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References


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Decreased plasma level of vitamin C in chronic kidney disease: comparison between diabetic and non-diabetic patients

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Abstract

Background. A decreased plasma level of vitamin C has been reported to be associated with an increased risk of cardiovascular morbidity and mortality. Here, we sought to determine the vitamin C status of patients with chronic kidney disease and the pathophysiological role of vitamin C in these patients.

Methods. We studied 58 patients and evaluated the relationship between renal function and plasma vitamin C concentration, as well as the effect of diabetes on this relationship.

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Endothelium-dependent flow-mediated dilation of brachial artery was measured to assess the endothelial function. Serum malondialdehyde low-density lipoprotein was measured as a marker for oxidative stress.

**Results.** Plasma vitamin C concentration had a positive linear relationship with eGFR in both diabetic and non-diabetic patients (P = 0.006 and P = 0.004, respectively). When vitamin C concentration and eGFR relationships were compared in the two groups, vitamin C concentration was significantly lower in diabetic patients at every eGFR (P = 0.006). Flow-mediated vasodilatation of the brachial artery was positively correlated with vitamin C concentration in non-diabetic patients (P = 0.047) but not in diabetic patients. There was a negative correlation between serum malondialdehyde low-density lipoprotein and vitamin C concentration in non-diabetic patients (P = 0.044) but not in diabetic patients.

**Conclusions.** Renal dysfunction was associated with a decrease in plasma vitamin C level. Moreover, decreased vitamin C may cause endothelial dysfunction via an increase in oxidative stress in non-diabetic chronic kidney disease patients.

**Keywords:** endothelial function; estimated glomerular filtration rate; flow-mediated dilatation; malondialdehyde low-density lipoprotein; vitamin C

**Introduction**

Chronic kidney disease (CKD) has been focused on as an independent risk factor for cardiovascular disease (CVD) [1], and the severity of renal disease is known to be associated with a graded increase in the risk for CVD and death [2]. One of the principal pathophysiological features involved in this association has been proposed to be endothelial dysfunction [3]. Many of the traditional and non-traditional cardiovascular risk factors that could affect endothelial function can be found in association with CKD [4]. Among these, oxidative stress is considered to predispose individuals to endothelial dysfunction and the development of atherosclerosis in patients with CKD [5]. The susceptibility to oxidative stress in CKD patients is mediated by abnormal oxidant or defective antioxidant production [6].

In unsupplemented CKD patients, several deficiencies in various components of antioxidant defence mechanisms have been demonstrated, including reduced plasma vitamin C concentration [7]. Vitamin C is a primary antioxidant that directly neutralizes radical species [8] as well as an essential nutrient required for the formation of collagen and normal immune function [9]. A large-scale study of an elderly population cohort demonstrated a continuous increase in the risk for all-cause and cardiovascular disease mortality with decreasing plasma vitamin C concentration [10]. Recently, Deicher et al. reported with a low plasma vitamin C level predicts fatal and major non-fatal adverse cardiovascular events among maintenance haemodialysis patients [11]. Moreover, experimental lines of evidence have been accumulated that vitamin C has beneficial effects on endothelial function and formation of atherosclerotic lesions [8]. However, the pathophysiological role of vitamin C in patients with CKD has not been fully elucidated.

Hyperglycaemia induces overproduction of reactive oxygen species in diabetic nephropathy, and an increase of oxidative stress is known to be important in the development of diabetic complications [12]. The balance between pro-oxidant and antioxidant activity, and the effect of vitamin C against oxidative stress should differ between diabetic and non-diabetic patients. The aim of the present study was to evaluate the relationship between renal function and plasma vitamin C concentration in patients with CKD on conservative therapy, and to further elucidate the relationships between vitamin C concentration and both endothelial function and oxidative stress in these patients. Moreover, we analysed the data separately in diabetic and non-diabetic patients to determine the effect of diabetes on the role of vitamin C in CKD patients.

**Materials and methods**

**Patients**

We prospectively studied 58 patients with CKD who visited the outpatient clinic of our hospital and met the following criteria for entry into the study: (i) estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m² determined using the relevant equations for Japanese subjects [13], and not receiving dialysis therapy; (ii) no previous history of myocardial infarction, heart failure or stroke within 6 months; and (iii) not taking vitamin C or multivitamins. Subjects were defined as having diabetes if diabetes of adult onset had been documented, and therapy was initiated prior to the study. None of the patients had a history of sudden onset of diabetes with ketotic acidosis. Ophthalmologic evaluation was performed within 3 months of the study, and all of the diabetic subjects had retinopathy. Information regarding the use of calcium–potassium exchange resins or diuretics was collected from the medical records. This study was approved by the ethical committee of our institution, and written informed consent was obtained from each patient.

