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Testosterone Levels And Dna Fragmentation Index In Male Undergoing Intrauterine Insemination After Vitamin D3 Supplementation

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ABSTRACT

Nutrition is one of the factors that contribute to male infertility. Vitamin D3 are known to affect spermatozoa and usually served in fertility supplementation in doses of 400 and 1000 IU. Low levels of vitamin D3 have been linked to decreased sperm quality and quantity. This research would like to know the effect of Vitamin D3 to the DNA Fragmentation Index (DFI) and Testosterone blood level in men undergoing intrauterine insemination (IUI). This was an analytical observational study with two groups and a pretest-posttest design. Seventeen men aged 20 to 45 who were undergoing the IUI program and did not have diabetes or hypertension are participate in this study. Sperm DFI was determined by using an ELISA method with a DNA Kit. Blood testosterone levels in ng/mL units was measure using ELISA. IBM-SPSS version 22 was used to perform univariate and bivariate analysis on the collected data. The blood level of Vitamin D3 was significantly increased after the intervention of six week Vitamin D3. Meanwhile, this study shows there are no differences in the level of blood testosterone before and after treatment. The DFI was significantly increased in the 400 IU groups.

Keywords: Blood Testosterone, DFI, Infertility, Vitamin D, Intrauterine Insemination.

1. Introduction

Vitamin D, a micronutrient consisting of D2 (egocalciferol) and D3 (cholecalciferol), has been shown to affect spermatozoa (Leslie, Soon-Sutton and Khan, 2023). Vitamin D3 supplementation has been shown to improve male reproductive function in both quantity and quality and low vitamin D3 levels also have been linked to lower sperm quality and quantity (Deng *et al.*, 2014; Özçelik *et al.*, 2018; Maghsoumi-Norouzabad *et al.*, 2021). Previous research found that administering vitamin D3 treatment for three months improved the average sperm concentration significantly from 19.72 to 28.95 million/ml, then to 33.63 million/ml after six months (Wadhwa *et al.*, 2020).

The sperm DNA Fragmentation Index (DFI) is an important element in determining sperm quality. The DNA fragmentation index reflects the degree of DNA fragmentation or damage in sperm. It is estimated that 10-15% of men without children have DNA damage in the sperm they produce, even if the sperm laboratory findings are normal. The DNA fragmentation test is used to determine whether sperm genetic material has been damaged. In other words, this test directly assesses the quality of sperm DNA. The effect of vitamin D levels on sperm DNA fragmentation index remains inconclusive. Several studies show that vitamin D levels have no effect on sperm DFI, but other studies find that vitamin

D levels and obesity affect sperm DNA fragmentation index (Derakhshan *et al.*, 2020; Maghsoumi-Norouzabad *et al.*, 2021).

Vitamin D is also known to affect blood testosterone levels. The vitamin D receptor (VDR) is almost universally expressed in human cells, highlighting the clinical importance of the vitamin D endocrine system. VDR and vitamin D metabolic enzymes are co-expressed throughout the male reproductive tract, including Leydig cells. In addition, vitamin D significantly increased testosterone production in a human primary testicular cell culture model. Thus, vitamin D may play a role in the production of male reproductive hormones (Lerchbaum *et al.*, 2019).

The Assisted Reproductive Technology program at the Sekar Clinic Surakarta have been in operation for decades with Intrauterine Insemination as the most frequent program. The Intrauterine Insemination (IUI) program is an assisted reproductive technology (ART) option for infertile couples suffering from fertility-related conditions such as ovulation disorders, polycystic ovarian syndrome (PCOS), mild endometriosis, and mild male fertility disorders. However, Dr Moewardi Hospital does not perform DFI or testosterone tests.

