The Association between Homocysteinemia and Mortality in Pre-dialysis CKD patients: A Propensity-Score Matched Analysis Using NHANES-National Death Index Link

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Abstract

Background: Hyperhomocysteinemia (HHcy) is considered a risk factor for cardiovascular disease (CVD) including chronic kidney disease (CKD). In this study, we investigated the association between serum homocysteine (Hcy) level and mortality according to the presence of CKD.

Methods: Our study included data of 9,895 participants from the 1996–2016 National Health and Nutrition Examination Surveys (NHANES). Moreover, linked mortality data were included and classified into four groups according to the Hcy level. Multivariable-adjusted Cox proportional hazards models using propensity-score were used to examine dose-response associations between Hcy level and mortality.

Results: Of 9,895 participants, 1032 (21.2%) participants were diagnosed with CKD. In a multivariate Cox regression analysis including all participants, Hcy level was associated with all-cause mortality, compared with the 1st quartile in Model 3 (2nd quartile: hazard ratio (HR) 1.751, 95% confidence interval (CI) 1.348-2.274, p<0.001; 3rd quartile: HR 2.220, 95% CI 1.726-2.855, p<0.001; 4th quartile: HR 3.776, 95% CI 2.952-4.830, p<0.001). In the non-CKD group, there was a significant association with all-cause mortality; however, this finding was not observed in the CKD group. The observed pattern was similar after propensity score matching. In the non-CKD group, overall mortality increased in proportion to Hcy concentration (2nd quartile: HR 2.195, 95% CI 1.299-3.709, p = 0.003; 3rd quartile: HR 2.607, 95% CI 1.570-4.332, p<0.001; 4th quartile: HR 3.720, 95% CI 2.254-6.139, p<0.001). However, the risk of all-cause mortality according to the quartile of Hcy level did not increase in the CKD group

Conclusion: This study found a correlation between the Hcy level and mortality rate only in the non-CKD group. This altered risk factor patterns may be attributed to protein-energy wasting or chronic inflammation status that is accompanied by CKD.

Background

The prevalence of chronic kidney disease (CKD) increases with age, along with the risk of cardiovascular disease (CVD) [1, 2]. Prior evidence suggest an association between CKD and CVD through various sharing mechanisms; moreover, another study presented an association between CVD and CKD among patients with advanced CKD [3] who were undergoing dialysis and also those with mild to moderate CKD [4]. There are several potential traditional mechanisms through which CKD could lead to CVD. For example, systemic inflammation might lead to uncontrollable levels of uremic metabolite, such as phosphorus, which leads to changes in cardiac remodeling [4].

Prior studies have examined homocysteine (Hcy) as a risk factor for CVD and cerebrovascular disease in the general population [5]. However, there is a lack of research on the risk factor of CVD in CKD patients. Hcy is used as an indicator of vascular disease, especially in Asian populations, who report insufficient daily folic acid intake, compared with that of Western populations [6]. Prior studies considered an increase in Hcy concentration as not being the cause of CKD, but as the result or coincidence of CKD, which was thought to be due to the decrease in glomerular filtration rate (GFR) [7, 8]. However, recent studies suggest that hyperhomocysteinemia (HHcy) may be a risk factor of CKD itself. The results of a meta-analysis of 41 studies showed that Hcy level was inversely correlated with the decrease in GFR [9]. Moreover, studies on elderly patients with hypertension in China reported that HHcy can predict a decline of kidney function [10]. Furthermore, CVD may also occur in addition to CKD through various mechanisms such as increased Hcy concentration, oxidative stress, and endothelial dysfunction [11]. However, results on the effect of HHcy on death in advanced CKD patients are inconclusive. In particular, reverse epidemiology was observed in the mortality of advanced CKD patients, compared with that of the general population group [12]. Further, this effect was highly associated with the malnutrition-chronic inflammation hypothesis for obesity using body mass index (BMI) [13, 14] and dyslipidemia [15] in CKD patients.

Notably, dialysis patients were associated with low Hcy and mortality [16, 17]. In a HOST-trial in 2007, a randomized trial announced vitamin combination therapy in 2,056 CKD and end-stage renal disease (ESRD) patients. In this trial, while the level of Hcy had a statistically significant decrease, it did not improve CVD outcome [18]. Moreover, as prior research has focused on traditional risk factors such as high blood pressure and Diabetes mellitus (DM), there is limited research on the factors influencing increased CVD risk among CKD patients. Therefore, in this study, we aimed to identify patients with CKD at each stage by using open-source nationwide data, while determining the change in mortality according to the Hcy level among CKD and non-CKD patients.

Materials And Methods

Study population and ethics statement

The National Health and Nutrition Examination Surveys (NHANES) is a large cross-sectional survey conducted for citizens of the United States; it provides basic and clinical information related to the prevalence and risk factors of chronic diseases. We used anonymized information of participants included in the NHANES from 1996–2016. This study protocol received approval by the National Center for Health Statistics (NCHS) Institutional Review Board of NHANES [19]. We included 9,895 out of a total of 92,062 participants by excluding those with missing GFR, Hcy level, and mortality data (Fig. 1). The National Center for Health Statistics (NCHS) Research Ethics Review Board (ERB) approved the NHANES protocols (protocol #98-12, #2005-06 and #2011-17, available site https://www.cdc.gov/nchs/nhanes/irba98.htm) and written informed consent was obtained from NHANES participants, and all procedures were approved by the NCHS Research Ethics Review Board. And this research has been carried out in accordance with the Declaration of Helsinki. The NHANES performance of adult specimens was evaluated based on a method described in another previous study [20].

