Association Between Serum Phosphate Levels and Stroke Risk in Patients Undergoing Hemodialysis

The Q-Cohort Study

Shunsuke Yamada, MD, PhD; Kazuhiko Tsuruya, MD, PhD; Masatomo Taniguchi, MD, PhD; Masanori Tokumoto, MD, PhD; Kiichiro Fujisaki, MD, PhD; Hideki Hirakata, MD, PhD; Satoru Fujimi, MD, PhD; Takanari Kitazono, MD, PhD

Background and Purpose—The contribution of serum phosphate levels to stroke risk in dialysis patients remains unclear. The present study aimed to elucidate the respective association between serum phosphate levels and the risk of brain hemorrhage or infarction in patients undergoing hemodialysis.

Methods—A total of 3437 patients undergoing hemodialysis were followed up for a median of 3.9 years. The primary outcome was the occurrence of brain hemorrhage or infarction. Patients were divided into 4 groups based on their baseline serum phosphate levels (Q1–Q4). Stroke risk was estimated using a Cox proportional hazards model.

Results—During the follow-up period, 75 patients experienced brain hemorrhage and 139 experienced brain infarction. The risk of brain hemorrhage was significantly higher in the highest (Q4) compared with the lowest quartile (Q1) as the reference value (multivariate-adjusted hazard ratio [95% confidence intervals]; Q1, 1.00; Q2, 1.76 [0.79–4.18]; Q3, 1.99 [0.92–4.67]; and Q4, 2.74 [1.27–6.47]; P=0.077 for trend; hazard ratio for every 1 mmol/L increase in serum phosphate level, 2.07 [1.10–3.81]; P=0.025). In contrast, the risk of brain infarction was significantly higher in Q1 (P=0.045) compared with Q3 as the reference value (Q1, 1.65 [1.01–2.73]; Q2, 1.35 [0.82–2.25]; Q3, 1.00; and Q4, 1.30 [0.77–2.20]).

Conclusions—Higher serum phosphate levels were associated with an increased risk of brain hemorrhage, whereas low levels were associated with an increased risk of brain infarction in hemodialysis patients. These results suggest the importance of managing serum phosphate levels within an appropriate range in hemodialysis patients.

Clinical Trial Registration—URL: http://www.umin.ac.jp/. Unique identifier: UMIN000000556.

Key Words: brain infarction ◼ hemodialysis ◼ intracerebral hemorrhage ◼ phosphate

Stroke is a major cause of mortality in patients with chronic kidney disease (CKD).1–4 The risk of stroke is increased in patients with CKD, and CKD patients, especially those undergoing hemodialysis, are at increased risks of hospitalization, disability, and death after stroke, which are associated with a negative impact on society.5–7 Although previous studies have identified traditional and nontraditional risk factors for stroke in hemodialysis patients, the incidence of stroke still remains unacceptably high.8–11

Increasing evidence suggests that CKD-related bone-mineral disorders are an important nontraditional risk factor for cardiovascular morbidity and mortality in CKD patients.12,13 Hyperphosphatemia is the hallmark of such CKD-related bone-mineral disorders.14,15 Multiple lines of evidence have indicated strong associations between higher serum phosphate levels and cardiovascular disorders in both the general and dialysis populations.16,17 Although several clinical studies examined the association between serum phosphate levels and the incidence of stroke in the general population,8–10 they assessed the risk of composite stroke, including both brain hemorrhage and brain infarction. They did not assess the relationships between phosphate levels and brain hemorrhage and infarction separately, potentially resulting in inconsistent results and a lack of association.

We aimed to elucidate the associations between serum phosphate levels and the incidences of stroke in patients...
undergoing hemodialysis, using the data from the prospective Q-Cohort Study in Japanese hemodialysis patients.21 Importantly, the present study examined the associations between serum phosphate levels and the risks of brain hemorrhage and brain infarction separately.

Methods

Design of the Q-Cohort Study

The Q-Cohort Study was a multicenter, prospective longitudinal observational study designed to identify risk factors for cardiovascular and noncardiovascular morbidities and mortalities in patients undergoing maintenance hemodialysis in Japan. The details of the study have been described elsewhere.23 The study population consisted of 3598 outpatients ≥18 years old, who underwent regular hemodialysis therapy between December 2006 and December 2007 at 39 dialysis facilities. All patients were scheduled to be followed up until December 2010. Among the 3598 patients, 161 patients were excluded from the analysis because of missing data on baseline characteristics, serum biochemistry, or medication. Data for 3437 patients were, thus, analyzed in the present study. The study was performed according to the Ethics of Clinical Research (Declaration of Helsinki). The study protocol was approved by the Kyushu University Hospital Institutional Review Board for Clinical Research (No. 20–31) and was registered in the clinical trial registry (University Hospital Medical Information Network, UMIN000000556). All patients provided written informed consent before study participation.

