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Assessment of Vitamin D_3 Level and its Relationship with Inflammatory Markers Pre -Albumin and C-reactive protein in Hemodialysis Patient

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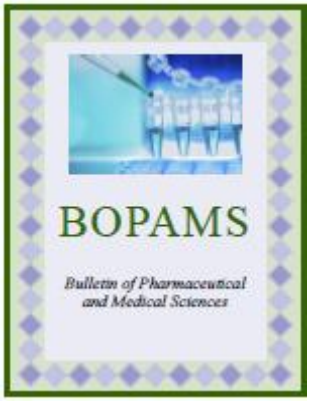

ABSTRACT

Recent studies propose that low vitamin D focuses are related with increased levels of inflammatory markers. In any case, there are restricted examinations researching relationship between vitamin D levels and inflammatory markers in the general population and quite a bit of this evidence in older adults is inconclusive. The present study focused to assess the vitamin D_3 in dialysis patients for people with diabetes and non diabetes patients, is find a possible association between vitamin D_3 and prealbumin is find a possible association between vitamin D_3 and has CRP in dialysis patients for people with diabetes and non diabetes patients.

The Study suggests that vitamin D deficiency is common in hemodialysis patients, Serum vitamin D level is positively associated with serum hsCRP level and negatively associated with prealbumin level in both groups diabetic and non diadetic patient, Vitamin D deficiency may play an important role in the increased inflammatory status in dialysis patients. The present study comes to an important conclusion that hsCRP is a useful independent predictor in CKD and correlated with vitamin D levels.

1.0 INTRODUCTION

Vitamin D (A steroid vitamin) which promotes the intestinal absorption and metabolism of calcium and phosphorus. Under normal conditions of sunlight exposure, dietary supplementation is necessary because sunlight promotes no adequate vitamin D synthesis in the skin. deficiency vitamin D can lead to bone deformity rickets in children and bone weakness osteomalacia in adults (Holick,2006). Cardiovascular disease and malnutrition are causes of the increased morbidity and mortality observed in hemodialysis patients. C-reactive protein (CRP), an acute-phase protein, is a predictor of cardiovascular mortality in chronic kidney disease patient population (Lloyd *et al*.,2006). That low vitamin D levels may result from diseases rather than causes disease (Autier et al.,2014).

Pre-albumin an acute-phase protein is a predictor of malnutrition (Shenkin,2006) the prevalence of an acute-phase response has been associated with an increased mortality. Chronic kidney disease (CKD) denotes the renal impairment for three or more months indicated by the presence of abnormalities in structure or function of the kidney with or without decreasing glomerular filtration rate (GFR) that is triggered by pathologic anomalies of renal structure , defects in the constituents of the blood or urine and /or abnormality in imaging or radiological investigations (Mindikoglu *et al*., 2014 ; Neil *et al*., 2016).

CRP is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells (Thompson,1999). CRP is synthesized by the liver (Pepys and Hirschfield,2003). In response to factors released by macrophages and fat cells (adipocytes) (Lau *et al.,*2005). It is a member of the pentraxin family of proteins (Pepys and Hirschfield,2003). t is not related to C-peptide (insulin) or protein C (blood coagulation). C-reactive protein was the first pattern recognition receptor (PRR) to be identified (Mantovani *et al.,*2008). CRP rises within two hours of the onset of inflammation, up to a 50,000-fold, and peaks at 48 hours. Its half-life of 18 hours is constant, and therefore its level is determined by the rate of production and hence the severity of the precipitating cause. CRP is thus a marker for inflammation that can be used to screen for inflammation.

CKD stage 5 is indicated by eGFR < 15 mL/min/1.73 m^2 with or without renal replacement therapy (RRP), while the term end stage renal disease (ESRD) implies kidney failure , where dialysis or kidney transplantation is necessary to tolerate renal survival. ESRD can be indicated by imaging study and laboratory tests (Neil *et al*., 2016; Fan *et al*., 2016).

