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Association between hippuric acid and left ventricular hypertrophy in maintenance hemodialysis patients

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ABSTRACT

Background: Left ventricular hypertrophy (LVH) is one of the most common cardiac abnormalities in patients with end-stage renal disease. Hippuric acid (HA), a harmful uremic toxin, is known to be elevated in patients with uremia, and serum HA levels are associated with neurological symptoms, metabolic acidosis, and accelerated renal damage associated with chronic kidney disease. However, the pathophysiological role of HA in patients with uremia remains unclear. We investigated the association between serum HA levels and echocardiographic measurements in patients undergoing hemodialysis (HD) treatment.

Methods: Eighty consecutive patients treated at a single HD center (44 males, 36 females; mean age 66 y, mean HD duration 6 y) were included in this study. Comprehensive echocardiography was performed after HD. Blood samples were obtained before HD.

Results: Pearson's correlation analysis revealed that serum HA levels were positively correlated with diastolic blood pressure, serum creatinine, left ventricular mass index, end diastolic interventricular septal thickness, left ventricular end-diastolic diameter, left ventricular end systolic diameter, end systolic left ventricular posterior wall thickness, and left atrium diameter, and negatively correlated with age. Furthermore, the HD patients with LVH had higher median serum HA levels than those without LVH (34.2 vs. 18.1 μg/ml, $p = 0.003$). Multiple logistic regression analysis revealed that HA was independently associated with LVH even after adjusting for known biomarkers. Moreover, the receiver operator characteristics curve of HA showed that a HA level of > 26.9 μg/ml was associated with LVH.

Conclusions: HA was significantly associated with LVH. HA could be a novel biomarker of left ventricular overload, which is closely associated with an increased risk of death in HD patients.

1. Introduction

Patients with end-stage renal disease (ESRD) have a substantially higher risk of mortality from cardiovascular diseases than the general population [1,2]. Moreover, cardiovascular disease is the leading cause of morbidity and mortality in patients with ESRD, accounting for > 50% of all deaths [3]. Although classical cardiovascular risk factors such as diabetes mellitus and hypertension are highly prevalent in ESRD patients and are associated with poor cardiovascular outcomes, such traditional risk factors do not fully account for the burden of cardiovascular disease in patients with ESRD [4].

Hippuric acid (HA), a protein-bound uremic retention solute, is known to accumulate during chronic renal failure [5]. Previous studies have suggested that HA plays a role in a variety of pathological conditions, including the inhibition of both plasma protein binding [6] and organic anion secretion by the kidneys [7], and stimulation of

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ammoniagenesis [8]. HA may also be involved in the development of muscular weakness in patients with uremia by inhibiting glucose utilization in muscles [9,10], and inducing neurological symptoms by inhibiting the blood-cerebrospinal fluid barrier [11] or organic anion transport at the blood-brain barrier [12]. Furthermore, HA is known to participate in the correction of metabolic acidosis by stimulating phosphate-independent glutaminase localized at desamidating glutamine and proximal luminal membrane with the formation of ammonia, a dominant elimination product of H^+ [13]. Previous studies have demonstrated that neuromuscular disorders are accompanied by a wide variety of cardiac disorders [14]. Furthermore, neuro-hormonal mechanisms have been shown to be involved in subsequent cellular and vascular dysfunction, leading to tissue ischemia, hypertrophy, and fibrosis [15]. Moreover, metabolic acidosis has been shown to increases levels of fibroblast growth factor 23 (FGF-23) in neonatal mice bones, and FGF-23 has been significantly associated with left ventricular mass index (LVMI) [16]. Furthermore, FGF-23 has been reported to potentially be a novel biomarker of left ventricular overload, which is closely associated with an increased risk of death in hemodialysis (HD) patients [17].

Left ventricular hypertrophy (LVH) is one of the most common cardiac abnormalities in patients with ESRD, and it has been reported to increase the risk of cardiovascular and all-cause mortality by 3.7 times in this population. Age, gender, hypertension, diabetes, increased body mass index (BMI), anemia, and hyperparathyroidism have all been reported to be risk factors for LVH in patients on dialysis. However, these traditional risk factors do not fully account for the burden of LVH in patients with ESRD [18,19]. In addition, echocardiographic measurements reflecting systolic and diastolic function have been shown to be able to predict mortality in HD patients [20]. However, no previous study has examined the relationships between HA and ESRD. Therefore, the aim of the present study was to investigate the role of HA in long-term HD patients by examining the associations between serum HA levels, other biomarkers, and echocardiographic parameters.

2. Materials and methods

2.1. Study population

All HD patients treated at Lee's Endocrinology Clinic in June 2016 were included in this study. All of the patients gave informed consent in accordance with the guidelines of the Human Research Ethics Committee of E-Da Hospital before participating in the study. We prospectively and consecutively enrolled patients with ESRD who had been on regular HD treatment, 3 times weekly on a 4–6-h schedule for at least 6 months. Data on demographic characteristics, medical history, medications (angiotensin-receptor blockers, statins, angiotensin-converting enzyme inhibitors, β-blockers, calcium channel-blockers, warfarin), and blood samples were collected for all subjects at the time of enrollment.