Definition of Outcomes and Covariate of Interest

Stroke was defined as a sudden-onset neurological impairment lasting for ≥24 hours. Stroke types were confirmed by brain imaging, including computed tomography and magnetic resonance imaging, and were classified as either brain hemorrhage or infarction by the attending physician. The primary outcomes were the first-ever incidence of either brain hemorrhage or infarction. The secondary outcome was the incidence of composite stroke, including both brain hemorrhage and infarction. In the present study, brain hemorrhage excluded subarachnoid hemorrhage and hemorrhage after brain infarction. The covariate of interest was serum phosphate level.

Biochemical Determinations

Routine biochemical parameters were measured with an auto-analyzer using standard procedures at multiple facilities depending on the demographic location of each dialysis center. Corrected serum calcium levels were adjusted to serum albumin level using Payne’s formula.22 Serum parathyroid hormone (PTH) levels were measured using whole or intact PTH assays, and the relationship between the 2 assays was determined according to the following equation: intact PTH (ng/L) = 1.7 × whole PTH (ng/L). Target ranges of serum calcium, phosphate, and PTH levels during the follow-up period were as follows: calcium, 2.10 to 2.50 mmol/L (8.5–10.0 mg/dL); phosphate, 1.13 to 1.94 mmol/L (3.5–6.0 mg/dL); and intact PTH, 60 to 180 ng/L (60–180 pg/mL).23

Statistical Analysis

Normally distributed continuous variables were described as mean±standard deviation, and non-normally distributed continuous variables were described as median (interquartile range). Categorical data were described as percentage. We divided the patients into 4 groups according to serum phosphate level (Q1–Q4): Q1 (serum phosphate ≤1.29 mmol/L), Q2 (1.30–1.54 mmol/L), Q3 (1.55–1.79 mmol/L), and Q4 (≥1.80 mmol/L) in SI units and Q1 (serum phosphate ≤4.0 mg/dL), Q2 (4.1–4.7 mg/dL), Q3 (4.8–5.5 mg/dL), and Q4 (≥5.6 mg/dL) in US unit. The cutoff values for each subgroup were set based on the 25th, 50th, and 75th percentiles of serum phosphate in our population and the reference ranges recommended by the Kidney Disease Outcome Quality Initiative.24 Age- and sex-adjusted or multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for each stroke type were estimated using a Cox proportional hazards model. The multivariate-adjusted model included the following covariates: age, sex, presence of diabetes mellitus, dialysis history, history of cardiovascular events, dry weight, systolic blood pressure, normalized protein catabolic rate, Kt/V for urea, blood hemoglobin level, serum levels of albumin, total cholesterol, C-reactive protein, creatinine, corrected calcium, phosphate, and PTH and use of phosphate binders, vitamin D receptor activators, and antihypertensive drugs. To determine the effect modifications, we examined the effects of baseline clinical characteristics on the associations between serum phosphate levels and each type of stroke. The effects of heterogeneity in serum phosphate on each type of stroke across clinical characteristics were tested by adding an interaction term to the relevant multivariate Cox proportional hazards model. A 2-tailed P value <0.05 was considered statistically significant in all analyses. Statistical analyses were performed using the JMP version 11.2 software program (SAS Institute Inc, Tokyo, Japan).

Results

Baseline Characteristics of the Study Population

All the data were stratified according to quartile of serum phosphate level (Table 1). Patients with higher serum phosphate levels had a lower mean age, longer median dialysis history, greater mean dry weight and normalized protein catabolic rate, and lower mean Kt/V for urea. Blood hemoglobin levels and mean serum albumin, creatinine, corrected calcium, and PTH levels were higher, whereas mean serum C-reactive protein levels were lower in subjects with higher serum phosphate levels. Patients with higher serum phosphate levels also used phosphate binders more frequently.

Association Between Baseline Serum Phosphate Level and Brain Hemorrhage

A total of 75 patients experienced new brain hemorrhage during a median observation period of 3.9 years, with a crude incidence rate of 7.1 per 1000 person-years and crude incidence rates per 1000 person-years of 4.0 (Q1), 7.0 (Q2), 7.3 (Q3), and 9.3 (Q4), respectively. In an age- and sex-adjusted model, patients with the highest serum phosphate levels (Q4) showed the highest HRs for the incidence of brain hemorrhage compared with the reference group (Q1) (HR [95% CI], 2.41 [1.17–5.47]; P=0.016; Table 2). The multivariate-adjusted model showed that higher serum phosphate levels were associated with increased risk of brain hemorrhage, with a HR for every 1 mmol/L increase in serum phosphate level of 1.26 (95% CI, 1.03–1.54; P=0.025; Table 2).

