



Total Vitamin B12 Vs Active B12 (Holo-Transcobalamin) In Chronic Kidney Disease Patients

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Abstract

Introduction: Anaemia, followed by development of left ventricular hypertrophy then cardiovascular failure, is the major cause of mortality in Chronic Kidney Disease (CKD) patients. B12 deficiency is one of the major contributors to anaemia in CKD. Nutritional deficiency due to dietary restrictions, deranged metabolism of vitamins and loss of vitamins in the dialysis procedure are important causes of vitamin b12 deficiency in CKD patients. Hence study of b12 deficiency and its active form in CKD is essential.

Aim: The aim of this is to assess performance of active vit B12 in comparison to total vit B12 for the correct diagnosis of true vitamin B12 status in CKD patients.

Study Design: An observational cross sectional study

Place and Duration of Study: Dept. of Biochemistry, Institute of Nephro-Urology, Bangalore, India from July 2022 to December 2022

Methodology: Study population consists of CKD patients attending Nephrology outpatient for follow up. They were categorized into 5 stages using eGFR values. Laboratory tests including. Total vit B12 and Active B12 also known as Holo-Transcobalamin (Holo-TC) were assayed in all the participants using Abbott chemistry analyzer.

Results: In our study, a statistically significant (P value = <0.0001) vitamin b12 deficiency was observed in severe stages of CKD (stage 4 & 5) than in earlier stages (stage 1, 2, & 3). Holo TC had better sensitivity, specificity, positive predictive value and negative predictive value in predicting vitamin b12 deficiency in CKD patients than total vitamin b12.

Conclusion: Holo TC is the better indicator of vitamin B12 deficiency in CKD patients in comparison with total vitamin b12.

Keywords: Chronic Kidney Disease; Vitamin B12; Anaemia

Introduction

A significant proportion of Chronic Kidney Disease (CKD) patients have physiological vitamin B12 deficiency. CKD patients are at higher risk of nutritional deficiency due to dietary restrictions, and malnutrition. Deranged metabolism in CKD patients leading to metabolic alterations and hormonal dysregulations is another contributor to vitamin b12 deficiency. Also dialysis procedure itself can cause loss of vitamins leading to vitamin b12 and folic acid deficiency [1]. It's well known that vitamin B12 along with folic acid is essential for the

homocysteine metabolism in the body. Hyperhomocystinemia, an immediate complication of vitamin B12 deficiency has grown as an important risk factor for cardiovascular disease in CKD patients [2]. Hence detecting vitamin B12 deficiency in CKD patients at the earliest is more important as administration of intravenous B-Complex vitamins is proven to be efficient in reducing serum homocysteine levels in CKD patients [3]. Vitamin B12 in the blood is basically protein-bound and approximately 20% of circulating B12 is bound to transcobalamin in which is the biologically active form that can be taken up into cells. That active form of vitamin b12 is also called Holo-transcobalamin

(Holo TC). CKD patients have an impaired tissue uptake of vitamin B12 because of increased transcobalamin losses in the urine and reduced absorption in the proximal tubule leading to a functional B12 deficiency which goes undetected by measuring only the Total b12 levels in CKD patients [4]. Thus it is very important to measure Active vitamin B12 (Holo TC) instead of total B12 levels in CKD patients. Also studies in this aspect in general population, have shown that Holo TC would be a better indicator of vitamin B12 status than total serum cobalamin, and may more accurately reflect functional B12 status.5,8. The aim of present study is to assess performance of Active vitamin B12 in comparison to Total vitamin B12 for the diagnosis of true VitB12 status in CKD patients of different stages.

Methodology

This was a retrospective cross-sectional study conducted in the Institute of Nephrourology, Bangalore, a tertiary care Centre for Nephro and urology care. Data was collected from the medical records and laboratory records of patients over a period of six months from July 2022 to Dec 2022.

Inclusion criteria

Adult patients both men and women of age group 20 to 60 year, diagnosed with CKD, attending routine nephrology outpatient department for follow-up were randomly selected and enrolled for the study.

