

Original Paper

Homocysteine and C-Reactive Protein as Useful Surrogate Markers for Evaluating CKD Risk in Adults

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Key Words

Chronic kidney disease • C-reactive protein • Homocysteine • Risk

Abstract

Background/Aims: This study aimed to evaluate the effectiveness of homocysteine and C-reactive protein (CRP) as potential markers for chronic kidney disease (CKD) in adults in Taiwan, and to identify associations between these factors and CKD, stratifying by gender.

Methods: This cross-sectional study analyzed multi-center data retrospectively. Data were collected from 22,043 adult Taiwanese at Chang-Gung Memorial Hospital from 2005 to 2011. Smoking/drinking history, personal medical/medication history, pregnancy, fasting times as well as laboratory parameters, including homocysteine and CRP were measured and analyzed.

Results: Significant differences were observed between four homocysteine and CRP quartiles in eGFR and CKD. For males, only one model showed significant associations between plasma homocysteine and CKD, while in females, all three models showed significant associations with CKD. On the contrary, the gender difference in the case of CRP was opposite. Combined homocysteine and CRP were associated with CKD in males but not in females. **Conclusion:** Among Taiwanese adults, plasma homocysteine is associated with CKD in females and plasma hsCRP is associated with CKD in males. High hsCRP/high homocysteine is associated with elevated CKD risk in male. Our results suggest that homocysteine and hsCRP may be useful surrogate markers for evaluating CKD risk in adults.

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Introduction

Chronic kidney disease (CKD) is a serious public health problem of reportedly epidemic proportions [1]. From 10% to 16% of adults in Asia [2], Australia [3], Europe [4] and the United States [5] are reported to have CKD. In the 2002 Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation defined CKD as the presence of kidney damage for more than three months with or without a decrease in glomerular filtration rate (GFR) [6]. Applying the classification and stratification of CKD described in K/DOQI guidelines points to large numbers of older adults with stage 3 CKD as the primary population contributing to increasing prevalence of the disease [1, 4]. Compiling data on estimated GFR (eGFR) and albuminuria from 46 cohorts, the Chronic Kidney Disease Prognosis Consortium (CKD-PC) evaluated the prognostic impact of these two measures of kidney function and indicated that 1) the full range of GFR and albuminuria contributes to increased risk of CKD, 2) low eGFR and high albuminuria confer clinical risk independently, and 3) these results are consistent across populations [7]. Such uniform analytic evidence is valuable for evaluating all-cause, cardiovascular and end-stage renal disease (ESDR) mortality and the impact of CKD on populations. However, outside of baseline kidney measures such as eGFR and urine albumin-to-creatinine ratios, the effectiveness of other CKD-linked parameters such as homocysteine and C-reactive protein in evaluating CKD risk in adult populations remains unclear.

Homocysteine is a sulfur-containing amino acid formed during the metabolic process of the essential amino acid, methionine [8]. Previous epidemiological studies have reported associations between homocysteine and atherosclerotic vascular disease [8]. Homocysteine may induce endothelial injury, decrease levels of adenosine in plasma and interstitial tissue, and induce proliferation and apoptosis of glomerular mesangial cells through effects of produced reactive oxygen species in vascular smooth muscle cells, which leads directly to renal vascular injury [8-11]. A few studies have also suggested that homocysteine may be a predictor of CKD [12, 13]. CRP has been shown to be an important predictor of future cardiovascular events in patients with cardiovascular disease and in an apparently healthy population [14, 15]. This acute phase protein also appears to play a positive role in the pathophysiology of atherosclerosis [16]. CRP is a pentraxin, an innate immunity effector protein that is typically synthesized by the liver after stimulation by cytokines, including IL-1, IL-6, and tumor necrosis factor [17]. It may also be generated by other types of cells, including smooth muscle cells [18], kidney cells [19], and fat cells [20]. Moderate to severe CKD patients with a CRP of more than 3 mg / L have a 90% increased independent risk of cardiovascular disease [21]. In that study, CRP and albumin were both predictive of all-cause and cardiovascular mortality in CKD. Few studies have investigated possible associations between plasma levels of homocysteine and CRP and risk of developing CKD. Further, the connecting mechanism between homocysteine and CRP is not fully understood and gender differences associated with the two factors are not known. In addition, physical examinations do not typically measure albuminuria as an indicator of CKD, and other indicators should be identified. Homocysteine and CRP are both associated with vascular inflammation and this association with large vessels has been confirmed in cardiovascular disease, stroke/ cardiovascular accident, diabetes mellitus, hypertension and metabolic syndrome. We hypothesized that homocysteine and CRP would correlate positively with the number of CKD cases, that they may be potential surrogate markers for increased risk of developing CKD, and that possible gender differences may be associated with these factors. Therefore, in this study, we aimed to test the above hypotheses in the adult population in Taiwan.