Measurement of homocysteine and covariates

Blood samples from NHANES participants are stored, processed, and analyzed according to standardized protocols. Total plasma Hcy were measured in blood samples of participants. The Abbott Homocysteine assay was used to measure Hcy in plasma. This laboratory method is a fully automated fluorescence polarization immunoassay from Abbott Diagnostics. Further, total plasma Hcy concentrations were calculated using a machine-stored calibration curve. In addition, other laboratory data such as serum creatinine, albumin, and uric acid were analyzed. Moreover, self-reports were used to collect information on demographic variables of participants including age, gender, race, education level, and smoking status.

Definitions of variables and outcomes

CKD was defined as a urine albumin creatinine ratio (UACR) $\geq$ 30 mg/g or an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2. Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg and/or a diastolic blood pressure (DBP) $\geq$ 90 mmHg. DM was defined as hemoglobin A1c $\geq$ 6.5%, self-reports of prior diagnosis of DM, or consumption of medications for DM. The primary outcome of the study was all-cause mortality, which was defined using the National Death Index (NDI) mortality data.

Statistical analysis

We divided study population into two groups (i.e., CKD and non-CKD group) on the value of eGFR and/or UACR. For group comparisons, t-test and $\chi 2$ test were used for continuous variables and proportions, respectively. Cox regression models were applied to calculate the hazard ratios (HRs). Further, 95% confidence intervals (CIs) were set for homocysteine and all-cause mortality. In Model 3, we included covariates that had a significant association (e.g., age, gender, serum albumin, UACR, smoking status, BMI, concurrent history of hypertension, and DM; $P < 0.05$). We used Kaplan-Meier survival curves to calculate all-cause mortality. Fig. 2 shows the methodology of propensity-score matched analysis. The propensity score and standardized differences were used to compare baseline characteristics between the two groups. The propensity score

is was used to balance covariates between the two groups to reduce biasing effects of between-group differences in baseline characteristics on measured effects of the CKD. The nearest neighbor matching method and 1:5 matching algorithm without replacement were used to select a match in the control group for each individual in the CKD group (Fig 2).

Results

Baseline characteristics of the study population

The average age of the study participants was 47 ± 20 years; further, the sample comprised 48.4% males. Among the study population, 3,771 (38.1/%) had hypertension, whereas 1,117 (11.3%) had DM. The CKD group was older in age (CKD: 73 ± 11 years; non-CKD: 44 ± 18 years, p<0.001) and had a lower level of education, compared with the non-CKD group. Moreover, in the CKD group, BMI, SBP, DBP, and the prevalence of hypertension and DM were high, whereas serum albumin level was low (Table 1).

Plasma homocysteine level according to the stage of Chronic Kidney Disease

For total plasma Hcy, the overall mean was 8.8 ± 4.7umol/L. Participants in the CKD-group had a higher Hcy level, compared with those in the non-CKD group (13.5 ± 5.1 versus 8.3 ± 4.3umol/L, p<0.001). Although an identical trend from CKD stage 4 to CKD stage 5 was not observed, Hcy levels in the CKD group according to the stage showed a tendency of gradual increase as the stage progressed from stage 1 to stage 4 (Fig. 3a). The same pattern was observed when analyzed separately by gender (Fig. 3b).

Analysis of all-cause mortality according to homocysteine level by quartile

We divided participants into quartiles according to the Hcy level. In overall population, mortality rate was increased as the level of Hcy increased (Figure 4). We used multivariate Cox regression analysis to adjust for age and gender in Model 2; further, we adjusted for age, gender, serum albumin, UACR, smoking status, BMI, concurrent history of hypertension, and DM in Model 3. In a multivariate Cox regression analysis comprising all participants, Hcy level was associated with all-cause mortality, compared with the 1st quartile in Model 3 (2nd quartile: HR 1.751, 95% CI 1.348-2.274, p<0.001; 3rd quartile: HR 2.220, 95% CI 1.726-2.855, p<0.001; 4th quartile: HR 3.776, 95% CI 2.952-4.830, p<0.001). In the non-CKD group, a significant association with all-cause mortality was observed; however, this was not observed in the CKD group (Table 2).

Association between homocysteine and chronic kidney disease

Through a multivariate logistic regression analysis, the odd ratios (ORs) of age, hypertension, and DM, which are the conventional risk factors for CKD were 1.096 (1.083-1.109, p<0.001), 1.699 (1.259-2.291, p<0.001), and 1.824 (1.264-2.633, p=0.001), respectively. However, factors such as obesity and smoking were not significantly correlated. Compared with the 1st quartile of Hcy level, the ORs were 2.439 (1.074-5.539, p=0.033) and 7.518 (3.342-16.911, p<0.001) in the 3rd and 4th quartile, respectively. Thus, we observed a significant correlation between the Hcy level and CKD (Table 3).

