Applied nutritional investigation

High homocysteine, low vitamin B-6, and increased oxidative stress are independently associated with the risk of chronic kidney disease

Cheng-Hsu Chen M.D., Ph.D. a,b,c,d, Wen-Ching Yang M.S., R.D. e, Yu-Hua Hsiao M.S. f,g, Shih-Chien Huang Ph.D. f,g, Yi-Chia Huang Ph.D., R.D. f,g,*

a Division of Nephrology, Taichung Veterans General Hospital, Taichung, Taiwan
b Department of Internal Medicine, Chiayi Branch, Taichung Veterans General Hospital, Taichung, Taiwan
c School of Medicine, China Medical University, Taichung, Taiwan
d Department of Life Science, Tunghai University, Taichung, Taiwan
e Department of Food and Nutrition, Taichung Veterans General Hospital, Taichung, Taiwan
f School of Nutrition, Chung Shan Medical University, Taichung, Taiwan
g Department of Nutrition, Chung Shan Medical University Hospital, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 25 April 2015
Accepted 17 August 2015

Keywords:
Homocysteine
Folate
Vitamin B-6
Oxidative stress
Antioxidant enzyme activities
Chronic kidney disease

ABSTRACT

Objective: Hyperhomocysteinemia, increased oxidative stress, and decreased antioxidant defense function have been found to be associated with the risk of chronic kidney disease (CKD). Deficiencies of folate and vitamin B-6 (pyridoxal 5'-phosphate, PLP) may cause hyperhomocysteinemia and increased oxidative stress. The purpose of this study was to determine the associations among homocysteine, folate, PLP, oxidative stress indicator, and antioxidant capacities in patients with stage 2 to 3 CKD, and to further analyze these relationships with respect to risk for CKD.

Methods: Ninety-seven patients with CKD and 135 healthy subjects were recruited.

Results: Patients with CKD had significantly higher levels of malondialdehyde and total antioxidant capacities, but had significantly lower antioxidant enzyme activities compared with healthy subjects. Serum folate but not plasma PLP was significantly negatively associated with plasma homocysteine. There were no significant associations of homocysteine, PLP, and folate with oxidative stress indicator and antioxidant capacities. High homocysteine (odds ratio [OR] = 1.11; 95% confidence interval [CI], 1.02–1.22) and malondialdehyde (OR = 34.24; 95% CI, 4.44–264.40) level increased the risk of CKD, whereas high plasma PLP (OR = 0.98; 95% CI, 0.97–0.99) and superoxide dismutase activity (OR = 0.82; 95% CI, 0.74–0.91) decreased the risk of CKD after adjusting all potential confounders.

Conclusion: High homocysteine, low PLP, increased oxidative stress, and decreased antioxidant enzyme activity (superoxide dismutase activity) were independent contributing factors in the development of early stage CKD.

© 2015 Elsevier Inc. All rights reserved.

Introduction

Chronic kidney disease (CKD) is a pathology characterized by progressive impairment of renal function over time and it has become a major health problem worldwide. CKD is now the 10th leading cause of death among men and women in Taiwan [1]. The early detection and treatment of CKD is important not only to prevent or delay CKD progression but also to reduce the risk of developing cardiovascular events and death.
Hyperhomocysteinemia is often seen in patients with CKD [2–7] and is associated with the later development of vascular disease in patients with CKD [8–10]. In homocysteine metabolism, methyltetrahydrofolate is an essential cosubstrate for homocysteine remethylation to methionine. When there is an excess of methionine, homocysteine is directed to the trans-sulfuration pathway. In the transsulfuration of homocysteine metabolism, homocysteine is converted to cystathionine and then to cysteine by enzymes dependent on pyridoxal 5′-phosphate (PLP, the physiologically coenzyme form of vitamin B-6). Studies have reported that low folate and vitamin B-6 were significantly associated with high homocysteine concentrations in patients with CKD and end-stage renal disease [11,12]. It seems that a negative link exists between folate, vitamin B-6, and homocysteine in patients with CKD; however, whether folate and vitamin B-6 are independently related to the risk of CKD or mediate the risk of CKD in connection with high homocysteine levels is unknown.

