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Original Article

Relationship Between Vitamin D Level and DNA Fragmentation Index of Sperm in Men Referred to Omid Infertility Center in Hamadan-Iran: A Cross-Sectional Study

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Abstract

Objective: Unfortunately, the prevalence of infertility in Iran and the world is increasing. There is limited research on the relationship between vitamin D and DNA fragmentation index (DFI). DFI is a measure of DNA damage in sperm cells and is used to evaluate male fertility. Some studies suggest that vitamin D may play a role in sperm health and fertility.

Materials and methods: In this cross-sectional study, 789 eligible men referred to the Omid Infertility Clinic in Hamadan-Iran in 2021-2023. Serum levels of vitamin D, DFI, spermogram indexes and demographic characteristics (occupation, age, and body mass index) were collected by face-to-face interview and records review in case files. Data were analyzed with SPSS version 27 software.

Results: There is no significant relationship between DFI and vitamin D before and after removing the effect of confounding variables. Clearly, increasing age has been associated with increasing (DFI 95% CI: 1.06-3.03; $p<0.05$) odds ratio (OR) = 1.79. The relationship between the increase in DFI and the decrease in normal sperm morphology (95% CI: 1.43-4.20; $p<0.01$) OR = 2.45 and the decrease in the progressive motility of sperm was seen (95% CI: 1.66-4.87; $p<0.05$) OR= 2.85 and also. The relationship between DFI and the decrease in sperm count after removing the effect Confounding variables were observed (95%CI: 1.06-8.38; $p<0.05$) OR=2.98.

Conclusion: No correlation between DFI and vitamin D serum level was found. However, a clear association was observed between increased DFI and advanced age, reduced progressive motility, abnormal sperm morphology, and decreased sperm count.

Keywords: DNA Fragmentation Index; Vitamin D; Spermogram

Introduction

Couples who fail to conceive after one year of unprotected intercourse are defined as infertile. About 15% of couples are infertile, and 50% of these cases are due to male infertility (1). Many factors are associated with male infertility, including anatomical abnormalities, varicoceles, oxidative stress, systemic

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diseases, endocrine disorders, and infections (2). Understanding the mechanisms underlying male infertility remains a significant challenge. Currently, semen analysis is the gold standard laboratory technique. This simple test evaluates several important parameters, including sperm motility, morphology, sperm concentration, liquefaction time, and semen volume (2-4). However, despite semen analysis and the advanced technique of intracytoplasmic sperm injection (ICSI), the issue of male infertility remains unresolved (5). Therefore, male factors should be better identified at the molecular level in assisted reproductive technology (ART) clinics to improve the live birth rate in ART. New molecular parameters are needed to provide more information about sperm quality, allowing for a more accurate assessment of sperm's ability to fertilize oocytes and support embryo development for a successful pregnancy (6). Thus, additional tests are recommended for male infertility, including the evaluation of anti-sperm antibodies, acrosomal reaction, sperm hyperactivation, penetration in the zona pellucida, and sperm DNA damage and fragmentation (7).

DNA fragmentation refers to damage or breakage of the sperm’s genetic material, which cannot be detected through routine semen analysis. This damage can result from intrinsic factors such as defective sperm maturation or increased oxidative stress, as well as external factors like environmental pollution, smoking, prior chemotherapy, elevated testicular temperature, and endocrine-disrupting compounds (7, 8). Human sperm chromatin is highly susceptible to structural changes during spermatogenesis, leading to DNA strand breaks (9). Sperm chromatin is repaired during the early stages of spermatogenesis, whereas in mature spermatids, this ability is highly limited. The strong chromatin condensation and reduction of cytoplasmic components prevent effective DNA repair (10, 11).

During fertilization, the genetic information in the sperm's DNA encounters and integrates with the oocyte's DNA. This chromatin integrity is essential for the accurate transmission of genetic information, which is crucial for embryonic development, implantation, and pregnancy (12-14). Sperm chromatin fragmentation appears to be associated with a reduced reproductive capacity of human sperm. A higher percentage of DNA damage is observed in semen samples from infertile patients (15). Sperm chromatin fragmentation is associated with apoptosis and mitochondrial membrane dysfunction. It also shows a negative correlation with certain semen parameters, such as sperm motility and morphology (16, 17).

