RELATIONSHIP AMONG SERUM HOMOCYSTEINE, SERUM FOLATE AND LEFT VENTRICULAR EJECTION FRACTION IN CHRONIC KIDNEY DISEASE

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Abstract

Background: Homocysteine (Hcy) a sulfur-containing, non-protein α-amino is now considered an independent non-traditional risk factor for vascular disease. In Chronic Kidney Disease (CKD) highly prevalent hyperhomocysteinemia which is often associated with decreased folate concentrations is thought to contribute to increased cardiovascular morbidity. So, the aim of this cross-sectional study was to investigate the relations among serum homocysteine, serum folate and Left Ventricular Ejection Fraction (LVEF) in the context of native CKD patients. **Materials and methods:** A total of one-thirty (130) participants were recruited between June and July 2016. Among them ninety (90) were CKD patients of stage III, IV & V and forty (40) were healthy controls.eGFR was calculated by MDRD formula. Different groups were created based on serum homocysteine level and eGFR. **Results:** In this study, hyperhomocysteinemia was seen in 97% CKD patients compared to 52.50% in controls. The CKD patients with elevated homocysteine levels had higher serum creatinine, LDL and systolic BP but they had lower eGFR, serum folateand LVEF than those with normal homocysteine concentrations. As the severity of homocysteinemia increased, eGFR and serum folate decreased. LVEFalso reduced significantly with increasing severity of hyperhomocysteinemia i.e. from 68% in those withouthyperhomocysteinemia to 41.33% in those with severe hyperhomocysteinemia. Chi-squared tests and odds ratios proved significant associations of hyperhomocysteinemia and folate deficiency with CKD. Higher homocysteine concentrations were also associated with low folate and low LVEF. Here, serum homocysteine correlated negatively with eGFR, serum folate and LVEF and positively with LDL and systolic BP. **Conclusion:** Inconclusion, this study revealed significant associationsamong hyperhomocysteinemia, low serum folate and low LVEF in the native CKD patients. The consistency of homocysteine-eGFR and homocysteine-LVEF correlations suggests that serum homocysteine may provide useful additional information about CKD and associated high cardiovascular morbidity.

Key words
Homocysteine; Folate; Chronic kidney disease; Left ventricular ejection fraction; Hyperhomocysteinemia.

Introduction

Homocysteine (Hcy) a sulfur-containing non-protein -amino acid is recently getting increasing attention because of its role in vascular pathology, especially cardiovascular disease. In the body Hcy is synthesized from methionine by methylation reaction. Hcy is converted back to methionine through folate and vitamin B_{12} dependent remethylation pathway or converted to cysteine through vitamin B_6 requiring transsulfuration pathway. So, deficiency of any of these vitamins, a common finding in CKD, can raise plasma Hcy concentration. Excess homocysteine in circulation is cleared by the kidney and liver. Renal pathways of homocysteine handling largely depend on filtration, reabsorption and metabolism (remethylation and transsulfuration) abilities of kidney[1]. But in CKD reduced glomerular filtration along with impaired homocysteine metabolism lead toplasma homocysteine accumulation[2].

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Hyperhomocysteinemia (HHcy) generally defined as aserum homocysteine level ≥ 15 µmol/L, is increasingly being viewed as anindependent risk factor for cardiovascular disease[3,4-6]. Study has found that each increment of plasma homocysteine level by 5 µmol/L increases the risk of Coronary Heart Disease (CHD) events by approximately 20%[7]. A 25% lower homocysteine level was associated with an 11% lower risk of coronary artery disease and a 19% lower risk of stroke[8]. Approximately 40% patients with cardiovascular disease are reported to have hyperhomocysteinemia[9]. Numerousretrospective and prospective studies revealed that elevated homocysteine levels are associated with atherosclerosis, Cardiovascular Disease (CVD) cerebrovascular complications, venous thromboembolism and hypertension[10-13]. This phenomenon may be even more important in CKD patients because cardiovascular disease is the leading cause of death amongst these patients[14]. The conventional cardiovascular risk factors such as smoking, hypertension, glucose intolerance / diabetes, and dyslipidemia despite their widespread prevalence, are relatively limited predictors of CVD-specific morbidity and mortality in the CKD patients[15]. So, there has been increasing emphasis on the role of homocysteine and other nontraditional risk factors. Prospective study of dialysis patients has shown that the risk of vascular disease rises 1% for each 1 µmol/L increase in total homocysteine concentration[16]. The cerebral and cardio-vascular complications of the vascular disease, and, in some studies, thrombosis of the vascular access, a common and costly complication in ESRD, correlated with high homocysteine levels[16-18]. Several studies have also found an inverse association between plasma homocysteine level and Left Ventricular Ejection Fraction (LVEF) an established marker of cardiovascular morbidity[19, 20]. For these reasons, serumhomocysteine has been suggested asa prognostic marker as well as an important risk factor for cardiovascular morbidity and mortality in patients with CKD. Yet, local studies in this area are scanty to date. So,this study was undertaken to evaluate the relations among serum homocysteine, serum folate and left ventricular ejection fraction in the context of native CKD patients.

