



DETERMINATION OF VITAMIN-D, CRP & ASSOCIATION WITH HEMOGLOBIN LEVEL IN HEMODIALYSIS PATIENTS

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Abstract:

Background: Chronic kidney disease (CKD) is becoming a major chronic illness in the world. In India, population >1 billion people facing renal disorders, the rising incidence of CKD is expected to pose major challenges to both the healthcare system and the economy in the coming years. The number of patients with chronic kidney disease (CKD) has been rising, impacting an estimated 843.6 million people globally in 2017. This increase can be partially attributed to the rise in risk factors, such as obesity, hypertension, and diabetes mellitus. Several human and animal studies have suggested that vitamin D deficiency may be a contributory factor in the pathogenesis of chronic kidney disease. The primary causes of anemia in CKD include low erythropoietin levels from loss of erythropoietin synthesis or from presence of inhibitors of erythropoietin production, iron deficiency, and decreased half-life of circulating red blood cells. CRP is also a major acute phase reactant which is elevated as a response to tissue damage and is considered as an important indicator of systemic inflammation

Materials and Methods: The total study group consists of 200 subjects, of which 100 Hemodialysis patients (cases) & 100 were healthy individuals (controls). Venous blood was used for analysis. Renal function test were done on Erba EM 200 chemistry analyzer, Serum electrolytes by Ion selective electrode on PSR ST 200, Serum VIT-D by Competitive Fluorescent Immunoassay & Serum CRP by Turbidimetric method. The data analysis was done by using mean, standard deviation & student t-test.

Results: Vit-D (10.79 ± 2.03 vs. 38.81 ± 5.90), Hb (7.13 ± 0.70 vs. 13.35 ± 0.87) were lower in Hemodialysis patients than control subjects. The Crp (90.92 ± 34.9 vs. 2.21 ± 1.23) was higher in Hemodialysis patients than control subjects. The level of serum Vit-D & Hb were significantly decreased in Hemodialysis patients as compared to healthy individuals.

Conclusion: The present study concluded that decrease activity of vitamin D, calcium, Hemoglobin & significant elevation of CRP, phosphorus & potassium in Hemodialysis patients as compared to healthy individuals.

Key words: Chronic kidney disease (CKD), Glomerular filtration rate (GFR), C-reactive protein (CRP)

Introduction:

Chronic kidney disease (CKD) is becoming a major chronic illness in the world. ¹ In the twenty-first century, chronic kidney disease (CKD) has become one of the leading causes of death and suffering. The number of patients with chronic kidney disease (CKD) has been rising, impacting an estimated 843.6 million people globally in 2017. This increase can be partially attributed to the rise in risk factors, such as obesity, hypertension, and diabetes mellitus. ² In India, population >1 billion people facing renal disorders, the rising incidence of CKD is expected to pose major challenges to both the healthcare system and the economy in the coming years. According to recent estimates, India's age-adjusted incidence rate of end-stage renal disease (ESRD) is 229 per million people (pmp), and more than 100,000 new patients enroll in renal replacement programs in each year. CKD patients are detected only at advanced stages due to lack of community-based screening programs. ^{3,4}

Vitamin D, a fat-soluble vitamin also known as an anti-ricketic factor or sunshine vitamin. Besides its pivotal role in calcium homeostasis and bone mineral metabolism, the vitamin D endocrine system is now recognized to be involved in a wide range of fundamental biological functions in cell differentiation, inhibition of cell growth, and immunomodulation. Several human and animal studies have suggested that vitamin D deficiency may be a contributory factor in the pathogenesis of chronic kidney disease (CKD).^{5,6} Vitamin D deficiency is a rising health issue in patients with CKD. ⁷ It can lead to serious rickets, periodontitis, osteoporosis, weakness, muscle ache, and depression⁸ several studies have identified an association between vitamin D deficiencies and reduced decreased glomerular filtration rate (GFR) in patients with CKD. ⁹

The incidence and prevalence of anemia increases as kidney function declines. The primary causes of anemia in CKD include low erythropoietin levels from loss of erythropoietin synthesis or from presence of inhibitors of erythropoietin production, iron deficiency, and decreased half-life of circulating red blood cells.^{10,11,12} Approximately 30-50% of dialysis patients show symptoms that indicate active inflammatory response.¹³ CRP is also a major acute phase reactant which is elevated as a response to tissue damage and is considered as an important indicator of systemic inflammation.¹⁴ The association of 25(OH) D levels and CRP level with hemoglobin concentrations in the setting of decreased kidney function has not been extensively examined. We examined the association between serum 25(OH) D levels, CRP and hemoglobin levels in Hemodialysis patients.

