An Insight to Hyperhomocysteinaemia in CKD Patients of a Tertiary Care Hospital, Karachi

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ABSTRACT

Objective: To evaluate the frequency and association of hyperhomocysteinaemia (HHcy) in different stages of chronic kidney disease (CKD) patients.

Study Design: Cross-sectional, descriptive study.

Place and Duration of the Study: Department of Chemical Pathology, Indus Hospital and Health Network Karachi, Pakistan, from July to December 2022.

Methodology: Blood samples of 100 known CKD patients were collected for this study. The estimated glomerular filtration rate (eGFR) was calculated from the CKD-EPI calculator for CKD staging. Plasma homocysteine (Hcy) and serum creatinine concentrations were analysed on Abbott Alinity I and C analyzers, respectively. The SPSS was used for data compilation and analysis. Descriptive statistics were calculated. The association of HHcy with CKD and other variables was assessed using the Chi-square test as appropriate. A p-value of ≤0.05 was considered as significant.

Results: Out of total 100, 52% males and 48% females known CKD patients were included in the study. The mean age of the patients was 50.62 ± 16.29 years. The median eGFR, serum creatinine, and Homocysteine were 18 ml/min/1.73m^2, 3.48mg/dl, and 20.07 µmol/l, respectively. The percentage of CKD patients in each stage was 7% in stage 3a, 18% in stage 3b, 30% in stage 4, and 45% in stage 5. HHcy was observed in 79% of the CKD patients and among them 7.6% patients were in stage 3a, 19.0% in stage 3b, 31.6% in stage 4, and 41.8 % in stage 5.

Conclusion: Patients with CKD were found to have HHcy indicating a very high level of prevalence in CKD patients, especially in the late stages. Hence, Hcy can be considered as a predictor of advancing disease. Timely interventions are required to prevent future adverse outcomes and improve the quality of life in CKD patients. However, a significant association was not seen between Hcy concentration and eGFR stages in the current study.

Key Words: *Hyperhomocysteinaemia, Estimated glomerular filtration rate, CKD-EPI calculator, Mortality, End-stage renal disease, Cardiovascular disease*.

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INTRODUCTION

Chronic kidney disease (CKD), a non-communicable disease known for its significant role in progressively increasing mortalities and morbidities, is considered an alarming and a serious health issue worldwide.[1] It is described as the presence of structural or functional abnormalities in kidneys for more than three months and any one of the two criteria: Either GFR less than 60 ml/min/1.73 square metres or the presence of markers of kidney damage, including albuminuria.[2] Bikbov *et al*. stated an estimate of 9.1% prevalence of CKD worldwide in 2017, with the highest number of CKD patients reported in China and India.

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A recent estimate shows that 434 million people have CKD across Asia.[3] In South Asia, the prevalence of CKD increases to one to four out of every ten people, and in Pakistan, this prevalence peaks at 21.2% with more than 10 million cases reported.[4] It is estimated that from 12.5 to 31.2% of Pakistanis suffer from this disease.[5] It has now been recognised as one of the established causes of mortality and morbidity in the 21st century. CKD has been associated with a number of adverse outcomes such as cardiovascular disease, hyperhomocysteinaemia (HHcy), uraemia, acidosis, bone disease, anaemia, and hyperparathyroidism.[6]

Hcy is a sulphur-enriched, non-essential amino acid which is obtained from the transmethylation of an amino acid, methionine. The primary function of Hcy is to act as a biochemical junction between Met metabolism and cysteine production, both of which play vital functions in the body.[7] It is a pro-inflammatory and prothrombotic agent, acting on impairment of vasodilation. In circulation, Hcy is 80-90% bound to protein, 10-20% exists as cysteine or disulphide (Hcy dimer) forms, and less than 1% occurs in the free state.[8] Hcy cannot be obtained

through diet in humans. Rather, Hcy is biosynthesised from methionine (Met) in a series of processes. Hcy is converted to cysteine *via* the trans-sulfuration pathway, in which Hcy combines with serine through cystathionine-beta-synthase (CBS) catalysed reaction, with the formation of cystathionine, which is converted by cystathionine-gamma-lyase (CTH) or by re-methylation pathway into cysteine and alpha-ketobutyrate or may resynthesise to methionine.[9] A key regulatory enzyme in the remethylation pathway, methylene tetrahydrofolate reductase (MTHFR), remethylates and converts Hcy into methionine in the folate-dependent Hcy pathway.[8] This process involves methionine synthase (MS) recycling Hcy to methionine, which links Hcy metabolism and folate cycle together. This process involves methionine synthase (MS) recycling Hcy to methionine, which connects Hcy metabolism to the folate cycle. For this process, methyl donors, cobalamin (vitamin B12), 5-methyltetrahydrofolate (active folate), folate (vitamin-B9), and the enzyme MTHFR, are required.[10]

