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ORIGINAL PAPERS

Serum Homocysteine and Folic Acid Levels as Risk Factors of Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) is a worldwide health problem. Homocysteine (Hcy) and folic acid (FA) have been found to correlate with intra-renal atherosclerosis, which leads to decreased renal perfusion pressure and may impair the endothelial function of renal arterioles and glomerular capillaries, thus being considered as new risk factors for CKD.
Objective: To investigate serum Hcy and FA as risk factors for CKD.
Methods: A cross-sectional study was conducted on 80 patients who underwent creatinine examination. Hcy and FA levels were examined using enzyme-linked immunosorbent assay (ELISA) method. Bivariate analysis to calculate the prevalence ratio (PR) using a 2x2 table and using Receiver Operating Characteristic (ROC) curves to determine the cut off value of HCY and FA in CKD.
Results: The mean Hcy level of CKD patients was 7.66±4.25 µmol/L and without CKD was 4.29 ± 2.71 µmol/L. The mean FA levels of CKD and non-CKD patients were 6.75±1.75 and 9.27±9.28 ng/mL, respectively. The prevalence ratio of Hcy to CKD was 2.17 (95%CI=1.35-3.45; p=0.001). The prevalence ratio of FA to CKD was 1.75 (95%CI=1.11-2.75; p=0.014).
Conclusion: Increased Hcy levels and decreased serum FA levels are risk factors for CKD.

Keywords: Chronic kidney disease, homocysteine, folic acid.

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INTRODUCTION

Chronic kidney disease (CKD) is a worldwide health problem. The prevalence of CKD is increasing as the population ages and the incidence of diabetes mellitus (DM) and hypertension increases. Approximately 1 in 10 of the global population has CKD at some stage and has continued to increase in recent years, with an estimated 843.6 million people worldwide suffering from CKD in 2017.[1,2] Data from the Centers for Disease Control and Prevention (CDC) in 2021 estimated that around 15% of adults in the United States, or around 37 million people, have CKD. 786,000 people in the United States, or 2 out of every 1,000 people have end-stage CKD.[3] The global prevalence of CKD in 2017 was 9.1% (8.5-9.8%), while Indonesia like other countries also suffers from a high burden of CKD incidence. Epidemiologic data on CKD in Indonesia are scarce and inconsistent. The Basic Health Research (Riskesdas) reported that the prevalence of CKD (estimated glomerular filtration rate, eGFR<60 ml/min/1.73M^2) was 2.0 per mil (‰) in 2013 and increased to 3.8‰ in 2018.[4]

CKD has also been clarified to be associated with an increased risk of cardiovascular disease morbidity and mortality.[5] The study of Stenvikel P, et al. showed that inflammation, oxidative stress, and suppression of nitric oxide (NO) activity together play a role in vascular endothelial dysfunction that ultimately leads to early atherosclerosis in end-stage renal disease patients.[6,7] Oxidative stress and suppression of NO activity can be caused by an excessive increase in serum homocysteine (Hcy) levels, commonly known as hyperhomocysteinemia (HHcy).[7]

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine, an essential amino acid, where Hcy metabolism is influenced by nutritional and genetic factors.[8] Homocysteine can induce endothelial injury, decrease adenosine levels in plasma and interstitial tissue, and induce proliferation and apoptosis of glomerular mesangial cells through the effect of reactive oxygen species (ROS) generated in vascular smooth muscle cells, and lead to renal vascular injury.[8] Mallamaci, et al. reported that there was a positive correlation between elevated total homocysteine (tHcy) and higher mortality rates in dialysis patients,[9] However, other studies have shown that elevated Hcy is not associated with chronic kidney disease, which contradicts existing theories.[10,11]

The conversion of Hcy to methionine is assisted by several cofactors, one of the cofactors that helps the conversion of Hcy to methionine is folic acid (FA). Folic acid is a vitamin that regulates amino acid metabolism, is a synthetic form of vitamin B9 and is very important in the conversion of Hcy to methionine, if FA intake is inadequate, it causes Hcy levels to increase.[12] Folic acid is biologically inactive and requires the activity of the enzyme methylenetetrahydrofolate reductase (MTHFR). MTHFR enzyme is the main regulator of Hcy remethylation.[13]

Research by Mallamaci, et al. showed that FA correlates with circulating Hcy in blood vessels.[9] A meta-analysis study by Quin, et al. from 1966-2011 showed a positive effect when Hcy levels decreased by >20%, with FA supplementation,[14] but the study of Jamison, et al. showed Hcy reduction secondary to high-dose FA supplementation did not correspond to improved survival or reduction in cardiovascular events in CKD patients.[15]

The inconsistent results of research on the relationship between serum Hcy and FA levels in CKD patients are the basis for this study, to determine the role of Hcy and FA as risk factors for CKD.