**Brachial artery vasodilatation**

Endothelium-dependent and endothelium-independent vasodilatations were assessed by flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) of brachial artery according to the guidelines for the ultrasound assessment of endothelium-dependent flow-mediated vasodilatation of the brachial artery [14]. Brachial artery diameter was measured by B-mode ultrasound imaging (UNEX EF 18G, UNEX Corporation, Aichi, Japan) with a 7.5-MHz linear array transducer while an electrocardiogram was simultaneously recorded. The right brachial artery was scanned in longitudinal sections 1–10 cm above the elbow after at least 5 min of rest in a supine position, the skin surface was marked and the arm was kept in the same position during the study. After baseline measurements of the brachial artery diameter, FMD was determined by scans during reactive hyperaemia. A pneumatic cuff placed around the forearm was inflated to 70 mmHg above the systolic pressure and was deflated after 5 min. The diameter of the brachial artery was scanned and recorded continuously from 30 s before to 2 min after cuff deflation to obtain a maximal diameter. After a 15-min rest, a second control scan of the diameter was recorded. Then, 0.075 mg of sublingual nitroglycerin was administered, and the diameter was scanned and recorded continuously from 30 s before to 20 min after nitroglycerin administration. The diameter of the artery was measured from one media-adventitia interface to the other at end-diastole, coincident with the R-wave on a continuously recorded electrocardiogram. Vasodilatation was expressed as the percent change in diameter from the baseline value after the release of occlusion and after the administration of nitroglycerin.

**Laboratory measurements**

Blood sampling was performed after 20–30 min of quiet resting in a sitting position. After overnight fasting, blood samples for measurement of vita-
min C, malondialdehyde low-density lipoprotein (MDA-LDL), creatinine, blood urea nitrogen, potassium, albumin, serum lipids, high-sensitivity C-reactive protein and haemoglobin were obtained from all patients. To determine vitamin C concentration, 500 μL of serum was immediately deproteinized with perchloric acid. Dehydroascorbic acid was then reduced to ascorbic acid by adding 0.3 M phosphate buffer containing 10 mM diithiothreitol. The concentration of vitamin C was determined as total ascorbic acid by adding 0.3 M phosphate buffer containing 10 mM dithiothreitol. The concentration of vitamin C was determined as total ascorbic acid by the serum level of MDA-LDL was measured by ELISA using monoclonal antibodies against MDA-LDL and apoB labelled with beta-galactosidase [15]. As a marker of oxidative stress, urinary protein excretion was estimated from urinary protein concentration divided by urinary creatinine concentration and expressed as gram per gram creatinine.

Statistical analysis
Because data are not normally distributed, numerical variables are shown as median and range. Mann–Whitney’s U-test was used to test the significance of numerical variables between diabetic and non-diabetic patients, and chi-square analysis was used to compare the categorical variables between the two groups. Multiple regression analysis was performed to determine the factors that affect plasma vitamin C concentration. Linear regression models were derived for the eGFR and plasma vitamin C concentration relationships in diabetic and non-diabetic patients. To test the difference in the relationship between the two groups, the two regression lines were analysed for equality of intercept and slope with analysis of covariance. The relationships between vitamin C and FMD, NMD and MDA-LDL were also tested using linear or non-linear regression analysis. A P-value of <0.05 was considered significant. All analyses were performed using StatView 5.0 statistical software (SAS Institute, Cary, NC, USA).

Results
Clinical characteristics
Twenty-seven of 58 patients had diabetes. All of the diabetic patients had >10 years of disease history and had been treated for diabetic retinopathy. Clinical characteristics of the study population are listed in Table 1. Body mass index and systolic blood pressure were higher in diabetic patients compared with non-diabetic patients. Urinary protein excretion was higher, and serum albumin concentration was lower in diabetic patients compared with non-diabetic patients. There was no significant difference in the use of calcium–potassium exchange resins between the two groups. Diuretics were more frequently used in diabetic patients compared with non-diabetic patients.

eGFR and plasma vitamin C concentration
We evaluated the relationship between eGFR and plasma vitamin C concentration in non-diabetic and diabetic patients with CKD (Figure 1). Plasma vitamin C concentration had a positive linear relationship with eGFR in both diabetic (P = 0.006) and non-diabetic (P = 0.004) patients. Using multiple regression analysis, eGFR and diabetes were found to be independently related to vitamin C concentration (Table 2). Therefore, to test the difference in the eGFR and plasma vitamin C concentration relationships in the two groups, two regression lines were analysed for equality of intercept and slope with analysis of covariance. As a result, vitamin C concentration was significantly lower in diabetic patients at every eGFR (P = 0.006).

