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Serum Alkaline Phosphatase Levels and Increased Risk of Brain Hemorrhage in Hemodialysis Patients: The Q-Cohort Study

Hiromasa Kitamura[1], Shunsuke Yamada[1], Hiroto Hiyamuta[1], Ryusuke Yotsueda[2], Masatomo Taniguchi[3], Masanori Tokumoto[4], Kazuhiko Tsuruya[5], Toshiaki Nakano[1] and Takanari Kitazono[1]

[1]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[2]Munakata Medical Association Hospital, Fukuoka, Japan
[3]Fukuoka Renal Clinic, Fukuoka, Japan
[4]Department of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan
[5]Department of Nephrology, Nara Medical University, Nara, Japan

Aim: Elevated serum alkaline phosphatase (ALP) levels have been associated with increased risks of all-cause and cardiovascular mortality in patients receiving hemodialysis. However, little is known about the impact of serum ALP levels on the development of stroke, such as brain hemorrhage and infarction.

Methods: A total of 3,497 patients receiving maintenance hemodialysis registered in the multicenter observational Q-Cohort Study were analyzed. The primary outcomes were the incidences of brain hemorrhage and infarction. The covariate of interest was serum ALP levels. Patients were divided into tertiles based on their serum ALP levels (U/L) at baseline (T1, <69.3; T2, 69.3–98.4; T3, >98.4). The risks of brain hemorrhage, brain infarction, and composite stroke were estimated using Cox proportional hazards models and competing risk models with all-cause death as a competing risk.

Results: A total of 89 patients developed brain hemorrhage and 195 patients developed brain infarction during the 4-year follow-up period. The risk of brain hemorrhage in the highest tertile (T3) was significantly higher than that in the lowest tertile (T1) (multivariable-adjusted hazard ratio [95% confidence interval], 1.93 [1.12–3.35], subdistribution hazard ratio, 1.91 [1.10–3.30]). However, there was no significant association between serum ALP levels and the risk of brain infarction or composite stroke.

Conclusions: Higher serum ALP levels are associated with an increased risk of brain hemorrhage, but not brain infarction, in patients receiving maintenance hemodialysis. High serum ALP level is thus an important risk factor for brain hemorrhage in hemodialysis patients.

Key words: Alkaline phosphatase, Brain hemorrhage, Brain infarction, Cohort study, Hemodialysis

Introduction/Aim

Chronic kidney disease (CKD) is an established risk factor for stroke[1]. Patients with CKD, especially those undergoing hemodialysis, are also more likely to have poor functional outcomes and death after stroke[2, 3]. Despite therapies aimed at the conventional risk factors for stroke, the incidence of stroke in hemodialysis patients remains unacceptably high[4-6]. There is thus an urgent need to clarify the pathogenesis of stroke and identify other modifiable risk factors in this population.

CKD-mineral and bone disorder is characterized by abnormal mineral metabolism, bone turnover, and vascular calcification and is a prominent feature of CKD and closely associated with elevated risks of morbidity and mortality[7, 8]. Alkaline phosphatase (ALP) is a membrane-bound enzyme that catalyzes the

Address for correspondence: Toshiaki Nakano, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 8128582, Japan. E-mail: toshink@med.kyushu-u.ac.jp

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conversion of pyrophosphate into inorganic phosphate and is used as a marker of high bone turnover in patients with CKD[9-11]. Accumulating evidence suggests that higher serum ALP levels are associated with a heightened risk of all-cause and cardiovascular deaths in hemodialysis patients[12, 13]. Previous studies have shown that serum ALP levels are associated with an increased incidence of stroke and outcomes after the incidence of stroke in the general population; however, little is known about these associations in hemodialysis patients[14-17]. Furthermore, the pathogeneses of brain hemorrhage and infarction as causes of stroke differ, suggesting that the relationships of serum ALP levels with brain hemorrhage and brain infarction should be separately examined; however, most previous studies have determined the association between serum ALP levels and the occurrence of composite stroke, including both brain hemorrhage and infarction[16, 17].

This study aimed to elucidate the associations between serum ALP levels and brain hemorrhage and brain infarction separately, using data for patients receiving maintenance hemodialysis registered in the Q-Cohort Study[18, 19].

Methods

Study Population

The Q-Cohort Study was a multicenter, longitudinal, observational cohort study of patients undergoing maintenance hemodialysis in Japan. The details of the study design have been described elsewhere[18, 19]. The study population consisted of 3,598 hemodialysis outpatients aged ≥ 18 years attending 39 hemodialysis facilities between December 2006 and December 2007. Participants were followed until December 2016. A total of 101 participants for whom baseline data or outcome information were not available were excluded. The remaining 3,497 patients were enrolled in the final study population. The study protocol was approved by the Clinical Research Ethics Committee of the Institutional Review Board at Kyushu University (Approval Number 20–31) and registered in the University Hospital Medical Information Network (UMIN) clinical trial registry (UMIN ID: 000000556). Written informed consent was obtained from all participants at the start of the study. This study was performed according to the Declaration of Helsinki. The ethics committees of all the participating institutions waived the requirement for written informed consent for additional follow-up surveys from 2011 to 2016 due to the retrospective nature of this study.

Outcomes and Covariates

The primary outcomes were the first occurrence of brain hemorrhage or brain infarction during the follow-up period. The definition of each type of stroke has been previously published[20, 21]. Briefly, stroke was defined as a sudden-onset neurological deficit lasting for >24 h. The type of stroke was confirmed by brain imaging, such as computed tomography and magnetic resonance imaging, and was classified as brain hemorrhage or brain infarction by local physicians. Subarachnoid hemorrhage and hemorrhage after ischemic stroke were excluded from the definition of brain hemorrhage. Patients who developed both brain hemorrhage and brain infarction during the follow-up period were classified according to the first type of stroke and were censored for the subsequent stroke. The secondary outcomes were the incidence of composite stroke and all-cause death. Composite stroke included both brain hemorrhage and brain infarction during the follow-up period. Death events were collected from the patients medical records. The covariate of interest was serum ALP level at baseline. Since the Q-Cohort Study was a long-term observational cohort study, right-censored data at a fixed time point of 4 years was used to estimate the risks of brain hemorrhage, brain infarction, and composite stroke. Four-year outcomes were regarded as the major results in the present study, and 10-year outcomes were additionally presented if significant associations were observed during the 4-year follow-up period.

