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# PLASMA HOMOCYSTEINE LEVELS IN CHRONIC KIDNEY DISEASE AND THEIR ASSOCIATION WITH DISEASE SEVERITY: AN ANALYTICAL CROSS-SECTIONAL STUDY

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## Abstract

***Background:*** Chronic kidney disease (CKD) is associated with a high burden of cardiovascular morbidity and mortality. Hyperhomocysteinemia is a non-traditional vascular risk factor that may increase with declining renal function, but Indian data on its relationship with graded CKD severity remain limited.**Objectives:** To estimate plasma homocysteine levels in patients with CKD and to assess the association of homocysteine levels with the severity of CKD.**Methods:** This analytical cross-sectional study was conducted in a tertiary care teaching hospital from January 2024 to December 2025. Adult patients with CKD fulfilling the selection criteria were enrolled by consecutive sampling. Plasma homocysteine was estimated by chemiluminescent immunoassay. Serum creatinine was measured by Jaffe method, estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation, and CKD was staged according to eGFR. Homocysteine levels >15 µmol/L were considered elevated. Data were analysed using descriptive statistics, chi-square test, comparison of means and correlation analysis.**Results:** A total of 120 CKD patients were included. Most patients were aged 60-79 years (41.7%) or 40-59 years (38.3%); 61.7% were male. Hypertension (68.3%), anaemia (63.3%) and diabetes mellitus (51.7%) were common comorbidities. Stage 5 CKD was present in 33.4%, stage 4 in 28.3%, stage 3B in 23.3% and stage 3A in 15.0%. Hyperhomocysteinemia was present in 86 patients (71.7%). Mean homocysteine increased progressively from stage 3A to stage 5 (18.6 +/- 6.2, 23.4 +/- 7.8, 29.8 +/- 10.5 and 36.9 +/- 14.1 µmol/L respectively; p<0.001). Elevated homocysteine was present in 50.0%, 64.3%, 79.4% and 80.0% of stages 3A, 3B, 4 and 5 respectively (p=0.018). Homocysteine showed a significant negative correlation with eGFR (r=-0.46, p<0.001) and positive correlations with serum creatinine, blood urea and blood urea nitrogen. Cardiovascular events were more frequent among patients with elevated homocysteine than among those with normal levels (30.2% vs 11.8%, p=0.028).**Conclusion:** Hyperhomocysteinemia was highly prevalent among CKD patients and increased significantly with advancing CKD stage. Plasma homocysteine may be a useful biochemical marker of renal dysfunction severity and cardiovascular risk in CKD.

**Keywords:** chronic kidney disease; homocysteine; hyperhomocysteinemia; estimated glomerular filtration rate; cardiovascular risk; CKD-EPI



## INTRODUCTION

Chronic kidney disease (CKD) is a progressive clinical syndrome characterized by persistent structural or functional abnormalities of the kidney and a gradual decline in glomerular filtration rate. Its public-health importance arises not only from the risk of progression to end-stage renal disease, but also from its strong association with premature cardiovascular disease, repeated hospitalization, reduced quality of life and high treatment cost. In India, the burden of CKD is particularly relevant because diabetes mellitus, hypertension, population ageing and late referral to nephrology services coexist with variable access to early screening and renal replacement therapy. Many patients are identified only when renal dysfunction is advanced, making risk stratification and detection of potentially modifiable biochemical abnormalities important components of clinical care.[1]

Cardiovascular disease remains the leading cause of mortality among patients with CKD. Traditional risk factors such as age, diabetes, hypertension, dyslipidemia and smoking do not completely explain the excess vascular risk seen in CKD. The uremic milieu is characterized by oxidative stress, endothelial dysfunction, chronic inflammation, vascular calcification, anaemia and accumulation of metabolites that are inadequately cleared as renal function declines. These non-

traditional risk factors interact with conventional cardiovascular risks and may accelerate atherosclerosis and thrombotic complications. Identification of such factors can improve understanding of CKD complications and may provide low-cost targets for monitoring or intervention.

