Correlation of Obesity and Serum Vitamin D Levels with Sperm DNA Integrity, Sperm Quality, and Sperm Viability in Normozoospermia Men

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Abstract

Background: Obesity, Vitamin D (VD) deficiency, and infertility are important ubiquitous issue; however, the association of obesity and serum VD levels with abnormal sperm is unclear and inconclusive. The current study investigated the correlation of obesity and serum VD levels with sperm DNA integrity and sperm parameters in normozoospermia men.

Materials and Methods: Semen and blood samples from 64 men were divided into two groups: obese and nonobese men based on body mass index (BMI). Sperm motility and viability were determined by computer-aided sperm analysis and eosin-nigrosin staining. DNA fragmentation, determined by sperm chromatin dispersion method. VD concentrations were assessed by the Elisa technique.

Results: Serum concentration of VD levels in the obese group was significantly lower than nonobese men ($P < 0.05$). Sperm motility was significantly reduced in the obese group in comparison to nonobese ($P < 0.05$). Rapid progressive motility was statistically lower in obese men compared with the nonobese group ($P < 0.05$). Sperm count and morphology were not statistically significant in both groups. Sperm viability in the nonobese group was significantly decreased in comparison to obese group ($P < 0.05$). DNA integrity was significantly higher in the obese group as compared with nonobese ($P < 0.01$).

Conclusion: VD deficiency in the obese group showed decreased sperm motility, increased DNA damage, and viability. Adverse consequences of obesity and the possible effect of BMI infertility treatment must be discussed with counseling couples interested in assisted reproductive techniques outcomes, especially in men without any unknown cause.

Keywords: DNA, obesity, sperm, viability, Vitamin D

Introduction

Obesity and low Vitamin D (VD) status have concomitantly become a major risk factor with epidemic levels worldwide. Research linking these two health issues has clinically increased over the last number of years.¹ Evaluation of body mass is used for body mass index (BMI). When BMI value is between 25 and 30 (kg/m²) are regarded as overweight, while values higher than 30 are considered as obese.²,³ Obesity and overweight have become very common in both sexes in Iran. Epidemiological studies reported overweight as a risk factor for the development of various health problems.⁴ Obesity raises the risk of type 2 diabetes, cardiovascular disease,
hypertension, hyperlipidemia, sleep apnea,\textsuperscript{[5,6]} and VD deficiency.

Several studies reported VD deficiency in most regions of Iran, to be alarmingly epidemiological.\textsuperscript{[7]} Males, mostly suffer from VD deficiency, due to lifestyle and geographical location. In Isfahan, VD deficiency was 50.8%, and in adults, it was 19.6% and was more common in young people and women.\textsuperscript{[8]} The VD receptors (VDRs) found in male reproductive parts, showing a link between the VDRs and reproductive system.\textsuperscript{[9]} Human spermatozoa express VDRs, in the postacrosome areas of mature spermatozoa’s mid-piece, neck, and head.\textsuperscript{[10]} Studies reported a connection between sperm parameters and blood VD concentrations.\textsuperscript{[11]} However, its role in reproductive activities is inconclusive\textsuperscript{[12]} and may affect the biological activities of spermatozoa.\textsuperscript{[13]} Obese people show low levels of total antioxidant capacity and higher concentrations of oxidative stress, such as reactive oxygen species (ROS).\textsuperscript{[14]} It is reported that a high level of ROS can cause DNA fragmentation.\textsuperscript{[15]}

VD deficiency showed serious health problems and obesity. Its relationship with infertility, sperm parameters, and obesity in fertile men has not been thoroughly explored. Keeping in mind the adverse effect of obesity on sperm function and the potent effect of VD, this study was considered as a basis to explore the possible compensable effect of VD against obesity. Although there is a likelihood of abnormal sperm in obese men, results of previous studies are inconclusive. The current study investigated the effect of serum VD levels in obese and nonobese males and their correlation to sperm DNA integrity, sperm parameters, and viability in normozoospermia men.

\section*{Materials and Methods}

\subsection*{Study population}

This study was approved by the ethics committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.568). Informed consent was obtained from men attending the Andrology Unit of Saint Maryam Fertility and Infertility Center, Shahid Beheshti Hospital, Isfahan, Iran. Semen samples were classified as normozoospermic category according to the World Health Organization (WHO) criteria 2010. In this study, 64 normozoospermic men were divided into nonobese \((n = 32)\) and obese groups \((n = 32)\).

\subsection*{Inclusion criteria}

Individuals with an age range of 25 and 55 years old with BMI values between 24.8 and 18.9 kg/m\(^2\) were considered as a nonobese group, whereas BMI values of higher than 25 kg/m\(^2\) as obese group. Patients were asked to have sexual abstinence of 3–4 days before the experiment.

\subsection*{Exclusion criteria}

Patients with a history of cryptorchidism, varicocele, vasectomy, substance abuse, consuming exogenous hormones (replacement therapy testosterone), and VD consumption were excluded. Biochemical factors such as normal serum inhibin B <50 ng/ml, HBA1c ≥6.5%, normal weight with triglycerides ≥2.3 mmol/l, and follicle-stimulating hormone level >12.4 IU/ml were also excluded.