Patients and Methods

Study sample

This study was approved by the institutional review board of Chang Gung Memorial Hospital. Data of 22,043 Taiwanese adults older than age 18 years who had undergone annual physical examinations at Chang-Gung Memorial Hospital in Linkou (northern Taiwan) and Chiayi and Kaohsiung (southern Taiwan)

between 2005 and 2011 were collected for retrospective analysis. All subjects had participated in annual physical examinations organized by their companies or communities. Included subjects had complete standard physical examination records, including smoking and drinking history, personal medical and medication history, pregnancy status at the time of examination, fasting times and fasting levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), fasting plasma glucose (FPG), homocysteine and high-sensitivity C-reactive protein (hs-CRP). Subjects were excluded if they had incomplete examination records, had fasting time less than 12 hours, were pregnant (which might affect waist circumference and levels of various indicators of metabolic syndrome), were taking diuretics or ACEI, or if chronic diseases were present that may significantly affect metabolism or body composition, including thyroid function abnormalities, tumor, resection, chronic hepatitis, cirrhosis, pituitary disease or adrenal disease.

Data collection

To obtain data of annual physical examinations, we first filed an application to collect the 2005-2011 data from each physical examination center that met the inclusion criteria (adults aged >18 years and complete record of examination). After excluding participants according to criteria above, statistical analysis was carried out. Subjects were stratified by gender and males and females were divided into four groups each by quartiles of homocysteine and hsCRP levels. Data included smoking and drinking history, personal medical history, medication history, pregnancy, and fasting times, which had been entered into the uniform physical examination forms of Chang Gung Memorial Hospital by the subjects themselves with the help of trained nurses at the time of physical examination. Data were inputted into computers and verified by nurses to establish personal history data of physical examination.

Anthropometric and biochemical measures

Anthropometric measurements, including height/weight and waist circumference, were measured on the same day as the physical examination, which included measuring blood pressure and biochemical parameters. All patients were fasted for > 12 hours prior to blood sample collection. Whole blood samples were collected from all included patients while they were in a sitting position, not supine. At least 2ml of whole blood was drawn into BD Vacutainer™ Plastic Blood Collection Tubes (Thermo Fischer Scientific, Hampton, NH, USA) containing K₂EDTA anticoagulant, which were immediately centrifuged and plasma was separated into aliquots for all assays. The resulting plasma samples were stored at 4 °C and sent to the clinical laboratory of the hospital for assay analyses to be performed as soon as possible, not exceeding within one week; hemolyzed samples were not used and corresponding patients were excluded from the study. Valid residual plasma samples were held for up to 20 days for repeat analysis if necessary.

Definitions and calculations

The eGFR calculation used the improved MDRD formula for Chinese subjects developed at Peking University by Ma et al. [22]: $eGFR = 175 \times (Scr)^{-1.234} \times (age)^{-0.179} \times (0.79 \text{ female})$. CKD staging was determined by eGFR and proteinuria based on the Kidney Disease Outcomes Quality Initiative (K/DOQI) definition (Ref). Specifically, CKD stage 1 was defined as an eGFR ≥ 90 mL/min/1.73 m² with kidney damage (proteinuria); stage 2 was an eGFR of 60–89 mL/min/1.73 m² with proteinuria; stage 3 was an eGFR of 30–59 mL/min/1.73 m²; stage 4 was an eGFR of 15–29 mL/min/1.73 m²; and stage 5 was an eGFR < 15 mL/min/1.73 m². Subjects were excluded if the eGFR was < 15 mL/min/1.73 m² or classified as stage 5 (because hemodialysis could affect eGFR). The patients with CKD stage 1~4 were included in this study because proteinuria was tested. Metabolic syndrome was determined according to the criteria of the Third Adult Treatment Panel of the National Cholesterol Education Program (NCEP/ATPIII) [23]. CKD was defined according to the K/DOQI definitions, classification and stratification [6].

Equipment and reagents

Homocysteine and hsCRP were measured on the Hitachi 7600 Modular Chemistry Analyzer (Hitachi, Tokyo, Japan) with corresponding reagents (Sekisui Inc., Osaka, Japan). Urine parameters were measured from 2005-2009 on the Urisys 2400 Automated UA analyzer (Roche Diagnostics, Basel, Switzerland, and from 2009 to end study date by the Siemens Atlas Urinalysis Analyzer (Siemens, Munich, Germany). Other laboratory parameters (i.e., HDL-C, TG, FPG) were also measured on the Hitachi 7600 Modular Chemistry Analyzer (Hitachi, Tokyo, Japan).

Table 1. Demographic and clinical characteristics of study subjects stratified by gender

	Males (n=14,874)	Females (n=4,498)	P-value
Age, years	37.0 (34.0,43.0)	35.0 (32.0,41.0)	<0.001*
BMI, kg/m ²	24.5 (22.5,26.7)	21.3 (19.6,23.7)	<0.001*
Waist-to-height ratio	0.49 (0.46,0.52)	0.45 (0.42,0.49)	<0.001*
Mean arterial pressure, mmHg	94.0 (87.7,101.0)	83.3 (77.3,90.0)	<0.001*
Fasting glucose, mg/dL	90.0 (85.0,95.9)	87.0 (83.0,92.0)	<0.001*
Total cholesterol, mg/dL	185.0 (165.0,207.0)	173.0 (156.0,195.0)	<0.001*
HDL cholesterol, mg/dL	49.0 (42.0,56.0)	60.0 (52.0,69.0)	<0.001*
Triglycerides, mg/dL	108.0 (77.0,157.0)	70.0 (55.0,96.0)	<0.001*
Metabolic syndrome	2401 (16.1)	308 (6.8)	<0.001*
Plasma homocysteine, umol/L	10.4 (8.9,12.4)	7.8 (6.7,9.2)	<0.001*
Plasma hsCRP, µg/mL	0.9 (0.5,1.8)	0.6 (0.3,1.4)	<0.001*