Homocysteine is a sulfur-containing, non-protein amino acid formed during the demethylation of methionine. Under physiological conditions, homocysteine is metabolized through remethylation to methionine or through trans-sulfuration to cysteine. These pathways require folate, vitamin B12 and vitamin B6 as important cofactors. Disturbance of these pathways, nutritional deficiencies, genetic polymorphisms and impaired renal handling may increase circulating homocysteine levels. Plasma concentrations above 15 µmol/L are generally classified as hyperhomocysteinemia, with higher ranges considered moderate, intermediate or severe according to the degree of elevation.[2]

The kidney has a central role in homocysteine metabolism. Declining renal function leads to impaired clearance and altered metabolism of homocysteine and related methylation intermediates. Consequently, plasma homocysteine levels frequently rise in CKD, even before dialysis dependency. Hyperhomocysteinemia has been implicated in endothelial injury, reduced nitric oxide bioavailability, oxidative vascular damage, smooth-muscle proliferation, platelet activation and a prothrombotic state. In patients with CKD, these mechanisms may be particularly important because renal dysfunction itself creates a pro-inflammatory and pro-atherogenic biological environment.

Previous studies have reported that homocysteine levels are inversely related to estimated glomerular filtration rate (eGFR) and are often markedly elevated in advanced CKD and dialysis populations. However, Indian evidence remains relatively limited, and many available reports focus on dialysis patients rather than evaluating homocysteine across graded CKD stages. Nutritional patterns, vitamin status, comorbidity burden and healthcare access differ across populations; therefore, local data are necessary before applying findings from other settings. A stage-wise assessment can clarify whether homocysteine rises progressively with renal dysfunction and whether it is associated with clinical indicators such as dialysis status and cardiovascular events.[3-4]

The present study was undertaken to estimate plasma homocysteine levels among CKD patients attending a tertiary care hospital and to analyse their association with CKD severity. The study also evaluated the relationship of homocysteine with renal biochemical parameters, dialysis status and cardiovascular event history. The central premise was that hyperhomocysteinemia would be more common in advanced CKD and would correlate inversely with eGFR.

## MATERIALS AND METHODS

**Study design and setting:** This was an analytical cross-sectional study conducted in the Department of Medicine of a tertiary care research and teaching hospital. The study was carried out over a two-year period from January 2024 to December 2025.

**Study population:** The study population included adult patients with CKD who were admitted to medicine wards, intensive care units, the dialysis unit or who attended the outpatient department during the study period. Patients were enrolled after confirming eligibility and obtaining written informed consent.

**Sample size and sampling:** The sample size was calculated using the formula for estimation of a single population proportion, n=Z^2p(1-p)/d^2. Based on an anticipated proportion of 17.2%, absolute precision of 7%, 95% confidence level and Z value of 1.96, the minimum sample size was 112. To account for possible non-response and incomplete records, the final sample size was rounded to 120. Consecutive eligible patients were included using convenience sampling.

**Selection criteria:** Patients aged 18 years and above with a diagnosis of CKD were included. Patients unwilling to participate were excluded. CKD was defined as reduced eGFR or evidence of kidney damage persisting for at least three months according to standard diagnostic principles. For analysis in the present study, patients were staged by eGFR into stage 3A, stage 3B, stage 4 and stage 5.

**Data collection:** Data were collected using a pre-designed structured proforma, laboratory reports and hospital records. Demographic details, clinical history, comorbidities, dialysis status, smoking status, alcohol intake and cardiovascular event history were recorded. A detailed clinical examination was performed for each participant. Routine investigations included blood and urine tests, chest radiography and electrocardiography as clinically indicated.