The dosage of vitamin D can affect vitamin D levels in a person's body, though the effective dosage is still being studied. From another research, supplementiation of 400 IU of vitamin D3, after 16 weeks, increases blood vitamin D levels by 31.2%, from 25.5 ng/ml to 31.2 ng/mL (Karaplis *et al.*, 2011). Supplementing with 1000 IU of vitamin D3 after 22 weeks has been shown to increase blood vitamin D3 levels by 15-25 nmol/l, or 6-10 ng/ml (Amrein *et al.*, 2020). The maximum dose limit for toxicity is still being investigated, but some sources state that the therapeutic toxicity dose is 4000 IU per day. In developing country where the infertility program policies is not covered by insurance, patients undergoing IUI usually receive vitamin D3 supplementation in a multivitamin preparation along with others vitamin with the doses of 400-800 IU to optimize men's preparation, with a 400 IU dose of vitamin D3 costing IDR 780 and a 1000 IU dose costing IDR 2750, while the above dose is obviously more expensive.

Based on these considerations, researchers are interested in conducting studies to determine whether the physician need to give more doses of vitamin D3 by finding the differences in spermatozoa quantity observed in DFI and blood testosterone level. In this case, the researchers studied the sperm DNA fragmentation index and blood testosterone levels before and after 400 IU and 1000 IU vitamin D supplementation in men undergoing IUI to optimize ART insemination outcomes at the Sekar Clinic, Dr Moewardi Hospital. Knowing the effect of vitamin D levels on the DNA Fragmentation Index and blood testosterone levels is expected to help couples conduct related examinations and improve the effectiveness and efficiency of vitamin D administration in preparation for IUI.

2. Methods

This was an analytical observational study with two groups and a pretest-posttest design. The study lasted for six weeks and was conducted at the Sekar Fertility Clinic, Dr. Moewardi General Hospital Surakarta.

The study included 17 men aged 20 to 45 who were undergoing the IUI program, did not have diabetes or hypertension, and were willing to participate. Men who had previously received infertility treatment, were diagnosed with varicocele or anatomical structural abnormalities and malignancy, and have not completed the treatment were excluded from the study.

Spermatozoa quantity was determined by measuring the sperm DFI using an ELISA method with a DNA Kit for patients undergoing IUI (measurement with the modified TUNEL assay in percentage units (%)). ELISA was used to measure blood testosterone levels in ng/mL units. Vitamin D (25-Hydroxyvitamin D) levels were measured in ng/mL units. IBM-SPSS version 22 was used to perform univariate and bivariate analysis on the collected data.

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3. Results

This study included 17 male patients diagnosed with infertility at Dr. Moewardi Regional Public Hospital, who were divided into two sample groups: 9 patients were given vitamin D at a dose of 400 IU, and 8 patients were given vitamin D at a dose of 1000 IU. Table 1 displays the characteristics of the study's respondents.

Table 1. Patient Charateristic

Characteristic		Groups			
		Vit D 400 IU		Vit D 1000 IU	
		f	%	f	%
Age (year old)	25 - 30	3	33.3%	1	12.5%
	31 - 40	3	33.3%	4	50%
	41 - 50	3	33.3%	3	37.5%
	Total	**9**		**8**	
BMI (kg/m2)	Normal	3	33.3%	5	62.5%
	Overweight	2	22.2%	2	25%
	Obesitas tipe 1	2	22.2%	0	0%
	Obesitas tipe 2	2	22.2%	1	12.5%
	Total	**9**		**8**	
Infertil Duration (years)	1 -2	3	33.3%	2	25%
	3 - 5	4	44.4%	2	25%
	> 5 tahun	2	22.2%	4	5%
	Total	**9**		**8**	
Sperm Result	Astenoteratozoospermia	2	22.2%	0	0%
	Astenozoospermia	0	0%	0	0%
	Normozoospermia	0	0%	0	0%
	Oligoastenoteratozoospermia	1	11.1%	1	12.5%
	Oligoteratozoospermia	1	11.1%	4	50%
	Teratozoospermia	5	55.55%	3	37.5%
	Total	**9**		**8**	

Table 2. Descriptive Analysis Results

Variable	Groups						Total Sample		
	Vit D 400 IU			Vit D 1000 IU					
	Mean	±	SD	Mean	±	SD	Mean	±	SD
VitD pre	23.30	±	4.35	23.09	±	6.32	23.20	±	5.20
VitD post	26.08	±	5.43	29.15	±	4.58	27.52	±	5.14
Testosteron pre	541.10	±	201.35	469.39	±	128.80	507.35	±	169.97
Testosteron post	674.13	±	276.80	618.99	±	347.31	648.18	±	303.13
DFI pre	23.04	±	12.00	21.58	±	6.90	22.35	±	9.67
DFI post	13.38	±	6.06	17.35	±	7.76	15.25	±	6.99

