

# Relationship between the serum levels of Vitamin D and inflammatory markers in ESRD patients

Abbas Etminan<sup>1</sup>, Seyed Mostafa Seyed Askari<sup>1</sup>, Ahmad Naghibzadeh Tahami<sup>2</sup>, Seyed Adel Mahdi<sup>1</sup>, Mina Behzadi<sup>3</sup>, Mohammad Shabani<sup>3\*</sup>

<sup>1</sup>Clinical Research Unit, Shafa Hospital, Kerman University of Medical Sciences, Kerman, Iran; <sup>2</sup>Physiology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran; <sup>3</sup>Kerman Neuroscience Research Center, Neuropharmacology Institute, Kerman University of Medical Sciences, Kerman, Iran

**Summary.** *Background and aim:* In patients with End-stage renal disease (ESRD), 25-(OH)-Vitamin D3 deficiency is a common problem and also the inflammatory responses increase in these patients. The present study aims to evaluate the relation of 25-(OH)-Vitamin D3 with the indirect inflammatory markers in patients on hemodialysis (HD) and peritoneal dialysis (PD). *Methods:* This study was done by cross-sectional method on 85 ESRD patients receiving renal replacement therapy (RRT), from one geographical area. 64 patients on HD and 21 patients on PD who were matched for age and sex were studied. Serum level of 25-(OH) Vitamin D3 was measured in each patient. ESR, CRP and the other routine blood tests were measured as well. *Results:* The level of 25-OH Vitamin D3 was significantly lower in PD group in comparison to HD group (P: 0/0012, 2/70±0/10 vs 2/05±0/14). Platelet (195/40±7/6 vs 265/52±15/6, P: 0/001) and ESR (46/80±6/89 vs 23/53±1/96, P: 0/003) were significantly higher in PD group. Considering total population of the study (PD and HD), there was a significant association between ESR and serum level of 25-(OH)-Vitamin D3 (r: 0.26, P: 0.036) but no correlation was seen between 25-(OH)-Vitamin D3 and hemoglobin (Hb) or duration of dialysis. On the other hand, in patients on HD, multiple regression analysis revealed a significant relationship between duration of dialysis (P: 0.02), Hb (P: 0.01) and ESR (P: 0.001) with 25-(OH)-Vitamin D3 level. Moreover, there was a relationship between vitamin D3 levels and inflammatory markers as well. *Conclusions:* The deficiency of 25-(OH)-Vitamin D3 was followed with increase of ESR as an inflammatory marker in patients on HD. (www.actabiomedica.it)

**Key words:** inflammation, 25-hydroxy vitamin D, renal replacement, dialysis

## Introduction

The role of vitamin D in the metabolism of Calcium, Phosphor, parathyroid hormone, development of neurons and the development of the immune cells is well known. 25-OH Vitamin D3 is an inactive form of vitamin D which regulates the hemostasis of Calcium and Phosphor (1). The deficiency of vitamin D is a risk factor for infectious, autoimmune, neurodegenerative and cardiovascular diseases, as it is widely seen in patients suffering from diabetes, osteoporosis and cancer (2). The vitamin D deficiency is also observed in patients with end-stage renal disease (ESRD) who

are under renal replacement therapy (RRT) (3). The metabolism of vitamin D in patients on hemodialysis (HD) is severely disrupted and the deficiency of vitamin D is a common finding among these patients (4-6). Ciccone and colleagues defined that vitamin D is related to cardiovascular mortality as a result of heart failure, myocardial infarction, sudden cardiac death in both general population, and patients on RRT (7).

Chronic kidney disease (CKD) is associated with oxidative stress and inflammation (8). The existence of cardiovascular diseases in patients without traditional risk factors, suggests the role of non-traditional risk factors or pathogenic mechanisms such as inflamma-

tion, oxidative stress and hormone changes. Although with inconsistent reports, the low grade inflammations and the low level of vitamin D were introduced as risk factors of cardiovascular diseases (9). Epidemiologic studies have shown that vitamin D has a potential anti-inflammatory effect by regulating the inflammatory mediators and has discrete relation with the increase of C-reactive protein (CRP) (10).

Encouraged by these reports, the present study was conducted with the aim of evaluating the correlation of vitamin D and inflammatory parameters in ESRD patients under RRT including HD and peritoneal dialysis (PD).

