

Study of Vitamin B12, Vitamin D Levels and Their Clinical Implications in Patients with Chronic Kidney Disease¹Dr. Sadananjali, Assistant Professor, Department of Biochemistry, RIMS, Raichur²Dr. Vijay P. M., Associate Professor, Department of Pathology, KRIMS, Karwar³Dr. Mahesh Kumar C. H., Associate Professor, Department of Pathology, RGSSH (OPEC), RIMS, Raichur**Corresponding Author:** Dr. Mahesh Kumar C. H., Associate Professor, Department of Pathology, RGSSH (OPEC), RIMS, Raichur**How to citation this article:** Sadananjali, Vijay P. M, Mahesh Kumar C. H., “Study of Vitamin B12, Vitamin D Levels and Their Clinical Implications in Patients with Chronic Kidney Disease”, IJMACR– September– 2026, Volume – 9, Issue – 5, P. No. 653 – 664.**Open Access Article:** © 2026 Dr. Mahesh Kumar C. H., et al. This is an open access journal and article distributed under the terms of the creative common’s attribution license (<http://creativecommons.org/licenses/by/4.0>). Which allows others to remix, tweak, and build upon the work non - commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.**Type of Publication:** Original Research Article**Conflicts of Interest:** Nil**Abstract**

Introduction: Chronic kidney disease (CKD) is a progressive disorder associated with declining renal function and multiple metabolic, endocrine, and nutritional abnormalities. Vitamin B12 and vitamin D are important micronutrients whose metabolism may be altered with advancing renal dysfunction. Their deficiencies may contribute to haematological, skeletal, and metabolic complications and may be associated with adverse clinical outcomes.

Aim: To evaluate serum vitamin B12 and vitamin D levels in patients with chronic kidney disease and assess their association with CKD severity.

Methodology: A hospital-based retrospective observational study was conducted over six months among 100 adult patients diagnosed with CKD/chronic renal failure at the Central Laboratory of Rajiv Gandhi Superspeciality Hospital, Raichur. Patients aged ≥ 18

years with documented CKD/chronic renal failure were included, while those with acute kidney injury, chronic liver disease, vitamin B12 or vitamin D supplementation, malabsorption syndromes, pregnancy, or chronic inflammatory diseases were excluded. Venous blood samples received at the Central Laboratory RGSSH(OPEC), Raichur, were processed according to standard laboratory, protocols. Serum vitamin B12 and 25-hydroxyvitamin D [25(OH)D] were estimated using an enzyme-linked fluorescence assay. Demographic and clinical information was retrieved from available medical records, and the six-month clinical course was assessed retrospectively from documented follow-up and hospital records. Data were analysed using SPSS. Appropriate statistical tests, including one-way analysis of variance (ANOVA), Student's independent-samples t-test, and chi-square test, were applied. A two-sided p-value < 0.05 was considered statistically significant.

Results: The study included 100 patients with a mean age of 53.6 ± 11.8 years; 62 (62.0%) were male and 38 (38.0%) were female. Diabetes mellitus was present in 54 (54.0%) patients, hypertension in 68 (68.0%), and current or past smoking exposure in 27 (27.0%). CKD Stage 3, Stage 4, Stage 5 non-dialysis, and Stage 5D accounted for 20 (20.0%), 25 (25.0%), 20 (20.0%), and 35 (35.0%) patients, respectively. Mean serum vitamin B12 decreased from 421.6 ± 162.4 pg/mL in Stage 3 to 309.7 ± 136.8 pg/mL in Stage 5D ($p=0.018$). Vitamin B12 deficiency increased from 10.0% in Stage 3 to 31.4% in Stage 5D ($p=0.041$), with an overall prevalence of 21.0% (21/100). Mean serum 25(OH)D declined from 22.4 ± 7.1 ng/mL in Stage 3 to 10.9 ± 4.8 ng/mL in Stage 5D ($p<0.001$), while vitamin D deficiency increased from 45.0% to 94.3% ($p<0.001$), with an overall prevalence of 76.0% (76/100). Of the study population, 68 (68.0%) had a favourable six-month outcome, whereas 32 (32.0%) experienced a composite adverse six-month clinical outcome. Patients with a composite adverse six-month clinical outcome had significantly lower mean serum vitamin B12 levels than those with a favourable outcome (283.5 ± 128.6 vs 377.4 ± 149.2 pg/mL; $p=0.003$) and lower mean serum 25(OH)D levels (11.4 ± 5.2 vs 17.0 ± 6.8 ng/mL; $p<0.001$). Vitamin B12 deficiency was present in 34.4% of patients with a composite adverse six-month clinical outcome compared with 14.7% of those with a favourable outcome ($p=0.026$), while vitamin D deficiency was present in 90.6% and 69.1%, respectively ($p=0.020$). Thus, lower serum vitamin B12 and 25(OH)D concentrations and a higher prevalence of corresponding deficiencies were significantly associated with the composite adverse six-month clinical outcome. These findings represent associations and should not be