Continuous variables are presented as medians and IQRs as examined by Mann-Whitney U test; categorical variables are expressed as counts and percentages as examined by Chi-square test. * indicates a significant difference between results of males and females

Statistical analysis

Continuous variables are presented as median and interquartile range (IQR, the range between the 25th and 75th percentile) according to non-normal distribution. Categorical variables are expressed as count and percentage. For comparisons between different genders, the Mann-Whitney U test was used to examine continuous variables and the Chi-square test was used to examine categorical variables. For comparisons between the four homocysteine or hsCRP groups, the Kruskal-Wallis test was used to examine continuous variables and the Chi-square test was used to examine categorical variables. When significant differences were noted between groups, multiple comparisons were performed using the Bonferroni procedure with type-I error adjustment. Point estimates and 95% confidence intervals (CIs) of crude and adjusted odds ratios (ORs) were calculated by univariate and multivariate logistic regression models. Quartile distributions of hsCRP and homocysteine were used for further analysis; the 1st and 2nd quartiles were defined as “low hsCRP” and “low homocysteine,” and the 3rd and 4th quartiles were defined as “high hsCRP” and “high homocysteine.” The Chi-square test was used to examine associations between hsCRP / homocysteine and CKD. All statistical analyses were performed using SAS software package, version 9.2 (SAS Institute Inc., Cary, NC, USA). All statistical assessments were evaluated at a two-sided α level of 0.05.

Results

Of the 22,043 subjects (≥ 18 years) whose data were collected from records of routine physical examinations, 2,671 subjects were excluded and 19,372 subjects were finally retained for analysis, including 14,874 males with median age of 37.0 (IQR: 34.0, 43.0) years and 4,498 females with median age of 35.0 (IQR: 32.0, 41.0) years.

Subjects' demographic and clinical characteristics

Significant differences were shown between males and females in all characteristics. Males had higher age, BMI, waist-to-height ratio, mean arterial pressure, FPG, TC, TG, homocysteine and hsCRP than females, while HDL-C was lower in males than females. The proportion of subjects with metabolic syndrome was significantly higher in males than in females (16.1% vs. 6.8%, $P < 0.001$) (Table 1).

Male subjects' characteristics analyzed by homocysteine and hsCRP levels

Significant differences were observed between low-to-high homocysteine groups in age, smoking, BMI, mean arterial pressure, FPG, TC, metabolic syndrome, eGFR and CKD distribution (Table 2). Mean arterial pressure and eGFR increased as homocysteine levels

Table 2. Baseline characteristics of male study subjects by quartiles of plasma homocysteine and hsCRP