Measurements

The details of risk factor measurements have been previously published[18, 19]. Data included age, sex, cause of end-stage kidney disease (diabetic nephropathy or other), history of stroke, parathyroidectomy, and bone fractures, hemodialysis duration, hemodialysis time per session, clinical data (e.g., blood hemoglobin, serum ALP, albumin, C-reactive protein [CRP], phosphate, corrected calcium, and parathyroid hormone [PTH] levels, body weight, single-pool Kt/V for urea, systolic blood pressure [BP]), and use of phosphate binders, vitamin D receptor activators, and antihypertensive drugs at baseline. Serum levels of ALP were measured using the Japan Society of Clinical Chemistry (JSCC) method and described using the International Federation of Clinical Chemistry (IFCC) method, using the following conversion equation: ALP (IFCC method, U/L) = 0.35 × ALP (JSCC method, U/L)[22]. The corrected serum calcium concentration was based on Paynes formula as follows: corrected calcium (mg/dL) = observed total calcium (mg/dL) + (4.0 – serum

albumin concentration (g/dL)), if the serum albumin level was <4 g/dL[23]. Serum PTH levels were measured using either whole or intact PTH assays depending on the hemodialysis institution. PTH levels in the present study were expressed as serum intact PTH using the following equation: intact PTH (pg/mL) = 1.7 × whole PTH (pg/mL)[11]. Body weight was measured in light clothing without shoes. BP measured before starting a hemodialysis session was collected from the hemodialysis records. Blood samples were collected via vascular access before starting a hemodialysis session.

Statistical Analysis

Patients were divided into tertiles based on their serum ALP levels at baseline: T1, <69.3 U/L; T2, 69.3–98.4 U/L; and T3, >98.4 U/L. Baseline characteristics, according to tertiles of serum ALP levels, were presented as median and interquartile range or number and percentage. Trends across tertiles of serum ALP levels were examined by the Cochran–Armitage and Jonckheere–Terpstra test for categorical and continuous variables, respectively.

Event-free survival probabilities for brain hemorrhage and brain infarction, respectively, were plotted using the Kaplan–Meier method and compared by log-rank tests. Unadjusted and multivariable-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for the occurrence of brain hemorrhage, brain infarction, and composite stroke according to serum ALP tertiles were estimated using a Cox proportional hazards model. The assumption of the proportional hazards analysis was confirmed graphically using log cumulative hazard plots for each outcome, stratified according to serum ALP levels, and tested using an analysis of Schoenfeld residuals. Multivariable-adjusted models were incrementally adjusted for the following potential confounders based on a priori theoretical considerations: Model 1 adjusted for age and sex; Model 2 adjusted for age, sex, the presence of diabetic nephropathy, history of stroke, body weight, systolic BP, blood hemoglobin level, and serum levels of phosphate, corrected calcium, and PTH; and Model 3 additionally included hemodialysis duration, Kt/V for urea, cardiothoracic ratio, serum levels of albumin and total cholesterol, log-transformed serum CRP levels, and use of antihypertensive agents, phosphate binders, and vitamin D receptor activators. Outcomes were also assessed using the Fine–Gray proportional subdistribution hazards model with all-cause death as a competing risk.

In addition, multivariable-adjusted associations between serum ALP levels at baseline and brain hemorrhage, brain infarction, and composite stroke (HRs and 95% CIs) were plotted using restricted cubic spline curves. Restricted cubic spline curves were plotted using four knots located at the 5th, 35th, 65th, and 95th percentiles of serum ALP levels. The multivariable-adjusted model was adjusted for the same variables as the fully adjusted Cox model. Age, hemodialysis duration, body weight, Kt/V for urea, cardiothoracic ratio, systolic BP, blood hemoglobin level, serum levels of albumin, total cholesterol, phosphate, corrected calcium, and PTH and log-transformed serum CRP levels were used as the spline terms. The value of 81.6 U/L as the median serum ALP level was chosen as the reference for each spline plot.

To obtain a reliable estimate for each subgroup, serum ALP levels at baseline were modeled as a continuous variable and HRs were determined for each 50-U/L increment in serum ALP levels. Potential interactions were tested by including relevant interaction terms. Heterogeneity in the association between subgroups was determined by adding a multiplicative interaction term to the relevant Cox model for each 50-U/L increase in serum ALP level.

Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA). A two-tailed p-value <0.05 was considered statistically significant.

Results

Baseline Characteristics of the Study Population

The baseline characteristics of the patients based on tertiles of serum ALP are listed in **Table 1**. Patients with higher ALP levels were significantly older, were more likely to be female, and showed a significantly higher prevalence of a history of bone fractures, longer hemodialysis, lower body weight, and higher cardiothoracic ratios (all $p<0.001$). Serum CRP and PTH levels were also significantly higher in patients with higher serum ALP levels (both $p<0.001$), while blood hemoglobin, serum albumin, total cholesterol, urea nitrogen, creatinine, and phosphate levels, and the use of phosphate binders, vitamin D receptor activators, and antihypertensive agents, were all significantly lower in patients with higher serum ALP levels ($p<0.001$).