In healthy humans, the normal total concentration of Hcy in plasma is 5.0 to 15.0 µmol/l.[11] When the concentration of homocysteine increases above 15 µmol/L in the blood, it is called Hyperhomocysteinaemia (HHcy).[3] It is not only recognised as a consequence of CKD but is also suggested as a risk factor of CKD.[12] The possible mechanisms in metabolism pathways that contribute to HHcy are compromised Met metabolism, genetic errors in the enzymes, or cofactors such as vitamins-B6, B12, and folate deficiency.[13] The risk factors of HHcy include ageing, diet, smoking, oxidative stress,[11] CKD, anaemia, malignancy, and thyroid disease.[10]

Globally, extensive research has been done on HHcy in CKD patients, but Pakistan still falls behind in its diagnosis and management of CKD patients.

The current study aimed to get an insight into the prevalence and association of HHcy in CKD patients of various stages and can safely advocate that Hcy testing should be incorporated on a regular basis in CKD patients to enhance their well-being and reduce future adverse outcomes.

METHODOLOGY

This cross-sectional study was conducted from July to December 2022 at the Department of Chemical Pathology, Indus Hospital and Health Network, Karachi, Pakistan. A total of 100 patients were incorporated into the study. The sample size was calculated on the OpenEpi sample size calculator using a prevalence of 21.2%[4] in accordance with the inclusion criteria. The patients recruited in this study were all known CKD patients aged 18-75 years, who had either attended OPD or were admitted to the Indus hospital whereas, pregnant women, patients with a known history of medicine abuse, addictions, smoking, use of corticosteroids, or hormones during the last six months were excluded from this study.

Ethical approval from the Ethical Committee of the Hospital (IHHN_IRB#_2022_06_004) was taken. Selected patients were informed about the study purpose and procedure of the test. Structured data were used for data collection, and written consent was taken and signed by each patient.

Blood samples were collected in a gel-top vacutainer for serum creatinine and in an EDTA vacutainer for plasma Hcy analysis. Plasma Hcy and serum creatinine were analysed on Abbott Alinity I (Chemiluminescence microparticle immunoassay) and Alinity C (Spectrophotometric technique) analysers, respectively, with valid quality control results. The estimated glomerular filtration rate (eGFR) was calculated from the CKD-EPI calculator. Results of Hcy concentration greater than 15 µmol/L in the blood were labelled as HHcy.

Non-probability consecutive sampling was done. SPSS was used for data compilation and analysis. Data were organised and entered on the SPSS version 22. Mean and standard deviation were calculated for height, weight, and body mass index (BMI). Median and IQR were calculated for quantitative variables such as age, eGFR, Hcy concentration, creatinine concentration, and blood pressure on the basis of the normality test. The normality test was checked by the Shapiro-Wilk's test. Frequency and percentage of categorical variables such as gender were reported. The association and correlation of HHcy with CKD and other comorbidities, including diabetes mellitus, hypertension, and cardiovascular disease, were assessed with the Chi-square test as applicable. Other effect modifiers such as age, gender, weight, and BMI were controlled through stratification. A p-value of ≤0.05 was regarded as statistically significant.

RESULTS

A total of 100 patients with CKD of both gender, age between 18 and 75 years, were included and evaluated to determine the frequency of HHcy. Among these CKD patients, 52% were males and 48% were females. Fifty-one percent of study subjects were in-patients (admitted) and 49% were from outpatient departments. The overall mean duration of CKD in the study subjects was 43.87 ± 29.48 months, the median eGFR was 18 ml/min/1.73m^2, and the median serum creatinine and Hcy were 3.48 mg/dl and 20.07µmol/l, respectively (Table I). The overall mean of height, weight, and body mass index was 1.57 ± 0.09 m, 61.03 ± 15.54 kg, and 24.43 ± 5.70 kg/m^2, respectively. The total percentage of patients in each stage of CKD comprises 7% of patients in stage 3a, 18% of patients in stage 3b, 30% of patients in stage 4, and 45% of patients in stage 5.

There was a high frequency of HHcy in the male gender (n = 43) along with an increased frequency of cardiovascular disease (Figure 1).

Seventy-nine percent of CKD patients had HHcy, and it was observed that among these CKD patients with HHcy, 7.6% (n = 6) were of stage 3a, 19.0% (n = 15) patients had stage 3b, 31.6% (n = 25) patients had stage 4, and 41.8% (n = 33) patients had stage 5 CKD (Table II).

Table I: Descriptive statistics of age, systolic and diastolic blood pressure, chronic kidney disease duration, eGFR, serum creatinine, and homocysteine (n = 100).

