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Original Research Article

Serum Homocysteine Levels in Various Stages of Chronic Kidney Disease

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ABSTRACT

Objectives: To study serum homocysteine levels in Chronic Kidney Disease (CKD) patients and co-relate it with the stages of CKD, Body Mass Index (BMI), cholesterol levels and cardiac function and to study effect of hemodialysis on homocysteine levels.

Settings & Design: Cross sectional study carried out at a tertiary care teaching hospital.

Methods: Thirty CKD patients as cases and 30 normal subjects as controls were randomly selected according to eligibility criteria. Clinical examination, blood investigations including serum homocysteine levels and 2D-ECHO were done for all patients. Data was analyzed using Mean, Standard Deviation and Test of Significance for quantitative data.

Results: Serum Homocysteine levels were significantly higher in 90% CKD patients as compared to non CKD group (P<0.01). Duration of CKD > 2 years was associated with higher serum homocysteine levels as compared to lesser duration (P<0.05). Serum homocysteine levels were higher with advanced stages of CKD, but it was not statistically significant (P>0.05). Other results that were not statistically significant were - Homocysteine levels were higher in nondialysed patient as compared to dialysed patients (P>0.05), Homocysteine levels were low in patients on hemodialysis with high flux dialyser compared to low flux dialyser, Homocysteine levels were not affected by BMI (P>0.05) and CKD patients with high homocysteine levels had high cholesterol (P>0.05)and lower Ejection fraction.(P>0.05).

Conclusions: Serum Homocysteine levels are high in CKD patients and its level increases with duration of CKD.

Key words: Homocysteine, Hyperhomocysteimia, CKD, Hemodialysis, Cardiac Function.

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INTRODUCTION

Hyperhomocysteinemia, defined as a serum homocysteine level of >15 µmol/l, is common in patients with Chronic Kidney Disease (CKD) as its levels in plasma increases with declining Glomerular filtration rate.[1] It is a major risk factor and predictor for atherosclerotic complications like ischemic heart disease and stroke.[2,3] It is an independent risk factor for the progression of CKD.[4] Treatment of hyperhomocysteinemia with high dose folic acid, is an important mode of renoprotective measures in CKD patients.[5] Studies have shown that hemodialysis using high-flux and super-flux membranes decreases the homocysteine levels in CKD patients.[6,7]

Importance of homocysteine levels is well highlighted in cardiology, but there are few studies in nephrology literature & in Indian CKD patients.Plasma homocysteine is strongly correlated with (estimates of) glomerular filtration rate (GFR). Patients with chronic kidney disease, especially end-stage renal disease (ESRD), exhibit many abnormalities in protein and amino acid metabolism. One of these alterations involves an increased plasma concentration of the sulphur-containing amino acid homocysteine.

The exact mechanism of hyperhomocysteinemia is not known. Two, not mutually exclusive hypotheses for high homocysteine levels in CKD are:

1) Homocysteine disposal in the kidneys itself is disturbed and
2) Extrarenal homocysteine metabolism is impaired.

Factors that cause renal dysfunction and hyperhomocysteinaemia by different mechanisms have not been identified.

This study was conducted to determine the levels of homocysteine in patients with CKD and to co-relate the homocysteine levels with the stage of CKD and other co-morbidities.

AIMS & OBJECTIVES

Primary

- To study the serum Homocysteine levels in patients with Chronic Kidney Disease and compare with normal population

Secondary

- To co-relate the Serum Homocysteine levels with the various stages of Chronic Kidney Disease, including CKD VD [dialysis dependent CKD STAGE V]
- To co-relate serum cholesterol levels, Body Mass Index (BMI) & cardiac function with Homocysteine levels

SUBJECTS & METHODS

This was a population based cross sectional study carried out at a teaching hospital after due permission from the Institutional Ethics committee. Sample size was 60:30 of CKD & 30 normal. The selection was as per rules of normal distribution law. Selection in both groups was independent & random.

Inclusion criteria

- Age > 18 years
- CKD stages II-V with GFR <90 ml/min for > 3 months and
- Either pathological abnormalities or markers of kidney damage i.e. abnormalities in blood, urine or imaging tests
- Patients willing to give informed written consent.

Exclusion criteria

- Pregnancy, Diabetes mellitus, Smokers, Metastatic cancer, Acute lymphoblastic lymphoma, Chronic liver disease,
- Treatment with methotrexate or anticonvulsants, Vitamin B12 or folic acid treatment, as these factors increase the serum homocysteine levels.
- Alcohol addict or excess coffee consumers.