Excessive free radicals might be gradually overloaded, and exhausted the line of antioxidant defense system during the progression of CKD. Therefore, increased oxidative stress and decreased antioxidant capacities have been found to be associated with the risk of CKD [13–17]. Elevated plasma homocysteine and reduced folate or PLP concentrations may induce excessive production of reactive oxygen species, thus leading to greater oxidative stress and decreased antioxidant enzyme activities [18–23]. It would then be reasonable to hypothesize that higher homocysteine and lower folate or PLP would affect oxidative stress and, as a consequence, the entire antioxidant defense system, possibly triggering the development of CKD. However, the associations of homocysteine, folate, and PLP with oxidative stress and antioxidant capacities in patients with CKD are unclear.

Although decreased serum folate and/or plasma PLP concentration might be associated with hyperhomocysteinemia and increased oxidative stress in patients with CKD, it is unclear whether folate, PLP, homocysteine, and oxidative stress are independently related to the risk of CKD or whether they mediate the risk of CKD in connection with each other. Therefore, the purpose of this study was to determine the associations among homocysteine, folate, PLP, oxidative stress indicators, and antioxidant capacities in patients with stage 2 to 3 CKD, and to further analyze these relationships with respect to the risk for CKD.

Materials and methods

Study design and sample size calculation

This study was designed as a case-control study. A previous study [2] found a non-significant difference of 1.6 ± 2 ng/mL for serum folate between subjects with chronic renal insufficiency and healthy controls. Therefore, our group size was based on power calculations by using a power of 90% and a two-sided test with an a of 0.05 on serum folate. We then needed a sample size of 86 subjects between two groups. With an expected difference of 1 ± 2 ng/mL for serum folate, we thus started with the recruitment of at least 95 patients and 95 control subjects, allowing for an approximate 10% dropout rate. However, the final recruitment number (n = 97 in the case group and n = 135 in the control group) was higher than our expectation.

Subjects

Consecutive patients were recruited at the outpatient clinic of the division of nephrology of Taichung Veterans General Hospital, Taiwan, if they had stage 2 (estimated glomerular filtration rate = 60–89 mL·min⁻¹·1.73 m²) or stage 3 (estimated glomerular filtration rate = 30–59 mL·min⁻¹·1.73 m²) CKD (case group). Patients’ diagnoses and CKD staging were confirmed by an experienced nephrologist. Patients were excluded if they were less than 20 y old or more than 80 y old, taking vitamin supplementation, clinically unstable, pregnant, or lactating; had a history of cardiovascular disease, cancer, or alcoholism; or were taking any medication that could influence folate or vitamin B-6 status. Healthy subjects (control group) with normal blood biochemical values were recruited from the health management center of Taichung Veterans General Hospital, Taiwan. Subjects in the control group were excluded if they were less than 20 y old or more than 80 y old or had a history of gastrointestinal disorder, cardiovascular diseases, liver or renal diseases, diabetes, cancer, alcoholism, or other metabolic diseases. Informed consent was obtained from each subject. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB approval number SF1222).

Data collection and biochemical measurements

All subjects’ age, sex, height, weight, smoking and drinking habits, and use of medications were recorded. Subjects’ height and weight were measured and their body mass index (BMI, kg/m²) was then calculated. Systolic and diastolic blood pressure was measured after a resting period of at least 5 min.