## Methods

This study was done by using cross-sectional method in HD and PD centers of Kerman. 85 patients diagnosed with ESRD, who were receiving RRT including 64 patients on HD and 21 patients on PD in Shafa and Javad-Al-Aeme HD centers and PD center of Shafa hospital, were involved in the study. Patients with malignancy, immunological diseases, immobility, obesity ( $BMI \geq 30$ ), smoking, severe anemia (hemoglobin (Hb)  $\leq 9$ ), acute inflammatory or infectious diseases within the past 3 months, and patients under treatment by immunosuppressive drugs were excluded from this study. The ethics committee of Kerman University of Medical Sciences confirmed this study and an informed consent was obtained from each patient. Blood sample (10 cc) was drawn from patients to examine the serum level of 25-OH vitamin D3, as well as CBC, Platelet, parathormone (PTH), alkaline phosphatase, Calcium (Ca), Phosphorus (P), Erythrocyte sedimentation rate (ESR) and CRP levels. The serum level of 25-OH vitamin D3 was measured by Enzyme Linked Immunosorbent Assay (ELISA) method in the laboratory. Assessment of ESR was done by Automated Analysis (Lena, Spain) and quantitative CRP was determined by PHOTOMETRIC (BIONIC, Iran).

### Statistical analysis

Statistical analysis was performed by SPSS 20.0 software (SPSS, Chicago, Illinois, USA). The para-

metric variables were compared with independent t-test. Nonparametric variables were compared with chi-square test. Spearman's rho correlation test was used to assess the relation among parametric variables. P value less than 0.05 considered significant.

## Results

64 patients receiving HD (23 women, 41 men) and 21 patients on PD (10 women, 11 men), who were matched on age and sex were included in the study. There were not significant differences in mean duration of PD and HD ( $33/61 \pm 6/17$  vs  $33/35 \pm 2/85$  months, Table 1). Of the 85 patients who entered this survey, 72 patients (72/94%) had vitamin D deficiency ( $20 \geq \text{ng/dl}$ ) and 11 patients (12/94%) had vitamin D insufficiency ( $20-29 \text{ ng/dl}$ ).

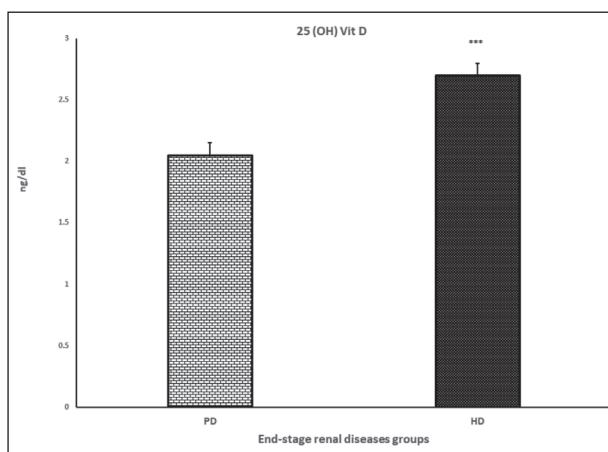
Among patients on PD, 19 patients (90/47%) had vitamin D deficiency and 2 (9/53%) had vitamin D insufficiency. In patients on HD, 43 patients (67/18%) had vitamin D deficiency and 9 (14/06%) had vitamin D insufficiency. The level of 25-OH Vitamin D3 was significantly lower in patients on PD ( $2/05 \pm 0/14$  vs  $2/70 \pm 0/10$ ,  $P < 0.001$ , Fig. 1). No meaningful difference was seen in level of vitamin D according to sex variability in the study population.

No significant difference in PD and HD groups was observed, in surveying levels of WBC, Hb, P, PTH, Albumin, Ferritin and CRP (Table 1), But Ca was significantly lower in HD group ( $9/09 \pm 0/11$ ) comparing to PD patients ( $9/78 \pm 0/22$ ,  $P < 0.01$ , Fig. 2). On the other hand, Alkaline Phosphatase was significantly higher in HD patients ( $389/50 \pm 34$ ) comparing to PD group ( $285/76 \pm 89$ ,  $P < 0.001$ , Fig. 3). While, in PD group, Platelet ( $195/40 \pm 7/6$  vs  $265/52 \pm 7/6$ ,  $P < 0/001$ , Fig. 4) and ESR ( $46/80 \pm 6/89$  vs  $23/53 \pm 1/96$ ,  $P < 0/01$ , Fig. 5) were significantly higher. Considering all the patients (HD and PD), a significant relation was seen between Vitamin D level and ESR ( $r = -0/262$ ,  $P < 0.01$ , Table 2). There was a near to significant relation between ESR and 25-OH Vitamin D in HD patients ( $r = -0/243$ ,  $P = 0.053$ , Table 2). In study of the whole population, the analysis of backward regression showed that the RRT duration, Hb and ESR had a significant effect on the level of vitamin D ( $P: 0/0001$ , Table 3).

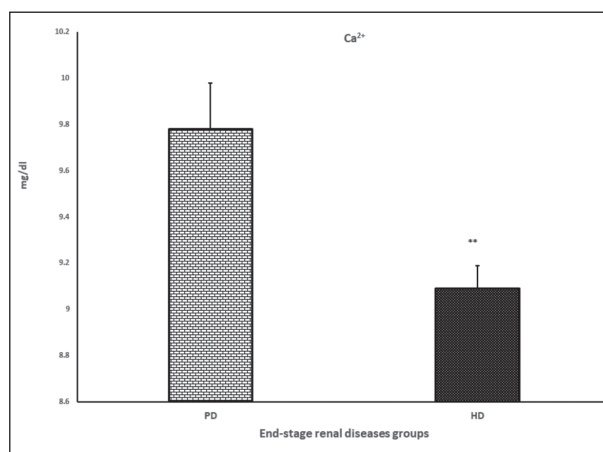
**Table 1.** The comparison of variables in the two groups of patients on hemodialysis (HD) and peritoneal dialysis (PD)

|                                   | PD           | HD           | P        |
|-----------------------------------|--------------|--------------|----------|
| 25 (OH) Vit D (ng/dl)             | 2/05±0/14    | 2/70±0/10    | 0/001*** |
| Duration of RRT                   | 33/61±6/17   | 33/35±2/85   | 0/966    |
| WBC                               | 7166/66±459  | 6756/25±265  | 0/452    |
| Hb                                | 11/30±0/38   | 11/58±0/15   | 0/409    |
| Plt (× 10 <sup>9</sup> per liter) | 265/52±15/6  | 195/40±7/6   | 0/001*** |
| Ca <sup>2+</sup> (mg/dl)          | 9/78±0/22    | 9/09±0/11    | 0/005    |
| P                                 | 4/73±0/34    | 5/02±0/14    | 0/385    |
| Ca <sup>2+</sup> × P              | 46/05±14/82  | 46/45±12/23  | 0/404    |
| PTH                               | 258/76±89    | 389/50±34    | 0/10     |
| Alk Ph                            | 212/85±26/31 | 338/25±24/10 | 0/006**  |
| Alb                               | 3/64±0/14    | 3/39±0/07    | 0/059    |
| Ferritin                          | 218/32±29    | 231/42±25    | 0/78     |
| ESR                               | 46/80±6/89   | 23/53±1/96   | 0/003**  |
| CRP                               | 1/65±0/20    | 1/93±0/09    | 0/17     |

\*p<0.05, \*\*p<0.01, and \*\*\*p<0.001 as compared to the PD group.



**Figure 1.** The comparison of 25-OH Vitamin D3 between two groups of End-stage renal disease (Patients on hemodialysis (HD) and peritoneal dialysis (PD)). \*p<0.05, \*\*p<0.01, and \*\*\*p<0.001 as compared to the PD group



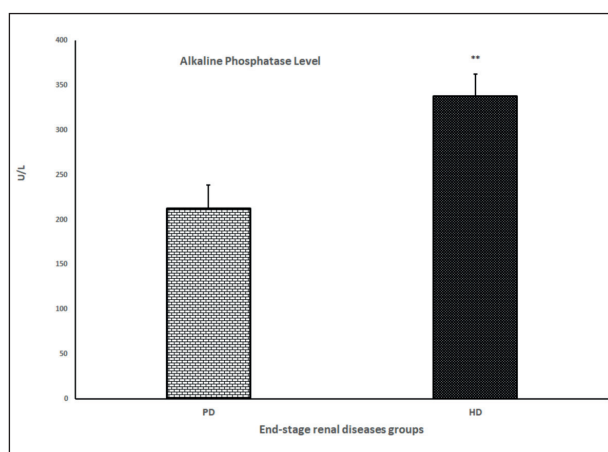
**Figure 2.** The comparison of Ca<sup>2+</sup> between two groups of End-stage renal disease. \*\*p<0.01 as compared to the PD group

Also evaluating of backward regression in patients on HD separately, showed that the duration of RRT, Hb and ESR had a significant effect on the level of vitamin D. In the model, ESR had the most effect on vitamin D (P: 0/001, Table 4).

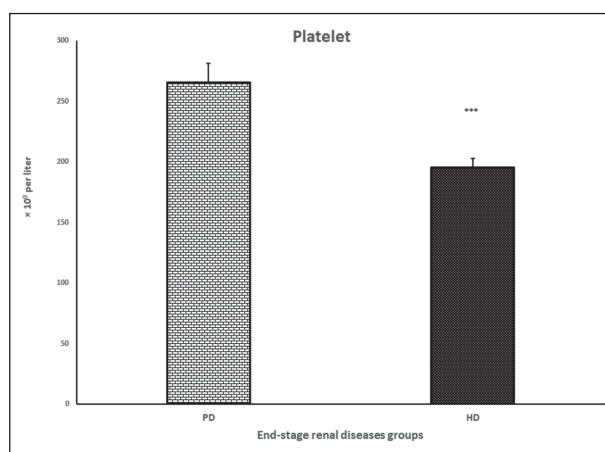
## Discussion

In our study the deficiency of vitamin D (considering all the patients in both groups) was significantly

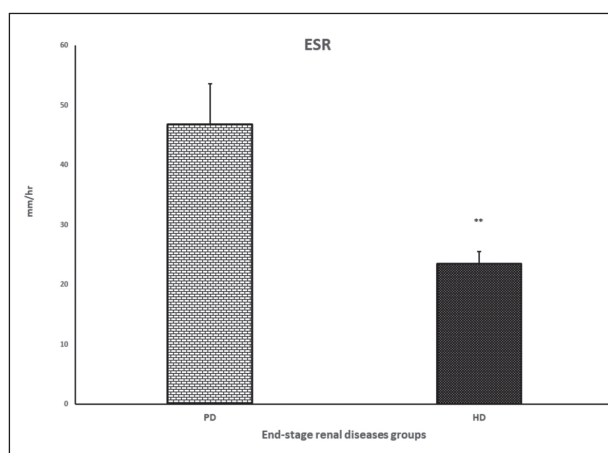
related to increase in ESR, but this kind of relation was not observed in comparing CRP and vitamin D. However previous studies have shown that, In general population there is a strong relationship between the low level of Vitamin D and CRP (11). Also the animal researches showed that the long term vitamin D deficiency is a cause of chronic inflammation (12). The activation of vitamin D, regulates the cytokines transcription and stops the overproduction of inflammatory cells (12). Vitamin D has stimulatory impact on anti-inflammatory cytokines, including: IL4, IL5



**Figure 3.** The comparison of Alkaline Phosphatase Level between patients on hemodialysis (HD) and peritoneal dialysis (PD). \*\* $p<0.01$  as compared to the PD group



**Figure 4.** The comparison of Platelet level between two groups of End-stage renal disease. \*\*\* $p<0.001$  as compared to the PD group, Values are represented as mean $\pm$  SEM



**Figure 5.** The comparison of Erythrocyte sedimentation rate (ESR) between two groups of End-stage renal disease. \*\* $p<0.01$  as compared to the PD group, Values are represented as mean $\pm$  SEM

**Table 2.** The relation of ESR with 25-Hydroxy Vitamin D considering both groups (PD and HD)

|     |    | R      | P      |
|-----|----|--------|--------|
| ESR | PD | -0/262 | 0/015* |
|     | HD | -0/243 | 0/053  |

\* $p<0.05$  as compared to the PD group

and IL10 and has inhibitory effect on pre-inflammatory cytokines, including: IL2, IL3, TNF-alpha (13). Vitamin D also facilitates the transformation of CD4 T cells to regulatory cells (13). Although CRP has a predictive role in mortality rate of patients on HD, but this role in patients on PD is not clear (14). The potential capability of vitamin D in reducing the inflammation in patients on HD is of high importance because the inflammation effects on malnutrition (15),

**Table 3.** The analysis of multiple regression to examine the effect of variables on 25-OH Vitamin D considering all patients (HD and PD)

|                 | B      | SE    | Beta   | T      | P value  |
|-----------------|--------|-------|--------|--------|----------|
| Constant        | 4/324  | 0/814 |        | 5/313  | 000***   |
| Duration of RRT | 0/007  | 0/003 | 2/007  | 0/207  | 0/048*   |
| Hb              | -0/141 | 0/066 | -0/236 | -2/138 | 0/036*   |
| ESR             | -0/013 | 0/004 | -0/377 | -3/389 | 0/001*** |

