

Correlation of Sperm DNA Fragmentation Index With Semen Parameters, Lifestyle and Clinical Pregnancy Outcome After Intracytoplasmic Injection

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Abstract

Objective: This study aimed to measure the correlation of sperm DNA fragmentation with semen parameters, lifestyle, and fertility outcomes after intracytoplasmic injection (ICSI).

Materials and methods: The partners who were candidates for ICSI with a history of one In vitro fertilization (IVF) failure or male factor were recruited in the study. Semen parameters including sperm count, motility, and morphology as well as DNA fragmentation index (DFI) (that were divided into 2 groups as high (>15%), and low (\leq 15%) fragmentation scales) were evaluated either. The correlation of DFI with semen parameters, lifestyle, and clinical pregnancy after ICSI were compared between groups.

Results: In 120 included couples, 59 men (49.2%) had DFIs \leq 15% and 61 (50.8%) cases had DFIs >15%. In the group with higher DFI, abnormal morphology ($p=0.010$) was higher whereas, progressive motility ($p=0.001$), total motility ($p<0.001$), and total count ($p<0.001$) of sperm were significantly lower. In addition, the DFI was significantly higher in the subgroup of male infertility (0.012). Logistic regression showed that a lower risk of DFI>15% was associated with higher values of progressive motility (OR=0.97, $p=0.001$), total motility (OR=0.96, $p<0.001$), count (OR=0.96, $p<0.001$) and even clinical pregnancy (OR=0.27, $p=0.011$). However, a history of testicular surgery was associated with a higher risk of DFI>15% (OR=3.37, $p=0.046$). Although no correlation was found between male age and lifestyle components with DFI, the number of embryos was lower in DFI \geq 15% ($p<0.001$).

Conclusion: DFI provide a clinically important measurement of sperm quality and have an impact on IVF outcomes; however, lifestyle components may not correlate with DFI.

Keywords: Sperm DNA Fragmentation Index; Assisted Reproductive Technology; Male Infertility; Embryo Quality; Semen Analysis

Introduction

Infertility is a globally personal and social issue

affecting 10-15% of couples of reproductive age (1). Approximately 50% of infertile couples have both male and female etiologies for infertility and about 20% are due to "purely male factor" (2). Currently, male infertility is mainly evaluated through

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traditional semen analysis, which includes volume, concentration, vitality, and morphology; however, infertility is diagnosed in approximately 15% of men with a normal semen analysis. Based on this, it seems that semen analysis alone can only provide limited information about male fertility, and it does not fully reflect potential sperm fertilization. It is crucial to develop better clinical indicators to determine the status of male infertility and its effects on reproduction (3).

In recent years, the molecular mechanism of male infertility and sperm DNA integrity has revived interest in sperm function tests. The impaired apoptotic process of the spermatogenic cells may lead to the fragmentation of the double-stranded DNA of the sperm (4). These structural changes may occur to ambient stresses such as chemical agents, infections, radiations, oxidative stress and high temperature (5). The development of sperm DNA fragmentation (SDF) assays which calculate the proportion of cells with DNA damage or fragmentation and the current evidence regarding the role of sperm DNA integrity on reproductive outcomes are opening a new horizon in clinical andrology (6).

Several procedures are introduced for measuring DFI and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is a method of choice using a bench flow cytometer (7). The accepted cut off for DFI is below 15% in some studies and higher DFI may associate with impairment in all seminal parameters (8-10).

Several studies have demonstrated that SDF assessment is a valuable tool for assessing male infertility (11), although the predictive value for outcomes of assisted reproductive technology (ART) remains controversial (12, 13).

This study aimed to evaluate the relationship between SDF and semen analysis parameters, as well as lifestyle factors such as age, cigarette smoking, alcohol consumption, male environmental chemical exposure, occupational heat exposure, and male sedentary job and ICSI outcomes to investigate the value of SDF in assessing male fertility and the impact of its associated factors on pregnancy outcomes following ART.