Fasting blood samples were drawn at an appointed day in the outpatient clinic for case subjects and in the health management center for control subjects. Blood specimens were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing an appropriate anticoagulant or no anticoagulant as required to estimate hematological and vitamin status. Hematological entities (i.e., serum albumin, creatinine, triacylglycerols, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol) were measured using an automated biochemical analyzer. High-sensitivity C-reactive protein concentration was determined with particle-enhanced immunonephelometry using an image analyzer. Serum folate was analyzed using standard competitive immuno-nochemiluminometric methods on a Chiron Diagnostics ACS:180 Automated Chemiluminescence System (Chiron Diagnostics Corporation, East Walpole, MA, USA). Folate deficiency was defined as serum concentrations of less than 3 ng/mL [24,25]. Plasma homocysteine was quantified by high-performance liquid chromatography using fluorescence detection according to the method of Araki and Sako [26]. The interassay variability was 2.61% (n = 17) for plasma homocysteine. Hyperhomocysteinemia was defined as a plasma homocysteine concentration ≥14 μmol/L [27]. Plasma PLP was determined by high-performance liquid chromatography as previously described [28]. The interassay variability of plasma PLP was 4.82% (n = 13). Vitamin B-6 deficiency was defined as a plasma PLP level <20 nmol/L [29]. Homocysteine and vitamin B-6 measurements were carried out under yellow light to prevent photodestruction. All analyses were performed in duplicate.

Plasma malondialdehyde (MDA) concentration was determined by thiobarbituric-acid-reactive substances as an indicator of oxidative stress [29]. The MDA level was measured at an excitation wavelength of 515 nm and an emission wavelength of 555 nm using a fluorescence spectrophotometer. Plasma total antioxidant capacity (TAC) was measured according to a 2,2′-azinoisobutyrimidino-5-ethylbenzothiazoline-6-sulfonate radial cation-based colorimetric and automated method described by Erol [30]. This method could determine the antioxidant effects of bilirubin, uric acid, vitamin C, polyphenols, and proteins [30]. Plasma antioxidant enzyme activities, including those of superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione S-transferase (GST), were determined by using the respective commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA).

Statistical analyses

The SAS statistical software package (version 9.3; Statistical Analysis System Institute Inc., Cary, NC, USA) was used for all data analyses. A Shapiro-Wilk test was performed to test the normal distribution. Demographic characteristics and biochemical data of case and control groups were compared for significance using Student’s t test or Mann-Whitney rank sum test. Chi-square or Fisher’s exact tests were used for the analysis of categorical variables. Partial Pearson’s correlation coefficient was used to assess the relationship among serum creatinine, homocysteine, folate, PLP, indicators of oxidative stress, and antioxidant capacities after adjusting for potential confounders in the case and control groups. Adjusted odds ratios with 95% confidence intervals for CKD risk were calculated from unconditional logistic regression models using homocysteine, folate, PLP, indicators of oxidative stress, and antioxidant capacities. Statistical significance was defined as a two-sided P < 0.05.

Results

Table 1 shows subjects’ demographic and health characteristics. There were a total of 232 subjects (155 men and 77 women): 33 patients with stage 2 CKD and 64 patients with stage 3 CKD in the case group, and 135 healthy subjects in the
control group. Subjects’ mean age was 52.37 ± 11.74 y, with a median age of 52 y. There were no significant differences in age, sex, BMI, diastolic blood pressure, high-sensitivity C-reactive protein values, triacylglycerol values, and high-density lipoprotein cholesterol values between the case and control groups. Case subjects had significantly higher systolic blood pressure and serum glucose and creatinine levels but lower serum albumin, total cholesterol, and low-density lipoprotein cholesterol values compared with control subjects.

Case subjects had significantly higher plasma homocysteine and oxidative stress (MDA level) but lower serum folate and plasma PLP concentrations than control subjects did (Table 2). More than half (56.57%) of case subjects had hyperhomocysteinemia (plasma homocysteine concentration ≥ 14 μmol/L). Although case subjects had significantly higher TAC, they had significantly lower GPx, GST, and SOD activities (Table 2).

