful fertilization and live birth in cases with sperm DFI lesser than 30% was high (11).

Regarding the presence of normal spermogram in percentage of infertile men and also the role of chromatin quality, especially the importance of sperm DNA fragmentation in preserving the male fertility potential, and the limitation of studies in this area, this study aimed to determine the sperm DNA fragmentation in infertile couples and clarify the necessity of determining sperm DFI in early infertility studies, as well as unsuccessful IVF due to the presence of normal spermograms. In addition, the role of chromatin quality, and in particular, the importance of sperm DNA in maintaining male fertility potential were investigated.

Materials and Methods

All infertile men who had referred to the Infertility Clinic of Ali-ibn-Abitalib hospital in Zahedan from June 2016 to December 2016 entered this cross-sectional study under the ethical code of IR.ZAUMS.REC.1395.47. After giving enough information about the study and the benefits that this study would bring for couples in the future, informed consent was obtained from the participants. The participants then completed the information form and were explained how to collect samples.

The result of the spermogram was recorded for each patient, on the basis of the sperm concentration ($\times 10^6$/mL), and sperm motility and morphology (%) in the information form. The interpretation of the results of the sperm analysis parameters was determined in accordance with the WHO (12) guidelines, both normal and abnormal (Table 1).

The WHO has published lower reference limits for semen analysis. The following parameters represent the generally accepted 5th percentile (lower reference limits and 95% Cls in parentheses), derived from a study of over 1900 men whose partners had a time-to-pregnancy of <12 months:

Volume: 1.5 mL, sperm concentration: 15 million spermatozoa/mL, total sperm number: 39 million spermatozoa per ejaculate, morphology: 4% normal forms using strict Tygerberg method, vitality: 58% live, progressive motility: 32%, total motility (progressive and non-progressive): 40% (Table 1).

The semen samples were collected by masturbation after a 2-5 days sexual abstinence period and were delivered to the laboratory of Ali-ibn-Abitaleb hospital. The percentage of sperm DNA fragmentation was measured by two laboratory staff using the Halosperm test kit. Using this method, the percentage of sperm DNA fragmentation can be checked by the presence of a halo around the sperm core and its size. Then the number of sperms with damaged DNA (sperm nucleus with small halo and no halo) and without damaged DNA (sperm nucleus with large halo and medium halo) were determined. To evaluate the DNA fragility in sperms, 200 cells were counted. The sperm DFI was determined by calculating the fractional percent that was obtained by dividing the number of sperms with DNA fragility by the total number of sperms (total sperm with and without DNA fragility).

The information was recorded in the information form for each participant by the laboratory staff in order to be assigned into two categories during the analysis based on Rafighdoost et al study (13):

1. High level ofsperm DFI (greater than 30%)
2. Low level of sperm DFI (equal to or lower than 30%).

Prevalence, percentage, mean and standard deviation were used as descriptive statistics. The dispersion of samples was investigated by Kolmogorov-Smirnov test. Moreover, to compare the mean of quantitative variables between two groups, independent *t* test (data with normal distribution) and Mann–Whitney U test (data with abnormal distribution) were used. The significance level was considered as $P < 0.05$.

Table 1. Interpretation of the Results of Sperm Analysis According to the WHO 2010 Guidelines

Parameter	Lower Reference Limit
Semen volume (mL)	1.5
Sperm concentration (10^6/mL)	15
Total sperm number (10^6/ejaculate)	39
Progressive motility (PR, %)	32
Total motility (PR+NP, %)	40
Vitality (live sperms, %)	58
Sperm morphology (NF, %)	4
pH	≥7.2
Leukocyte (10^6/mL)	<1
MAR/Immunobead test (%)	<50

Results

A total of 100 couples with male factor infertility (regardless of whether or not they had female factor infertility), who had referred to the Infertility Clinic of Ali-ibn-Abitalib hospital of Zahedan during a 6-month period (from June 2016 to December 2016), were studied. Inclusion criteria were: history of infertility and abnormal semen analysis. While exclusion criteria were: Klinefelter syndrome, X deletion syndrome, hypogonadotropic hypogonadism, and cryptorchidism. The mean age of patients was 34.21 ± 4.14 and the mean sperm DFI was 34.69 ± 19.13%. In addition, 45 patients (45%) had sperm DFI greater than 30%. Semen analysis was also done. The results of this study showed that the mean age, sperm concentration, normal morphology, and sperm motility in patients with DFI> 30% were lower than that in patients with DFI < 30%. This difference was statistically significant only for normal morphology (Table 2).