Hemodialysis, ordinarily called kidney dialysis or just dialysis, is a procedure of decontaminating the blood of a man whose kidneys are not working typically. This kind of dialysis accomplishes the extracorporeal expulsion of waste items, for example, creatinine and urea and free water from the blood when the kidneys are in a condition of kidney disappointment. Hemodialysis is one of three renal substitution treatments (the other two being kidney transplant and peritoneal dialysis). An option strategy for extracorporeal detachment of blood segments, for example, plasma or cells is apheresis (National Kidney,2004; Hugh, 2016). Hemodialysis can be an outpatient or inpatient treatment. Routine hemodialysis is led in a dialysis outpatient office, either a reason manufactured room in a healing facility or a devoted, remain solitary center. Less every now and again hemodialysis is done at home. Dialysis medications in a facility are started and overseen by specific staff made up of attendants and professionals; dialysis medicines at home can act naturally started and oversaw or done mutually with the help of a prepared partner who is generally a relative (Daugirdas,2007).

Aim of the study

The aim of this study was to assess vitamin D3 in dialysis patients for people with diabetes and non diabetes patients, is find a possible association between vitamin D3 and prealbumin is find a possible association between vitamin D3 and has CRP in dialysis patients for people with diabetes and non diabetes patients.

2.0 Experimental

2.1 Subjects and Study protocol

This study was carried out at Medical City Complex, Baghdad Teaching Hospital, Iraqi Center of Kidney Dialysis under the supervision of consultant nephrologist from December 2016 to August 2017 One hundred and four subjects were selected to participate in this study. Only (90) subjects completed the courses of the study successfully. All patients (49 male & 41 female) . Mean age of the subjects was 53.2 ± 13.5 years. These subjects were recruited into the following groups. It classified in to two groups.

•Group A : included 45 persons as diabetic nephropathy

•Group B : included 45 persons non diabetic nephropathy

The study protocol was approved by the local ethical committee of the college of medicine Karbala University, with verbal informed consent from patients

2.1.1 Inclusion Criteria: The primary inclusion criteria involved patients with chronic renal failure on maintenance hemodialysis for at least six months, Secondary inclusion criteria involved patients diabetic nephropathy and non diabetic nephropathy

2.1.2. Exclusion Criteria

- Acute renal failure.
- Patients with hepatitis B virus
- Patients with hepatitis C virus

- Inadequate data.
- Patients with arthritis and Rheumatoid
- Peritoneal dialysis
- Renal carcinoma.
- Heart disease and bone disease

2.2 Data Collection

Information on demographic characteristics (such as age, weight, height, occupation, handedness, consanguinity and current smoking and alcoholic status) were obtained through patient interview at baseline (Appendix A). Causes and family history of CKD and the presence of co morbidities including coronary artery disease, hypertension, diabetes mellitus and congestive heart failure, as well as the medication history, duration of dialysis and dialysis treatment, were reported by the patients' nephrologists. Blood pressure, mean arterial blood pressure (MABP), and pulse rate were recorded from the dialysis machine directly when the patient in a supine position during a dialysis session. Body mass index (BMI) was calculated as weight in kilogram divided by height in meter squared

2.3 Sample Collection and Preparation

Five to eight milliliters of venous blood were obtained from patients and control group, Blood samples were collected in the morning by venipuncture using 10 mls disposable syringes. Blood was divided into 2 parts **First part**: 6ml was put in the plain tube and left to clot for 30 minin room temperature and then separated by centrifugation at (3000 rpm) for (10 min), then the serum was divided into three parts, and put into three eppendorff tubes then stored in the freezer at -70˚C until use then collected in tubes without anticoagulants.

Second part: 3ml blood was put into EDTA tube, tube with anticoagulants blood group mixed gently and put on shaker for measurements HbA1C.