\subsection*{Fertility assessment}

It includes blood sampling, a general questionnaire, and semen analysis. The questionnaire form was related to body weight, medical history, height, and lifestyle factors, use of cigarettes, narcotics, alcohol, and nutritional supplements. Fresh semen samples were collected in sterile containers for semen volume, appearance, PH, viscosity, sperm concentration, and motility tests.

\subsection*{Sperm preparation}

After liquefaction of specimens in an incubator at 37°C for 20–30 min, initially washed with Ham’s F10 buffer with 5% human serum albumin (Irvine Scientific, Santa Ana, California). Sperm analysis was conducted according to the guidelines of WHO, using computer-aided sperm analysis (CASA) system (VT-SPERM Test. 2.3 model-company of Video Test-Finland).

\subsection*{Serum isolation}

Blood samples from both groups were centrifuged at 856 g for 12 min to isolate serum of specimens. The resulting sera were frozen at −70°C until analysis of VD.

\subsection*{Vitamin D measurement}

The serum concentration of 25-OH VD was evaluated in the groups by a commercial kit (DIA source-Immuno Assays S. A., Belgium). 5 ml of blood specimens obtained from all patients without utilizing anticoagulants. Serum samples were separated by centrifugation. The optical absorbance was measured by microplate reader RT-6000, Lorderan11, at a wavelength of 450 nm.

\subsection*{Assessment of sperm viability and motility}

Sperm viability was determined by eosin-nigrosin staining. A 5 μl of sperm sample mixed with eosin (1%, prepared in distilled water, Merck Company, Germany) and nigrosin (10% in distilled water, Merck company, Germany) was prepared and placed on microscopic slide and studied by light microscopy (magnification1000x). In each specimen, 200 sperms were studied. Sperms with red or dark pink heads were nonviable (membrane damaged), whereas sperms showing no color were considered alive (membrane-intact).\textsuperscript{[16]} Results of eosin-nigrosin staining were reported as a percentage.

Sperm motility, percentage of rapid progressive sperm (RPS), progressive motile sperm (PMS), nonprogressive sperm, and nonmotile sperm were evaluated by light microscopy (Olympus, Tokyo, Japan) connected to a CASA system.\textsuperscript{[17]}

\subsection*{Assessment of sperm DNA using sperm chromatin dispersion method}

Halosperm kit (Idevarzan-e-Farda Co. Tehran, Iran) was used to detect sperm chromatin dispersion (SCD). Initially, each sample was washed twice with PBS, 50 μl of sperm suspension added to microtubes containing low melting point agarose. From each sample, 30 μl was placed on a glass...
slide, covered with a coverslip, and refrigerated for 5 min to solidify. Each slide immersed in denaturation and lysis solutions and rinsed with distilled water for 5 min before being dehydrated with rising gradients of ethanol (70%, 90%, and 100%). The stained sample was washed and dried. In each slide, 200 sperms were studied by light microscopy to analyze the halos (magnification × 1000). Sperm with small/no halos was considered to have fragmented DNA, whereas sperm with medium/large halos was considered to have intact DNA.[18]

Statistical analysis
Statistical analysis was carried out by SPSS software version 20 (IBM corporation, Armonk, NY, USA). Student’s t-test was used for comparison of obtained values between obese and nonobese groups. Pearson test used to analyze the correlation between variables. Data were presented as the means and standard deviation (mean ± standard deviation). P < 0.05 was considered statistically significant.

RESULTS
Evaluation of Vitamin D
The VD level was significantly different (P < 0.05) between the experimental groups. Average VD serum concentration in obese subjects was 22.52 ± 6.45, while it was 30.93 ± 11.80 in the nonobese group. VD level was higher in the nonobese group than obese subjects [Figure 1].

Evaluation of sperm motility by computer-aided sperm analysis
The rate of RPS and PMS in nonobese men, in comparison to obese group, was statistically significant (P < 0.05) [Figure 2a and b]. Sperm morphology and count in obese and nonobese groups were not statistically significant. Sperm of normal morphology was high in both groups, but no statistically significant difference was seen between groups in terms of number of sperm and normal morphology. Sperm count was higher than 15 × 10⁶/ml (normal range); therefore, no statistically significant discrepancies were seen between obese and nonobese groups [Figure 2c and d].

Evaluation of sperm viability
Sperm viability in the obese group in comparison to a nonobese group showed a significant reduction (P < 0.05). In the nonobese group, average viability of sperm was 67.71 ± 15, obese group was 52.17 ± 19.28 [Figure 3a]. Percentage of viable sperm was higher in a nonobese group [Figure 4].

Evaluation of DNA fragmentation
Figure 3b shows the DNA integrity status of obese and nonobese groups. In the SCD method, the percentage of DNA fragmented sperm in a nonobese group (21%) was significantly decreased (P < 0.01), compared with obese individuals (33%). SCD method showed the adverse effect of obesity on DNA integrity, nonobese group seem to compensate the effect of obesity on DNA of sperm [Figure 5].

