ORIGINAL RESEARCH

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Serum alkaline phosphatase level is correlated with the incidence of cerebral small vessel disease

Abstract

Background: Vascular calcification is one of the mechanisms underlying the pathogenesis of cerebral small vessel disease (CSVD). Whether higher levels of serum alkaline phosphatase (ALP), a predictor of vascular calcification, are independently associated with CSVD remains inconclusive.

Purpose: The present study explored the link between levels of circulating ALP and CSVD in a Chinese population.

Methods: A total of 568 participants were recruited from the healthcare center at the affiliated Zhongshan Hospital of Dalian University and the Fifth People Hospital of Dalian between January 2010 and December 2016. The subjects were divided into three groups based on the infarct and severity of white matter hyper-intensities (WMH) as categorized by brain magnetic resonance imaging (MRI) analysis and Fazekas rating scales: no/mild cerebral WMH (nm-WMH); moderate-to-severe WMH (MS-WMH); and silent lacunar infarct (SLI). The subjects were also divided into three tertiles based on circulating levels of ALP: ≤ 64 , 65–105 and ≥ 106 (IU/L).

Information regarding the risk factors, such as coronary artery disease, diabetes mellitus, hypertension and serum ALP level, C-reactive protein, homocysteine (HCY), and other laboratory results, were collected. The associations of ALP with WMH and SLI were evaluated using logistic regression analysis.

Results: After adjustment for vascular risk factors, subjects with MS-WMH and SLI were more likely to have ALP levels ≥ 106 IU/L than ≤ 64 IU/L. The mean circulating level of ALP was substantially increased in patients with MS-WMH or SLI compared with patients with nm-WMH. The multivariate model revealed that this significant difference remained when MS-WMH or SLI was added to the model, after adjustment for confounding factors.

Conclusion: The circulating level of ALP was positively correlated with a high risk of silent lacunar infarct and white matter hyperintensity; important indicators of small vessel disease.

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Alkaline phosphatase (ALP), an enzyme that catalyzes the hydrolysis of organic pyrophosphate [1], is generally recognized as a biomarker of diseases related to bone and liver as well as obesity [1]. Recent studies have shown a correlation between serum ALP level and the risk of atherosclerosis [2], which involves vascular calcification in its pathogenesis [3]. Moreover, several studies have suggested that serum ALP was correlated to clinical outcomes of vessel diseases. For example, higher serum ALP levels were positively associated with the all-cause mortality of patients with cardiovascular diseases [4]; higher serum ALP levels were also linked to the functional outcomes and mortality of patients after stroke [5-7]. Hence, serum ALP is linked to cerebrovascular diseases.

Cerebral small vessel disease (CSVD)—a vascular disorder that affects small arteries, arterioles, venules and capillaries in the brain—has long been associated with cognitive impairment and dementia. Characteristics of CSVD include white matter damage with enlarged perivascular spaces, lacunar infarction, and deep microbleeds that coexist with lipohyalinosis [8]. In addition, cerebral white matter hyper-intensities (WMH) and silent lacunar infarct (SLI) are radiological indicators of CSVD occasionally observed in healthy populations [9].

The potential correlation between the circulating levels of ALP and CSVD has been previously explored; however, the findings were inconsistent. For instance, Kim et al. reported that a higher circulating level of ALP was a potential predictor of prognosis of cerebral infarction and not correlated with CSVD [10], while other studies indicated that elevated serum ALP was linked to more severe white matter damage and might serve as an indicator of CSVD [11,12].

In the present study, we aimed to explore a possible link between the circulating ALP levels and CSVD indicators in a neurologically healthy Chinese population.

Methods

Subjects

From January 2010 to December 2016, a total of 568 Chinese Han individuals in Dalian City were recruited from the healthcare center at the affiliated Zhongshan Hospital of Dalian University, or from the Fifth People Hospital of Dalian. All subjects displayed normal neurological function and had either a history of silent lacunar infarct or potential

risk factors. Only those subjects who had received brain magnetic resonance imaging (MRI) examinations were registered in this study.

Demographic and baseline clinical characteristics

For each patient, gender, age, and vascular risk factor (including coronary artery disease, diabetes mellitus, hypertension, smoking and alcohol history) data were collected. Serum ALP levels were measured and these ranged from 35–135 IU/L. To assess the factors correlated to ALP level, the subjects were divided into three tertiles according to the circulating level of ALP (first tertile, ALP $\leq$ 64 IU/L; second tertile, 65–105 IU/L; and third tertile, $\geq$ 106 IU/L).