Discussion

Around 15% to 20% of couples are infertile. Furthermore, 30%-40% of the infertility cases are related to male factor infertility. The majority of male infertility is associated with abnormal sperm parameters. On the other hand, the health of sperm DNA is one of the factors that directly affects the quality and quantity of sperm. An increased DNA damage is associated with infertility.

Sperm selection based on normal parameters (concentration, morphology, and motility) cannot indicate the health of sperm DNA. This study was conducted to investigate the frequency of sperm DFI in infertile men. In this study, DFI was evaluated by Halosperm test and an acceptable threshold of 30% was considered. The mean and standard deviation of DFI in the study participants was 34.69 ± 19.13%, and sperm DFI was greater than 30% in 45 patients (45%).

The results of this study revealed that the mean age of patients in the sperm DFI group > 30% was higher than that of patients with sperm DFI <30%. Additionally, mean concentration, normal morphology, and sperm motility were lower in patients with DFI> 30% compared to the patients with DFI <30%. However, statistical tests did not show a significant difference between the two groups except for normal sperm morphology.

Rafighdoost et al found a significant reverse relationship between DFI and the sperm parameters of concentration, mobility, and morphology (13).

Oleszczuk et al reported that sperm DFI was higher than 30% in over 8.4% of infertile men, whereas it was reported for 45% of infertile men in this study (14). In a study by Tandara et al, this rate was 28%, which was lower than the present study (15). Moreover, the results of Heidari et al in Tehran showed that the mean sperm DFI in infertile men (73.77%) was higher than that of the present study (16). Perhaps this difference in results is related to the difference in the method used for measuring sperm DFI and the effect of environmental and genetic factors on the extent of sperm DNA damage.

Changes including the function of the testicles, the glands and sex hormones, the production of semen fluid, sexual activity, fertility and sperm DNA damage occur in the male reproductive system with increasing age. Kühnert and Nieschlag investigated the fertility changes with age and found that fertility rate in men reduces after the third decade of life (17). Moreover, sperm DNA fragility has been reported to be higher in men older than 35 years than in young men. It has been shown that aging influences sperm chromatin integrity and consequently increases sperm DNA damage (18). The results of a study by Das et al also showed a positive significant relationship between age and sperm DNA fragmentation (19). Although in the present study, patients with high DFI were older, unlike previous studies, no significant association was found between sperm DNA damage and age. Perhaps this result could be due to the young age of the patients in the present study or the difference in the normal and abnormal DFI threshold in various researches.

Many researchers believe that morphology is a predictive factor for fertility (20). The results of this study showed a significant inverse correlation between normal morphology and sperm DFI. Normal morphology also appears to represent normal function, and any factor that contributes to DNA damage, could affect sperm morphology. As a result, increased DNA damage would probably be accompanied by poor sperm morphology. In infertile men with reduced normal morphology, the increase in the DNA damage should, therefore, be considered. In this respect, the administration of antioxidants to these patients, with the aim of resolving one of the main causes of sperm DNA damage, can

Table 2. Comparison of Age and Semen Parameters of Patients Based on Sperm DNA Fragmentation Index

Variable	Number	DFI	Mean	Standard Deviation	Max	Min	*P* value
Age	45	DFI >30 %	34.91	4.47	44	25	0.18*
	55	DFI ≤ 30%	33.63	5.03	45	19	
Concentration	45	DFI >30 %	21.04	13.55	50	4	0.07**
	55	DFI ≤ 30%	29.40	22.09	90	3	
Morphology	45	DFI >30 %	18.88	13.13	60	5	0.04
	55	DFI ≤ 30%	22.90	11.85	50	5	
Motility	45	DFI >30 %	44	17.17	70	20	0.1
	55	DFI ≤ 30%	49.27	14.02	75	20	

* Dependent t test; **Mann–Whitney U test.

increase the fertility chances.

The results of this study and its comparison with the results of most of the similar studies indicated that there was a correlation between sperm DFI and spermogram parameters, however it seems that differences in sample volume, age of patients, genetic and environmental factors, performing semen analysis by different laboratories, the use of different methods for measuring sperm DFI and differences in sperm morphology threshold, mobility, and threshold sperm DFI in comparison with the results of other studies should be approved by further studies.

The results of this study indicated the high rate of sperm DFI in infertile men. The higher levels of sperm DFI were also associated with quantitative and qualitative reduction in spermogram parameters, especially normal morphology.

Therefore, it can be concluded that men with higher sperm DNA damage represent poor results in sperm analysis; this necessitates doing the sperm DFI in infertile men with abnormal spermogram results (20,21).