interpreted as evidence of causality or independent prognostic prediction.

Conclusion: Vitamin D deficiency was highly prevalent and increased across more advanced CKD stages, while vitamin B12 deficiency affected 21.0% of patients and was more frequent in advanced disease. Lower serum vitamin B12 and 25(OH)D concentrations and a higher prevalence of corresponding deficiencies were significantly associated with the composite adverse six-month clinical outcome. Given the retrospective observational design, these associations should not be interpreted as evidence of causality or independent prognostic prediction.

Keywords: Chronic Kidney Disease; Vitamin B12; Vitamin D; 25-Hydroxyvitamin D; Dialysis

Introduction

Chronic kidney disease (CKD) is a progressive and irreversible disorder characterised by persistent impairment of renal function and accumulation of uraemic toxins and represents an important cause of morbidity and mortality.¹ The consequences of CKD extend well beyond reduced glomerular filtration and include nutritional, metabolic, endocrine, mineral, and cardiovascular abnormalities. Progressive renal dysfunction is frequently accompanied by anaemia, mineral and bone disorders, cardiovascular disease, and nutritional disturbances, making assessment of clinically relevant micronutrient abnormalities an important component of comprehensive CKD care.^{1,2}

Vitamin B12 and vitamin D are particularly relevant micronutrients in patients with CKD because of their essential roles in haematological, neurological, skeletal, and metabolic functions. Vitamin B12 is required for DNA synthesis, normal erythropoiesis, and maintenance of neurological integrity. Deficiency may result in

megaloblastic anaemia, neurological dysfunction, and altered homocysteine metabolism.³ The relationship between renal dysfunction and cardiovascular disease is particularly important because cardiovascular complications contribute substantially to morbidity and mortality in CKD.⁴

Interpretation of vitamin B12 status in CKD, however, is complex. Renal dysfunction can influence cobalamin metabolism, while circulating vitamin B12 concentrations may not always accurately represent functional intracellular availability.⁵ Consequently, an apparently adequate serum concentration does not necessarily exclude functional cobalamin abnormality in patients with renal disease. This consideration is particularly relevant in advanced CKD and dialysis-dependent patients, in whom nutritional restrictions, altered metabolism, and dialysis-related factors may influence vitamin status.

Vitamin D has a distinct but equally important role in CKD. It contributes to calcium and phosphorus homeostasis and normal skeletal metabolism. With progressive renal dysfunction, abnormalities in vitamin D metabolism become increasingly prominent and contribute to secondary hyperparathyroidism, renal osteodystrophy, and other manifestations of CKD-mineral and bone disorder.⁶ Holick emphasized the broad clinical importance of vitamin D deficiency, while Levin et al. demonstrated a high prevalence of abnormal vitamin D concentrations among patients with CKD.^{7,8}

Vitamin D inadequacy is also common in the general population and may compound the metabolic vulnerability associated with CKD. Holick described the widespread prevalence of vitamin D inadequacy and its potential health consequences, while Harinarayan and Joshi highlighted the substantial burden of inadequate

vitamin D status and its clinical implications.^{9,10} Thus, patients with CKD may experience the combined effects of impaired renal vitamin D metabolism and pre-existing nutritional or environmental determinants of vitamin D status.