2.3.1 Chemicals: All chemicals and reagents were of the highest available purity and needed no more purification. Specific diagnostic kits and chemicals used in this study are listed in table 2-1 with their suppliers.

2.3. Methods

2.3.1. Determination of pre-albumin

Principle: This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human prealbumin(PA). Add prealbumin(PA) to wells that are pre-coated with prealbumin(PA) monoclonal antibody and then incubate. After incubation, add anti PA antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of Human prealbumin(PA) are positively correlated

Washing method

A. Washing by hand:Shake off liquids in the wells of the ELISA plate. Lay several bibulous papers on the test bed and pat the ELISA plate hard several times downward. Then inject at least 0.35ml of diluted washing concentrate and soak for 1-2 minutes. Repeat as needed.
B. Washing by automatic plate washer: If an automatic plate washer is available, it should only be used in the test by those proficient with its function

Assay procedure

A. Dilution of standard solutions: This kit provides one standard original concentration. Users may independently dilute in small tubes following the chart below:

Standard solution **No.5** **No.4** **No.3** **No.2** **No.1**

B. The number of stripes needed is determined by that of samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

C. Sample injection: 1) Blank well: Do not add sample, anti PA antibody labeled with biotin and streptavidin-HRP; add chromogen reagent A & B and stop solution, each other step operation is the same. 2) Standard solution well: Add 50µl standard and streptomycin-HRP 50µl (biotin antibodies have united in advance in the standard so no biotin antibodies are added). 3) Sample well to be tested: Add 40µl sample and then 10µl PA antibodies, 50µl streptavidin-HRP. Then cover it with seal plate membrane. Shake gently to mix. Incubate at 37℃ for 60 minutes.

D. Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

E. Washing: carefully remove the seal plate membrane, drain liquid and shake off the remainder. Fill each well with washing solution, let stand for 30 seconds, then drain. Repeat this procedure five times then blot the plate.

F. Color development: First add 50µl chromogen reagent A to each well, and then add 50µl chromogen reagent B to each well. Shake gently to mix. Incubate for 10 minutes at 37℃ away from light for color development.

G. Stop: Add 50µl Stop Solution to each well to stop the reaction (color changes from blue to yellow immediately at that moment).

H. Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450 nm wavelength, which should be conducted within10 minutes after having added stop solution.

i) According to standards concentrations and corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Statistical software could also be employed

Determination of high-Sensitivity C-Reactive Protein

Principle: The principle of (ichroma™ hsCRP) uses a sandwich immuno fluorescence assay. A fluorescence conjugated Anti-CRP in a detection buffer binds to CRP in a sample to form an antigen- antibody complex. These antigen-antibody complexes are then captured by another Anti-CRP that has been immobilized on a test strip, as the sample mixture migrates through a nitrocellulose matrix. The more CRP in a sample, the more antigen-antibody complexes are accumulated on a test strip, resulting in higher signal intensities of fluorescence. ichroma™ Reader analyzes and reads the fluorescence intensity, and shows the CRP concentration in a sample (Oh sw and Moon JD et al., 2005).

Procedure (ichroma™ hsCRP Test) :

i. Taked out one tube of Detection Buffer from refrigerator and leave it at room temperature.
ii. Make a puncture on the top of the detection buffer tube by inserting an empty sample collector.
iii. Draw 10 µL of sample with a sample collector.

iv. If necessary, wipe out the excess blood outside of the capillary on the sample collector with paper towel.
v. Assemble the sample collector and the tube into one.
vi. Shake the 10 times or more until the sample out of the sample collector by inversion. The mixture of buffer and the sample has to be used within 30 seconds.
vii. Remove the cap off the top of assembled tube. Discard two drops of reagent onto the paper towel before applying to the cartridge
viii. Apply only two drops of the mixture onto the sample well of the cartridge
ix. Leave the Cartridge at room temperature for 3 minutes before inserting the device into the holder.
x. For scanning the sample-loaded Test Cartridge, insert it into the Test Cartridge holder in the ichroma™ Reader. Ensure proper orientation of the Test Cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.
xi. Press "Select" button on the ichroma™ Reader to start the scanning process.
xii. The ichroma™ Reader will immediately scan the sample- loaded Test Cartridge.
xiii. Read the test result on the display screen of the ichroma™ Reader.