Conflict of Interests
None.

Ethical Issues
This research was approved by the Ethics Committee of Zahedan University of Medical Sciences under the ethical code of IR.ZAUMS.REC.1395.4.

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References
1. Rowe PJ, Comhaire FH, Hargreave TB, Mahmoud AM. WHO manual for the standardized investigation and diagnosis of the infertile male: Cambridge university press; 2000. https://apps.who.int/iris/handle/10665/42437.
2. Sakkas D, Tomlinson M. Assessment of sperm competence. Semin Reprod Med. 2000;18:133-140. doi:10.1055/s-2000-12552
3. Lewis SE, Agbaje I, Alvarez J. Sperm DNA tests as useful adjuncts to semen analysis. Syst Biol Reprod Med. 2008; 54(3):111-125. doi: 10.1080/19396360801957739
4. Oehninger S. Strategies for the infertile man. Semin Reprod. Med. 2001;19:231-7.
5. Ali AA, Bilodeau JF, Sirard MA. Antioxidant requirements for bovine oocytes varies during inVitro maturation, fertilization and development. Theriogenology. 2003;59(3-4):939-949. doi: 10.1016/s0093-691x(02)01125-1
6. Yang HW, Hwang KJ, Kwon HC, Kim HS, Choi KW, Oh KS. Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos. Hum Reprod. 1998;13(4):998-1002. doi: 10.1093/humrep/13.4.998
7. Donnelly ET, McClure N, Lewis SE. The effect of ascorbate and alpha-tocopherol supplementation in vitro on DNA integrity and hydrogen peroxide-induced DNA damage in human spermatozoa. Mutagenesis; 1999:505-12. doi: 10.1093/mutage/14.5.505
8. Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod. 2004;19(3):611-615. doi: 10.1093/humrep/deh127
9. BungumM, Humaidan P, Axmon A, SpanoM, BungumL, Erenpreiss J, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod. 2007;22:174-9. doi: 10.1093/humrep/del326
10. Evenson DP, Jost LK, Marshall D, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod. 2009;14(4):1039–1049. doi: 10.1093/humrep/14.4.1039
11. Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril. 2000;73(1):43–50. doi: 10.1016/s0015-0282(99)00462-8
12. World Health Organization: WHO laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge University Press; 1999.
13. Rafighdoost H, Farsi MM, Javadi M, Khafri, S. Relationship between sperm parameters and DNA Fragmentation using a Halosperm kit. Anatomical Sciences Journal 2013;10(2):79-85.
14. Oleszczuk K, Augustinsson L, Bayat N, Giwercman A, Bungum M. Prevalence of high DNA fragmentation index in male partners of unexplained infertile couples. Andrology. 2013;1:357-360. doi: 10.1111/j.2047-2927.2012.00041.x
15. Tandara M, Bajić A, Tandara L, Šunj M, Jurišić Z, Jukić M. Correlation between proportions of sperm with DNA fragmentation assessed by Halosperm test and values of standard quality parameters of semen and possible impact on embryo quality. Zdrav Vestn. 2013;82:298-307.
16. Heidari M, Salehi M, Akbari H, Allahezn A, Novin M, Ghaffari H. Evaluation role of sperm parameters and disturbies of sperm chromatin with infertility by CASA and chromatin diffraction test. Investiagation of Medicine. 2012;4(36):176-82. (Persian).
17. Kühnert B, Nieschlag E. Reproductive functiones of ageing male. Hum Reprod. 2004;10:327–339. doi: 10.1093/humupd/dmh030
18. Plastira K, Msaouel P, Angelopoulou R, et al. The effects of age on DNA fragmentation, chromatin packaging and conventional semen parameters in spermatozoa of oligo astheno teratozoospermic patients. J Assist Reprod Genet. 2007;24:437–443. doi: 10.1007/s10815-007-9162-5
19. Das M, Naif AH, San-Gabriel M, et al. High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age. J Assist Reprod Genet. 2013;30:843–848. doi: 10.1007/s10815-013-0015-0
20. Behnoud N, Bahrami R, Kordafshari G, Farzaneh, F., Kenari HM. Management of early menopause using traditional Persian medicine. Int J Womens Health Reprod Sci. 2019;7(2):231-236. doi: 10.15296/ijwhr.2019.39
21. Behnoud N, Farzaneh F, Ershadi S. The effect of clomiphene citrate versus letrozole on pregnancy rate in women with polycystic ovary syndrome: a randomized clinical trial. Crescent J Med Biol Sci. 2019;6(3):335-340.