Association Between Baseline Serum ALP Levels and Brain Hemorrhage

A total of 89 patients developed brain hemorrhage during the 4-year follow-up period, with an incidence rate of 7.5 per 1,000 person-years. The event-free survival probability for brain hemorrhage

Table 1. Baseline patient characteristics according to tertiles of serum alkaline phosphatase level

		Tertile of serum ALP level, U/L			
	Total (n = 3497)	T1 <69.3 n = 1158	T2 69.3–98.4 n = 1169	T3 >98.4 n = 1170	p for trend
Demographics and comorbidities					
Age, years	64.2 (55.9–72.8)	60.3 (52.2–69.9)	65.3 (57.0–73.6)	66.3 (57.9–74.4)	<0.001
Female sex, %	1424 (40.7)	377 (32.6)	450 (38.5)	597 (51.0)	<0.001
Diabetic nephropathy, %	1024 (29.3)	326 (28.2)	359 (30.7)	339 (29.0)	0.68
History of stroke, %	543 (15.5)	150 (13.0)	211 (18.1)	182 (15.6)	0.09
History of PTX, %	275 (7.9)	86 (7.4)	90 (7.7)	99 (8.5)	0.36
History of bone fracture, %	345 (9.9)	81 (7.0)	127 (10.9)	137 (11.7)	<0.001
Hemodialysis duration, years	5.3 (2.1–11.3)	4.4 (2.1–9.3)	4.9 (1.9–10.9)	7.0 (2.7–13.8)	<0.001
Hemodialysis time per session, h	5.0 (4.0–5.0)	5.0 (4.5–5.0)	5.0 (4.0–5.0)	5.0 (4.0–5.0)	0.009
Body weight, kg	53.0 (45.8–60.5)	55.3 (48.2–63.0)	52.7 (45.7–60.9)	50.3 (43.7–58.3)	<0.001
Kt/V for urea	1.56 (1.42–1.71)	1.56 (1.40–1.67)	1.56 (1.41–1.69)	1.56 (1.45–1.78)	<0.001
Systolic blood pressure, mmHg	152 (138–168)	153 (140–168)	152 (138–169)	152 (137–167)	0.10
Cardiothoracic ratio, %	50.0 (46.7–53.7)	49.0 (45.9–52.4)	50.0 (47.0–53.5)	51.2 (47.6–55.2)	<0.001
Laboratory tests					
Blood hemoglobin, g/dL	10.6 (9.8–11.3)	10.6 (9.9–11.3)	10.6 (9.9–11.3)	10.4 (9.7–11.2)	<0.001
Serum albumin, g/dL	3.8 (3.6–4.1)	3.9 (3.7–4.2)	3.8 (3.6–4.1)	3.8 (3.5–4.0)	<0.001
Serum C-reactive protein, mg/dL	0.13 (0.06–0.30)	0.10 (0.04–0.23)	0.13 (0.07–0.30)	0.17 (0.09–0.43)	<0.001
Serum total cholesterol, mg/dL	152 (131–178)	158 (136–184)	151 (130–176)	149 (127–176)	<0.001
Serum urea nitrogen, mg/dL	66 (55–76)	67 (57–76)	65 (55–76)	64 (54–75)	0.001
Serum creatinine, mg/dL	10.1 (8.4–12.0)	10.9 (9.0–12.7)	10.0 (8.3–11.9)	9.5 (7.9–11.3)	<0.001
Serum phosphate, mg/dL	4.9 (4.1–5.6)	5.0 (4.3–5.7)	4.8 (4.1–5.7)	4.8 (4.0–5.5)	<0.001
Serum corrected calcium, mg/dL	9.4 (8.9–9.9)	9.4 (9.0–9.9)	9.3 (8.9–9.8)	9.4 (8.9–9.9)	0.06
Serum PTH (intact assay), ng/L	106 (48–216)	79 (36–150)	105 (53–207)	145 (67–301)	<0.001
Medication					
Use of phosphate binders, %	2839 (81.2)	995 (85.9)	961 (82.2)	883 (75.5)	<0.001
Use of VDRAs, %	2450 (70.1)	853 (73.7)	801 (68.5)	796 (68.0)	0.003
Use of antihypertensive agents, %	2191 (62.7)	778 (67.2)	754 (64.5)	659 (56.3)	<0.001

Baseline data expressed as median (interquartile range) or number (percentage). A two-tailed p-value <0.05 was considered significant. Conversion factors for units: hemoglobin in g/dL to g/L, ×10; albumin in g/dL to g/L, ×10; C-reactive protein in mg/dL to nmol/L, ×9.524; cholesterol in mg/dL to mmol/L, ×0.0259; urea nitrogen in mg/dL to mmol/L, ×0.357; creatinine in mg/dL to mmol/L, ×88.4; phosphate in mg/dL to mmol/L, ×0.323; corrected calcium in mg/dL to mmol/L, ×0.25. Abbreviations: ALP, alkaline phosphatase; T, tertile according to serum ALP level; PTH, parathyroid hormone; PTX, parathyroidectomy; VDRA, vitamin D receptor activator.

according to baseline serum ALP levels is shown in **Fig. 1**. The 4-year incidence rate was significantly higher in patients with higher serum ALP levels (log-rank test, p=0.02). Patients in T3 had higher HRs for brain hemorrhage than those in T1 in the unadjusted and multivariable-adjusted Cox proportional hazards models (fully adjusted HR [95% CI], 1.93 [1.12–3.35], p for trend <0.05) **(Table 2)**. Similarly, patients in T3 had a significantly higher risk of brain hemorrhage than those in T1 according to the multivariable-adjusted Fine–Gray model with all-cause death as a competing risk, where 626 patients died of any cause during the 4-year observation period (subdistribution HR [95% CI], 1.91 [1.11–3.30], p< 0.05). The HRs and subdistribution HRs (95% CIs) for each 50-U/L increase in serum ALP levels were 1.22 (1.04–1.44) in the Cox model (p<0.05) and 1.21 (1.04–1.41) in the Fine–Gray model (p<0.05). Multivariable-adjusted restricted cubic spline analysis revealed that HRs for brain hemorrhage were significantly increased in patients with higher serum ALP levels **(Fig. 2)**.