Materials and methods

This cross-sectional comparative study was carried out inDepartment of Biochemistry andDepartment of Nephrology of Chittagong Medical College Hospital (CMCH) between June and July 2016.Permission for the study was taken from concerned departments as well as from ethical review committee. Adults aged between 18 to 60 yearsfulfilling the undermentioned enrollment criteria were included by nonprobability consecutive sampling. Considering the cost, duration and nature of study, the sample size was limited to 130.

Inclusion criteria for CKD patients: CKD patients (Stages III, IV & V) aged from 18 to 60 years attending the Department of Nephrology, CMCH. CKD was defined as either kidney damage or GFR < 60 mL/min/1.73 m^2 for three or more months.

Exclusion criteria for CKD patients: Patients with acute kidney injury, obstructive uropathy, end-stage liver disease, clinical cardiovascular disease, cancer, pituitary, thyroid or adrenal function abnormalities, fever and any acute pathology, patients on dialysis, patients with malabsorption disorders, those on particular diet (eg. vegetarian) on vitamin B_3, B_6, B_{12} or folic acid supplementation, on drugs that may affect serum folate level, pregnant patients,malnourished patients.

Inclusion criteria for controls: Healthy individuals from community aged from 18 to 60 years with an eGFR 60 ≥ mL/min/1.73 m^2 and without proteinuria (Dip-stick test negative).

Exclusion criteria for controls: Those on particular diet (eg. vegetarian), on vitamin B_3, B_6, B_{12} or folic acid supplementation, pregnant women.

eGFR was calculated by MDRD formula[21]. Fasting serum total homocysteine and fasting serum folate were estimated on The ADVIA Centaur system which employsa competitive immunoassaywith direct, chemiluminescent technology. Serum creatinine was measured using CRE2 method which uses a modified kinetic Jeffe technique on a Siemens Dimension clinical chemistry system. Fasting serum LDL was determined by ALDL method on same machine. Proteinuria was determined by simple dipstick test.

All the data were processed and analyzed using computer-based statistical software. p value < 0.05 was considered to be statistically significant. Quantitative data were expressed as mean ± SD, and to measure the significance of their difference

t-test or ANOVA were used. Qualitative data were expressed in frequency and percentage, and to estimate the significance of association chi-squared tests or odds ratios were used.To test the correlation amongst different parameters, Pearson correlation coefficient was used.

Results

Table I : Characteristics of controls and stage III, IV, V CKD patients (n = 130)

Characteristics	Controls	CKD patients		
		Stage III	Stage IV	Stage V
Age, years	42.25± 13.07	38.82 ± 16.41	45.18 ± 14.71	44.71 ± 11.9
Male sex, %	57.5	58.82	54.55	50.98
Hypertensive, %	NA	41.18	59.09	94.12
Diabetic, %	NA	29.41	40.91	41.18
LDL 100 mg/dL, %	62.50	70.59	90.91	96.08
Hyperhomocysteinemic, %	52.50	82.35	100	100
Low folate, %	10	17.65	36.36	76.47
Proteinuric, %	NA	29.41	77.27	95.74
Low LVEF, %	Not done	0	9.09	48

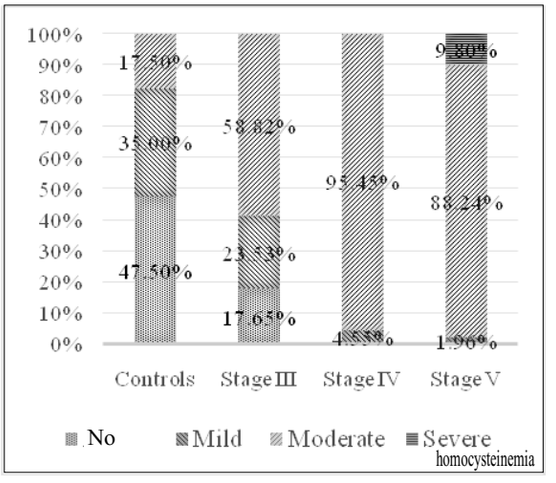

Fig 1 : 100% stacked column showing patterns of homocysteinemia in controls and stage III, IV, V CKD patients, n = 130.