Effect Modifications by Baseline Clinical Characteristics Regarding Association Between Serum Phosphate Level and Brain Hemorrhage

There was a significant interaction between serum phosphate levels and serum total cholesterol levels regarding brain hemorrhage (P for interaction 0.011). Higher phosphate levels increased the risk of brain hemorrhage in patients with lower serum cholesterol levels (<270 mg/dL [150 mg/dL]), but this effect was not significant in patients with higher serum total
cholesterol levels (≥270 mmol/L [150 mg/dL]). There were no significant interactions between serum phosphate levels and other baseline characteristics regarding brain hemorrhage (P for interaction 0.150–0.979; Figure 1).

**Other Risk Factors for Brain Hemorrhage**
In addition to serum phosphate levels, male sex, history of cardiovascular events, higher systolic blood pressure, and lower blood hemoglobin levels were also identified as significant risk factors for brain hemorrhage.

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Table 1. Baseline Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Q1 (Pi≤1.29), (n=773)</th>
<th>Q2 (1.30≤Pi≤1.54), (n=776)</th>
<th>Q3 (1.55≤Pi≤1.79), (n=930)</th>
<th>Q4 (1.80≤Pi), (n=958)</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66.9±12.3</td>
<td>65.7±12.7</td>
<td>62.9±12.2</td>
<td>60.1±12.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (male), %</td>
<td>58.2</td>
<td>59.7</td>
<td>59.2</td>
<td>59.2</td>
<td>0.784</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>30.0</td>
<td>32.3</td>
<td>28.7</td>
<td>25.6</td>
<td>0.014</td>
</tr>
<tr>
<td>Dialysis history, y</td>
<td>4.8 (1.7–10.7)</td>
<td>5.2 (2.0–11.1)</td>
<td>6.0 (2.4–12.3)</td>
<td>6.3 (2.4–11.9)</td>
<td>0.004</td>
</tr>
<tr>
<td>History of cardiovascular events, %</td>
<td>37.6</td>
<td>33.6</td>
<td>34.1</td>
<td>30.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Dry weight, kg</td>
<td>51.1±10.0</td>
<td>53.8±11.0</td>
<td>54.1±10.9</td>
<td>55.7±12.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>152.1±25.2</td>
<td>154.6±22.8</td>
<td>153.0±23.2</td>
<td>152.5±22.7</td>
<td>0.827</td>
</tr>
<tr>
<td>nPCR, g/kg/d</td>
<td>0.90±0.21</td>
<td>0.95±0.19</td>
<td>0.96±0.17</td>
<td>1.01±0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kt/V for urea</td>
<td>1.60±0.28</td>
<td>1.58±0.27</td>
<td>1.57±0.28</td>
<td>1.56±0.28</td>
<td>0.001</td>
</tr>
<tr>
<td>Blood hemoglobin, g/L</td>
<td>103.3±11.5</td>
<td>104.7±11.3</td>
<td>106.2±11.5</td>
<td>106.6±11.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Serum biochemistry**

| Albumin, g/L                          | 36.6±4.9               | 38.2±4.0                    | 38.5±3.9                    | 38.8±4.4               | <0.001      |
| Total cholesterol, mmol/L             | 270 (229–313)          | 275 (234–322)               | 275 (239–319)               | 274 (238–326)          | 0.911       |
| C-reactive protein, mmol/L            | 1.43 (0.67–4.10)       | 1.24 (0.57–2.86)            | 1.24 (0.48–2.86)            | 1.24 (0.57–2.86)       | <0.001      |
| Creatinine, μmol/L                    | 790±219                | 885±229                     | 923±212                     | 995±241                | <0.001      |
| Corrected calcium, mmol/L             | 2.34±0.19              | 2.35±0.18                   | 2.36±0.19                   | 2.37±0.20              | <0.001      |
| Pi, mmol/L                            | 1.10±0.18              | 1.42±0.06                   | 1.65±0.07                   | 2.06±0.25              | <0.001      |
| PTH (intact assay), ng/L              | 78 (36–148)            | 102 (53–197)                | 117 (53–238)                | 133 (57–275)           | <0.001      |

**Medications**

| Use of Pi-binders, %                  | 72.2                   | 81.7                        | 82.6                        | 86.8                   | <0.001      |
| Use of VDRAs, %                       | 64.2                   | 73.1                        | 71.7                        | 70.7                   | 0.013       |
| Use of antihypertensive drugs, %      | 58.1                   | 66.4                        | 63.3                        | 62.7                   | 0.176       |

Data are expressed as the mean±standard deviation or percentages. Dialysis history and serum levels of total cholesterol, C-reactive protein, and PTH are shown as the median (interquartile range). A 2-tailed P value <0.05 was considered statistically significant. Ranges of serum Pi levels in each quartile in US units were as follows: Q1, ≤4.0 mg/dL; Q2, 4.1–4.7 mg/dL; Q3, 4.8–5.5 mg/dL; and Q4, ≥5.6 mg/dL. Conversion factor for SI to US units: Pi in mmol/L to mg/dL, ×3.095. nPCR indicates normalized protein catabolic rate; Pi, phosphate; PTH, parathyroid hormone; and VDRAs, vitamin D receptor activators.