**Laboratory methods:** Blood samples were collected for biochemical analysis. Plasma total L-homocysteine was estimated by chemiluminescent immunoassay using the ADVIA Centaur XP immunoassay system. The laboratory reference range for homocysteine was 5-15 µmol/L, and values >15 µmol/L were considered elevated. Homocysteine levels were categorized as normal (5-15 µmol/L), moderate hyperhomocysteinemia (16-30 µmol/L), intermediate

hyperhomocysteinemia (31-100 µmol/L) and severe hyperhomocysteinemia (>100 µmol/L). Serum creatinine was measured by the kinetic colorimetric Jaffe method. eGFR was calculated using the CKD-EPI equation. Blood urea and blood urea nitrogen (BUN) were recorded from routine biochemical testing.

**Outcome variables:** The primary study variables were plasma homocysteine level and CKD stage. Secondary variables included prevalence of hyperhomocysteinemia, eGFR, serum creatinine, blood urea, BUN, dialysis status and history of cardiovascular events. Severe CKD was defined as stage 4 or stage 5 for quartile-based analysis.

**Statistical analysis:** Data were entered into Microsoft Excel and analysed using STATA version 10.1. Quantitative variables were expressed as mean and standard deviation. Qualitative variables were expressed as frequency and percentage. Differences between groups were evaluated using appropriate tests of significance for categorical variables and comparison of means. Correlation analysis was performed to assess the relationship between plasma homocysteine and renal parameters. A p value <0.05 was considered statistically significant.

**Ethical considerations:** The study was conducted after approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants before enrolment. Confidentiality of patient identity and clinical information was maintained throughout the study, and the study followed the ethical principles of the Declaration of Helsinki.

## RESULTS

A total of 120 patients with CKD were included in the study. The age distribution showed predominance of middle-aged and elderly patients. Patients aged 60-79 years constituted the largest group (41.7%), followed by those aged 40-59 years (38.3%). Patients aged 18-39 years and those aged 80 years or above accounted for 10.0% each. Males constituted 61.7% of the study population and females 38.3%, indicating male predominance among hospital-presenting CKD patients.
Body mass index distribution showed that 43.3% of patients had normal BMI, 26.7% were overweight, 15.0% were obese and 15.0% were underweight. Thus, a substantial proportion had either excess body weight or undernutrition, reflecting the heterogeneous nutritional and metabolic profile of CKD patients. Hypertension was the most common comorbidity, present in 68.3% of patients, followed by anaemia in 63.3% and diabetes mellitus in 51.7%. Dyslipidemia was present in 28.3%, coronary artery disease in 23.3%, and prior stroke or transient ischemic attack in 8.3%. With respect to lifestyle factors, 65.0% were never smokers, 21.7% were current smokers and 13.3% were former smokers. Alcohol use was absent in 71.7%, occasional in 21.7% and regular in 6.6%. Baseline characteristics are summarized in Table 1.

CKD stage distribution demonstrated a high burden of advanced renal dysfunction. Stage 5 CKD was the largest category, seen in 40 patients (33.4%), followed by stage 4 in 34 patients (28.3%), stage 3B in 28 patients (23.3%) and stage 3A in 18 patients (15.0%). Overall, 46 patients (38.3%) were on maintenance hemodialysis and 74 (61.7%) were not on dialysis.

The distribution of CKD stage, dialysis status and homocysteine categories is presented in Table 2.
Normal homocysteine levels were observed in only 34 patients (28.3%). Moderate hyperhomocysteinemia was the most common category, present in 58 patients (48.3%), followed by intermediate hyperhomocysteinemia in 26 patients (21.7%). Severe hyperhomocysteinemia was rare and was observed in 2 patients (1.7%). Overall, elevated homocysteine levels >15 µmol/L were present in 86 patients, giving a hyperhomocysteinemia prevalence of 71.7%.

Mean plasma homocysteine levels increased progressively with advancing CKD stage. Patients with stage 3A CKD had a mean level of 18.6 +/- 6.2 µmol/L, which increased to 23.4 +/- 7.8 µmol/L in stage 3B, 29.8 +/- 10.5 µmol/L in stage 4 and 36.9 +/- 14.1 µmol/L in stage 5. This stepwise increase was statistically significant (p<0.001). Similarly, the proportion of patients with elevated homocysteine increased from 50.0% in stage 3A to 64.3% in stage 3B, 79.4% in stage 4 and 80.0% in stage 5. The association between CKD stage and elevated homocysteine was statistically significant (p=0.018) (Table 3).