The clinical importance of these abnormalities extends beyond biochemical measurement. Vitamin D status has been associated with cardiovascular risk, although observational associations should be distinguished from evidence of therapeutic benefit.¹¹ Similarly, treatment of vitamin D deficiency in CKD requires individualized consideration of CKD stage, mineral metabolism, and clinical context. Nigwekar et al. evaluated vitamin D-related treatment and outcomes in CKD, illustrating the continuing uncertainty surrounding the relationship between biochemical correction and hard clinical outcomes.¹²

Vitamin B12 abnormalities may be less routinely recognized in CKD than vitamin D deficiency. Wu and Wang described the complex relationship between vitamin B12 metabolism and CKD and emphasized the importance of understanding altered vitamin metabolism in renal disease.¹³ Clinical evidence among dialysis populations further demonstrates that vitamin B12 deficiency can occur despite the complexity of its biochemical interpretation. Mushtaq et al., in 135 chronic haemodialysis patients, reported vitamin B12 deficiency in 30 patients (22.2%) and found a significant association between deficiency and duration of haemodialysis ($p=0.013$).¹⁴

The potential relationship between micronutrient status and clinical outcomes has also attracted increasing attention. Wu et al. examined serum vitamin B12 and circulating methylmalonic acid among individuals with CKD and demonstrated the importance of distinguishing

serum vitamin B12 concentration from functional markers of cobalamin metabolism when evaluating mortality risk.¹⁵ Chazot, Steiber, and Kopple emphasized that dietary intake, metabolism, urinary excretion, and vitamin requirements may be substantially altered in kidney failure, with differences between functional assessment and circulating vitamin concentrations.¹⁶ More recently, Wang et al. emphasized that patients with CKD may be vulnerable to vitamin deficiency because of poor dietary intake, restricted diets, protein-energy wasting, dialysis losses, and reduced sun exposure, while also stressing that supplementation should be individualized.¹⁷

Against this background, simultaneous assessment of vitamin B12 and vitamin D across different stages of CKD is clinically relevant. The present study was undertaken to evaluate serum vitamin B12 and 25-hydroxyvitamin D concentrations in patients with CKD, determine their distribution according to CKD severity, and assess their association with a predefined composite adverse six-month clinical outcome.

Aim

To evaluate serum vitamin B12 and 25-hydroxyvitamin D [25(OH)D] levels in patients with chronic kidney disease, determine their distribution according to CKD severity, and assess their association with a predefined composite adverse six-month clinical outcome.

Objectives

1. To determine serum vitamin B12 levels and the prevalence of vitamin B12 deficiency among patients with chronic kidney disease.
2. To determine serum 25-hydroxyvitamin D [25(OH)D] levels and the prevalence of vitamin D deficiency among patients with chronic kidney disease.

3. To assess the association of serum vitamin B12 and 25(OH)D levels with the predefined composite adverse six-month clinical outcome among patients with chronic kidney disease.

Materials and Methods

Study Design and Setting

A hospital-based retrospective observational study was conducted over a period of six months at the Central Laboratory of Rajiv Gandhi Superspeciality Hospital(OPEC), Raichur. The study included adult patients with a documented diagnosis of chronic kidney disease/chronic renal failure attending the outpatient or inpatient Nephrology Dept

of the RGSSH hospital, including patients receiving maintenance dialysis, whose blood samples were received at the Central Laboratory, RGSSH (OPEC), Raichur for routine clinical investigation.

Study Population and Sample Size

A total of 100 eligible patients with chronic kidney disease/chronic renal failure were included in the study. The sample size was determined on the basis of feasibility and patient flow at the tertiary-care hospital during the study period. Patients were classified according to their documented CKD stage as Stage 3, Stage 4, Stage 5 not receiving dialysis, and Stage 5D receiving maintenance dialysis.

Inclusion Criteria

Patients aged ≥ 18 years with a documented diagnosis of chronic kidney disease/chronic renal failure were included.

Exclusion Criteria

Patients with acute kidney injury, chronic liver disease, current vitamin B12 or vitamin D supplementation, malabsorption syndromes, pregnancy, or chronic inflammatory diseases were excluded.

Data Collection and Retrospective Outcome Assessment
Demographic and clinical information was retrieved retrospectively from available hospital records and laboratory documentation. The recorded variables included age, sex, diabetes mellitus, hypertension, smoking status, and CKD stage.

For outcome assessment, the date of the vitamin B12 and 25(OH)D laboratory assessment was considered the index date. The subsequent six-month clinical course was retrospectively assessed using available hospital records and documented follow-up information.

A favourable six-month outcome was defined as survival without documented unplanned hospitalization during the six-month period following the index laboratory assessment. A composite adverse six-month clinical outcome was defined as the occurrence of one or more documented unplanned hospitalizations and/or all-cause mortality during the same six-month period.

Thus, the outcome analysis was based on retrospectively documented clinical events, and no additional intervention or prospective follow-up was undertaken as part of the study.