<table>
<thead>
<tr>
<th>Non-diabetic patients</th>
<th>Diabetic patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>CKD stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>12 (39%)</td>
<td>9 (33%)</td>
</tr>
<tr>
<td>Stage 4</td>
<td>7 (22%)</td>
<td>8 (30%)</td>
</tr>
<tr>
<td>Stage 5</td>
<td>12 (39%)</td>
<td>10 (37%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69 (49–85)</td>
<td>68 (46–82)</td>
</tr>
<tr>
<td>Men/women</td>
<td>20/11</td>
<td>18/9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.2 (17.9–30.4)</td>
<td>24.5 (16.2–31.4)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>139 (115–199)</td>
<td>154 (124–187)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76 (52–103)</td>
<td>81 (65–99)</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.9 (8–16.5)</td>
<td>10.7 (6.5–14.3)</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>35 (15–92)</td>
<td>37 (3–126)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>2.27 (0.84–8.09)</td>
<td>2.36 (0.80–5.72)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>23 (5–57)</td>
<td>18 (9–58)</td>
</tr>
<tr>
<td>Serum potassium (mEq/L)</td>
<td>5.0 (3.7–6.0)</td>
<td>4.6 (3.1–6.2)</td>
</tr>
<tr>
<td>Haemoglobin A1C (%)</td>
<td>5.1 (4.7–6.4)</td>
<td>6.2 (4.5–9.6)</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>4.0 (2.2–4.8)</td>
<td>3.5 (1.7–4.6)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>113 (53–179)</td>
<td>122 (57–362)</td>
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<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>50 (32–145)</td>
<td>49 (33–80)</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>0.111 (0.020–1.105)</td>
<td>0.126 (0.020–2.621)</td>
</tr>
<tr>
<td>Vitamin C (µg/mL)</td>
<td>6.2 (1.4–19.8)</td>
<td>4.5 (0.6–13.0)</td>
</tr>
<tr>
<td>MDA-LDL (mg/dL)</td>
<td>95.9 (54.7–208.2)</td>
<td>116.9 (44.0–216.0)</td>
</tr>
<tr>
<td>Urinary protein (g/g creatinine)</td>
<td>0.32 (0.05–11.5)</td>
<td>2.95 (0.05–13.6)</td>
</tr>
<tr>
<td>Use of calcium-potassium exchange resins</td>
<td>2 (6%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Use of diuretics</td>
<td>9 (29%)</td>
<td>15 (56%)</td>
</tr>
</tbody>
</table>

Media and range are shown for numerical variables.
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; MDA, malondialdehyde.
used in stage 5 patients compared with stage 3 and 4 patients (P = 0.016). On the other hand, diuretics were administered in 4 of 9 stage 3 CKD patients, 5 of 8 stage 4 patients and 4 of 10 stage 5 patients in the diabetic group. There were no significant differences in the use of diuretics among the three stages.

eGFR and urinary protein excretion

We evaluated the relationship between eGFR and urinary protein excretion. Urinary protein excretion was inversely correlated with eGFR in both non-diabetic and diabetic patients: urinary protein excretion = 3.937 − 0.087 × eGFR for non-diabetic patients (P = 0.009) and urinary protein excretion = 6.403 − 0.088 × eGFR for diabetic patients (P = 0.047).

Plasma vitamin C concentration and endothelial function

We measured FMD to evaluate the possible role of vitamin C in endothelial function in CKD patients. In non-diabetic patients, FMD was significantly correlated with plasma vitamin C concentration, but there was no significant relationship between NMD and vitamin C concentration (Figure 2). On the other hand, neither FMD nor NMD had a significant relationship with vitamin C concentration in diabetic patients (Figure 3).

Plasma vitamin C concentration and oxidative stress

To evaluate the effect of vitamin C on oxidative stress, the relationship between plasma vitamin C concentration and MDA-LDL was examined (Figure 4). There was a significant negative exponential correlation between vitamin C concentration and MDA-LDL in non-diabetic patients, but not in diabetic patients.

Discussion

This study demonstrated that renal dysfunction was associated with a continuous decrease in plasma vitamin C concentration in both diabetic and non-diabetic patients with CKD. Moreover, vitamin C concentration was significantly lower at any given eGFR in diabetic patients compared with non-diabetic patients. Dialysis patients are known to be at high risk for vitamin C deficiency [7,17,18]. However, the decrease in plasma vitamin C concentration in CKD on conservative therapy has not been well established.