The following laboratory data were collected: total cholesterol, triglycerides, blood glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), C-reactive protein (CRP), high sensitivity C-reactive protein (hs-CRP), homocysteine (HCY), aspartate aminotransferase (AST), alanine transaminase (ALT), creatinine (CREA), and urea. Demographic characteristics as well as radiological and laboratory results of all subjects were collected from the medical records of the two hospitals.

Brain MRI measurements

The MRI examinations were performed using 1.5 T MR systems. The MRI protocol included a T2-weighted sequence, a T1-weighted sequence, and a fluid attenuated inversion recovery (FLAIR) sequence. Cerebral infarct was assessed on the FLAIR and T1-weighted sequences. SLI was defined as a small [3–15 mm in diameter) cavitated lesion in an area supplied by deep perforating arteries, which presented high signal strength on T1-weighted imaging and low signal strength with a hyperintense rim on FLAIR imaging as reported [8].

To assess the WMH severity, quantification of the deep subcortical and periventricular WM on MRI FLAIR images was performed with the Fazekas scale; the Fazekas scores obtained above were added together to define the degree of WMH as follows: no WMH (0 score), mild WMH (1 or 2 score), moderate WMH (3 or 4 score), and severe WMH (5 or 6 score). Participants were classified into three groups according to the MRI measurement: no/mild cerebral WMH (nm-WMH), moderate-to-severe WMH (MS-WMH), and SLI.

TABLE 1. Demographic and basal clinical characteristics of the subjects based on circulating levels of ALP

	Alkaline phosphatase tertiles				
	1 (n=193)	2 (n=185)	3 (n=190)	H/ χ^2	P
Age (year)	61 (55, 69)	63 (56, 72)	60.5 (55.75,70)	4.082	0.13
Gender (male)	121 (62.7)	111 (60.0)	96 (50.5)	6.38 1	0.041
Hypertension	112 (58.0)	110 (59.5)	37 (19.5)	78.630	<0.001
Diabetes mellitus	51 (26.4)	51 (27.6)	36 (18.9)	4.507	0.105
Coronary artery disease	40 (20.7)	28 (15.1)	43 (22.6)	3.610	0.164
Smoking	76 (39.4)	58 (31.4)	41 (21.6)	14.267	0.001
Alcohol drinker	45 (23.3)	48 (25.9)	47 (24.7)	0.353	0.838
Blood glucose (mmol/L)	5.6 (5.15, 6.55)	5.7 (5.23, 7.06)	5.59 (5.1, 6.05)	7.858	0.02
Fibrinogen	3.25 (2.86, 3.81)	3.41 (2.92, 4.05)	3.27 (2.79, 3.71)	4.452	0.108
Triglycerides (mmol/L)	1.36 (1.01, 1.98)	1.25 (0.9, 1.75)	1.14 (0.87, 1.67)	6.959	0.031
Cholesterol (mg/dL)	4.69 (4.12, 5.42)	4.57 (4.04, 5.13)	4.46 (3.84, 5.17)	8.129	0.017
LDL (mmol/L)	3.14 (2.56, 3.8)	3.01 (2.57, 3.52)	2.92 (2.38, 3.48)	8.598	0.014
HDL (mmol/L)	1.09 (0.93, 1.32)	1.07 (0.9, 1.31)	1.08 (0.94, 1.31)	0.19	0.909
AST (IU/L)	28 (22, 31)	28 (23, 31.5)	27.5 (23, 33)	0.768	0.681
ALT (IU/L)	29 (24, 33)	30 (24, 36)	31 (24, 39)	5.931	0.052
CRP (mg/L)	5.16 (3.37, 6.3)	5.49 (4.79, 6.77)	5.01 (4.02, 5.77)	13.071	0.001
hs-CRP (mg/L)	1.15 (0.93, 1.45)	1.31 (1, 2.17)	2.03 (1.22, 3.16)	70.673	<0.001
HCY (umol/L)	9.32 (7.68, 11.18)	10.12 (9.09, 12.17)	9.65 (8.08, 11.39)	15.421	<0.001
CREA (umol/L)	65.9 (55.6, 80.4)	70.1 (58.9, 86.35)	70.55 (61.5, 87.9)	10.378	0.006
Urea (mmol/L)	5.78 (4.43, 6.92)	5.74 (4.93, 6.73)	5.89 (4.91, 7.01)	1.386	0.5
SLI	17 (8.8)	21 (11.4)	29 (15.3)	3.886	0.143
MS-WMH	38 (19.7)	40 (21.6)	49 (22.4)	2.138	0.343