Thirty patients suffering from CKD and fulfilling the above mentioned inclusion criteria were included and 30 normal individuals were taken as controls. Controls were selected so as to match age, sex & nutritional status of the CKD population. Only those with normal blood pressure [≤ 130/80 mm of Hg] , normal fasting & post prandial blood sugar & GFR > 90 ml/min {estimated by Modification of Diet in Renal Disease [MDRD] equation} were included. [Table 1]

All patients were evaluated with detailed history, complete systemic examination, Investigations- Hemoglobin (Hb), WBC, Blood Urea Nitrogen (BUN), Serum Creatinine, Serum Electrolytes, Serum Proteins, Lipid profile, Serum Homocysteine, Urine routine, Ultrasonography- Kidney, Urter & Bladder (USG-KUB), Electrocardiography (ECG), Two dimensional Echocardiography (2D Echo) and X ray chest. Kidney biopsy was done when required.

The Homocysteine levels were measured by Fluorescent Polarised Immuno Assay (FPIA) method. The sample was collected after 12 hours of fasting and sent to the laboratory in ice pack or stored in refrigerator at 4^0 Celsius and then processed. The normal values of serum Homocysteine by this method are - males: 5.9 – 16 µmol/lit and Females: 3.36 – 20.44 µmol/lit.

According to K-DOQI guidelines[8] CKD was defined as:

1) Kidney damage for > 3 months with structural or functional abnormalities of the kidney, with or without decreased GFR, manifested by either pathological abnormalities or markers of kidney damage i.e. abnormalities in blood or urine investigation or imaging

2) Glomerular filtration rate of < 60 ml/min/1.73 m2 for > 3months with or without kidney damage

GFR was calculated using MDRD equation:

MDRD equation:

186 X (sr Creat) -1.154 x age - 0.203 x 0.742 (if female) X 1.210 (if black)

Stages CKD were defined according to Kidney Disease Outcomes Quality Initiatives (K-DOQI) guidelines:

Stage I: GFR ≥90ml/min
Stage II: GFR 60-89 ml/min
Stage III: GFR 30-59ml/min
Stage IV: GFR 15-29 ml/min
Stage V: GFR <15ml/min (or dialysis : Stage V D)

Kidney biopsy was performed in 8 patients.

All patients were classified in two groups according to CKD duration < 2 years and > 2 years.

For statistical analysis Serum Homocysteine levels in CKD patients were grouped as follows:

Normal (≤ 15 µmol/L)
Moderately high (>15 & ≤ 30µmol/L)
Intermediately high (>30 & ≤100 µmol/L)
Severely high (> 100 µmol/L).

The type of dialyser used by the patients on dialysis and its effect on the homocysteine levels were studied. BMI was calculated as – Weight (kg) / Height (m^2). For statistical analysis - BMI was divided into 2 classes as < 20 & >20, serum cholesterol levels were divided as: High (>200mg/dl) and Normal (≤200mg/dl) and ejection fraction on 2D-echo, which is an indicator of cardiac function was classified as follows: >40 % & <40 %.

Statistical Analysis

Data was analyzed using Mean, Standard Deviation and Test of Significance for quantitative data. P value <0.05 was considered to be significant.

RESULTS

Demography of normal group & CKD patients has been detailed in Table 1. Both the groups were comparable for age, sex & BMI. CKD group had significantly lower GFR, Hb & higher serum phosphate, cholesterol & triglycerides compared to controls. Commonest cause of CKD was Chronic Glomerulonephritis, followed by obstructive uropathy. (Table 2)

Serum Homocysteine levels were higher in CKD patients (mean: 25.26 µmol/lit) than normal controls (mean: 13.01µmol/lit). The difference was statistically significant (P<0.01). The incidence of hyperhomocysteinemia in our CKD population was 90% (Table 3).

Serum Homocysteine showed statistically significant rise when duration of CKD was more than 2 years as compared to those with less than 2 years. (Mean Serum Homocysteine: 30.25 µmol/lit Vs 20.96 µmol/lit) -P value < 0.05. (Fig 1)

Rising trend was seen in Serum Homocysteine levels with higher stages of CKD. (Mean Homocysteine levels in Stage II: 23.48 µmol/lit, Stage III: 24.20 µmol/lit, Stage IV: 28.53 µmol/lit). However, the positive co-relation was not significant stastistically (P > 0.05). (Fig 2)

Homocysteine levels were higher in non-dialysed patients (Mean Homocysteine- 25.59 µmol/lit) as compared to dialysed patients (24.48 µmol/lit) but the difference [P value > 0.05] was

not significant (Table 4). Homocysteine levels were lower (Mean: 17.74 µmol/lit) in patients dialysed with high flux dialysers as compared to those dialysed with low flux dialysers (26.74 µmol/lit), but sample size was small for statistical analysis.

