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High serum alkaline phosphatase in relation to cerebral small vessel disease

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ABSTRACT

Objectives: Vascular calcification is related with cerebral small vessel disease. We investigated whether alkaline phosphatase (ALP), a marker of vascular calcification, is related to cerebral small vessel disease.
Methods: We included 1082 neurologically healthy subjects who underwent brain magnetic resonance image for a routine health checkup. ALP levels were divided into quartiles. We used quantile regression and logistic regression to evaluate the associations of ALP with white matter hyperintensities (WMH), cerebral infarct and cerebral microbleeds.
Results: Subjects with higher ALP were more likely to have a large WMH volume. The adjusted difference of WMH volume between the highest and the lowest quartiles was 0.27 mL (95% confidence interval [CI]; 0.22–0.31 mL). In addition, cerebral infarct was more prevalent in subjects with higher ALP. Compared to the lowest quartile, adjusted odds ratios of having cerebral infarct for the highest quartile was 2.60 (95% CI, 1.10–6.10). No association was found between ALP and cerebral microbleeds. In addition, we found a conjoint effect of ALP and C-reactive protein(CRP) on cerebral small vessel disease. Compared with subjects with low ALP (≤63 IU/L) and low CRP (≤0.5 mg/dl), those with high ALP (>63 IU/L) and high CRP (>0.5 mg/dl) had larger WMH volume (adjusted difference 0.39 mL; 95% CI 0.37–0.42 mL) and a 3-fold (adjusted OR. 3.37; 95% CI, 1.61–7.03) risk of cerebral infarct.
Conclusion: We found that higher serum levels of ALP are independently associated with WMH and cerebral infarct, but not with cerebral microbleeds.

1. Introduction

Alkaline phosphatase (ALP) is generally recognized as a marker of bony or hepatic disease, such as vitamin D deficiency, renal osteodystrophy, or cholestasis. In addition to its clinical application, ALP has been found to contribute to vascular calcification through catalyzing the hydrolysis of organic pyrophosphate, an inhibitor of vascular calcification [1].

A number of studies have demonstrated the impact of ALP on the prediction of clinical outcomes in various diseases. Higher serum levels of ALP are positively associated with an adverse outcome in dialysis patients [2], and an all-cause or cardiovascular mortality in a general population [3]. In relation to stroke, higher levels of ALP were associated with functional outcome and mortality after stroke [4,5]. Furthermore, a study from a large prospective cohort showed an independent association between ALP and risk of stroke [6]. It is hypothesized that these associations may be largely mediated by a crucial role of ALP in vascular calcification which is associated with incident myocardial infarction [7], cardiovascular morbidity and mortality [8], and an all-cause of death [9]. In addition, a calcification of the aortic arch as well as coronary artery has been associated with a risk of stroke [10].

White matter hyperintensities (WMH), cerebral infarct, and cerebral microbleeds are surrogate markers of vascular brain disease. Chronic ischemia due to arteriosclerosis is a major pathological mechanism of cerebral small vessel disease [11]. However, there is a large variability in cerebral small vessel disease among subject with similar vascular risk factor [12,13]. Hence, more unknown mechanisms are thought to participate in development of cerebral small vessel disease. Two large population studies recently

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demonstrated an independent association between calcification of various vessel beds and cerebral small vessel disease [14,15]. Considering a salient role of ALP in vascular calcification, we hypothesized that higher levels of ALP are related to cerebral small vessel disease.

2. Methods

2.1. Study participants

From October 2003 through December 2004, we enrolled a consecutive series of healthy adult subjects (aged 20 or more) who visited the Seoul National University Hospital Healthcare System Gangnam Center (Seoul, Republic of Korea) and who underwent brain magnetic resonance image (MRI) as part of their routine health check. Subjects without symptoms or signs of organic neurological disease (e.g., stroke, parkinson disease, dementia, etc.) before and at the time of study enrollment were considered neurologically healthy [16]. A total of 1588 subjects were eligible, and subjects without serum ALP ($n = 184$) were excluded. In addition, because ALP is closely associated with cholestatic diseases, we further excluded subjects with total bilirubin $\geq$1.3 mg/dL or reporting to have cholestatic disease ($n = 322$), leaving 1082 subjects for analyses. Clinical information was gathered by a personal interview, and a physical examination was performed by physicians. All subjects provided informed consent. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Seoul National University Hospital institutional board review.

2.2. Demographic characteristics and laboratory data

For each patient, vascular risk factors, including hypertension, diabetes mellitus, hypercholesterolemia, current smoking and coronary artery disease, were obtained. Blood samples were drawn after at least 8 h of fasting, and these were examined for glucose, lipids, and a standard battery of biochemical and hematological tests. Serum ALP levels were tested on unfrozen samples by an automated enzymatic method (Boehringer Mannheim, Mannheim, Germany) using a Hitachi 747 analyzer. Glomerular filtration rate was calculated using a 4-item Modification of Diet in Renal Disease formula [17].