METHODS

Analytical observational study with a cross-sectional approach to determine risk factors in chronic kidney disease patients by calculating the prevalence ratio of Hcy and FA conducted at the outpatient polyclinic of Dr. Kariadi Hospital Semarang from February 2023 to May 2023. The study subjects were male and female patients aged > 18 years who performed creatinine examination. This study excluded patients taking vitamin B6, B12 or FA supplements, pregnant women and women using hormonal contraceptives.

All study subjects were asked for their consent by signing a written informed consent before the study. This research protocol was approved by the Health Research Ethics Commission of Dr. Kariadi Semarang Central General Hospital (No. 1375/ECKEPK-RSDK/2022).

Data were obtained from the calculation of CKD-EPI eGFR values based on the 2021 CKD-EPI formula using MDCalc and serum creatinine levels using

the Jaffe method from Siemens Dimension RxL. The diagnosis of chronic kidney disease was obtained with an eGFR value <60 mL/min/1.73 m^2. Serum homocysteine levels were measured using the enzyme-linked immunosorbent assay (ELISA) method using the Hcy ELISA kit (Wuhan Fine Biotech Co., Ltd., Wuhan, China), while serum FA was measured using the ELISA method of the Elabscience kit, and the results were read using a Biotek ELX800. Hcy and FA cut-off values were determined based on Receiver Operating Characteristic (ROC) Curves. Bivariate analysis using Chi Square was performed to determine the prevalence ratio (RP). Statistical tests were considered significant if $p < 0.05$. SPSS software version 26.0 (IBM Corporation, Armonk, NY, USA) was used for data analysis.

RESULTS

The results of the study obtained 80 adult patients who met the inclusion and exclusion criteria at Dr. Kariadi Hospital Semarang, 34 patients with CKD (eGFR < 60 mL/min/1.73M2) and 46 patients without CKD (eGFR ≥ 60 mL/min/1.73M2), and consisted of 39 men and 41 women.

Calculation of cut off levels of Hcy and FA for the incidence of CKD with ROC curves can be seen in Figure 1. The cut off value of Hcy level was 4.8 µmol/L (AUC, area under curve=76.1% (moderate); sensitivity 70.6%; and specificity 67.4%). The cut off value of FA level was 6.95 ng/mL (AUC=65.8% (moderate); sensitivity 63%; and specificity 70.6%).

The result of Hcy level examination was considered positive if it was more than or equal to the cut off value, and considered negative if it was less than the cut off value. FA level was considered positive if it was less than or equal to the cut off value, and negative if it was more than the cut off value. Based on the cut off value, 39 samples (48.8%) had Hcy levels ≥4.8 µmol/L, and 41 samples (51.2%) had FA levels <4.8 µmol/L. FA levels ≤6.95 ng/mL were 39 samples (48.8%), and those >6.95 ng/mL were 41 samples (51.2%).