	Homocysteine				p-value	hsCRP				p-value
	1 st quartile (≤ 8.19 umol/L) (n=2,048)	2 nd quartile (8.20~9.84 umol/L) (n=3,891)	3 rd quartile (9.85~11.81 umol/L) (n=4,297)	4 th quartile (≥ 11.82 umol/L) (n=4,638)		1 st quartile (≤ 0.41 μg/mL) (n=3,114)	2 nd quartile (0.42~0.81 μg/mL) (n=3,806)	3 rd quartile (0.82~1.66 μg/mL) (n=4,026)	4 th quartile (≥ 1.67 μg/mL) (n=3,928)	
Age, years	36.0 (33.0, 42.0)	37.0 (33.0, 43.0) †	37.0 (34.0, 43.0) †	37.0 (34.0, 44.0) ††	<0.001*	36.0 (33.0, 41.0)	37.0 (34.0, 43.0) †	38.0 (34.0, 44.0) †	38.0 (34.0, 44.0) ††	<0.001*
Smoking					<0.001*					0.714
Former smoker	190 (9.3)	342 (8.8)	378 (8.8)	340 (7.3)		238 (7.6)	321 (8.4)	358 (8.9)	333 (8.5)	
Current smoker	523 (25.5)	977 (25.1)	1156 (26.9)	1427 (30.8)		863 (27.7)	1037 (27.2)	1103 (27.4)	1080 (27.5)	
BMI, kg/m ²	24.3 (22.4, 26.4)	24.4 (22.4, 26.7)	24.5 (22.5, 26.7)	24.6 (22.5, 26.9) †	0.009*	22.6 (20.9, 24.3)	24.0 (22.3, 25.8) †	25.1 (23.2, 27.2) ††	26.1 (23.9, 28.8) ††§	<0.001*
Waist-to-height ratio	0.49 (0.46, 0.52)	0.49 (0.46, 0.52)	0.49 (0.46, 0.52)	0.49 (0.46, 0.53)	0.483	0.46 (0.43, 0.49)	0.48 (0.45, 0.51) †	0.50 (0.47, 0.53) ††	0.52 (0.48, 0.55) ††§	<0.001*
Mean arterial pressure, mmHg	92.3 (85.7, 98.7)	92.7 (87.0, 99.7) †	94.0 (87.7, 101.0) ††	95.7 (89.0, 102.7) ††§	<0.001*	91.3 (85.3, 97.7)	93.0 (87.0, 99.7) †	94.3 (88.0, 101.3) ††	96.3 (89.3, 103.7) ††§	<0.001*
Fasting glucose, mg/dL	90.0 (85.0, 95.0)	90.0 (85.0, 95.0)	90.0 (85.0, 96.0)	91.0 (85.7, 96.0) ††	<0.001*	89.0 (85.0, 94.0)	90.0 (85.0, 95.0) †	90.0 (85.0, 96.0) †	91.0 (86.0, 97.0) ††§	<0.001*
Total cholesterol, mg/dL	183.0 (164.0, 205.5)	184.0 (165.0, 205.0)	185.0 (165.0, 208.0)	186.0 (166.0, 208.0) ††	0.005*	177.0 (158.0, 198.0)	183.0 (164.0, 204.0) †	189.5 (169.0, 210.0) ††	189.0 (169.0, 212.0) ††	<0.001*
HDL cholesterol, mg/dL	48.0 (42.0, 56.0)	48.0 (42.0, 56.0)	49.0 (42.0, 56.0)	49.0 (43.0, 56.0)	0.292	54.0 (47.0, 61.0)	50.0 (44.0, 57.0) †	48.0 (42.0, 54.0) ††	45.0 (40.0, 52.0) ††§	<0.001*
Triglycerides, mg/dL	108.0 (78.0, 156.0)	106.0 (76.0, 156.0)	107.0 (77.0, 156.0)	110.0 (78.0, 159.0)	0.064	86.5 (65.0, 119.0)	105.0 (75.0, 145.0) †	118.0 (85.0, 169.0) ††	125.0 (88.0, 179.0) ††§	<0.001*
Metabolic syndrome	296 (14.5)	602 (15.5)	701 (16.3)	802 (17.3)	0.017*	144 (4.6)	381 (10.0)	744 (18.5)	1132 (28.8)	<0.001*
eGFR, ml/min/1.73 m ²	114.6 (98.0, 131.2)	120.5 (104.1, 136.8) †	129.0 (110.5, 145.4) ††	135.3 (118.6, 152.8) ††§	<0.001*	130.4 (111.7, 147.9)	127.8 (108.8, 145.4) †	126.4 (108.0, 144.2) †	125.5 (107.8, 143.6) †	<0.001*
Proteinuria					0.091					<0.001*
(-)/trace	2008 (98.0)	3801 (97.7)	4188 (97.5)	4494 (96.9)		3072 (98.7)	3745 (98.4)	3925 (97.5)	3749 (95.4)	
1+	30 (1.5)	65 (1.7)	85 (2.0)	99 (2.1)		35 (1.1)	48 (1.3)	70 (1.7)	126 (3.2)	
2+	8 (0.4)	18 (0.5)	13 (0.3)	27 (0.6)		4 (0.1)	9 (0.2)	19 (0.5)	34 (0.9)	
≥3+	2 (0.1)	7 (0.2)	11 (0.3)	18 (0.4)		3 (0.1)	4 (0.1)	12 (0.3)	19 (0.5)	
Chronic kidney disease	40 (2.0)	90 (2.3)	109 (2.5)	144 (3.1)	0.024*	42 (1.3)	61 (1.6)	101 (2.5)	179 (4.6)	<0.001*

Continuous variables are presented as medians and IQRs as determined by Kruskal-Wallis test; categorical variables are expressed as counts and percentages as determined by Chi-square test. * indicates significant differences among the four homocysteine/hsCRP quartile groups; † significant difference compared to 1st quartile group; † significant difference compared to 2nd quartile group; § significant difference compared to 3rd quartile group.

Continuous variables are presented as medians and IQRs as determined by Kruskal-Wallis test; categorical variables are expressed as counts and percentages as determined by Chi-square test. * indicates significant differences among the four homocysteine/hsCRP quartile groups; † significant difference compared to 1st quartile group; †† significant difference compared to 2nd quartile group; ††§ significant difference compared to 3rd quartile group

increased. Ages in the 2nd, 3rd and 4th quartiles were significantly higher than in the 1st quartile, and ages in the 4th quartile group were significantly higher than those in the 2nd quartile group. BMI in the 4th quartile group was significantly higher than in the 1st quartile group. FPG and TC levels of the 4th quartile groups were significantly higher than those in the 1st and 2nd quartile groups. Significant differences were found among the four hsCRP groups in age, BMI, waist-to-height ratio, mean arterial pressure, FPG, TC, HDL-C, TG, metabolic syndrome, eGFR, proteinuria and CKD (Table 2). BMI, waist-to-height ratio, mean arterial pressure and TG increased with increased hsCRP levels, and HDL-C decreased with increased hsCRP levels. FPG levels in the 2nd, 3rd and 4th quartile groups were significantly higher than in the 1st quartile group, and significantly higher in the 4th quartile group than in the 2nd and 3rd quartile groups. TC in the 3rd and 4th quartile groups was significantly higher than in the 1st and 2nd quartile groups, and was significantly higher in the 2nd than in the 1st quartile group. The 2nd, 3rd and 4th quartile groups had significantly higher eGFR than the 1st quartile groups.

