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Correlative Study between Vitamin D3 Level and Sperm Mitochondrial Activity

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Abstract

Background Mitochondria play a significant plays a function in sperm metabolism and energy generation. The cellular integrity of sperm evaluated by examination of mitochondrial membrane potential (MMP), which may be achieved by using fluorescent dyes like JC-1 probe (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolyl carbocyanin iodide. Vitamin D3 (VitD3) increases men sterility can be improved by modifying hormonal balance through genomic as well as non-genomic mechanisms, while semen quality can be improved mostly through non-genomic effects. VitD3 effect a component of sperm motility, capacitation, and acrosome response, have been demonstrated.

Objective To study the correlation between red MMP, VitD3 level and sperm motility.

Methods The current experimental trial study was included 42 semen and blood samples. The sample of the study was subdivided into 4 groups according to sperms motility and VitD3 level the semen samples from 42 subjects of normozoospermic, asthenozoospermia according to World Health Organization (WHO, 2021). MMP Assay Kit (with JC-1) was performed.

Results There was a positive significant correlation between serum VitD3 level and red fluorescence MMP ($P < 0.001$). There was no significant correlation between serum VitD3 level and sperm motility percentage ($P > 0.05$).

Conclusion This study revealed a noticeable association between VitD3 level and sperm mitochondrial MMP assessed by fluorescent staining, which could be applied as a valid approach to evaluate the motility of sperm in Iraqi fertility centers.

Keywords Infertility, vitamin D3, sperm motility, mitochondrial membrane potential, MMP Assay Kit (with JC-1).

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List of abbreviations: JC-1 = (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolyl carbocyanine iodide) dye, MMP = Mitochondrial membrane potential assay , PTH = Parathyroid hormone, VitD2 = Ergocalciferol, VitD3 = Cholecalciferol, UV = Ultraviolet irradiation

Introduction

Infertility is defined by the World Health Organization as the inability to conceive after at least 12 months of unprotected intercourse [(1)]. Sperm motility is one of the most important factors to consider when assessing the quality of sperm. Sperm motility can be measured in a variety of ways, including using wet preparation slides or computer assisted semen analysis [(2)]. Increase sperm motility is generally considered to be an important aspect of typical male fertility. Males with immotile or poorly motile sperm are

typically sterile unless assisted reproductive technology is used [3].

A relevance of the mitochondrion in male fertility cannot be overstated: it is essential not only for sperm movement but also for hyperactivation, capacitation, acrosome response, and fertilization. As a result, contemporary sperm physiology research is focused on this key organelle as a biomarker of sperm health and [4]. Although multiple studies demonstrating a link between sperm motility and mitochondrial function, the effects of antioxidant treatment on the mitochondrial membrane potential (MMP) have received little attention. The initial goal of such research was to assess the effectiveness of antioxidants on mitochondrial activity and, as a result, sperm motility in male infertile patients [5,6].

Vitamin D (VitD) is a group of fat-soluble steroids that play an important role in regulating calcium and phosphorus balance in the body. One of most significant substances in this category of components in humans are steroid of vitamin D3 (VitD3) (cholecalciferol) and vitamin D2 (VitD2) (ergocalciferol).

Ultraviolet (UV) irradiation of the yeast sterol ergo sterol yields vitamin D2, which is present naturally in sun-exposed mushrooms. VitD3 is generated in the skin and found in oily fish such as salmon, mackerel, and herring [7]. Both VitD2 and VitD3 are hydrogenated in the liver to produce 25(OH)D, the predominant form of VitD in circulation and the best signal for measuring VitD levels due to its circulating half-life of 2-3 weeks. 25(OH)D is delivered to the kidney, where it undergoes another hydroxylation process to generate the physiologically active form 1,25(OH)2D, which has a half-life of roughly 4 hours and is strictly controlled by serum levels of parathryoid hormone (PTH), calcium, and phosphate [8].

This study aimed to study the correlation between Red MMP, VitD3 level and sperm motility.

Methods

The current experimental trial study was included 42 semen and blood samples. The sample of the study was subdivided into 4 groups according to sperms motility and VitD3 level: Group 1: was 11, patients with normal motility and normal VitD3 (Control), groups 2 was 10 patients with the normal sperm's motility and low VitD3, group 3 was 10 patients with normal VitD3 and low sperms motility and group 4 was 11 patients with low VitD3 and low sperms motility was specified for the main experimental study.

Semen samples from 42 subjects of normozoospermic, asthenozoospermia according to World Health Organization (WHO, 2021) obtained after 2-7 days abstinence, detailed questionnaire was designed depending on history and physical examination. Semen samples that used in this study were processed as described below. Semen parameters (fluid volume, sperm concentration, motility and morphology) of all samples fell within the lower reference limit criteria of the fifth edition of WHO manual of human seminal fluid analysis [9].