Analysis before and after propensity score matching

We performed propensity score matching by dividing the CKD and non-CKD group. Participants were allocated to each group in a 1:5 ratio. We used variables including age, gender, race, BMI, DM, and hypertension for propensity score matching. Baseline characteristics of the study population after propensity score matching are presented in Table 4. After propensity score matching, we included 2,015 and 741 participants in the non-CKD group and CKD group, respectively. After adjusting for variables, the differences in socioeconomic factors such as race, education level, BMI, and smoking history were offset between the groups. Moreover, we found a decrease in the differences in factors such as the prevalence of DM, hypertension, SBP, and serum uric acid level (Table 4). The risk of mortality according to Hcy level after propensity score

matching is presented in Table 5. In the multivariate Cox regression analysis, we found similar results compared to those observed in the crude analysis. The risk of all-cause mortality according to the quartile of Hcy level was not found to increase in the CKD group. However, in the non-CKD group, the Hcy level was associated with all-cause mortality (Table 5).

Discussion

This study analyzed the association between serum Hcy concentration and all-mortality in CKD and non-CKD patients using NHANES data. An increased survival rate was observed in patients with high levels of Hcy in the non-CKD group only. Moreover, we confirmed a significant correlation between the Hcy level and mortality risk; this risk was found to have a similar pattern after adjusting for confounding factors including age, gender, and inflammation or nutrition in the non-CKD group. However, in the CKD group, even in cases where the concentration of homocysteine was high, no association to mortality was observed. Therefore, this is a result consistent with the reverse epidemiology hypothesis of Hcy and mortality in CKD patients [12].

Although, it is well known that patients with CKD have a high CVD risk [21], there is little research on the mechanism through which this risk increases. Therefore, studying and identifying contributing factors other than these accompanying diseases are an important part of improving the prognosis of CKD patients. Further, vascular calcification related to mineral bone disease in CKD patients (medial calcification) [22] and inflammation such as C-reactive protein (CRP) or interleukin and endothelial dysfunction and metabolic disorder associated with adiponectin or FGF-23 are consider early biomarkers and etiologies causing excessive CVD occurrence of CKD [23].

The high prevalence of HHcy in patients with CKD [24, 25] has generated interest in the potential role of total homocysteine (tHcy) as a risk factor for the excess risk of CVD that is evident in this population [26]. In 1969, Macully first announced in a post-mortem case report that HHcy may be associated with atherosclerosis [27], which led to the concept of the “Hcy hypothesis,” that considered Hcy a potential risk factor for CVD, contributing to vascular physiology. Since then, observational studies have confirmed the association between HHcy and CVD. In the general population, HHcy is associated with arteriosclerosis, increased CVD risk, and mortality. In addition, even in patients with coronary artery disease [5], it is associated with poor prognosis and is considered a prognostic factor of poor outcome in patients with Type 2 DM [28]. While there have been cumulated hypotheses on the association between CVD occurrence and HHcy, Hcy is known to activate the associated pathway of atherogenesis or thrombosis by endothelial dysfunction, inflammation, and oxidative stress [24, 25, 29]. In addition, studies have reported an association between patients with gene variance and metabolism in Hcy, such as methylenetetrahydrofolate reductase (MTHFR) 677C raise CVD risk with elevated Hcy [23, 26]. Finally, there have also been reports that the administration of folic acid or vitamin B12 can lower the Hcy level and reduce mortality [22, 30]. Owing to this evidence, HHcy has been recognized as a risk factor for CVD occurrence. Although BMI, serum cholesterol, and high blood pressure are traditional risk factors for cardiovascular disease and mortality in the general population, in patients with CKD with regards to Hcy, the results of prior studies reveals the effect of reverse epidemiology in these traditional risk factors [18, 31]. Although HCY is known to be increased in CKD patients [32, 33], findings regarding whether it is an independent risk factor for increasing CVD risk in CKD patients are inconclusive [34-37]. In two studies with 367 and 88 hemodialysis patients, respectively, the low Hcy level in ESRD patients was observed to increase CVD outcome [38, 39]; moreover, Hcy also showed a reverse epidemiology pattern as mentioned above. In addition, the control over risk factors can reversely prove whether the risk factors reduce disease incidence. Since folate and vitamin B12 act as cofactors in Hcy metabolism, insufficiency of these cofactors could cause HHcy [40]. Therefore, there have been several studies on the improvement in CVD outcome after administration of VitB12 and folate for the treatment of HHcy. In contrast, a systematic review and meta-analyses revealed that Hcy-lowering treatment did not improve CVD outcomes in patients with a high level of Hcy among the ESRD population [31, 32].

The results of our study show that HHcy was highly correlated with mortality in the general population; moreover, the effect was maintained consistently when various confounding factors were calibrated. However, in CKD patients, the level of Hcy

was not found to be associated with mortality before and after propensity matching, indicating a pattern corresponding to the reverse epidemiology.