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Table 2. Age- and Sex-Adjusted or Multivariate-Adjusted Hazard Ratios for the Incidence of Brain Hemorrhage

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Brain Hemorrhage/No. of Patients</th>
<th>Age- and Sex-Adjusted</th>
<th>Multivariate-Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Pi, mmol/L</td>
<td></td>
<td>HR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>Q1 (Pi≤1.29)</td>
<td>9/773</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>Q2 (1.30≤Pi≤1.54)</td>
<td>17/776</td>
<td>1.75 (0.80–4.10)</td>
<td>0.167</td>
</tr>
<tr>
<td>Q3 (1.55≤Pi≤1.79)</td>
<td>21/930</td>
<td>1.84 (0.87–4.25)</td>
<td>0.114</td>
</tr>
<tr>
<td>Q4 (Pi≥1.80)</td>
<td>28/958</td>
<td>2.41 (1.17–5.47)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Multivariate-adjusted model adjusted for age, sex, diabetes mellitus, dialysis history, history of cardiovascular events, dry weight, systolic blood pressure, normalized protein catabolic rate, Kt/V for urea, blood hemoglobin level, serum levels of albumin, total cholesterol, C-reactive protein, creatinine, corrected calcium, and parathyroid hormone and use of phosphate binders, vitamin D receptor activators, and antihypertensive drugs. A 2-tailed P value <0.05 was considered statistically significant. Ranges of serum Pi levels in each quartile in US units were as follows: Q1, ≤4.0 mg/dL; Q2, 4.1–4.7 mg/dL; Q3, 4.8–5.5 mg/dL; and Q4, ≥5.6 mg/dL. Conversion factor for SI to US units: Pi in mmol/L to mg/dL, ×3.095. CI indicates confidence interval; HR, hazard ratio; N, number; and Pi, phosphate.
Association Between Baseline Serum Phosphate Levels and Brain Infarction

A total of 139 patients developed brain infarction during the observation period. The crude incidence rate was 13.2 per 1000 person-years, and the crude incidence rates per 1000 person-years in each quartile were 20.2 (Q1), 14.8 (Q2), 9.3 (Q3), and 10.2 (Q4), respectively. In the age- and sex-adjusted model, lower serum phosphate levels (Q1) were significantly associated with a higher risk of brain infarction, compared with Q3 used as a reference (HR [95% CI], 1.81 [1.13–2.96]; P=0.014). Even after adjusting for multiple potential confounding factors, the risk of brain infarction in Q1 was significantly higher than in Q3 (HR [95% CI], 1.65 [1.01–2.74]; P=0.045; Table 3).

Effect Modifications by Baseline Clinical Characteristics Regarding Association Between Serum Phosphate Levels and Brain Infarction

There were significant interactions between serum phosphate levels and sex (P for interaction 0.016) and between serum phosphate levels and systolic blood pressure (P for interaction 0.007) in relation to brain infarction risk. Lower phosphate levels increased the risk of brain infarction in female patients, but not in male patients, and in patients with lower systolic blood pressure (<150 mm Hg), but not in those with higher systolic blood pressure (≥150 mm Hg). There were no significant interactions between serum phosphate levels and other baseline characteristics regarding brain infarction (P for interaction 0.187–0.963; Figure 2).

![Figure 1. Multivariate-adjusted hazard ratios and 95% confidence intervals for the incidence of brain hemorrhage for every 1 mmol/L increase in serum phosphate levels in subgroups stratified according to baseline characteristics and treatment assignment. Results were adjusted using the final selected model, which included age, sex, diabetes mellitus, dialysis history, history of cardiovascular events, dry weight, systolic blood pressure, nPCR, Kt/V for urea, blood hemoglobin, serum levels of albumin, total cholesterol, C-reactive protein, creatinine, corrected calcium, and PTH and use of phosphate binders, VDRAs, and antihypertensive drugs. A 2-tailed P value <0.05 was considered to be statistically significant. CI indicates confidence interval; CRP, C-reactive protein; DM, diabetes mellitus; HR, hazard ratio; nPCR, normalized protein catabolic rate; PTH, parathyroid hormone; SBP, systolic blood pressure; and VDRAs, vitamin D receptor activators.](http://stroke.ahajournals.org/ by guest on August 11, 2017)
Table 3. Age- and Sex-Adjusted or Multivariate-Adjusted Hazard Ratios for the Incidence of Brain Infarction

