LABORATORY STUDY

Homocysteine as a predictive biomarker in early diagnosis of renal failure susceptibility and prognostic diagnosis for end stages renal disease

Hatem K. Amin\textsuperscript{a,b}, Mohamed-I Kotb El-Sayed\textsuperscript{a} and Ola F. Leheta\textsuperscript{c}

\textsuperscript{a}Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University, Cairo, Egypt; \textsuperscript{b}Cell Cycle Control Group, Center for Chromosome Biology, National University of Ireland, Galway, Ireland; \textsuperscript{c}Department of Clinical Pathology, Faculty of Medicine, University Hospital, Suez Canal University, Ismailia, Egypt

ABSTRACT

Glomerular filtration rate and/or creatinine are not accurate methods for renal failure prediction. This study tested homocysteine (Hcy) as a predictive and prognostic marker for end stage renal disease (ESRD). In total, 176 subjects were recruited and divided into: healthy normal group (108 subjects); mild-to-moderate impaired renal function group (21 patients); severe impaired renal function group (7 patients); and chronic renal failure group (40 patients) who were on regular hemodialysis. Blood samples were collected, and serum was separated for analysis of total Hcy, creatinine, high sensitive C-reactive protein (CRP), serum albumin, and calcium. Data showed that Hcy level was significantly increased from normal-to-mild impairment then significantly decreases from mild impairment until the patient reaches severe impairment while showing significant elevation in the last stage of chronic renal disease. Creatinine level was increased in all stages of kidney impairment in comparison with control. CRP level was showing significant elevation in the last stage. A significant decrease in both albumin and calcium was occurred in all stages of renal impairment. We conclude Hcy in combination with CRP, creatinine, albumin, and calcium can be used as a prognostic marker for ESRD and an early diagnostic marker for the risk of renal failure.

Introduction

The National Kidney Foundation, USA, Clinical Practice Guide for chronic kidney disease (CKD) divided the CKD into Stages I–V. Stage I and II CKD are characterized by normal or mildly reduced glomerular filtration rate (GFR) linked to markers of kidney damage, while Stages III–V GFR are below 60 mL/min/1.73 m\textsuperscript{2} with or without known etiology of kidney damage.\textsuperscript{1}

Cardiovascular disease (CVD) constitutes the leading cause of mortality in CKD patients,\textsuperscript{2} and the degree of reduction of GFR is an independent risk factor for CVD development. Moderate renal insufficiency (GFR <60 mL/min/1.73 m\textsuperscript{2}) will increase cardiovascular risk, while the risk is about 10–20 times higher in dialysis patients and end-stage renal disease (ESRD).\textsuperscript{3–5}

In a comprehensive review regarding the diagnosis and prognosis of CKD, the authors Fassett et al.\textsuperscript{6} stated there is no single biomarker can predict CKD progression when encompassing all primary renal diseases due to specific pathophysiological mechanisms linked to the primary renal diseases underpinning the diagnosis of CKD.\textsuperscript{6}

Homocysteine (Hcy) is a thiol-containing amino acid formed in the metabolic conversion of methionine to cysteine. Hcy undergoes either to be metabolized to cysteine by trans-sulfuration or converted back to methionine through re-methylation.\textsuperscript{7–9}

Deficiency of 5,10-methylenetetrahydrofolate reductase, the enzyme involved in folate-dependent re-methylation of Hcy to methionine, causes severe hyperhomocysteinemia (HHcy) and can result in several CVDs such as thrombotic disease and premature atherosclerosis.\textsuperscript{10–12} The multicenter European project including 750 patients and 800 controls revealed that HHcy represents an independent risk of developing occlusive arterial disease similar to smoking and hypercholesterolemia.\textsuperscript{13}

