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Original Research Article

A comparative study of total vitamin B12 and active B12 (holotranscobalamin) in patients with chronic kidney disease

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ABSTRACT

Introduction: Vitamin B12 deficiency is a serious disorder that can lead to severe neurological symptoms, especially if not detected and treated effectively. Nutritional deficiency due to dietary restrictions, deranged metabolism, and subsequent vitamin loss during dialysis are important causes of vitamin B12 deficiency in CKD patients. Hyperhomocysteinemia, a complication of vitamin B12 deficiency, has grown as an important risk factor for cardiovascular disease and the leading cause of mortality in patients with CKD.
Methodology: Serum samples were randomly selected from 124 patients (46 females, 78 males; age range 18-65 years) referred to the Dept. of Biochemistry, Institute of Nephrourology, Bangalore, India for the assessment of vitamin B12 status. For each patient, serum total vitamin B12 level and active B12 (holoTC) level were determined by chemiluminescent microparticle immunoassay on Architect ci1000 analyzer.
Results: Out of the total 124 patients, 17 CKD patients were excluded from the study, and in the remaining 107 patients, 13.08% showed a deficiency of both Total vitamin B12 and Active B12. In the 107 patients, the mean total vitamin B12 level was 604.85 ± 495.2 pg/mL, and the mean Active B12 level (holoTC) was 67.1 ± 32.75 pmol/L, with a strong positive correlation (r=0.501, p <0.01) between total B12 and active B12 levels. A significant deficient level of B12 was found in the patients on hemodialysis for more than three years.
Conclusion: Active B12 can aid vitamin B12 measurements for diagnosis of B12 deficiency and can be a potential indicator of B12 deficiency in patients with CKD.

1. Introduction

Chronic kidney disease (CKD) patients are at risk of vitamin and mineral deficiencies, which may contribute to comorbidities such as anemia, cardiovascular disease, and metabolic imbalances.[1] Studies have shown that impairment of B12 metabolism is an emerging nontraditional risk factor for poor outcomes linked to CKD, thus provoking further interest in the effect of vitamin B12 levels on improved clinical outcomes.

Vitamin B12, or Cobalamin, is a water-soluble metal enzyme needed for crucial methyl transfer reactions, while Transcobalamin (TC-II), the principal cellular transporter of B12, is necessary for the entry of vitamin B12 into tissues. TC-II binds to free Vitamin B12 with high affinity and releases B12 to bone marrow and other tissues. This complex is referred to as 'active B12 or holotranscobalamin (holoTC)' to distinguish it from complexes with transcobalamin I and III, which bind the vitamin tightly and do not release it to tissues.[2]

Although CKD patients display increased transcobalamin levels, they show an impaired vitamin tissue uptake of B12, and a significant proportion of CKD patients have physiological vitamin B12 deficiency. Moreover, in uremic patients, a functional vitamin B12

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deficiency can be observed because of increased TC-II losses in the urine and reduced absorption in the proximal tubule. Deranged metabolism in CKD patients leading to metabolic alterations and hormonal dysregulations is another contributor to vitamin B12 deficiency. Together these derangements lead to a "paradoxical" increase in cellular homocysteine levels, a modifiable cardiovascular disease risk factor.[3,4]

Hyperhomocysteinemia, an immediate complication of vitamin B12 deficiency, has grown as an important risk factor for cardiovascular disease in CKD patients. Thus detecting vitamin B12 deficiency in CKD patients at the earliest is of importance for a better outcome.[2–4] Studies have shown that Active B12 (holoTC) would be a better indicator of vitamin B12 status than total B12, and may more accurately reflect functional B12 status. Therefore, it may be crucial to measure Active B12 (holoTC) instead of total B12 levels, especially in CKD patients.[5,6] Thus, this study was undertaken to compare the usefulness of the determination of total vitamin B12 and Active B12 as a clinical approach for detecting vitamin B12 deficiency in patients with CKD.

2. Materials and Methods

This was a cross-sectional study conducted over six months from July 2022 to December 2022 on patients with primary diagnosis of CKD who attended the Institute of Nephrourology, Bangalore, a tertiary care referral center for nephrology and urology care.

A total of 124 patients with CKD (46 females and 78 males, aged between 18–65 years, median age of 44 years) as per KDOQI criteria were included in the study.[7]

Chronic kidney disease (CKD) definition: Kidney damage for ≥3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR or GFR <60 mL/min/1.73m2 for ≥3 months, with or without kidney damage. The cases were further categorized into stages as per KDOQI guidelines, using eGFR values, calculated by the CKD Epidemiology Collaboration (CKD-EPI) equation.[8]

Stage 1	Normal or high GFR	GFR > 90 mL/min
Stage 2	Mild CKD	GFR = 60-89 mL/min
Stage 3	Moderate CKD	GFR = 30-59 mL/min
Stage 4	Severe CKD	GFR = 15-29 mL/min
Stage 5	End-stage CKD	GFR <15 mL/min

All study participants were selected after having a preliminary evaluation consisting of a detailed medical history and clinical data. All the necessary clinical data was collected from patients' medical and laboratory records..

