

## The Effect of Vitamin D Administration on Intracellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1 Levels in Hemodialysis Patients: A Placebo-controlled, Double-blinded Clinical Trial

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### INTRODUCTION

Cardiovascular diseases (CVDs) are the primary cause of mortality in chronic kidney disease (CKD) patients. The prevalence of CVD is 3.5 times higher in these patients compared to normal population, and CKD is considered an independent factor for CVD.<sup>[1,2]</sup>

Atherosclerosis is the main cause of CVD. Microinflammation, oxidative stress, and endothelial dysfunction are the main factors related to atherosclerosis in CKD patients.<sup>[3]</sup> The atherosclerotic process begins with changes in endothelial cell function. Inflammatory response leads to overexpression of vascular endothelial adhesion molecules and secretion of selectins from endothelial tissue which causes leukocyte adhesion to the vascular membrane.<sup>[4-6]</sup>

Cellular adhesion molecules such as intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) then cause firm adhesion of leukocytes to the vascular membrane and facilitate permeation of these cells to vascular intima layer.<sup>[7,8]</sup>

ICAM and VCAM are subgroups of Ig-like superfamily gene secreted from the endothelial tissue. The inflammatory process increases secretion of these compounds and hence leads to an escalation of the atherosclerotic process.<sup>[9]</sup> Secretion of these compounds rises their plasma level in such a manner that serum

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### ABSTRACT

**Objective:** Vitamin D deficiency is quite common among end-stage renal disease (ESRD) patients, and Vitamin D administration could reduce morbidity and mortality in these patients through different mechanisms. Cardiovascular diseases are the most common cause of mortality in these patients that are caused by vascular injuries. Intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) are vascular inflammation indicators. The goal of this study is to find the effect of Vitamin D administration on ICAM-1 and VCAM-1 serum levels in ESRD patients on hemodialysis.

**Methods:** The current study is a double-blind, randomized, placebo-controlled clinical trial on 64 patients in two groups of control and treatment. Serum levels of Vitamin D, ICAM-1, and VCAM-1 were measured before and after the study. Treatment group was treated with Vitamin D pearls while control group underwent treatment with placebo pearls. Average serum levels of Vitamin D, ICAM, and VCAM were measured in both groups before and after the study and were analyzed by ANOVA, paired *t*-test, and Chi-square test using SPSS software.

**Findings:** Sixty-four ESRD patients were recruited for this study consisting of 32 male and 32 female subjects within the ages of 18 and 76 years. The change in serum level of Vitamin D was significant in treatment group ( $P = 0.001$ ) but not in control group ( $P > 0.05$ ). Serum levels of ICAM and VCAM also changed significantly in treatment group ( $P = 0.001$ ) but not in control group ( $P > 0.05$ ).

**Conclusion:** Based on the findings of this study, it could be said that Vitamin D administration in ESRD patients may increase serum level of Vitamin D up to four times. It also reduces serum levels of ICAM and VCAM which might improve the vascular condition of these patients.

**KEYWORDS:** End-stage renal disease, hemodialysis, intracellular adhesion molecule-1, vascular cell adhesion molecule-1, Vitamin D deficiency

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levels of these compounds could be an indicator of endothelial cell damage and endothelial activation.<sup>[10,11]</sup> In patients with end-stage renal disease (ESRD) or CKD, serum levels of ICAM and VCAM are risen so that ICAM could be a predicting factor for mortality of these patients<sup>[12,13]</sup> while hemodialysis (HD) has no significant effect on the levels of these compounds.<sup>[14]</sup>

Vitamin D deficiency is rather common among ESRD patients (80%–90%) and is related to atherosclerosis, endothelial dysfunction, and left ventricle hypertrophy in these patients.<sup>[15–18]</sup> Vitamin D deficiency is also tightly correlated with the level of inflammatory biomarkers such as interleukin-6, ICAM, and VCAM.<sup>[19]</sup> Vitamin D also extensively modulates both innate and adaptive immune responses and could reduce systemic inflammation in patients.<sup>[20]</sup> Vitamin D administration could reduce morbidity and mortality in CKD and ESRD patients through reduction of inflammatory mechanisms. Herein, we have studied the effect of administration of Vitamin D supplements on ICAM and VCAM levels in ESRD patients undergoing HD.