Female subjects' characteristics analyzed by homocysteine and hsCRP quartiles

Significant differences were observed between the four female homocysteine groups in age, smoking, mean arterial pressure, FPG, total cholesterol, HDL-C, metabolic syndrome, eGFR and CKD distribution (Table 3). Age in the 2nd quartile group was significantly higher than in the 1st quartile group. Mean arterial pressure and FPG in the 2nd, 3rd and 4th quartile groups were significantly higher than in the 1st quartile group. TC levels in the 2nd and 3rd quartile groups were significantly higher than in the 1st quartile group. HDL-C in the 2nd quartile group was significantly higher than in the 1st quartile group. The eGFR in the 3rd and 4th quartile groups was significantly higher than in the 1st and 2nd quartile groups, and significantly higher in the 2nd quartile than in the 1st quartile group. Significant differences were found between the four female hsCRP groups in age, BMI, waist-to-height ratio, mean arterial pressure, FPG, TC, HDL-C, TG, metabolic syndrome, eGFR, proteinuria and CKD distribution (Table 3). BMI, waist-to-height ratio, mean arterial pressure, FPG and TG increased with increased hsCRP levels, and HDL-C decreased with increased hsCRP levels. Ages in the 2nd, 3rd and 4th quartile groups were significantly higher than in the 1st quartile group, and age in the 3rd quartile group was significantly higher than in the 2nd quartile group. TC levels in the 2nd, 3rd and 4th quartile groups were significantly higher than in the 1st quartile group, and was significantly higher in the 4th quartile group than in the 2nd quartile group. The 2nd, 3rd and 4th quartile groups had significantly higher eGFR than the 1st quartile group.

Associations between homocysteine and CKD stratified by gender

The 4th quartile of homocysteine for males was significantly associated with elevated risk for developing CKD in univariate logistic regression Model 1 compared to the 1st quartile, but no associations were found between homocysteine and CKD in multivariate logistic regression Models 2 and 3. For females, the 4th quartile of homocysteine was significantly associated with elevated risk for developing CKD in all three models compared to the 1st quartile (Table 4).

Associations between hsCRP and CKD stratified by gender

For males, univariate logistic regression analysis (Model 1) showed that the two highest quartiles of hsCRP were significantly associated with elevated risk for developing CKD compared to the 1st quartile. However, after multivariate logistic regression analysis, after adjusting for age, smoking, waist-to-height ratio, and mean arterial pressure, only the 4th quartile of hsCRP was significantly associated with elevated risk for developing CKD. For females, univariate logistic regression analysis (Model 1) showed that the 4th quartile of hsCRP was significantly associated with elevated risk for developing CKD compared to the 1st quartile, but no associations were found between hsCRP and CKD in multivariate logistic regression Models 2 and 3 (Table 5).

Associations between combined homocysteine/CRP and CKD stratified by gender

For males, compared with low hsCRP/low homocysteine, the two groups with high

Table 3. Baseline characteristics of female study subject by quartiles of plasma homocysteine and hsCRP