Laboratory Investigations

Venous blood samples received at the Central Laboratory, RGSSH(OPEC) Raichur were processed according to standard laboratory protocols. Serum vitamin B12 and

serum 25-hydroxyvitamin D [25(OH)D] were estimated using an enzyme-linked fluorescence assay (ELFA).

Vitamin B12 status was categorized as deficient (<200 pg/mL), borderline (200–299 pg/mL), and adequate ($\geq$ 300 pg/mL). Vitamin D status was categorized as deficient (<20 ng/mL), insufficient (20–29.9 ng/mL), and sufficient ($\geq$ 30 ng/mL), according to the classification used in the study dataset.

Statistical Analysis

Data were entered into a computerized database and analysed using SPSS software. Continuous variables were expressed as mean $\pm$ standard deviation (SD), while categorical variables were expressed as frequency and percentage.

For comparison of continuous variables among the four CKD-stage groups, one-way analysis of variance (ANOVA) was used. For comparison of continuous variables between the favourable and composite adverse six-month clinical outcome groups, Student's independent-samples t-test was applied. Categorical variables were compared using the chi-square test.

Where appropriate, post-hoc comparisons were performed following statistically significant ANOVA results. A two-sided p-value <0.05 was considered statistically significant.

Result

Table 1: Baseline Demographic, Clinical and Renal Characteristics of the Study Population (N=100)

Parameter	Study Population (N=100)
Age (years), mean $\pm$ SD	53.6 $\pm$ 11.8
Male, n (%)	62 (62.0%)
Female, n (%)	38 (38.0%)
Diabetes mellitus, n (%)	54 (54.0%)
Hypertension, n (%)	68 (68.0%)
Current/past smoking, n (%)	27 (27.0%)

CKD Stage 3, n (%)	20 (20.0%)
CKD Stage 4, n (%)	25 (25.0%)
CKD Stage 5, not on dialysis, n (%)	20 (20.0%)
CKD Stage 5D/on maintenance dialysis, n (%)	35 (35.0%)

The study population comprised 100 patients with a mean age of 53.6 ± 11.8 years, including 62 (62.0%) males and 38 (38.0%) females. Diabetes mellitus was present in 54 (54.0%) patients, hypertension in 68 (68.0%), and current or past smoking exposure in 27 (27.0%). CKD Stage 3 accounted for 20 (20.0%) patients, Stage 4 for 25 (25.0%), Stage 5 not on dialysis for 20 (20.0%), and Stage 5D/on maintenance dialysis for 35 (35.0%). Overall, 55 (55.0%) participants had Stage 5 or Stage 5D disease.

Table 2: Serum Vitamin B12 Levels and Vitamin B12 Status According to CKD Severity (N=100)

Vitamin B12 Parameter	Stage 3 (n=20)	Stage 4 (n=25)	Stage 5, Non-Dialysis (n=20)	Stage 5D (n=35)	p-value
Serum vitamin B12 (pg/mL), mean $\pm$ SD	421.6 $\pm$ 162.4	378.2 $\pm$ 148.7	334.8 $\pm$ 140.5	309.7 $\pm$ 136.8	0.018
Deficient (<200 pg/mL), n (%)	2 (10.0%)	4 (16.0%)	4 (20.0%)	11 (31.4%)	0.041
Borderline (200–299 pg/mL), n (%)	2 (10.0%)	4 (16.0%)	4 (20.0%)	7 (20.0%)	0.728
Adequate ($\geq$ 300 pg/mL), n (%)	16 (80.0%)	17 (68.0%)	12 (60.0%)	17 (48.6%)	0.047
Overall vitamin B12 deficiency	—	—	—	—	21/100 (21.0%)

Serum vitamin B12 demonstrated a progressive decline across the CKD-stage groups, from 421.6 ± 162.4 pg/mL in Stage 3 to 309.7 ± 136.8 pg/mL in Stage 5D ($p=0.018$). Vitamin B12 deficiency was observed in 2 (10.0%), 4 (16.0%), 4 (20.0%), and 11 (31.4%) patients, respectively ($p=0.041$), giving an overall deficiency prevalence of 21/100 (21.0%). Borderline vitamin B12 status occurred in 10.0%, 16.0%, 20.0%, and 20.0%, respectively ($p=0.728$), while adequate concentrations were present in 80.0%, 68.0%, 60.0%, and 48.6%, respectively ($p=0.047$).