Patients with elevated homocysteine had significantly lower mean eGFR than patients with normal homocysteine levels (27.9 +/- 12.3 vs 32.6 +/- 11.8 mL/min/1.73 m²; p=0.041). Serum creatinine was significantly higher in the elevated homocysteine group (3.2 +/- 1.1 vs 2.8 +/- 0.9 mg/dL; p=0.036). Blood urea and BUN were also significantly higher among patients with elevated homocysteine, with p values of 0.048 and 0.043 respectively. Correlation analysis showed a statistically significant moderate negative correlation between homocysteine and eGFR (r=-0.46, p<0.001). Significant positive correlations were observed with serum creatinine (r=0.39, p<0.001), blood urea (r=0.31, p=0.002), BUN (r=0.33, p=0.001) and age (r=0.18, p=0.048). These findings are presented in Table 4.

Hyperhomocysteinemia was more frequent among patients on maintenance hemodialysis than among non-dialysis patients (82.6% vs 64.9%; p=0.032). Cardiovascular events were also more common among patients with elevated homocysteine

compared with those having normal homocysteine levels (30.2% vs 11.8%; p=0.028). In quartile-based analysis, the proportion of patients with severe CKD (stage 4 or 5) increased from 40.0% in the lowest homocysteine quartile to 83.3% in the highest quartile (p<0.001). These results suggest a dose-response pattern between increasing homocysteine concentration and CKD severity (Table 5).

**Table 1. Baseline demographic, anthropometric, comorbidity and lifestyle profile of CKD patients (n=120)**

| Variable | Category | n | % |
|---|---|---|---|
| Age group | 18-39 years | 12 | 10.0 |
| | 40-59 years | 46 | 38.3 |
| | 60-79 years | 50 | 41.7 |
| | >=80 years | 12 | 10.0 |
| Sex | Male | 74 | 61.7 |
| | Female | 46 | 38.3 |
| BMI category | Underweight (<18.5 kg/m²) | 18 | 15.0 |
| | Normal (18.5-24.9 kg/m²) | 52 | 43.3 |
| | Overweight (25-29.9 kg/m²) | 32 | 26.7 |
| | Obese (>=30 kg/m²) | 18 | 15.0 |
| Comorbidity | Hypertension | 82 | 68.3 |
| | Diabetes mellitus | 62 | 51.7 |
| | Anaemia | 76 | 63.3 |
| | Dyslipidemia | 34 | 28.3 |
| | Coronary artery disease | 28 | 23.3 |
| | Prior stroke/TIA | 10 | 8.3 |
| Smoking | Never smoker | 78 | 65.0 |
| | Current smoker | 26 | 21.7 |
| | Former smoker | 16 | 13.3 |
| Alcohol use | None | 86 | 71.7 |
| | Occasional | 26 | 21.7 |
| | Regular | 8 | 6.6 |

**Table 2. Distribution of CKD stage, dialysis status and homocysteine category (n=120)**

| Variable | Category | n | % |
|---|---|---|---|
| CKD stage | Stage 3A (eGFR 45-60) | 18 | 15.0 |
| | Stage 3B (eGFR 30-44) | 28 | 23.3 |
| | Stage 4 (eGFR 15-29) | 34 | 28.3 |
| | Stage 5 (eGFR <15) | 40 | 33.4 |
| Dialysis status | On maintenance hemodialysis | 46 | 38.3 |
| | Not on dialysis | 74 | 61.7 |
| Homocysteine category | Normal (5-15 µmol/L) | 34 | 28.3 |
| | Moderate HHcy (16-30 µmol/L) | 58 | 48.3 |
| | Intermediate HHcy (31-100 µmol/L) | 26 | 21.7 |
| | Severe HHcy (>100 µmol/L) | 2 | 1.7 |
| Homocysteine status | Normal (<=15 µmol/L) | 34 | 28.3 |
| | Elevated (>15 µmol/L) | 86 | 71.7 |

