Nutrition, Metabolism & Cardiovascular Diseases (2016) **26**, 683–688

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Serum phosphorus levels are associated with endothelial dysfunction in hypertensive patients

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Received 2 November 2015; received in revised form 2 February 2016; accepted 5 February 2016
Available online 22 February 2016

KEYWORDS
Phosphorus;
Endothelium;
Essential hypertension;
Atherosclerosis;
Cardiovascular risk factors

Abstract *Background and aims:* Recent data demonstrated that serum phosphorus, within the normal range, is an independent predictor of atherosclerotic cardiovascular disease, independently of renal function. Traditional cardiovascular risk factors are important mediators of endothelial dysfunction, the early step of atherosclerosis. We designed this study to evaluate a possible correlation between serum phosphorus and endothelium-dependent vasodilation, evaluated by the strain-gauge plethysmography, in naïve hypertensives.
Methods and results: We investigated by strain-gauge plethysmography, the relationship between forearm blood flow (FBF) response to acetylcholine (ACh) and serum phosphorus in 500 patients with uncomplicated, never-treated, essential hypertension, divided by phosphorus tertiles. There were no significant differences among tertiles with the exclusion of forearm blood flow (FBF). Phosphorus ($\beta = -0.454$; $P = 0.0001$), estimated-glomerular filtration rate (e-GFR, by CKD-EPI formula) ($\beta = 0.261$; $P = 0.0001$), gender ($\beta = 0.215$; $P = 0.0001$), BMI ($\beta = -0.086$; $P = 0.018$), HDL-cholesterol ($\beta = 0.077$; $P = 0.036$) were significantly related to endothelium-dependent vasodilation. In an additional analysis including serum high sensitivity C-reactive protein (hs-CRP) (measured in 400 patients) in the same model, the link between serum phosphorus and ACh-stimulated FBF did not change ($\beta = -0.422$; $P = 0.0001$). Clinically relevant, 0.1 mg of phosphorus increase is associated with a reduction of 22% of ACh-stimulated FBF. On multiple logistic regression analysis, the risk of endothelial dysfunction was about twice higher in patients in the second (OR = 1.754, 95% CI = 1.055–2.915; $P = 0.030$) and three-fold higher in the third tertile (OR = 2.939, 95% CI = 1.598–5.408; $P = 0.0001$) in comparison with those in the first tertile of phosphorus.
Conclusion: An impaired ACh-stimulated FBF is associated with serum phosphorus levels, within the normal range, in hypertensives.

Abbreviations: CV, cardiovascular; e-GFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C reactive protein; BP, blood pressure; ACh, acetylcholine; SNP, sodium nitroprusside; FBF, forearm blood flow.

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http://dx.doi.org/10.1016/j.numecd.2016.02.003

Introduction

Traditional cardiovascular (CV) risk factors, promoting an increased oxidative stress that reduces the nitric oxide bioavailability, are important mediators of endothelial dysfunction [1–8] that is an early event in the atherogenic process [9]. In addition, the biologic relevance of endothelial dysfunction is supported by the evidence that it is able to also predict the progression of subclinical renal [10], cardiac [11] and vascular [12] damage. Thus, on the basis of these evidences, endothelial dysfunction plays a pivotal role in the pathogenetic mechanisms of CV diseases.

It is well known that the pathogenesis of endothelial dysfunction is multifactorial, involving many of the clinical conditions leading to an increased oxidative stress and subclinical inflammation [13,14]. Recent published data reported that elevated serum phosphorus concentrations, even if within the normal range, emerged as a non-traditional risk factor for CV events in patients with high CV risk conditions such as chronic kidney disease [15,16], coronary or peripheral artery diseases [17,18], diabetes [19] as well as in the general population [20,21]. Finally, recent data reported that acute postprandial phosphorus load is inversely correlated with endothelium-dependent vasodilation in young healthy men, evaluated by brachial flow-mediated vasodilation [22].