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Brain Infarction/No. of Patients</th>
<th>Age- and Sex-Adjusted HR (95% CI)</th>
<th>P Value</th>
<th>P for Trend</th>
<th>Multivariate-Adjusted HR (95% CI)</th>
<th>P Value</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Pi, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (Pi ≤1.29)</td>
<td>45/773</td>
<td>1.81 (1.13–2.96)</td>
<td>0.014</td>
<td></td>
<td>1.65 (1.01–2.74)</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>Q2 (Pi 1.30–1.54)</td>
<td>36/776</td>
<td>1.38 (0.84–2.29)</td>
<td>0.207</td>
<td></td>
<td>1.35 (0.82–2.25)</td>
<td>0.242</td>
<td></td>
</tr>
<tr>
<td>Q3 (Pi 1.55–1.79)</td>
<td>27/930</td>
<td>1.00 (reference)</td>
<td>0.102</td>
<td></td>
<td>1.00 (reference)</td>
<td>0.256</td>
<td></td>
</tr>
<tr>
<td>Q4 (Pi ≥1.80)</td>
<td>31/958</td>
<td>1.26 (0.75–2.12)</td>
<td>0.382</td>
<td></td>
<td>1.30 (0.77–2.20)</td>
<td>0.330</td>
<td></td>
</tr>
<tr>
<td>Every 1 mmol/L decrease in serum Pi level</td>
<td>1.49 (0.93–2.40)</td>
<td>0.095</td>
<td></td>
<td></td>
<td>1.30 (0.80–2.13)</td>
<td>0.287</td>
<td></td>
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</table>

Multivariate-adjusted model adjusted for age, sex, diabetes mellitus, dialysis history, history of cardiovascular events, dry weight, systolic blood pressure, normalized protein catabolic rate, Kt/V for urea, blood hemoglobin level, serum levels of albumin, total cholesterol, C-reactive protein, creatinine, corrected calcium, and parathyroid hormone and use of phosphate binders, vitamin D receptor activators, and antihypertensive drugs. A 2-tailed P value <0.05 was considered statistically significant. Ranges of serum Pi levels in each quartile in US units were as follows: Q1, ≤4.0 mg/dL; Q2, 4.1–4.7 mg/dL; Q3, 4.8–5.5 mg/dL; and Q4, ≥5.6 mg/dL. Conversion factor for SI to US units: Pi in mmol/L to mg/dL, ×3.095. CI indicates confidence interval; HR, hazard ratio; N, number; and Pi, phosphate.

Other Risk Factors for Brain Infarction

Multivariate-adjusted Cox proportional hazards analysis (Table II in the online-only Data Supplement) also identified older age, history of cardiovascular events, higher normalized protein catabolic rate, and lower serum PTH levels as being significantly associated with the risk of brain infarction. Diabetes mellitus, higher systolic blood pressure, and lower serum albumin levels were marginally associated with the increased risk of brain infarction.

Association Between Baseline Serum Phosphate Level and Risk of Composite Stroke

We also determined the association between serum phosphate levels and the risk of composite stroke, including both brain hemorrhage and infarction. An age- and sex-adjusted multivariate model found no significant association between serum phosphate levels and the risk of composite stroke (Table III in the online-only Data Supplement).

Discussion

The present observational study demonstrated the associations between serum phosphate levels and brain hemorrhage and infarction, respectively, in hemodialysis patients. Higher serum phosphate levels were significantly associated with an increased risk of brain hemorrhage, even after adjusting for potential confounding factors, whereas a low serum phosphate level (Q1) was significantly associated with an increased risk of brain infarction. These results indicate the importance of managing serum phosphate levels to prevent both brain hemorrhage and infarction in patients undergoing hemodialysis.

The present study provides the first evidence for an association between higher serum phosphate levels and an increased risk of brain hemorrhage in the dialysis population. Although previous studies focused on the association between serum phosphate levels and stroke in the general population, the results were inconsistent. One possible reason for this is that they examined the association between serum phosphate levels and composite stroke, rather than assessing the effects of serum phosphate on brain hemorrhage and brain infarction separately. The results of the present study notably revealed differential effects of serum phosphate on brain hemorrhage and brain infarction; higher serum phosphate levels increased the risk of brain hemorrhage, whereas low levels (Q1) increased the risk of brain infarction. Notably, we found no association between serum phosphate levels and the risk of composite stroke, highlighting the importance of focusing on the respective associations between serum phosphate levels and brain hemorrhage and infarction separately in hemodialysis patients.

To the best of our knowledge, only one previous 1-year study investigated the association between stroke and serum parameters related to CKD-related bone-mineral disorders in a dialysis population. They reported an increased risk of brain hemorrhage in patients with serum intact PTH >500 ng/L (500 pg/mL) and indicated that higher serum phosphate levels also tended to increase the risk of brain hemorrhage. However, the current study found no association between serum PTH levels and brain hemorrhage. This apparent discrepancy may be partly explained by the different study populations; only 5.1% of patients in our study had serum PTH levels ≥500 ng/L (500 pg/mL), and serum PTH levels were controlled below 300 ng/L (300 pg/mL) in 85% of patients. Serum PTH levels may, thus, not have become a risk factor in our population. Further studies are, therefore, needed to clarify the relationship between serum phosphate levels and the risk of brain hemorrhage.