As mentioned above, in particular, CKD is known as a representative disease in which reverse epidemiology occurs [12]. Several researchers explain this inverse correlation using several mechanisms. First, inflammation and protein-energy wasting (PEW), which occur in advanced CKD patients, have been discussed. In fact, previous studies show that tHcy decreased when CKD patients were accompanied by inflammation or PEW [16, 39, 41, 42]. In addition, prior studies report an association between inflammations, PEW, and poor CVDs [43, 44]. Similarly, a reduction in albumin and Hcy level was observed in advanced CKD patients in this study. Therefore, PEW can offset the effect of HHcy on the clinical outcome including mobility. The second possible explanation is selection bias, which included survival bias. This is also a pattern that is observed in epidemiology studies among elderly [45] and advanced CKD patients, including ESRD patients. As these patients have high morbidity and mortality, compared to that of the general population, they may be viable targets for cross-section studies. In addition, even among CKD patients, there is an immense difference in epidemiological characteristics between patients who have just begun dialysis and those who have elapsed time [46]. Finally, in describing reverse epidemiology, time discrepancy is a competitive risk factor that can affect mortality. This can be explained first with the obesity paradox, as overweight or obesity is known to be highly related to CVD outcomes in developed countries such as the United States and Europe with high average life expectancy [47, 48]. Nevertheless, in developing countries, under-nutrition is known to be a powerful factor that can predict a poor clinical outcome, including mortality [49]. Therefore, for CKD patients with low life expectancy, strict management of weight, blood pressure, DM, etc., and low serum cholesterol and Hcy maintenance, which are known to be highly associated with long-term survival, maybe less important to improve clinical outcome, compared with the general population. Thus, the presence of reverse epidemiology may not necessarily imply that the principles of vascular pathophysiology are different in CKD patients. However, it might indicate that other superimposed factors, such as PEW and inflammation, are more important.

In conclusion, this study did not find a correlation between the Hcy level and mortality rate among the CKD group, unlike in the non-CKD group. This altered risk factor patterns may be attributed to protein-energy wasting or chronic inflammation status that is accompanied by CKD.

Abbreviations

Body Mass Index = BMI

Cardiovascular disease = CVD

Chronic Kidney Disease = CKD

Confidence Interval = CI

C-reactive protein = CRP

Diabetes Mellitus = DM

Diastolic blood pressure = DBP

End-stage renal disease = ESRD

Hazard ratio = HR

Hyperhomocysteinemia = HHcy

Homocysteine = Hcy

Methylenetetrahydrofolate Reductase = MTHFR

National Center for Health Statistics = NCHS

National Health and Nutrition Examination Surveys = NHANES

Odd ratios = ORs

Estimated Glomerular Filtration Rate = eGFR,

The National Center for Health Statistics = NCHS

Protein-energy wasting = NHANES

Ethics Review Board = ERB

Systolic Blood Pressure = SBP

Total homocysteine = tHcy)

Urine albumin creatinine ratio = UACR

Declarations

Ethics approval and consent to participate

This study protocol received approval by the National Center for Health Statistics (NCHS) Institutional Review Board of NHANES

Consent for publication

N/A

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

None

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None

Authors' contributions

Research idea and study design: KDY, GSK; data acquisition: JHS, HH, KDY, GSK; data analysis/interpretation: HH, JHS, GSK, KDY; supervision or mentorship: EB, JL, JPL, JSL. Each author contributed important intellectual content during manuscript drafting or revision. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline characteristics of the study population according CKD

BMI, body mass index; DBP, diastolic blood pressure; ESC, European Society of Cardiology; GFR, glomerular filtration ratio; IDH, isolated diastolic hypertension; ISH, isolated systolic hypertension; NICE, National Institute for Health and Care Excellence; SBP, systolic blood pressure; and SDH, systolic and diastolic hypertension.

Table 2. Hazard ratios of all-cause mortality according to homocysteine level (quartile)

	All (n=9895)			CKD (n=1025)			Non-CKD (n=8870)		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
	All-cause mortality								
Model 1									
Q2	2.073	1.633-2.632	<0.001	0.833	0.293-2.365	0.731	2.071	1.261-2.646	<0.001
Q3	3.602	2.885-4.498	<0.001	1.155	0.467-2.857	0.756	3.176	2.517-4.009	<0.001
Q4	11.361	9.244-13.961	<0.001	2.183	0.905-5.269	0.082	7.608	6.097-9.493	<0.001
Model 2									
Q2	1.470	1.155-1.872	0.002	0.767	0.270-2.178	0.618	1.434	1.118-1.840	0.005
Q3	1.857	1.474-2.339	<0.001	0.960	0.388-2.379	0.930	1.657	1.298-2.116	<0.001
Q4	3.841	3.073-4.800	<0.001	1.706	0.706-4.127	0.236	2.962	2.328-3.768	<0.001
Model 3									
Q2	1.751	1.348-2.274	<0.001	0.882	0.280-2.775	0.830	1.707	1.301-2.240	<0.001
Q3	2.220	1.726-2.855	<0.001	1.231	0.449-3.373	0.687	1.992	1.523-2.604	<0.001
Q4	3.776	2.952-4.830	<0.001	1.931	0.720-5.182	0.191	3.016	2.308-3.941	<0.001