Many potential confounding factors might affect oxidative stress or antioxidant enzyme activities. Therefore, age, sex, BMI, albumin, glucose, smoking, and drinking status were adjusted to rule out any possible influences of these confounding factors on the relationships among homocysteine, folate, PLP, indicators of oxidative stress, and antioxidant capacities and the risk of CKD. Results of partial Pearson’s correlation coefficient analyses showed that serum creatinine concentration was significantly correlated with plasma homocysteine level in both case and control groups, but there were significantly negative correlations of serum creatinine with GST activity in the case group and with GST in the control group (Table 3). Plasma homocysteine was negatively correlated with serum folate level in the case group (Table 3). Plasma PLP and serum folate concentrations were significantly positively associated with each other in both groups (Table 3). Plasma homocysteine, PLP, and serum folate had no association with indicators of oxidative stress and antioxidant capacities in both groups (Table 3). In addition, there was no significant correlation between TAC and antioxidant enzyme activities (i.e., GPx, GST, and SOD) in either case or control group (data not shown).

Table 4 and Figure 1 show the association of serum plasma homocysteine, folate, PLP, indicators of oxidative stress, and antioxidant capacities with the risk of CKD. Homocysteine, folate, PLP, MDA, GST, and SOD activities were significantly associated with the risk of CKD after adjusting for age, sex, BMI, systolic blood pressure, serum albumin, glucose, and smoking and drinking habits. However, the associations of folate level and GST activity with the risk of CKD disappeared, whereas homocysteine, PLP, MDA, and SOD activities were still associated with the risk of CKD when all the potential confounders were simultaneously adjusted in the unconditional logistic regression model.

Discussion

High plasma homocysteine concentration and decreased renal function have been found to be significantly related [3,7,31]. Although the exact mechanism by which decreased renal function is associated with plasma homocysteine concentration has not been definitely established, a significant relationship between high plasma homocysteine and decreased renal function (serum creatinine as an indicator) was found in the present study. In agreement with previous studies [2–7], our study found that patients with CKD not only had a significantly higher plasma homocysteine concentration but also had a higher percentage of hyperhomocysteinemia (≥ 14 μmol/L) than healthy controls did. In spite of the possible association between renal function and plasma homocysteine, among factors (i.e., age, enzyme deficiencies and mutations, vitamin deficiencies, diseases, and drugs) that might contribute to the increased homocysteine concentration, folate and vitamin B-6 status have received the most attention. Folate is the cosubstrate in the remethylation of homocysteine metabolism; thus, it was not surprising to observe a significant relationship between high plasma homocysteine and low serum folate in the present study. Because serum folate is inversely correlated to plasma homocysteine, folate acid supplementation has been suggested for treatment of hyperhomocysteinemia in patients with CKD. Folic acid supplementation was beneficial in reducing plasma homocysteine levels. However, it had no further
effects on the reduction of cardiovascular risk and death in patients with CKD [6,32,33]. Serum folate seems to have no independent effect but does mediate the risk of CKD in connection with plasma homocysteine. This might explain why the association between decreased serum folate and increased risk of CKD disappeared after additionally considering plasma homocysteine in the logistic regression model.

Although our stage 2 to 3 patients with CKD had significantly lower plasma PLP concentrations than the healthy controls, their vitamin B-6 status was not deficient. In a previous study [5], patients with stage 2 to 4 CKD had mean plasma PLP levels 8.8 times higher than our patients. It seems that, in the early stage, patients with CKD might still have adequate vitamin B-6 status, and the deficiency may develop later in patients receiving hemodialysis [34,35]. Unlike the significantly negative association between serum folate and plasma homocysteine, our results as well as those of previous studies [2,5] reported no association between plasma PLP and plasma homocysteine concentration. In homocysteine metabolism, the remethylated pathway is preferential in the fasting state [36,37]. When there is an excess of methionine, homocysteine is directed to the transsulfuration pathway. Therefore, plasma PLP concentration might be associated more closely with plasma homocysteine concentration after methionine loading. However, a previous study indicated that plasma PLP concentration did not correlate with postmethionine loading of homocysteine in subjects with chronic renal insufficiency [2]. We did not perform methionine loading, and so the association between plasma PLP and postmethionine loading of homocysteine concentration was not addressed in this study. Because no relationship between plasma PLP and fasting plasma homocysteine was found, it is not surprising that low plasma PLP was associated with an increased risk of CKD independent of plasma homocysteine. However, the role vitamin B-6 plays in the development of CKD is unclear. Previous studies indicated that vitamin B-6 deficiency could cause microscopic renal lesions in rats [38] and increase the occurrence of renal oxalate stones [39, 40]. Further study is warranted to investigate the role plasma PLP plays in the development of early stage CKD.