## METHODS

This double-blind, randomized, placebo-controlled clinical trial was carried out between July 2013 and August 2014 on ESRD patients undergoing HD in Noor and Amin Hospitals of Isfahan, Iran.

Sixty-four ESRD patients aged between 18 and 80 years on HD suffering from Vitamin D deficiency were recruited. Patients were divided into treatment and control groups by paired randomization method, and each group received drug or placebo by HD staffs that were blinded to agent. Patients were selected for groups that there were 16 men and 16 women in each group [Figure 1]. Baseline serum levels of 25(OH) Vitamin D, ICAM, and VCAM were measured before the intervention in Baradaran Laboratory, Isfahan, Iran, using Boster ELISA kits. Patients in treatment group were prescribed with 50,000 IU Vitamin D per oral weekly for 12 weeks and then the treatment was continued for 3 months with the same dose every 3 weeks independent to the meal.<sup>[14,17]</sup> Control group was treated with oral placebo pearls with the same regimen. Vitamin D and placebo pearls were supplied from Zahravi Pharmaceutical Co. Iran. Serum levels of 25(OH) Vitamin D, ICAM, and VCAM were determined after the study. Demographic data of subjects such as age and sex were extracted from patients' hospital documents. Body mass index (BMI) and background cause of ESRD were also recorded. Participation criteria for patients were as follows:

1. Age of over 18 years
2. Patients undergoing HD for at least 3 months
3. Vitamin D level of below 30 ng/ml
4. Patient's consent for participating in the study.

While exclusion criteria were:

1. Very severe obesity (BMI > 40 based on dry weight after HD)
2. Noncompliance to treatment or being uninterested in continuing the study

3. History of malignancy or recent chemotherapy
4. Other infection or inflammatory diseases
5. Undergoing treatment with antiseizure medication.

Average serum levels of Vitamin D, ICAM, and VCAM were found in both groups before and after the study and were analyzed by ANOVA, paired *t*-test, and Chi-square test using SPSS 20<sup>th</sup> version (SPSS Inc, Chicago, Ill., USA).

This clinical trial was approved by the Research Ethics Committee and Nephrology Research Center of Isfahan University of Medical Sciences. Subjects were fully informed about the process of the study, and possible side effects of treatment were fully explained. Patients were required to sign a written consent to get recruited before randomization. The present study was also registered in Iran's official clinical trial portal by the code IRCT201505152417N17. All subject information will remain confidential and will not be used for any other study.

## RESULTS

A total of 64 ESRD patients with Vitamin D deficiency on HD were recruited for the study. Patients were categorized into two 32 subject groups consisting of 16 male and 16 female subjects. Demographic data such as age and sex were similar between the two groups with age of the subjects ranging from 20 to 74 years and with 24 subjects (37.5%) between 51 and 60 years. The primary cause of ESRD in patients was either hypertension or diabetes mellitus [Table 1].

Chi-square test with appropriate exponential ratio showed that the likelihood ratios of the frequency distribution for ESRD

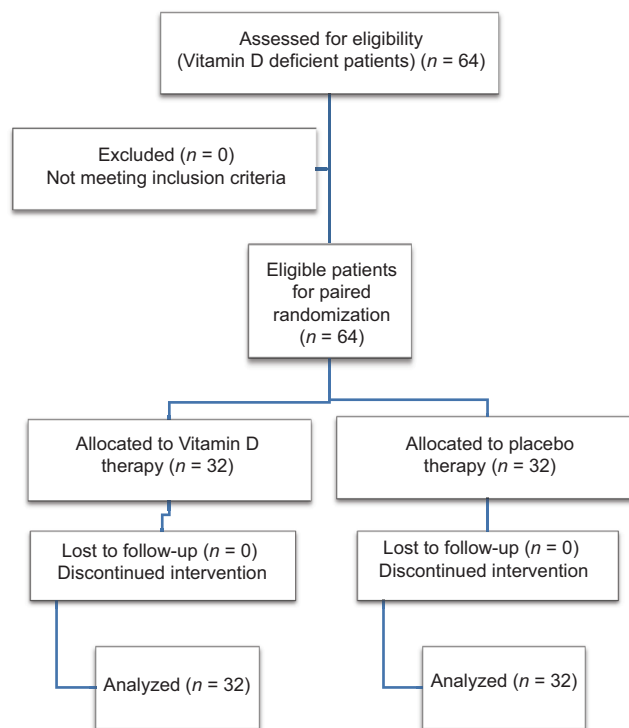


Figure 1: Flow diagram of the study process and patient selection

causes were not different between the two groups ( $P = 0.246$ ). Independent  $t$ -test showed that mean  $\pm$  standard deviation (SD) for age and BMI were not significantly different between the two groups ( $P$  values = 0.23 and 0.37, accordingly).