**Table 3. Association of plasma homocysteine with CKD stage**

| CKD stage | n | Mean Hcy (µmol/L) +/- SD | Elevated Hcy n (%) | Normal Hcy n (%) | p value |
|---|---|---|---|---|---|
| Stage 3A | 18 | 18.6 +/- 6.2 | 9 (50.0) | 9 (50.0) | <0.001*; 0.018† |
| Stage 3B | 28 | 23.4 +/- 7.8 | 18 (64.3) | 10 (35.7) | |
| Stage 4 | 34 | 29.8 +/- 10.5 | 27 (79.4) | 7 (20.6) | |
| Stage 5 | 40 | 36.9 +/- 14.1 | 32 (80.0) | 8 (20.0) | |

HHcy: hyperhomocysteinemia; Hcy: homocysteine. *p value for comparison of mean homocysteine across CKD stages; †p value for association of elevated homocysteine with CKD stage.

**Table 4. Renal function parameters and correlation of plasma homocysteine with renal markers**

| Parameter | Normal Hcy (n=34) Mean +/- SD | Elevated Hcy (n=86) Mean +/- SD | p value | Correlation with Hcy (r) | Correlation p value |
|---|---|---|---|---|---|
| eGFR (mL/min/1.73 m²) | 32.6 +/- 11.8 | 27.9 +/- 12.3 | 0.041 | -0.46 | <0.001 |
| Serum creatinine (mg/dL) | 2.8 +/- 0.9 | 3.2 +/- 1.1 | 0.036 | 0.39 | <0.001 |
| Blood urea (mg/dL) | 96 +/- 31 | 108 +/- 36 | 0.048 | 0.31 | 0.002 |
| BUN (mg/dL) | 44 +/- 16 | 51 +/- 19 | 0.043 | 0.33 | 0.001 |
| Age (years) | -- | -- | -- | 0.18 | 0.048 |

**Table 5. Dialysis status, cardiovascular events and homocysteine quartiles in relation to hyperhomocysteinemia and CKD severity**

| Analysis | Category | Finding | p value |
|---|---|---|---|
| Dialysis status and elevated Hcy | On hemodialysis (n=46) | Elevated Hcy: 38 (82.6%); Normal Hcy: 8 (17.4%) | 0.032 |
| | Not on dialysis (n=74) | Elevated Hcy: 48 (64.9%); Normal Hcy: 26 (35.1%) | |
| Cardiovascular event by Hcy status | Normal Hcy (n=34) | Event present: 4 (11.8%); absent: 30 (88.2%) | 0.028 |
| | Elevated Hcy (n=86) | Event present: 26 (30.2%); absent: 60 (69.8%) | |
| Homocysteine quartile and severe CKD | Q1 <=18.0 µmol/L (n=30) | Severe CKD stage 4-5: 12 (40.0%) | <0.001 |
| | Q2 18.1-25.0 µmol/L (n=30) | Severe CKD stage 4-5: 17 (56.7%) | |
| | Q3 25.1-35.0 µmol/L (n=30) | Severe CKD stage 4-5: 20 (66.7%) | |
| | Q4 >35.0 µmol/L (n=30) | Severe CKD stage 4-5: 25 (83.3%) | |

## DISCUSSION

The present analytical cross-sectional study evaluated plasma homocysteine levels among 120 CKD patients and assessed their association with the severity of renal dysfunction. The main findings were that hyperhomocysteinemia was highly prevalent, mean homocysteine levels increased progressively with advancing CKD stage, elevated homocysteine was associated with lower eGFR and higher uremic biochemical markers, and patients with elevated homocysteine had a higher frequency of cardiovascular events. The quartile analysis further demonstrated a graded relationship between increasing homocysteine concentration and severe CKD. Together, these findings support the role of plasma homocysteine as a biochemical marker linked to renal dysfunction severity and cardiovascular risk burden in CKD.