Hcy has an inflammatory response by enhancing the production of several preinflammatory cytokines. Mild-to-moderate HHcy has an oxidative stress and impair endothelium-dependent vasodilatation and could be overcome by vitamin B which indicates the involvement of reactive oxygen species (ROS). Misfolding of endoplasmic reticulum proteins could occur because Hcy can...
disrupt di-sulfide bond formation. In addition, Hcy increases the activity of factor V and inhibits protein C that enhances the coagulation process. Hcy can upregulate collagen, direct cell injury, growth arrest, and cellular methylation.\(^{14}\)

HHcy and increased oxidative stress with decreased superoxide dismutase enzyme antioxidant activity are independent contributing factors in the development of early-stage CKD.\(^{15}\)

C-reactive protein (CRP) is a plasma pentraxins protein family and comprised of five identical subunits of approximately 23 kDa. It is a major component in human acute-phase response due to its ability to activate the complement system and also because of its upregulation during tissue injury, inflammation, and infection cytokine-mediated response.\(^{16–18}\)

The binding of Ca\(^{2+}\) to CRP induces a large conformational hiding the exposed site of proteolysis.\(^{15–17}\) Two groups of ligands have been reported to interact with CRP: one group that requires Ca\(^{2+}\) such as phosphocholine-containing compounds and a second group whose binding is inhibited by Ca\(^{2+}\) such as polycation and fibronectin.\(^{17,19,20}\) CRP circulates in blood in a fully Ca\(^{2+}\)-loaded form with Ca\(^{2+}\) affinity of 0.06 mM. Consequently, the function of Ca\(^{2+}\) is both to coordinate the binding of phosphocholine and to stabilize the protein structure.\(^{21}\)

Aim of the current case–control study was using highly established markers with proven correlation and linearity such as homocysteine (representing circulatory impairments due to renal problems), creatinine (representing the renal excretion capacity), high sensitive C-reactive protein (representing inflammatory process within the kidney and the whole body because of renal problems), albumin (co-indicator for kidney function, dehydration, and inflammation due to renal problems), and calcium to test their capability to make an early prediction for renal failure and/or chronic renal disease prognosis.

**Patients and methods**

**Study design**

The tested markers were very well-established biomarkers; therefore, Priori test was applied with anticipated effect size of 0.6 and desired statistical power level of 0.8 to detect the statistical accepted minimum required total sample size for the main two groups namely: control group and patients group.

The numbers of recruited patient's subgroups were matching with similar studies conducted by Zhai et al.\(^{22}\), Fung et al.\(^{23}\), and Gilardini et al.\(^{24}\) who recruited 18, 39, and 85 subjects (cohorts), respectively, and concluded highly significant diagnostic and/or prognostic effect of copeptin as a marker for aneurysmal subarachnoid hemorrhage and glucose intolerance as a marker in obstructive sleep apnea and LAPTMB4 allele 2 as a risk factor associated with poor prognosis in patients with gallbladder carcinoma, respectively.\(^{22–24}\)

**Exclusion criteria**

All selected patients were free of diabetes, history of myocardial infarction or angina, and not administered any treatment for cancer or any autoimmune disease nor any steroidal antiinflammatory.

**Study limitations**

We did not consider any correction factors for the used medications such as antihypertensive, diuretics, and anti-hyperlipidemia and did not subgroup the recruited patients according to their blood pressure.

**Study groups**

In total, 176 subjects were recruited for the current study and divided into: Group 1: normal subjects (108 subjects) with mean age of 42.5 ± 12.2 (25 females and 83 males) with an average creatinine clearance (male: 105 mL/min; female: 88 mL/min) and no history of diabetes, hypertension, renal, or cardiac diseases. Group 2: Mild-to-moderate impaired renal function group: (21 patients) with mean age of 50.3 ± 20.3 (6 females and 15 males) with serum creatinine <1.5 mg/dL. Group 3: Severe impaired renal function group: (7 patients) with mean age of 50.3 ± 20.3 (4 females and 3 males) with serum creatinine <2.5 mg/dL. Group 4: chronic renal failure group: (40 patients) with mean age of 36.5 ± 16.3 years (21 females and 19 males) who were on regular hemodialysis therapy three times per week and creatinine clearance less than 10 mL/min.