The average Vitamin D levels in patients before and after the study were determined in both groups. The level of Vitamin D was significantly increased in treatment group ( $P = 0.001$ ) while this change was not statistically significant in control group ( $P > 0.05$ ). Twelve patients (seven in control group and five in treatment group) had Vitamin D levels below ten ng/ml before the study while this level was between 10 and 30 ng/ml for the rest of the subjects. None of the patients reached hypervitaminosis D level during the study [Table 2].

The mean  $\pm$  SD of ICAM levels before the study in the two group were compared by independent  $t$ -test, and no significant difference was detected ( $P = 0.94$ ). Analysis with ANOVA showed that after the study, the serum level of ICAM was lower in treatment group ( $P < 0.001$ ). While paired  $t$ -test demonstrated no significant change in the level of ICAM in control group ( $P = 0.96$ ), this amount had significantly dropped in treatment group ( $P < 0.001$ ) [Table 3].

Similar statistical analyses showed that VCAM levels before the study were not significantly different between the two groups while ANOVA showed a significantly lower VCAM level in treatment group after the study ( $P < 0.001$ ). Similarly, serum levels of VCAM after the study had not changed significantly in control group ( $P = 0.97$ ), whereas in treatment group, this amount was significantly lower ( $P < 0.001$ ) [Table 3].

## DISCUSSION

The present study shows that Vitamin D administration in ESRD patients on HD leads to decreased serum levels of ICAM-1 and VCAM-1. Treatment with 50,000 IU Vitamin D weekly for 12 weeks and then continued with the same dose every 3 weeks for 3 months resulted in significant increase of Vitamin D serum level. None of the patients suffered from hypervitaminosis D during the course of the study. This study also showed that age, BMI, gender, and primary cause of ESRD do not have an effect on ICAM and VCAM levels.

CVDs are the main cause of morbidity and mortality in CKD patients. The first stage of CVD is vascular atherosclerosis. The atherosclerotic process begins with changes in vascular endothelial cell function followed by secretion of endothelial factors that cause adhesion of leukocytes to the vascular membrane, their attachment, and finally their permeation to intima layer of the blood vessels. Selectins secreted from vascular endothelial cells known as cellular adhesion molecules such as ICAM and VCAM cause tight attachment of leukocytes to the vascular membrane. Systemic and local inflammation increases serum levels of these molecules and hence accelerates atherosclerotic process.<sup>[3-8,21]</sup>

ICAM and VCAM are compounds from Ig-like supergene family that are secreted into serum from endothelial cells. It is

shown that serum levels of these molecules are indicator and predictors for endothelial cell damage, endothelial activation, and monocyte adhesion.<sup>[10,11]</sup>

Levels of these indicators are higher in CKD patients<sup>[22,23]</sup> but HD does not affect their levels.<sup>[24]</sup> Therefore, it is safe to say that these molecules could be used as indicators for endothelial activation in ESRD patients on HD.

Several studies have been conducted on Vitamin D supplements and their effects on ESRD patients. It is found that this vitamin could reduce morbidity and mortality in these patients. Vitamin D affects different parts of the immune system and modulates it and finally leads to a decrease in inflammatory response. Reduction of inflammation, especially systemic inflammation, is one of the benefits of this vitamin for ESRD patients.<sup>[18]</sup>

**Table 1: Basic characteristics of the study population**

Variable	Control group	Treated group
Age (year)	62 $\pm$ 21 (21-78)	60 $\pm$ 19 (18-76)
BMI (kg/m <sup>2</sup> )	26.2 $\pm$ 6 (18.1-35.1)	27.4 $\pm$ 8 (17.2-34.8)
Hb (mg/dL)	9.19 $\pm$ 1.42 (7.2-11.2)	9.93 $\pm$ 1.65 (7.4-12.2)
Comorbidities		
DM (%)	14 (43.7)	10 (31.25)
HTN (%)	12 (37.5)	17 (53.12)
Others (%)	6 (18.8)	5 (15.62)

