Increased Serum Alkaline Phosphatase in Patients with Acute Ischemic Stroke

Yang Liu, MS,*,† Xue Liang, MS,*,† Xiaomin Xu, MS,‡ Meixue Dong, DR,*,† Shiyu Jia, MS,*,† Changqi Lu, MS,*,† and Youdong Wei, DR*,†

Background: Stroke is one of the most common causes of disability and death. Higher alkaline phosphatase (ALP) levels have been associated with poor functional outcomes and mortality in previous studies. We investigated alterations in serum ALP concentrations and functional outcomes in patients with acute ischemic stroke (AIS). *Methods:* Patients with first-ever AIS were recruited to participate in the study. Serum ALP levels were measured using a Cobas Integra 400 Plus automatic biochemical analyzer, and severity of stroke was evaluated using the National Institutes of Health Stroke Scale (NIHSS) score on admission. Functional outcome was measured using the modified Rankin scale 1 year after admission. *Results:* Serum ALP concentration was increased in patients with AIS (81.75 ± 20.49 versus 69.93 ± 16.12 U/L, $P = .000$) and the optimal ALP cutoff point for diagnosing patients with AIS was 81.50 U/L, with a sensitivity of 49.5% and specificity of 78.9%. However, there was no significant correlation between ALP and NIHSS scores ($r = .170$, $P = .085$) and ALP was not significantly different between favorable and unfavorable functional outcomes (81.76 ± .60 versus 81.70 ± 20.54 U/L, $P = .802$). *Conclusions:* Serum ALP concentration, which was increased in patients with AIS, might represent a low-potency biomarker for the diagnosis of AIS. However, this was not significantly correlated with NIHSS scores or the functional outcome after 1 year.

Key Words: Alkaline phosphatase—biomarker—acute ischemic stroke—functional outcome

Introduction

Stroke is one of the most common causes of disability and death.[1] In fact, stroke is the third most common cause of global disability. In the United States, stroke causes 1 out of every 20 deaths and is the fifth leading cause of death.[2] The use of blood biomarkers to predict stroke outcomes is attractive; however, despite years of research in this area, there are currently no blood biomarkers for stroke in clinical practice.

Alkaline phosphatase (ALP) is generally considered as a clinical marker of bone or liver disease, such as vitamin D deficiency and cholestasis.[3] However, ALP can catalyze the hydrolysis of organic pyrophosphate and promote vascular calcification.[3] Previous studies have found that higher levels of ALP are associated with poorer functional outcomes and mortality in patients with stroke.[4,5] Serum is a reliable clinical specimen and its collection is relatively noninvasive. The aim of this small prospective study was

From the *Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China; †Chongqing Key Laboratory of Neurobiology, Chongqing, China; and ‡Department of Neurology, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China.

Received May 25, 2018; revision received July 17, 2018; accepted September 2, 2018.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Financial Disclosure: This study was supported by Chongqing Health and Family Planning Commission under grant numbers 2017MSXM023 and 2017ZBXM046.

Address correspondence to Youdong Wei, Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China. E-mail: havonewei@163.com

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.09.011

to determine the change in serum ALP activity in Chinese patients with acute ischemic stroke (AIS).

Methods and Materials

A total of 103 patients with clinically confirmed AIS were enrolled in the Department of Neurology at the Affiliated Hospital. During the same enrollment period, 57 healthy controls were included in the physical examination department. Diagnosis of AIS was made according to the World Health Organization standard[6] and verified by magnetic resonance imaging. Exclusion criteria for all participants were as follows: (1) admitted to hospital more than 3 days after the stroke onset; (2) aged less than 41 years or more than 80 years; (3) a history of previous stroke; (4) a history of previous diagnosis of cancer, brain injury, or brain surgery; (5) a history of neurodegenerative disease or another central nervous system disease; and (6) a history of liver disease, cholestatic disease, or another end-stage disease. All subjects provided written informed consent prior to inclusion in this study. This study was approved by the ethics committee of the Affiliated Hospital, and was conducted in accordance with ethical principles for human studies.

Serum samples were obtained by centrifugation of blood specimens. The levels of high-sensitivity C-reactive protein, leucocytes, albumin, alanine transaminase (ALT), aspartate aminotransferase (AST), direct bilirubin, total bilirubin, calcium, and ALP activity were measured using a Cobas Integra 400 Plus automatic biochemical analyzer with matched reagent kits (Roche, Basel, Switzerland). The laboratory benchmarks were quantified per the manufacturer's specifications.