**Laboratory investigations**

Blood samples (3 mL) were collected in plain tubes. Serum was separated and frozen at –20 °C until analysis of creatinine, total Hcy, high sensitive C-reactive protein (hs-CRP), serum albumin, and serum calcium. Urine was collected every hour, and uncorrected creatinine clearance was calculated using Cornell University, USA online calculator.

Serum creatinine (Cre) (normal: 0.6–1.2 mg/dL) and urine creatinine measured using two-point kinetic Jaffe reaction.\(^{25}\)
Total Hcy: normal values for male and female are 6–16 and 3.4–20.4 μmol/L, respectively and measured by fluorescence polarization immunoassay kit (Abbott Diagnostics, Abbott Park, IL) on the Abbott AxSYM analyzer (Abbott Diagnostics, Abbott Park, IL) by reducing bound oxidized Hcy form to free Hcy that is enzymatically converted to S-adenosyl-L-homocysteine. The AxSYM homocysteine assay file must be installed on the AxSYM system from the software disk, No. 7G53-01 or higher, prior to performing the assay. Six different calibrator concentrations were used (0, 2.5, 5, 10, 20, 50 μmol/L) and three controls (lows = 5.25–8.75, normals = 10–15, highs = 20–30 μmol/L) used if Hcy concentration is more than 50 μmol/L serum is diluted to 1:10 ratio by the zero calibrator.

hs-CRP: normal value is less than 3 μg/mL and measured by microplate immunoenzymometric sandwich technique using Accu-Bind ELISA micro wells using manufacturer protocol (Monobind Inc., Lake Forest, CA). The essential reagents required include high affinity and specificity antibodies (enzyme and immobilized) with different epitope recognition and native antigen. The immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CRP antibody.

Serum albumin was estimated by bromocresol green methods where albumin binds with bromocresol green dye in an acidic environment, causing an increase in absorbance at 630 nm, and the resulted increase in absorbance is directly proportional to the concentration of albumin in the sample.26

Serum calcium was estimated by cresol phthalein complex one reaction where cresol phthalein complex one reacts with calcium to form a purple-colored complex that causes an increase in absorbance at 570 nm which is directly proportional to the concentration of calcium in the sample.27

Statistical evaluation

Data were collected and analyzed using SPSS version 22 (IBM, USA), because all tested parameters are closely interacting; therefore, their statistical effect should be probed in-between each other, consequently one-way ANOVA was avoided. Multivariate test and “Tukey” post-hoc test were used to test subgroups homogeneity and the statistical significance of difference between subgroups means, and “F”-value tested the multivariate effect of investigated subjects using multivariate Pillai’s trace, Wilks’s lambda, Hoteling’s trace, and Roy’s largest root tests. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means. Observed difference was considered to be significant at \( p < 0.05 \).

Results

In brief, Figure 1(A–E) and Tables 1 and 2 illustrate the following: the early and middle stages of chronic renal disease (Stages I, II, III), the patients’ profiles showing significant elevated Hcy and Cre with significant decrease in albumin and calcium and normal value of CRP.

In ESRD, Hcy (with its sex independent unique pattern) significantly increased from normal-to-mild impairment then significantly decreases from mild impairment until the patient reaches severe impairment. Hcy level at severe impairment is insignificant in comparison with the Hcy level in control subjects.

In the late stage of chronic renal disease (Stage IV), the patients’ profiles show minor insignificant decrease in Hcy values compared to normal values in control group (changed from significant elevated level in early stages) accompanied with normal CRP values, more significant decrease in albumin and calcium and then more significant increase of Cre.