3.0 Results

3.1 Demographic and disease characteristics

Mean age of the subjects was 53.2 ± 13.5 years, with mean BMI of 26.6 ± 5.3 Kg/m^2, female and male had similar distribution (49; 54.4% female and 41; 45.6% males), mean duration of ESRD was 9.9 ± 4.7 years, 50 patients (55.6%) had fistula access and 40 patients (44.4%) had graft catheter access, mean arterial blood pressure was 107.6 ± 18.5

There was no significant difference in age, BMI, Gender, vascular access and MABP between DM and non DM patients. While the duration of disease was significantly longer in non DM patients as illustrated in table 3-1 and figure 3-1

Table 3-1: Demographic and disease characteristics

	Non diabetic	Diabetic	All	P value
Number	45	45	90	-
Age, mean ± SD	50.5 ± 13.7	55.7± 13.0	53.2 ± 13.5	0.068
BMI, mean ± SD	26.0 ± 5.5	27.2 ± 5.1	26.6 ± 5.3	0.279
Gender, number (%)				0.672
Female	22 (48.9%)	19(42.2%)	41 (45.6%)	
Male	23 (51.1%)	26(57.8%)	49 (54.4%)	
Disease duration,mean± SD	10.9 ± 5.0	8.9 ± 4.1	9.9 ± 4.7	0.046 [Sig.]
Vascular access, number (%)				0.671
Fistula	26 (57.8%)	24(53.3%)	50 (55.6%)	
Graft catheter	19 (42.2%)	21(46.7%)	40 (44.4%)	
MABP, mean ± SD	107.5 ± 18.5	107.7±18.7	107.6 ± 18.5	0.938

Independent t test and chi square was used

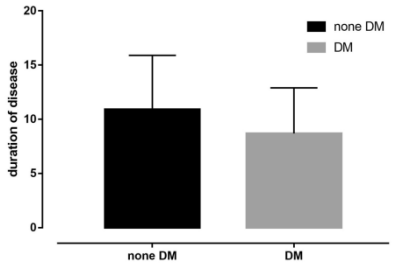

Figure 3-1: Duration of disease

3.2. Association between demographic data and DM with vitamin D_3 levels

There were no significant differences between vitamin D_3 and diabetic and non-diabetic patients in there, Age, BMI, Gender(Female and Male) as illustrated in table 3-2.

Table 3-2: Association between demographic data and DM with vitamin D_3 levels

Variables	Vitamin D levels			P value
	5 - 15 ng/mL	16 - 30 ng/mL	30 - 100 ng/mL	
Age	53.62 ± 13.76	48.73 ± 14.12	60.43 ± 4.86	0.159
BMI	26.60 ± 5.46	27.63 ± 5.29	24.01 ± 3.55	0.335
Gender				0.874
Female	32 (47.1%)	6 (40.0%)	3 (42.9%)	
Male	36 (52.9%)	9 (60.0%)	4 (57.1%)	
DM				0.670
Non DM	33 (48.5%)	9 (60.0%)	3 (42.9%)	
DM	35 (51.5%)	6 (40.0%)	4 (57.1%)	
ANOVA and chi square test used				

3.4 Electrolyte and serum proteins

Serum albumin was significantly lower in diabetic patients (3.4 ± 0.5 vs. 3.6 ± 0.6), also sodium was significantly lower in DM patients compared to DM (135.5 ± 5.1 vs. 138.4 ± 8.2), the rest of the variables including pre-albumin did not differ significantly between groups, as illustrated in table3-3