Materials and Methods

Study Design:

The present study was hospital based cross sectional observational study, which had carried out in the Department of Biochemistry, Rama Medical College, Hapur. The subjects for the study included from Nephrology OPD of Rama Medical College, Hapur. The written consents were taken from the patients prior to the study & the objectives of the study were fully explained. The written informed consent was taken from the subjects to be included in the study.

Study groups

The study included a total 200 subjects; which was divided in to two groups. The first group has 100 cases & second group has 100 controls. Selection of cases was done on the basis of Renal functions tests markers. CKD is defined as abnormalities structure or function of a kidney that has been present for more than three months and has health implications (not graded)

Group I (Cases): This group had 100 Patients of Hemodialysis

Group II (Controls): This group had 100 Age & gender matched healthy individuals

Exclusion criteria for cases

1. Any other Acute /Chronic inflammatory disorder
2. Smoking & Alcoholism
3. Recent use of lipid lowering drugs & corticosteroids
4. Pregnant or lactating women.

Exclusion criteria for Controls

1. Any other Acute /Chronic inflammatory disorder
2. Smoking & Alcoholism
3. Pregnant or lactating women.

Objectives:

- Estimation of Renal function test markers in Hemodialysis patients & controls
- Compare the result of Renal function test markers in Hemodialysis patients & controls
- Estimation of Vit-D, crp & Hb level in Hemodialysis patients & controls
- Compare the result of Vit-D, Crp & Hb in Hemodialysis patients & controls

Sample collection:

Five ml blood will be collected from the patients as well as controls after taking appropriate aseptic precaution. The sample was collected in EDTA & plain vacutainer for the estimation of various parameters.

Methods:

- Estimation of Serum Urea by Urease, commercially available kit from ERBA Diagnostics Mannheim, Germany ¹⁵
- Estimation of Serum Creatinine by Jaffe’s method, commercially available kit from ERBA Diagnostics Mannheim, Germany ¹⁶
- Estimation of Serum Uric acid by modified Trinder peroxidase method, commercially available kit from ERBA Diagnostics Mannheim, Germany ¹⁷
- Estimation of Serum electrolytes by Ion selective electrode on PSR ST 200 ¹⁸
- Estimation of VIT-D by Competitive Fluorescent Immunoassay Technique ¹⁹
- Estimation of Serum CRP by Turbidimetric method on ERBA EM 200 Machine ²⁰

Statistical Analysis

Data and various parameters will be analyzed on SPSS software (USA inc.) version 23. Mean and standard deviation of all parameters will be calculated. Chi square test will be applied to non-parametric variables. Student t-test will used to compare averages in two groups

Results:

Table 1: Comparison of Renal function test in Cases & Controls Group

Parameters	Group-I (Cases) Mean± SD	Group -II (Controls) Mean± SD	P value
Serum Urea(mg/dl)	205.88±104.45	29.0 ±5.95	<0.001
Serum Creatinine (mg/dl)	8.78±4.02	0.85 ±0.19	
Serum Uric Acid (mg/dl)	7.688±2.09	4.43±0.85	
Serum Calcium (mg/dl)	8.3±0.27	9.42 ±0.55	
Serum Phosphorus (mg/dl)	6.70±0.73	4.0±0.52	

Table 2: Comparison of Serum Electrolytes in Cases & Control Groups

Parameters	Group-I (Cases) Mean± SD	Group -II (Controls) Mean± SD	P value
Sodium (Na) (meq/l)	139.57±4.62	139.5±2.77	<0.941
Potassium (K) (meq/l)	5.32±0.64	3.87±0.21	<0.001