Exclusion criteria

CKD patients, on vitamin b12 supplementation were excluded from the study. Cases were categorized into 5 groups CKD stage 1, to stage 5 as per KDOQI guidelines [5], using eGFR (estimated glomerular filtration rate) values. Different stages of CKD and their eGFR values included stage 1(eGFR >90), stage 2 (eGFR 60-90), stage 3 (eGFR 30- 60), stage 4 (eGFR 15-30), and stage 5 (eGFR <15).Laboratory tests mainly Urea, creatinine, total vitamin B12 and active b12 (Holo TC) were done in all the patients enrolled in the study. eGFR was calculated by the CKD Epidemiology Collaboration (CKD-EPI) equation [6].

Sample Collection and lab Analysis

Blood sample was collected randomly by standard venepuncture technique into plain plastic tubes using aseptic precautions. Complete clot formation was ensured prior to centrifugation. Serum was separated after centrifuging for 15 minutes, and was analysed for all the parameters on the same day. Analysis of serum was done by Abbott ci4100 chemistry and immunoassay auto analyser in biochemistry laboratory. Ready to use kits from Abbott architect ci systems were used for the analyses. Urea by colorimetric method using urease method and creatinine using

alkaline picrate method. Vitamin b12 was assayed by Chemiluminescent Micro particle Immunoassay (CMIA) method with reportable range of 148 pg/ml to 2000 pg/ml. Active vitamin b12 (Holo TC) was assayed based on Micro particle Enzyme Immunoassay (MEIA) technology with reportable range of 19 pg/ml to 128 pg/ml.

Statistical Analysis

Data were analysed by Statistical Package for Social Science (SPSS) version 17. Results were presented as mean \pm Standard Deviation (SD) for quantitative variables. The significance level, or p-value, was calculated using the unpaired t-test. A P value <0.005 was considered significant. Sensitivity, specificity, positive predictive value and negative predictive value for the tests were calculated using formulas.

Results

A total of 103 patients with CKD were enrolled into the study as per the inclusion criterion. Patients were categorized into 5 stages of CKD using eGFR values as per KDOQI guidelines, mentioned above. Total vitamin B12 and Holo TC were analysed in all the patients. Biochemical data of the patients enrolled in the study are listed in Table 1. Among 103 CKD patients (n=103), 29 patients (29%) were in stage 5, 23 patients (23%) were in stage 4, 19 patients (19%) were in stage 3, 16 patients (16%) were in stage 2 and 1 of CKD. Both vitamin 12 and Holo TC values were lower in stage 5 CKD patients followed by stage 4, 3, 2 and 1 (Table 1).

Vitamin B12 Deficiency

Out of 103 CKD patients, around 36 patients (37%) had total vitamin B12 deficiency and 52 patients (53%) had Active B12 (Holo Tc) deficiency. On clinical examination, only 26% (n= 25) had symptoms of B12 deficiency like tingling and numbness, burning feet, particularly at night time

Effect of dialysis onB12 deficiency

Total Vitamin B12 and Holo TC were predominantly lower in CKD patients on dialysis for more than five years duration compared to those on dialysis for less than five years which was significant with p value (<0.0001) at 95% confidence interval (Table 2) (Figure 1).

Total Vitamin B12 vs Holo TC

From the methodology of both Total b12 and Holo TC the upper cut off and the lower cut offs were defined. (Table 3). Seven patients had lower cut off value (<148) for total vitamin B12 and twelve had lower cut off for (<19) for Holo TC. None had higher

cut off (>2000) for vitamin B12 and six had higher cut off for HoloTC. Based on these results and also on the clinical features of B12 deficiency in CKD patients, sensitivity, specificity of the tests were calculated for vitamin B12 and Holo TC. Holo TC

showed high sensitivity and specificity compared to vitamin b12 which was significant with p value of <0.005. Also positive and negative predictive values were higher for Holo TC than Total vitamin B12 (Table 4).

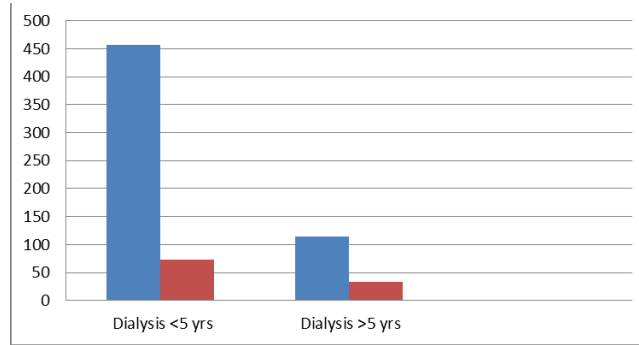


Figure 1: Effect of dialysis on vit B12 & HoloTC.