In patients with AIS, the National Institutes of Health Stroke Scale (NIHSS) score was evaluated during blood sampling. NIHSS scores were used to evaluate the degree of neurological deficit. The modified Rankin scale (mRS) scores were assessed 1 year after admission using a structured follow-up telephone interview by a researcher who was blind to the ALP levels. An mRS score of 3-6 points indicates an unfavorable functional outcome. Two observers evaluated all scores, and any differences were resolved through discussion.

We used nonparametric Mann-Whitney *U* tests and chi-square tests to analyze basic demographics and clinical features of all participants. Categorical and continuous data are shown as absolute numbers and percentages (%) and the mean value of the standard deviation. We explored the risk factors for participants' ALP concentrations using univariate and multivariate linear regression analyses, and investigated the relationship between ALP concentrations and NIHSS scores using Pearson's correlation coefficients and scatter plots. Finally, receiver operating characteristic (ROC) curves were used to assess the ability of serum ALP concentrations to distinguish AIS from healthy controls. A available software package (SPSS version 21.0, IBM, Armonk, NY) was used and statistical significance was set at $P < .05$.

Results

The clinical characteristics of all participants are shown in Table 1. We enrolled 160 participants and there were no differences in age between the AIS group (mean age = 62.36 ± 9.94 years) and control group (mean age = 61.28 ± 10.15 years). All patients were treated according to the guidelines of the American Heart Association. The levels of serum ALP were significantly higher in the AIS group (81.75 ± 20.49 U/L) compared with the control group (69.93 ± 16.12 U/L; $P = .000$). Figure 1 shows the ROC curves of serum ALP, which indicates the ability of serum ALP to distinguish between the AIS and control groups; the area under the curve for AIS was .674. According to the ROC, the optimal cutoff point with maximum sensitivity 49.5% plus specificity 78.9% of serum ALP concentration for detecting AIS was 81.50 U/L.

Table 2 presents the univariate linear regression results for serum ALP concentrations in participants. Serum ALP was positively correlated with ALT (beta value = .185, $P = .019$), AST (beta value = .322, $P = .000$), total bilirubin (beta value = .206, $P = .009$), Hs-CPR (beta value = .297, $P = .000$), atrial fibrillation (beta value = .178, $P = .024$), and AIS (beta value = −.286, $P = .000$). Multivariate linear regression analysis to modify revealed that serum ALP concentration was correlated with AST (beta value = .230, $P = .031$), Hs-CPR (beta value = .217, $P = .005$), and AIS (beta value = −.189, $P = .020$).

Serum ALP concentration was not significantly correlated with NIHSS score ($r = .170$, $P = .085$, Fig 2). The AIS group was divided into favorable and unfavorable functional outcome subgroups according to mRS scores. There was no difference in ALP concentration between the favorable functional group and unfavorable functional outcome group (81.76 ± 20.60 versus 81.70 ± 20.54 U/L, $P = .802$, Table 3).

Discussion

The brain contains microvessels rich in ALP, which are therefore used as markers for the blood brain barrier.[7] Two previous similar perfusion experiments found that there was more intense ALP activity in the walls of unperfused small cerebral arteries than in perfused arteries.[8] In experimentally induced cerebral swelling, ALP activity was found to decrease on capillary walls.[9] ALP might participate in the control of transport mechanisms across the capillary wall and glial cell membranes.[10] This study found significantly increased ALP activity in patients with AIS compared with healthy controls. Furthermore, based on the ROC, the optimal cutoff point with maximum sensitivity 49.5% plus specificity 78.9% of serum ALP concentration for detecting AIS was 81.50 U/L. This result suggests that ALP concentration might represent a