In the last stage of chronic renal disease (Stage V), the patients’ profiles show significant elevation in Hcy (elevated again after its major decrease in Stage IV) and significant elevation in CRP (only elevated in the last stage) accompanied with more significant increase in Cre, then more significant decrease in albumin while calcium decrease in male patients and illustrating significant increase in female patients.
Figure 1. Serum levels of Hcy (A), Cre (B), CRP (C), albumin (D), and calcium (E) in different studied groups. Data expressed as (M ± SE). *Compares all renal disease stages versus normal, †compares among severe renal impairment and renal failure versus mild–moderate, while #compares renal failure versus severe renal impairment. hs-CRP: high sensitive C-reactive protein; Hcy: homocysteine; Cre: Creatinine. ‭***, ‭###, ‭#### p < 0.001; ‭**, ‭†, ‭##, ‭# p < 0.01.
Clinical status versus renal failure. Mild–moderate renal impairment, while severe renal impairment.

hs-CRP: high sensitive C-reactive protein; Hcy: homocysteine.

Clinical status* Sex

| Clinical status | Sex | Variables | Sex | Homocysteine (µmol/L) | Creatinine (mg/dL) | hs-CRP (µg/mL) | Albumin (g/dL) | Calcium (mg/dL) |
|-----------------|-----|-----------|-----|-----------------------|-------------------|---------------|----------------|----------------|
| Normal (n = 83) | Male | 1.72 ± 0.49 | 0.99 ± 0.195 | 2.2024 ± 0.635 | 3.9747 ± 0.279 | 9.3145 ± 0.462 |
|                  | Female | 1.19 ± 0.29 | 0.85 ± 0.086 | 2.3880 ± 0.652 | 3.9680 ± 0.267 | 9.5000 ± 0.460 |
|                  | Total (n = 108) | 1.19 ± 0.29 | 0.85 ± 0.086 | 2.3880 ± 0.652 | 3.9680 ± 0.267 | 9.5000 ± 0.460 |
| Mild–moderate | Male (n = 15) | 1.7260 ± 0.456 | 1.7667 ± 0.255 | 3.1533 ± 1.073 | 3.9687 ± 0.379 | 8.9953 ± 0.531 |
| renal impairment | Female (n = 6) | 1.5033 ± 0.98 | 1.7500 ± 0.314 | 2.7500 ± 0.989 | 3.8667 ± 0.216 | 8.8333 ± 0.612 |
|                  | Total (n = 21) | 1.6638 ± 4.42 | 1.7619 ± 0.256 | 3.0381 ± 1.042 | 3.9524 ± 0.340 | 8.9476 ± 0.579 |
| Severe renal | Male (n = 3) | 8.8000 ± 2.96 | 2.6333 ± 0.230 | 3.1000 ± 1.153 | 3.4667 ± 0.305 | 8.5667 ± 0.450 |
| impairment | Female (n = 4) | 12.1250 ± 6.23 | 2.7500 ± 0.173 | 2.9750 ± 1.493 | 3.9250 ± 0.543 | 8.6500 ± 0.493 |
|                  | Total (n = 7) | 9.8429 ± 5.51 | 2.7000 ± 0.191 | 3.0281 ± 1.249 | 3.7286 ± 0.488 | 8.6143 ± 0.437 |
| Renal failure | Male (n = 19) | 25.7632 ± 18.06 | 9.0632 ± 3.29 | 13.4632 ± 12.64 | 3.5316 ± 0.457 | 9.1421 ± 0.744 |
|                  | Female (n = 21) | 20.7714 ± 25.00 | 8.7619 ± 2.12 | 16.8667 ± 23.14 | 3.5905 ± 0.499 | 8.6381 ± 0.857 |
|                  | Total (n = 40) | 23.1425 ± 14.39 | 10.950 ± 2.71 | 15.2500 ± 18.75 | 3.5625 ± 0.474 | 8.8775 ± 0.835 |

Data expressed as (M ± SE). *Compares all renal disease stages versus normal, ** Compares among severe renal impairment and renal failure versus mild–moderate, while # compares renal failure versus severe renal impairment. hs-CRP: high sensitive C-reactive protein; Hcy: homocysteine.