CKD, chronic kidney disease; HR, hazard ratio

Model 1: crude

Variables	Total (N =9,895)	CKD (N=1,025)	Non-CKD (N =8,870)	*P*
Age (years)	47±20	73±11	44±18	<0.001
Men, %	4789 (48.4)	487 (47.5)	4302 (48.5)	0.553
Race/ethnicity, %				<0.001
Non-Hispanic white	5005 (50.6)	640 (62.4)	4365 (49.2)	
Non-Hispanic black	2145 (21.7)	253 (24.7)	1892 (21.3)	
Other-Hispanic	309 (3.1)	14 (1.4)	295 (3.3)	
Mexican-American	2033 (20.5)	86 (8.4)	1947 (22.0)	
Other	403 (4.1)	32 (3.1)	371 (4.2)	
Education level, %				<0.001
<12 years	2615 (28.0)	378 (36.9)	2237 (27.0)	
12-15 years	2277 (24.4)	272 (26.6)	2005 (24.1)	
≥16 years	2615 (28.0)	212 (20.7)	2403 (28.9)	
BMI (kg/m^2)	28.5±6.5	29.0±6.2	28.4±6.6	0.012
SBP (mmHg)	125±21	142±26	123±19	<0.001
DBP (mmHg)	69±14	66±19	70±13	<0.001
Smoking status, %				<0.001
Never	4783 (51.3)	503 (49.1)	4280 (51.5)	
Ex-smoker	2457 (26.3)	408 (39.8)	2049 (24.7)	
Current	2081 (22.3)	112 (10.9)	1969 (23.7)	
Albumin (g/dL)	4.2±0.4	4.0±0.4	4.2±0.4	<0.001
Cholesterol (mg/dL)				
Uric acid (mg/dL)	5.4±1.4	6.5±1.6	5.2±1.4	<0.001
Estimated GFR (mL/min/1.73m^2)	92±25	46±12	98±20	<0.001
Homocysteine (umol/L)	8.8±4.7	13.5±5.1	8.3±4.3	<0.001
Albumin creatinine ratio (mg/g)	43.1±341.4	215.5±926.5	24.1±175.8	<0.001
Hypertension, %	3771 (38.1)	810 (79.0)	2961 (33.4)	<0.001
Diabetes, %	1117 (11.3)	296 (28.9)	821 (9.3)	<0.001

Model 2: adjusted for age over 65, gender

Model 3: adjusted for age over 65, gender, serum albumin, urine albumin creatinine ratio, smoking status, body mass index, concurrent history of hypertension and diabetes mellitus

CKD was defined as estimated GFR < 60 ml/min/1.73m^{2,} Reference was Quartile 1 in model 1, 2, 3

Table 3. Odds ratios of chronic kidney disease using logistic regression

Variables	Univariate		Multivariate	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age	1.114 (1.107-1.121)	<0.001	1.096 (1.083-1.109)	<0.001
Gender (male)	0.961 (0.844-1.094)	0.549	0.460 (0.350-0.605)	<0.001
Hypertension	7.518 (6.428-8.794)	<0.001	1.699 (1.259-2.291)	0.001
Diabetes mellitus	3.981 (3.416-4.639)	<0.001	1.824 (1.264-2.633)	0.001
Obesity (BMI>30)	1.343 (1.135-1.589)	<0.001	0.942 (0.663-1.339)	0.739
Uric acid	1.807 (1.724-1.893)	<0.001	1.583 (1.435-1.746)	<0.001
Fasting glucose	1.006 (1.004-1.008)	<0.001	0.997 (0.992-1.001)	0.152
HCY (Ref Q1)				
Q2	2.797 (1.515-5.164)	0.001	0.588 (0.229-1.506)	0.268
Q3	13.85 (8.017-23.925)	<0.001	2.439 (1.074-5.539)	0.033
Q4	81.94 (48.132-139.5)	<0.001	7.518 (3.342-16.911)	<0.001
Current smoking	0.395 (0.323-0.484)	<0.001	0.877 (0.592-1.300)	0.514

adjusted for age , gender, smoking status, body mass index, hypertension and diabetes mellitus, serum glucose, uric acid and HCY quartile.

CKD was defined as estimated GFR < 60 ml/min/1.73m^2

Table 4. Baseline characteristics of the study population according CKD before and after propensity score matching.

Variables	No CKD (N = 8870)	CKD (N = 1025)	*P*	SD	No CKD (N =2015)	CKD (N =741)	*P*	SD
Age (years)	44±18	73±11	<0.001	2.439	65±15	71±12	<0.001	0.157
Age over 65 years, %	1405 (15.8)	806 (78.6)	<0.001	1.504	1116 (55.4)	537 (72.5)	<0.001	0.066
Men, %	4302 (48.5)	487 (47.5)	0.553	0.023	1031 (51.2)	367 (49.5)	0.465	0.000
Race/ethnicity, %			<0.001	0.373			0.059	0.000
Non-Hispanic white	4365 (49.2)	640 (62.4)			1182 (58.7)	460 (62.1)		
Non-Hispanic black	1892 (21.3)	253 (24.7)			425 (21.1)	173 (23.3)		
Other-Hispanic	295 (3.3)	14 (1.4)			40 (2.0)	12 (1.6)		
Mexican-American	1947 (22.0)	86 (8.4)			275 (13.6)	72 (9.7)		
Other	371 (4.2)	32 (3.1)			93 (4.6)	24 (3.2)		
Education level, %			<0.001				0.116	
<12 years	2005 (22.6)	272 (26.5)			509 (25.3)	202 (27.1)		
12-15 years	2403 (27.1)	212 (20.7)			523 (26.0)	153 (20.6)		
≥16 years	1652 (18.6)	157 (15.3)			350 (17.4)	129 (17.4)		
BMI (kg/m^2)	28.4±6.6	29.0±6.2	0.012	0.056	29.2±6.4	28.8±6.1	0.116	-0.031
Weight (kg)	80.4±20.7	79.5±19.7	0.191	-0.068	82.0±20.5	79.8±19.7	0.013	-0.036
SBP (mmHg)	123±19	142±26	<0.001		134±23	141±25	<0.001	
DBP (mmHg)	70±13	66±19	<0.001		70±15	67±19	<0.001	
Smoking status, %			<0.001	0.156			0.313	-0.012
Never	4280 (51.5)	503 (49.1)			932 (46.3)	351 (47.4)		
Ex-smoker	2049 (24.7)	408 (39.8)			788 (39.1)	296 (39.9)		
Current	1969 (23.7)	112 (10.9)			295 (14.6)	94 (12.7)		
Albumin (g/dL)	4.2±0.4	4.0±0.4	<0.001	-0.301	4.1±0.3	4.1±0.4	0.017	-0.005
Glucose (mg/dL)	97.1±33.4	110.3±41.2	<0.001		107.3±39.9	108.2±41.8	0.608	
Uric acid (mg/dL)	5.2±1.4	6.5±1.6	<0.001	0.798	5.8±1.4	6.1±1.5	<0.001	0.050
Estimated GFR (mL/min/1.73m^2)	98±20	46±12	<0.001		81±15	48±12	<0.001	
Albumin	24.1±175.8	215.5±926.5	<0.001		42.3±161.3	221.8±981.0	<0.001	