Table 3: Serum 25-Hydroxyvitamin D Levels and Vitamin D Status According to CKD Severity (N=100)

25(OH)D Parameter	Stage 3 (n=20)	Stage 4 (n=25)
Serum 25(OH)D (ng/mL), mean $\pm$ SD	22.4 $\pm$ 7.1	17.3 $\pm$ 6.2
Deficient (<20 ng/mL), n (%)	9 (45.0%)	17 (68.0%)
Insufficient (20–29.9 ng/mL), n (%)	9 (45.0%)	7 (28.0%)
Sufficient ($\geq$ 30 ng/mL), n (%)	2 (10.0%)	1 (4.0%)
Overall vitamin D deficiency	—	—

25(OH)D Parameter	Stage 3 (n=20)	Stage 4 (n=25)
Serum 25(OH)D (ng/mL), mean $\pm$ SD	22.4 $\pm$ 7.1	17.3 $\pm$ 6.2
Deficient (<20 ng/mL), n (%)	9 (45.0%)	17 (68.0%)
Insufficient (20–29.9 ng/mL), n (%)	9 (45.0%)	7 (28.0%)
Sufficient ($\geq$ 30 ng/mL), n (%)	2 (10.0%)	1 (4.0%)

Mean serum 25(OH)D declined significantly across the CKD-stage groups, from 22.4 $\pm$ 7.1 ng/mL in Stage 3 to 10.9 $\pm$ 4.8 ng/mL in Stage 5D ($p < 0.001$). Vitamin D deficiency occurred in 9 (45.0%), 17 (68.0%), 17 (85.0%), and 33 (94.3%) patients, respectively ($p < 0.001$), with an overall prevalence of 76/100 (76.0%). Vitamin D insufficiency decreased from 45.0% in Stage 3 to 5.7% in Stage 5D ($p < 0.001$), while sufficient levels were present in 10.0%, 4.0%, 0%, and 0%, respectively ($p = 0.048$).

Table 4: Association of Serum Vitamin B12 and 25(OH)D Status With the Composite Adverse Six-Month Clinical Outcome in Patients With Chronic Kidney Disease (N=100)

Parameter	Favourable Six-Month Outcome (n=68)	Composite Adverse Six-Month Clinical Outcome (n=32)	p-value
Serum vitamin B12 (pg/mL), mean $\pm$ SD	377.4 $\pm$ 149.2	283.5 $\pm$ 128.6	0.003
Vitamin B12 deficiency, n (%)	10 (14.7%)	11 (34.4%)	0.026
Serum 25(OH)D (ng/mL), mean $\pm$ SD	17.0 $\pm$ 6.8	11.4 $\pm$ 5.2	<0.001
Vitamin D deficiency, n (%)	47 (69.1%)	29 (90.6%)	0.020
Advanced CKD/Stage 5 or 5D, n (%)	31 (45.6%)	24 (75.0%)	0.006
Diabetes mellitus, n (%)	32 (47.1%)	22 (68.8%)	0.043

Data are presented as mean $\pm$ SD or n (%). A favourable six-month outcome was defined as survival without documented unplanned hospitalization during the six-month period following the index laboratory assessment. A composite adverse six-month clinical outcome was defined as one or more documented unplanned hospitalizations and/or all-cause mortality during the same six-month period. Student's independent-samples t-test and chi-square test were used as appropriate; $p < 0.05$ was considered statistically significant. The study

included 100 patients with a mean age of 53.6 $\pm$ 11.8 years; 62 (62.0%) were male and 38 (38.0%) were female. Diabetes mellitus was present in 54 (54.0%) patients, hypertension in 68 (68.0%), and current or past smoking exposure in 27 (27.0%). CKD Stage 3, Stage 4, Stage 5 non-dialysis, and Stage 5D accounted for 20 (20.0%), 25 (25.0%), 20 (20.0%), and 35 (35.0%) patients, respectively. Mean serum vitamin B12 decreased from 421.6 $\pm$ 162.4 pg/mL in Stage 3 to 309.7 $\pm$ 136.8 pg/mL in Stage 5D ($p = 0.018$). Vitamin B12 deficiency increased

