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Serum levels of novel adipokines in patients with acute ischemic stroke: Potential contribution to diagnosis and prognosis☆

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ABSTRACT

This study evaluated serum levels of novel adipokines in acute ischemic stroke (AIS) and their association with prognosis. We enrolled 168 patients with AIS and 58 stroke-free age- and sex-matched individuals (controls). Clinical parameters, carotid ultrasound, metabolic profile, vaspin, apelin, visfatin, and ghrelin were assayed. Stroke-patients were sampled at hospital admission and were prospectively followed-up (median 16 months) for the cardiovascular endpoint (cardiovascular death/stroke/myocardial infarction). At admission, stroke-patients appeared with higher levels of systolic blood pressure, hsCRP and worse metabolic profile ($p < 0.05$), ($p > 0.05$). Compared to controls, AIS group had significantly higher serum concentrations of visfatin (22.92 ± 9.72 ng/ml vs 16.56 ± 7.82 ng/ml, $p = 0.006$) and lower of vaspin (0.94 ± 0.43 ng/ml vs 1.84 ± 0.82 ng/ml, $p = 0.019$) and ghrelin (3.47 ± 1.44 ng/ml vs 5.93 ± 2.78 ng/ml, $p < 0.001$), while apelin did not differ between groups. Similar differences in adipokines were found between stroke subgroups with and without significant ipsilateral carotid stenosis (>50%) ($p < 0.05$). In stepwise logistic regression analysis adjusted for diabetes, hypertension, dyslipidemia and age, visfatin ($p = 0.026$) and ghrelin ($p = 0.012$) proved to be independent predictors of AIS. During follow-up, 27 patients achieved cardiovascular endpoint. In addition to coronary artery disease and NIHSS score, visfatin serum levels was associated with cardiovascular endpoint (HR: 1.255, 95% CI: 1.025–1.576). Our results suggested the association of AIS with higher visfatin and lower vaspin and ghrelin serum levels. Visfatin levels can be a predictor of cardiovascular mortality and morbidity in AIS.

Introduction

Stroke is the second leading cause of death worldwide and a major cause of long-term disability [1]. Because inflammation-driven atherosclerosis contributes to a large proportion of stroke cases, several inflammatory biomarkers have been linked to stroke incidence [4]. Adipose tissue produces several cytokines (adipokines), like leptin, adiponectin, which modulate insulin sensitivity and appear to play an important role in the pathogenesis of diabetes, dyslipidemia, inflammation, coronary artery disease (CAD) and carotid atherosclerosis [11,24]. However, the role of novel members of adipokines in atherosclerosis-related cerebrovascular events has not been well established.

Emerging evidence suggests the interaction of visceral adipose tissue-derived serpin, vaspin, with obesity and human atherosclerosis [13]. A single previous study has documented increased vaspin serum levels in the acute phase of ischemic stroke [6]. In parallel, apelin was firstly detected in human adipocytes, while its biologically active fraction, apelin-12, has been identified in myocardial and vascular tissues, implicating its involvement in cardiovascular diseases [20]. We and other investigators have recently supported the inverse relationship of serum apelin with atherosclerosis [15,23]. However, data concerning the interplay between apelin and ischemic cerebro-vascular events are missing. Unambiguously, more clinical data are required to shed more light into the precise role of apelin and vaspin in cerebro-vascular ischemic events.

Another novel member of adipokines, visfatin, was originally identified as derivative of lymphocytes. Upregulated serum levels

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of visfatin have been found in diabetes, obesity, CAD and carotid atherosclerosis [11,16]. Notably, plasma visfatin levels may be useful for 6-month prognosis after stroke [28]. Ghrelin, a potent gastric, orexigenic factor, exerts anti-inflammatory and atheroprotective actions, as adipokine [6,27]. Although ghrelin is predominantly modified by body-weight changes, weight-independent interventions may also affect ghrelin per se leading to direct suppression of inflammation [12]. Those novel neuroprotective properties of ghrelin require further research [18].

The aim of the present study was to investigate the association between serum levels of novel adipokines and acute ischemic stroke (AIS) in patients admitted to hospital. We also examined the association between their serum levels and post-stroke cardiovascular death, stroke and myocardial infarction (MI).

Materials and methods

Study participants

AIS was defined as a sudden focal neurologic defect lasting for more than 24 h and diagnosed on the basis of clinical history, neurologic examination, and brain imaging study by computed tomography or magnetic resonance imaging. Neurological status was continuously evaluated according to the National Institutes of Health Stroke Scale (NIHSS) [19]. Brain scan was repeated after 48 h according to our hospitals' protocol. Non-selection criteria involved primary intra-cerebral hemorrhage, onset of symptoms >24 h before admission and probable sources of cardio-embolism, (e.g. atrial fibrillation, moderate-to-severe cardiac valvulopathies). Exclusion criteria included infection on admission, autoimmune rheumatic diseases, malignancies, heart failure (NYHA I–IV), severe hepatic (AST > twofold upper normal limit) and renal impairment (creatinine > 2 mg/dl), and cardiovascular ischemic events within the last 1 month, to overcome potential bias concerning inflammatory conditions. Among a pool of 146 patients admitted to our hospitals with AIS 118 patients were considered eligible and entered the study.