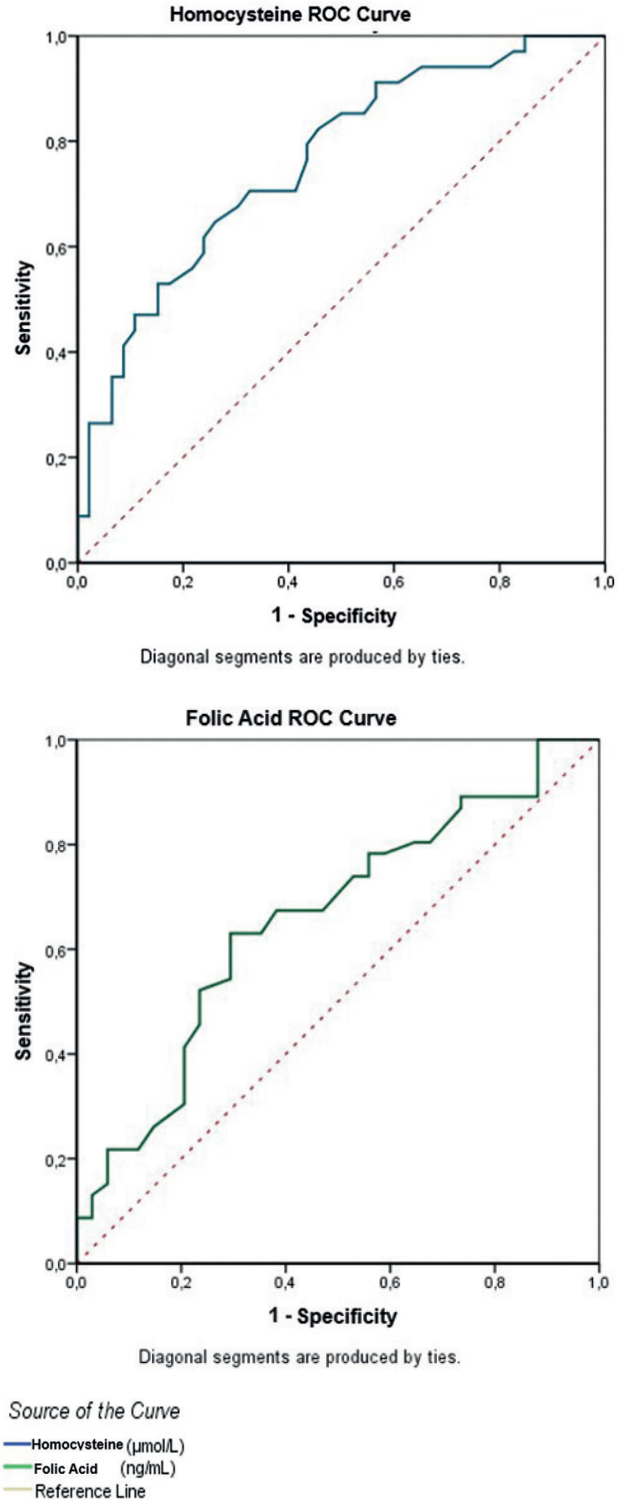

Figure 1. ROC curve of Hcy and FA levels for diagnosis of CKD
Notes: CKD, chronic kidney disease; FA, folic acid; Hcy, homocysteine.

The calculation of the prevalence ratio of Hcy levels to CKD can be seen in Table 1. The value of RP=2.17 (p=0.001; 95% CI: 1.35-3.45), indicating that patients with Hcy levels above the cut off value have a 2.17-fold risk of CKD than patients with Hcy levels below the cut off value.

Table 1. Prevalence ratio of Hcy levels to CKD

Hcy	CKD		Total	PR (95%CI)	*p*
	Yes	No			
≥4,8 µmol/L	24 (88,2%)	15 (32,6%)	39 (48,8%)	2,17 (1,35 – 3,45)	0,001
<4,8 µmol/L	10 (50%)	31 (67,4%)	41 (51,2%)		
Total	34 (100%)	46 (100%)	80 (100%)		

Notes: CKD, chronic kidney disease; Hcy, homocysteine; PR, prevalence ratio; CI, confidence interval.

The calculation of the prevalence ratio of FA levels to CKD can be seen in Table 2. The value of RP=1.75 (p=0.014; 95% CI: 1.11-2.75), indicating that patients with FA levels below the cut off value have a risk of CKD 1.75 times that of patients with FA levels above the cut off value.

Table 2. Prevalence ratio of FA levels to CKD

FA	CKD		Total	PR (95%CI)	*p*
	Yes	No			
≤6,95 ng/mL	22 (64,7%)	17 (37%)	39 (48,8%)	1,75 (1,11 – 2,75)	0,014
>6,95 ng/mL	12 (35,3%)	29 (63%)	41 (51,2%)		
Total	34 (100%)	46 (100%)	80 (100%)		

Notes: CKD, chronic kidney disease; FA, folic acid; PR, prevalence ratio; CI, confidence interval.