Table II : Comparison of parameters among controls and stage III, IV, V CKD patients (n = 130)

Parameter	Control	Stage III CKD	Stage IV CKD	Stage V CKD	p value
Creatinine (mg/dL)	0.87 ±0.13	1.63 ± 0.31	2.91 ± 0.53	10.75 ± 5.27	<0.0001
eGFR (mL/min)	87.60 ± 15.62	44.41 ± 8.75	21.50 ± 3.86	5.90 ± 3.36	<0.0001
Hcy (µmol/L)	17.80 ± 9.5	33.51 ± 15.19	49.46 ± 13.04	72.06 ± 23.93	<0.0001
Folate (nmol/L)	16.45 ± 7.17	11.01 ± 4.53	7.47 ± 2.16	4.72 ± 2.09	<0.0001
LDL (mg/dL)	108.18 ± 21.11	111.88 ± 14.64	114.82 ±12.62	124.37 ±12.84	<0.0001
SystolicBP (mmHg)	117.55 ± 10.87	128.88 ± 11.34	138.95 ± 11.65	148.22 ± 18.33	<0.0001
LVEF (%)	Not done	63.89 ± 5.18	57.18 ± 6.48	50.28 ± 9.05	< 0.001

Table III : Characteristics of hyperhomocysteinemic CKD patients; n = 90

	Grades of hyperhomocysteinemia			
	No <15 µmol/L	Mild 15–30 µmol/L	Moderate 30–100 µmol/L	Severe >100 µmol/L
Hypertensive,%	33.33	50	77.63	100
Diabetic, %	33.33	50	36.84	60
LDL ≥100 mg/dL, %	100	50	92.11	100
Low folate, %	0	0	59.21	100
Proteinuric, %	66.67	50	79.45	100
Low LVEF, %	0	0	27.03	100

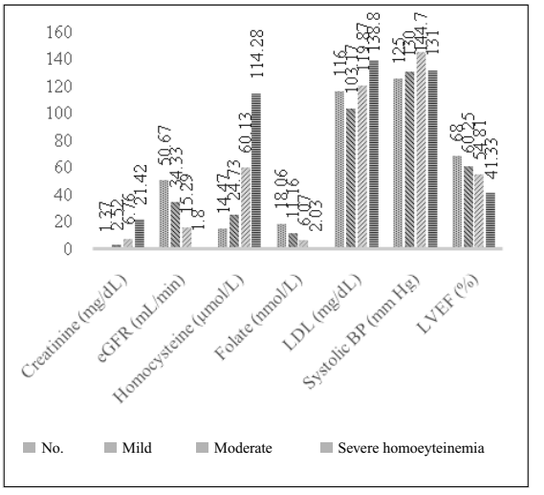

Fig 2 : Clustered column showing mean values of different parameters in four homocysteine groups (n = 90)

Table IV : Associations found in this study

Association	Test of	n	p value
between CKD & hyperhomocysteinemia	significance $\chi 2$ value = 38.42 Odds ratio =26.24	130	<0.0001 <0.0001

CKD & low folate levels	$\chi 2$ value = 23.67 Odds ratio = 11.25	130	<0.0001 <0.0001
Hyperhomocysteinemia & low folate levels	$\chi 2$ value = 3.88	90	<0.05
Elevated homocysteine levels & low LVEF	Odds ratio =9.75 (Homocysteine concentration in upper tertile vs. lower two tertiles)	45	<0.01

Table V : Correlations observed in this study

Correlation between	r value	p value
eGFR & serum homocysteine	r = -0.81, n = 130	< 0.00001
eGFR & serum folate	r = +0.84, n = 130	< 0.00001
Serum homocysteine & serum folate	r = -0.80, n = 130	< 0.00001
Serum homocysteine & LVEF	r = -0.70, n = 45	< 0.00001
Serum homocysteine & LDL	r = +0.62, n = 130	< 0.00001
Serum homocysteine & systolic BP	r = +0.59, n = 130	< 0.00001

Age and sex among groups were evenly matched. Percentages of hyperhomocysteinemia, low folate and low LVEF increased with increasing CKD stages (Table I).

Table II shows significant differences in parameters amongst four eGFR groups. Serum homocysteine increased whereas serum folate and LVEF decreased with increase in severity of CKD.

Hypertension was more common in homocysteinemic CKD patients. Low folate concentration and low LVEF were found only in moderate and severely homocysteinemic patients (Table III).

CKD was associated with hyperhomocysteinemia and low folate concentrations. Again, hyperhomocystein-emia was associated with low folate and low LVEF (Table IV).

Serum homocysteine correlated negatively with eGFR, serum folate & LVEF but positively LDL and systolic BP (Table V).

Discussion

Of the 130 subjects included in this study, forty (40)were controls and ninety (90) were CKD patients.Among the cases, stage III, IV & V CKD patients were 19% (n = 17) 24% (n = 22) & 57% (n = 51) respectively. Ages and sex ratios of different groups and subgroups were evenly matched and the differences were not statistically significant (Table I).