Fifty-eight stroke-free subjects, deriving from routine check-up in our outpatient departments were also included as controls for the assessment of adipokines values. Absence of evident previous ischemic cerebro-vascular events was assessed using the Questionnaire for Verifying Stroke-Free Status (QVSFS) [9]. Control subjects were unrelated to study patients, and balanced with them for age and sex by a 2:1 ratio. Moreover, they were free from overt cardiac origin symptoms, while history, electrocardiographic, echocardiographic and functional ischemic tests (within the last 2 years) were negative for CAD or heart failure. At study beginning, all participants or their legal representatives gave their informed consent, and study protocol was approved by the local ethical committee.

All AIS-patients were followed-up for at least 6 months. Information on vital status and the achievement of composite endpoint (cardiovascular death, non-fatal stroke, and non-fatal MI) after the discharge was obtained from medical records of outpatient department or with telephone interview.

Clinical examination

Anthropometrical parameters, like body-mass index (BMI) and waist-to-hip ratio (WHR), were calculated in all participants by a single operator. The percentage of body fat-mass was also measured using the body composition analyzer (Bodystat 1500, Bodystat Ltd, Isle of Man, British Isles). Blood pressure (BP) was assessed at admission. It was measured twice, after keeping participants at a sitting position for 15 min. There was a 5 min interval between the two measurements and the mean value was estimated. Moreover, current medications and cardiovascular risk factors, including hypertension (blood pressure ≥ 140/90 mm Hg or use of antihypertensive agents), smoking status (patients who had stopped smoking more than 1 year before the examination were considered former/nonsmokers), dyslipidemia (LDL-C ≥130 mg/dl or use of lipid-lowering drugs) and diabetes (fasting plasma glucose–FPG ≥ 126 mg/dl or use of anti-diabetic medications) were documented and collected through structure questionnaire. All the above data were obtained at admission.

Carotid ultrasound examination

These ultrasound examinations were performed at admission by single experienced operator, with the use of ultrasound linear array 7.5 MHz transducer (General Electric LogiqE, Riverside, USA). Peak systolic velocity (PSV) and ICA/CCA PSV ratio were calculated and thereafter the percentage of arterial stenosis was graded according to the recommendations of the Society of Radiologists in Ultrasound [8]. Significant carotid stenosis was defined on Doppler ultrasound as an occlusion or at least a 50% atherosclerotic stenosis of each carotid artery corresponding to clinical symptoms. All participants underwent echocardiographic examination (Vivid 5, General Electric, Ohio, OH, USA) to evaluate left ventricular function.

Blood assays

For both patients and controls, blood sampling was performed in the morning after an overnight fast, between 8.00 and 10.00 am. FPG and lipid parameters were all measured in an automatic enzymatic analyzer (Olympus AU560, Hamburg, Germany). In case of AIS group, additional blood samples were also obtained at hospital admission to measure the following adipokines and inflammatory markers: serum apelin (human apelin-12) and vaspin (ALPCO Diagnostic, Salem NH), ghrelin and visfatin (Phoenix Pharmaceuticals, Belmont, CA, USA) were assayed using quantikine enzyme immunoassay (EIA) commercially available kits. The intra-assay CVs were 5% for apelin, 1.31% for vaspin, <5% for visfatin, and <14% for ghrelin, while the inter-assay CVs for the latter variables were 14%, 3.2%, 4% and 7.5%, respectively. The high-sensitivity C-Reactive Protein (hsCRP) levels were measured with latex-enhanced nephelometry (Dade Behring, Marburg, Germany). Samples were frozen and stored (−80 °C) until analysis in the same assay.

Statistical analysis

Results of normally distributed continuous variables were expressed as the mean value ± SD. Normality of distribution was assessed with Kolmogorov–Smirnov test. Comparisons of continuous and categorical variables were analyzed with the student's *t*-test and chi-square test, respectively. To test whether adipokines levels were associated with any of the study population characteristics, a Pearson correlation for normally distributed variables was performed. Variables showing a significant correlation with adipokines levels were then entered in a multiple linear regression analysis, to check for independent associations within stroke patients. A multiple logistic regression analysis was carried out to test whether adipokines were associated with AIS presence, after consecutive adjustments for potential confounders like age, diabetes mellitus, hypertension, dyslipidemia and CAD. Independent predictors of the composite cardiovascular endpoint were calculated using Cox proportional hazards modeling. All covariates that were significant in the univariate analysis and those with important clinical significance or confounding were entered into the final model. A two-tailed p value < 0.05 was considered to be statistically significant. The computer software package SPSS