DISCUSSION

The results of this study showed that the prevalence ratio of Hcy levels with CKD was 2.17 (95%CI=1.36 - 3.46; p=0.001), this indicates that 34 individuals with Hcy levels ≥ 4.8 µmol/L have a 2.17-fold risk of CKD compared to individuals who have Hcy levels < 4.8 µmol/L. Homocysteine is an amino acid derived from methionine metabolism and converted into cysteine to be excreted through the kidneys, the metabolic disorder causes an increase in Hcy levels in the blood.[16,17]

Hyperhomocysteinemia is known to play a role in endothelial dysfunction by triggering increased oxidative stress and disrupting the balance of vascular tone-regulating mediators such as NO, endothelin, and thromboxane..[18] Research by Amiin, et al. states that kidney disease patients have increased plasma levels of S-Adenosylmethionine (SAM), S-Adenosylhomocysteine (SAH), and low serine levels due to blocks in Hcy re-methylation that block the expression of the enzyme betaine-homocysteine methyltransferase in the kidneys, so CKD patients show increased Hcy levels.[19]

Several studies have shown a correlation between elevated Hcy levels and the incidence of CKD.[19–22] The results of data analysis that increased Hcy levels are a risk factor for CKD are in accordance with research conducted by Chuang, et al. in 2013 which reported patients with elevated Hcy levels had a 2.21 times greater risk of developing CKD.[22] Research by Lai, et al. in 2018 reported that the CKD group had higher Hcy levels compared to the non-CKD group, where patients with increased Hcy levels had a 1.38-fold risk of suffering from CHD.[23] Chuang, et al. found that an increase in Hcy levels ≥11.82 µmol/L had a risk of developing CKD 1.61 times greater in men and 2.21 times greater in women compared to the group with Hcy levels <11.82 µmol/L.[22] The conclusions of these studies are in line with this study, where increased Hcy levels are a risk factor for CKD. These results are consistent with the mechanism of impaired NO synthesis and bioavailability due to increased Hcy levels. Endothelial cells increase NO synthesis and release to prevent cell damage and detoxify excess Hcy levels.[24]

Increased Hcy levels are associated with oxidative injury to vascular endothelial cells and inhibition of endothelial mediators such as NO; generation of superoxide radicals that inhibit vascular relaxation; increased proliferation of vascular smooth muscle

cells; and decreased adenosine production, which are thought to be associated with vasodilation and vascular remodeling, meanwhile, under HHcy conditions, Hcy load leads to the expression of endoplasmic reticulum stress genes causing cellular injury in cultured podocytes showing an association with renal damage and eventually leading to focal/global glomerulosclerosis, tubular atrophy, interstitial fibrosis and reduced GFR.[25]

The results of this study also showed the prevalence ratio of serum FA levels with CKD was 1.75 (95%CI=1.11 - 2.75; p=0.014), indicating that 34 individuals with FA levels ≤6.9 ng/ml had a 1.75-fold risk of CKD compared to individuals with FA levels >6.9 ng/ml. The results of this study are in accordance with several studies showing a correlation between FA levels and the incidence of CKD.[26–28] Folic acid, vitamin B6, and B12 are necessary cofactors in Hcy metabolism.[29] FA deficiency can interfere with MS activity where Met is converted to Hcy via SAM and SAH which are released in various methylation reactions.[30] Genetic alterations of MER metabolizing enzymes and deficiencies of FA, vitamin B6, and B12 determine Hcy levels. FA consumption can reduce plasma Hcy levels, so FA is considered protective against vascular disease. Folic acid has been known to have antioxidant activity, and it has been shown in vitro that FA can act as an antioxidant by protecting LDL from oxidation.[29] Plasma LDL penetrates and oxidizes into the subendothelial space of renal arterioles and glomerular capillaries. Ox-LDL substances injure endothelial cells, and disrupt the endothelial function of renal arterioles and glomerular capillaries, with decreased GFR rates indicating renal impairment.[31]

CONCLUSIONS

Individuals with Hcy levels ≥4.8 μmol/L have a risk of suffering from CKD 2.17 times more than individuals who have Hcy levels <4.8 μmol/L and individuals with FA levels ≤6.95 ng/mL have a risk of suffering from CKD 1.75 than individuals who have FA levels >6.95 ng/mL.

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AUTHOR CONTRIBUTION

BR and NW were involved in research review, data analysis, script preparation. EL was involved in research planning, measurement, data analysis, and script preparation. All authors were involved in the discussion of the results, the editing process, and approving the final script. There is no conflict of interest in this study.

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