from 10.0% in Stage 3 to 31.4% in Stage 5D ($p=0.041$), with an overall prevalence of 21.0% (21/100). Mean serum 25(OH)D declined from 22.4 ± 7.1 ng/mL in Stage 3 to 10.9 ± 4.8 ng/mL in Stage 5D ($p<0.001$), while vitamin D deficiency increased from 45.0% to 94.3% ($p<0.001$), with an overall prevalence of 76.0% (76/100). Regarding the predefined six-month clinical outcome, 68 (68.0%) patients had a favourable outcome, whereas 32 (32.0%) experienced a composite adverse six-month clinical outcome. Patients with a composite adverse six-month clinical outcome had significantly lower mean serum vitamin B12 levels than those with a favourable outcome (283.5 ± 128.6 vs 377.4 ± 149.2 pg/mL; $p=0.003$) and lower mean serum 25(OH)D levels (11.4 ± 5.2 vs 17.0 ± 6.8 ng/mL; $p<0.001$). Vitamin B12 deficiency was present in 34.4% of patients with a composite adverse six-month clinical outcome compared with 14.7% of those with a favourable outcome ($p=0.026$), while vitamin D deficiency was present in 90.6% and 69.1%, respectively ($p=0.020$). Thus, lower serum vitamin B12 and 25(OH)D concentrations and a higher prevalence of corresponding deficiencies were significantly associated with the composite adverse six-month clinical outcome. These findings represent associations and should not be interpreted as evidence of causality or independent prognostic prediction.

Discussion

The present study evaluated serum vitamin B12 and 25(OH)D concentrations among 100 patients with CKD, examined their distribution across CKD severity, and assessed their association with a predefined composite adverse six-month clinical outcome. The study population had a mean age of 53.6 ± 11.8 years, with males constituting 62.0% and females 38.0%. Diabetes mellitus was present in 54.0%, hypertension in 68.0%, and current

or previous smoking exposure in 27.0%. Notably, 55.0% of participants had Stage 5 or Stage 5D disease, including 35.0% who were receiving maintenance dialysis. This distribution represents a clinically advanced CKD cohort in which nutritional and metabolic abnormalities are particularly relevant. Jha et al. emphasized the substantial morbidity associated with CKD, while Kalantar-Zadeh and Fouque highlighted the importance of nutritional and metabolic disturbances as renal function progressively declines.^{1,2}

The principal finding concerning vitamin B12 was that patients in more advanced CKD stages had lower serum concentrations and a greater frequency of biochemical deficiency. Mean serum vitamin B12 declined from 421.6 ± 162.4 pg/mL in Stage 3 to 378.2 ± 148.7 pg/mL in Stage 4, 334.8 ± 140.5 pg/mL in Stage 5 non-dialysis, and 309.7 ± 136.8 pg/mL in Stage 5D, with a statistically significant difference across groups ($p=0.018$). Correspondingly, the prevalence of biochemical vitamin B12 deficiency increased from 10.0% in Stage 3 to 16.0% in Stage 4, 20.0% in Stage 5 non-dialysis, and 31.4% in Stage 5D ($p=0.041$), giving an overall prevalence of 21.0%. The proportion with adequate vitamin B12 concentrations decreased from 80.0% in Stage 3 to 48.6% in Stage 5D ($p=0.047$). Thus, within this cohort, more advanced CKD stages were associated with progressively lower circulating vitamin B12 concentrations and a greater frequency of biochemical deficiency.

The observed pattern is consistent with the established complexity of cobalamin physiology in renal disease. O'Leary and Samman described vitamin B12 as essential for DNA synthesis, erythropoiesis, and neurological integrity, while Obeid and Herrmann emphasized that renal dysfunction can complicate interpretation of circulating vitamin B12 because serum concentrations

may not always correspond to functional intracellular availability.^{3,5} The present study demonstrated a difference of approximately 111.9 pg/mL between Stage 3 and Stage 5D. Mushtaq et al. reported vitamin B12 deficiency in 30 of 135 chronic haemodialysis patients (22.2%), with a mean serum vitamin B12 concentration of 411.61 ± 224.95 pg/mL, and found a significant association between deficiency and duration of haemodialysis ($p=0.013$).¹⁴ The overall deficiency prevalence in the present study, 21.0%, was therefore comparable to that reported by Mushtaq et al., although the Stage 5D subgroup demonstrated a higher deficiency frequency of 31.4%. Differences in dialysis duration, nutritional status, dietary intake, patient characteristics, and laboratory methodology may account for variation between cohorts.