Table 1. Comparison of serum total Hcy, creatinine, hs-CRP, albumin, and calcium levels among normal group and different stages of renal impairment.

Discussion

According to the National Kidney Foundation, USA, GFR test via creatinine clearance is the differential diagnostic test for CKD Stages I–V, where GFR below 60 mL/min/1.72 m² indicates Stages III–V. Although creatinine is the most common biochemical test for kidney disease, it shows limitations in CKD differential diagnosis in particular for the first two stages and Stage IV with ESRD, and cannot predict the renal failure. Creatinine production is proportional to muscle mass and is higher in men than in women.

CKD is an inflammatory disease; therefore, inflammatory markers such as Hcy and hs-CRP in addition to serum albumin and serum calcium level were studied together in relation to serum creatinine. Kidney plays a major role in amino-thiol metabolism; thus, Hcy and S-adenosyl-homocysteine could be used as a mirror image for kidney sufficiency. The kidney contains more beta-adenosyl-homocysteine could be used as a mirror image for kidney sufficiency. The kidney contains more beta-

and methionine synthase (30 and 50%, respectively) in comparison with the liver. The kidney keeping constant Hcy amount returned to plasma through the compensation of changes in glomerular filtration by up- or down-regulating these biochemical pathways. Hcy is linearly correlated to creatinine, creatinine clearance, and uric acid suggesting that renal function is an important determinant of Hcy.

Serum albumin and hs-CRP are independent predictors of death in Tunisian renal patients; serum albumin can predict mortality in ESRD patients and this might confirm the suggestion that chronic inflammation may be the missing link or factor that usually ties hypo-albuminemia to morbidity and mortality.

Serum calcium was determined because it regulates the physiological functions of CRP by stimulating and inhibiting CRP–ligands interaction.

Vupputuri et al. tested CKD markers for differential estimation (diagnosis) and did not make or considered any classifications for the reasons or used drugs, and on the other hand Fung et al. evaluated the prognosis value of the tested marker; therefore, they mentioned the causes and their classification.

Hcy, CRP, creatinine, albumin, and serum calcium were measured, and their selection were based upon
the ESRD pathophysiology as these markers will cover the pathological changes due to inflammation, nitric oxide level change and its related endothelial stress, and the renal function efficiency.

The study outcome illustrated that some of the tested biomarkers are increasing by the deterioration of the chronic renal disease stage like creatinine and hs-CRP, and other biomarkers were decreasing like albumin and calcium then Hcy with its sex independent unique pattern where it increases in mild and moderate renal impairment then decreases while severe renal impairment is progressing then increases again upon renal failure (Table 3).

This interesting outcome of Hcy could be explained as Hcy resulted from irreversible trans-methylation from methionine via S-adenosyl-homocysteine (Figure 2) then undergoes irreversible trans-sulfuration in the presence of serine to yield cysteine which metabolites to sulfites. Hcy is eliminated from the body by reconverting it into methionine through irreversible re-methylation in the presence of tetrahydrofolate and betaine. Human kidney tissue can metabolize Hcy because it has Hcy trans-sulfuration and re-methylation enzymes.38

Half of betaine synthesis is in liver44 therefore, upon HHcy liver is compensating this elevation by expression of betaine-homocysteine methyltransferase until the depletion of the choline precursor and/or the depletion of the betaine-homocysteine methyltransferase enzyme and decreased betaine cellular transfer.43,45 This compensatory reaction resulted in significant decrease in plasma Hcy during severe stage of chronic renal disease through re-methylation to methionine. As a result of decrease of plasma Hcy, the transmethylation pathway increased and lead to high level of S-adenosyl-methionine which inhibits betaine-homocysteine methyltransferase enzyme expression in kidney46 which ends the compensatory mechanism to HHcy.