The blood obtained from 42 voluntary patients; blood was sampled from the antecubital vein with 5 ml or less than drawn into a Gel Activator tubes were used to collect blood for clinical biochemistry and immunology and drug testing. Gel and Activator tubes have micronized silica particles, which help clot the blood before centrifugation, and a gel at the bottom which separates whole blood cells from serum. These tubes would greatly shorten the clotting time they can improve serum surface and prevent blood substance exchange between blood cell and serum.

MMP Assay Kit (with JC-1)

The reduction in the MMP is a hallmark of the early stages of apoptosis. JC-1 is a popular fluorescent probe for measuring MMP. When the MMP is high in normal cells, JC-1 aggregates the mitochondrial matrix to create a polymer that can produce red fluorescence.

The change of JC-1 fluorescence color from red to green may be utilized to identify a reduction in cell MMP, and the transition of JC-1 fluorescence color can be used as an early detection signal of cell apoptosis.

Statistical analysis

The statistical package for social sciences (SPSS) version 23.0 and Microsoft Office 2010 were used to analyze the data. To characterize the data, descriptive statistics like frequency, range, mean, and standard deviation were calculated. The categories were examined by applying independent sample t-test (Unpaired t-test for comparison between two groups) and analysis of variance (ANOVA for comparison of more than two different groups). The coefficient of Pearson's correlation (r) was used to determine the level of relationship between continuous variables, and the findings were considered statistically significant when the p value was lower than to 0.05.

Results

Correlation between serum VitD3 levels and sperms motility percentage of patients involved in the present study. There was no significant correlation between patient's serum VitD3 level and sperms motility percentage (r = 0.082; P = 0.606) as demonstrated in figure (1).

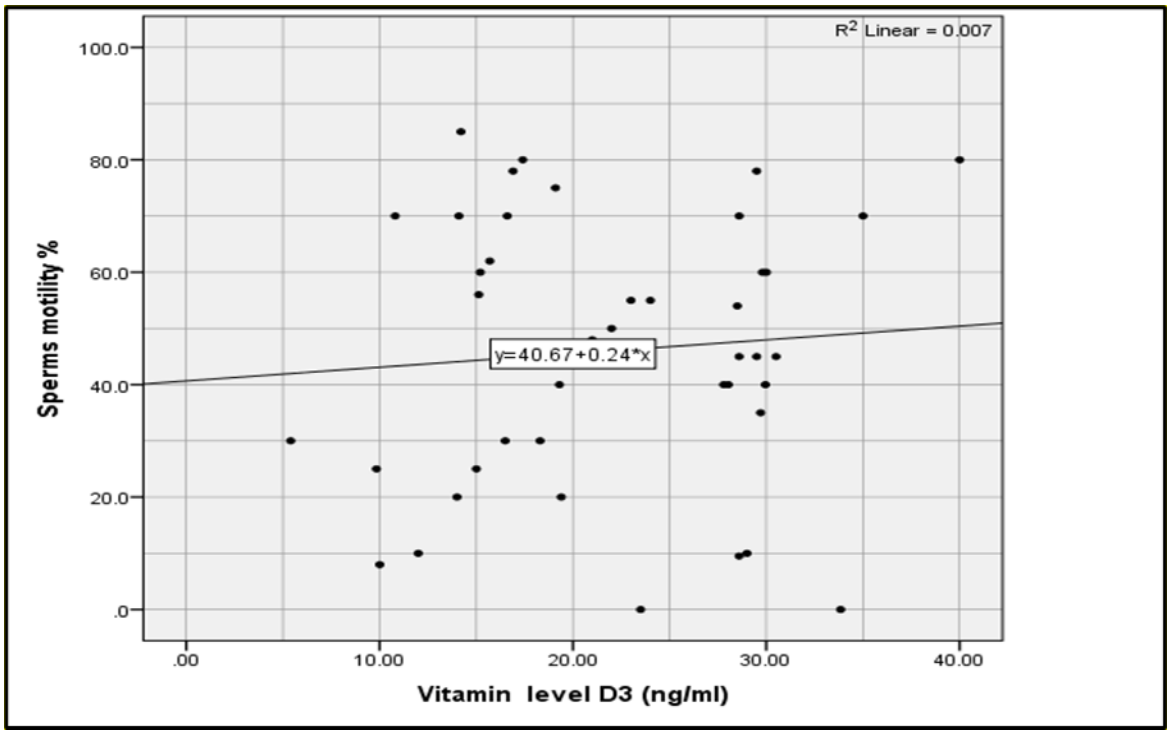

Figure 1. Correlation between patient's serum vitamin D3 level and sperms motility percentage

Comparison of MMP red fluorescence (Red MMP) according to serum VitD3 levels, Red MMP values were higher in patients with normal VitD3 levels (9.75±1.65 versus 5.17±3.88) with significant difference (P = 0.018) between two groups of patients (normal and low VitD3 groups) as presented in table 1.