Table 3: Comparison of Vit-D, Crp & Hb level in Cases & Controls Group

Parameters	Group-I (Cases) Mean± SD	Group -II (Controls) Mean± SD	P value
Vitamin D (ng/ml)	10.79±2.03	38.81 ±5.90	<0.001
CRP (mg/l)	90.92±34.9	2.21 ±1.23	
Hemoglobin (Hb) (g/dl)	7.13±0.70	13.35 ±0.87	

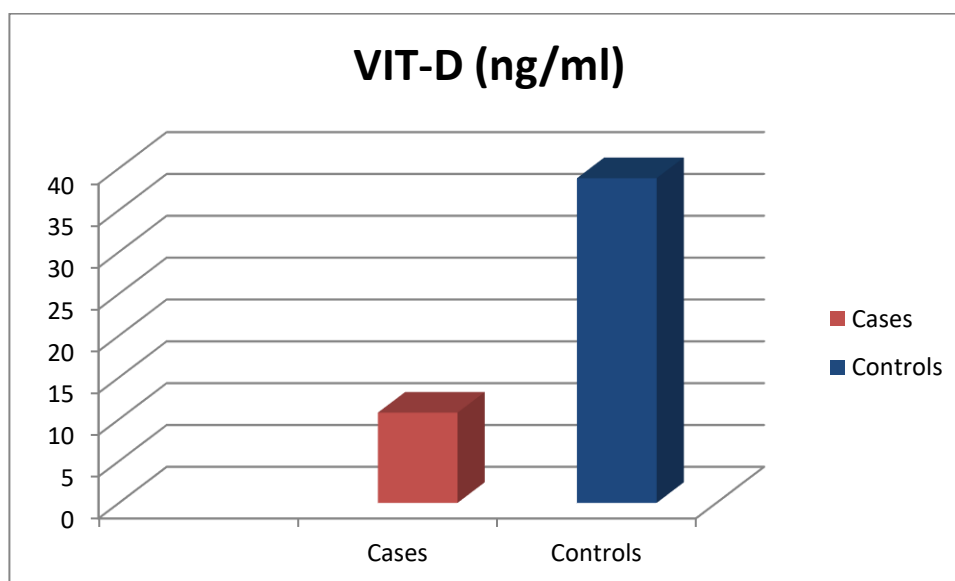


Fig 1: Shows Vitamin –D level in cases & Controls group