The vitamin B12 findings should, however, be interpreted specifically as abnormalities in serum vitamin B12 concentration. Wu and Wang emphasized the altered metabolism of vitamin B12 in CKD and the potential discrepancy between circulating concentration and functional cobalamin status.¹³ The present study measured serum vitamin B12 according to the methodology specified in the thesis and did not include methylmalonic acid or homocysteine measurements. Consequently, the study cannot determine the prevalence of functional intracellular vitamin B12 deficiency. Wu et al. further demonstrated the relevance of circulating methylmalonic acid when examining associations between cobalamin metabolism and mortality among individuals with CKD.¹⁵ Accordingly, the 21.0% prevalence observed in the present study should be interpreted as serum vitamin B12 deficiency according to the predefined study threshold rather than as definitive evidence of functional cobalamin deficiency.

The findings for vitamin D demonstrated a considerably greater burden of abnormality. Mean serum 25(OH)D declined progressively from 22.4 ± 7.1 ng/mL in Stage 3 to 17.3 ± 6.2 ng/mL in Stage 4, 13.8 ± 5.4 ng/mL in Stage 5 non-dialysis, and 10.9 ± 4.8 ng/mL in Stage 5D ($p<0.001$). Vitamin D deficiency increased from 45.0% in Stage 3 to 68.0% in Stage 4, 85.0% in Stage 5 non-dialysis, and 94.3% in Stage 5D ($p<0.001$). Overall, 76.0% of the study population was vitamin D deficient. In contrast, sufficient vitamin D concentrations were present in 10.0% of Stage 3 patients and 4.0% of Stage 4 patients, while no patients in Stage 5 non-dialysis or Stage 5D had sufficient concentrations.

The magnitude and direction of this finding are compatible with the recognized disturbances of vitamin D and mineral metabolism accompanying CKD. KDIGO guidance identifies abnormalities of mineral metabolism as a central component of CKD-mineral and bone disorder, while Holick described the broad clinical significance of vitamin D deficiency.^{6,7} Levin et al. reported a high prevalence of abnormal vitamin D concentrations among patients with CKD, supporting the progressive decline observed in the present cohort.⁸ Harinarayan and Joshi likewise documented a substantial burden of vitamin D inadequacy and emphasized its clinical relevance.¹⁰ In the present study, the proportion of patients with vitamin D deficiency increased by 49.3 percentage points, from 45.0% in Stage 3 to 94.3% in Stage 5D. This marked gradient demonstrates that biochemical vitamin D deficiency was particularly prominent among patients with advanced renal disease.

The Stage 5D findings are especially noteworthy. Of the 35 patients receiving maintenance dialysis, 33 (94.3%) were vitamin D deficient and 2 (5.7%) were insufficient; none had a sufficient 25(OH)D concentration. Therefore,

100% of the Stage 5D group had either deficient or insufficient vitamin D status. Chazot, Steiber, and Kopple emphasized that kidney failure can substantially alter vitamin metabolism and that nutritional requirements and assessment may vary according to the stage of renal disease and dialysis status.¹⁶ Wang et al. similarly discussed restricted dietary intake, nutritional vulnerability, dialysis-related factors, and reduced sunlight exposure as potential contributors to vitamin abnormalities among patients with CKD.¹⁷ The present retrospective dataset cannot establish the contribution of each of these factors; nevertheless, the pronounced stage-wise decline in 25(OH)D is consistent with the greater metabolic vulnerability observed in advanced CKD.

The analysis of the composite adverse six-month clinical outcome provided an additional clinically relevant observation. Among the 100 participants, 68 (68.0%) had a favourable six-month outcome, whereas 32 (32.0%) experienced the predefined composite adverse six-month clinical outcome. Mean serum vitamin B12 was significantly lower among patients with a composite adverse six-month clinical outcome than among those with a favourable outcome (283.5 ± 128.6 vs 377.4 ± 149.2 pg/mL; $p=0.003$). Similarly, vitamin B12 deficiency was present in 34.4% of patients with a composite adverse six-month clinical outcome compared with 14.7% among those with a favourable outcome ($p=0.026$). These findings indicate that lower circulating vitamin B12 concentrations and a greater frequency of biochemical vitamin B12 deficiency were significantly associated with the composite adverse six-month clinical outcome.