In kidney failure, patients showed elevated Hcy levels again which explained that kidney failure patients have elevated plasma levels of S-adenosyl-methionine, S-adenosyl-homocysteine, and low serine levels due to block in Hcy re-methylation47 which blocks the expression of the betaine-homocysteine methyltransferase enzyme in the kidney. In addition, in this study all recruited renal failure patients are under routine dialysis which is lowering the Hcy clearance and induce more HHcy.48

Hcy interesting unique change pattern throughout the disease progress could be used in correlation with other tested biomarkers for the diagnosis of the ESRD and prediction for the risk to undergo renal failure.

| Table 3. Schematic presentation for comparison of measured markers among different studied groups. |
|---------------------------------------------------------------|
| **Clinical status** | **Biochemical marker** | **Mild–moderate chronic renal disease stages (I, II, III)** | **Severe chronic renal disease stage (IV)** | **Severe chronic renal disease stage (V)** | **Renal failure** |
|---------------------------------------------------------------|
| **Hcy** | Normal | ↑ | ↓ | ↑ | ↑ |
| **CRP** | Normal | Normal | Normal | Normal | ↑ | ↑ |
| **Cre** | Normal | ↑ | ↓ | ↑ | ↑ |
| **Albumin** | Normal | ↓ | ↓ | ↓ | ↓ |
| **Calcium** | Normal | ↓ | ↓ | ↓ | ↓ |

↑ = increase, ↓ = decrease.

and these phenomena could be explained as HHcy is compensated inside the body by two reactions namely: reversible re-methylation to S-adenosyl-homocysteine and irreversible trans-sulfuration to cystathionine. Upon the Hcy elevation, an upregulatory mechanism by the kidney is initiated through the higher expression of SLC6A12 gene in the kidney to express the cellular betaine transporter BGT1 and increased expression of betaine-homocysteine methyltransferase enzyme to overcome the plasma HHcy and to protect the kidney tissue.43
In ESRD, Hcy significantly increased from normal-to-mild impairment then significantly decreased from mild impairment until the patient reaches severe impairment. Hcy level at severe impairment is insignificant with the normal level of Hcy in the control group; therefore, other biomarkers are needed for determining the patient’s real disease stage. To diagnose ESRD, Hcy level was about normal value but albumin and calcium significantly decreased than control group normal values, and creatinine (Cre) increased significantly than control group normal values while CRP increased in severe renal impairment significantly than the control group normal values. The current study illustrates the early and middle stages of chronic renal disease (Stages I, II, and III); the patients’ profiles show significant elevated Hcy and Cre values, with significant decrease in albumin and calcium and normal value of CRP. In the late stage of chronic renal disease (Stage IV), the patients’ profiles show minor insignificant decrease in Hcy values compared to normal values in control group (changed from significant elevated level in early stages) accompanied with normal CRP values, more significant decrease in albumin and calcium then more significant increase of Cre. In the last stage of chronic renal disease (Stage V), the patients’ profiles are showing significant elevation in Hcy (elevated again after its major decrease in Stage IV) and significant elevation in CRP (only elevated in the last stage) accompanied with more significant increase in Cre then more significant decrease in albumin while calcium decreased in male patients and illustrating significant increase in female patients.

The results of current study in agreement with Foley et al., Bostom et al., and Hoogeveen et al., whom stated that Hcy levels moderately elevated as renal function declines and markedly elevated in ESRD, with the vast majority (≤85%) of dialysis patients ultimately experiencing mild-to-moderate HHcy and this case is a risk factor for albuminuria.

We conclude that Hcy in combination with hs-CRP, albumin, calcium and creatinine can be used as a
prognostic marker for ESRD and an early diagnostic marker for the risk of renal failure.

Acknowledgements

We thank our colleagues in Nephrology Department, Suez Canal University, for their help in collecting patients’ samples.

