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The Effectiveness of Vitamin D Supplementation on Oxidative and Inflammatory Markers in Patients Suffering from End-stage Renal Disease, a Randomized Controlled Trial

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ABSTRACT

Vitamin D insufficiency is common in patients suffering from end-stage renal disease (ESRD). In contrast, vitamin D supplementation could improve the status of ESRD patients (ESRDP). However, this effect's molecular mechanism is not fully understood. Therefore, this study aimed to assess vitamin D supplementation's impact on inflammation and oxidative signaling pathways in ESRDP. 104 ESRDP were divided into placebo (53) and vitamin D (51) groups. They were also categorized into four subgroups based on the severity of vitamin D deficiency. The dose of vitamin D3 (0.25-0.5mg/day) supplementation was determined based on plasma levels of calcium and parathyroid hormone (PTH). Vitamin D supplementation was performed for eight weeks. Serum levels of calcium, phosphorus, PTH, albumin, creatinine, ALP, and glomerular filtration along with antioxidant enzymes, malondialdehyde, and pro-inflammatory factors were measured. Moreover, the Nrf2 and NF-κB expression was evaluated in whole blood. According to the results, vitamin D supplementation improved the status of patients with ESRD significantly as compared with the placebo group ($p<0.05$). In addition, the expression of NF-κB and the serum levels of pro-inflammatory factors and malondialdehyde were significantly reduced. Finally, the expression of Nrf-2 and the serum of antioxidant enzymes were raised in the vitamin D group as compared with the placebo group ($p<0.05$). Vitamin D reduces clinical and metabolic symptoms in ESRDP by modulating gene expression (in oxidative stress and inflammation).

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Introduction

Vitamin D is of fat-soluble secosteroids, such as vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) (1). In order to define the vitamin D grade, 25-hydroxy vitamin D [25(OH)D] is evaluated in serum. A biologically active form of vitamin D is1, 25-dihydroxycholecalciferol (2). It functions as a hormone and is responsible for maintaining the homeostasis of calcium, phosphate, and magnesium in order to help the healthy development and bone remodeling. It also participates in many biological functions including immune functions, cell proliferation and differentiation, and the synthesis of neurotrophic factors, nitric oxide synthase, glutathione (3,4) and reduction of inflammation (5,6). Therefore, deficiency of this vitamin has widespread harmful effects.

About one billion people worldwide suffer from vitamin D deficiency, prevalent in children, the elderly, and adults (4,7). It may be due to sun avoidance and a diet with inadequate vitamin D. Further, its severity leads to weakened bone mineralization and its damage, resulting in bone-softening conditions, such as osteomalacia in adults and rickets in children (8). Vitamin D deficiency is recognized as a potential cause of the development of many illnesses such as cancer, depression, autoimmune diseases, cardiovascular disease, diabetes mellitus (DM), kidney failure, and ESRD (9,10).

ESRD could be caused by a wide range of kidney problems. Nowadays, 90 percent of ESRD patients have chronic hyperglycemia, hypertension, and glomerulonephritis, (11). The inability of the kidneys to eliminate waste, preserve fluid and electrolyte balance, and create hormones is connected with ESRD. ESRD needs either a kidney transplant or hemodialysis (HD) (12). Cases that use HD are 3.5 to 4 times more likely to die than those with normal

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kidney function. This rate is increasing by 8% per year (13).

Latest clinical and experimental data shows that ESRD is associated with chronic inflammation, oxidative stress, malnutrition, and endothelial dysfunction. Oxidative stress is defined as an imbalance between the formation of oxygen and nitrogen free radicals and the capability of antioxidant mechanisms to detoxify or repair the damage caused by them. Because decreasing levels of plasma antioxidants, including glutathione, vitamins, and other antioxidants, lead to an increase in low-density lipoprotein (LDL) oxidation and malondialdehyde, oxidative stress may be involved in the pathophysiology of chronic renal failure (CRF) (MDA). This research revealed that inflammation and oxidative stress are important in ESRD pathophysiology.

Moreover, new research suggests that hypovitaminosis D could well be connected to the advancement of kidney failure and several cardiovascular problems. Patients with renal failure have an unusually high prevalence of severe vitamin D deficiency, aggravated by a decreased ability to convert 25-(OH) D into the active form, 1, 25 dihydroxy vitamins D. However, animal research has indicated 25 (OH) D supplementation ability in improving this condition through its antioxidant properties. Large-scale clinical trials on vitamin D supplements are still being conducted. Nevertheless, there is little evidence linking serum 25(OH)D levels to oxidative and inflammatory indicators in ESRD patients. As a result, the goal of this study was to see how vitamin D supplementation affected inflammation and oxidative signaling pathways in ESRD patients.