\* $p<0.05$  and \*\*\* $p<0.001$  as compared to the PD group

**Table 4.** The analysis of multiple regression to examine the effect of variables on 25-OH Vitamin D in HD patients

|                   | B      | SE    | Beta   | T      | Sig      |
|-------------------|--------|-------|--------|--------|----------|
| Constant          | 5/308  | 1/035 |        | 5/131  | 000***   |
| Dialysis duration | 0/010  | 0/004 | 0/273  | 2/318  | 0/024*   |
| Hb                | -0/208 | 0/082 | -0/313 | -2/528 | 0/014*   |
| ESR               | -0/022 | 0/006 | -0/430 | -3/413 | 0/001*** |

\* $p < 0.05$  and \*\*\* $p < 0.001$  as compared to the PD group.

resistance to erythropoietin (16, 17), atherosclerosis (18), immune response disorder (19, 20) and mortality rate of the population.

In patients on PD, no connection was observed between ESR, CRP and the level of vitamin D. In these patients, contact of peritoneal cells with the dialysis fluid which includes glucose, promotes leukocytes infiltration and causes the production of the inflammatory cytokines (21). Peritoneal mesothelial cells are defined as a part of RAAS (Renin–Angiotensin–Aldosterone System) which will be activated in acute inflammation which is suggestive of the relation of inflammation with the fluid and electrolyte balance (22). Uremia can stimulate the inflammation of peritoneum in patients on HD without a PD background which shows the importance of the adequacy of dialysis (23).

In the study of Eleftheriadis and colleagues in patients receiving HD an inverse relation was detected between the level of vitamin D, CRP and IL-6 levels (24). contrarily, in another study Esen et al, reported that there is a connection between vitamin D deficiency and rising of ESR and CRP in patients on PD (25). These controversies are probably due to different circumstances and different factors effecting vitamin D, CRP and IL-6 levels including, the size of the population of the study, illness stage and length of exposure to drugs. also The duration of renal disease and RRT have a certain impact on the inflammatory markers (26). An increase in the duration of RRT, results in reduction in the function of remaining parts of kidneys, decrease in the status of nutrition volume, and increase in atherosclerotic disorders, all together prove that all these items are related to an activated immune system (14). In our study a direct relationship was shown between the RRT duration and the level of vitamin D in HD patients so that, as the level of vitamin D was higher, the duration

of RRT would increase. this relation may be because of this matter that the more prolonged the dialysis, the more improvement in uremia status is seen. but this was not observed in patients on PD group. Patients with chronic inflammation mostly suffer from anemia and low level of vitamin D and high level of CRP because of the inhibitory effect of inflammatory mediators on the iron supply to bone marrow (27). Consistent with this data, In the study of Esen and colleagues, an association was seen between the deficiency of vitamin D and anemia, but contrarily, in our study evaluating the patients receiving hemodialysis and all the patients rather than receiving PD or HD, showed an inverse result between deficiency of vitamin D and anemia.

Aging, obesity, vegetarian diet, living in areas with high UV radiation and avoiding sun exposure, are common related risk factors that lead to decrease in production and reuptake of vitamin D (28). in most populations, women are two times more at the risk of vitamin D deficiency (28). As the adipose tissue defines as a source of subclinical inflammation and adipocytes define as receptors for the inflammatory mediators (29) , so patients with BMI over 30, did not enter this study. Since smoking may concur with inflammation, Smokers did not enter as well.

The main limitation in our study was the small sample size; it was especially visible in patients on PD. In this study we could not omit the role of malnutrition and inflammatory markers in vitamin D deficiency, although the examined cases were generally well and most of them had a normal level of albumin. Finally we came to the conclusion that in patients on HD, the deficiency of vitamin D is related to rising of ESR as an inflammatory marker. Studies with larger sample sizes with more precisely examination of the inflammatory markers are recommended.

## Acknowledgment

Funding for this study was provided by Kerman University of Medical Sciences as a grant (#:95000164) for the MD thesis conducted by Seyed Adel Mahdi.

**Declaration of transparency and scientific rigor:** This Declaration acknowledges that this paper adhered to the principles for transparent reporting and scientific rigor of preclinical research recommended by funding agencies, publishers and other organizations engaged with supporting research.

**Conflict of interest:** None to declare

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Received: 16 February 2019

Accepted: 15 April 2019

Correspondence:

Mohammad Shabani

Kerman Neuroscience Research Center,

Islamic Republic of Iran

E-mail: shabanimoh@yahoo.com