A total of 157 patients developed brain hemorrhage when the survival analysis was extended to 10 years **(Supplementary Table 1)**. Patients in T3 still had a significantly higher HR for brain hemorrhage than those in T1 in multivariable-adjusted Cox models, even over an observation period

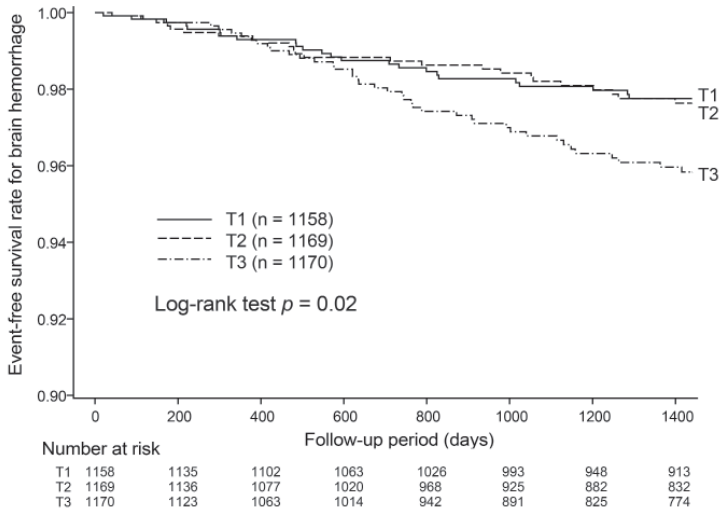

Fig. 1. Event-free survival probabilities for brain hemorrhag during a 4-year follow-up period, according to tertiles of serum alkaline phosphatase (ALP) levels at baseline

Abbreviations: T, tertile of serum ALP level at baseline

Table 2. Hazard ratios for the incidence of brain hemorrhage by tertiles of serum alkaline phosphatase level during a 4-year follow-up period

	Tertile of serum ALP level, U/L				
	T1 <69.3	T2 69.3–98.4	T3 >98.4	*p* for trend	Each 50-U/L increase in serum ALP levels
Number of events (%)	24 (2.1)	24 (2.1)	41 (3.5)		
Unadjusted model	1.00 (reference)	1.04 (0.59–1.84)	1.83 (1.10–3.02)	0.02	1.16 (1.02–1.32)
Model 1	1.00 (reference)	1.05 (0.59–1.86)	1.97 (1.18–3.30)	0.008	1.20 (1.05–1.36)
Model 2	1.00 (reference)	1.05 (0.59–1.88)	2.00 (1.18–3.41)	0.02	1.24 (1.06–1.45)
Model 3	1.00 (reference)	1.05 (0.59–1.88)	1.93 (1.12–3.35)	0.01	1.22 (1.04–1.44)
Competing risk model	1.00 (reference)	1.06 (0.60–1.88)	1.91 (1.11–3.30)	0.02	1.21 (1.04–1.41)

Values given as HR (95% CI) or subdistribution HR (95% CI). Model 1 adjusted for age and sex; Model 2 adjusted for the variables included in Model 1, presence of diabetic nephropathy, history of stroke, body weight, systolic blood pressure, blood hemoglobin level, serum levels of phosphate, corrected calcium, and parathyroid hormone; Model 3 adjusted for the variables included in Model 2, hemodialysis duration, Kt/V for urea, cardiothoracic ratio, serum levels of albumin and total cholesterol, log-transformed serum C-reactive protein levels, use of phosphate binders, vitamin D receptor activators, and antihypertensive agents; competing model adjusted for the variables included in Model 3 with all-cause death as a competing risk. A two-tailed *p*-value <0.05 was considered significant. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio; T, tertile according to serum ALP level.

of 10 years (fully adjusted HR [95% CI], 1.63 [1.09–2.46]).

Effects of Higher Serum ALP Levels on Brain Hemorrhage in Subgroups Stratified by Baseline Clinical Characteristics

There were significant interactions between serum ALP levels and the cause of end-stage kidney disease in relation to the risk of brain hemorrhage during the 4-year follow-up period ($p<0.05$) **(Fig. 3)**. Specifically, the association between serum ALP levels and the risk of brain hemorrhage was significantly augmented in patients with diabetic nephropathy than in those without diabetic nephropathy.

Association Between Baseline Serum ALP Levels and Brain Infarction

A total of 195 patients experienced brain infarction during the 4-year follow-up period, with an incidence rate of 16.4 per 1,000 person-years. The event-free survival probability for brain infarction according to serum ALP levels was almost significant across the tertiles (log-rank test, $p=0.06$) **(Fig. 4)**, but there was no significant difference in the incidence of brain infarction among the tertiles according to multivariable-adjusted risks **(Table 3)** or multivariable-

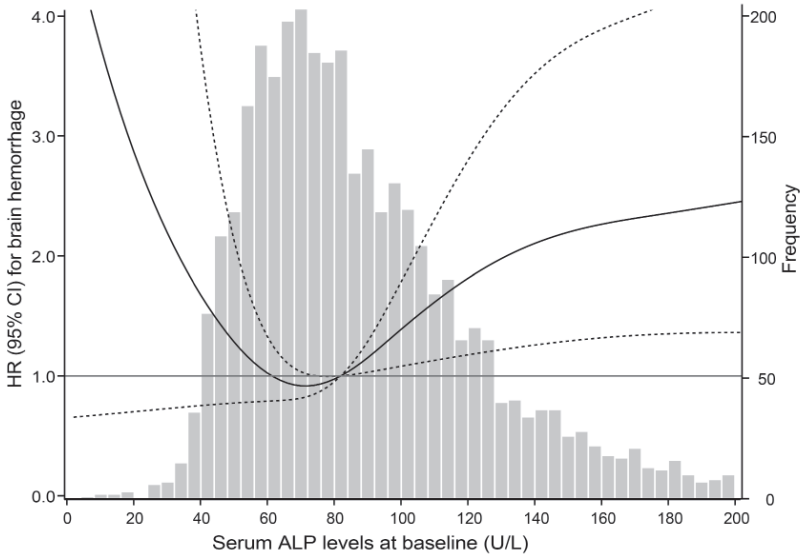

Fig. 2. Multivariable-adjusted restricted cubic spline plots of hazard ratios (HRs) for brain hemorrhage during a 4-year follow-up period, according to serum alkaline phosphatase (ALP) levels at baseline