2.1. Inclusion criteria

Patients with different stages of chronic kidney disease (CKD), who underwent routine B12 investigation were randomly selected and enrolled for the study.

2.2. Exclusion criteria

1. Patients were already receiving multivitamin supplements or therapy that has been shown to influence the level of B12.
2. Patients who have acute kidney injury, acute illness, other known malignancies, and cardiovascular events, transplantation.
3. Patients younger than 18 years.

2.3. Assays

Serum total vitamin B12 levels and active B12 (holoTC) were measured using ARCHITECT system kits (Abbott Diagnostics, Abbott Park, IL, USA) according to the manufacturer's instructions.

Vitamin B12 and Active B12 (HoloTC) were assayed by the Chemiluminescent Microparticle Immunoassay (CMIA) method with a reportable range of 148 to 2000 pg/ml and 5 to 128 pmol/L, respectively.

The Active B12 (holoTC) assay is a two-step immunoassay where the sample and anti-holoTC-coated paramagnetic microparticles are combined and incubated. The Active B12 (holoTC) present in the sample binds to the anti-holoTC-coated microparticles. Anti-transcobalamin acridinium-labeled conjugate is added to create a reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of holoTC in the sample and the RLUs detected.

Based on literature reports or standard clinical concentrations the following cutoff levels were used.[9,10]

B12 levels were classified as:

1. Above 300 pg/mL is interpreted as normal.
2. Between 200 and 300 pg/mL is considered borderline.
3. Below 200 pg/mL are considered deficient.

Active B12 levels < 35 pmol/L are considered deficient.

3. Statistical Analysis

Statistical analyses were performed with Graphpad Prism 10.2.3 (Graphpad Software, San Diego, CA). Spearman correlation analysis between different variables was calculated and a *p*-value less than 0.05 was considered statistically significant.

4. Results

A total of 124 patients with CKD (46 females and 78 males, aged between 18–65 years, median age of 44 years) were

included in the study. The CKD patients were into 5 stages of CKD using eGFR values as per KDOQI guidelines.

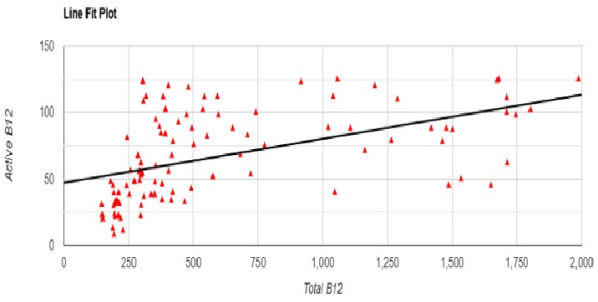
From the total 124 patients, 11 CKD patients who had total B12 levels more than 2000 pg/dL and 06 patients with Active B12 > 128 pmol/L were excluded from the study. In the remaining 107 patients, 14 patients (13.08%) showed a deficiency of both Total vitamin B12 and Active B12. While 7 patients showed a deficiency of only Active B12 with normal total B12 levels and 04 patients showed a deficiency of total B12 with normal Active B12 levels. The demographic data of the patients are shown in Table 1.

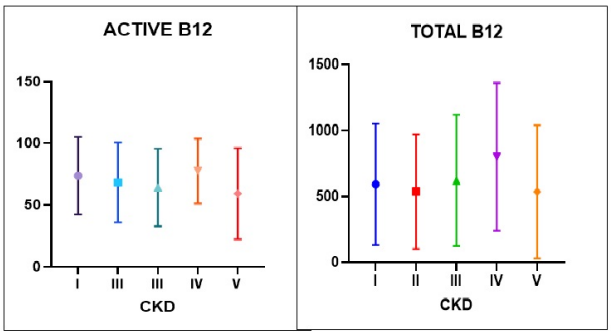
Twenty-one patients had B12 levels between 200 and 300 pg/mL, considered as borderline deficiency, and the remaining patients had B12 levels above 300 pg/mL, which is interpreted as normal. On the contrary, the folate concentration was within the normal range (mean 7.16 ± 3.38 ng/ml) in all of the patients. In the 107 patients, the mean total vitamin B12 level was 604.85 ± 495.2 pg/mL, and the mean Active B12 level (holoTC) was 67.1 ± 32.75 pmol/L (Table 2).

Table 2: Comparison data between total vitamin B12, active B12, and folate

	Total vitamin B12	Active B12	Folate
Range	146-1987	8.1-125	3.2-16.3
Mean ± SD	604.85 ± 495.2	67.1 ± 32.75	7.16 ± 3.38

Pearson correlation study of total vitamin B12 with active vitamin B12 indicated a strong positive correlation (r=0.501, p <0.01) between total B12 and active B12, as shown in figure (below).





4.1. *Effect of dialysis on B12 deficiency*

Total Vitamin B12 and Active B12 were predominantly lower in CKD patients on hemodialysis for more than three years duration compared to those for less than three years, which was significant with a p-value (<0.01) at 95% confidence interval (Table 3).

Table 3: Comparison of total vitamin B12 and active B12 with duration of dialysis

Duration of dialysis	Less than 3 years	More than 3 years
Number of patients	28	17
Total Vitamin B12	549.24 ± 323.4	509.41 ± 413.7
Active B12	66.24 ± 35.78	54.25 ± 30.52

5. Discussion

Vitamin B12 deficiency can be a major medical problem in CKD, particularly in ESRD and hemodialysis patients. However, B12 deficiency may not manifest with symptoms but can present with neurological complications in the later stages of CKD. It is also possible that, in many cases, functional B12 deficiency is not reflected in the blood tests, and the levels may remain borderline low or within normal reference range. Patients with CKD have a high risk of developing vitamin B12 deficiency, and disturbance of homeostasis may be directly linked to cardiovascular risk and the advancement of CKD. [11,12]

Active B12 (HoloTC) is an important marker that can override the clinical dilemma associated with Total B12 deficiency. It helps in the early detection of vitamin B12 deficiency due to its faster cellular uptake and short half-life period as compared to Total B12. Studies have shown that HoloTC levels reflect vitamin B12 status, independent of recent absorption of the vitamin. [13,14] The study by Barnali et al. shows that active B12 assay is accurate, reliable, and efficient and can be used for the diagnosis of vitamin B12 deficiency. Dastidar et al. also reported that active B12 is a much more reliable biomarker than total B12 for early

Table 1: Demographic data of the patients with different stages of CKD

Parameters	Stage 1	Stage 2	Stage3	Stage 4	Stage 5
Cases (*n*)	18	16	23	17	33
Age (years)	41.0 ±13.4	38.8 ±8.07	50.8 ±16.4	43.7 ±12.2	47.6 ±14.9
Creatinine	1.11 ±0.34	2.24 ±0.87	3.74 ±1.21	7.71 ±2.72	8.46 ±4.12
Total B12	590.4 ±460.7	536.1 ± 434.1	620.1 ± 498.2	798.6 ± 561.0	534.6 ± 505.4
Active B12	73.86 ±31.34	68.47 ±32.31	64.27 ±31.49	77.69 ±26.19	59.27 ±36.89

diagnosis of vitamin B12 deficiency.[6,12]

In our study, we compared Total B12 and Active B12 levels in patients with CKD, in all five stages, the values of Total B12 and Active B12 followed each other i.e. both parameters were lowest in stage 5 of CKD compared to the other stages of CKD, though there were no statistically significant differences noted in between the different stages.

It has been shown that B12 deficiency in healthy adults is uncommon, even if a healthy person consumed insufficient amounts of vitamin B12 for 3 years. Hence, we assessed the vitamin B12 and active B12 status in CKD patients on hemodialysis for duration of three years. The duration of dialysis had a significant effect on vitamin B12 deficiency as shown by decreased levels of both total and active B12 in patients with dialysis duration for more than three years. The B12 deficiency indicates that dialysis procedures can cause loss of vitamins, leading to B12 deficiency in these CKD patients.[15,16]

The appropriate range of B12 levels in CKD remains to be defined adequately and also there are no data on the storage levels of the vitamin in these patients. At present, there is no 'gold standard' method for the diagnosis of vitamin B12 deficiency, as B12 in plasma is not a sensitive indicator of overall B12 levels. As a consequence, the diagnosis requires consideration of both the clinical state of the patient and the results of various investigations. Downstream metabolites, such as methylmalonic acid[MMA] and homocysteine, may more accurately reflect functional B12 status in uremic patients. However, renal insufficiency itself can cause elevated serum MMA concentrations, leading to difficulty in interpretation. Plasma homocysteine levels rise quickly as vitamin B12 levels decrease, have poor specificity, and are influenced by other factors, such as low folate levels and, especially, by declined kidney function.[16–19]

Studies have stated that Active B12 and total vitamin B12 have equal diagnostic accuracy in screening for metabolic vitamin B12 deficiency. Thus measurement of both holoTC and total vitamin B12 could provide a better screen for vitamin B12 deficiency. Our study had limitations such as a small sample number, selection bias, and lack of MMA/homocysteine levels for confirmation of vitamin B12 deficiency which could not be determined due to financial restraints.[20,21]

6. Conclusion

Active B12 can supplement vitamin B12 measurements, especially in CKD patients and aid in the diagnosis of B12 deficiency. Active B12 (holoTC) can act as a potential marker for diagnosing B12 deficiency, especially in patients with CKD.

7. Source of Funding

None.

8. Conflict of interest

None.

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