Materials and methods

Study design: This cross-sectional study was recruited from March 2020 to March 2021 on infertile couples candidates for ICSI at the IVF Unit of an academic hospital.

Eligibility criteria: The infertile couples between 18-42 years old (men and women) old who were candidates for ICSI were recruited in the study. The inclusion criterion was one previous IVF failure due to embryonic arrest. The cases who were candidates for ICSI due to poor-quality oocytes, (preimplantation genetic testing) PGT candidates were excluded from the study (14). Moreover, males who had previously undergone surgeries on their genitalia were also excluded from the study.

Semen analysis: Human semen was examined and processed following the WHO laboratory manual (15). The male collected the semen in a sterile container by masturbating three to seven days after abstinence. Following semen liquefaction, 10 milliliters of the sample were taken and counted in a Makler chamber (Sefi medical instruments), and sperm concentrations and viabilities were recorded. Following Kruger's strict criteria, sperm morphology assessment (SMA) was carried out using H-E staining analysis within 1 hour after sperm collection.

DFI detection: The detection of DNA fragmentation index (DFI) was based on the terminal-deoxynucleotidyl-transferase (TdT)-mediated dUTP nick end labelling (TUNEL) method. This process involved assessing the vulnerability of sperm DNA to fragmentation by staining the sperm with a fluorescent dye called acridine orange, without the need for prior denaturation of the sperm chromatin. Subsequently, the sperm DFI was determined by measuring the fluorescence signals using a flow cytometer. During this analysis, the single-stranded DNA fragments, when combined with acridine orange, emitted red fluorescence. Conversely, the intact double-stranded DNA emitted green fluorescence when combined with acridine orange. At least 5,000 sperm were counted with flow cytometry. The proportion of the sperm with red fluorescence of the total number of sperms was calculated as DFI (16).

ART protocol: An antagonist regimen was used for ovulation induction. The initial regimen contained recombinant human follicle stimulating hormone Cinnal-f (Cinnagen Co., Iran), and the amount of gonadotropin was adjusted according to the follicular development, which was monitored by vaginal ultrasound and estradiol levels. When at least 2 dominant follicles reached an average diameter of at least 17 mm, as monitored by ultrasound, gonadotropin usage was stopped, and 10,000 IU of Ovidrel (choriogonadotropin alfa) (manufactured by Merck Global) was injected intramuscularly. At

35-37 h after injection, eggs were collected with the guidance of vaginal ultrasound. After oocyte injection with spermatozoa, embryos were cultured to day 3, and embryonic development was monitored. Good quality embryos were extracted and frozen for further transfer (17).

In one or two cycles, either natural menstruation or progesterone-induced withdrawal bleeding, the administration of estradiol valerate (2 mg RPSI) was initiated on the second or third day of menstruation. Endometrial thickness was assessed by ultrasound. Vaginal progesterone supplements (Fertigest 400MG Supp) were added if endometrial thickness reached ≥ 7 mm. Embryo transfer was planned on the fourth day of progesterone administration.

Outcome measures: A questionnaire was designed to collect the couple's demographic information, duration of infertility, and male lifestyle characteristics including chemical and heat exposure, sedentary jobs, tobacco and alcohol habit as well as clinical varicocele and the history of varicolectomy. According to the National Health Interview Survey (NHIS), a smoker was defined as someone who smokes more than 100 cigarettes during their lifetime (18). A question about alcohol consumption was asked to assess whether male participants had a history of consuming alcoholic beverages. Answer options were "Yes" or "No." Exposure to pesticides and herbicides such as dichlorodiphenyltrichloroethane (DDT), dibromochloropropane (DBCP), organophosphates and atrazine, endocrine disrupting compounds including plastic compounds phthalates and bisphenol A (BPA), heavy metals, natural gas/oil, non-ionizing radiation were asked to determine whether they had a history of environmental chemical exposure: "Have you ever been exposed to any of the above conditions?" The response options were "Yes" or "No."

By definition of the U.S. Department of Labor's Dictionary of Occupational Titles, sedentary jobs were those in which employees lift no more than ten pounds per day. An employee with a sedentary job is likely to carry only small items. In addition, sedentary jobs can be performed sitting down. It is generally considered sedentary to have a job that requires you to sit for 6 of your 8 hours a day. Walking or standing for an extended period is not considered sedentary work.

The primary outcome was comparing clinical pregnancy that was confirmed by intrauterine pregnancy with or without a normal fetal heart rate

seen on ultrasound 7 weeks after embryo transfer (19). The secondary outcomes were evaluating the relationship between DFI and age, lifestyle and routine semen parameters.

Ethical approval: This study has been approved by the Medical Ethics Committee of Tehran university of medical sciences (Reference Number: IR.TUMS.MEDICINE.REC.1400.1134). All authors adhered to the 1975 Declaration of Helsinki and its next revisions. All participants signed and agreed with informed consent.

Statistical analysis: Data analysis was performed by software SPSS version 24. Mean and standard deviation (SD) were used to describe quantitative data indices and in describing qualitative data frequency (%) was used. The Kolmogorov-Smirnov test was utilized to evaluate the normality of the data. Variables such as male age, BMI, duration of infertility, abnormal sperm morphology, progressive sperm motility, total sperm count, and the number of embryos did not exhibit a normal distribution in the study. The two groups were assessed by Mann-Whitney U. The chi-square test or Fisher's exact test was employed for comparing categorical variables. The Spearman correlation coefficient was utilized to assess the relationship between variables. In addition, both univariate and multivariate logistic regression analyses were employed to assess the impact of associated factors on DFI. The significance level was determined to be statistically significant, with a value below 0.05.

Results

In a study involving 120 men, the distribution of cases with respect to DFI was as follows: 59 cases (49.2%) had DFIs less than or equal to 15%, while 61 cases (50.8%) had DFIs greater than 15%. The characteristics of the male participants, along with their clinical data and laboratory test results, are summarized in Table 1. Upon analyzing the data, it was found that there were no significant differences in terms of male age ($p=0.394$) and BMI ($p=0.431$) between the two groups. However, notable differences were observed between the groups in relation to several factors.

These factors included abnormal sperm morphology ($p=0.010$), progressive motility ($p=0.001$), total motility ($p<0.001$), and total count ($p<0.001$) of sperm. Additionally, it was found that the DFI was significantly higher in cases where male factor infertility was identified ($p=0.012$).

Table 1: Patient characteristics by DFI category

Variables		Median (Q1, Q3)/n (%)			P value
		Total (N=120)	DFI≤15% (N=59)	DFI>15% (N=61)	
Male age		38.0 (33.8, 42.0)	35.0 (32.0, 42.0)	38.0 (35.0, 42.0)	0.394
Body mass index		27.6 (4.4)	27.1 (4.3)	28.1 (5.1)	0.431
Duration of infertility (year)		5.0 (4.0, 7.0)	5.0 (4.0, 7.0)	5.0 (4.0, 8.0)	0.480
Sperm abnormal morphology		94.0 (87.0, 97.0)	90.0 (85.0, 95.0)	95.0 (90.0, 98.0)	0.010
Sperm progressive motility		44.0 (20.0, 55.3)	50.0 (36.5, 60.0)	36.0 (20.0, 50.0)	0.001
Sperm total motility		54.0 (32.0, 68.0)	62.0 (49.5, 76.0)	46.0 (29.8, 58.5)	<0.001
Sperm count		23.0 (12.0, 40.0)	32.0 (20.0, 50.0)	15.0 (10.0, 30.0)	<0.001
Number of embryos		4.0 (3.0, 6.0)	5.0 (4.0, 7.0)	4.0 (2.0, 5.0)	0.012
Type of infertility	Primary	110 (91.67)	55 (93.22)	55 (90.16)	0.743
	Secondary	10 (8.33)	4 (6.78)	6 (9.84)	
Cause of infertility	Male	52 (43.33)	19 (32.20)	33 (54.10)	<0.001
	Female	36 (30.00)	29 (49.15)	7 (11.48)	
	Male + female	32 (26.67)	11 (18.64)	21 (34.43)	
Environmental toxins	No	106 (88.33)	54 (91.53)	52 (85.25)	0.396
	Yes	14 (11.67)	5 (8.47)	9 (14.75)	
Occupational heat exposure	No	99 (82.50)	49 (83.05)	50 (81.97)	1.000
	Yes	21 (17.50)	10 (16.95)	11 (18.03)	
Sedentary work	No	93 (77.50)	48 (81.36)	45 (73.77)	0.384
	Yes	27 (22.50)	11 (18.64)	16 (26.23)	
Disease history - Male	No	94 (78.33)	44 (74.58)	50 (81.97)	1.000
	Yes	14 (11.67)	7 (11.86)	7 (11.48)	
Varicocele	No	90 (75.00)	48 (81.36)	42 (68.85)	0.141
	Yes	30 (25.00)	11 (18.64)	19 (31.15)	
Testicular surgery	No	104 (86.67)	55 (93.22)	49 (80.33)	0.058
	Yes	16 (13.33)	4 (6.78)	12 (19.67)	
Use of medication	No	111 (92.50)	54 (91.53)	57 (93.44)	1.000
	Yes	8 (6.67)	4 (6.78)	4 (6.56)	
Tobacco use - Male	No	91 (75.83)	45 (76.27)	46 (75.41)	0.831
	Yes	28 (23.33)	13 (22.03)	15 (24.59)	
Alcohol use- Male	No	110 (91.67)	53 (89.83)	57 (93.44)	0.526
	Yes	10 (8.33)	6 (10.17)	4 (6.56)	

DFI: DNA fragmentation index

Moreover, the number of embryos was lower in cases where DFI > 15% ($p < 0.001$). No significant differences were found between the two groups in terms of lifestyle factors.

In the next step, the impact of demographic variables, clinical data, and laboratory tests on the DFI category is presented. According to the univariate analyses, lower odds of DFI>15% versus DFI≤15% were significantly associated with the higher values of progressive motility (B: 0.97, SE: 0.95-0.99), total motility (B: 0.96, SE: 0.94-0.98), total count (B: 0.96, SE: 0.94-0.98), number of embryos (B: 0.79, SE: 0.66-0.94), cause of infertility (Female vs. Male, OR: 0.14, 95% CI: 0.05-0.38), and clinical pregnancy (OR: 0.27, 95% CI: 0.09-0.71).

Based on the multivariate analyses, the adjusted impact of total motility ($P=0.028$), total count

($P=0.016$), and number of embryos ($P=0.043$) on the odds of DFI > 15% was significant (Table 2).

Table 3 shows the Spearman correlation coefficient between the DFI score and numeric variables. A positive and significant correlation ($r=0.29$, $p=0.001$) was found between DFI and abnormal morphology. However, the DFI score was negatively correlated with variables including progressive motility ($r=-0.32$, $p < 0.001$), total motility ($r=-0.37$, $p < 0.001$), and total count ($r=-0.41$, $p < 0.001$).

Discussion

In this study, half of the men had DFI >15%. Also, the semen parameters, including sperm concentration, and progressive and total sperm motility were significantly higher in high DFI, but no correlation was found among DFI, lifestyle components, and the number of embryos.

Table 2: The impact of variables on DFI

Variables	Univariate		Multivariate	
	B coefficient (SE)/ Odds ratio (95% CI)	P-value	B coefficient (SE)	P-value
Male age* (year)	1.03 (0.97-1.09)	0.381		
Duration of infertility* (year)	1.04 (0.95-1.16)	0.423		
Sperm abnormal morphology*	1.05 (1.00-1.11)	0.060		
Sperm progressive motility*	0.97 (0.95-0.99)	0.001		
Sperm total motility*	0.96 (0.94-0.98)	<0.001	0.98 (0.95-0.99)	0.028
Sperm count*	0.96 (0.94-0.98)	<0.001	0.97 (0.95-0.99)	0.016
Number of embryos*	0.79 (0.66-0.94)	0.010	0.80 (0.64-0.99)	0.043
Cause of infertility**				
Male	Ref			
Female	0.14 (0.05-0.38)	<0.001		
Male+female	1.10 (0.44-2.77)	0.841		
Environmental toxins**	1.87 (0.59-5.95)	0.290		
Occupational heat exposure**	1.03 (0.42-2.77)	0.876		
Sedentary work**	1.55 (0.65-3.70)	0.322		
Disease history–Male**	1.97 (0.86-4.74)	0.117		
Varicocele**	1.97 (0.86-4.74)	0.117		
Testicular surgery**	3.37 (1.09-12.67)	0.046		
Use of medication**	0.95 (0.21-4.19)	0.941		
Tobacco use- Male**	1.13 (0.48-2.67)	0.780		
Alcohol use- Male**	0.62 (0.15-2.29)	0.477		
Clinical pregnancy**	0.27 (0.09-0.71)	0.011		

DFI: DNA fragmentation index, CI: Confidence interval, SE: standard error, *B coefficient (SE), **Odds ratio (95% CI)

Table 3: The correlation between variables and DFI

Variables	Spearman's rho (95% CI)	P value
Male age	0.13 (-0.05, 0.31)	0.146
Duration of infertility (year)	0.15 (-0.04, 0.33)	0.104
Sperm abnormal morphology	0.29 (0.12, 0.45)	0.001
Sperm progressive motility	-0.32 (-0.48, -0.14)	<0.001
Sperm total motility	-0.37 (-0.52, -0.20)	<0.001
Sperm total count	-0.41 (-0.55, -0.24)	<0.001
Number of embryos	-0.12 (-0.30, 0.07)	0.198

In this study, lower values of progressive motility, total motility, count, and number of embryos, as well as testicular surgery, and lack of clinical pregnancy were potential risk factors for risk of higher DFI.

The sperm DFI is used to assess the DNA damage and directly reflects the degree of sperm DNA destruction. Damage to sperm chromatin can directly affect the sperm's normal function (12) that was seen in our study that most of the semen parameters were disturbed in higher DFI. The inflammation in the external genital tracts and varicocele can also increase the risks of SDF by inducing reactive oxygen species in the sperm (20). We also see a positive correlation between high DFI and varicocele but this finding was not significant.

Yong et al. showed that DFI rises significantly with age, and unhealthy lifestyle habits such as smoking and alcohol consumption can lead to an elevated risk of DFI (21). In our study, lifestyle indicators and men's age were not correlated with higher DFI, but probably due to the small sample size, the differences were not significant.

High sperm DFI is not only related to reduced fertilization rate and poor embryo quality in IVF but also it is associated with decreased rates of pregnancy (22). Although, the study by Niu et al. indicated that high DFIs affect embryo quality but have no impact on oocyte fertilization rates or pregnancy outcomes following IVF (23). Also, Yang et al. did not find a correlation between DFI and clinical pregnancy, early abortion, oocyte fertilization, or good-quality embryos in IVF or ICSI (21). We also did not find a correlation between DFI and the number of embryos.

It has been reported that among infertile couples, DFI is associated with the rate of pregnancy, and improving DFI can increase the likelihood of achieving pregnancy (24). Additionally, Wdowiak et al. suggested that the rate of DFI growth is a valuable predictor of the pregnancy rate in couples undergoing intracytoplasmic sperm injection (ICSI) (25).

The limitation of our study was the small sample

size which may lead to research bias, especially in the comparison of pregnancy and early abortion rates. In future studies, the sample size should be expanded, and the male and female subjects' various interference indicators should be matched and grouped strictly, to allow more accurate conclusions.

Conclusion

Overall, the study confirms that DFI provides a clinically important measurement of sperm quality and may have an impact on IVF outcomes.

Conflict of Interests

Authors declare no conflict of interests.

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