Solid lines represent HRs and dotted lines represent 95% confidence intervals. Horizontal gray lines correspond to the reference HR (1.0). Histogram of serum ALP levels is overlaid. The median serum ALP level of 81.6 U/L was chosen as the reference value. Covariates used in the multivariable model are described in the Methods section. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio

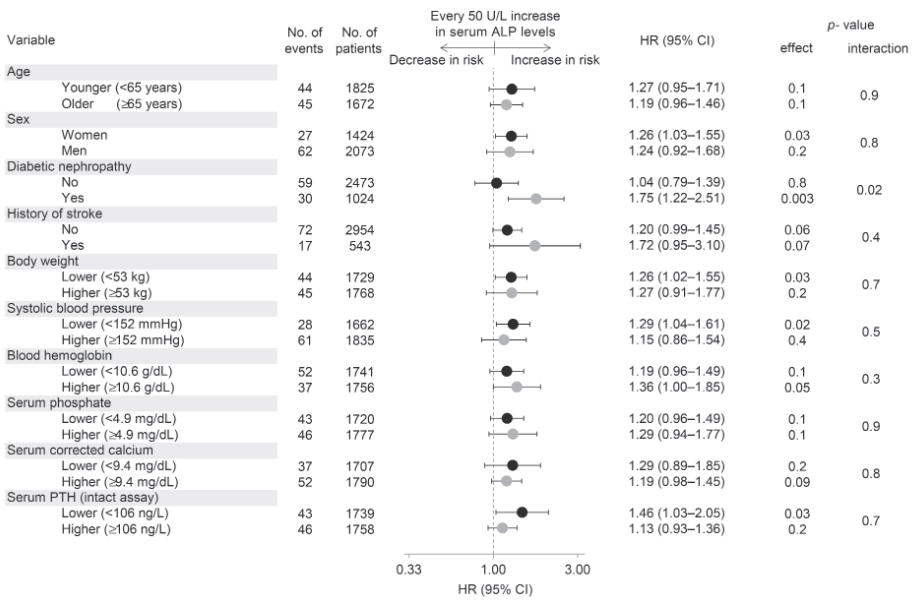

Fig. 3. Multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for brain hemorrhage for a 50-U/L increase in serum ALP levels according to subgroups during a 4-year follow-up period

Covariates used in the multivariable model are described in the Methods section. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio; PTH, parathyroid hormone

adjusted restricted cubic spline analysis **(Fig. 5)**.

Association Between Baseline Serum ALP Levels and Composite Stroke

We confirmed the validity of the separate analyses of stroke subtype by determining the association between serum ALP levels and the risk of composite stroke, including both brain hemorrhage and brain infarction. A total of 284 patients developed composite stroke during the 4-year follow-up period,

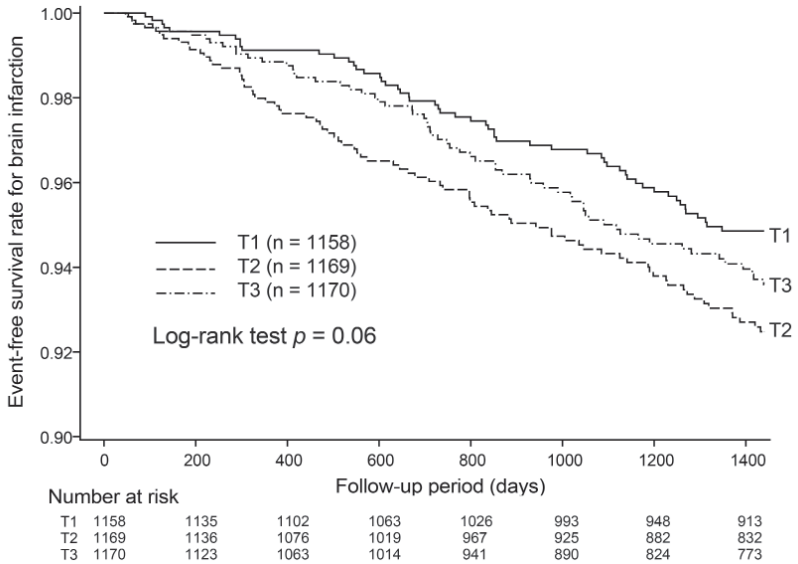

Fig. 4. Event-free survival probabilities for brain infarction during a 4-year follow-up period, according to tertiles of serum alkaline phosphatase levels at baseline

Abbreviations: T, tertile of serum alkaline phosphatase level at baseline

Table 3. Hazard ratios for the incidence of brain infarction by tertiles of serum alkaline phosphatase level during a 4-year follow-up period

	Tertile of serum ALP level, U/L				
	T1 <69.3	T2 69.3–98.4	T3 >98.4	*P* for trend	Each 50-U/L increase in serum ALP levels
Number of events (%)	54 (4.7)	78 (6.7)	63 (5.4)		
Unadjusted model	1.00 (reference)	1.51 (1.07–2.14)	1.26 (0.87–1.81)	0.22	1.06 (0.94–1.20)
Model 1	1.00 (reference)	1.25 (0.88–1.78)	1.02 (0.70–1.47)	0.99	1.02 (0.88–1.19)
Model 2	1.00 (reference)	1.28 (0.90–1.82)	1.13 (0.77–1.66)	0.56	1.12 (0.95–1.31)
Model 3	1.00 (reference)	1.23 (0.86–1.76)	1.06 (0.72–1.58)	0.79	1.12 (0.94–1.32)
Competing risk model	1.00 (reference)	1.24 (0.86–1.78)	1.03 (0.69–1.53)	0.93	1.08 (0.92–1.28)