Vascular inflammation is one possible explanation for the association between hyperphosphatemia and brain hemorrhage. Vascular inflammation has been shown to induce brain hemorrhage in both humans and animal models. Phosphate loading directly induced inflammation in vascular smooth muscle cells and increased the expression of matrix metalloproteinases II and IX and cathepsin S, which degrade extracellular matrix and disrupt the integrity of vascular layers. Furthermore, phosphate loading also induced the apoptosis of vascular smooth muscle cells and endothelial cells via oxidative stress and inflammation. Taken together, these results suggest that inflammation induced by phosphate loading promotes the rupture of atherosclerotic small vessels that have already been damaged by various factors in the uremic milieu, resulting in brain hemorrhage.
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Vascular calcification may also play a role in the association between serum phosphate levels and brain hemorrhage. Phosphate overload induces vascular calcification via phenotypic change of vascular smooth muscle cells into osteoblast-like cells, apoptosis of vascular smooth muscle cells, degradation of extracellular matrix in the arterial wall, and release of matrix vesicles.\textsuperscript{31–33} Furthermore, clinical studies indicated that deposition of vascular calcification in the artery in a spotty pattern enhanced wall stress and promoted wall rupture.\textsuperscript{34} These results collectively suggest that high phosphate–induced small-vessel calcification in the brain may lead to rupture of the vessels after exposure to a multitude of arteriosclerotic risk factors in the uremic milieu.

In the present study, lower serum phosphate levels were associated with an increased risk of brain infarction, even after adjusting for serum levels of albumin and C-reactive protein and for dry weight. To the best of our knowledge, no study has examined the direct effects of lower serum phosphate levels on brain metabolism and brain vascular biology. Low serum phosphate levels are often a manifestation of decreased food intake and advanced underlying comorbidities. A recent study showed that malnutrition and inflammation were risk factors for brain infarction.\textsuperscript{35} Further studies are, therefore, needed to determine if lower serum phosphate levels promote brain infarction directly or are just a manifestation of malnutrition in the dialysis population.

Previous studies showed that low serum total cholesterol was associated with a risk of brain hemorrhage in dialysis patients.\textsuperscript{36} In the present study, the effects of serum phosphate on brain hemorrhage were enhanced in patients with lower serum total cholesterol, suggesting that phosphate control may...
be particularly important in patients with low total cholesterol levels. Low serum total cholesterol also increased the risk of cardiovascular mortality in dialysis patients during inflammation and malnutrition, identifying inflammation and malnutrition as confounding factors for low serum cholesterol.

Although our data were adjusted for parameters related to inflammation and malnutrition, we were unable to exclude the possibility that these factors may have confounded our findings. Experimental studies are, therefore, needed to explain the precise mechanisms involved in the interactive effects of cholesterol and phosphate metabolism on vascular biology.

Sex and blood pressure also affected the association between serum phosphate levels and brain infarction risk. Although the biological explanation for these interactions remains unclear, our findings suggest that lower serum phosphate levels may increase the risk of brain infarction, especially in female patients and patients with lower systolic blood pressure.

We also identified several risk factors for stroke, other than serum phosphate levels. Male sex, history of cardiovascular events, higher systolic blood pressure, and lower hemoglobin levels were associated with increased brain hemorrhage risk, consistent with previous reports. Similarly, the identified associations of older age and history of cardiovascular events with increased risk of brain infarction were consistent with previous observations. However, the mechanisms whereby lower serum PTH levels and higher normalized protein catabolic rate enhance the risk of brain infarction remain unclear. On the other hand, other investigators showed that higher serum PTH tended to decrease the risk of brain infarction. Given that malnourished subjects often show lower serum PTH levels, low PTH might, thus, be a manifestation of malnutrition, which is in turn associated with brain infarction.

The protein catabolic rate is often increased during inflammation, and a higher normalized protein catabolic rate might reflect systemic inflammation, which is in turn associated with an increased risk of brain infarction.