Table 1. *Clinical characteristics of stroke patients and healthy controls*

Variable (SD/%)	Stroke patients (103)	Control (57)	*P* value	Variable (SD/%)	Stroke patients (103)	Control (57)	*P* value
Age (year)	62.36 ± 9.94	61.28 ± 10.15	.523	Hs-CRP (mg/L)	4.63 ± 5.95	2.47 ± 4.25	.000
Gender, male	78 (75.7%)	27 (47.4%)	.000	Leucocytes (10^9/L)	7.85 ± 2.58	6.44 ± 2.48	.000
Albumin (g/L)	39.93 ± 3.51	40.25 ± 3.31	.747	Smoking history	58 (56.3%)	20 (35.1%)	.010
ALT (U/L)	24.61 ± 13.08	20.70 ± 11.31	.040	Alcohol consumption	41 (39.8%)	7 (12.3%)	.000
AST (U/L)	24.98 ± 12.26	20.61 ± 6.12	.012	Hypertension	65 (63.1%)	26 (45.6%)	.032
Direct bilirubin (μmol/L)	5.42 ± 2.78	4.24 ± 1.37	.005	Diabetes mellitus	29 (28.2%)	11 (19.3%)	.215
Total bilirubin (μmol/L)	16.52 ± 9.55	10.66 ± 4.36	.000	Hypercholesterolemia	40 (38.8%)	21 (36.8%)	.804
ALP (U/L)	81.75 ± 20.49	69.93 ± 16.12	.000	CHD	12 (11.7%)	6 (10.5%)	.829
Calcium (mmol/L)	2.26 ± 0.10	2.27 ± 0.10	.000	Atrial fibrillation	14 (13.6%)	0 (.0%)	.004

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; CHD, coronary heart disease; Hs-CRP, high-sensitivity C-reactive protein; SD, standard deviation.

low-potency biomarker for the diagnosis of AIS. It is consistent with the above opinion.

We found that that age, serum ALT, AST, total bilirubin, Hs-CPR, atrial fibrillation, and AIS were significantly correlated with ALP concentration. After multivariate linear regression analysis, only serum AST, Hs-CPR, and AIS were positively correlated with serum ALP concentration.

ALP can inactivate organic pyrophosphate, which is an important vascular calcification inhibitor; intravascular calcification can result arteriosclerosis and eventually cause cerebral ischemia.[11] Several previous clinical studies have shown that elevated serum ALP levels are independent predictors of future cardiovascular events.[12-14] In a prospective follow-up of 923 days, studies showed the highest quintile of ALP deaths. The risk increased 2.5-fold compared with the first quintile of patients with ischemic

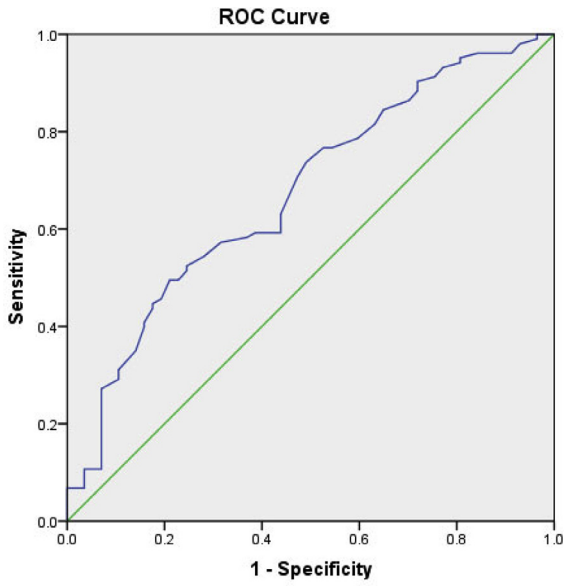

Figure 1. *ROC curve of serum ALP activities for diagnosing ischemic stroke patients. Abbreviations: ALP, alkaline phosphatase; ROC, receiver operating characteristic.*

Table 2. *Univariate and multivariate linear regression analysis of correlation factors for serum Sirtuin 1 activity*

Variable	Univariate		Multivariate	
	Beta value	*P* value	Beta value	*P* value
Age	.065	.416	/	/
Gender, male	−.002	.977	/	/
Albumin	.058	.469	/	/
ALT	.185	.019	—	—
AST	.322	.000	.23	.031
Direct bilirubin	.137	.083	/	/
Total bilirubin	.206	.009	—	—
Calcium	.101	.245	/	/
Hs-CRP	.297	0	.217	.005
Leucocytes	.117	.144	/	/
Smoking history	−.051	.525	/	/
Alcohol consumption	0	.999	/	/
Hypertension	−.034	.672	/	/
Diabetes mellitus	.069	.386	/	/
Hypercholesterolemia	.029	.712	/	/
CHD	.029	.713	/	/
Atrial fibrillation	.178	.024	—	—
AIS	−.286	0	−.189	.02

Abbreviations: AIS; acute ischemic stroke; ALT, alanine transaminase; AST, aspartate aminotransferase; beta value, adjusted regression coefficient; CHD, coronary heart disease; Hs-CRP, high-sensitivity C-reactive protein.