Values given as HR (95% CI) or subdistribution HR (95% CI). Model 1 adjusted for age and sex; Model 2 adjusted for the variables included in Model 1, presence of diabetic nephropathy, history of stroke, body weight, systolic blood pressure, blood hemoglobin level, serum levels of phosphate, corrected calcium, and parathyroid hormone; Model 3 adjusted for the variables included in Model 2, hemodialysis duration, Kt/V for urea, cardiothoracic ratio, serum levels of albumin and total cholesterol, log-transformed serum C-reactive protein levels, use of phosphate binders, vitamin D receptor activators, and antihypertensive agents; competing model adjusted for the variables included in Model 3 with all-cause death as a competing risk. A two-tailed *P*-value <0.05 was considered significant. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio; T, tertile according to serum ALP level.

with no significant difference in the multivariable-adjusted risks for composite stroke among the serum ALP tertiles **(Supplementary Table 2)**. The HR (95% CIs) for each 50-U/L increase in serum ALP levels was 1.16 (1.03–1.31) in the Cox model ($p<0.05$); however, the HR for composite stroke was not significant in the multivariable-adjusted restricted cubic spline analysis **(Supplementary Fig. 1)**.

Discussion

This study provides the first evidence for a significant association between higher serum ALP levels and an increased risk of brain hemorrhage, even after adjusting for potential confounders. This association remained consistent independent of the length of the observation period, even after considering all-cause death as a competing risk. In contrast, there was no significant association between serum ALP levels and the risk of brain infarction or composite stroke. These findings suggest that elevated serum ALP levels are an important risk factor for brain hemorrhage in patients receiving maintenance hemodialysis.

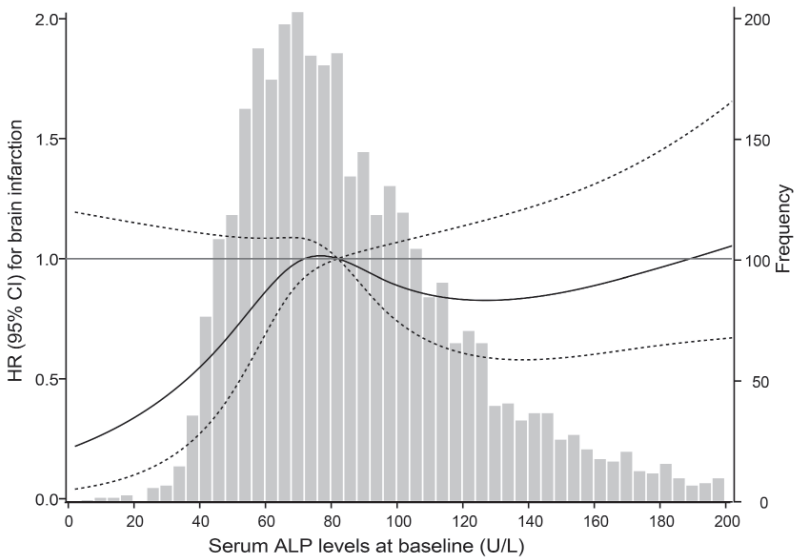

Fig. 5. Multivariable-adjusted restricted cubic spline plots of hazard ratios (HRs) for brain infarction during a 4-year follow-up period, according to serum alkaline phosphatase (ALP) levels at baseline

Solid lines represent HRs and dotted lines represent 95% confidence intervals. Horizontal gray lines correspond to the reference HR (1.0). Histogram of serum ALP levels is overlaid. The median serum ALP level of 81.6 U/L was chosen as the reference value. Covariates used in the multivariable model are described in the Methods section. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio

To the best of our knowledge, this is the first report to demonstrate an association between high serum ALP levels and an increased risk of brain hemorrhage in hemodialysis patients. A previous population-based cohort study showed that elevated serum ALP levels were associated with an increased risk of stroke[16]; however, the primary outcome was composite stroke, and whether serum ALP levels were associated with brain hemorrhage, brain infarction, or both is unclear. Another prospective community-based study showed that high serum ALP levels in the general population were associated with the incidence of brain hemorrhage in men and brain infarction in women, and low serum ALP levels were associated with the incidence of brain infarction in women[15]. One possible explanation for the inconsistent result compared with the current hemodialysis cohort might be the difference in patients backgrounds. Participants in the previous study had fewer risk factors for stroke, such as a relatively younger mean age, and only 15% of participants had concomitant CKD, compared with our study. Therefore, the association between increased serum ALP levels and an elevated risk of brain hemorrhage in the hemodialysis population should be cautiously interpreted until consistent results are obtained by future studies.

Several mechanistic explanations have been postulated to explain the association between elevated serum ALP levels and an increased risk of brain hemorrhage. Patients receiving hemodialysis are reported to be at higher risk for brain hemorrhage than the general population[3]. The reason for the higher risk for brain hemorrhage in hemodialysis patients is that the effect of uremia-related factors (e.g., uremic toxins, intravascular volume overload, renal anemia, and malnutrition in the vascular wall) becomes more apparent as CKD progresses, accelerating damage and rupture of the diseased vascular wall[1]. However, increased serum ALP levels are considered to reflect high bone turnover in patients with CKD, especially those undergoing hemodialysis[9]. Basic research has also shown that tissue-nonspecific ALP hydrolyzes pyrophosphate and simultaneously liberated phosphate[24]. In addition, phosphate loading has been shown to induce apoptosis of vascular smooth muscle cells and endothelial cells *in vivo*[25], and phosphate loading degrades the extracellular matrix[26]. These results collectively indicate that elevated serum ALP levels in patients undergoing hemodialysis may accelerate rupture of potentially necrotized vessel walls or pseudoaneurysms. This could occur via cell death and degradation of the extracellular matrix in the presence of other concomitant risk factors (e.g., traditional and non-traditional risk factors, including the uremic milieu), resulting in the development of brain hemorrhage. Further studies are required to determine whether high serum ALP levels in hemodialysis patients affect vascular cell damage.