Vitamin D plays a crucial role in calcium metabolism and skeletal health, as well as pleiotropic genomic and non-genomic effects in various organs, including the reproductive system. The expression of the vitamin D receptor (VDR) and vitamin D-metabolizing enzymes in male reproductive tissues—such as the testes, prostate, seminal vesicles, epididymis, Leydig cells, and even sperm—highlights the significant role of vitamin D in male fertility (18, 19). The antioxidant and anti-inflammatory roles of vitamin D have been demonstrated in various studies. Vitamin D may improve oxidative stress (OS) and protect macromolecules, such as DNA and cell membranes, from oxidative damage (20, 21).

This cross-sectional study examined the relationship between serum vitamin D levels and DNA fragmentation index (DFI). The study assessed vitamin D levels in eligible men visiting an infertility center and its correlation with DFI, controlling for confounding factors. The goal was to achieve better outcomes in infertility treatment by emphasizing the correction of vitamin D levels if positive results are found. This is important because DFI is one of the key criteria for selecting sperm for fertilization in ART cycles, and improving it can significantly aid in infertility treatment.

Materials and methods

Study population: This cross-sectional study was conducted on 783 infertile men (18-45 years old) referring to Omid Infertility Clinic, Hamadan in 2011-2013. A demographic questionnaire was completed by the participants. The inclusion criteria were as follows: history of infertility for at least 1 year, idiopathic infertility, drug or alcohol abuse, men with a history of drug treatment in the past 12 weeks or less, history of systemic or chronic diseases, or occupational or environmental exposure to toxins were excluded from the study.

Semen collection and analysis: After 2–7 days of sexual abstinence, semen samples were collected from the enrolled participants and immediately transported to the laboratory. These were analyzed for semen volume, sperm count, progressive and total sperm motility, and normal sperm morphology parameters according to the 2010 World Health Organization criteria (22). Examination of the semen

samples was performed by trained clinical technicians using the methods described in our previous article (23).

Sperm DNA fragmentation: Sperm DNA integrity was determined using a Sperm DNA Fragmentation Assay Kit (SDFA; ACECR, Tehran, Iran) according to the manufacturer's instructions (25). Sperm with fragmented DNA had no halos or small halos, and sperm with intact DNA (26) had medium or large halos. At least 200 sperm per sample were observed and counted under a light microscope at 100× magnification.

Blood sampling: 5 mL blood sample was taken from subjects in the morning and then was sent to the laboratory to assess serum 25(OH) D (Ideal Tashkhis Atieh 25-OH Vitamin D ELISA Kit). Participants were then divided by 25(OH) D levels into sufficient (25[OH]D > 30 ng/ml), insufficient (25[OH]D 20-30 ng/ml) and deficient (25[OH]D < after 30ng/ml) (24).

Ethical considerations: The study was approved by the Ethical Committee of Hamadan University of Medical Sciences, Iran (IR.UMSHA.REC.1401.065).

Statistical analysis: For reporting descriptive statistics of quantitative data, the mean and standard deviation were used, and for describing qualitative variables, absolute and relative frequency tables were employed. If the normality assumption of quantitative variables was met, parametric tests such as independent t-test, one-way analysis of variance (ANOVA), logistic regression, and chi-square were used to compare results between two or more groups. Additionally, multiple linear regression was used to control for the effects of confounding variables. A 95% confidence level was considered for all tests, and all analyses were performed using SPSS version 27.

Results

As shown in Table 1, the average age of men was 38.5 years, and their mean BMI was 26.5 (Kg/m²). The highest percentage of occupations among visitors, after the "self-employed and other" category, was "driving" (15%). A total of 35% of men had insufficient serum vitamin D levels (20 ≤ D < 30 ng/ml), while 20% had a vitamin D deficiency (D < 20 ng/ml). Additionally, 19% of the individuals were smokers. Semen analysis showed normal sperm count, progressive motility, and normal morphology at rates of 96.2%, 65.1%, and 74.7%, respectively.