Variables	Age (years)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Chronic kidney disease duration (months)	eGFR (ml/min/m2)	Serum creatinine (mg/dl)	Plasma homocysteine (µmol/l)
Median	55.50	145	90	36	18	3.48	20.070
Interquartile range	27	24	15	36	21.3	3.8	12.4
Range	57	90	50	116	54	15.2	43.7
Minimum	18	100	60	4	3	1.3	6.3
Maximum	75	190	110	120	57	16.5	50

Table II: Frequency and association of hyperhomocysteinaemia according to eGFR stages (n = 100).

		Hyperhomocysteinaemia			p-value
		Yes	No	Total patients	
eGFR stages	Stage 3a mildly decreased	6 (7.6%)	1	7	0.806
	Stage 3b moderately decreased	15 (19%)	3	18	
	Stage 4 severely decreased	25 (31.6%)	5	30	
	Stage 5 kidney failure	33 (41.8%)	12	45	

Chi-square test was applied. p-value of <0.05 was considered significant.

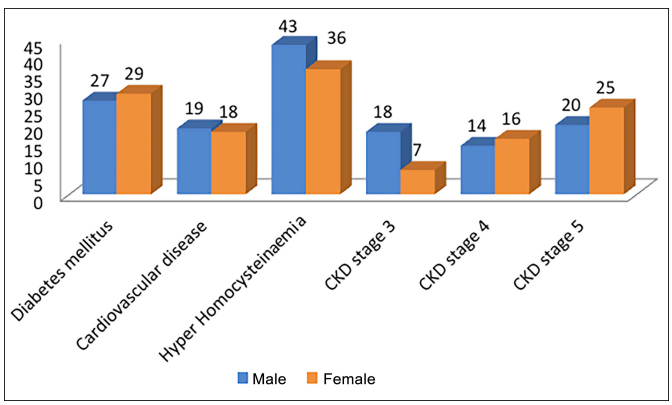

Figure 1: Gender-wise distribution of variables.

There was no significant association of HHcy with gender (p = 0.345), age group (p = 0.141), weight (p = 0.596), BMI (p = 0.526), in/out-patients (p = 0.068), hypertension (p = 0.236), diabetes mellitus (p = 0.540), dialysis (p = 0.230), CVD (p = 0.257), medication use (p >0.99), and eGFR stage (p = 0.806). It was observed that 89% of patients had hypertension (mean duration = 7.17 ± 4.88 years) and 56% had diabetes (mean duration = 8.53 ± 6.40 years). Out of all study patients, 32% were on dialysis due to CKD, while 37% of patients were found with cardiovascular disease. Eighty-two percent were on medications for their underlying illnesses.

DISCUSSION

Hyperhomocysteinaemia, a metabolic condition which features an increase in Hcy concentration in the blood, is now recognised as a potential risk factor for many diseases, including stroke and CVD[14] and also one of the lethal consequences of CKD.[12] In Hcy metabolism, kidneys have an important function to perform, and any decline in renal Hcy clearance is one of the causes of HHcy in CKD patients, leading to the development of end-stage renal disease (ESRD). Mild-to-moderate HHcy has been seen in about 85% of dialysis patients.[15] HHcy exerts oxidative damage and decreases the vasodilation effect of nitric oxide, contributing to the loss of endothelial function and contributing to various cytokines production involved in the inflammatory pathway.[16] It also plays a part in the reduction of vascular tone through increased activity of metalloproteinases and collagen synthesis and also stimulates atherosclerosis by promoting the smooth-muscle cells proliferation and formation of oxidised LDL by macrophages.[17]

The current study attempted to ascertain the prevalence of HHcy in various stages of CKD patients of the tertiary care hospital and can emphasise the early recognition and treatment of this condition to prevent future mortalities. The prevalence of HHcy amongst CKD patients in the present study was 79%, which is similar to the studies done by Badri *et al*. and Cianciolo *et al*., which demonstrated a high prevalence of HHcy of 89-100%,[18] and 85%,[19] respectively, among CKD patients. This observation has similar concordance and agreement to Long and Nie *et al*. who stated the 85-100% prevalence of HHcy and Hcy levels were 3 to 5 times higher than normal in patients with ESRD.[20] Chen *et al*. reported a high prevalence of HHcy in increasing CKD stages and haemodialysis patients had a 2-fold higher prevalence.[21]