creatinine ratio (mg/g)								
Diabetes, %	821 (9.3)	296 (28.9)	<0.001	0.422	430 (21.3)	184 (24.8)	0.056	-0.014
Hypertension, %	2961 (33.4)	810 (79.0)	<0.001	1.093	1356 (67.3)	565 (76.2)	<0.001	0.033
Homocystein (umol/L)	8.3±4.3	13.5±5.1	<0.001		9.8±4.5	13.1±5.2	<0.001	

BMI, body mass index; DBP, diastolic blood pressure; ESC, European Society of Cardiology; GFR, glomerular filtration ratio; IDH, isolated diastolic hypertension; ISH, isolated systolic hypertension; NICE, National Institute for Health and Care Excellence; SBP, systolic blood pressure; and SDH, systolic and diastolic hypertension.

Table 5. Hazard ratios of all-cause mortality according to homocysteine level (quartile) after PSM

	All (n=2756)			CKD (n=741)			Non-CKD (n=2015)		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
	All-cause mortality								
Model 1									
Q2	1.800	1.159-2.796	0.009	0.568	0.189-1.736	0.321	2.099	1.292-3.412	0.003
Q3	2.599	1.720-3.927	<0.001	0.989	0.396-2.472	0.982	2.805	1.766-4.455	<0.001
Q4	5.013	3.361-7.478	<0.001	1.646	0.680-3.982	0.269	4.975	3.166-7.820	<0.001
Model 2									
Q2	1.442	0.928-2.241	0.104	0.530	0.173-1.622	0.266	1.622	0.997-2.639	0.051
Q3	1.821	1.202-2.759	0.005	0.830	0.332-2.077	0.691	1.875	1.176-2.990	0.008
Q4	3.128	2.087-4.688	<0.001	1.296	0.534-3.146	0.566	2.961	1.872-4.685	<0.001
Model 3									
Q2	1.875	1.166-3.014	0.010	0.592	1.173-2.028	0.404	2.195	1.299-3.709	0.003
Q3	2.402	1.530-3.773	<0.001	1.050	0.380-2.904	0.924	2.607	1.570-4.332	<0.001
Q4	3.674	2.360-5.719	<0.001	1.490	0.553-4.017	0.430	3.720	2.254-6.139	<0.001

CKD, chronic kidney disease; HR, hazard ratio

Model 1: crude

Model 2: adjusted for age over 65, gender

Model 3: adjusted for age over 65, gender, serum albumin, urine albumin creatinine ratio, smoking status, body mass index, concurrent history of hypertension and diabetes mellitus