Homocysteine levels in CKD patients were not affected by BMI, (Homocysteine levels: Mean: 25.34 µmol/lit with BMI < 20 & 25.20 µmol/lit with BMI > 20, p > 0.05). CKD patients with higher cholesterol levels were associated with high homocysteine levels but it was not statistically significant (P value- P>0.05). Marginally higher values of Homocysteine were seen in CKD patients with lower EF (Ejection Fraction). (Mean Homocysteine: 26.06 µmol/lit in CKD pts with LVEF (Left Ventricular Ejection Fraction) < 40 % Vs 25.06 µmol/lit in those with LVEF > 40 %). But it was not statistically significant (P value- >0.05)

Table 1: Clinical demography: Control Vs CKD patients

	Controls	CKD patients	P value
No.	30	30	
Serum HCM µmol/lit	13.01(±3.07)	25.30(±10.01)	< 0.001
M:F	24 :6	20:10	> 0.05
Age (Years)	38.5(±13.28)	40.17(±12.90)	> 0.05
BMI	20.70(±2.82)	19.19(±3.02)	> 0.05
Duration of CKD (Years)	-	4.07(±3.72)	_
Hypertension	Nil	26	_
Diabetes	Nil	Nil	_
Hb gm%	13.9(±1.03)	10.33(±2.36)	< 0.001
GFR ml/ min	104.6(±5.01)	32.26(±21.22)	< 0.001
S Na meq/lit	137.70(±1.90)	137.33(±4.60)	< 0.001
S K meq/lit	4.22(±0.42)	4.78(±0.58)	> 0.05
S Ca mg/dl	9.32(±0.41)	8.52(±0.55)	> 0.05
Sphosphate mg/dl	4.12(±0.40)	4.53(±1.05)	< 0.001
S cholesterol mg/dl	157.23(±20.09)	198.8(±51.96)	< 0.001
S Triglyceride mg/dl	87.83(±17.23)	138.13(±83.84)	< 0.001

Table 2: CKD: Aetiology

Cause of CKD	No. of patients		Total
	Male	Female	
Chronic Glomerulonephritis	9	3	12
Obstructive uropathy	5	2	7
Autosomal Dominant Polycystic Kidney Disease	1	2	3
Hypertensive Nephrosclerosis	3	0	3
Chronic Tubulointerstitial disease	2	1	3
Ischemic Nephropathy	0	1	1
Thrombotic Microangiopathy	0	1	1
Total	**20**	**10**	**30**

Table 3: Serum homocysteine levels in normal and CKD patients

Serum homocysteine levels µmol/L	<15	16-30	31-100	>100
Normal	30	0	0	0
CKD	3	21	6	0

Significant difference in homocysteine levels in normal and CKD patients i.e P value <0.01

Table 4: Homocysteine levels µmol/L in dialysed and non-dialysed patients.

	Dialysed	Non dialysed
Mean	24.48	25.59
SD	6.9	11.19
No. of patients	8	22

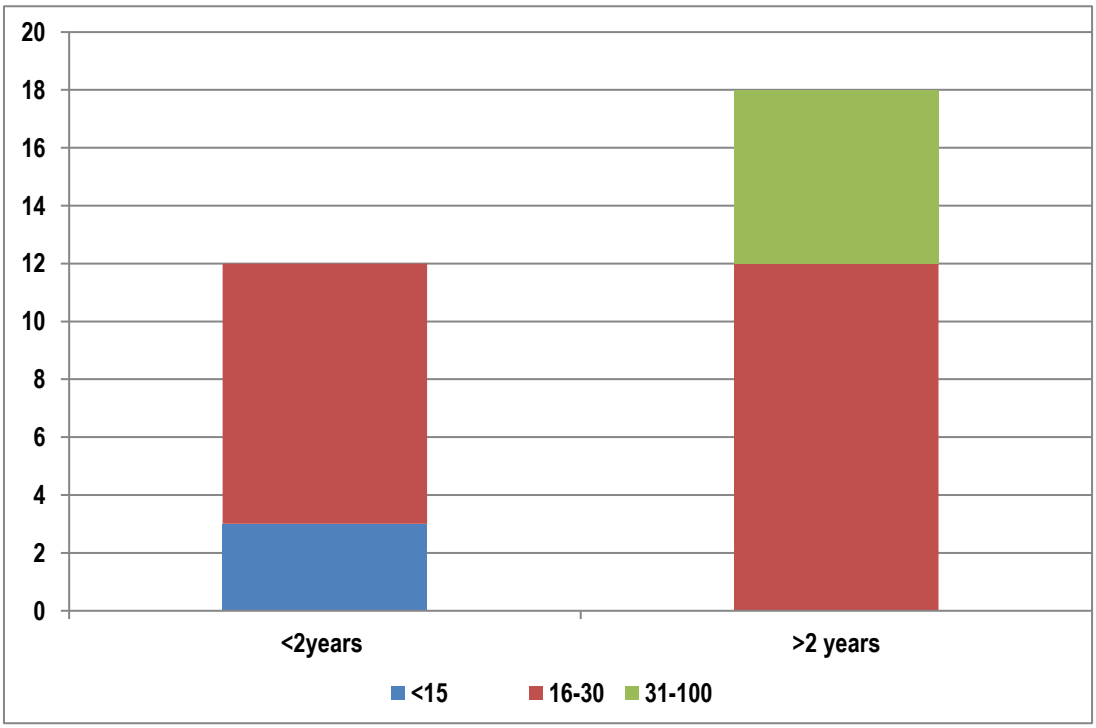
Homocysteine levels in dialysed patients was less than non-dialysed CKD patients.