Table 3-3: Various laboratory markers in both groups

	Non Diabetic	Diabetic	All	P value
Number	45	45	90	-
Albumin, mean ± SD	3.6 ± 0.6	3.4 ± 0.5	3.5 ± 0.6	0.035
Low.Albumin (<3.9g/L)	32 (71.1%)	36 (80.0%)	68 (75.6%)	
Normal Albumin(3.9–4.9 g/L)	13 (28.9%)	9 (20.0%)	22 (24.4%)	
Pre-albumin, mean ± SD	30.3 ± 20.5	35.2 ± 26.1	32.8 ± 23.5	0.319
Protein, mean ± SD	6.9 ± 1.9	6.7 ± 1.3	6.8 ± 1.6	0.476
Globulin, mean ± SD	3.3 ± 1.7	3.3 ± 1.2	3.3 ± 1.4	0.941
HsCRP, median (IQR)	6.88 (2.12–11.21)	5.55 (1.81– 10.98)	5.81 (1.95 – 11.07)	0.537
Uric acid	4.1 ± 1.6	4.7 ± 2.3	4.4 ± 2.0	0.145
K	5.1 ± 1.1	5.6 ± 1.0	5.4 ± 1.1	0.053
Na	138.4 ± 8.2	135.5 ± 5.1	136.9 ± 6.9	0.042
Independent t test, chi square and Mann Whiney U test used				

3.5 Renal and bone disease

Median PTH was 196.8 with interquartile range (2% to 75% of the patients) 87.0 – 312.4, of those 71.7% with PTH level ≥ 300, total calcium was 8.4 ± 0.9, while ionized calcium was 4.3 ± 0.6, phosphate serum level 5.5 ± 1.9, and calcium phosphate product 46.2 ± 16.3 with 31.1% had ≥55 calcium phosphate product in the all the patients, as illustrated in table 3-4.

PTH, corrected total serum calcium, ionized calcium, phosphate, and calcium phosphate product was no statistically different between DM and non DM, as illustrated in table 3-8

Table 3-4: Renal bone disease markers in both groups

	Non diabetic	**Diabetic**	**All**	**P value**
Number	45	45	90	-
PTH,median (IQR)	229.8(130.9– 376.0)	153.7(72.1– 297.9)	196.8(87.0– 312.4)	0.102
PTH groups				0.352
≥300	30 (66.7%)	34 (75.6%)	64 (71.1%)	

<300	15 (33.3%)	11 (24.4%)	26 (28.9%)	
Calcium corrected	8.4 ± 0.8	8.5 ± 1.1	8.4 ± 0.9	0.511
Ionized calcium	4.3 ± 0.6	4.2 ± 0.6	4.3 ± 0.6	0.508
Phosphate	5.3 ± 2.1	5.7 ± 1.7	5.5 ± 1.9	0.305
Ca *x* PO_4	43.9 ± 17.0	48.5 ± 15.5	46.2 ± 16.3	0.187
Ca *x* PO_4 groups				0.222
<55	34 (75.6%)	28 (62.2%)	62 (68.9%)	
≥55	11 (24.4%)	17 (37.8%)	28 (31.1%)	
Independent t test and chi square used				

3.5 Vitamin D3

Overall median vitamin D3 levels was 8.76 ng/ml with interquartile range from 7.34 to 15.1 ng/ml, patients with diabetic had lower median vitamin D compared to non-diabetic 7.99 vs. 10.55 ng/ml. Mild deficiency was in 75.6% of total patients with serum vitamin D3 between 5 – 15 ng/ml, about 16.7% of total patients presented as insufficiency (16 – 30 ng/ml), and finally only 7.8% was in the normal range (31 – 100 ng/ml) as illustrated in table 3-5 and figure 3-3.