Dietary restrictions of fresh fruits and vegetables to avoid hyperkalaemia are likely to be responsible for the decrease in plasma vitamin C concentration, because vitamin C cannot be synthesized endogenously and plasma vitamin C levels are largely dependent of dietary intake of the vitamin. Possible impairment of enzymatic or non-enzymatic recycling of ascorbate from dehydroascorbate is suspected in CKD patients, since the recycling is largely glutathione dependent and glutathione level is decreased in these patients [19]. Moreover, CKD patients lose vitamin C ex...
cessively into urine due to its water solubility [17]. Mydlík et al. reported that the administration of furosemide led to a further increase in the urinary excretion of vitamin C in patients with chronic renal failure [20]. Because diuretics were more frequently administered as renal function deteriorates in non-diabetic patients, increased diuretic-induced urinary loss of vitamin C could explain the relationship between decreased vitamin C concentration and renal dysfunction in this patient group. On the contrary, there were no significant differences in the use of diuretics among the three stages of CKD patients in diabetic patients. Proteinuria is another important factor that causes an increase in urinary loss of vitamin C [21]. As there were significant relationships between eGFR and urinary protein excretion in both non-diabetic and diabetic groups, these could explain the relationship between decreased vitamin C concentration and renal dysfunction.

This study also demonstrated that vitamin C concentration was significantly lower at any given eGFR in diabetic patients compared with non-diabetic patients. Diabetic patients exhibited more excretion of urinary protein compared with non-diabetic patients. In addition, the presence of oedema due to lower serum albumin might have caused more frequent use of diuretics. Therefore, although we cannot exclude the difference in the amount of dietary vitamin C intake between the two groups, proteinuria and more frequent use of diuretics would explain lower vitamin C concentration in diabetic CKD patients.

The pathophysiological role of decreased vitamin C in CKD patients is not fully elucidated. A large-scale study of an elderly population reported a continuously increased risk for all-cause and cardiovascular disease mortality with lower plasma vitamin C concentration [10]. Interestingly, other antioxidants, plasma alpha-tocopherol, beta-carotene and retinol, had no impact on the outcomes. Recently, Deicher et al. reported that low plasma vitamin C level predicts fatal and the major non-fatal adverse cardiovascular events among maintenance haemodialysis patients and speculated on the existence of a link between decreased vitamin C level and progression of coronary atherosclerosis [11]. These findings raise the possibility that vitamin C may inhibit the progression of atherosclerosis.

In this study, we hypothesized that decreased vitamin C level affects endothelial function which is the first step towards atherosclerosis [22]. To test this hypothesis, we evaluated endothelial function using FMD of brachial artery. We found that a decreased level of vitamin C was associated with impaired FMD in non-diabetic CKD patients which suggested an association of decreased vitamin C with endothelial dysfunction in this patient group. Vitamin C stimulates the proliferation of endothelial cells [23] and prevents apoptosis [24], thereby contributing to the preservation of endothelial function. Vitamin C also enhances nitric oxide (NO) generation by increasing endothelial NO synthase activity [25]. As vitamin C is essential to preserving endothelial function, CKD patients with a decreased vitamin C level may be susceptible to endothelial dysfunction and progression of atherosclerosis. Vitamin C is one of the most important water-soluble antioxidants in plasma, and vitamin C deficiency has been reported to cause an increase in oxidative stress [26]. However, evidence for a causal link between low vitamin C level and increases of
oxidative stress parameters is limited. We found an inverse relationship between plasma vitamin C concentration and MDA-LDL, a marker for oxidized and degenerated LDL, in non-diabetic CKD patients. Vitamin C acts against lipid peroxidation and also has hypochlorous acid scavenging ability. The relationship between malondialdehyde level and the development of atherosclerosis has been demonstrated in dialysis patients [27]. Therefore, our data suggest that decreased plasma vitamin C increases oxidative stress and leads to endothelial dysfunction in non-diabetic CKD patients.

There were no significant relationships between vitamin C and FMD or MDA-LDL in diabetic patients. In diabetes, additional mechanisms may trigger endothelial dysfunction. Advanced glycation end products were shown to quench NO and impair endothelial function as evidenced by the inhibition of advanced glycosylation with aminoguanidine [28]. Hyperglycaemia induces overproduction of reactive oxygen species, and an increase of oxidative stress is known to be important in the development of diabetic complications [12]. Therefore, the effects of decreased vitamin C might be somewhat concealed by the presence of a powerful pro-oxidant system and metabolic factors affecting endothelial dysfunction and increased oxidative stress. Although diabetic patients showed a lower vitamin C level compared with non-diabetic patients, the significance of decreased vitamin C is yet to be elucidated.

In conclusion, renal dysfunction was associated with a decreased level of plasma vitamin C in patients with CKD, and diabetic patients showed a lower level of vitamin C at any given eGFR compared with non-diabetic patients. Decreased vitamin C may contribute to endothelial dysfunction via an increase of oxidative stress and oxidative modification of low-density lipoprotein in non-diabetic patients with CKD. Further investigations are needed to clarify whether vitamin C supplements ameliorate endothelial dysfunction and prevent adverse cardiovascular events in CKD patients.

Conflict of interest statement. None declared.

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