As shown in Table 2, the likelihood of DFI impairment in infertile men over the age of 40 was significantly 1.79 times higher (1.06, 3.03) than in those under 40. The likelihood of DFI impairment in infertile men with abnormal progressive motility was significantly 2.85 times higher (1.66, 4.87) than in those with normal motility. Similarly, in infertile men with abnormal morphology, the likelihood was significantly 2.45 times higher (1.43, 4.20) than in those with normal morphology.

Table 1: Distribution of Demographic and Spermogram indicators in infertility men

Variables	Category	Number/%
Age	≤40	507/65
	>40	269/35
	Total	776/100
Job	Driver	117/15
	Employee	158/20.3
	Worker& Farmer	130/16.7
	Other	373/48
	Total	778/100
Body mass index	BMI<25	308/39
	25≤BMI<30	338/45
	30≤BMI	128/16
	Total	774/100
Smoking	Non-smoker	631/81
	Smoker	144/19
	Total	775/100
DNA fragmentation index	Normal	728/92.3
	Abnormal	61/7.7
	Total	789/100
Serum vitamin D levels	Sufficient	352/45
	Insufficient(20≤D<30ng/ml)	274/35
	Deficiency (D<20ng/ml)	162/20
	Total	788/100
Concentration	Normal Sperm Count	756/96.2
	Abnormal Sperm Count	30/3.8
	Total	786/100
Progressive movement of sperm	Progressive	512/65.1
	Non progressive	274/34.9
	Total	786/100
Normal morphology	Normal	589/74.7
	Abnormal	199/25.3
	Total	788/100

No significant association was observed between other variables and DFI impairment. According to the adjusted logistic regression results in Table 3, after adjusting for other variables, the likelihood of DFI impairment in infertile men over the age of 40 was significantly 2.11 times higher (1.22, 3.67) than in those under 40.

Table 2: Association of Demographic and Spermogram indicators with Serum vitamin D levels using unadjusted logistic regression

Variable	DF Abnormal	DF normal	Odds ratio	Confidence interval 95%	p-value
Age					
≤40	32(52)	475(67)	1	-	-
>40	29(48)	240(33)	1.79	1.06	3.035
Job					
Driver	6 (9.8)	111 (15.5)	0.52	0.21	1.27
Employee	10 (16.4)	148 (20.6)	0.65	0.31	1.35
Worker& Farmer	10 (16.4)	120 (16.7)	0.80	0.39	1.67
Other	35 (57.4)	338 (47.1)	-	-	-
BMI					
BMI<25	19(31.2)	289(40)	1	-	-
25≤BMI<30	30(49.2)	308(43)	1.48	0.81	2.69
30≤BMI	12(19.6)	116(17)	1.57	0.74	3.34
Smoking					
Non-smoker	51(83.6)	10(16.4)	1	-	-
Smoker	580(81.3)	134(18.7)	0.84	0.42	1.71
Vitamin D					
Sufficient (30≤Vit D)	30(49.2)	322(44.2)	1	-	-
Insufficient (20≤Vit D<30)	21(34.4)	253(34.8)	0.89	0.49	1.59
Deficient (VitD<20)	9(14.8)	153(21)	0.63	0.29	1.36
Concentration					
Normal	55(91.6)	701(96.3)	1	-	-
Abnormal	5(8.4)	25(3.7)	2.54	0.93	6.91
Progressive movement					
PR	25(41)	487(67)	1	-	-
Non PR	35(59)	239(33)	2.85	1.66	4.87
Morphology					
Normal	34(56.6)	555(76.2)	1	-	-
Abnormal	26(43.4)	173(23.8)	2.45	1.43	4.20

The likelihood of DFI impairment in infertile men with abnormal non-progressive motility was significantly 2.69 times higher (1.54, 4.68) than in those with normal motility. Similarly, in infertile men with abnormal morphology, the likelihood was significantly 2.19 times higher (1.25, 3.85) than in those with normal morphology.

The results suggest that individuals with a BMI over 30, compared to those with a BMI below 25, may have an increased likelihood of DFI impairment, although this association was border-line significant. No significant association was observed between other variables and DFI impairment.