For the multivariate linear regression analysis using a backward method based on the results from univariate linear regression analysis, the values were "/" if variables were excluded before the analysis, and the values were "—" if variables were excluded after the analysis.

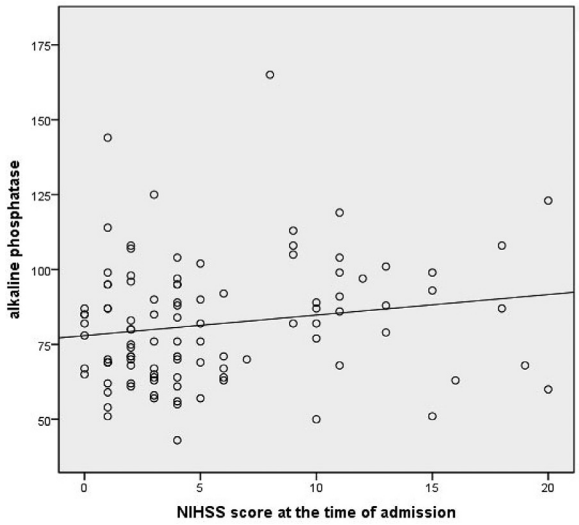

Figure 2. *Linear correlation between serum ALP concentration and NIHSS score. Serum ALP concentration was not significantly correlated with NIHSS score (r = .170, P = .085). Abbreviations: ALP, alkaline phosphatase; NIHSS, National Institutes of Health Stroke Scale.*

stroke.[5] A retrospective observational study found that patients with elevated ALP levels 3 months after stroke onset had poor functional outcomes.[4] The role of ALP in vascular disease has been emphasized and its activity is often used as an early indicator of molecular markers and vascular calcification.[15] ALP may be related to the occurrence or prognosis of vascular disease by accelerating vascular calcification.[5] Given the confirmed association between vascular calcification and ALP, ALP is clearly associated with acute stroke, and may be useful for its diagnosis and prognosis assessment.

At present, evidence for the association between ALP and incident vascular diseases is insufficient. However, unexpectedly, we found that serum ALP concentrations were no different between patients with AIS with favorable functional outcomes and those with unfavorable functional outcomes. Furthermore, there was no correlation between serum ALP concentrations and NIHSS scores. This suggests that ALP has no effect on functional outcomes after 1 year, which differs from some previous reports.

Unlike the 2 previous reports, our results suggest that the concentration of ALP is not related to the outcomes of ischemic stroke, and some relevant factors should be considered. First, the correlation between ALP and the prognosis of acute stroke is unclear. So far, there is no clear pathophysiological mechanism to explain. Second, the grouping of ALP is different. Our study is based on the ALP grouping of the ending, while previous research has been based on grouping by the ALP value. Third, participants were recruited from a single center in China. Small prospective studies have some differences in demographic characteristics, such as sex composition, ALT, AST, direct bilirubin, total bilirubin, smoking status, and alcohol intake. This shows that our group is not quite satisfactory. Fourth, ALP measurements performed after a stroke may not accurately reflect prestroke exposure, and we did not perform a series of measurements of circulating ALP levels to accurately assess prognosis. Fifth, ALP levels can be affected by many factors, which will inevitably interfere with the results. Finally, this study lacks data on the use of antihypertensive and statin therapy.

Therefore, the current findings suggest that ALP may be partially used for the diagnosis of AIS, but may be meaningless for the assessment of prognosis. Because of the limited sample size, we cannot determine the validity of the result; a larger sample size would be needed to better address this issue.

Table 3. *Clinical characteristics of stroke patients with favorable and unfavorable functional outcome*