Disclosure statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

1. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Ann Intern Med. 2003;139:137–147.
2. Foley RN, Collins AJ. End-stage renal disease in the United States: An update from the United States Renal Data System. J Am Soc Nephrol. 2007;18:2. 644–2648.
3. Tonelli M, Wiebe N, Culleton B, et al. Chronic kidney disease and mortality risk: A systematic review. J Am Soc Nephrol. 2006;17:2034–2047.
4. Manjunath G, Tighiouart H, Coresh J, et al. Level of kidney function as a risk factor for cardiovascular outcomes in the elderly. Kidney Int. 2003;63:1121–1129.
5. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: A statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Hypertension. 2003;42:1050–1065.
6. Fassett RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. Biomarkers in chronic kidney disease: A review. Kidney Int. 2011;80:806–821.
7. Loscalzo J. The oxidant stress of hyperhomocysteinemia. J Clin Invest. 1996;98:5–7.
8. Chambers JC, McGregor A, Jean-Marie J, Obeid OA, Kooner JS. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: An effect reversible with vitamin C therapy. Circulation. 1999;99:1156–1160.
9. Eberhardt RT, Forgione MA, Cap A, et al. Endothelial dysfunction in a murine model of mild hyperhomocyst(e)inemia. J Clin Invest. 2000;106:483–491.
10. Kokame K, Agarwala KL, Kato H, Miyata T. Herp, a new ubiquitin-like membrane protein induced by endoplasmic reticulum stress. J Biol Chem. 2000;275:32846–32853.
11. Kokame K, Kato H, Miyata T. Homocysteine-responsive genes in vascular endothelial cells identified by differential display analysis. GRP78/BIP and novel genes. J Biol Chem. 1996;271:29665.
12. Outinen PA, Sood SK, Liaw PC, et al. Characterization of the stress-inducing effects of homocysteine. Biochem J. 1998;332:213–221.
13. Mudd SH, Levy HL, Skovby F. Disorders of transsulfa-
tion. In: Scriver CR, Bead et AL, Sly WS, Valle D, eds. The Metabolic Basis for Inherited Diseases. New York, NY: McGraw-Hill; 1989:693–734.
14. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med. 1998;338:1042–1050.
15. Chen CH, Yang WC, Hisao YH, Huang SC, Huang YC. High homocysteine, low vitamin B-6, and increased oxidatve stress are independently associated with the risk of chronic kidney disease. Nutrition. 2016;32:236–241.
16. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure. 1999;7:169–177.
17. Volanakis JE. Human C-reactive protein: Expression, structure, and function. Mol Immunol. 2001;38:189–197.
18. Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem. 2004;279:48487–48490.
19. DiCamelli R, Potempa LA, Siegel J, Suyehira L, Petras K, Gewurz H. Binding reactivity of C-reactive protein for polyacrylamide. J Immunol. 1980;125:1933–1938.
20. Suresh MV, Singh SK, Agrawal A. Interaction of calcium-bound C-reactive protein with fibronectin is controlled by pH: In vivo implications. J Biol Chem. 2004;279:52552–52557.
21. Kinoshita CM, Ying SC, Hugli TE, et al. Elucidation of a protease-sensitive site involved in the binding of calcium to C-reactive protein. Biochemistry. 1989;28: 9840–9848.
22. Zhai G, Yan K, Ji X, et al. LAPTM4B allele *2 is a marker of poor prognosis for gallbladder carcinoma. PLoS One. 2012;7:Art. No. e45290.
23. Fung C, De Marchis GM, Katan M, et al. Copeptin as a marker for severity and prognosis of aneurysmal subarachnoid hemorrhage. PLoS One. 2013;8:Art. No. e53191.
24. Gildardini L, Lombardi C, Redaelli G, et al. Glucose tolerance and weight loss in obese women with obstructive sleep apnea. PLoS One. 2013;8:Art. No. e61382.
25. Strzelak K, Misztal J, Tymeczki L, Koncki R. Bianalyte multicommutated flow analysis system for microproteinurics diagnostics. Talanta. 2016;148:707–711.
26. McGinlay JM, Payne RB. Serum albumin by dye-binding: Bromocresol green or bromocresol purple? The case for controlled by pH: In vivo implications. J Biol Chem. 1989;279:48487–48490.
27. Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney Int. 1999;55:1028–1035.
32. Michaela M, Sonja B, Stefano d. Plasma homocysteine and cardiovascular risk in heart failure with and without cardiorenal syndrome. *Int J Cardiol.* 2010;141:32–38.