This study had several strengths, including a large sample size and the homogeneous patient characteristics in terms of first-ever stroke. Furthermore, the data were collected prospectively, and possible confounders were adjusted for. However, the study also had several limitations. First, a single serum phosphate measurement may not account for intraindividual variability in levels over time and may, thus, lead to misclassification of study subjects into inappropriate phosphate-level categories. Furthermore, determination of the biochemical parameters, including serum phosphate levels at multiple facilities, may have resulted in measurement and misclassification biases. These biases could be reduced by performing all the measurements at a single center. Another potential source of error was the lack of information regarding the subtype of brain infarction and location of brain hemorrhage, given that the effects of serum phosphate levels may differ across stroke subtypes. In addition, the nature of our study design meant that we did not consider the cause of stroke (hypertensive crisis, presence of aneurysm, stenosis of carotid artery, arrhythmia, etc.). Future studies including more detailed information may differentiate between the effects of serum phosphate on the risks of each stroke subtype and, thus, aid the management of dialysis patients based on established risks. Third, although we adjusted for potential confounders considered likely to affect the association between serum phosphate levels and stroke incidence, the effects of unmeasured confounders, such as history of smoking and alcohol, low-density lipoprotein, presence of atrial fibrillation, and the use of anticoagulants, antiplatelet drugs, and lipid-lowering agents were not examined in our study.

Conclusions
This study demonstrated that higher serum phosphate levels increased the risk of brain hemorrhage, whereas low serum phosphate levels increased the risk of brain infarction in hemodialysis patients. These results will improve our understanding of the mechanisms responsible for the increased incidence of stroke in dialysis patients. Interventional trials are required to determine if managing serum phosphate levels within the appropriate range can lower the risk of stroke in patients undergoing hemodialysis.

Acknowledgments
We thank all the doctors and medical staff involved in the Q-Cohort Study.

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Disclosures
None.

References


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Supplemental Material

Association between Serum Phosphate Levels and Stroke Risk in Patients Undergoing Hemodialysis: The Q-Cohort Study

Shunsuke Yamada, MD, PhD; Kazuhiko Tsuruya, MD, PhD; Masatomo Taniguchi, MD, PhD; Masanori Tokumoto, MD, PhD; Kiichiro Fujisaki, MD, PhD; Hideki Hirakata, MD, PhD; Satoru Fujimi, MD, PhD; Takanari Kitazono, MD, PhD;

1 Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
2 Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
3 Department of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan
4 Division of Nephrology and Dialysis Center, Japanese Red Cross Fukuoka Hospital, Fukuoka, Japan
5 Fukuoka Renal Clinic, Fukuoka, Japan

Address for correspondence:
Kazuhiko Tsuruya, MD, PhD
Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 8128582, Japan,
Tel.: +81-92-642-5843 Fax: +81-92-642-5846
E-mail: tsuruya@intmed2.med.kyushu-u.ac.jp
### Supplemental Table I. Other Risk Factors for Brain Hemorrhage Analyzed by Multivariable-Adjusted Cox Proportional Hazard Model

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>2.38 (1.27–4.56)</td>
<td>0.007</td>
</tr>
<tr>
<td>History of cardiovascular events</td>
<td>1.98 (1.22–3.20)</td>
<td>0.006</td>
</tr>
<tr>
<td>Systolic blood pressure, per 10 mmHg increase</td>
<td>1.18 (1.06–1.31)</td>
<td>0.002</td>
</tr>
<tr>
<td>Blood hemoglobin level, per 10 g/L increase</td>
<td>0.69 (0.56–0.85)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The HR was estimated using Cox proportional hazard model. In the multivariable model, age, sex, presence of diabetes mellitus, dialysis history, history of cardiovascular events, dry weight, systolic blood pressure, normalized protein catabolic rate, Kt/V for urea, blood hemoglobin level, serum levels of albumin, total cholesterol, C-reactive protein, creatinine, corrected calcium, phosphate, and parathyroid hormone, and use of phosphate-binders, vitamin D receptor activators, and antihypertensive drugs are included. Parameters that showed P <0.1 other than serum phosphate level are shown. A two-tailed P-value less than 0.05 was considered statistically significant. CI, confidence interval; HR, hazard ratio.
Supplemental Table II. Other Risk Factors for Brain Infarction Analyzed by Multivariable-Adjusted Cox Proportional Hazard Model

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per 10 years increase</td>
<td>1.44 (1.21–1.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.40 (0.95–2.06)</td>
<td>0.088</td>
</tr>
<tr>
<td>History of cardiovascular events</td>
<td>2.21 (1.56–3.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, per 10 mmHg increase</td>
<td>1.07 (0.99–1.16)</td>
<td>0.075</td>
</tr>
<tr>
<td>Normalized protein catabolic rate, per 1 g/kg/day increase</td>
<td>2.65 (1.23–5.38)</td>
<td>0.014</td>
</tr>
<tr>
<td>Serum albumin level, per 10 g/L increase</td>
<td>0.63 (0.39–1.02)</td>
<td>0.060</td>
</tr>
<tr>
<td>Serum intact PTH level, per 100 ng/L increase</td>
<td>0.84 (0.72–0.96)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