Variable (SD/%)	Favorable outcome (20)	Unfavorable outcome (83)	*P* value	Variable (SD/%)	Favorable outcome (20)	Unfavorable outcome (83)	*P* value
Age (year)	67.95 ± 7.67	61.01 ± 9.99	.006	Hs-CRP (mg/L)	6.75 ± 6.58	4.13 ± 5.72	.025
Gender, male	17 (85.0%)	61 (73.5%)	.281	Leucocytes (10^9/L)	9.15 ± 2.90	7.53 ± 2.41	.027
Albumin (g/L)	39.10 ± 3.54	40.13 ± 3.50	.417	Smoking history	16 (80.0%)	42 (50.6%)	.017
ALT (U/L)	23.05 ± 11.81	24.99 ± 13.41	.425	Alcohol consumption	12 (60.0%)	29 (34.9%)	.04
AST (U/L)	26.10 ± 16.51	24.71 ± 11.11	.818	Hypertension	14 (70.0%)	51 (61.4%)	.477
Direct bilirubin (μmol/L)	7.19 ± 4.00	5.00 ± 2.18	.006	Diabetes mellitus	7 (35.0%)	22 (26.5%)	.448
Total bilirubin (μmol/L)	20.74 ± 12.16	15.50 ± 8.59	.064	Hypercholesterolemia	5 (25.0%)	35 (45.2%)	.157
ALP (U/L)	81.70 ± 20.54	81.76 ± 20.60	.802	CHD	4 (20.0%)	8 (9.6%)	.195
Calcium (mmol/L)	2.27 ± 0.14	2.26 ± 0.09	.705	Atrial fibrillation	3 (15.0%)	11 (13.3%)	.838
Admission NIHSS	9.45 ± 6.42	4.58 ± 4.25	.002				

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; CHD, coronary heart disease; Hs-CRP, high-sensitivity C-reactive protein; NIHSS, National Institutes of Health Stroke Scale; SD, standard deviation.

Conclusions

Serum ALP concentration was increased in patients with AIS, but was not significantly correlated with NIHSS scores; furthermore, there was no difference in serum ALP concentration between patients with favorable and unfavorable functional outcomes. These results are only partly in agreement with some previous reports; therefore, more multicenter, large-scale investigations are necessary to investigate the connection between ALP and AIS.

References

1. Feigin VL, Forouzanfar MH, Krishnamurthi R, et al. Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet 2014;383:245-254.
2. Bustamante A, Simats A, Vilarbergua A, et al. Blood/brain biomarkers of inflammation after stroke and their association with outcome: from C-reactive protein to damage-associated molecular patterns. Neurotherapeutics 2016;13:671-684.
3. Harmey D, Hessle L, Narisawa S, et al. Concerted regulation of inorganic pyrophosphate and osteopontin by akp2, enpp1, and ank: an integrated model of the pathogenesis of mineralization disorders. Am J Pathol 2004;164:1199.
4. Kim J, Song TJ, Song D, et al. Serum alkaline phosphatase and phosphate in cerebral atherosclerosis and functional outcomes after cerebral infarction. Stroke 2013;44:3547.
5. Ryu WS, Lee SH, Kim CK, et al. Increased serum alkaline phosphatase as a predictor of long-term mortality after stroke. Neurology 2010;75:1995-2002.
6. Hatano S. Experience from a multicentre stroke register: a preliminary report. Bull World Health Org 1976;54:541-553.
7. Lawrenson JG, Reid AR, Finn TM, et al. Cerebral and pial microvessels: differential expression of γ-glutamyl transpeptidase and alkaline phosphatase. Anat Embryol 1999;199:29-34.
8. Bannister RG, Romanul FC. The localization of alkaline phosphatase activity in cerebral blood vessels. J Neurol Neurosurg Psychiatry 1963;26:333-340.
9. Shinonaga Y, Kondo Y, Ogawa K, et al. Alkaline phosphatase activity in normal rodent brain and experimentally induced cerebral swelling. Arch Histol Jpn 1962;22:193.
10. Masuzawa T, Shimabukuro H, Sato F. Cytochemical study of alkaline phosphatase activity in microvessels of rat cerebral cortex. Neurol Med Chir 1983;23:511-514.
11. Ryu WS, Lee SH, Chi KK, et al. High serum alkaline phosphatase in relation to cerebral small vessel disease. Atherosclerosis 2014;232:313-318.
12. Tonelli M, Curhan G, Pfeffer M, et al. Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. Circulation 2009;120:1784.
13. Wannamethee SG, Sattar N, Papcosta O, et al. Alkaline phosphatase, serum phosphate, and incident cardiovascular disease and total mortality in older men. Arterioscler Thromb Vasc Biol 2013;33:1070-1076.
14. Park JB, Kang D, Yang HM, et al. Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. Eur Heart J 2013;34:920-931.
15. Johnson RC, Leopold JA, Loscalzo J. Vascular calcification: pathobiological mechanisms and clinical implications. Circ Res 2006;99:1044.