Abbreviations: ALP: alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; CREA, creatinine; CRP, C-reactive protein; HCY, homocysteine; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MS-WMH, moderate-to-severe cerebral white matter hyper-intensities; SLI, silent lacunar infarct;

P values from Kruskal–Wallis H test or χ^2 test.

TABLE 2. Determination of correlation of SLI and MS-WMH with the circulating ALP levels

	SLI		MS-WMH	
	OR (95%CI)	P	OR (95%CI)	P
Unadjusted				
1 st tertile	Ref		Ref	
2 nd tertile	1.865 (0.988, 3.521)	0.055	1.418 (0.876, 2.293)	0.155
3 rd tertile	1.407 (0.770, 2.569)	0.267	1.260 (0.781, 2.031)	0.343
Adjusted Model 1				
1 st tertile	Ref		Ref	
2 nd tertile	2.569 (1.261, 5.231)	0.009	1.757(1.032 , 2.994)	0.038
3 rd tertile	2.016 (1.029, 3.949)	0.041	1.581(0.935 , 2.672)	0.087
Adjusted Model 2				
1 st tertile	Ref		Ref	
2 nd tertile	8.471 (3.642, 19.707)	<0.001	5.695 (2.982, 10.874)	<0.001
3 rd tertile	3.820 (1.793, 8.138)	0.001	3.028 (1.652, 5.547)	<0.001

Adjusted Model 1 for age, gender, coronary artery disease, diabetes, hypertension, smoking and drinking. Adjusted Model 2 for age, gender, coronary artery disease, diabetes, hypertension, smoking, drinking [alcohol], blood glucose, triglyceride, cholesterol, LDL, CRP, hs-CRP, HCY, and CREA level.

Abbreviations: CI: confidence interval; OR, odds ratio; Ref, reference group for statistical analysis

Statistical analyses

The SPSS v24.0 software was used to perform statistical analyses. Data are presented as the median or percent frequency. The Kruskal–Wallis H test was used for analyzing continuous variables and the Chi-squared (χ^2) test was used

for categorical variables between groups. Logistic regression analyses were carried out to assess the factors linked to WMH or SLI. A p value <0.05 was considered statistically significant.

Results

The demographic and basal clinical characteristics of the participants according to their serum ALP levels are shown in Table 1. The prevalence of MS-WMH and SLI were 22.4% and 11.8%, respectively. There were no differences of MS-WMH, SLI, some risk factors (age, gender, diabetes, coronary heart disease) and some other lab parameters examined in three groups classified according to ALP tertiles except hypertension, smoking, CRP, hs-CRP and HCY levels (Table 1).

After adjustment for risk factors (including age, gender, coronary artery disease, diabetes, hypertension, smoking, and drinking [alcohol]), the multivariate analysis revealed that SLI (OR: 2.016; 95%CI: 1.029–3.949; $p=0.041$) or MS-WMH (OR: 1.581; 95%CI: 0.935–2.672, $p=0.087$) were more likely to occur in the participants in the third ALP tertile compared to those in the first tertile (Table 2).

Following further adjustment for Glu, cholesterol, triglyceride, LDL, CRP, hs-CRP, HCY, and CREA levels in this multivariate model, the circulating level of ALP was significant for SLI (OR: 3.82; 95%CI: 1.79–8.14; $p=0.001$) and MS-WMH (OR: 3.03; 95%CI: 1.65–5.55; $p<0.001$) (Table 2).

Discussion

In the present study, we provided evidence showing that higher levels of circulating ALP were independently associated with SLI and WMH. Previously, Ryu and colleagues reported that the study subjects in the highest ALP quartile had a larger volume of WMH and an increased risk for SLI compared with those in the lowest ALP quartile.¹¹ In addition, Lee et al reported that participants in the third ALP tertile had increased risk for SLI and WMH compared to those in the first tertile,¹² thus suggesting a positive correlation between the circulating level of ALP and CSVD indices, such as WMH and SLI. These findings were strongly supported by our findings from the present study. Taken together, we argue that an elevated plasma ALP level may serve as a valuable predictor for CSVD.