CKD was defined as estimated GFR < 60 ml/min/1.73m^2

Reference was Quartile 1 in model 1, 2, 3

Figures

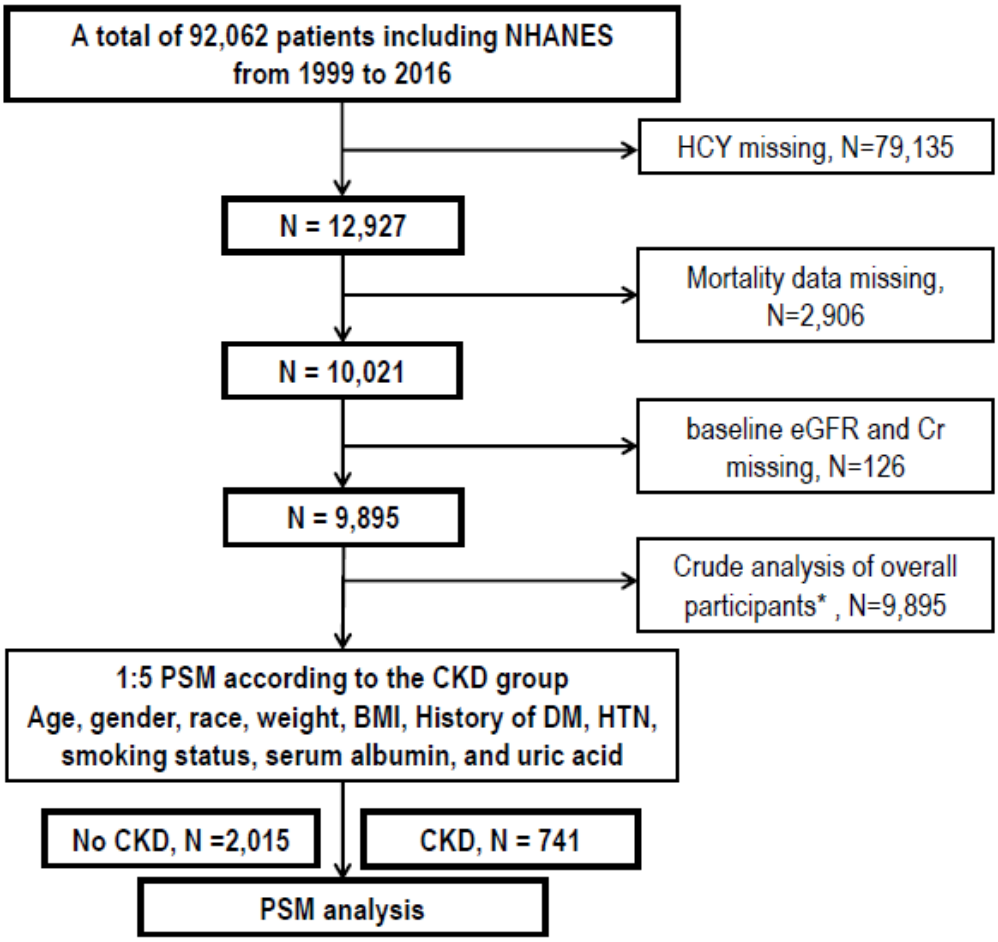

Figure 1

Study flow chart

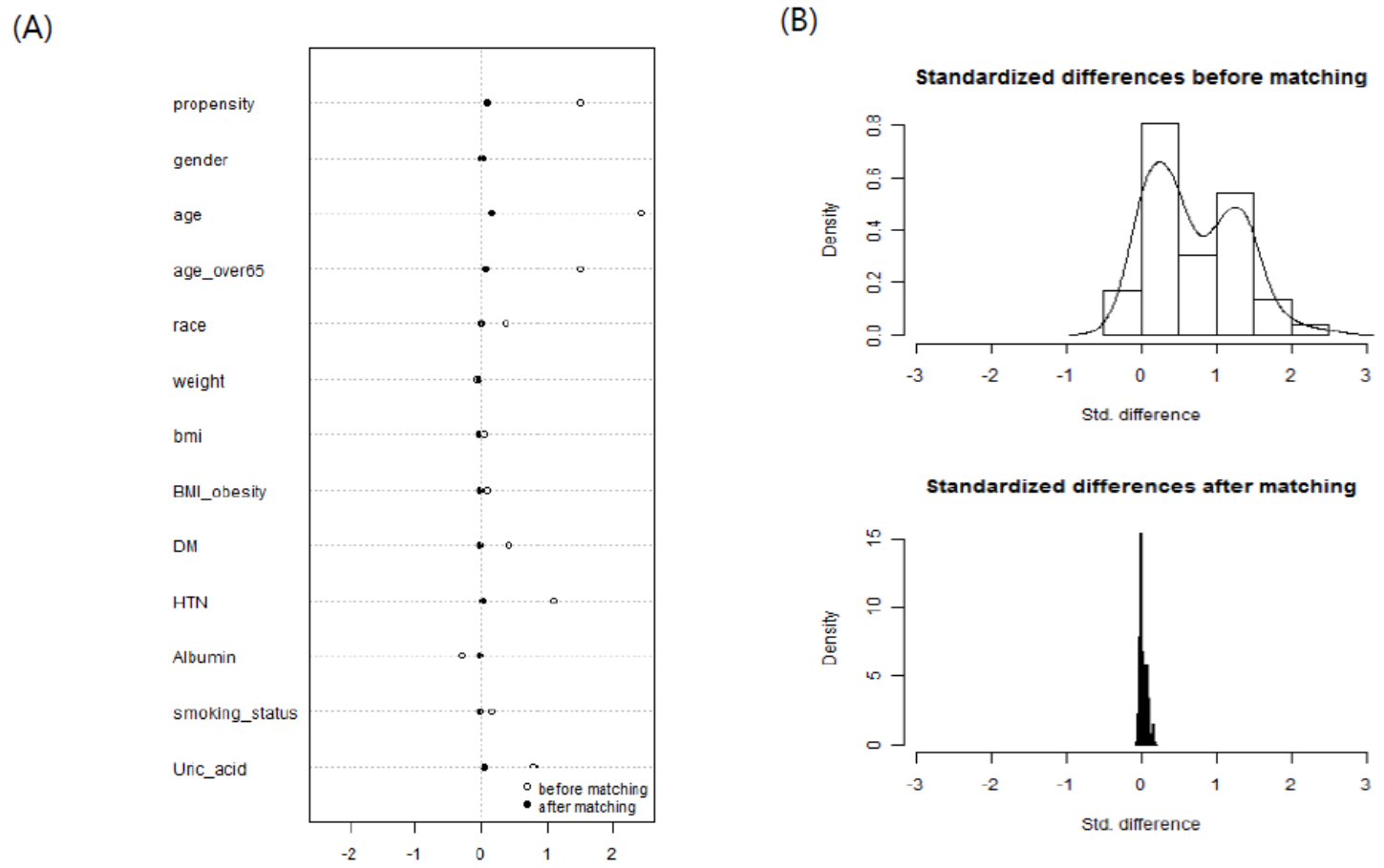

Figure 2

Standardized difference (SD) before and after propensity-score matching (A) Change of SD value for before and after propensity-score matching by variables (B) Distribution of SD for before and after propensity-score matching