Besides the independent association of high homocysteine and low plasma PLP with the risk of CKD, increased oxidative stress and decreased antioxidant activities were associated with the acceleration of renal injury progression [13–17]. We also observed that our patients with CKD had higher oxidative stress and lower antioxidant enzyme activities compared with the healthy controls, and that the increased oxidative stress (MDA level as the indicator) and decreased antioxidant capacities

---

### Table 3
Partial Pearson’s correlation coefficients (r) among serum creatinine, homocysteine, folate, vitamin B-6, indicators of oxidative stress, and antioxidant capacities in both case and control groups (n = 232)

<table>
<thead>
<tr>
<th></th>
<th>Case (n = 97)</th>
<th>Control (n = 135)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homocysteine (µmol/L)</strong></td>
<td>0.5*</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Pyridoxal 5’-phosphate (nmol/L)</strong></td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Folate (ng/mL)</strong></td>
<td>-0.1</td>
<td>-0.0</td>
</tr>
<tr>
<td><strong>Oxidative stress indicator</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>-0.1</td>
<td>-0.1</td>
</tr>
<tr>
<td>Antioxidant capacities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total antioxidant capacity (µmol/L)</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Glutathione peroxidase (nmol·mL⁻¹·min⁻¹)</td>
<td>-0.3</td>
<td>-0.2</td>
</tr>
<tr>
<td>Glutathione S-transferase (nmol·mL⁻¹·min⁻¹)</td>
<td>-0.1</td>
<td>-0.2</td>
</tr>
<tr>
<td>Superoxide dismutase (U/mL)</td>
<td>0.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values shown are Partial Pearson’s correlation coefficient (r)

Adjusted for age, sex, body mass index, systolic blood pressure, serum albumin and glucose, and smoking and drinking habits

* P < 0.001.
† P < 0.05.
‡ P < 0.01.

### Table 4
Odds ratios for risk of chronic kidney disease

<table>
<thead>
<tr>
<th></th>
<th>No factors adjusted</th>
<th>Factors adjusted for†</th>
<th>Factors adjusted for‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI P</td>
<td>OR 95% CI P</td>
<td>OR 95% CI P</td>
</tr>
<tr>
<td>Plasma homocysteine (µmol/L)</td>
<td>1.16 1.08–1.25 &lt;0.01</td>
<td>1.17 1.08–1.28 &lt;0.01</td>
<td>1.11 1.02–1.22 0.02</td>
</tr>
<tr>
<td>Plasma PLP (nmol/L)</td>
<td>0.99 0.98–0.99 &lt;0.01</td>
<td>0.99 0.98–0.99 &lt;0.01</td>
<td>0.98 0.97–0.99 0.01</td>
</tr>
<tr>
<td>Serum folate (ng/mL)</td>
<td>0.94 0.91–0.98 &lt;0.01</td>
<td>0.92 0.88–0.97 &lt;0.01</td>
<td>0.95 0.92–0.96 0.65</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>14.46 4.31–48.48 &lt;0.01</td>
<td>56.27 10.93–289.85 &lt;0.01</td>
<td>34.24 4.44–264.40 &lt;0.01</td>
</tr>
<tr>
<td>Total antioxidant capacity (µmol/L)</td>
<td>1.00 1.00–1.00 0.08</td>
<td>1.00 1.00–1.00 0.08</td>
<td>1.00 1.00–1.00 0.46</td>
</tr>
<tr>
<td>Glutathione peroxidase (nmol·mL⁻¹·min⁻¹)</td>
<td>0.99 0.99–1.00 0.03</td>
<td>0.99 0.99–1.00 0.05</td>
<td>0.99 0.99–1.00 0.22</td>
</tr>
<tr>
<td>Glutathione S-transferase (nmol·mL⁻¹·min⁻¹)</td>
<td>0.98 0.97–1.00 0.01</td>
<td>0.99 0.97–1.00 0.03</td>
<td>0.99 0.97–1.00 0.12</td>
</tr>
<tr>
<td>Superoxide dismutase (U/mL)</td>
<td>0.78 0.72–0.84 &lt;0.01</td>
<td>0.80 0.73–0.87 &lt;0.01</td>
<td>0.82 0.74–0.91 &lt;0.01</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio; PLP, pyridoxal 5’-phosphate