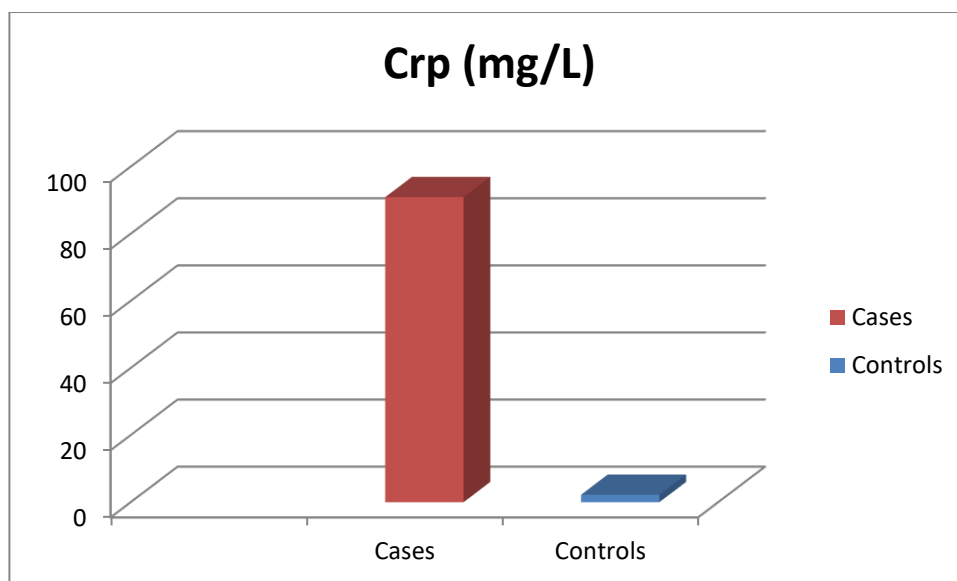


Fig 2: Shows serum CRP level in cases & Controls group

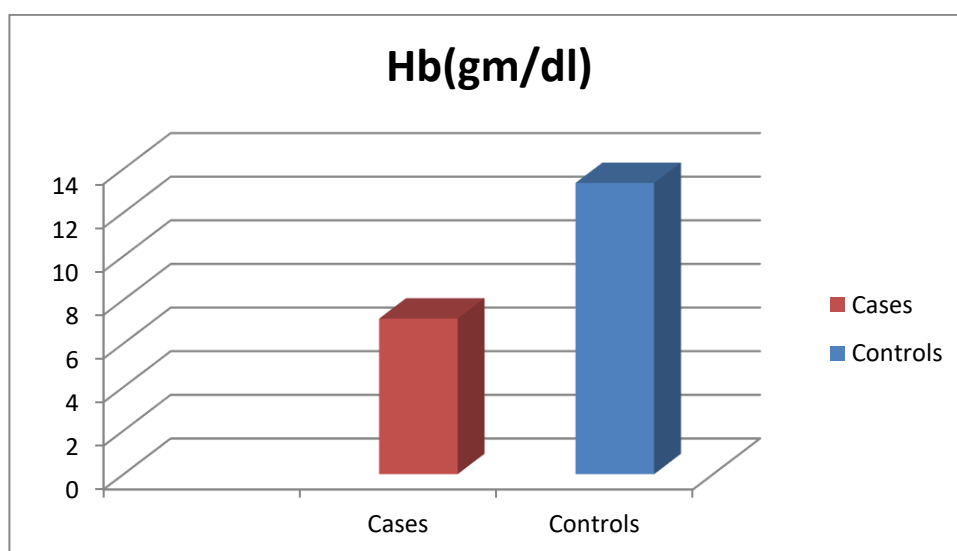


Fig 3: Shows Hemoglobin level in cases & Controls group

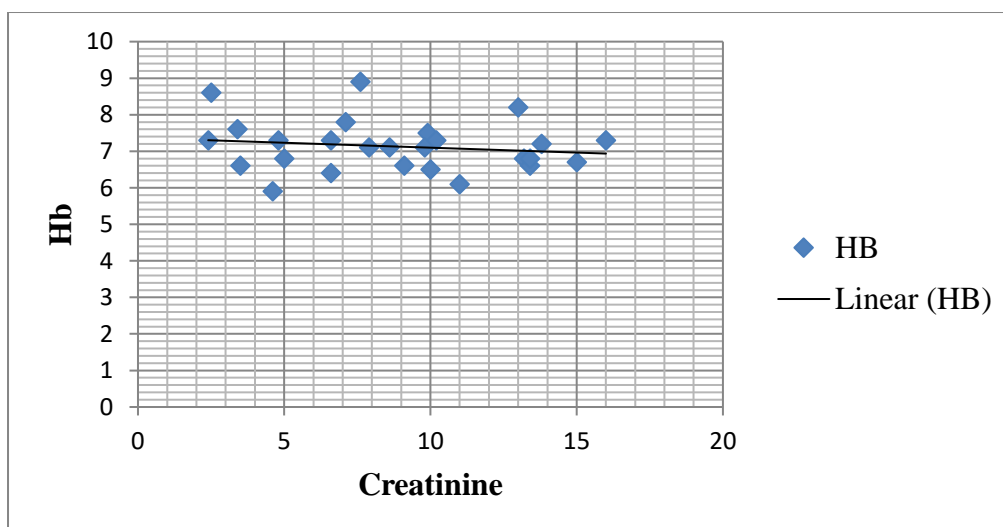


Fig 4: Shows Significant ($r = -0.154$) negative correlation between serum Creatinine & Hemoglobin level