Table 1. Comparison of mitochondrial membrane potentials red fluorescence (Red MMP) between patients with normal and low vitamin D3 levels

Parameter	Vitamin D3 (ng/ml) Normal N=21 Mean±SD	Low N=21 Mean±SD	P value
Red MMP (nm)	9.75±1.65	5.17±3.88	0.018 ₸S

Red MMP: Mitochondrial membrane potential red fluorescence; S: significant (P <0.05); ₸: Independent sample ttest

The comparison of Red MMP between the studied groups were illustrated in table (2), that showed highest red fluorescence value (13.5±2.1) in group 1 patients (normal VitD3 and normal sperms motility) and lowest red fluorescence value (3.8±1.70) in group 2 patients (low VitD3 and low sperms motility) with significant differences between the 4 studied groups (P = 0.001).

Table 2. Comparison of mitochondrial membrane potential red fluorescence between the studied groups

Parameter	Group 1	Group 2	Group 3	Group 4	P value
Red MMP (nm)	13.5±2.1	3.8±1.7	5.6±1.8	6.6±1.6	0.001 ⍰

S: Significant (P ≤0.05); ⍰: Analysis of variance (ANOVA); Red MMP: Mitochondrial membrane potential red fluorescence

The results showed a positive significant correlation between patient's serum VitD3 level and patient's red fluorescence MMP (r = 0.405, P = 0.009) as demonstrated in table (3).

Table 3. Correlation between patient's vitamin D3 level and Red MMP

Parameter	Red MMP (nm) Pearson's correlation coefficient (r)	P value
Vitamin D3 (ng/ml)	0.405	0.009 S

S: Significant (p ≤ 0.05); Red MMP: Mitochondrial membrane potential red fluorescence

Discussion

The connection between blood VitD3 levels and semen qualities has been the subject of several studies. Generally speaking, they found that almost all infertile men had much lower VitD3 levels than fertile controls. While other research has supported this finding, it is unclear how semen characteristics (concentration, motility, and morphology) and VitD3 levels are related. While some researchers discovered no discernible relationship between VitD3 level and sperm concentration or even motility, others discovered a favorable association [11,12].

The current study did not elicit a significant positive correlation between VitD3 level and sperm motility and this could be because most patients were selected in the current study

from non-homogenous patient groups with already abnormal semen parameters from idiopathic or other etiology [11,12]. The role of VitD in the regulation of testis activities, such as hormone synthesis and spermatogenesis, has been studied; according to experimental investigations, VitD increases male fertility by regulating hormone synthesis through both genomic and non-genomic activities [13]. In fact, rather than altering total testosterone, VitD3 appears to influence the bioavailable testosterone [14]. However, although an increased prevalence or risk for testosterone deficiency was reported in men with VitD deficiency in observational studies, despite observational research finding an increased incidence or risk for testosterone deficiency in males with vitamin D3 deficiency. A study had demonstrated that VitD3 has direct effects on spermatozoa, including non-genomic control of intracellular calcium homeostasis and activation of sperm motility-related biochemical pathways [8]. Since the VitD3 has a very short half-life, the amount of circulation VitD3 level in the serum at the time of measurement may be lowered physiologically so to find a precise accurate. Correlation between VitD3 level and sperm motility is to perform a separate prospective study for group of patients and should emphasize on inclusion and exclusion crateria in reaction to VitD3 level and sperm motility only i.e patients should be excluded for the study if they have any disease or habit or even work that may have effect on semen parameters, in addition to that, the induced patient in the study should be followed for more than 3 months in term of serum VitD3 level and semen fluid analysis in order to see precisely the in time at comelation between increasing or decreasing VitD3 level on semen parameter, particularly the sperm motility [15].

VitD restored the MMP and ATP production [16]. VitD promotes mitochondrial homeostasis and prevents protein oxidation, lipid peroxidation, and DNA damage caused by oxidative stress. Autophagy, mitochondrial malfunction, inflammation, oxidative stress, epigenetic modifications, DNA abnormalities, and calcium and ROS signaling changes are all known to be regulated by VitD. This will leads confidently to the postulation that VitD3 has a cruicial association and positive correlation with the cell mitochondrial activity and functional stability. The current study went with such hypothesized conclusion [17].

In conclusions, There is a positive significant correlation between patient's serum VitD3 level and patient's red fluorescence MMP. Normalization of the serum level of VitD3 level will stabilize the sperm mitochondrial function.

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Author contribution

Dr. Farhan: was responsible for designing the study, read and approved the final version of the manuscript. Fiza: wrote the whole manuscript and collected the data from the patients and followed the results with biostatician and put them in the text of the article.

Conflict of interest

The authors declare that there is no conflict of interest.

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