The present results also highlighted that the frequency of HHcy increased with the advancing disease i.e., the frequency of HHcy in stage 3a was 7.6%, stage 3b was 19%, stage 4 was 31.6%, and stage 5 was 41.8%, serving as a good predictor as CKD advances. This result shows conformity with the study of Ye *et al*. who also stated the same in the study conducted on 1,042 patients with CKD, where the prevalence of HHcy increased as the CKD progressed. Their reported frequency of HHcy was 10.73%, 29.22%, 58.71%, 75.23%, and 83.75% in CKD stage 1, stage 2, stage 3, stage 4, and stage 5 patients, respectively. With further compromised renal function, its pooled frequency of HHcy reached higher from 10.73% in stage-1 to 83.75% in stage-5 of patients with CKD. This analysis showed that since eGFR was used to determine plasma Hcy, the decline in kidney func-

tion has an immense contribution in an adverse increase of Hcy concentrations in the blood.[6]

In another study, Postovitenko *et al*. suggested that adverse structural and functional changes in the heart and blood vessels are associated with an increased concentration of serum homocysteine levels. In particular, increased homocysteine levels were associated with lower ejection fraction indices in CKD patients with HHcy, which is closely associated with diseases of the cardiovascular system (endothelial dysfunction, structural and functional myocardium remodelling) and may be an important risk factor for the development of vascular lesions.[22] The frequency of HHcy in patients with ESRD spans from 85 to 100% globally.[18]

It has been shown to be extremely common among South Asians, particularly among seemingly healthy Pakistanis. Lack of folate and vitamin B6 are considered important factors in contributing to the increasing prevalence of HHcy. Generally, Pakistanis lack fresh fruits and vegetables in their diet; high prevalence of parasitic gastrointestinal diseases (particularly amoebiasis and giardiasis) in Pakistan contributing to malabsorption also promotes the increasing frequency of HHcy.[23] There is considerable evidence to suggest that the increasing rate of CKD, CVD, and other pathological conditions, along with dietary insufficiency of low-blood folate, vitamin B-12, and vitamin B-6 levels, are responsible for high-plasma Hcy levels in the Pakistani population.[24] A study on the Hcy level in young Pakistani patients with cardiovascular disease showed a strong association between the two, suggesting that in these patients, Hcy is regarded as an independent risk factor which can be used as a marker to predict the future risk of CVD onset.[25] Since it has a pro-inflammatory and thrombotic nature, especially in Asian populations with inadequate folate intake, nephrologists are seen as more interested in Hcy metabolism, its pathogenicity, and its role in potentiating the risk of cardiac events in CKD,[12] has drawn more attention in the area of research. The CKD patients with comorbidities such as CVD, hypertension, diabetes mellitus, and dialysis in the current study were not on any folic acid, or vitamin supplements. Lamuno *et al*. reported the beneficial effect of vitamin B6, folic acid, and vitamin- B12 on patients with cardiovascular disease, as it limits the atherosclerosis process.[14] Thus, highlighting the need for initiation of these treatments alongside their prescribed treatment of their underlying illness in CKD patients.

Although Hcy concentrations were seen to increase with increasing stages of CKD, significant association was not found between Hcy concentration and eGFR stages in the patients of the present research study, which could possibly be due to nutritional deficiencies, genetic variations or underlying illnesses masking the association between the Hcy and eGFR. This emphasises a need for extensive work to be done on its relationship and confounders affecting it.

This study strengthens the importance of early recognition and implication of Hcy testing in CKD patients and timely management to prevent future increases in mortalities due to this pathogenic factor. As there is no significant amount of work done in Pakistan on HHcy in CKD patients and the application of its testing is currently limited in many centres of Pakistan, this study stands as a good contribution in highlighting the increasing prevalence of HHcy in CKD patients of various stages and understanding the need to incorporate Hcy assay testing in CKD patients so that this disease can be curbed with timely prompt diagnosis and management.

However, the shortcoming of this research was the small sample size, and therefore, these findings might not be generalisable to larger populations. This can be improved upon by a larger sample size and multicentre research on this topic.

CONCLUSION

The current study results corroborate a high prevalence of seventy-nine percent (79%) of HHcy among various stages of CKD patients. This prevalence has increased with progressive rise in CKD stages, with 7.6% in stage 3a, followed by 19, 31.6%, and 41.8% in stage 3b, 4, and 5 CKD, respectively. However, this study did not find significant association between eGFR and HHcy. The regular inclusion of Hcy assay testing in CKD patients may effectively help in reducing the adverse outcomes associated with it and improving better quality of life of the patients.

ETHICAL APPROVAL:

Ethical approval was taken from the Ethical Review Board of the Indus Hospital and Health Network, Karachi, Pakistan (No: IHHN_IRB_2022_06_004).

PATIENTS' CONSENT:

Informed consent was obtained from the patients.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

NY: Designing, acquiring, data collection, analysis, and drafting of the manuscript.
AMZ: Conceptualising, data analysing, interpreting, critically reviewing, and final approval of the manuscript.
FK, MZ: Results and reviewing of the manuscript.
AI: Manuscript reviewing and data collecting.
All authors approved the final version of the manuscript to be published.

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