Journal of Diabetes and Its Complications xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Angiopoietin-related growth factor is independently associated with lower extremity peripheral arterial disease

Hao Nie [a], Yue Liang [a], Hong-Xia Wang [b], Hua-Liang Ren [a], Yue-Wei Wang [a], Fang-Da Li [a], Yue-Hong Zheng [a,*]

[a] Department of Vascular Surgery, Peking Union Medical Hospital, Beijing, 100000, PR China
[b] Department of Physiology and Physiopathology, School of Basic Medical Sciences, Capital Medical University, Beijing, 100000, PR China

ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 22 September 2016
Accepted 16 October 2016
Available online xxxx

Keywords:
Angiopoietin-related growth factor
Type 2 diabetes mellitus
Lower extremity
Peripheral artery disease
Angiogenic factor

ABSTRACT

Aims: The present study investigated the association of serum levels of angiopoietin-related growth factor (AGF) with lower extremity peripheral arterial disease (LEPAD).
Methods: The study group is comprised of 105 patients with lower extremity peripheral arterial disease. The control group consisted of 80 individuals without lower extremity peripheral arterial disease. Serum AGF concentrations were determined by enzyme-linked immunosorbent assay. The relationship between AGF and clinical and biochemical parameters was studied. Besides, this study analyzed AGF levels in LEPAD patients according to disease severity and evaluated the prognostic value of AGF for amputation and mortality in LEPAD patients after a follow-up period of 1.7 years.
Results: Median serum AGF levels were significantly higher in LEPAD group (103.70 ± 64.69 ng/mL) as compared with control group (53.83 ± 37.87 ng/mL) ($P < 0.001$). In addition, T2DM patients with LEPAD exhibited markedly higher serum AGF concentrations (118.7 ± 60.90 ng/mL) than those without LEPAD (60.23 ± 32.62 ng/mL) ($P < 0.0001$). Moreover, LEPAD positively predicted AGF concentrations in multivariate linear regression analysis ($P < 0.0001$). Serum AGF levels were independently associated with LEPAD in binary logistic regression analysis model. Among LEPAD patients, those with critical limb ischemia (n = 43) showed higher AGF levels (124.9 ± 73.9 vs. 88.98 ± 53.26 ng/mL, $P = 0.01$) compared with those with intermittent claudication (n = 62). Furthermore, patients with the highest AGF tertile had an increased all-cause mortality and cardiovascular mortality ($P = 0.033$ and $P = 0.025$, respectively).
Conclusions: Our results suggested that lower extremity peripheral artery disease was positively associated with AGF serum levels. High serum AGF level was a potential risk factor for LEPAD and associates with disease severity and poor outcome in LEPAD patients.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a dynamic and complex disease affecting multiple organs and leading to numerous downstream sequelae. Lower extremity peripheral artery disease (LEPAD) is highly associated with T2DM, with a threefold increased risk in T2DM patients compared with normal population (American Diabetes A, 2003; Selvin & Erlinger, 2004). LEPAD is the atherosclerotic stenosis and occlusion of lower extremity arteries, which increases the risk of functional limitation and amputation. The clinical manifestations of LEPAD are intermittent claudication (IC) and critical limb ischemia (CLI). CLI is a more severe manifestation of LEPAD and associated with high risk of total and cardiovascular mortality (Subherwal et al., 2015). LEPAD tends to have poorer outcomes in T2DM patients than nondiabetic patients (Jude, Oyibo, Chalmers, & Boulton, 2001). Additionally, LEPAD is a marker for atherothrombosis in cardiovascular and cerebrovascular beds. Patients with LEPAD therefore have an increased risk of myocardial infarction, stroke and death (American Diabetes A, 2003; Criqui et al., 2010; O'Hare, Katz, Shlipak, Cushman, & Newman, 2006). Early detection and treatment of LEPAD are critical to minimize the risk of adverse outcomes. Therefore, it is vital for diabetic patients to recognize LEPAD and control its risk factors as early as possible. Several traditional cardiovascular risk factors for LEPAD have been well established, including age, race, current smoking, T2DM, hypertension, and hypercholesterolemia (Fowkes et al., 1992; Selvin & Erlinger, 2004). However, few existing studies have explored serum biomarkers as risk factors for incident LEPAD in T2DM patients.

Angiopoietin-related growth factor (AGF), also known as angiopoietin-like protein 6 (Angptl 6), was first recognized as a novel angiogenic factor (Oike et al., 2004). AGF is a multimeric glycoprotein secreted predominantly from the liver (Oike et al., 2003). Oike and co-workers found that the chemotactic activity of vascular endothelial

Competing interests: The authors declare that they have no conflict of interest.
* Corresponding author. Tel.: +86 010 69152502; fax: +86 010 69152502.
E-mail address: yuehongzheng@yahoo.com (Y-H. Zheng).