Discussion

This study was conducted to investigate the association between DFI, as an indicator of fertilization capacity in semen samples, and vitamin D**.** Assess the relationship between age and DFI**.** Examine the relationship between sperm count, progressive motility, and sperm morphology with DFI after adjusting for confounding variables. Male fertility is influenced by various factors, and vitamin D is one of the potential factors that may impact DFI. In the present study, no significant association was found between serum Vitamin D levels and DFI. However, a significant relationship was observed between age and DFI, indicating that as age increases, DFI also increases, suggesting a decline in male fertility with age**.** A significant association was also observed between sperm morphology, progressive motility, sperm count, and DFI after adjusting for confounding variables. Specifically, increased DFI was associated with a decrease in normal morphology, reduced progressive motility, and a lower sperm count. This finding highlights the critical importance of improving DFI in the treatment of male infertility.

The results of this study were compared with similar studies. In all observational and interventional studies reviewed, no significant independent association was found between DFI and vitamin D.

Table 3: Association of Demographic and Spermogram indicators with Serum vitamin D levels using uadjusted logistic regression

Variable	Odds ratio	Confidence limits 95%		p-value
		Lower Limit	Upper Limit	
Age				Reference
≤40	1	-	-	-
>40	2.11	1.22	3.67	0.008
BMI				
25>BMI	1	-	-	
25≤BMI<29.99	1.60	0.73	3.52	0.242
30≤BMI	1.75	0.94	3.26	0.078
Vitamin D				
Sufficient (30≤Vit D)	1	-	-	-
Insufficient (20≤Vit D<30)	0.73	0.33	1.61	0.434
Deficient (Vit D<20)	0.84	0.48	1.61	0.688
Concentration				
Normal Sperm Count	1	-	-	-
Abnormal Sperm Count	1.87	0.64	5.49	0.256
Progressive movement				
Progressive	1	-	-	-
Non progressive	2.69	1.54	4.68	<0.001
Morphology				
Normal	1	-	-	-
Abnormal	2.19	1.25	3.85	0.006

A randomized, triple-blind, placebo-controlled clinical trial also reported results consistent with the present study, showing no statistically significant association between DFI and vitamin D(27). Similarly, in another study involving 102 participants, individuals were divided into three groups based on vitamin D levels: Deficient (<20 ng/ml; n=24), Insufficient (20-30 ng/ml; n=43), Sufficient (>30 ng/ml; n=35).

No statistical differences were observed between vitamin D levels, DFI, and spermogram parameters across these groups (18). However, another study reported that lifestyle changes and the consumption of various antioxidants significantly reduced DFI. In that study, multiple antioxidants, including multivitamins, coenzyme Q10, omega-3, and oligo-elements, were prescribed to patients. However, the individual effects of each antioxidant were not separately analyzed, suggesting that the observed results could be due to the combined effect of all antioxidants. Given that vitamin D is also classified as an antioxidant, it may play a crucial role in this process (28).

Additionally, a 2018 clinical trial in Denmark involving participants found no significant differences in spermogram parameters between those who received vitamin D and the control group. However, an increase in pregnancy rates was observed in the vitamin D group. This suggests that vitamin D may influence pregnancy through other mechanisms (29). Finally, a systematic review on the relationship between vitamin D and male fertility factors indicated that there is still no strong evidence supporting the use of vitamin D to improve sperm parameters and hormonal disorders. Although no significant association was found between serum vitamin D levels and DFI in the present study, the role of vitamin D in spermatogenesis has been well established (30).

Vitamin D is a fat-soluble vitamin that functions both as a vitamin and a hormone. Its chemical structure is similar to steroid hormones, and it acts through nuclear VDR receptors, similar to those hormones (31, 32). The expression of vitamin D receptors and metabolizing enzymes in the testes, seminal vesicles, prostate, epididymis, and germ cells including spermatogonia, spermatocytes, and Sertoli cells indicates its key role in the male reproductive system (33, 34).

The antioxidant and anti-inflammatory properties of vitamin D have also been demonstrated in various studies. It may reduce oxidative stress (OS) and protect macromolecules such as DNA and cell membranes from oxidative damage (35).

Moreover, vitamin D has been reported to have a positive impact on male fertility by improving semen quality through non-genomic effects, modifying hormone synthesis via genomic and non-genomic mechanisms, regulating intracellular calcium homeostasis, enhancing sperm motility and capacitation, and facilitating molecular pathways involved in the acrosome reaction in sperm (36).