Correlation between variables
According to Pearson correlation analysis, no statistically significant correlation was seen in serum VD levels with sperm parameters, viability, and DNA fragmentation in obese and nonobese groups.[19]

DISCUSSION
Cellular and molecular aspects of spermatogenesis play a critical role in male infertility. Obesity seems to be related with impaired sperm DNA integrity, showing negative effects on key sperm parameters and viability in normozoospermic men, but no statistically significant correlation was found between serum VD levels and study variables [Table 1]. Conversely, nonobese group seems to compensate sperm DNA damage, showing improved sperm motility. Obesity affects male fertility, increases testicular temperature, and alterations in sperm parameters.[20] Our results are in agreement with this study, about serum VD levels in nonobese and obese men. Obesity showed lower concentrations of VD levels, which normalized after weight loss.[21] This may be due to less exposure of obese men to ultraviolet radiation, which stimulates biosynthesis of cholecalciferol (VD3) from cholesterol.[22] Indeed, the synthesis of an active metabolite of VD known as 1,25-dihydroxy VD is enhanced, and high levels showed negative feedback impact

![Figure 1: The comparison of Vitamin D levels (mean ± standard deviation) between obese and nonobese groups. ***P ≤ 0.001](image-url)
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The role of VD was seen in the male reproductive system, spermatogenesis; but, the mechanism of VD in the male reproductive system remains obscure. VD concentration plays an essential role in the biology of human spermatozoa. Several studies reported correlation of semen parameters with VD levels, although the results were contradictory. Relationship between sperm motility and serum concentrations of VD levels was seen with oligo-asthenoteratozoospermia, but no association was found in normozoospermic men. Moreover, no relationship was seen between sperm motility and VD concentrations. In contrast, a study found a negative link between sperm parameters and VD levels. Analogously another study reported a negative effect of VD deficiency on sperm motility. A study found a relationship between serum 25-OH VD and sperm motility. One study showed that low concentrations of VD were not related to motility, counts of spermatozoa, and normal morphology. Men with sufficient serum levels of 25-OH VD showed higher sperm motility, counts, and normal morphology than those with VD deficiency. These results are in agreement with the present study, whereas sperm motility (rapid progressive and progressive) in nonobese group was higher than obese group, only RPS was significantly different in nonobese men. VD initiates the biosynthesis of estrogen, accumulation of amino acids in testes affecting spermatogenesis, causing poor sperm motility and morphology in obese men. It was observed that VD function in spermatozoa is not limited to genomic activity. It has numerous biological functions, like regulation of intracellular calcium levels through inositol triphosphate-receptor activation found in the neck region of spermatozoa and lipid metabolism which influences sperm motility. Several studies found no link between VD levels and sperm counts. Consequently, it was found that sperm count is positively connected to VD levels. Correlation between VD levels, sperm morphology, and the count is a challenging issue. Majority of researches showed no link between circulating 25-OH VD and sperm morphology. Despite partial data on protective role of VD, interestingly, results from previous studies are inconclusive to serum VD levels and sperm morphology. In the current study, no significant differences in spermatozoa morphology and count were found between the two groups. Addition of VD to freeze/thaw medium increased spermatozoa motility, viability,
The generation of ROS in plasma is mostly due to adipose tissue. Excessive levels of ROS showed DNA damage and immotile sperm. VD showed antioxidant activity against OS and reduced the rate of DNA damage in spermatozoa. Epigenetic alterations by obesity and lifestyle have been identified as an factor influencing sperm parameters in obese males. DNA methylation and noncoding RNA changes lead to epigenetic alterations disrupting sperm parameters. Obesity decreased testosterone by disrupting the hypothalamic-pituitary-gonadal axis and increased testicular temperature due to accumulation of fat in testicular area causing decreased spermatogenesis, increased ROS generation, and SCD. The overall results of this study found that sperm DNA fragmentation in obese males with low serum VD levels is more susceptible than the nonobese group. However, no significant correlation was seen between serum VD levels, sperm DNA integrity, viability, and sperm parameters in the obese and nonobese groups.

Conclusion

This study explored the effect of obesity with low serum VD level concentration on sperm DNA integrity, motility, and viability of normozoospermic men. Similarly, the role of VD, in ameliorating the side effects of obesity in normozoospermic men. The adverse consequences of obesity and the possible effect of BMI in fertility treatment, based on the above discussion, should be considered for improving sperm function and quality in assisted reproductive techniques outcomes. Further research is suggested by VD supplement therapy in obese men with poor sperm motility, viability, and high DNA fragmentation without any unknown cause.

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Conflicts of interest

There are no conflicts of interest.

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Figure 5: SCD test. Spermatozoa with a large halo (a), a medium halo (b), a tiny halo (c), and no halo (d). Sperm cells with large/medium halos were considered to have normal DNA. Sperm with small or absent halos was considered to have abnormal DNA (Light microscopy, magnification, ×1000). (A) nonobese group; (B) obese group
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