Table 5: Homocysteine levels and cardiac function.

Ejection fraction	Serum Homocysteine levelsμmol/L			Total	Mean	SD
	<15	15-30	30-100			
< 40%	0	5	2	7	26.06	6.63
>40%	3	16	4	23	25.06	11.08

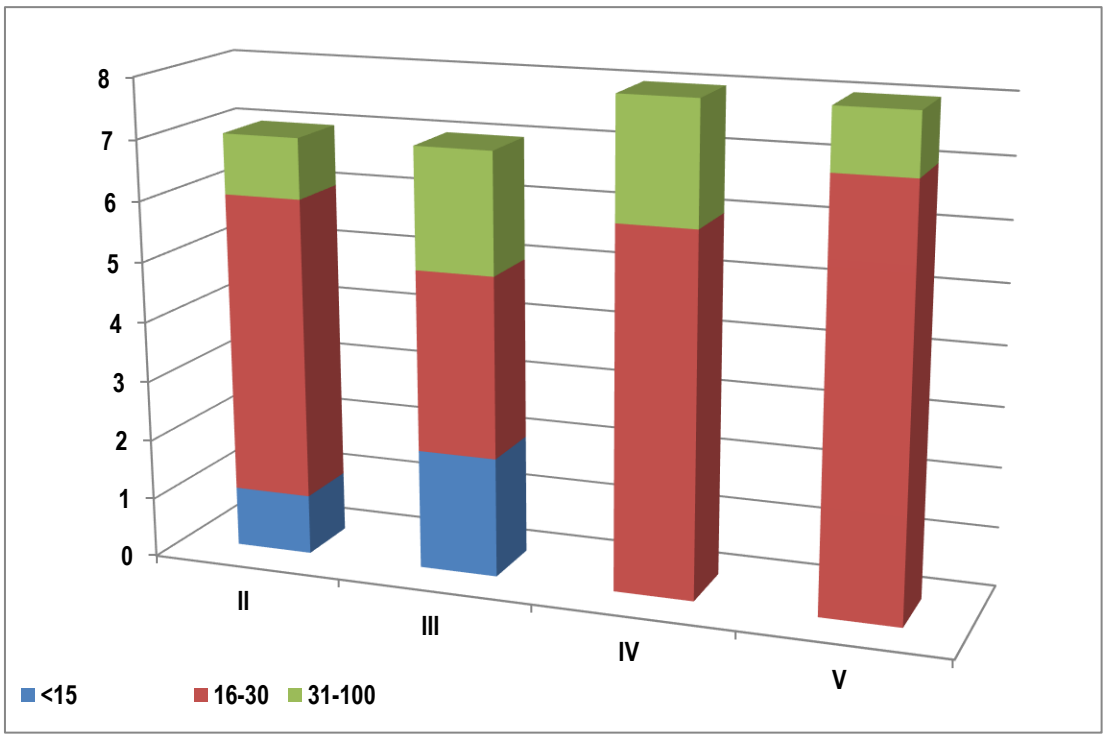
Patients with high homocysteine levels had poor ejection fraction. (P value- P>0.05)- not significant.

Fig 1: Duration of CKD and homocysteine levels.