Another objective of the present study was to assess the relationship between age and DFI. The results indicated a significant association between age and DFI, showing that as age increases, DFI also rises, consequently leading to a decline in male fertility. In this study, the likelihood of DFI impairment in infertile men over the age of 40 was significantly 1.79 times higher (1.06, 3.03) than in those under 40. This factor alone can reduce fertility potential in this group, especially if one or more sperm parameters such as count, motility, or morphology are also abnormal.

In line with this, a 2017 study titled "The Relationship Between Sperm DNA Fragmentation Index, Age, and Semen Parameters in Infertile Men" demonstrated a direct association between increasing age and reduced sperm motility and concentration, along with higher DFI levels (37). As men age, paternal DNA integrity becomes increasingly compromised, leading to greater DNA damage (38). Mature sperm cells are unable to repair their damaged DNA on their own and rely on the oocyte's repair mechanisms immediately after fertilization. This places an additional burden on the oocyte to correct paternal DNA damage, which can have significant consequences for reproductive success (39, 40).

Measuring %DFI is valuable for predicting the likelihood of unsuccessful pregnancy outcomes. Previous studies have shown that semen samples with a %DFI lower than 27–30% have a significantly higher chance of successful pregnancy compared to those with DFI >30%, including: Natural conception (6.5 to 10 times higher success rate) Intrauterine insemination (IUI) (7.0 to 8.7 times higher) Conventional in vitro fertilization (IVF) (2 times higher) Intracytoplasmic sperm injection (ICSI) (1.4 times higher). If one or more semen parameters are abnormal, the chances of IVF/ICSI success are lower, and the risk of miscarriage increases (41, 42).

In this study, a significant association was found between sperm morphology, progressive motility, sperm count (after adjusting for confounding variables), and DFI. The likelihood of DFI impairment was significantly: 2.85 times higher (1.66, 4.87) in infertile men with abnormal sperm progressive motility. 2.45 times higher (1.43, 4.20) in infertile men with abnormal sperm morphology. In other words, increased DFI was associated with a decrease in normal morphology, reduced progressive motility, and lower sperm count. This finding highlights the critical importance of improving DFI in male infertility treatment.

Similarly, a retrospective study with 2,567 participants categorized individuals into five groups based on DFI levels (ranging from <5% to >30%). The study found a significant association between increased DFI and reduced sperm count, sperm morphology, and progressive motility. Although this study included three times more participants than the present study, the results were consistent (43).

A 2019 cross-sectional study in Vietnam with 318 participants set a DFI threshold of 30% and found a significant association between increased DFI, reduced sperm progressive motility and defects in sperm head morphology. However, no significant correlation was observed with other semen parameters (44). Overall, the association between DFI and various semen parameters—either individually or collectively—has been widely reported, reinforcing the hypothesis that DFI is linked to all spermogram indices (44, 45).

It is important to note that even sperm with normal morphology may have fragmented DNA. In such cases, fertilization may still occur due to the oocyte's ability to repair the damaged DNA. However, this can negatively impact post-fertilization processes and embryo development. If a high percentage of morphologically normal sperm still carry fragmented DNA, there is an increased risk of injecting abnormal chromatin into the oocytes or even treatment failure. In such cases, antioxidant therapy should be considered. Among antioxidants, vitamin D holds particular significance due to the presence of its receptors on sperm cells and reproductive tissues.

Conclusion

The relationship between DFI and independent variables can vary depending on specific conditions and research studies. It is important to note that the association between DFI and these independent variables may not be consistent across studies, and further research is needed to fully understand this relationship. In this study, after considering demographic characteristics and spermogram indices,

no significant association was found between DFI and serum vitamin D levels after adjusting for con-founding factors among men attending Omid Clinic in Hamadan. However, a clear association was observed between increased DFI and advanced age, reduced progressive motility, abnormal sperm morphology, and decreased sperm count, even after eliminating confounding effects. Numerous studies have demonstrated a link between antioxidants and DFI, suggesting the need for further complementary research on vitamin D as well as additional studies on other antioxidants to confirm these findings.

Conflict of Interests

Authors declare no conflict of interests.

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