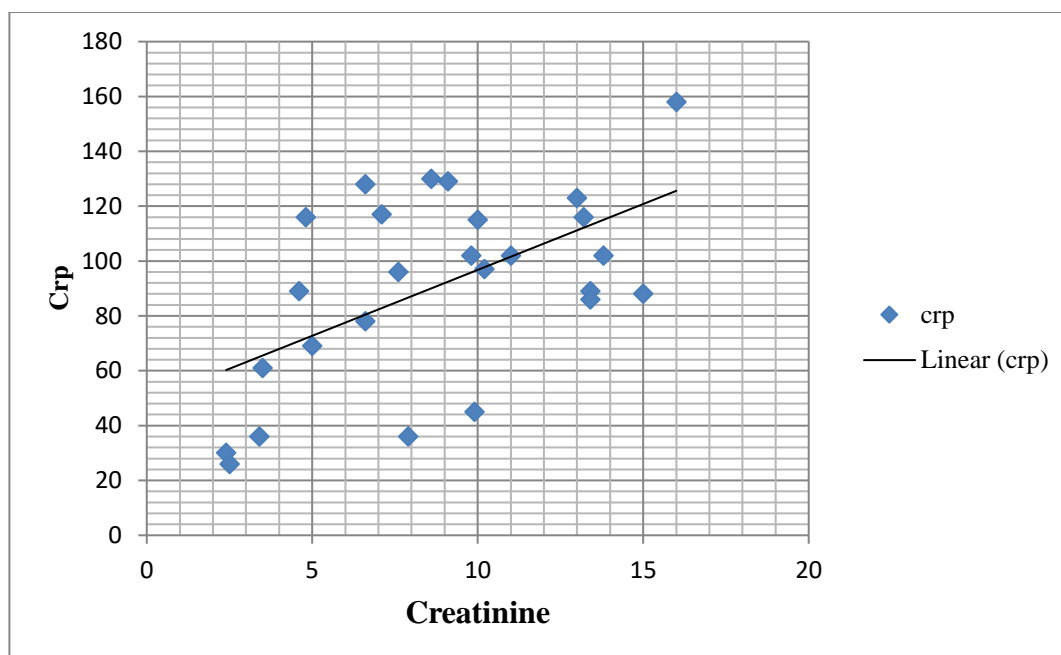


Fig 5: Shows Significant ($r = 0.554$) Positive correlation between serum Creatinine & CRP level

Discussion:

CKD has grown to be a significant public health concern worldwide, especially in developing nations. Among CKD patients, diabetes and hypertension are frequent comorbidities. Ninety percent of end-stage kidney disease patients receiving regular hemodialysis experience anemia. The primary clinical outcomes of chronic kidney disease (CKD) include a gradual decline in residual renal function, cardiovascular issues, cognitive decline, and a decreased quality of life for hemodialysis patients.

The result of renal function test in cases & controls is shown in Table 1. The levels of serum Urea, creatinine & Uric acid in the cases were significantly higher than in the controls. In this study, we have also compared the calcium, phosphorus levels in Hemodialysis patients. The findings showed a deficiency of calcium level in advanced stages of CKD; while phosphorus has significantly higher level.

The result of serum electrolyte in cases & control is shown in Table 2. The levels of potassium in the cases were significantly higher than in the controls.

The results shown in Table 3 of Vitamin-D, CRP & Hemoglobin in cases & controls. Serum CRP was significantly increased; whereas Vitamin -D, Hemoglobin level was decreased in Haemodialysis Patients as compared to healthy controls.

Kantas T et al 2021; Conducted a study of 513 patients with CKD were included in the study. The results of the study clearly shown that vitamin D deficiency, calcium deficiency, and hyperphosphatemia are more common in patients with CKD.²¹

Kendrick J 2008; Conducted a study among 16,301 kidney disease participants by National Health and Nutrition Examination Survey (NHANES). The study provides evidence that lower 25(OH)D and higher CRP levels are independently associated with lower hemoglobin concentrations in kidney disease subjects not requiring dialysis.²²

Our results for serum Vitamin-D, CRP & Hemoglobin are supported by **Kendrick J.** The decrease level of Vitamin-D, Hemoglobin & higher CRP level along with kidney profile test will help in diagnosis of CKD patients.