Materials and Methods

Patients and Trial Design

Individuals with ESRD who were admitted to the Division of Nephrology's outpatient clinic at Shar Teaching Hospital in Sulaimani city in 2019 and 2020 were recruited. All procedures followed the Declaration of Helsinki criteria and were authorized and monitored by the Local Medical Ethics Committee of the Sulaimani Governorate's Directorate of Health (approval no: 60344). In addition, patients with the inclusion criteria were fully explained about the research method and were enrolled after signing an informed consent form. First, 121 female and male cases (range: 18–60 years) were examined by a nephrologist and found to be stable in ESRD. All participants underwent 3-weekly 4-hour hemodialysis treatments. Dialysis lasted an average of 6 years (range: 2–21 years). No patients had inflammatory disorders, intestinal resection, or malabsorption, and no medicines that interfered with vitamin D absorption were used. Patients who had received a renal transplant, patients with a systemic disease, hyperparathyroidism (intact parathyroid hormone > 3 upper limits), active infection (hepatitis, tuberculosis) and malignancy, undergoing immunosuppressive therapy, and patients on a lipid-lowering drug were also excluded in this study.

In a randomized, double-blind placebo-controlled clinical trial, 104 individuals with ESRD and inclusion criteria accepted into the research were randomly assigned to one of two groups: placebo (53) or vitamin D (51) (Figure 1).

The groups were then subdivided into four categories based on the severity of vitamin D insufficiency. Randomization was assigned using computer-generated random numbers. Data on base features, smoking behaviors, causes of kidney diseases, diabetes, clinical cardiovascular (CV) diseases such as acute coronary syndrome or CV congestion, and history of hypertension were collected at the first visit (Table 1). Patients were regularly followed up each time on dialysis for two months. The primary dose of 0.25 mg/day for vitamin D3 was determined on the basis of the levels of plasma calcium and parathyroid hormone (PTH). Dose adjustment for subsequent visits was determined accordingly up to a dose of 0.5 mg daily. Participants in the placebo group were given identical placebo (paraffin oil) pills in the same sequence. All subjects were recommended to follow a previous diet and physical activity, avoid changing their medications type and dosage and to be exposed to the same amount of time as before to sunlight to avoid confounding factors. Food frequency questionnaires (FFQs) were used to examine long-term dietary consumption, a significant risk factor for disorders including cardiovascular disease. In order to follow up, patients who did not attend dialysis sessions were contacted by telephone to find out about their

medication, dialysis, and survival status. Based on all available medical information, the cause of each death was assessed and determined by 3 doctors not engaged in this research. If a death occurred outside of the hospital, family members and the general practitioner were called via phone to determine the cause. Two cases in the vitamin D group and five in the control group died due to COVID-19 and myocardial infarction after the trial period.