Elevated circulating ALP levels may also increase

the risk of brain hemorrhage via inflammation and oxidative stress. Serum ALP levels represent systemic inflammatory reactions, and patients with inflammation are at increased risk of brain hemorrhage, irrespective of the presence of CKD[27, 28]. Recent studies have also demonstrated that calciprotein particles (nanoparticles composed of calcium, phosphate, magnesium, and proteins including fetuin-A) are increased in response to calcium and phosphate overload and directly induce inflammation, oxidative stress, and calcification in vascular smooth muscle cells, leading to cardiovascular events[29, 30]. High bone turnover increases calcium and phosphate influx into the blood and accelerates formation of calciprotein particles. Therefore, higher ALP levels may reflect high bone turnover, followed by increased formation of calciprotein particles. This process eventually triggers rupture of strained vessels in the brain as a result of vascular damage caused by inflammation and oxidative stress[31]. However, the association between ALP levels and brain hemorrhage in the present study remained significant even after adjusting for serum CRP levels. Therefore, further studies are required to determine the mechanisms responsible for the putative association between increased serum ALP levels and an enhanced risk of brain hemorrhage.

Intracranial microbleeds, which are detected on T2*-weighted magnetic resonance imaging, appear to predict future brain hemorrhage in the general population and hemodialysis patients[32, 33]. Intracranial microbleeds on T2*-weighted images correspond histologically to hemosiderin deposition in the perivascular space in association with severe microangiopathy[34]. Intracranial microbleeds are correlated with older age and hypertension in patients undergoing hemodialysis[35]. A previous observational study showed that high serum ALP levels were associated with increased intracranial microbleeds in patients with acute ischemic stroke[36]. These reports support the hypothesis that high serum ALP levels are associated with increased intracranial microbleeds and subsequent events of brain hemorrhage. However, whether serum ALP levels are associated with intracranial microbleeds in hemodialysis patients remains unclear. In addition, whether the location of intracranial microbleeds, such as the cortico-subcortical area, brainstem, basal ganglia, and cerebellum, differs according to serum ALP levels remains unclear. Therefore, further studies are necessary to determine these associations.

Subgroup analysis showed that the effect of higher serum ALP levels on the risk of brain hemorrhage was significantly enhanced in patients with diabetic nephropathy. Persistent hyperglycemia promotes atherosclerosis and vascular calcification through oxidative stress, advanced glycation end products, inflammation, and decreased nitric oxide. This suggests that patients with diabetic nephropathy can suffer from advanced atherosclerosis and may thus be prone to brain hemorrhage when exposed to increased serum ALP levels[37].

Notably, this study showed no significant association between serum ALP levels and brain infarction in patients receiving maintenance hemodialysis. This study might have failed to detect an association between serum ALP levels and the stroke subtype of brain infarction because the three different pathogeneses of brain infarction, namely, large artery atherosclerosis, small vessel occlusion, and cardioembolism, were not differentiated. Therefore, future studies should focus on the specific associations between increased circulating ALP levels and brain infarction subtypes in hemodialysis patients.

In this study, when composite stroke was separated into brain hemorrhage and brain infarction, there was an overt association between serum ALP levels and brain hemorrhage. This finding is consistent with our previous report on the association between serum phosphate levels and the risk of stroke[20]. These results further highlight the importance of considering brain hemorrhage and infarction separately in terms of risk factors for stroke.

This study has some limitations. First, ALP levels were based on a single measurement at each facility, which could have resulted in misclassification of the exposure. Second, the source of increased circulating ALP levels was not determined. There were no data on the patients histories of liver disease, serum liver enzymes, and bone metabolism markers, including bone-type ALP. Therefore, effective approaches for reducing serum ALP levels could not be identified. Third, we lacked important information on management of stroke, such as the use of lipid-lowering agents, anticoagulants, and antiplatelet drugs, the presence of atrial fibrillation, and smoking and drinking habits. Although potential confounders were rigorously adjusted for, these unmeasured and unknown factors might have caused residual confounding. Finally, there was no information regarding the location of the brain hemorrhage and the subtype of brain infarction. Therefore, further studies are required to address these clinical issues.

Conclusion

This study shows that high serum ALP levels are significantly associated with an increased risk of brain

hemorrhage, but not brain infarction, in patients undergoing maintenance hemodialysis. Our results indicate that brain hemorrhage and brain infarction should be separately analyzed for identifying the risk factors for stroke. Serum ALP levels are an important risk factor for brain hemorrhage in hemodialysis patients. Further research is required to determine if interventions aimed at lowering serum ALP levels can reduce the risk of brain hemorrhage in these patients.

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Conflict of Interest

The authors declare that they have no relevant financial interests.

Authors Contributions

Hiromasa Kitamura contributed to the study design, statistical analysis, interpretation of data, and drafting of the manuscript. Shunsuke Yamada contributed to the study design, statistical analysis, interpretation of data, and drafting of the manuscript. Hiroto Hiyamuta contributed to the study design, data cleaning, interpretation of data, and drafting of the manuscript. Ryusuke Yotsueda contributed to the interpretation of data and drafting of the manuscript. Masatomo Taniguchi contributed to the acquisition of data, and critical revision of the manuscript. Masanori Tokumoto contributed to the critical revision of the manuscript. Kazuhiko Tsuruya contributed to the data cleaning and critical revision of the manuscript. Toshiaki Nakano contributed to the funding, acquisition of data, the study design, and drafting of the manuscript. Takanari Kitazono contributed to critical revision of the manuscript and supervision of the study. All authors provided critical reviews of the draft and approved the final version.