Conclusion

Our study shows decrease activity of vitamin D, calcium, Hemoglobin & significant elevation of Crp, phosphorus & potassium are more common in patients with CKD, but their severity is more common

in advanced stages of CKD. The elevated activity of CRP leading to accelerated systemic inflammation and causes renal related complications.

References:

1. Ruggenti P, Schiepati A, Remuzzi G. Progression, remission, regression of chronic renal diseases. *Lancet* 2001; 357(9268):1601-8.
2. Jager KJ, Kovesdy C, Langham R, et al. A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases. *Kidney Int.* 2019; 96:1048-50.
3. Modi GK, Jha V. The incidence of end-stage renal disease in India: a population-based study. *Kidney Int.* 2006; 70(12):2131-33.
4. Kher V. End-stage renal disease in developing countries. *Kidney Int.* 2002; 62(1):350-62.
5. RE Weishaar, SN Kim, D E Saunders, R U Simpson. Involvement of vitamin D3 with cardiovascular function. III. Effects on physical and morphological properties. *American Journal of Physiology-Endocrinology and Metabolism.*1990; 258(1): 134–142.
6. I Loftus, M Thompson. Role of matrix metalloproteinase in vascular disease. *Vascular Medicine.* 2016; 7(2): 117–133.
7. Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007; 357:266-81.
8. Wang CJ, McCauley LK. Osteoporosis and periodontitis. *Curr Osteoporosis Rep.* 2016; 14:284-91.
9. UrenaTorres P, Metzger M, Haymann JP et al. Association of kidney function, vitamin D deficiency, and circulating markers of mineral and bone disorders in CKD. *American J Kidney Dis.* 2011; 58:544-53.
10. McGonigle RJ, Wallin JD, Shadduck RK, Fisher JW. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. *Kidney International.* 1984;25:437–444
11. Radtke HW, Claussner A, Erbes PM, Scheuermann EH, Schoeppe W, Koch KM. Serum erythropoietin concentration in chronic renal failure: relationship to degree of anemia and excretory renal function. *Blood.* 1979; 54: 877–884.
12. Erslev AJ, Besarab A. The rate and control of baseline red cell production in hematologically stable patients with uremia. *J Lab Clin Med.* 1995;126:283-286.
13. YaoQ, Lindholm B, Stenvinkel P. Inflammation as a Cause of Malnutrition, Atherosclerotic Cardiovascular Disease, and Poor Outcome in Hemodialysis Patients. *Hemodial Int.* 2004; 8:118-29.
14. Pearson, TA et al. AHA/CDC Scientific Statement: Markers of Inflammation and Cardiovascular Disease-Application to Clinical and Public Health Practice. *Circulation.*2003; 107:499- 511.
15. Wachtel M. et al, Creation and Verification of Reference Intervals. *Laboratory Medicine* 1995; 26: 593-97.
16. Kroll MH, Elin RJ. *Clin Chem*, 1983 : 29 : 2044
17. Searcy, RL .*Diagnostic Biochemistry.* Mc Graw-Hill, New York, 1969.
18. Fogh Anderson et al. Determination of sodium & potassium with ion selective electrodes. *Clin Chem.* 1984; 30(3): 433-36.
19. Holick MF. Vitamin D deficiency .*The New England journal of medicine* 2007; 357 (3): 266-81.
20. Chetana Vaishnavi. *Immunology & infectious disease* 1996; 6:139-144.
21. Theodosios kantas et al. Relationship between Chronic Kidney Disease Staging and Vitamin D Deficiency: A Retrospective Study. *Cureus* 2022; 14(1): 1-8.
22. Kendrick J, Targher G ,Smits G Chonchol M. 25-Hydroxyvitamin D Deficiency and Inflammation and Their Association with Hemoglobin Levels in Chronic Kidney Disease. *Am J Nephrol* 2009;30:64-72