Vascular calcification of arterial vessels in the brain causes vessel walls to become stiff, thereby diminishing the microcirculation distant to the stiffened arteries. In turn

[13,14], vessel wall stiffness may cause CSVD and cognitive impairment, as revealed by a systematic meta-analysis of the correlation between stiffness, CSVD, and cognitive impairment, which showed a good correlation across various cross-sectional studies between markers of CSVD and greater arterial stiffness [15].

Other studies found that vascular calcification contributed to the development of a variety of cerebral ischemia, including incidences of ischemic stroke, WMH and cerebral micro-bleeds [16,17]. Furthermore, two large population-based studies showed an independent association between calcification in various arterial beds and CSVD, including WMH and cerebral infarct [18,19].

Given the important role of vascular calcification in the generation of stiff vessel walls and the enzymatic function of ALP in calcification, it is reasonable to assume a link between ALP and CSVD; indeed, it is highly likely that the serum ALP levels are associated with the vessel wall calcification, leading to vessel wall stiffness and CSVD. These findings also support the rationale for targeting arterial stiffness for the prevention of microvascular brain disease and (possibly) cognitive impairment.

Other mechanisms underlying the association between ALP and CSVD, such as inflammation, have also been proposed; for example, Van Dijk et al. found a correlation between CRP (a well-defined inflammation marker) and the initiation and progression of WMH and cerebral infarct [20]. Interestingly, recent studies showed the relationship between serum ALP and CRP in both cardiovascular disease and chronic kidney disease [21,22].

In addition, increased serum HCY (which plays a major role in the development of cerebral small vessel ischemia [23] and is considered an independent risk factor for SVD) was linked to the inflammation markers in the cardiovascular diseases [24]. The ALP is possibly involved in inflammatory processes that promote the pathogenesis of CSVD, although the correlation between the ALP and some inflammatory factors such as CRP, hs-CRP and HCY levels still needs to be determined. In our study, we found the association between ALP and CSVD might be through inflammation as ALP is an acute phase reactant and its activity is correlated with C reactive protein (CRP), high sensitivity C-reactive protein (hs-CRP) and homocysteine (HCY) levels (Table 1). But after adjustment for confounding factors included CRP, hs-CRP and HCY levels, the circulating level of ALP was still significant for SLI and MS-WMH (Table 2). Thus, we believed that some multiple complex mechanisms including

vascular calcification, endothelial dysfunction, as well as inflammation and oxidative stress may together play a role in ALP and CSVD.

There are several limitations in our study. First, this was a retrospective study, so it had a potential selection bias; it was difficult to find a large study cohort who had undergone brain MRI and MRA. Second, the participants in our study were neurologically normal but with potential vascular risk factors. Hence, our subjects exhibited a higher level of vascular risk factors than healthy controls. Third, limited information on cholestatic disease including g-glutamyl transpeptidase would bias our results although there was scarce evidence supporting the association between cholestatic disease and CSVD. Fourth, the mechanisms underlying the link between CSVD and plasma ALP remain elusive; whether a high circulating ALP level was a risk factor or just a marker for CSVD was unclear. Finally, to consider our analysis robust, many risk factors and laboratory findings were collected and adjusted for statistical analysis; however, we did not have clinical data regarding other disorders (including liver disease and bone disease), and ALP is implicated.

Conclusion

We demonstrated that higher serum levels of ALP are independently associated with WMH and silent lacunar infarct in a Chinese population, and that the serum alkaline phosphatase level is potentially a predictor of CSVD. Future studies using a large cohort are needed to further strengthen our findings.

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References

1. Harmey D, Hessle L, Narisawa S, Johnson KA, Terkeltaub R, Millan JL. 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: 1199-1209.
2. Panh L, Ruidavets JB, Rousseau H, et al. Association between serum alkaline phosphatase and coronary artery calcification in a sample of primary cardiovascular prevention patients. *Atherosclerosis*. 2017; 260: 81-86.