	Homocysteine				hsCRP			
	1 st quartile (≤ 8.19 umol/L) (n=2,619)	2 nd quartile (8.20~9.84 umol/L) (n=1,128)	3 rd quartile (9.85~11.81 umol/L) (n=544)	4 th quartile (≥ 11.82 umol/L) (n=207)	1 st quartile (≤ 0.41 µg/mL) (n=1,707)	2 nd quartile (0.42~0.81 µg/mL) (n=1,046)	3 rd quartile (0.82~1.66 µg/mL) (n=817)	4 th quartile (≥ 1.67 µg/mL) (n=928)
Age, years	35.0 (32.0, 40.0)	36.0 (32.0, 42.0)†	35.0 (32.0, 41.5)	35.0 (31.0, 43.0)	34.0 (31.0, 39.0)	36.0 (32.0, 41.0)†	37.0 (33.0, 43.0)†	37.0 (32.0, 43.0)†
Smoking								
Former smoker	36 (1.4)	24 (2.1)	6 (1.1)	5 (2.4)	32 (1.9)	12 (1.1)	13 (1.6)	14 (1.5)
Current smoker	123 (4.7)	56 (5.0)	38 (7.0)	19 (9.2)	102 (6.0)	51 (4.9)	30 (3.7)	53 (5.7)
BMI, kg/m ²	21.4 (19.6, 23.7)	21.2 (19.5, 23.7)	21.2 (19.5, 23.6)	21.6 (19.3, 24.0)	20.1 (18.8, 21.5)	21.3 (19.8, 23.2)†	22.4 (20.7, 24.7)†	24.4 (21.5, 27.6)†
Waist-to-height ratio	0.45 (0.42, 0.49)	0.45 (0.41, 0.49)	0.44 (0.41, 0.48)	0.44 (0.41, 0.49)	0.42 (0.40, 0.45)	0.45 (0.42, 0.48)†	0.47 (0.44, 0.50)†	0.50 (0.45, 0.54)†
Mean arterial pressure, mmHg	82.7 (76.7, 89.0)	84.0 (78.0, 91.3)†	84.3 (78.3, 91.3)†	85.7 (79.7, 92.3)†	81.3 (76.3, 87.3)	82.3 (77.0, 89.7)†	84.7 (79.0, 90.7)†	87.0 (80.0, 95.7)†
Fasting glucose, mg/dL	87.0 (82.0, 92.0)	88.0 (83.0, 93.0)†	88.0 (84.0, 93.0)†	89.0 (84.0, 95.0)†	86.0 (82.0, 90.0)	87.0 (82.0, 92.0)†	88.0 (83.0, 93.0)†	89.0 (84.0, 95.0)†
Total cholesterol, mg/dL	172.0 (155.0, 193.0)	176.0 (157.0, 197.5)†	176.5 (158.0, 199.0)†	174.0 (152.0, 193.0)	168.0 (152.0, 188.0)	174.0 (156.0, 194.0)†	177.0 (158.0, 198.0)†	180.0 (161.0, 204.0)†
HDL cholesterol, mg/dL	60.0 (52.0, 68.0)	61.0 (52.0, 70.0)†	60.5 (52.0, 70.0)	60.0 (51.0, 70.0)	64.0 (56.0, 73.0)	61.0 (53.0, 69.0)†	57.0 (49.0, 65.0)†	54.5 (47.0, 62.0)†
Triglycerides, mg/dL	70.0 (54.0, 96.0)	69.0 (54.0, 92.0)	71.0 (56.0, 96.0)	73.0 (57.0, 101.0)	61.0 (49.0, 76.0)	71.0 (55.0, 93.0)†	79.0 (61.0, 109.0)†	89.0 (64.0, 128.0)†
Metabolic syndrome	153 (5.8)	101 (9.0)	36 (6.6)	18 (8.7)	8 (0.5)	37 (3.5)	77 (9.4)	186 (20.0)
eGFR, ml/min/1.73 m ²	72.7 (57.2, 88.0)	82.9 (65.5, 98.9)†	93.0 (73.5, 106.7)†	95.4 (79.6, 111.3)†	82.7 (66.6, 98.1)	76.4 (60.0, 94.4)†	74.9 (56.1, 93.0)†	74.0 (57.0, 91.7)†
Proteinuria								
(-)/trace	2554 (97.5)	1101 (97.6)	536 (98.5)	196 (94.7)	1667 (97.7)	1026 (98.1)	804 (98.4)	890 (95.9)
1+	53 (2.0)	24 (2.1)	4 (0.7)	9 (4.3)	33 (1.9)	18 (1.7)	12 (1.5)	27 (2.9)
2+	9 (0.3)	2 (0.2)	2 (0.4)	2 (1.0)	5 (0.3)	2 (0.2)	1 (0.1)	7 (0.8)
≥3+	3 (0.1)	1 (0.1)	2 (0.4)	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	4 (0.4)
Chronic kidney disease	65 (2.5)	27 (2.4)	8 (1.5)	11 (5.3)	40 (2.3)	20 (1.9)	13 (1.6)	38 (4.1)
Continuous variables are presented as medians and IQRs as determined by Kruskal-Wallis test; categorical variables are expressed as counts and percentages as determined by Chi-square test. * indicates significant differences among the four homocysteine/hsCRP quartile groups; † significant difference compared to 1 st quartile group; ‡ significant difference compared to 2 nd quartile group; § significant difference compared to 3 rd quartile group								

Table 4. Association analyses between quartiles of plasma homocysteine and chronic kidney disease stratified by gender

	Model 1		Model 2		Model 3	
	Crude OR (95%CI)	p value	Adjusted OR (95%CI)	p value	Adjusted OR (95%CI)	p value
Males						
1 st Quartile	1		1		1	
2 nd Quartile	1.19 (0.82,1.73)	0.370	1.12 (0.77,1.64)	0.549	1.19 (0.81,1.75)	0.388
3 rd Quartile	1.31 (0.91,1.88)	0.153	1.14 (0.79,1.65)	0.487	1.24 (0.85,1.81)	0.263
4 th Quartile	1.61 (1.13,2.29)	0.009*	1.28 (0.89,1.83)	0.185	1.41 (0.97,2.03)	0.071
Females						
1 st Quartile	1		1		1	
2 nd Quartile	0.96 (0.61,1.52)	0.873	0.98 (0.62,1.55)	0.936	0.95 (0.6,1.51)	0.830
3 rd Quartile	0.59 (0.28,1.23)	0.158	0.58 (0.28,1.22)	0.152	0.58 (0.27,1.22)	0.149
4 th Quartile	2.21 (1.15,4.25)	0.018*	2.17 (1.12,4.21)	0.022*	2.15 (1.1,4.18)	0.025*

Values expressed as OR (95% CI). Model definitions are: Model 1, crude odds ratios; Model 2, adjusted for age, smoking, waist-to-height ratio, and mean arterial pressure; Model 3, Model 2+ fasting glucose level, HDL cholesterol level, triglycerides level. * indicated an association between homocysteine and chronic kidney disease

Table 5. Association analyses between quartiles of plasma hsCRP and chronic kidney disease stratified by gender