33. Fellah H, Hammami MB, Feki M, et al. Predictors for cardiovascular morbidity and overall mortality in Tunisian ESRD patients: A six year prospective study. *Clin Biochem.* 2009;7:648–653.

34. de Borst MH, Nauta FL, Vogt L, Laverman GD, Gansevoort RT, Navis G. Indomethacin reduces glomerular and tubular damage markers but not renal inflammation in chronic kidney disease patients: A post-hoc analysis. *PLoS One.* 2012;7:Art. No. e37957.

35. Honda H, Qureshi AR, Heimburger O, et al. Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis.* 2006;47:139–148.

36. Christopeit T, Gossas T, Danielson UH. Characterization of Ca2+ and phosphocholine interactions with C-reactive protein using a surface plasmon resonance biosensor. *Anal Biochem.* 2009;391:39–44.

37. Vupputuri S, Fox CS, Coresh J, Woodward M, Muntner P. Differential estimation of CKD using creatinine-versus cystatin C-based estimating equations by category of body mass index. *Am J Kidney Dis.* 2009;53:993–1001.

38. House JD, Brosnan ME, Brosnan JT. Renal uptake and excretion of homocysteine in rats with acute hyperhomocysteinemia. *Kidney Int.* 1998;54:1601–1607.

39. Veldman BA, Vervoort G, Blom H, Smits P. Reduced plasma total homocysteine concentrations in Type 1 diabetes mellitus is determined by increased renal clearance. *Diabet Med.* 2005;22:301–305.

40. Ninomiya T, Kiyohara Y, Kubo M, et al. Hyperhomocysteinemia and the development of chronic kidney disease in a general population: The Hisayama study. *Am J Kidney Dis.* 2004;44:437–445.

41. van Guldener C. Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering? *Nephrol Dial Transplant.* 2006;21:1161–1166.

42. Bayes B, Pastor MC, Bonal J, et al. Homocysteine, C-reactive protein, lipid peroxidation and mortality in haemodialysis patients. *Nephrol Dial Transplant.* 2003;18:106–112.

43. Kempson SA, Zhou Y, Danbolt NC. The betaine/GABA transporter and betaine: Roles in brain, kidney, and liver. *Front Physiol.* 2014;5:Art. No. 159.

44. Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. *J Biol Chem.* 1984;259:9508–9513.

45. Imbard A, Benoist JF, Esse R, et al. High homocysteine induces betaine depletion. *Biosci Rep.* 2015;35:Art. No. e00222.

46. Ou X, Yang H, Ramani K, et al. Inhibition of human betaine-homocysteine methyltransferase expression by S-adenosylmethionine and methylthioadenosine. *Biochem J.* 2007;401:87–96.

47. Nakanishi T, Otaki Y, Hasuike Y, et al. Association of hyperhomocysteinemia with plasma sulfate and urine sulfate excretion in patients with progressive renal disease. *Am J Kidney Dis.* 2002;40:909–915.

48. Tamura T, Johnston KE, Bergman SM. Homocysteine and folate concentrations in blood from patients treated with hemodialysis. *J Am Soc Nephrol.* 1996;7: 2414–2418.

49. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis.* 1998;32:5112–5119.

50. Bostom AG, Kronenberg F, Jacques PF, et al. Proteinuria and plasma total homocysteine levels in chronic renal disease patients with a normal range serum creatinine: Critical impact of true glomerular filtration rate. *Atherosclerosis.* 2001;159:219–223.

51. Hoogeveen EK, Kostense PJ, Bekx PJ, et al. Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: A population-based study. *Atherosclerosis Thromb Vasc Biol.* 1998;18:133–138.