2.2. Blood sampling and laboratory measurements

All laboratory measurements were performed before HD. Blood samples for measurements of HA were drawn, centrifuged, and stored at-80 °C until subsequent assay. Serum levels of calcium, phosphate, Na, K, creatinine, hemoglobin, albumin, glucose, uric acid, ferritin, parathyroid hormone, total-cholesterol, and triglycerides were measured using routine automated laboratory procedures. Serum HA was measured in an uncoated fused-silica capillary (Polymicro Technologies) with an effective length of 50 cm (total length: 40 cm, 50 μm, i.d.). Data acquisition was performed using a Beckman MDQ 32 Karat software system. HA was from Sigma-Aldrich. Acetone, sodium dihydrogen phosphate (NaH_2PO_4), dodecyl sodium sulfate (SDS), sodium hydroxide (NaOH), hydrogen chloride (HCl) and methanol (MeOH) were from Merck. Deionized distilled water (dd-water) was used to prepare buffer solutions and reconstitute samples. All reagents were analytically pure and used without further purification. The capillary electrophoresis apparatus was rinsed with MeOH, dd-water, 1 mol/l NaOH and 1 mol/l HCl for 10 min at 30 psi each for activation. The separation buffer consisted of 100 mM phosphate (pH 4.0) containing 150 mM SDS and 10% MeOH. Samples were introduced into the capillary by hydrodynamic injection at 5 psi for 20 s. Reverse separation was achieved at 20 kV, and the temperature was maintained at 25 °C. The detector was set at 214 nm. The concentration of plasma C-reactive protein (CRP) was measured using a high sensitivity system (IMMAGE; Beckman Coulter) with a detection limit of 0.2 mg/l. The intra-assay CV was 4.2%–8.7% for high-sensitivity (hs)-CRP. All measurements were performed in duplicate in a single experiment.

2.3. Echocardiographic examination

All patients underwent standard echocardiography after the HD session. Standard examinations were performed by the same experienced physician under blind conditions, and all of the echocardiographic measurements followed the recommendations of the American Society of Echocardiography (ASE), with at least three cycles being analyzed for each variable [21,22].

Echocardiography was performed during a resting period, in the morning, and in the left lateral position. The echocardiography machine used in our study was a GE Healthcare, General Electric Company, model Vivid 7 Systemwith 3–7 MHz transducers and features to obtain M-mode, two-dimensional and Doppler (pulsed, continuous, color and tissue) echo modalities. All examinations of the long and short parasternal and apical axes, as well as for the 2, 3, 4, and 5 chamber views were performed. Cardiac structure and function were assessed in M-mode guided by 2-dimensional imaging to obtain the following variables: end diastolic interventricular septal thickness, end systolic left ventricular posterior wall thickness, left atrium diameter, interventricular septum thickness, and posterior wall thickness.

LV mass was calculated using a two-dimensional method and indexed to the body surface area. LVMI of $> 131\ g/m^2$ for men and $> 100\ g/m^2$ for women was accepted as LVH [23]. Fractional shortening (FS) was calculated using the formula: FS = (left ventricular end diastolic diameter (LVEDD) –left ventricular end systolic diameter (LVESD)/LVEDD × 100, where LVEDD and LVESD represent the diameter of the left ventricle at end-diastole and end-systole, respectively. Left ventricular ejection fraction (LVEF) was calculated from the apical 4-chamber view using the modified Simpson method. Mitral flow was assessed in the apical 4-chamber view using pulsed Doppler. The sample was positioned between the distal extremities of the mitral valve leaflets, and then the following variables were obtained: early (E) and late diastolic mitral velocities (A), and the E/A ratio.

2.4. Statistical analysis

Data normality was assessed using the Kolmogorov-Smirnov test. Continuous, normally distributed variables are presented as mean ± SD, and non-normally distributed variables as median (interquartile range). Statistical differences in variables were compared using unpaired Student's *t*-tests for normally distributed variables. Categorical variables were recorded as frequencies and/or percentages, and intergroup comparisons were analyzed using the χ^2 test. Since the distributions of serum ferritin, parathyroid hormone, triglycerides, hs-CRP, and HA were skewed, logarithmically transformed values were used for statistical analysis. Pearson's correlation coefficient and simple linear regression analysis were used to examine the correlations and independence between serum HA and the values of the other parameters. The association between HA and LVH was examined using multivariate logistic regression analysis that contained: 1) HA, age, and gender; 2) HA, age, gender, and BMI; 3) HA, age, gender, BMI, systolic blood pressure (SBP), 4) HA, age, gender, BMI, SBP, and triglycerides;