	Model 1		Model 2		Model 3	
	Crude OR (95%CI)	p value	Adjusted OR (95%CI)	p value	Adjusted OR (95%CI)	p value
Males						
1 st Quartile	1		1		1	
2 nd Quartile	1.19 (0.80,1.77)	0.386	0.92 (0.62,1.38)	0.691	0.95 (0.63,1.42)	0.799
3 rd Quartile	1.88 (1.31,2.71)	0.001*	1.22 (0.84,1.77)	0.303	1.22 (0.84,1.79)	0.296
4 th Quartile	3.49 (2.49,4.9)	<0.001*	1.79 (1.24,2.59)	0.002*	1.74 (1.19,2.53)	0.004*
Females						
1 st Quartile	1		1		1	
2 nd Quartile	0.81 (0.47,1.40)	0.453	0.83 (0.48,1.44)	0.514	0.85 (0.49,1.48)	0.559
3 rd Quartile	0.67 (0.36,1.27)	0.220	0.68 (0.35,1.31)	0.248	0.66 (0.34,1.28)	0.218
4 th Quartile	1.78 (1.13,2.79)	0.012*	1.65 (0.97,2.81)	0.063	1.52 (0.88,2.64)	0.137

values expressed as OR (95% CI). Model definitions are: Model 1, crude odds ratios; Model 2, adjusted for age, smoking, waist-to-height ratio, and mean arterial pressure; Model 3, Model 2+ fasting glucose level, HDL cholesterol level, triglycerides level. * indicated an association between hsCRP and chronic kidney disease

hsCRP were significantly associated with elevated risk for developing CKD in multivariate logistic regression Models 1 and 2. However, in Model 3, only high hsCRP/high homocysteine was significantly associated with elevated risk for developing CKD. For females, no association was shown between the combination of homocysteine and C-reactive protein and CKD in all models (Table 6).

Discussion

The present study is the first to show that homocysteine and CRP are positively associated with CKD and that the associations include gender differences. Based on the main findings of this multicenter study, we concluded that plasma homocysteine is associated with CKD in females and plasma hsCRP is associated with CKD in males. Notable also was that, as values of homocysteine increased across quartile groups, eGFR also increased. However, eGFR did not increase with CRP quartile groups. The gender effect was in evidence starting with CKD distribution. In females, the highest level of homocysteine was significantly associated with elevated risk for developing CKD in all three models when compared to the lowest level. In males, the associations were not found between homocysteine and CKD in multivariate logistic regression. The situation was reversed with CRP relationships. When homocysteine and CRP were combined and stratified by gender, in males, compared with

Table 6. Association analyses between the combination of homocysteine and C-reactive protein and chronic kidney disease stratified by gender

	Model 1		Model 2		Model 3	
	Crude OR (95%CI)	p value	Adjusted OR (95%CI)	p value	Adjusted OR (95%CI)	p value
Males						
Low hsCRP/Low homocysteine	1		1		1	
Low hsCRP/High homocysteine	1.26 (0.84,1.91)	0.266	1.10 (0.72,1.66)	0.662	1.12 (0.74,1.70)	0.600
High hsCRP/Low homocysteine	2.31 (1.56,3.43)	<0.001*	1.52 (1.01,2.28)	0.043*	1.43 (0.94,2.15)	0.091
High hsCRP/High homocysteine	3.16 (2.18,4.57)	<0.001*	1.75 (1.20,2.57)	0.004*	1.75 (1.19,2.58)	0.005*
Females						
Low hsCRP/Low homocysteine	1		1		1	
Low hsCRP/High homocysteine	1.18 (0.62,2.24)	0.608	1.17 (0.61,2.22)	0.641	1.17 (0.62,2.24)	0.624
High hsCRP/Low homocysteine	1.42 (0.94,2.15)	0.095	1.30 (0.82,2.05)	0.262	1.21 (0.75,1.94)	0.430
High hsCRP/High homocysteine	1.24 (0.55,2.76)	0.603	1.10 (0.47,2.55)	0.825	1.01 (0.43,2.37)	0.988

Values expressed as OR (95% CI). Model definitions are: Model 1, crude odds ratios; Model 2, adjusted for age, smoking, waist-to-height ratio, and mean arterial pressure; Model 3, Model 2+ fasting glucose level, HDL cholesterol level, triglycerides level. * indicated an association between the combination of homocysteine and C-reactive protein and chronic kidney disease.

low hsCRP/low homocysteine, the higher/highest hsCRP/homocysteine combination was significantly associated with elevated risk for developing CKD in multivariate logistic regression models. However, after adjusting for confounders only the high hsCRP/high homocysteine combination was significantly associated with elevated CKD risk. In females, however, no association was shown between the combination of homocysteine and CRP and CKD across all models. Clinically, these results indicate that attention should be paid to renal function changes in males with concurrent high homocysteine and CRP, although not females in whom no association was shown between homocysteine/CRP and CKD.

Although associations between baseline CKD and risk of developing cardiovascular disease have been reported in community-based studies [24], it has been uncertain whether cardiovascular disease is a risk factor for CKD or its progression. In one community-based study, cardiovascular disease was independently associated with subsequent decline in kidney function and eventual development of CKD using serum creatinine levels and eGFR to assess kidney function [12]. In cardiovascular disease, CRP appears to be a marker for early inflammatory processes related to high lipid concentrations, which may predispose the kidney to glomerular hyperfiltration-related renal function loss [25]. Another study suggested that cardiovascular risk factors, including homocysteine levels, explain attributable mortality risk in CKD [26]. In a review of 85 studies, all but three supported the link between CKD and cardiovascular risk based on varying GFR cut-off points and varying definitions of cardiovascular disease; however, the impact of kidney dysfunction was undeniable [27]. These findings indicate that CKD and cardiovascular disease may be bidirectional, which adds credence to evidence from our study and previous studies [17, 28] that homocysteine and CRP could possibly be biomarkers for both diseases.