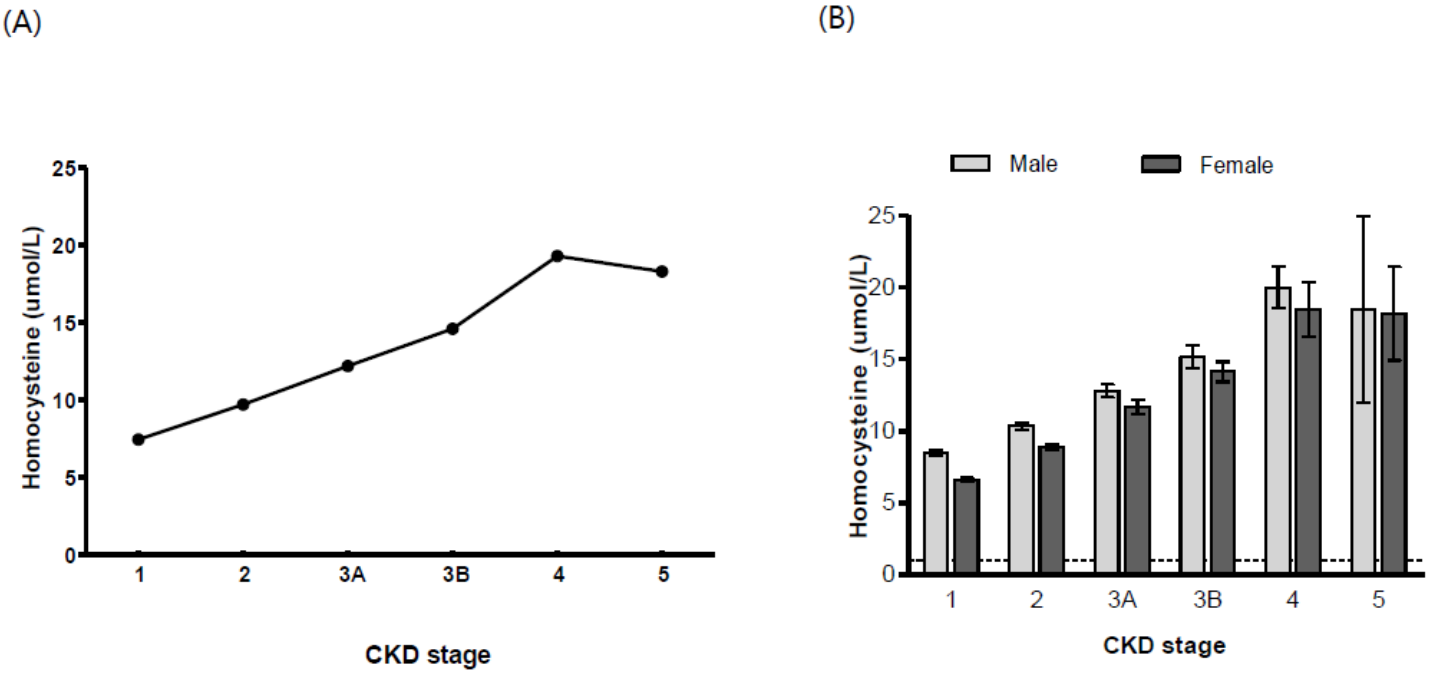

Figure 3

Serum levels of homocysteine according to CKD stage (A) Levels of Homocysteine according to CKD stage by GFR grade (B) Levels of Homocysteine according to CKD stage by gender

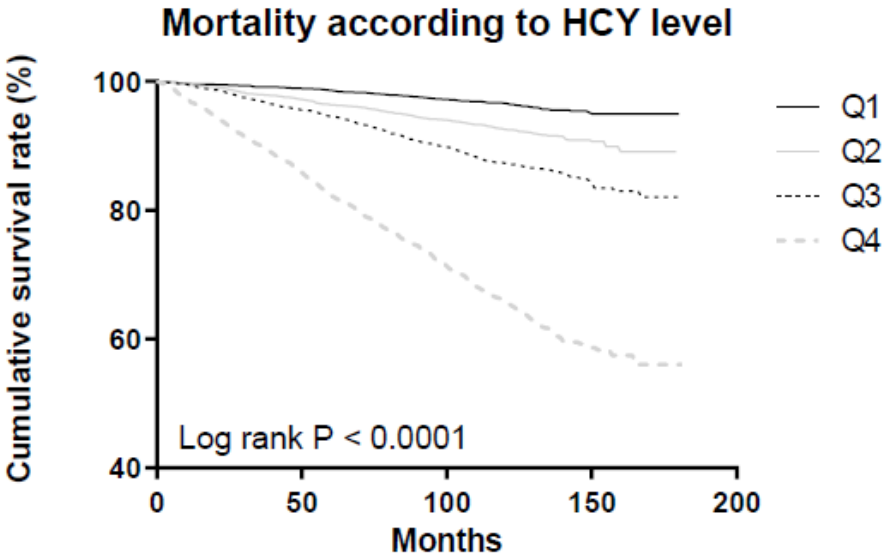

Figure 4

Comparison of the cumulative survival rate between the homocysteine quartile group