The study population was predominantly middle-aged and elderly, with nearly four-fifths of patients aged 40 years or above. This is consistent with the natural history of CKD, which becomes more common with advancing age due to cumulative nephron loss, greater prevalence of diabetes and hypertension, and longer exposure to vascular and metabolic risk factors. Male predominance was observed in the present cohort. Hospital-based CKD studies from India often show a similar male preponderance, which may reflect true disease burden, health-seeking behaviour, socioeconomic factors and referral patterns rather than a purely biological difference. The age and sex profile therefore appears compatible with a tertiary-care CKD population.

Hypertension and diabetes mellitus were the major comorbidities in this study. Hypertension was present in more than two-thirds of patients and diabetes in more than half. These two conditions are among the most important drivers of CKD progression worldwide and are especially relevant in India, where metabolic diseases are increasing at a relatively younger age. Anaemia was also common, reflecting reduced erythropoietin production, inflammation, nutritional deficiencies and advanced renal dysfunction. The high frequency of dyslipidemia and coronary artery disease further highlights that CKD patients represent a metabolically and vascularly vulnerable group. This background is important when interpreting homocysteine because it may act in combination with these traditional risk factors rather than in isolation.

A substantial proportion of the cohort had advanced CKD: 28.3% had stage 4 and 33.4% had stage 5 disease. In addition, 38.3% were on maintenance hemodialysis. This distribution suggests late presentation to tertiary care and a high referral burden of advanced renal disease. Late detection remains a major challenge in many Indian settings because early CKD is frequently asymptomatic and screening among high-risk groups is inconsistent. The high prevalence of advanced CKD in the present study created an appropriate clinical context for studying homocysteine across a wide gradient of renal

dysfunction, although it also means that results may not be fully representative of community-based early CKD populations.

Hyperhomocysteinemia was observed in 71.7% of patients. Moderate elevation was the most frequent category, while severe hyperhomocysteinemia was uncommon. This pattern is clinically plausible because CKD usually produces persistent moderate to intermediate elevation through impaired clearance and altered metabolism, whereas very high concentrations are more typical of inborn errors or severe vitamin-related defects. Menon et al. reported elevated homocysteine levels in CKD populations and emphasized that renal dysfunction strongly influences circulating homocysteine concentrations [1]. Bostom and Lathrop also described hyperhomocysteinemia as highly prevalent in end-stage renal disease and discussed its possible relationship with arteriosclerotic outcomes [2]. The prevalence observed in the present study lies within the range expected for a cohort enriched with advanced CKD and dialysis patients.

The most important finding was the statistically significant stepwise rise in mean homocysteine from stage 3A to stage 5 CKD. Mean values increased from 18.6 µmol/L in stage 3A to 36.9 µmol/L in stage 5. This increase is consistent with the biological role of the kidney in homocysteine metabolism. Friedman et al. reviewed the kidney-homocysteine relationship and described the kidney as an important organ in homocysteine handling [3]. Garibotto et al. further highlighted altered metabolism and impaired disposal of methylation intermediates in CKD [4,5]. As nephron function declines, both filtration-dependent removal and extrarenal metabolic regulation may be affected, resulting in progressive accumulation. Therefore, the stage-wise gradient observed in the present study is mechanistically credible.

The proportion of patients with elevated homocysteine also rose with CKD stage, from 50.0% in stage 3A to 80.0% in stage 5. Postovitenko et al. similarly found that hyperhomocysteinemia was common in CKD and was related to cardiovascular functional changes [6]. Patel and Bidri, in an Indian study comparing CKD patients with and without dialysis, reported higher homocysteine levels in CKD and particularly among dialysis patients [7]. Singh et al. also observed that serum homocysteine levels were associated with renal function among CKD patients [8]. The present data add to these reports by showing both a mean-level gradient and a categorical increase in hyperhomocysteinemia across CKD stages.