The evaluation of metabolic syndrome (MetS) involved data from a questionnaire, an examination at the healthcare center, and laboratory results. We applied the condition-specific cut points for MetS contained in a recent NCEP-ATP III report [18], with minor modifications. MetS is present when $\geq$3 of following determinants are met: Impaired fasting glucose, elevated blood pressure, hypertriglyceridemia, low HDL-C, and abdominal obesity defined by a large waist circumference.

2.3. Brain MRI measures

MRI examinations were performed at 1.5 T using a CHORUS (ISOL Technology Inc.). The MRI protocol included a T2-weighted sequence, a T1-weighted sequence, a fluid attenuated inversion recovery (FLAIR) sequence, and a T2*-weighted gradient echo sequence. Twenty-seven transaxial slices per scan of images were obtained. Cerebral infarct was assessed on the FLIAR and T1-weighted sequences. Cerebral infarct was defined as a focal lesion of $\geq$3 mm in diameter [19], with signal intensity corresponding to liquor (i.e., hyperintense on T2-weighted images and hypointense on FLAIR images). Quantitative WMH were assessed using MIPAV software package (Medical Image Processing, Analysis, and Visualization, version 4.3; National Institutes of Health) as previously described [20]. In brief, WMH on FLIAR sequence were outlined slice-by-slice using a semiautomatic threshold approach. WMH volumes were calculated by multiplying slice thickness with the region of interest. To account for head size, WMH volumes were adjusted for total intracranial volume determined by T1-weighted sagittal image [21]. Interrater reliabilities for WMH volume and total intracranial volume were 0.81 and 0.91, respectively. Cerebral microbleeds were rated on the T2*-weighted gradient echo sequence. Cerebral microbleeds were categorized into one of three locations: lobar (cortical gray and subcortical or periventricular white matter), deep (deep gray matter: basal ganglia and thalamus; and the white matter of the corpus callosum, internal, external, and extreme capsule), and infratentorial (brainstem and cerebellum) [22]. The κ value of agreement for cerebral infarct and cerebral microbleeds were 0.89 and 0.84, respectively. The raters were blind to the subjects' data.

2.4. Statistical analyses

ALP levels were divided into quartiles with cut points of 53, 63, and 77 IU/L. Baseline demographic and clinical characteristics across quartiles were compared using analysis of variance, chi-square test, and Kruskal–Wallis test as appropriate. The relation between ALP levels and components of MetS was assessed by Spearman rank correlation test. Because of the non-Gaussian distribution for WMH volume, quantile regression was used to examine the relationship between ALP levels and WMH volume, because it is less sensitive than parametric regression methods to extreme outliers [23]. To gain further insight on the linear association between ALP levels and WMH volume, generalized additive models with splines was used [24]. This model provides a method of identifying departures from linearity in exposure–response relationships. The relations of ALP quartiles with cerebral infarct and cerebral microbleeds were examined by multivariable logistic regression analyses. For these analyses, the presence of cerebral infarct and cerebral microbleeds were designated as a dependent variable. Baseline demographic and clinical covariates to be examined were preselected based on prior studies of factors related to cerebral small vessel disease. Given that the impact of ALP on vascular calcification is prominent in patients with chronic kidney disease, we reran the analyses after excluding those with chronic kidney disease [25]. Then, we performed multivariable analyses above with accounting for MetS. Furthermore, considering intercorrelation between ALP, osteoporosis, and stroke, we carried out the analyses after dividing subjects by sex. We further explored the associations between combinations of ALP and C-reactive protein (CRP) and cerebral small vessel disease after dividing the subjects into four mutually exclusive categories defined by the median of ALP (63 IU/L) and CRP (0.5 mg/dl). All analyses were performed using the statistical software package SAS Version 9.1 (SAS Institute).

3. Results

Compared with excluded subjects, included subjects were more likely to be women (51.0% vs. 26.3%), younger (mean $\pm$ SD, 52,4 $\pm$ 10.6 vs. 54.5 $\pm$ 10.5), and diabetic (13.1% vs. 9.7%). Other risk factor profiles and laboratory profiles were not different. In addition, there was no significant difference in relation to cerebral small vessel disease. The mean age of the 1082 subjects was 52 years (range 20–82), and mean ALP was 63 IU/L (range 25–150 IU/L). The subjects in higher quartiles of ALP tended to be older and hypertensive (Table 1). Fasting glucose, total cholesterol, CRP, and phosphate levels increased with increasing quartiles. In addition,