† Adjusted for age, sex, body mass index, systolic blood pressure, serum albumin and glucose, and smoking and drinking habits.
‡ Adjusted for age, sex, body mass index, systolic blood pressure, serum albumin and glucose, and smoking and drinking habits, and/or homocysteine, PLP, folate, malondialdehyde, total antioxidant capacities, activities of glutathione S-transferase, glutathione peroxidase, and superoxide dismutase.
(especially for SOD activity) were significantly associated with the risk of CKD independent of homocysteine and PLP. Although elevated plasma homocysteine and reduced folate or PLP concentrations might be associated with high oxidative stress and low antioxidant enzyme activities [18–23], they had independent effects on the risk of CKD in the present study. MDA level and SOD activity were more likely to enhance or reduce the risk of CKD compared with homocysteine and PLP. Oxidative stress and antioxidant enzyme activity seem to have more dominant roles in the pathogenesis of early stage CKD. Uremic toxins, activated leukocytes and macrophages, chronic infections, and the hemodialysis process might cause excess free radicals and increase oxidative stress in subjects with end-stage renal disease [41]. However, factors that may increase oxidative stress and decrease antioxidant enzyme activity in early stage (stage 2 to 3) CKD need further investigation. An interesting finding of this study was that our patients with CKD had lower antioxidant enzyme activities and higher TAC than our healthy controls did. It is difficult to explain this observation in our patients with CKD and healthy controls. However, the method of TAC is more sensitive for determining the antioxidative effects of bilirubin, uric acid, vitamin C, polyphenols, and proteins [30]. We observed no relationships between TAC and antioxidant enzyme activities (i.e., GPx, GST, and SOD) in both case and control groups. The results of TAC thus might not completely reflect GPx, GST, or SOD activities in our subjects. In addition, compared with healthy controls, either increased or stable TAC with CKD progression in patients with CKD has been observed in previous studies [42–44]. The TAC level might not be a reliable indicator of antioxidant capacity for patients with CKD because it might be confounded mainly by uric acid levels [42,43]. This might be the other reason that our patients with CKD had high TAC but low antioxidant enzyme activities. The reliable marker of antioxidant capacity for patients with CKD and the relationship between TAC and antioxidant enzyme activities are worth further study.

The strength of this study was that we simultaneously assessed associations of homocysteine, folate, PLP, oxidative stress, and antioxidant capacities with the risk of CKD. However, the limitation was that we measured all biochemical variables at only one time point, and single measurements may not be reflective of the true association between serum folate and the risk of CKD.

The data herein indicated that high plasma homocysteine, low PLP, increased oxidative stress (MDA level), and decreased antioxidant enzyme activity (SOD activity) are independent contributing factors in the development of early stage CKD. However, the mechanistic factors underlying the relationship among homocysteine, PLP, oxidative stress, and SOD antioxidant enzyme activity in the risk of CKD warrant further investigation.

References


[39] Data from: Chen C-H, et al., High homocysteine, low vitamin B-6, and increased oxidative stress are independently..., Nutrition (2015), http://dx.doi.org/10.1016/j.nut.2015.08.016