Table 3-5: vitamin D_3 (ng/ml) in both groups

	Non diabetic	Diabetic	All	P value
Number	45	45	90	-
Vitamin D, median (IQR)	10.55 (7.44–16.65)	7.99 (7.27–13.90)	8.76 (7.34 – 15.10)	0.319
Vitamin D levels				0.480
5 – 15 ng/ml	33 (73.3%)	35 (77.8%)	68 (75.6%)	
16 – 30 ng/ml	9 (20.0%)	6 (13.3%)	15 (16.7%)	
31 – 100 ng/ml	3 (6.7%)	4 (8.9%)	7 (7.8%)	

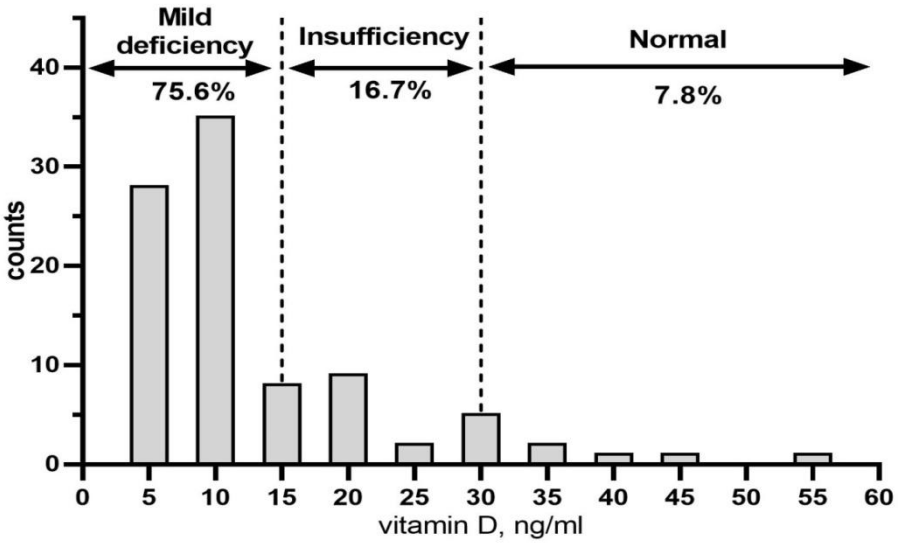

Figure 3-3: Distribution of vitamin D_3 in dialysis patients

3.6 Relationship between vitamin D_3 and various variables

Vitamin D_3 correlated significantly and inversely with protein globulin, FBS, and HsCRP (the strongest relationship was HsCRP), no significant relationship was observed with the rest of the variables in table 3-11 and figure 3-4

Table 3-6: Correlation between vitamin D_3 and various variables in all subjects

Variables	r	P value
Age	0.034	0.746
BMI	0.062	0.557
Disease Duration	0.068	0.522
MABP	-0.151	0.152
Albumin	0.015	0.883
Pre-albumin	0.044	0.677
Protein	-0.220	0.035 [Sig.]
Globulin	-0.289	0.005 [Sig.]
HsCRP	-0.343	0.001 [Sig.]
HbA1c	0.015	0.885
FBS	-0.208	0.047 [Sig.]
PTH	-0.162	0.122
Calcium corrected	0.058	0.586
PO_4	0.024	0.818
CaxPO4	0.055	0.604
Urea	0.128	0.223
Creatinine	0.119	0.258
GFR	-0.113	0.283
r: correlation coefficient		