A similar but more pronounced pattern was observed for vitamin D. Mean serum 25(OH)D was 11.4 ± 5.2 ng/mL among patients with a composite adverse six-month

clinical outcome compared with 17.0 ± 6.8 ng/mL among those with a favourable outcome ($p<0.001$). Vitamin D deficiency was present in 90.6% of patients with a composite adverse six-month clinical outcome compared with 69.1% of those with a favourable outcome ($p=0.020$). Thus, both micronutrients demonstrated significantly lower concentrations among patients experiencing the composite adverse six-month clinical outcome, with the difference being particularly marked for 25(OH)D.

The marked difference in 25(OH)D concentrations between the outcome groups is clinically noteworthy. Vitamin D has recognized roles in mineral metabolism and has been associated with cardiovascular health. Pilz et al. described associations between low vitamin D status and cardiovascular disease, although such observational relationships do not establish that vitamin D deficiency itself causes cardiovascular events or mortality.¹¹ Nigwekar et al. similarly examined vitamin D-related treatment and outcomes in CKD and highlighted the distinction between biochemical deficiency and demonstrable improvement in hard clinical outcomes.¹² Therefore, the present finding that lower 25(OH)D concentrations and vitamin D deficiency were associated with the composite adverse six-month clinical outcome should be interpreted as an observational association rather than evidence that vitamin D deficiency caused the outcome.

The clinical context of the composite outcome is also important. Advanced CKD/Stage 5 or 5D was present in 75.0% of patients with a composite adverse six-month clinical outcome compared with 45.6% of those with a favourable outcome ($p=0.006$). Diabetes mellitus was also more frequent among patients with a composite adverse six-month clinical outcome, occurring in 68.8%

compared with 47.1% among patients with a favourable outcome ($p=0.043$). These findings demonstrate that lower micronutrient concentrations occurred alongside greater renal disease severity and a higher prevalence of diabetes. Accordingly, vitamin B12 or vitamin D deficiency should not be considered in isolation when interpreting the observed six-month clinical associations. This issue is particularly important for vitamin B12 because renal disease itself may influence its biochemical interpretation. Wu et al. demonstrated that functional cobalamin-related markers, particularly methylmalonic acid, may provide additional information when examining mortality associations in CKD.¹⁵ The present finding of lower serum vitamin B12 among patients with a composite adverse six-month clinical outcome is therefore clinically relevant but does not establish an independent prognostic relationship. Greater CKD severity, dialysis dependence, diabetes, nutritional vulnerability, and other unmeasured clinical factors may coexist with lower vitamin B12 concentrations and adverse clinical outcomes. Since the present study was retrospective and observational and did not perform multivariable prognostic modelling, the findings should be interpreted as associations rather than independent prediction.

Overall, the study demonstrates a consistent stage-related pattern of micronutrient abnormality. Serum vitamin B12 declined from 421.6 ± 162.4 pg/mL in Stage 3 to 309.7 ± 136.8 pg/mL in Stage 5D, while vitamin B12 deficiency increased from 10.0% to 31.4%. Serum 25(OH)D declined from 22.4 ± 7.1 ng/mL to 10.9 ± 4.8 ng/mL, while vitamin D deficiency increased from 45.0% to 94.3%. Overall deficiencies were 21.0% for vitamin B12 and 76.0% for vitamin D. Furthermore, lower serum concentrations and higher frequencies of both

deficiencies were significantly associated with the composite adverse six-month clinical outcome. These findings support the clinical relevance of assessing vitamin B12 and vitamin D status in patients with CKD, particularly in advanced and dialysis-dependent disease. However, given the retrospective observational design, these findings should not be interpreted as evidence of causality or independent prognostic prediction.

Conclusion

The present study demonstrated a substantial burden of micronutrient abnormalities among patients with CKD. Vitamin B12 deficiency affected 21.0% of participants, whereas vitamin D deficiency affected 76.0% and increased across more advanced CKD stages. Lower serum vitamin B12 and 25(OH)D concentrations and a higher prevalence of corresponding deficiencies were significantly associated with the composite adverse six-month clinical outcome. Given the retrospective observational design, these associations should not be interpreted as evidence of causality or independent prognostic prediction.

Recommendations

Assessment of vitamin D and consideration of vitamin B12 evaluation may be useful, particularly in patients with advanced CKD and dialysis dependence. Larger prospective multicentre studies incorporating functional vitamin B12 biomarkers and longer follow-up are warranted to clarify the clinical significance of these deficiencies and determine whether targeted correction is associated with improved patient-centred outcomes.

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