The HR was estimated using Cox proportional hazard model. PTH value is expressed as that of intact assay. In the multivariable model, age, sex, presence of diabetes mellitus, dialysis history, history of cardiovascular events, dry weight, systolic blood pressure, normalized protein catabolic rate, Kt/V for urea, blood hemoglobin level, serum levels of albumin, total cholesterol, C-reactive protein, creatinine, corrected calcium, phosphate, and parathyroid hormone, and use of phosphate-binders, vitamin D receptor activators, and anti-hypertensive drugs are included. Parameters that showed P <0.1 other than serum phosphate level are shown. A two-tailed P-value less than 0.05 was considered statistically significant. CI, confidence interval; HR, hazard ratio; PTH, parathyroid hormone.
<table>
<thead>
<tr>
<th>Variable</th>
<th>N of composite stroke/ N of patients</th>
<th>Age- and sex-adjusted</th>
<th>Multivariable-adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P for trend</td>
</tr>
<tr>
<td>Serum Pi (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (Pi≤1.29)</td>
<td>54/773</td>
<td>1.29 (0.87–1.92)</td>
<td>0.198</td>
</tr>
<tr>
<td>Q2 (1.30≤Pi≤1.54)</td>
<td>53/776</td>
<td>1.20 (0.81–1.78)</td>
<td>0.358</td>
</tr>
<tr>
<td>Q3 (1.55≤Pi≤1.79)</td>
<td>48/930</td>
<td>1.00 (reference)</td>
<td>0.505</td>
</tr>
<tr>
<td>Q4 (Pi≥1.80)</td>
<td>59/958</td>
<td>1.30 (0.89–1.91)</td>
<td>0.179</td>
</tr>
<tr>
<td>Every 1 mmol/L increase in serum Pi level</td>
<td>0.92 (0.29–2.89)</td>
<td>0.887</td>
<td></td>
</tr>
</tbody>
</table>

Composite stroke includes both brain hemorrhage and infarction. Multivariable-adjusted model was adjusted for age, sex, presence of diabetes mellitus, dialysis history, history of cardiovascular events, dry weight, systolic blood pressure, normalized protein catabolic rate, Kt/V for urea, blood hemoglobin level, serum levels of albumin, total cholesterol, C-reactive protein, creatinine, corrected calcium, and parathyroid hormone, and use of phosphate-binders, vitamin D receptor activators, and anti-hypertensive drugs. A two-tailed P-value less than 0.05 was considered statistically significant. CI, confidence interval; HR, hazard ratio; N, number; Pi, phosphate.
Supplemental appendix
We would like to thank all the doctors and medical staff who are participating in the Q-Cohort Study. The following personnel (institutions) are participating in this trial: Takashi Ando (Hakozaki Park Internal Medicine Clinic); Takashi Ariyoshi (Ariyoshi Clinic); Kouichiro Goto (Goto Clinic); Fumitada Hattori (Nagao Hospital); Harumichi Higashi (St Mary’s Hospital); Tadashi Hirano (Hakujyuuji Hospital); Kei Hori (Munakata Medical Association Hospital); Takashi Inenaga (Ekisaikai Moji Hospital); Hidetoshi Kanai (Kokura Memorial Hospital); Shigemi Kiyama (Kiyama Naika); Tetsuo Komota (Komota Clinic); Hiromasa Kuma (Kuma Clinic); Yoshiro Maeda (Kozenkai-Maeda Hospital); Junichi Makino (Makino Clinic); Dai Matsuo (Hirao Clinic); Chiaki Miishima (Miishima Naika Clinic); Koji Mitsuiki (Japanese Red Cross Fukuoka Hospital); Kenichi Motomura (Motomura Naika Clinic); Sadatoshi Nakamura (Kokura Daiichi Hospital); Hidetoshi Nakamura (Kokura Daiichi Hospital); Koichi Nakashima (Ohashi Internal Circulatory Clinic); Nobumitsu Okita (Shiroishi Kyoritsu Hospital); Shinichiro Osato (Osato Jin Clinic); Sakura Sakamoto (Fujiyamato Spa Hospital); Keiko Shigematsu (Shigematsu Clinic); Kazumasa Shimamatsu (Shimamatsu Naika Iin); Yoshito Shogakiuchi (Shin-Ai Clinic); Hiroaki Takamura (Hara Hospital); Kazuhiro Takeda (Iizuka Hospital); Asuka Terai (Chidoribashi Hospital); Hideyoshi Tanaka (Mojiko-Jin Clinic); Suguru Tomooka (Hakozaki Park Internal Medicine Clinic); Jiro Toyonaga (Fukuoka Renal Clinic); Hiroshi Tsuruta (Steel Memorial Yawata Hospital); Ryutaro Yamaguchi (Shiseikai Hospital); Taihei Yanagida (Saiseikai Yahata General Hospital); Tetsuro Yanase (Yanase Internal Medicine Clinic); Tetsuhiko Yoshida (Hamanomachi Hospital); Takahiro Yoshimitsu (Gofukumachi Kidney Clinic, Harasanshin Hospital); Koji Yoshitomi (Yoshitomi Medical Clinic).