Patients with elevated homocysteine had worse renal function parameters than those with normal homocysteine. eGFR was significantly lower, whereas serum creatinine, blood urea and BUN were significantly higher. Correlation analysis demonstrated a moderate negative correlation between homocysteine and eGFR and positive correlations with creatinine and uremic markers. These findings support the interpretation that homocysteine behaves as a biochemical marker of renal impairment severity. Levi et al. reported that elevated serum homocysteine predicted accelerated decline in renal function and incident CKD in a historical prospective study [9]. Although the cross-sectional design of the present study does not establish temporal causality, the consistent association with multiple renal markers strengthens the inference that homocysteine accumulation parallels declining renal function.

The correlation coefficient between homocysteine and eGFR was -0.46, indicating a moderate inverse relationship rather than a weak association. This is clinically relevant because eGFR is the principal staging marker for CKD. A moderate correlation suggests that homocysteine is not merely a nonspecific abnormality but is meaningfully related to renal filtration status. At the same time, the association is not perfect, implying that other factors such as age, nutritional status, folate and vitamin B12 levels, inflammation, dialysis adequacy, genetic polymorphisms and medication use may also influence homocysteine levels. This multifactorial regulation is important for clinical interpretation; homocysteine should complement, not replace, standard renal function assessment.

Hyperhomocysteinemia was significantly more common among patients on maintenance hemodialysis. Dialysis-dependent patients represent a group with severe renal dysfunction, altered metabolism, dietary restrictions, inflammation and potential deficiency of water-soluble vitamins. Standard hemodialysis may lower circulating homocysteine transiently but generally does not normalize levels because homocysteine is partly protein-bound and because the underlying metabolic disturbance persists. Robinson et al. demonstrated that hyperhomocysteinemia in end-stage renal disease was linked to folate and pyridoxine concentrations and conferred increased atherosclerotic risk [10]. The present finding that 82.6% of dialysis patients had elevated homocysteine is therefore consistent with the wider ESRD literature.

The association between elevated homocysteine and cardiovascular event history is clinically important. Cardiovascular events were present in 30.2% of patients with elevated homocysteine compared with 11.8% among those with normal levels. CKD patients already have high cardiovascular risk because of hypertension, diabetes, dyslipidemia, anaemia, mineral-bone disorder and inflammation. Homocysteine may add to this risk by promoting endothelial dysfunction, oxidative stress, vascular smooth-muscle proliferation and thrombosis. Nappo et al. demonstrated impairment of endothelial function during acute hyperhomocysteinemia and reversal with antioxidant vitamins [11]. Tyagi et al. and Steed and Tyagi described oxidative stress and cardiovascular remodeling mechanisms in hyperhomocysteinemia [12,13]. These mechanisms provide biological plausibility for the observed association.

Prior clinical studies have also linked homocysteine with vascular outcomes in CKD and ESRD. Robinson et al. reported an independent increased risk of atherosclerosis among ESRD patients with hyperhomocysteinemia [10]. Elias et al. found interactions between homocysteine and arterial stiffness in individuals with CKD, suggesting that homocysteine may contribute to vascular ageing and reduced arterial compliance [14]. Muntner et al. identified nontraditional risk factors, including homocysteine, as common among patients with CKD [15]. The current study cannot determine whether homocysteine caused cardiovascular events; however, its association with event history supports its value as a risk marker in a high-risk clinical population.

The quartile analysis further strengthens the study findings. Severe CKD increased progressively across homocysteine quartiles, from 40.0% in the lowest quartile to 83.3% in the highest quartile. A dose-response relationship is an important epidemiological feature because it reduces the likelihood that the association is purely incidental. Similar graded associations have been described in studies evaluating homocysteine and renal function decline. Ninomiya et al. reported that kidney disease and blood pressure contribute substantially to cardiovascular disease risk in Asian populations [16], and other cohorts have suggested that higher homocysteine predicts renal function deterioration [9]. The present quartile-based finding is therefore aligned with both renal and vascular risk literature.