Table 1: Biochemical data.

Parameters	Stage 1 CKD (eGFR <15) n=16 Mean ± sd	Stage 2 CKD (eGFR 15-30) n=16 Mean ± sd	Stage3 CKD (eGFR30- 60) n=19 Mean ± sd	Stage 4 CKD (eGFR 60-90), n=23 Mean ± sd	Stag 5 CKD (eGFR ,>90) n=29 Mean ± sd
Age(yrs)	51 ± 13	42 ± 11	41 ± 12	39 ± 14	37 ± 18
Urea (10-44mg/dl)	35 ± 8	42 ± 7.1	79 ± 26	125 ± 35	158 ± 60
Creatinine (0.57-1.1mg/dl)	0.8 ± 0.2	1.6 ± 0.1	2.2 ± 0.2	4.3 ± 0.2	12 ± 4.2
Vit B12 (191-663 pg/ml)	688 ± 55	539 ± 137	390 ± 133	165 ± 55	140 ± 68
Holo Tc (35-128pg/ml)	120 ± 12	98 ± 14	66 ± 15	33 ± 15	21 ± 6

Holo TC- Holo Transcobalamin, vit B12- total vitamin B12 , CKD- chronic kidney disease

Table 2: Comparison of total vitamin B12 and Holo Tc with duration of Dialysis.

Duration of Dialysis	number of CKD patients	Total Vit B12 (Mean ± sd)	Active B12 (HoloTc) (Mean ± sd)
< 5 YEARS	52	456 ± 63	73 ± 13
> 5 YEARS	51	115 ± 12	33 ± 9
P value		0.0001	0.0001

Table 3: Comparison of Total B12 and Holo Tc Cutoff Values.

	Cut Offs	Number of CKD patients(n)
Total Vit B12	lower cut off (<148pg/ml)	7
	higher cut off (>2000 pg/ml)	Nil
Holo TC	lower cut off (<19 pg/ml)	12
	higher cut off (>128 pg/ml)	6

Discussion

Vitamin B deficiency is a major public health problem, particularly among older persons. Patients with CKD have high risk of developing vitamin B12 deficiency in early life and hence

the cardiovascular complications associated with it. All patients with vitamin b12 deficiency will not show the symptoms of B12 deficiency. But they develop metabolic complications of B12 deficiency which will be evident in their later life. It is also possible that, in many cases, functional vit B12 deficiency is not

reflected in the blood tests and it remains borderline low or within normal reference range [7]. Active B12 (Holo-TC) is important marker which can override the clinical dilemma associated with total vit B12 deficiency. It helps in early detection of vit B12 deficiency due to its faster cellular uptake and short half-life period as compared to total vit B12 [8]. In this study we compared Total B12 and Active vit B12 5 different groups of CKD patients.

Table 4: Comparison of the validity tests of Total b12 and Holo TC in the diagnosis of functional vitamin b12 deficiency.

Validity tests	Vit B12	HoloTC
Sensitivity	76.4%	85.4%
Specificity	62%	72%
Positive predictive value	79%	88%
Negative predictive value	42%	56%

In all the five groups, the values of TotalB12 and Active B12 were in accordance with each other ie both parameters were lowest in stage 5 followed by stage 4, 3, 2 and 1 of CKD. Both the parameters were in normal range in stage 1 and 2 of CKD. Duration of dialysis had an effect on vitamin b12 deficiency. CKD patients with dialysis duration for >5 years has predominant b12 deficiency indicating that dialysis procedure can cause loss of vitamins leading to B12 deficiency in CKD patients. In all stages of CKD Holo TC had better diagnostic accuracy than Total b12 with significantly higher sensitivity and specificity.

Conclusion

Active B12 has better diagnostic accuracy than vitamin b12 especially in CKD patients. Thus Active B12 (Holo TC) should be considered for diagnosing and treating B12 deficiency in CKD patients in order to reduce the risk of cardiovascular morbidity and mortality in these patients.

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