3. Doherty TM, Asotra K, Fitzpatrick LA, et al. Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. *Proc Natl Acad Sci U S A*. 2003; 100: 11201-11206.
4. Tonelli M, Curhan G, Pfeffer M, et al. Relation Between Alkaline Phosphatase, Serum Phosphate, and All-Cause or Cardiovascular Mortality. *Circulation*. 2009; 120: 1784-1792.
5. Ryu WS, Lee SH, Kim CK, Kim BJ, Yoon BW. Increased serum alkaline phosphatase as a predictor of long-term mortality after stroke. *Neurology*. 2010; 75: 1995-2002.
6. 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: 3547-3549.
7. Shimizu Y, Imano H, Ohira T, et al. Alkaline Phosphatase and Risk of Stroke Among Japanese: The Circulatory Risk in Communities Study (CIRCS). *Journal of Stroke & Cerebrovascular Diseases*. 2013; 22: 1046-1055.
8. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. *Lancet Neurol*. 2013; 12: 483-497.
9. Ihara M, Yamamoto Y. Emerging Evidence for Pathogenesis of Sporadic Cerebral Small Vessel Disease. *Stroke*. 2016; 47: 554-560.
10. 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: 3547-3549.
11. Ryu WS, Lee SH, Kim CK, Kim BJ, Kwon HM, Yoon BW. High serum alkaline phosphatase in relation to cerebral small vessel disease. *Atherosclerosis*. 2014; 232: 313-318.
12. Lee HB, Kim J, Kim SH, Kim S, Kim OJ, Oh SH. Association between Serum Alkaline Phosphatase Level and Cerebral Small Vessel Disease. *Plos One*. 2015; 10.
13. Sigrist MK, McIntyre CW. Vascular calcification is associated with impaired microcirculatory function in chronic haemodialysis patients. *Nephron Clinical Practice*. 2008; 108: C121-C126.
14. Shroff RC, McNair R, Figg N, et al. Dialysis Accelerates Medial Vascular Calcification in Part by Triggering Smooth Muscle Cell Apoptosis. *Circulation*. 2008; 118: 1748-1757.
15. van Sloten TT, Protogerou AD, Henry RMA, Schram MT, Launer LJ, Stehouwer CDA. Association between arterial stiffness, cerebral small vessel disease and cognitive impairment: A systematic review and meta-analysis. *Neuroscience and Biobehavioral Reviews*. 2015; 53: 121-130.
16. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation*. 2006; 113: 657-663.
17. Ohmine T, Miwa Y, Yao H, et al. Association between arterial stiffness and cerebral white matter lesions in community-dwelling elderly subjects. *Hypertension Research*. 2008; 31: 75-81.
18. Vidal JS, Sigurdsson S, Jonsdottir MK, et al. Coronary Artery Calcium, Brain Function and Structure The AGES-Reykjavik Study. *Stroke*. 2010; 41: 891-897.
19. Bos D, Ikram MA, Elias-Smale SE, et al. Calcification in major vessel beds relates to vascular brain disease. *Arterioscler Thromb Vasc Biol*. 2011; 31: 2331-2337.
20. van Dijk EJ, Prins ND, Vermeer SE, et al. C-reactive protein and cerebral small-vessel disease: the Rotterdam Scan Study. *Circulation*. 2005; 112: 900-905.
21. Kunutsor SK, Bakker SJ, Kootstra-Ros JE, Gansevoort RT, Gregson J, Dullaart RP. Serum Alkaline Phosphatase and Risk of Incident Cardiovascular Disease: Interrelationship with High Sensitivity C-Reactive Protein. *PLoS One*. 2015; 10: e0132822.
22. Damara S, Raphael KL, Baird BC, Cheung AK, Greene T, Beddhu S. Serum alkaline phosphatase levels associate with elevated serum C-reactive protein in chronic kidney disease. *Kidney Int*. 2011; 79: 228-233.
23. Park SY, An SA, Lee HB, et al. Different impact of hyperhomocysteinemia on cerebral small vessel ischemia and cervico-cerebral atherosclerosis in non-stroke individuals. *Thromb Res*. 2013; 131: e12-16.
24. Oudi ME, Aouni Z, Mazigh C, et al. Homocysteine and markers of inflammation in acute coronary syndrome. *Exp Clin Cardiol*. 2010; 15: e25-28.