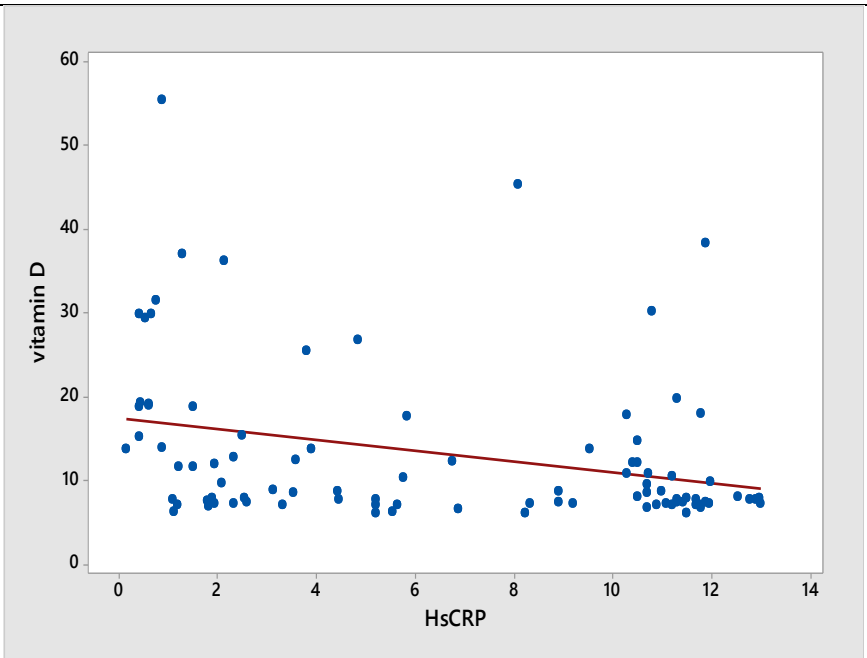

Figure 3-4: Correlation between HsCRP and vitamin D_3

There was significant inverse relationship between HsCRP and vitamin D in both groups (DM or not), while in DM only group protein and globulin also significantly and inversely correlated with vitamin D, as illustrated in table 3-7

Table 3-7: Correlation between vitamin D_3 and various variables in both groups

	r	P value	r	P value
Variables	Non DM		DM	
Age	0.002	0.992	0.112	0.455
BMI	0.165	0.279	-0.012	0.937
Disease Duration	-0.022	0.888	0.061	0.685
MABP	-0.055	0.722	-0.224	0.130
Albumin	0.071	0.643	-0.126	0.400
Pre-albumin	-0.007	0.962	0.137	0.358
Protein	-0.005	0.973	-0.409	0.004 [Sig.]
Globulin	-0.173	0.257	-0.363	0.012 [Sig.]
HsCRP	-0.352	0.018 [Sig.]	-0.327	0.025 [Sig.]
HbA1c	0.269	0.074	0.026	0.865
FBS	-0.241	0.111	-0.187	0.209
PTH	-0.196	0.198	-0.190	0.201
Calcium corrected	0.090	0.559	0.059	0.692
PO_4	0.101	0.509	-0.008	0.955
CaxPO4	0.126	0.411	0.054	0.719
Urea	0.331	0.026 [Sig.]	-0.001	0.997
Creatinine	0.094	0.541	0.165	0.267
GFR	-0.053	0.728	-0.163	0.274
Linear regression analysis				

Discussion

In this study only 7.8% of total patients had normal serum vitamin D, 16.7% had insufficiency and 75.6% of had mild deficiency, despite that there is no significant difference in vitamin D levels when the patients divided into two groups based upon if they had diabetic or not; more patients with diabetic had mild deficiency compared to non-diabetic (77.8% vs. 73.3%), overall median vitamin D was 8.76 ng/ml, also vitamin D in patients with diabetic was lower than those without diabetic (7.99 vs. 10.55 ng/ml), our results agreed with a cross sectional study in Spain 2010 involved 115 patients in which 7% of the dialysis patients had serum vitamin level in the normal range (>30 ng/ml), but disagree with them by having higher rate of vitamin D deficiency with 51% in the mild deficiency range (5 – 15 ng/ml) and 42% had vitamin D insufficiency (16 – 30 ng/ml) (Iguacel *et al*., 2010).

The results of the current study show no significant relationship between vitamin D with pre-albumin in all the patients (r=0.044, p=0.677), in diabetic patients (r=0.137, p=0.358) and in non-diabetic patients (r=-0.007, p=0.962), also in the current study no significant correlation observed between vitamin D with serum albumin in all the patients (r=0.015, p=0.883), with diabetic patients only (r=-0.126, p=0.400) and in non-diabetic patients (r=0.071, p=0.643) (Iguacel *et al*., 2010).