Data are presented as mean $\pm$ SD (range), or  $n$  (%). SD=Standard deviation, BMI=Body mass index, Hb=Hemoglobin, DM=Diabetes mellitus, HTN: Hypertension

**Table 2: Values of Serum 25-hydroxyvitamin D levels before and after the intervention**

Groups	Sex	$n$	Before study	After study	$P$
Control	Male	16	20.9 $\pm$ 6.53	21.74 $\pm$ 5.98	>0.05
	Female	16	21.64 $\pm$ 6.5	23.06 $\pm$ 6.49	
	Total	32	21.44 $\pm$ 6.51	22.98 $\pm$ 6.35	
Treated	Male	16	22.12 $\pm$ 7.72	79.24 $\pm$ 7.59	<0.001
	Female	16	20.04 $\pm$ 8.52	79 $\pm$ 9.41	
	Total	32	21.88 $\pm$ 8.32	79.1 $\pm$ 8.23	

Data are presented as Mean $\pm$ SD. SD=Standard deviation

**Table 3: Values of serum intracellular adhesion molecule levels in two groups before and after the intervention**

Variable	Treated group	Control group	$P$
ICAM levels			
Before intervention	1451.7 $\pm$ 326.3	1444 $\pm$ 481.5	0.94
After intervention	1292.5 $\pm$ 316.7	14445.6 $\pm$ 481.3	<0.001
$P$	<0.001	0.56	
VCAM levels			
Before intervention	9416.3 $\pm$ 1874.5	9344.9 $\pm$ 1465.4	0.87
After intervention	8843.3 $\pm$ 1858.8	9345.7 $\pm$ 1465	<0.001
$P$	<0.001	0.97	

Data are presented as Mean $\pm$ SD. SD=Standard deviation, ICAM=Intracellular adhesion molecule, VCAM=Vascular cell adhesion molecule

The effect of Vitamin D administration on ICAM and VCAM has been studied. In a placebo-controlled clinical trial on patients with coronary artery disease, Sokol *et al.* showed that Vitamin D administration lowers serum level of VCAM in treatment group while serum level of ICAM remained unaffected in treatment group but had a significant drop in control group.<sup>[25]</sup>

Dobnig *et al.* also found a relationship between serum levels of ICAM and VCAM and low Vitamin D level.<sup>[19]</sup> It was also shown that administration of Vitamin D in overweight and obese patients reduced ICAM level.<sup>[26]</sup>

Nevertheless, studies on the relationship between serum Vitamin D and ICAM and VCAM levels in ESRD patients are rather primary and limited. Chitalia *et al.* showed that treatment with Vitamin D for 16 weeks leads to a decrease in serum level of E-selectin, ICAM, and VCAM in predialysis CKD patients, which confirms the results of the present study.<sup>[25]</sup> Moreover, Assimon *et al.* showed that Vitamin D administration in patients on HD lowers serum levels of P-selectin, ICAM, and VCAM.<sup>[14]</sup>

Since Vitamin D improves vascular function, its insufficiency is related to CVD, and treatment with its supplements corrects serum levels of inflammatory biomarkers and vascular function indicators, it could be said that administration of Vitamin D in ESRD patients with Vitamin D deficiency is critical.

One of the downsides to the current study is its short duration which limits the results to short-term effects of Vitamin D in subjects. Furthermore, the study was done in two HD centers with a rather small sample size which calls for more extensive studies to confirm these primary results. Nonetheless, this study is distinctive for being a double-blind, randomized, placebo-controlled study.

The present study showed that administration of Vitamin D in ESRD patients undergoing HD leads to reduction in serum levels of ICAM-1 and VCAM-1. The results also demonstrated that treatment with 50,000 IU Vitamin D weekly for 12 weeks and then every 3 weeks up to 3 months could increase serum level of this vitamin to four times in ESRD patients. Having very few side effects and several benefits to quality and quantity of life in ESRD patients, administration of Vitamin D in these patients might be critical.

#### AUTHORS' CONTRIBUTION

All authors participated in design, experiments, and gathering information and all of them have read and approved the content of the manuscript.

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#### CONFLICTS OF INTEREST

There are no conflicts of interest.

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