CRP is a reliable biomarker for systemic inflammation and nephrologists consider CRP to be a predictor of overall mortality in CKD [17]. Plasma CRP actually divides CKD patients into different subgroups related to progression [17], as our study also indicated. CRP has been reported to be associated with atherogenesis through macrophage receptor concentration, which is associated with vascular risk; this suggests that CRP could be either a cause or a consequence of cardiovascular disease [28]. Elevated urinary albumin levels are associated with CRP in diabetic and non-diabetic subjects and both markers are associated with many of the same risk factors. CRP and microalbuminuria have been proposed as independent predictors of cardiovascular disease and are suggested to be independent correlates of CKD as well [25].

Nephrology patients, including those with CKD and ESRD, have elevated levels of homocysteine [9, 10], as also shown in the present study. The overall negative impact of high homocysteine levels has been demonstrated, especially in ESRD patients [29]. However, when the prognostic value of homocysteine was evaluated in long-term follow-up of ESRD

patients, homocysteine levels could not predict major adverse cardiovascular events [30]. A meta-analysis of 41 studies reporting correlations between GFR and homocysteine plasma levels showed that homocysteine levels significantly depend on renal function, but before homocysteine is established as an independent risk factor for CVD, accurate adjustments for renal function were essential [31]. The present study evaluated data retrospectively from a one-time examination and, although cardiovascular events were not included, cardiac parameters such as mean arterial pressure and metabolic syndrome were associated with increasing homocysteine levels across four quartiles. In a large community-based study, elevated homocysteine levels were shown to be a significant risk factor for development of CKD in the general population [13], which corresponds with our study purpose and agrees with our results. On the other hand, Sarnak et al. [32] found that elevated homocysteine levels and other factors associated with atherosclerosis (i.e., cysteine, folate, B vitamins) were not independent risk factors for progression of nondiabetic kidney disease. However, adjusting for renal function eliminates the relationship between homocysteine and vascular risk, supporting the idea that elevated homocysteine is a marker for renal impairment rather than a risk factor for cardiovascular disease [33].

The application of cardiovascular biomarkers in CKD is reasonable given that inflammation and vascular calcification are prevalent in both diseases and cardiovascular disease is the major cause of mortality in CKD. However, no single outstanding cardiovascular biomarker has been established as providing sufficient prognostic information to identify risk for CKD. Currently, detecting CKD in the general population depends on multiple measures such as albuminuria, sequential monitoring of GFR and evaluation of clinical risk conditions [1]. A review of cardiovascular biomarkers that could potentially be used for evaluating CKD risk suggests that a multimarker approach, including markers of inflammation such as CRP combined with markers of vascular calcification (e.g., interleukin-6 [IL-6], soluble receptor of advance glycation end products [sRAGE], osteoprotegerin [OPG] and fibroblast growth factor 23 [FGF-23]) provides a better stratification of risk [34]. Another review suggested that among emerging biomarkers for CKD, CRP was the only one that met the methodology requirements to be recommended for clinical practice [35]. When seven circulating biomarkers were evaluated in more than 2000 subjects, homocysteine was significantly associated with CKD incidence and microalbuminuria [36]. In accord with results of the present study, it appears that CRP and homocysteine are potential biomarkers that could shed light on pathways associated with development of CKD and possible therapeutic targets, but that clinical trials are necessary to establish their utility as biomarkers for CKD.

Strength and limitations

The strengths of the study include its large sample (approximately 20,000 people) and stratification by gender to indicate possible gender differences. In addition, data included smoking and drinking habits, medical and medication history; exclusion of participants with medication and medical history with possible impact on renal function; and reliability of our blood sample processing (i.e., separated immediately and stored at 4°C and same-day laboratory analysis). Also, proteinuria was examined and participants with possible stages 1 and 2 CKD were included for analysis. On the other hand, this study has certain limitations. First, the use of cross-sectional design limits making causal relationships between homocysteine, CRP and CKD. Secondly, data were retrospectively analyzed using a secondary database and only data from one physical examination were used to evaluate CKD in study subjects, while CKD is usually identified by K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification, which defines CKD as presence of kidney damage or GFR of $<60\text{mL/min/1.73m}^2 \geq 3$ months [6]. Menopause data were not collected so any impact is unknown. Also, herbal medicines were long term by some participants and possible effects on renal function are not known. Future study should involve prospective case-control evaluation of a large sample of adults in the general population in order to verify results of the present study and to help establish homocysteine and CRP as useful surrogate markers for elevated risk of CKD.

Conclusion

Among adults in Taiwan, plasma homocysteine is associated with CKD in females and plasma hsCRP is associated with CKD in males. The combination of high homocysteine and high hsCRP levels was significantly associated with an elevated risk for developing CKD in male. Our results suggest the usefulness of homocysteine and hsCRP as surrogate markers for evaluating CKD risk in the adult general population.

Conflict of Interests

The authors declared no conflict of interests in this study.

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