As previously reported, also essential hypertension is associated with endothelial dysfunction but, at this moment, no data are available regarding a possible relationship between serum phosphorus concentrations and endothelial function in this setting of patients. Thus, we designed this study to evaluate a possible correlation between serum phosphorus levels and endothelium-dependent vasodilation, evaluated by the strain-gauge plethysmography, in a population of newly diagnosed never treated hypertensive patients.

Methods

In this cross-sectional study we analyzed data from 500 Caucasian newly diagnosed never treated hypertensive outpatients (256 men and 244 women; mean age 47.2 + 11.0 years) referred to the Hypertension Clinic of the University Hospital of Catanzaro. Data from this well-characterized study population were previously published by our group [10,11,23]. All patients had a normal renal function evaluated by estimated-glomerular filtration rate (e-GFR) > 60 mL/min/1.73 m^2 and without proteinuria on the dipstick test. None of the patients had a history or clinical evidence of angina, myocardial infarction, valvular heart disease, diabetes mellitus, hypercholesterolemia, peripheral vascular disease, calcium-phosphorus metabolism disorders (particularly, patients with osteoporosis or hyperparathyroidism), coagulopathies, or any disease predisposing to vasculitis or Raynaud's phenomenon. Other exclusion criteria were history of alcohol, drug abuse and use of drugs interfering with phosphorus concentrations. Secondary forms of hypertension were excluded by systematic testing according to a standard clinical protocol, which included measurement of plasma renin activity, aldosterone, Doppler studies of the renal arteries, and/or renal scintigraphy or renal angiography. At the time of the first evaluation, we performed routine blood tests, assessment of atherosclerosis risk factors, and evaluation of vascular function.

Serum creatinine was measured in the routine laboratory by use of the Jaffe methodology and the uricase peroxidase (uricase/POD; Boehringer Mannheim, Mannheim, Germany) method implemented in an auto-analyzer. Values of e-GFR were calculated with the new equation proposed by investigators in the Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration [24]. Microalbuminuria was measured by a turbidimetric inhibition immunoassay (Boehringer Mannheim) in 164 patients whom demographic and anthropometric characteristics and risk factors were comparable to the whole study population.

The quantitative concentration of inorganic phosphate was measured by spectrophotometric determination based on the formation of ammonium molybdophosphate with subsequent reduction to molybdenum blue, using the diagnostic assay COBAS INTEGRA Phosphate 2 (Roche diagnostics, normal range = 2.7–4.5 mg/dL).

C-reactive protein (hs-CRP) was measured by a high-sensitivity turbidimetric immunoassay (Behring) in 400 patients who were comparable to the whole study population with regard to the variables listed in Table 1.

The local Ethics Committee approved the study, and all participants gave written informed consent for all procedures. The study was conducted in accordance with the Declaration of Helsinki.

Forearm blood flow measurement

All studies were performed at 09:00 AM after an overnight fast with the subjects lying supine in a quiet, air-conditioned room (22–24 °C). Subjects were instructed to continue their regular diet and were asked to refrain from alcohol and smoking for 24-h before the test. Forearm volume was determined by water displacement. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter (Vasculon 2, Baxter Healthcare, Deerfield, Ill) was inserted into the brachial artery of the non-dominant arm of each subject for evaluation of blood pressure (BP) and for drug infusion. This arm was elevated slightly above the right atrium, and a mercury-filled Silastic strain gauge was placed on the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-4, DE Hokanson, Issaquah, Wash) calibrated to measure the percent change in volume; this was connected to a chart recorder to obtain forearm blood flow (FBF) measurements. A cuff placed on the upper arm was inflated to 40 mmHg with a rapid cuff inflator (model E-10, DE Hokanson) to exclude venous outflow from the extremity. FBF was measured as the slope of the change in forearm volume; the mean of 3 measurements was obtained at each time point.