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Supplementary Table 1. Hazard ratios for the incidence of brain hemorrhage by tertiles of serum ALP level during a 10-year period

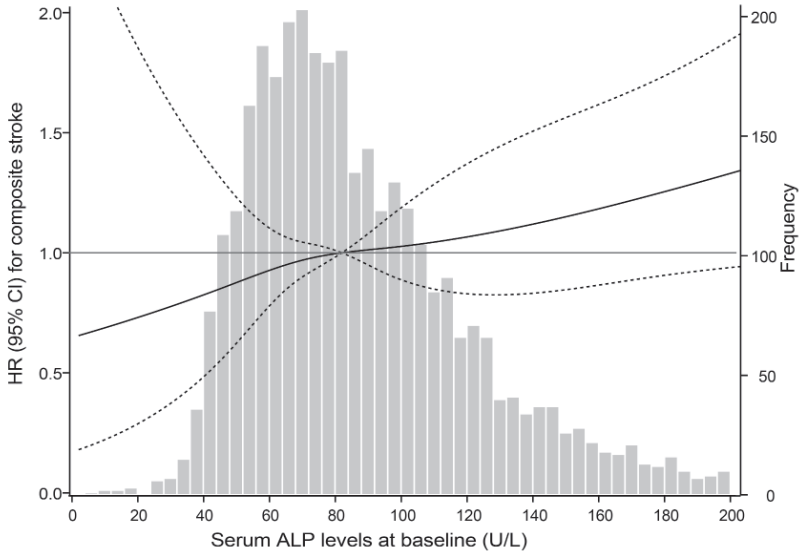
	Tertile of serum ALP levels, U/L				
	T1 <69.3	T2 69.3–98.4	T3 >98.4	*p* for trend	Each 50-U/L increase in serum ALP levels
Number of events (%)	49 (4.2)	44 (3.8)	64 (5.5)		
Unadjusted model	1.00 (reference)	0.99 (0.66–1.49)	1.53 (1.05–2.22)	0.02	1.13 (1.00–1.27)
Model 1	1.00 (reference)	0.99 (0.65–1.49)	1.58 (1.08–2.32)	0.02	1.15 (1.02–1.30)
Model 2	1.00 (reference)	0.98 (0.65–1.49)	1.63 (1.10–2.42)	0.01	1.21 (1.05–1.39)
Model 3	1.00 (reference)	0.98 (0.64–1.48)	1.63 (1.09–2.46)	0.02	1.21 (1.05–1.39)
Competing risk model	1.00 (reference)	0.96 (0.64–1.45)	1.56 (1.04–2.35)	0.03	1.17 (1.03–1.33)

Values given as HR (95% CI) or subdistribution HR (95% CI). Model 1 adjusted for age and sex; Model 2 adjusted for the variables included in Model 1, presence of diabetic nephropathy, history of stroke, body weight, systolic blood pressure, blood hemoglobin level, serum levels of phosphate, corrected calcium, and parathyroid hormone; Model 3 adjusted for the variables included in Model 2, hemodialysis duration, Kt/V for urea, cardiothoracic ratio, serum levels of albumin and total cholesterol, log-transformed serum C-reactive protein levels, use of phosphate binders, vitamin D receptor activators, and antihypertensive agents; competing model adjusted for the variables included in Model 3 with all-cause death as a competing risk. A two-tailed *P*-value <0.05 was considered significant. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio; T, tertile according to serum ALP level.

Supplementary Table 2. Hazard ratios for the incidence of composite stroke by tertiles of serum ALP level during a 4-year period

	Tertile of serum ALP level, U/L				
	T1 <69.3	T2 69.3–98.4	T3 >98.4	*p* for trend	Each-50 U/L increase in serum ALP levels
Number of events (%)	78 (6.7)	102 (8.7)	104 (8.9)		
Unadjusted model	1.00 (reference)	1.37 (1.02–1.84)	1.43 (1.07–1.92)	0.02	1.10 (1.01–1.20)
Model 1	1.00 (reference)	1.20 (0.89–1.62)	1.27 (0.94–1.71)	0.13	1.09 (0.99–1.21)
Model 2	1.00 (reference)	1.19 (0.88–1.61)	1.34 (0.98–1.83)	0.12	1.17 (1.04–1.30)
Model 3	1.00 (reference)	1.18 (0.87–1.59)	1.29 (0.94–1.78)	0.12	1.16 (1.03–1.31)
Competing risk model	1.00 (reference)	1.19 (0.88–1.62)	1.27 (0.92–1.74)	0.15	1.14 (1.02–1.28)

Composite stroke included both brain hemorrhage and brain infarction. Values given as HR (95% CI) or subdistribution HR (95% CI). Model 1 adjusted for age and sex; Model 2 adjusted for the variables included in Model 1, presence of diabetic nephropathy, history of stroke, body weight, systolic blood pressure, blood hemoglobin level, serum levels of phosphate, corrected calcium, and parathyroid hormone; Model 3 adjusted for the variables included in Model 2, hemodialysis duration, Kt/V for urea, cardiothoracic ratio, serum levels of albumin and total cholesterol, log-transformed serum C-reactive protein levels, use of phosphate binders, vitamin D receptor activators, and antihypertensive agents; competing model adjusted for the variables included in Model 3 with all-cause death as a competing risk. A two-tailed *P*-value <0.05 was considered significant. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio; T, tertile according to serum ALP level.



Supplementary Fig. 1. Multivariable-adjusted restricted cubic spline plots of hazard ratios (HRs) for composite stroke during a 4-year follow-up period according to serum alkaline phosphatase (ALP) levels at baseline

Solid lines represent HRs and dotted lines represent the 95% confidence intervals. Horizontal gray lines correspond to the reference HR (1.0). A histogram of serum ALP levels was overlaid. The overall median serum ALP level of 81.6 U/L was chosen as the reference value. The multivariable-adjusted model was adjusted for age, sex, presence of diabetic nephropathy, history of stroke, hemodialysis duration, body weight, Kt/V for urea, systolic blood pressure, cardiothoracic ratio, blood hemoglobin level, serum levels of albumin, total cholesterol, phosphate, corrected calcium, and parathyroid hormone, log-transformed serum C-reactive protein levels, and use of phosphate binders, vitamin D receptor activators, and antihypertensive agents. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio.