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Evaluative Study Of Inflammatory Markers Crp, Homocysteine And Vitamin- B12 In All Stages Of Ckd

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ABSTRACT:
Introduction: Chronic kidney disease (CKD) is identified by the presence of kidney damage, either structural or functional, or by a decline in glomerular filtration rate (GFR) below 60 mL/min/1.73 m2 of body surface area for more than 3 months.
Aim: To do a comparative study of Homocysteine, CRP and Vitamin B12 in different stages of chronic kidney disease.
Method: All the parameters serum CRP, Vitamin-B12 and Serum Homocysteine were estimated by VITROS 3600 immunodiagnostic system and VITROS 5600 integrated system using intellicheck technology.
Statistical analysis: The collected data was entered into MS excel sheet in proper format and analyzed for p-value, student t-test, mean value and standard deviation with the help of SPSS's latest software (version 29.0) and Microsoft excel. p-value <0.05 was considered as significant.
Result: Serum CRP and Homocysteine levels are lower in CKD stage 1 and statistically significantly higher in CKD stage 5 and Vitamin-B12 level is higher in CKD stage 1 and reduced in CKD stage 5, which is statistically significant.
Conclusion: In the study, statistically high homocysteine and low vitamin B12 levels were observed in different stages of chronic kidney disease. Hyperhomocysteinemia is associated with increase stages of kidney failure mostly in stages 4 and stage 5, therefore supplementation of vitamins should be done in these stages which will correct both Hyperhomocysteinemia and low vitamin-B12.

Keywords: Chronic kidney disease, C-reactive protein, Homocysteine and Vitamin-B12

INTRODUCTION

According to the kidney disease improving global outcomes (KDIGO) guidelines, chronic kidney disease (CKD) is identified by the presence of kidney damage, either structural or functional, or by a decline in glomerular filtration rate (GFR) below 60 mL/min/1.73 M^2 of body surface area for more than 3 months[1]. Three most common causes are Diabetes Mellitus, Hypertension, and Glomerulonephritis [2]. One of three adults with Diabetes and one of five adults with Hypertension have CKD [2]. Symptoms may include pedal edema, fatigue, vomiting, loss of appetite, and confusion. Complications include an increased risk of heart disease, high blood pressure, bone disease, and anemia. Signs of damage seen in blood, urine, or imaging studies which includes lab albumin/creatinine ratio (ACR) ≥ 30 is defined as Kidney damage [3–5]

Stages:

Stage 1: Slightly diminished function; kidney damage with normal or relatively high GFR (≥90 ml/min/1.73 m2) and persistent albuminuria. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies[6].
Stage 2: Mild reduction in GFR (60–89 ml/min/1.73 m2) with kidney damage. [3,5]
Stage 3: Moderate reduction in GFR (30–59 ml/min/1.73 m2). British guidelines distinguish between stage 3A (GFR 45–59) and stage 3B (GFR 30–44) for purposes of screening and referral. [6]
Stage 4: Severe reduction in GFR (15–29 ml/min/1.73 m2) Preparation for kidney replacement therapy. [6]

Stage 5: Established kidney failure (GFR <15 ml/min/1.73 m2), permanent kidney replacement therapy, or end-stage kidney disease. [6]

Homocysteine (Hcy) is a non-proteogenic amino acid. It is an amino acid homologue of cysteine, differing from cysteine by a second methylene bridge (-CH2-). It is produced biologically from methionine by removing the terminal methyl group. With the help of vitamin B-12 and folic acid the body may recycle homocysteine into methionine or convert it to cysteine [7]. The patients with end stage of real disease (ESRD) have Hcy levels 3-5 times higher than normal. Every study shows that concentration of creatinine and Hcy have positive significant correlation between them. Plasma Hcy levels are inversely correlated with GFR values estimated from serum creatinine or calculated creatinine clearance. Elevated levels of Hcy in renal diseases is powerful evidence linked to kidney function [7].

C-reactive protein is used as an inflammation marker. It is a pentameric protein found in plasma, whose circulating concentrations rise in response to inflammation [8,9].In kidney disease, CRP is significantly produced by various inflammatory cells, likely macrophages and intrinsic kidney cells, including tubular and endothelial cells. Continuing high levels of CRP may cause chronic inflammation, as seen in individuals with chronic kidney failure or end-stage renal disease[10].

Cobalamin, generally known as vitamin B12, is a water-soluble vitamin essential to every human cell metabolism. It plays a crucial part in the production of myelin, as well as the maturation of growing red blood cells in the bone marrow, which are crucial for the correct operation of the neurological system [11]. Along with co-morbidities and multiple medications, various metabolic changes in CKD patients such as acidosis, systemic inflammation, and hormone dysregulation may result in malnutrition and a consequent folic acid and vitamin B12 shortage. Under typical circumstances, the conversion of methyl cobalamin to cyanocobalamin is necessary to remove cyanide from the bloodstream. However, the decreased cyanide clearance in CKD patients hinders the conversion of cyanocobalamin to the active form, making supplementation less effective [12].

The aim of this study was to do a comparative study of Homocysteine, CRP and Vitamin-B12 in different stages of chronic kidney disease. To estimate serum homocysteine, CRP and serum Vitamin-B12.

MATERIALS AND METHODS

This is a cross sectional study and conducted in the Department of Biochemistry, and Nephrology department of shri Mahant Indresh Hospital attached with SGRRIM&HS, Dehradun, duration of 2 years from February 2022 to February 2024. Sample size was calculated with the help of Cochran's formula ($n=z^2\times pq/e^2$, where- z-1.96 is a confidence value, p-4%-20%[13], q=1-p). 150 Subjects were recruited according to selection criteria after the approval of institutional ethical clearance (Ref. No. SGRR/IEC/01/22) from institutional ethical committee (Reg. No. ECR/710/Inst/UK/2015/RR-21).

Inclusion criteria: Patients of Age 20-65 years, Patients having chronic renal diseases of other causes like hypertension, Diabetes mellitus, Glomerulonephritis and Patients having GFR <30 ml\min were included in the study.

Exclusion Criteria: Patients of age < 20 and >65 years, Patient with history of kidney transplant and Patients HbsAg positive, HCV positive and HIV positive were excluded.

Serum samples taken for serum CRP, Homocysteine and Vitamin B12 from patients came to Nephrology OPD and analyzed by fully automated biochemistry analyzer such as VITROS XT 7600/ VITROS 5600 Integrated system.

Statistical Analysis

The collected data was entered into MS excel sheet in proper format and analyzed for p-value, student t-test, mean value and standard deviation with the help of SPSS's latest software (version 29.0) and Microsoft excel. p-value <0.05 was considered as significant.

RESULT

All the 150 samples were analyzed for serum homocysteine, serum CRP and serum Vitamin B12 and subjects were categorized based on chronic kidney diseases stages. 30 patients were included in each stage.

Serum CRP (6.56 ± 4.45 mg/dl) and Homocysteine (10.28 ± 3.07 µmol/l) levels are lower in CKD stage 1 than CKD stage 5 (serum CRP-14.20± 12.68 mg/dl, Homocysteine-28.39 ± 10.34 µmol/l) and Vitamin B-12 (574.57 ± 254.01 pg/ml) level is higher in CKD stage 1 than CKD stage 5 (369.40 ± 163.36 pg/ml) [Table/fig-1].

Table/fig-1: Comparison of serum CRP, Homocysteine (HCY) and Vitamin-B12 in different stages of chronic kidney diseases.[1]

CKD Stages	CRP (mg/dl) (mean ± SD)	Homocysteine (µmol/l) (mean ± SD)	VIT-B12 (pg/ml) (mean ± SD)
CKD-1	6.56 ± 4.45	10.28 ± 3.07	574.57 ± 254.01
CKD-2	5.12 ± 2.96	12.38± 4.61	493.83 ± 232.85
CKD-3	10.86 ± 9.64	14.10 ± 4.19	456.17 ± 188.53

CKD-4	13.49 ± 9.08	19.70 ± 7.75	450.55 ± 210.06
CKD-5	14.20± 12.68	28.39 ± 10.34	369.40 ± 163.36

Table/fig-2: t-test and p-value of serum CRP, Homocysteine and Vit-B12 between CKD-1 with different stages of chronic kidney diseases.[1]

CKD STAGES	t-test Value			p-Value		
	CRP	HCY	VIT-B12	CRP	HCY	VIT-B12
CKD-1 V/s CKD-2	4.53	4.73	4.03	0.0001	0.000064	0.0004
CKD-1 v/s CKD-3	4.05	6.05	2.66	0.0003	0.000015	0.012
CKD-1 v/s CKD-4	2.42	6.11	2.70	0.02	<0.01	0.011
CKD-1 v/s CKD-5	4.66	7.28	6.66	<0.01	<0.01	<0.01

t-test value of serum CRP is 4.53 (p value <0.0001), 4.05 (p value <0.0003), 2.42 (p value 0.02) and 4.66 (p value <0.01) respectively in between CKD-1 to CKD-2, CKD-3, CKD-4 and CKD-5, which was statistically significant. T test value of serum Homocysteine (HCY) is 4.73 (p value- 0.000064), 6.05 (p value-0.0000015), 6.11 (p value-0.01) and 7.28 (<0.01) respectively in between CKD-1 to CKD-2, CKD-3, CKD-4 and CKD-5. T test value of serum Vitamin B12 is 4.03 (p value-0.00043), 2.66 (p value- 0.012), 2.70 (p value- 0.011) and 6.66 (p value- <0.01) respectively in between CKD-1 to CKD-2, CKD-3, CKD-4 and CKD-5 [Table/fig-2].

Table/fig-3: t-test and p-value of serum CRP, Homocysteine and Vit-B12 between CKD-2 with different stages of chronic kidney diseases. [1]

STAGES	t-test Value			p-Value		
	CRP	HCY	VIT-B12	CRP	HCY	VIT-B12
CKD-2 v/s CKD-3	5.86	5.69	2.65	<0.01	<0.01	0.013
CKD-2 v/s CKD-4	4.47	4.95	3.94	**0.00011**	0.00031	0.00048
CKD-2 v/s CKD-5	5.0	6.91	5.13	<0.01	**<0.01**	<0.01

t-test value of serum CRP is 5.86 (p value <0.01), 4.47 (p value <0.00011), 5.0 (p value 0.01) respectively in between CKD-2, to CKD-3, CKD-4 and CKD-5, which was statistically significant. T test value of serum Homocysteine (HCY) is 5.69 (p value- 0.01), 4.95 (p value-0.000031), 6.91 (p value- 0.01) and respectively in between CKD-2, CKD-3, CKD-4 and CKD-5. T test value of serum vitB12 is 2.65 (p value- 0.013), 3.94 (p value -0.00048), 5.13 (p value -<0.01) respectively in between CKD-2 to CKD-3, CKD-4 and CKD-5 [Table/fig-3].

DISCUSSION

According to our observations, Homocysteine level was significantly higher (28.39±10.34 µmol/L) in CKD-5 which was statistically significant (P value- 0.04), and CRP level was 14.20±12.68 mg/dl, also higher in CKD-5, Vitamin B-12 level was lower in CKD-5 (369.40±163.36 pg/ml) (p value). This indicates all stages of CKD shows increasing trend of CRP and HCY, and decreasing trend of Vitamin B-12.

Lalramenga P. et. al**;** reported the increased level of serum CRP in CKD patients [14]. Adejumo O et. al; found statistically significant level of serum CRP level in chronic renal failure [15]. Annuk M. et. al; also found raised value of serum c reactive protein in the patients of CKD which was statistically significant and p value was <0.01 (12). Pravin et. al; (2013) reported higher level of serum CRP level in the patients of chronic kidney disease than normal subjects [16]. Varughese S. et. al; all these studies show that in CKD patients, increasing CRP levels were decreasing renal function [17].

In our study serum homocysteine levels are higher in CKD stage 5 than other CKD stages, which is statistically significant. In 2014, Levi A et. al; reported significantly higher level of serum homocysteine in CKD patients [18]. Hyperhomocysteinemia with declining kidney function in individuals with preexisting renal disease have been the subject of two prior investigations (Samuelsson O, et. al; & Mark J. Sarnak, et. al;) [19,20]. Ninomiya T et. al; also found elevated homocysteine level in patients of chronic renal diseases [21]. Remacha A et. al; also reported the elevated level of homocysteine in CKD patients [22]. Andrea A et. al; reported higher serum level of Homocysteine than the general subjects [23]. McMahon G et. al; observed borderline increased level of Hcy in renal diseases with increased vitamin B12 in ESRD patients [24]. Anurag A. reported statistically significant high level of Hcy in CKD patients [25]. Yonova D et. al; described statistically higher level of Hcy in CKD patients [26].

In our study we have observed decreased level of serum vitamin B-12 in last stage of chronic renal failure (CKD-stage 5). Farrington D K et al; also described lower level of serum vit. B-12 level in CKD patients [27]. Remacha A et. al; also reported the lower level of Vit. B-12 level in CKD patients [22]. Hitesh H. Shah et.al; were also described lower level of serum vitamin B-12 in end stage of renal disease, which is one of the reasons of anaemia

[1] (p-value <0.05 was considered as significant, p-value <0.01 was considered as highly significant and p-value >0.05 was considered as statistically not significant)

[28]. Matthews D and Beckett A. Gordon have reported impaired high level of serum vitamin B12 in CKD patients [29]. Andrea A et. al; also reported statistically lower level of serum vitamin B-12 level in chronic kidney disease patients with ESRD [23]. Anurag A. observed lower level of serum vitamin B12 in CKD patients [25]. Yonova D et. al; have reported statistically significant reduced level of serum vitamin B12 in chronic renal failure [26]. Wu H. and Wang A. Yee-Moon have observed lower level of cobalamin in different stages of chronic renal failure [30]. Pastore A. et. al; also reported statistically significant reduced serum vitamin b12 level in CKD patients [31].

CONCLUSION

Across the various phases of chronic renal disease, our research found that homocysteine levels were considerably higher and vitamin B-12 levels were much lower. Vitamin supplements may treat Hyperhomocysteinemia and low vitamin B-12 levels; this is especially important in the latter stages of renal failure, when Hyperhomocysteinemia is more common. All phases of chronic kidney disease (CKD) exhibit an upward trend in CRP and HCY and a downward trend in vitamin B-12 when compared to CKD-1. Measuring CRP, HCY, and vitamin B-12 becomes crucial when patients' GFR drops below 60 or CKD stage 3 and beyond, particularly in patients with diabetes mellitus and related coronary artery disorders. Patients with microvascular and macrovascular problems from uncontrolled diabetes mellitus may be at an even higher risk.

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