Table 3. Paired Comparative Hypothesis Tests on Pretests and Posttests Values

Variabel	Mean	±	SD	Sig
VitD pre	23.20	±	5.20	**0.001[a]***
VitD post	27.52	±	5.14	
Testosteron pre	507.35	±	169.97	0.055[b]
Testosteron post	648.18	±	303.13	
DFI pre	22.35	±	9.67	**0.033[a]***
DFI post	15.25	±	6.99	

[a]Paired t-test
[b]Wilcoxon signed ranks test
*Significant in $p \leq 0.05$

Table 4. Paired Comparative Hypothesis Tests on Pretest and Posttest Values for Vitamin D 400 IU and Vitamin D 1000 IU Groups

Variabel	Groups							
	Vit D 400 IU				Vit D 1000 IU			
	Mean	±	SD	Sig	Mean	±	SD	Sig
VitD pre	23.30	±	4.35	0.054[a]	23.09	±	6.32	**0.007[a]***
VitD post	26.08	±	5.43		29.15	±	4.58	
Testosteron pre	541.10	±	201.35	0.240[a]	469.39	±	128.80	0.123[b]
Testosteron post	674.13	±	276.80		618.99	±	347.31	
DFI pre	23.04	±	12.00	**0.025[b]***	21.58	±	6.90	0.279[a]
DFI post	13.38	±	6.06		17.35	±	7.76	

[a]Paired t-test
[b]Wilcoxon signed ranks test
*Significant in $p \leq 0.05$

This study collected data on vitamin D3, testosterone levels, and DFI before and after treatment. Table 2 shows descriptive results for research variables. The descriptive data demonstrate that the average levels of vitamin D and testosterone in the post-test were greater than the pretest, both in individuals who received vitamin D supplementation at a dosage of 400 IU or 1000 IU. Meanwhile, the average DFI score on the posttest decreased when compared to the pretest.

A paired comparative hypothesis test was used to determine differences in vitamin D levels, testosterone levels, and DFI before and after administered by 400 IU and 1000 IU of vitamin D in table 3. The paired t-test on data with a normal distribution and the Wilcoxon signed ranks test on data with a non-normal distribution were used for comparison. The paired t-test statistical test revealed significant differences in vitamin D and DFI levels in all research samples before and after treatment ($p<0.05$). Meanwhile, the results of the Wilcoxon test on testosterone levels revealed that there was no significant difference in testosterone levels before and after treatment.

Table 4 shows a significant difference in vitamin D levels before and after Vitamin D 1000 IU treatment ($p=0.007$). The DFI data showed a significant difference before and after intervention of the Vitamin D in 400 IU group ($p=0.025$). The testosterone examination found no significant differences in testosterone levels between the two treatment groups before and after therapy ($p>0.05$).

Based on the statistical analysis results, it can be concluded that the empirical research findings shows significant differences in vitamin D and DFI levels before

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and after administration of Vitamin D. Although, the significant result was obtained from the intervention of D3 1000 IU. Meanwhile, the DFI decreased in the 400 IU groups, other than in 1000 IU group, shows us there were probably other factor influencing this result. In the other hand, there were no significant differences in testosterone levels between the two groups before and after receiving 400 and 1000 IU of vitamin D for 6 weeks.

4. Discussion

This is an analytical observational study involving two groups and a pretest-posttest design with the measurements taken twice, before and after therapy. This study included 17 patients diagnosed with infertility at Dr. Moewardi Regional Public Hospital, who were divided into two treatment groups: 9 patients who received vitamin D at a dose of 400 IU and 8 patients who received vitamin D therapy at 1000 IU.