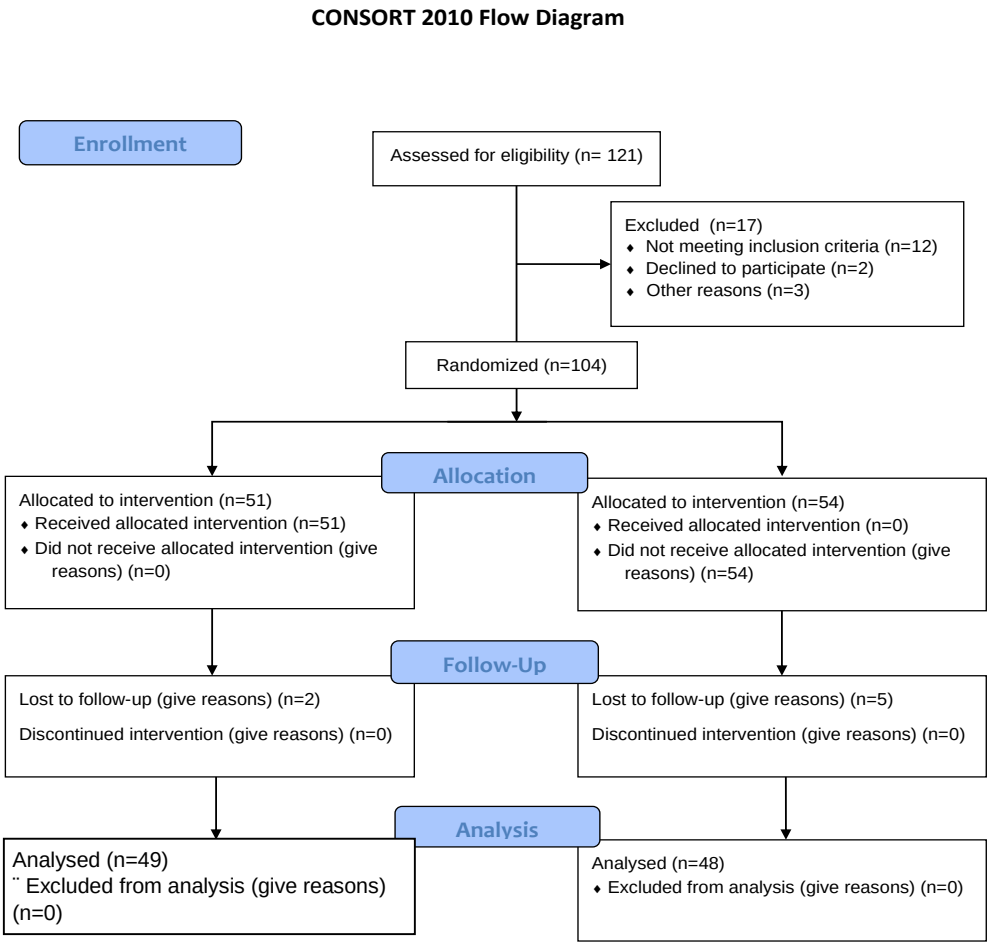

Figure 1. The CONSORT flowchart of the trial

Measurements

Before starting treatment with vitamin D3 and at each visit, blood samples were obtained to measure 25-dihydroxy vitamin D ($25(OH)_2D$) and other biochemical levels. Samples of plasma after centrifugation (6000 rpm at room temperature for 10 minutes) were kept at -80 C. IRMA (SC antibodies Laboratory Inc., Santee, CA, USA; normal range 14–65 pg/ml) was used to assess total intact PTH. Radioimmunoassay was used to determine the level of ($25(OH)_2D$) (DiaSorin Inc., USA). The four-variable diet modification in renal disease formula was used to calculate the glomerular filtration rate (GFR).

Alkaline phosphatase (ALP) serum level was calculated by colorimetric technique with a commercial kit (Pars Azmun Co., Iran). Serum level of TNF-α and IL-1β was measured by ELISA kits (Shanghai Crystal Day Biotech Co., Shanghai, China). The photometric approach was used to quantify creatinine (Cr) using standard kits (BioSystems Co., Spain). The levels of CAT and MDA were determined using Aebi (14) and thiobarbituric acid (TBA) modified colorimetric methods. Serum GPx activity was tested using the method published by Paglia and Valentine (15), and SOD activity was assessed using the method reported by Marklund and Marklund (16). Latex-enhanced nephelometry was applied to quantify serum C-reactive protein (CRP) using a Behring Nephelometer Analyzer and reagents from Behring Diagnostics (Somerville, NJ, USA).

The extraction of total RNA was performed by isolation total RNA kit (Bioneer, Exi Prep TM Tissue total RNA kit, South Korea).) For cDNA synthesis, the Ipsogen RT Kit (Qiagen, GmbH, Hilden, Germany) was utilized. The forward and reverse primers of genes NF-κB and Nrf-2 were extracted from the study of Zhao et al., (2018) (17) and Kanamori et al., (2015) (18), respectively. A Sybr green reaction of Real-Time PCR by the ABI PRISM 7000 (Applied Biosystems, Carlsbad, CA, US) was utilized to assess the expression of target and β-actin genes. The Nrf2 and NF-κB's relative expression was standardized to β-actin and computed using the $2^{-\Delta\Delta CT}$ equation.

Statistical analysis

IBM SPSS Statistics 22 was used to examine the data (IBM Corp., Armonk, USA). The Kolmogorov-Smirnov statistical test was used to determine the distribution of data in terms of normality in order to select the optimal statistical analysis approach. Frequency (%) and mean ±Standard Deviation (SD) were reported to define qualitative and quantitative data. In order to compare the qualitative variables between the two groups, the chi-Square test was employed. The one-way ANOVA following the Tukey test was utilized in order to compare the means in different severity of vitamin D deficiency. The paired sample t-test was used to compare the within-group means before and after the intervention. Non-parametric tests were utilized when data had a non-normal distribution. The level of significance was set at less than 0.05.

Results and discussion

Clinical characteristics of patients in this study

The average age of the study population at enrollment was 56±15.2 years in the control and 59±11.1 years in the vitamin D groups; 60.7% and 54.9% of the patients were male in the control group and vitamin D, respectively. In the control and vitamin D groups, 53% and 45% had hypertension, respectively. Also, in the control and vitamin D groups, 45% and 49% had diabetes, respectively; 33% and 31% had atherosclerotic disease, respectively too. 49% in the control group and 45% in the vitamin D group smoked. The major drugs used in these two groups were Antiplatelet Drugs, Statins and RAS inhibitors (Table 1).

Vitamin D level (primary)

To better evaluate vitamin D status, its serum level was divided into four categories: Vitamin D deficient [25(OH)D < 20 ng/ml], Vitamin D insufficient [25(OH)D 21–30 ng/ml], Severe vitamin D deficiency [25(OH)D < 10 ng/ml], and Vitamin D sufficient [25(OH)D > 30 ng/ml]. The majority of participants in the placebo group (58.82 percent) and the vitamin D group (60.78 percent) were in the vitamin D deficiency class. Below 9.8% in both groups were in the Vitamin D sufficient [25 (OH) D> 30 ng / ml] classification. The results showed that vitamin D supplementation for eight weeks could significantly improve serum levels in these categories. However, in the placebo group, there was an insignificant change in this period (Table 2).

Table 1a. Clinical features of the patients

Clinical characteristics	Placebo				Vitamin D				*p*-Value between two groups	Statistical test
	Basal	8th week	*p*-value	Statistical test	Basal	8th week	*p*-value	Statistical test		
Age (years)	56 ± 15.2	-	-	Paired t-test	59 ± 11.1	-	-	-	0.89	-
Male: female	31:20	-	-	-	28:23	-	-	-	-	-
BMI (kg/m2)	24.33 ± 3.69	24.23 ± 3.69	0.78	Paired t-test	25.36 ± 3.78	24.33 ± 3.69	0.281	Paired t-test	0.94	Independent t-test
Weight (kg)	68.96 ± 7.26	69.3 ± 8.32	0.71	Paired t-test	70.31 ± 8.68	69.35 ± 7.68	0.132	Paired t-test	0.98	Independent t-test
Systolic BP (mmHg)	152.3 ± 17.2	161.2 ± 28.3	0.34	Paired t-test	153.13 ± 12.3	138.3 ± 13.6	0.034	Paired t-test	0.033	Independent t-test
Diastolic BP (mmHg)	78.3 ± 7.6	75.6 ± 5.8	0.48	Paired t-test	85.39 ± 6.7	78.13 ± 6.3	0.046	Paired t-test	0.09	Independent t-test
Median Duration of Dialysis (years)	8.6 ± 2.5 (1-16)	8.6 ± 2.5 (1-16)	0.98	Paired t-test	7.8 ± 2.3 (1-18)	7.8 ± 2.3 (1-18)	0.98	Paired t-test	0.049	Independent t-test

*p<0.05

Table 1b. Clinical results of the patients

Basic diagnosis	Placebo		Vitamin D	
	Basal	8th week	Basal	8th week
Diabetic Nephropathy (%)	23/51 (45)	-	25/53 (49)	-
Glomerulonephritis (%)	13/51 (25)	-	16/53 (31)	-
Interstitial Nephritis (%)	11/51 (21)	-	10/53 (20)	-
Hypertension (%)	27/51 (53)	-	23/53 (45)	-
Atherosclerotic Disease (%)	17/51 (33)	-	16/53 (31)	-
Acute Coronary Syndrome (%)	13/51 (25)	-	12/53 (23)	-
CV congestion (%)	7/51 (14)	-	5/53 (10)	-
Others (smoke) (%)	28/51 (49)	-	23/53 (45)	-
Drug's use				
Antiplatelet Drugs	36/51(70)	-	33/51(65)	-
Statins	23/51(45)	-	27/51(53)	-
RAS inhibitors*	23/51(55)	-	19/51(37)	-

*Renin-angiotensin system (RAS)

Laboratory characteristics of hemodialysis patients in the vitamin D and placebo groups

On the basis of the serum, cases in the placebo and the vitamin D groups were divided into four groups and the levels of some laboratory parameters in these patients were monitored throughout the research. According to the results, the levels of ALP, albumin, CRP, creatinine, URR, GFR and Kt / V were high in patients with vitamin D deficiency and this condition worsened over time during the study period. The findings further indicated cases with severe vitamin D deficiency, PTH, and phosphorus levels to be inversely and significantly higher than other patients who were in a better condition in terms of vitamin D (Table 3). While calcium levels significantly reduced in line with vitamin D. However, the results showed that over time not only does not improve the serum levels of these parameters, but also their condition worsens. However, the results showed that vitamin D supplementation during eight weeks could significantly improve the levels of these parameters compared to before the start of treatment and compared with the placebo group. Additionally, vitamin D supplementation improved serum levels of PTH, phosphorus, and calcium significantly (Table 3).

Table 2. Vitamin D status of patients

Vitamin D status	Placebo (51)			Vitamin D (53)			*p*-Value between two groups++
	Basal	8th week	*p*-Value*	Basal	8th week	*p*-Value+	
Severe vitamin D deficiency (SVDD) [25(OH)D < 10 ng/ml]	10 (8.36±0.35)	9 (8.66±0.18)	0.36	11 (8.61±0.8)	6 (8.91±0.6)	0.02*	0.06
Vitamin D deficient (VDD) [25(OH)D < 20 ng/ml]	30 (12.70±2.6)	30 (12.61±1.8)	0.59	31 (12.66±1.6)	20 (19.66±1.6)	0.03*	0.01*
Vitamin D insufficient (VDI) [25(OH)D 21–30 ng/ml]	6 (21.72±1.3)	4 (22.32±2.7)	0.21	6 (22.01±2.36)	14 (28.68±3.76)	0.01*	0.005*
Vitamin D sufficient (VDS) [25(OH)D > 30 ng/ml]	5 (30.46±1.6)	3 (31.12±2.3)	0.21	5 (31.25±1.6)	8 (38.68±2.87)	0.06	0.06

*p<0.05 +Paired t-test, ++ Independent t-test

Table 3. Laboratory characteristics of hemodialysis patients in the vitamin D and control groups

Parameters	Placebo Group							
	Basal				Final (After 8 weeks)			
	SVDD	VDD	VDI	VDS	SVDD	VDD	VDI	VDS
ALP (mg/dL)	132 ± 7.89a	129 ± 9.63a	126 ± 8.93ab	124 ± 9.22bc	133 ± 10.3a	131 ± 9.65a	124 ± 9.75ab	126 ± 9.36b
Albumin (g/dL)	3.03 ± 0.21a	3.16 ± 0.12a	3.35 ± 0.29ab	3.56 ± 0.36bc	3.12 ± 0.22a	3.15 ± 0.34a	3.23± 0.12a	3.59 ± 0.65b
CRP (mg/dL)	1.98±0.01a	1.76±0.02 a	1.56±0.02b	1.35±0.01bc	1.86 ±0.02a	1.73 ±0.01a	1.60 ±0.01b	1.43±0.02 c
Creatinine (mg/dL)	17.4±3.21a	16.8±2.31	16.08±1.67ab	15.7±2.58bbc	18.7±2.36aA	17.36±3.6bB	16.17±2.979cC	15.36±1.38dD
GFR (ml/min/1.73m^2)	12.9±1.1a	14.3±1.3b	17.38±1.7c	19.85±1.03d	13.05±1.5a	16.5±1.7bB	18.3±2.3cC	21.3±2.1dD
Calcium(mg/dL)	8.2 ± 0.23a	7.98 ± 1.02a	8.06 ± 1.22ab	8.68 ± 1.12bc	8.02 ± 1.32a	8.31± 1.02aB	8.67 ± 1.2bC	8.95 ± 1.11c
Phosphorus (mg/dL)	5.23 ± 0.7a	4.98 ± 0.67a	4.62 ± 0.42ab	4.31 ± 0.11bc	5.27 ± 0.7a	5.06 ± 0.16a	4.65± 0.36b	4.13 ± 0.17c
PTH (ng/L)	18.17 ±1.3a	17.26±1.3b	16.75±1.7c	15.31±1.2d	19.36±1.5aA	18.35±1.3bB	16.67±1.7c	15.62±1.3d
	Vitamin D Group							
	Basal				Final (After 8 weeks)			
	SVDD	VDD	VDI	VDS	SVDD	VDD	VDI	VDS
ALP (U//dL)	135.7±8.5a	127.6 ±9.6a	122.3 ±8.9ab	113.3±9.2c	133.6 ± 9.7a	120.8 ± 8.5a	111.3 ± 9.3ab	101.5±9.8bc
Albumin (g/dL)	3.6 ± 0.62a	3.92 ± 0.51a	4.07 ± 0.12ab	4.55 ± 0.62c	4.15± 0.26aA	4.91 ± 0.39bB	5.58± 0.12cC	5.98 ± 0.38dD
CRP (mg/dL)	1.78±0.11a	1.67±0.15a	1.32±0.13ab	1.12±0.12bc	1.26±0.11aA	1.021±0.10bB	1.01±0.1cC	0.73±0.09dD
Cr (pg/mL)	18.21±1.3a	16.3±1.12b	14.17±0.9c	13.09 ±1.02d	15.98±1.29aA	14.6±1.2bB	13.1±1.1cC	12.15±1.7dD
GFR (ml/min/1.73m^2)	13.6±5.87a	14.08±3.16a	15.15±5.68b	16.6±2.3c	16.15±1.9aA	17.95±1.8bB	18.85±2.1cC	20.5±1.3dD
Calcium (mg/dL)	9.01±1.02a	8.75 ± 1.1a	8.12 ±0.9b	7.89 ±0.18b	9.57 ± 0.64a	9.72±0.75aB	10.05 ±0.87aC	10.12±1.03aD
Phosphorus (mg/dL)	4.78 ± 0.8a	4.81 ±0.9a	5.08 ± 0.7a	5.68 ± 0.6b	5.87 ± 0.6aA	6.13 ± 0.7aB	6.53± 0.6bC	6.46 ± 0.8cD
PTH (ng/L)	18.6±3.67a	17.63±2.67b	16.87±3.16c	15.32±2.65d	15.69±1.36aA	14.98±1.07bB	13.46±1.03cC	12.98±2.31dD

*Unexplained lowercase letters specify a significant difference among subgroups. Mismatched uppercase letters specify a significant difference among groups (p<0.05).

Vitamin D significantly improved oxidative stress and inflammation in hemodialysis patients

The results of oxidative and inflammatory markers are shown in Table 4. According to the table, the MDA, as a marker for lipid peroxidation, as well as IL-1β and TNF-α as the pro-inflammatory cytokines, increased significantly throughout the research in the placebo group. In contrast, their levels could significantly improve by vitamin D compared with the placebo group and prior to the vitamin D supplementation use. The finding further indicated the activity of antioxidant enzymes to be significantly reduced in hemodialysis patients in both placebo and vitamin D groups, while vitamin D supplementation for eight weeks could significantly improve their activity vitamin D group ($p<0.05$). According to the findings, the NF-κB expression was lower significantly, and Nrf2 expression was significantly higher in hemodialysis patients who had vitamin D supplementation as compared with the placebo group ($p<0.05$, Figure 2).

Table 4. Oxidative stress and inflammation biomarkers in hemodialysis patients*

Oxidative/ Inflammatory biomarkers	Placebo							
	Basal				Final (After 8 weeks)			
	SVDD	VDD	VDI	VDS	SVDD	VDD	VDI	VDS
MDA (nmol/mL)	8.61±0.3a	7.28±0.8b	5.06±0.7c	4.12 ± 0.3d	6.98 ± 0.37aA	6.52 ± 0.2aB	4.32 ±0.7cC	3.44±0.3dD
SOD (U/mL)	5.36±1.08a	7.97±1.25b	9.2 ± 0.9c	11.2 ± 1.5d	7.71 ±0.87aA	8.86 ± 0.81bB	10.06± 0.6cC	12.06 ± 1.11dD
GPX (U/mL)	19.6±3.87a	24.76±5.37b	38.67±4.9c	46.38±3.87d	28.9 ± 2.12aA	38.65±3.78bB	46.73±3.68cC	49.66 ± 9.7cD
CAT (U/mL)	6.01 ± 1.77a	8.21 ± 1.32b	11.08±1.76c	13.89±2.13d	8.76±1.2aA	10.75±1.61bB	13.02±1.23cC	16.35±2.41dD
TNF-α (pg/mL)	11.82±1.81a	10.07±2.7b	7.61±1.71c	5.47 ±1.11d	8.7±0.88aA	7.63±1.01bB	6.51±1.22cC	5.31 ± 1.78d
IL-1β(pg/mL)	8.43 ± 1.36a	7.58 ±1.37b	6.35 ± 1.01c	6.06±.61d	7.01±1.62aA	7.68 ± 0.87a	6.12 ±0.77b	4.26±1.07cD
Oxidative/ Inflammatory biomarkers	Vitamin D							
	Basal				Final (After 8 weeks)			
	SVDD	VDD	VDI	VDS	SVDD	VDD	VDI	VDS
MDA (nmol/mL)	9.12 ± 1.21a	7.25 ± 1.03b	5.27±0.7c	4.31 ± 0.3d	6.76 ±0.1a A	6.01± 0.2bB	5.41 ± 0.5c	4.71±0.1d
SOD (IU/mL)	6.81 ± 0.7a	8.31 ± 1.05b	10.98±1.23c	12.62 ± 1.25d	10.01±0.38a	13.31±0.1b	15.37 ±2.87c	19.68 ± 3.1d
GPX (IU/mL)	18.62±3.67a	25.71±2.67b	36.6 ± 3.68c	47.61 ± 0.85d	25.61 ±3.6aA	38.87± 4.12bB	49.78 ± 3.57cC	59.78 ± 7.5dD
CAT (IU/mL)	5.93 ± 1.28a	7.98 ± 1.2b	10.98±1.01c	15.01±2.3d	16.97±2.35aA	23.7±2.97bB	29.87±2.97cC	41.08±3.69dD
TNF-α(pg/mL)	12.01±1.61a	10.51±1.01b	6.7± 1.27c	5.61 ±1.28d	7.58± 1.25aA	7.12±1.31bB	6.01± 0.17c	5.36±0.18d
IL-1β(pg/mL)	9.62±1.28a	8.19±1.07b	6.97± 1.27c	5.31± 0.85d	6.78±0.61aA	6.31±0.38aB	5.97±0.75bC	5.35±0.37b

*Unexplained lowercase letters specify a significant difference among subgroups. Mismatched uppercase letters specify a significant difference among groups ($p<0.05$).

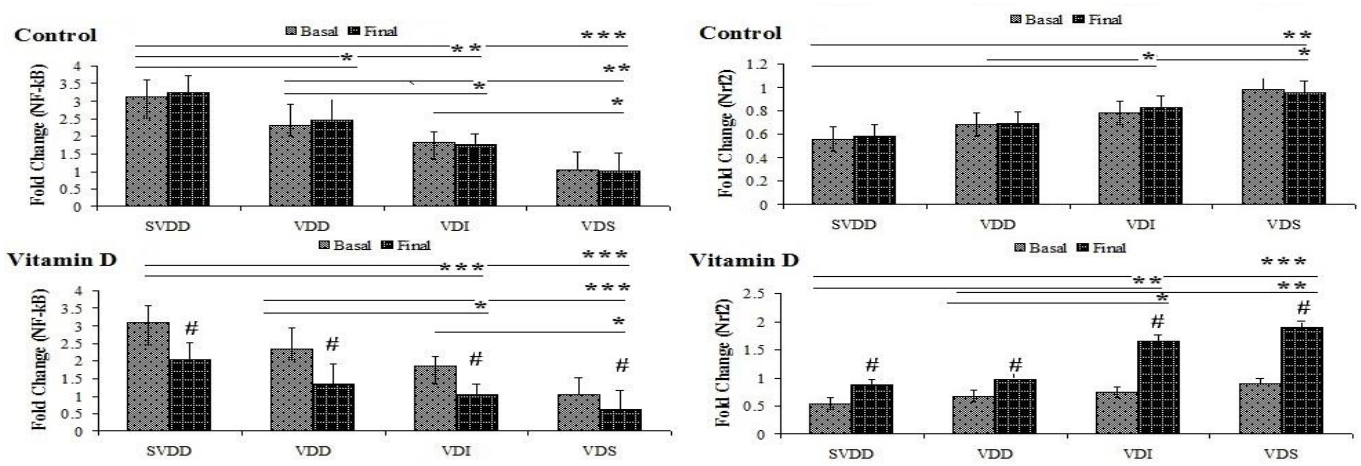

Figure 2. Mean ± SD of the NF-κB and Nrf-2 expression in control and vitamin D groups; #p < 0.05 significant difference as compared with base level. *, ** and *** for $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

Vitamin D treatment improved the status of patients with ESRD in this research by reducing inflammation and oxidative stress. Consistent with our results, extensive investigations have indicated that other supplements such as vitamin C and silymarin (19,20) may affect kidney function and the patients' condition.

Our results showed that vitamin D supplementation could control the serum state of inflammation and oxidative damage in patients by regulating the NF-κB and Nrf2 expression. In line with our findings, Haddad Kashani et al. (2018) and Schleithoff et al. (2006) indicate vitamin D supplementation to be able to improve oxidative stress and inflammation status in cases with congestive heart failure (21) and diabetic hemodialysis (22). According to Müller et al. (1992), 1,25- (OH) 2D3 blocked the IL-α, IL-6, and TNF-α production by LPS -stimulated monocytes, with no effect on uperoxide production dose-dependently (23). Schleithoff et al. (2006), in line with our findings, showed that vitamin D supplementation (for nine months) inhibited TNF-α production in cases of congestive heart failure (21). Haddad Kashani et al. (2018) showed that vitamin D supplementation (for three months) improved the condition of these patients through inflammation and oxidative stress signaling pathway in diabetic hemodialysis (22). In our study, in line with this study (22), the expression of the NF-κB and Nrf2 was evaluated as the main genes controlling the inflammatory and oxidative damage status, respectively. Following these signaling pathways, TNF-α and IL-1β serum were assessed as inflammatory elements, and antioxidant enzymes (SOD, GPx, CAT) and MDA were evaluated as oxidative factors. Haddad Kashani et al. (2018) found that vitamin D Supplements reduced gene expression of transforming growth factor (TGF) -, protein kinase C (PKC), and mitogen-activated protein kinases 1 (MAPK1), IL-1β, TNFα, and IFN- γin diabetic HD patients' PBMCs as compared with the placebo. They also found no significant variations in the expression of NF-κB, IL-4, IL-6, and vascular endothelial growth factor (VEGF) in diabetic hemodialysis patients' PBMCs as compared with the placebo group. In fact, this difference in results can be due to differences in the study protocol or in the number of people who experienced more severe degrees of vitamin D deficiency. Our findings demonstrated that NF-κB expression is related to changes in vitamin D serum levels or the severity of vitamin D insufficiency in individuals. Willis et al., (2012) found an inverse connection between serum vitamin D and serum TNF-α levels in healthy endurance-trained runners, which is consistent with these findings (24). 1,25- (OH) 2D3 inhibits lymphocyte proliferation and antibodies and lymphokines production (IL-2 & interferon-γ) (23,25) The lymphocyte functions depend on cytokines, e.g., IL-1α, IL-1β, IL-6, and TNF-α formed by antigen-presenting cells (25,26). Moreover, the inhibition of oxidative stress has a key role in controlling inflammation (27). We know that activation of macrophages, monocytes, and leukocytes improves free radicals' production and lead to oxidative damage. Therefore, inhibiting their activation by inhibiting the production of pro-inflammatory factors, including interleukin-1, has a key role in controlling inflammation and oxidative damage. In addition, our results showed that the severity of oxidative damage is associated directly with the severity of vitamin D deficiency and treatment with this vitamin can significantly inhibit this damage. In agreement with these results, Ji et al., (2020) and Chen et al., (2019) showed that vitamin D supplementation through various cellular pathways could improve oxidative damage (28,29). These studies showed that the expression of Nrf2 was upregulated by vitamin D. Our results also showed that vitamin D supplementation could significantly improve serum levels of ALP and albumin. In this regard, many studies have shown that chronic inflammation, malnutrition and impaired renal function in hemodialysis patients lead to hypoalbuminemia and have a central role in causing mortality in these cases (30,31). Furthermore, the major reason for morbidity and mortality in hemodialysis patients is atherosclerotic cardiovascular disease (32). Vitamin D supplementation possibly is able to alleviate these complications through its healing effects on liver functions such as lipid metabolism. Therefore, improving liver function greatly enhances the quality of life and reduces mortality from this disease. Moreover, although we did not measure IL-6 levels in this study, vitamin D supplementation is reported to decrease this inflammatory factor (33), and it is directly related to depression, which itself It is one of the main reasons for death in hemodialysis patients (34). According to Okereke and Singh, (2016), vitamin D deficiency can be a depression risk factor in late life (35-36); thus, it is vital to note that cases with ESRD and hemodialysis are mostly middle-aged and older, and supplement therapy for these patients can have a central role in improving quality of their life.

Conclusions

It can be stated that vitamin D supplementation has anti-inflammatory and antioxidant effects in individuals with ESRD by enhancing the expression of genes involved in oxidative stress and inflammation. Additionally, vitamin D could be beneficial in reducing clinical and metabolic symptoms in diabetic patients with ESRD due to its beneficial impacts.

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Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

Author's contribution

Dana Ahmed Sharif did all the work alone.

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