Homocysteinemia and its severity increased with increasing CKD stages. In stage III, homocysteinemia was seen in 82.35%, whereas all of the stage IV & V CKD patients were hyperhomocysteinemic. These were significantly higher compared to that of controls (52.5%) (Table I, Figure 1). Most of the CKD patients were moderately homocysteinemic (30 to 100 μmol/L). Severe homocysteinemia (>100 μmol/L) was seen only in 9.8% of stage V CKD patients (Figure 1). A recent systemic review and meta-analysis reported similar prevalence (36–89%) of hyperhomocysteinemia in CKD patients depending on severity[22]. Percentage of low folate concentration also increased in an ascending pattern from 10% in controls to 76.47% in stage V of CKD patients (Table I).

ANOVA showed highly significant differences of serum homocysteine and serum folate amongst the controls and stage III, IV & V CKD patients (Table II)Comparison of LVEF amongst stage III, IV, V CKD patients (n=45) also yielded significant differences(Table II). However, mean serum LDL concentration did not vary much amongst controls, stage III and stage IV CKD patients except in stage V (Table II). This phenomenon was also observed in other studies[23, 24].

There were significant differences in mean values of different parameters amongst four homocysteine groups. As evident in Figure 2, average eGFR, serum folate & LVEF were much lowerin hyperhomocysteinemic groups.With increase in severity of hyperhomocysteinemia, mean values of eGFR and serum folate decreased. LVEF also reduced significantly with increasing severity of hyperhomocysteinemiai.e. from 68% in those without hyperhomocysteinemia to 41.33% in those with severe hyperhomocysteinemia.

The study has found strong associationsof CKD with both hyperhomocysteinemia and low folate concentrations (Table IV). In addition, hyperhomocysteinemia was also associated with low folate (χ^2 value = 3.88, $p < 0.05$) and low LVEF

(Odds ratio = 9.75, $p < 0.01$) (Table IV). In this study serum homocysteine correlated positively with LDL and systolic BP, and negatively with eGFR, serum folate and LVEF (Table V). As predicted, serum folate was found to correlate positively with eGFR.

To test the relationsamong serum homocysteine, serum folate and LVEF in CKD, independent of hypertension and diabetes mellitus, subjects without these disorders were analyzed separately. Although that reduced the sample size significantly, the above-mentioned relationships remained substantially unchanged.

Previous studies also reported similar associations of both hyperhomocysteinemia and folate concentrations with CKD[12,25, 26]. However, the occurrence of hyperhomocysteinemia in these healthy subjects and mean homocysteine concentrations both in cases and controls were much higher than the values generally reported. These variations may be due to small sample size, deficiency of vitamin B_{12} (Not assessed in the present study), lack of food-fortifications with folate or traditional methods of prolonged cooking.It may also be due to higher rates of MTHFR polymorphism as seen in Indian populations[27]. Compared to other studies, much higher homocysteine concentrations among the CKD patients of this study can be explained by the fact that most of these patients belonged to stage V and none were on dialysis or vitamin supplementation.

Several lines of recent evidence confirm a two-way relationship between hyperhomocysteinemia and CKD. That is, rather than only being a consequence of CKD, hyperhomocysteinemia can also be involved as a cause of renal atherosclerosis and renal damage leading to a vicious cycle with accelerated deterioration of renal function[27, 28].

The adverse effect of hyperhomocysteinemia on cardiovascular function especially in CKD patients has also drawn significant attention. Apart from its established role in endothelial dysfunction, hypertension and atherosclerotic vascular disorders, elevated homocysteine is known to directly affect cardiac function[28, 29]. Homocysteine induced increased matrix accumulation in the myocardium leads to deposition of extracellular matrix between endothelium and myocyte causing endothelium-myocyte uncoupling. This causes prevention of nitric oxide to pass through the matrix barrier and impairs left ventricular diastolic dysfunction. This might explain the inverse association between plasma homocysteine level and Left Ventricular Ejection Fraction (LVEF) observed in the present study and similar others[19, 20].

Contribution of authors

PB - Conception, design, acquisition of data, drafting and final approval.

MH - Analysis, interpretation of data, critical revision of content and final approval.

MJK - Acquisition of data, drafting and final approval.

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Conclusion

In conclusion, this study revealed significant associations of hyperhomocysteinemia and low folate concentrations with CKD. Moderate and severe homocysteinemia in the CKD patients were also found to be associated with low LVEF. Similar to the findings of previous studies, these associations appeared to be independent of hypertension and diabetes mellitus. These observations support the growing consensus that serum homocysteine is an additional prognostic marker of CKD that may also be used to predict cardiovascular morbidity and folate status in these patients. The much higher rate and severity of homocysteinemia and folate deficiency reported by this study may be indicative of greater risk of vascular disease in Bangladeshi CKD patients. Hence, further studies are needed in this area to better validate these findings and to determine whether reduction in serum homocysteine results in improved prognosis.

Disclosure

All the authors declared no competing interest.

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