Duration of CKD >2years was associated with high homocysteine levels. P value <0.05

Fig 2: Homocysteine levels and grades of CKD.



Homocysteine levels were higher with high stages of CKD. There was a decline in levels in stage V CKD, possibly due to effect of hemodialysis.(P value- >0.05)- not significant.

DISCUSSION

In our study, serum homocysteine levels were higher in CKD patients than in the normal subjects and the difference was statistically significant. The incidence of hyperhomocysteinaemia in CKD patients was 90 %. Patients with end-stage renal disease (CKD V) usually have 2–3 times higher levels of homocysteine than in normal population, and the prevalence of hyperhomocysteinaemia in CKD patient is >90%.[9] Dennis et al also reported a threefold higher homocysteine levels in CKD patients.[10] In our study the homocysteine levels were two times higher than the general population.

Homocysteine levels in this study, showed a statistically significant rise when the duration of CKD was more than 2 years as compared to those with less then 2 years duration. A similar co-relation between homocysteine levels and duration of CKD was reported by Bostom et al.[11] However, Muscheites J et al have shown that high homocysteine levels had no relation with the duration of CKD.[12]

A rising trend of serum homocysteine was seen with higher stages of CKD, with a marginal decline in CKD stage V as compared to the CKD stages II-IV. This was possibly due to the effect of hemodialysis and malnutrition on homocysteine level. Suliman ME et al showed that malnutrition, hypoalbuminemia, inflammation and diabetes mellitus may lead to lower levels of homocysteine in patients with CKD V.[13] The positive relation of serum homocysteine and stages of CKD was not statistically significant in our study. We attribute this to the small sample size .There is evidence that even mild reduction in GFR (e.g., >60 ml/min) results in higher homocysteine levels in the absence of significant accumulation of uremic toxins.[1] Arnadottir M et al have shown that the reduction in Homocysteine levels during dialysis is not due to direct homocysteine clearance but results from the removal of uremic toxins which have an inhibitory effect against one or more of remethylation enzymes and/ or the transsulphulation pathway.[14] There was no difference in homocysteine levels between those who had residual renal function and those who did not.[14] Therefore, hemodialysis did not seem to contribute significantly to homocysteine removal.

The homocysteine levels were lower in patients who were dialysed with High flux dialysers as compared to those dialysed with low flux dialysers, but statistical analysis could not be done as the number was small. A three month study by House et al showed that the greater intra-dialytic reduction of homocysteine on high-flux dialysis, did not translate into a significant difference in its pre-dialysis levels[6] whereas Vriese et al showed low-flux and high-flux dialysis did not affect homocysteine values, while super-flux dialysers decreased homocysteine levels.[7]

In our CKD patients the homocysteine levels were not affected by BMI which is an indicator of nutritional status of the patients. Menon V et al highlighted that homocysteine was negatively correlated with measures of body fat.[15] On the contrary Suliman et al suggested that malnutrition may lower circulating levels of homocysteine.[9] Malnutrition has been co related with lower serum homocysteine level than patients with normal nutritional status.[4,16] CKD patients with higher cholesterol levels were associated with high homocysteine levels but it was not statistically significant. Similar findings were reported by Huseyin et al.[17] Mayer et al[18] also reported a positive correlation between homocysteine and cholesterol. Marginally higher values of homocysteine were seen in CKD patients with lower Ejection fraction. But it was not statistically significant. Studies have shown that high homocysteine levels are associated with increased cardiovascular morbidity and mortality.[19]

In contrast to the well documented association between homocysteine levels and cardiovascular disease in the general population, the association between homocysteine levels and risk for atherothrombotic disease is not a consistent finding in CKD V. Some cross-sectional studies report higher levels of homocysteine in CKD V patients with cardiovascular disease,[20] others report no difference in homocysteine levels[21] or even paradoxically lower homocysteine levels in cardiovascular disease patients.[3,16] Prospective evidence from Moustapha et al,[22] demonstrated a 1% increase in the relative risk of subsequent cardiovascular events for each 1 μmol/l increase in homocysteine in patients with end-stage renal disease, suggests that such a small absolute difference would probably have little clinical impact. The relationship between hyperhomocysteinaemia and CVD in CKD V patients is explained by endothelial dysfunction (a common phenomenon in this patient group), may be one of the most important factor.[23]

We conclude from this study that homocysteine levels are higher in CKD patients than in the general population and the homocysteine levels are twice the levels in the general population. The levels of homocysteine are higher in patients of CKD with duration of more than 2 years compared to those with duration less than 2 years. It is recommended that serum homocysteine levels in Indian CKD population be studied on larger scale.

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