The clinical implications of the study are several. First, homocysteine estimation may help identify CKD patients with advanced renal dysfunction and higher vascular risk. Second, because homocysteine metabolism depends on folate and B-vitamins, abnormal levels may point to nutritional issues that are potentially correctable. Third, documenting the high prevalence of hyperhomocysteinemia in Indian CKD patients supports the need for larger studies assessing vitamin status, dietary patterns, dialysis adequacy and cardiovascular outcomes. However, the use of homocysteine as a therapeutic target remains complex. Some trials of vitamin supplementation have reduced homocysteine concentrations without consistently reducing cardiovascular events. Therefore, clinical management should be individualized and should not assume that biochemical lowering automatically translates into outcome benefit.[17-21]

From a pathophysiological standpoint, homocysteine may be both a marker and a mediator. It reflects reduced renal clearance and altered metabolism, but it may also contribute directly to vascular and renal injury. Experimental studies have shown that homocysteine can induce mesangial cell apoptosis, glomerular injury, podocyte damage, inflammatory signalling and oxidative stress [22-25]. These mechanisms suggest that elevated homocysteine could potentially worsen renal microvascular injury. Nonetheless, human observational studies cannot fully separate cause from consequence. In the present study, the cross-sectional design demonstrates association, not causation. Longitudinal studies are required to determine whether baseline homocysteine predicts CKD progression or cardiovascular events independently of eGFR and comorbidities.

This study has several strengths. It included a clinically relevant CKD population from a tertiary-care setting, used a standardized assay for plasma homocysteine, calculated eGFR using CKD-EPI, and analysed homocysteine both as a continuous and categorical variable. It also evaluated dialysis status, cardiovascular event history and quartile-based severity trends, allowing a multidimensional assessment of the relationship between homocysteine and CKD. The use of 5 concise tables allows the key findings to be presented in a journal-appropriate format without excessive fragmentation of results.

The limitations should also be acknowledged. The study was conducted at a single tertiary-care hospital and used convenience-based consecutive sampling, which may limit generalizability. The cross-sectional design prevents inference about temporal direction or causality. Folate, vitamin B12, vitamin B6, inflammatory markers, albumin, dietary intake, dialysis adequacy and genetic polymorphisms were not incorporated into the present analysis, although they may influence homocysteine levels. Cardiovascular events were assessed as history rather than prospectively adjudicated incident outcomes. Finally, the cohort had a high proportion of advanced CKD, so findings may not fully represent early-stage CKD in the community. Despite these limitations, the study provides useful Indian tertiary-care data on the association between homocysteine and CKD severity.

## CONCLUSION

Hyperhomocysteinemia was highly prevalent among patients with CKD in this tertiary-care cohort. Plasma homocysteine levels increased significantly and progressively with advancing CKD stage, and elevated homocysteine was associated with lower eGFR, higher serum creatinine, higher blood urea and higher BUN. A moderate inverse correlation between homocysteine and eGFR confirms that homocysteine levels rise as renal filtration declines.

Elevated homocysteine was more frequent among patients on maintenance hemodialysis and was associated with a higher prevalence of cardiovascular events. The proportion of severe CKD increased across rising homocysteine quartiles, demonstrating a dose-response association. Plasma homocysteine may therefore serve as a useful biochemical marker of

renal dysfunction severity and cardiovascular risk in CKD. Larger longitudinal studies incorporating vitamin status, nutritional assessment and prospective cardiovascular outcomes are needed to clarify whether homocysteine is only a marker or also a modifiable mediator of adverse outcomes.

## DECLARATIONS

**Ethics approval and consent to participate:** The study was conducted after Institutional Ethics Committee approval. Written informed consent was obtained from all participants.

**Confidentiality:** Patient identity and clinical records were kept confidential.

**Funding:** No external funding was reported.

**Conflicts of interest:** The authors declare no conflicts of interest.

**Data availability:** Data may be made available by the corresponding author on reasonable request, subject to institutional permission.

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