Low serum levels of the circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D), are associated with low testosterone levels. The systematic review and meta-analysis conducted by D'Andrea et al. (2021) revealed a positive relationship between 25(OH)D and total testosterone levels. Furthermore, recent research has discovered that vitamin D deficiency reduces testosterone production, which may affect male fertility, though the relationship is debatable. Thus, the relationship between vitamin D and testosterone levels is important in clinical practice. Vitamin D hypovitaminosis is extremely common in populations all over the world, especially in Indonesia, despite the fact that vitamin D deficiency has been linked to a number of chronic diseases.

There are currently conflicting statements about the optimal serum 25(OH)D concentration for overall health. The Institute of Medicine recommends vitamin D levels of 20 ng/mL (≥50 nmol/L) or higher for bone health. The Endocrine Society defines vitamin D adequacy as serum 25(OH) equal to or greater than 30 ng/mL.

In this study, vitamin D levels in the 400 IU group increased from 23.30 ± 4.35 ng/ml before treatment to 26.08 ± 5.43 ng/ml after. There was no significant increase in the vitamin D 400 IU group ($p>0.05$), while vitamin D levels in the 1000 IU group increased significantly ($p<0.05$), from 23.09 ± 6.32 ng/ml before treatment to 29.15 ± 4.58 ng/ml after treatment. However, the vitamin D levels found in this study did not exceed the Endocrine Society's recommended level of 30 ng/ml.

In vitro studies found that treating human adrenocortical cells with 1α,25-Dihydroxyvitamin D3 reduced androstenedione secretion by inhibiting steroidogenic enzymes. After controlling for age, smoking status, and BMI, it was discovered that 25(OH)D remained associated with androstenedione. This demonstrates the research's limitations in that confounding variables such as BMI, age, and smoking status were not controlled, resulting in less statistically specific data (Damas-Fuentes *et al.*, 2022).

A previous study discovered that lower 25-hydroxyvitamin D [25(OH)D] levels were associated with lower testosterone levels and an increased odd ratio of hypogonadism, which is consistent with the findings of other cross-sectional studies and revealed associations with seasonal variations in one study. Furthermore, one trial found that vitamin D supplementation increased testosterone levels in overweight subjects, while another study found that vitamin D supplementation did not increase testosterone concentrations in men with normal baseline testosterone levels (Chen *et al.*, 2019).

The results of comparative statistical tests on each group to compare the results before and after treatment revealed that post-treatment testosterone levels increased in both groups, but not significant ($p=0.240$ in the vitamin D 400 IU group and $p=0.123$ in the vitamin D 1000 IU group). After treatment, the mean testosterone level in the Vitamin D 400 IU group was 674.13 ± 276.80, whereas in the Vitamin D 1000 IU group it was 618.99 ± 347.31.

This study found significant DFI changes for all subjects before and after treament ($p<0.05$, $p=0.33$). The DFI values before and after treatment were significant in the vitamin D 400 IU group ($p=0.025$), but not in the vitamin D 1000 IU group ($p>0.05$, $p=0.279$). This result shows us the probability of DFI are influenced indirectly by vitamin D blood level.

Blomberg Jensen et al. (2011) conducted a cross-sectional association study on 300 men from the general population to demonstrate the role of activated vitamin D in human spermatozoa and hypothesize a link between serum vitamin D levels and sperm quality. Men with low vitamin D levels (<25 nM) had a lower proportion of motile ($p = 0.027$), progressively motile ($p = 0.035$), and morphologically normal spermatozoa ($p = 0.044$) spermatozoa compared to those with high levels (>75 nM). Unexpectedly, high vitamin D levels were associated with lower mean total sperm counts and rates of normal sperm morphology; specifically, men with high vitamin D ($n = 101$) had approximately 31% lower total sperm count and a 23% lower percentage of normal morphology when compared to those with low ($n = 103$) and moderate ($n = 103$) vitamin D levels (Calagna *et al.*, 2022).

Several factors can influence male infertility, including endocrine disorders, sperm transport disorders, primary testicular defects, and idiopathic conditions. Giving vitamin D3 is known to improve the quantity and quality of male reproductive function through many factors. We find that this study need larger samples to determine more about the effectiveness of vitamin D supplementation to blood testosterone level and also DFI. To determine the role of vitamin D in increasing sperm DFI as testosterone levels rise, more samples and a longer study period are required.

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