Reported that a similar non-significant correlation between vitamin D with pre-albumin and vitamin D in their study that involved HD patients, a possible explanation of no relationship with albumin or pre-albumin is that vitamin D change affect it binding protein (Vitamin D-binding protein), known as globulin which is responsible on 85 – 90% of vitamin D2 in the circulation while 10 – 15% is albumin bound <1% in the free found (Powe *et al*., 2000). So the low binding capacity could explain this no association between vitamin D and albumin and pre-albumin.Vitamin D inversely correlation with high sensitivity CRP (r= -0.343, p=0.001) in all the patients, and this relationship remain the same in diabetic and non-diabetic patients, (Iguacel *et al.,* 2010).

In various studies that involved inflammatory disease like rheumatoid arthritis (Patel *et al*., 2007). vitamin D have been shown to have effect on inflammation (Levin *et al*., 2005) like inhibition of antigen-presenting cell maturation, modulation of cytokine production that leads to anti-inflammatory effect, and down-regulation of NF-κB (by increased IL-10 and also decreasing IL-6, IL-12, and TNF-α), on the other hand;

there is a possibility that inflammation leads to low vitamin D levels. Since this study is observational it is difficult to determine which is the cause of the inverse correlation between vitamin D and HsCRP (i.e. inflammation), however depending on the results of in vitro studies that shown vitamin D have causal effect on inflammation, indicating that low vitamin D levels leads to the proinflammatory condition in dialysis patients. Studies performed of patients that do not have renal impairment revealed vitamin D supplementation had suppressor effect on serum TNF- α and also increase IL-10 levels (Muller *et al*.,1992).

In the current study no significant correlation between vitamin D and with various variables was observed like age, BMI, disease duration, albumin, prealbumin, HbA1c, calcium, PO4, our findings was in agreement with (Iguacel *et al.,* 2010). in which they found no correlation with these parameters also they found inverse relationship between vitamin D and PTH ($r = -0.24$, $p < 0.05$) which is similar to our findings ($r = -0.162$) but in the current study the magnitude of the relationship was lower compared to (Iguacel *et al.,* 2010).

Result was in disagreement in this regards in which no such relationship was observed; this disagreement could be caused by different type of CRP analytical methods (since in the current study we use high sensitivity CRP), different in sample size , or their study included different modality of dialysis while in which only hemodialysis was used all these can explain this differences. On the other hand (Shroff *et al*., 2008). was in agreement with our findings of the inverse correlation between HsCRP with vitamin D ($r= -0.29$, $p < 0.0001$) and this correlation was associated with vascular calcification. The enzyme 1-α hydroxylase expressed on various cell types like macrophages, dendritic cells, and endothelial cells, in which it perform autocrine/paracrine function independent of the PTH–bone axis and this function is unaffected by renal failure but immune stimuli have regulatory function (Mathieu *et al*., 2002).

Conclusion

According to the findings of the current study, it can be concluded that:

- Serum level of vitamin D_3 was lower in patients with graft catheter Reverse patients with fistula however it did not reach statistical significance.
- Vitamin D_3 correlated significantly and inversely with protein globulin, FBS, and hsCRP (the strongest relationship was hsCRP), no significant relationship was observed with the rest of the variables
- There was significant inverse relationship between hsCRP and vitamin D_3 in both groups (diabetic nephropathy or Non diabetic nephropathy), while in diabetic nephropathy only group protein and globulin also significantly and inversely correlated with vitamin D_3.
- There was no significant relationship between vitamin D with pre-albumin in all the patients. in diabetic nephropathy or Non diabetic nephropathy.
- There was direct and significant relationship between disease duration and pre-albumin, and also significant and inverse relationship with protein in diabetic patients only.

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