http://dx.doi.org/10.1016/j.jdiacomp.2016.10.019
1056-8727/

cells and the expression of angiogenic factors were increased in mice with transgenic overexpression of AGF, indicated a positive impact of AGF on angiogenesis (Oike et al., 2004). Besides its important role in angiogenesis, AGF has additional novel effects on energy expenditure and insulin sensitivity. In animal studies, AGF deficiency has been suggested to cause hyperglycemia, hyperinsulinemia and insulin resistance (Oike et al., 2005). Increased serum AGF concentrations gave rise to a significant body weight loss and increases insulin sensitivity in mice fed a high-fat diet (Oike et al., 2005). These observations suggested that AGF plays an essential role in regulating cardiovascular development and energy metabolism. Namkung et al. found that metabolic syndrome can be a predictor of serum AGF level which suggests that AGF can serve as a useful marker of diabetes mellitus (Namkung, Koh, Kong, Choi, & Yeh, 2011). A study from Ebert et al. shown that renal dysfunction was negatively associated with AGF serum levels, while it was positive as for T2DM (Ebert et al., 2009). These results support the hypothesis that AGF can be a valuable circulating biomarker in diabetes mellitus and its downstream complications.

Despite these above findings that AGF was associated with diabetes mellitus and the well-established link between diabetes mellitus and cardiovascular disease, no clinical studies have determined the potential connection between AGF and atherosclerotic disease especially LEPAD. Thus, the aim of our study was to measure the level of AGF in patients with LEPAD, and further analysis its association with clinical outcomes.

2. Material and methods

2.1. Patient selection

This was a prospective study, and 105 participants with diagnosed LEPAD, who were hospitalized in the Department of Vascular Surgery in Peking Union Medical College Hospital (Beijing, China) from January 2013 to October 2013, were recruited in this study. Bilateral ankle–brachial index (ABI) was measured and the diagnosis of LEPAD was based on an ABI at rest of less than 0.9. Patients were classified according to the disease severity: IC (n = 62), with a history of IC (Fontaine class II) diagnosed by hemodynamic study (Doppler ultrasound); and CLI (n = 43), with lower limb rest pain or trophic lesions (Fontaine class III–IV) confirmed by imaging studies. In order to investigate whether there were differences in the serum AGF level in individuals with or without LEPAD, 80 individuals without LEPAD were designated as control group. Patients with type 1 diabetes, acute complications of diabetes, acute coronary syndrome and liver failure were excluded. The general background information including present and previous illness, medication, smoking status and alcohol consumption were collected. During the health check-up, height, weight and blood pressure were assessed and body mass index (BMI) was calculated. Patients currently smoking or having discontinued smoking within 5 years were considered smokers. The diagnostic criteria of diabetes mellitus was based on the American Diabetes Association standards (American Diabetes A, 2014). Hypertension was defined as a systolic blood pressure (SBP) of ≥140 mmHg and/or a diastolic blood pressure (DBP) of ≥90 mmHg or receiving antihypertensive treatment (Mancia et al., 2013). Informed consent was obtained, and the protocol was approved by the local ethics committee.

2.2. Follow-up

Patients were followed up at the outpatient service of the Department of Vascular Surgery every 3 or 6 months, depending on the severity of LEPAD. At those regular checkups, patients were tested for biochemical parameters and underwent physical examination and ABI measurement. No patient was lost to follow-up. For outcome evaluation of LEPAD patients, amputation, cardiovascular events and death were recorded. No follow-up of the control population was recorded.

2.3. Laboratory analysis

Following an overnight fast, blood samples were collected from anterior cubital vein in all subjects. All samples were immediately centrifuged for 15 min at 1000 rpm and the isolated plasma was stored at −80 °C before analysis. Serum angiopoietin-related growth factor (AGF) and adiponectin (ADP) were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits from CUSABIO (Wuhan, China). The coefficient of variation was <10%. A variety of biochemical markers such as hemoglobin A1c (HbA1c), fast blood glucose (FBG), creatinine, triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting insulin (FINS), high sensitivity C-reactive protein (hsCRP) were measured by routine techniques. The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated by the following formula: HOMA-IR = (FBG × FINS)/22.5.

2.4. Statistical analysis

Continuous variables were shown as mean ± SD in case of normally distributed parameters or median ± interquartile range in case of non-normally distributed parameters. For categorical parameters, the total number (percentage) of patients was shown. Comparative analysis for measurement data use two-sample Student test, chi-squared test for enumeration data. Both univariate and multivariate analyses were performed, linear regression for AGF while logistic for LEPAD. Here, parameters were included as independent variables that showed a significant correlation with circulating AGF levels in univariate analyses. Because HbA1c, fasting glucose, insulin and HOMA-IR depend largely on T2DM, ABI is closely related to LEPAD, these variables were not included in the same model. Before performing multivariate analyses, distribution was tested for normality using Shapiro–Wilk W test. Kaplan–Meier estimates were used to compare time to event differences across AGF tertiles by the log-rank test. $P < 0.05$ was considered as statistically significant. All statistical analyses were performed using SAS 9.3. All the statistic tests were based on logarithm translated data.

3. Results

3.1. Circulating AGF levels are increased in LEPAD patients

A total of 105 patients with LEPAD and 80 normal healthy controls were recruited. The clinical characteristics of LEPAD and control patients are shown in Table 1. There were no significant differences in age, hypertension, diabetes mellitus, and lipid profile among the two groups. Thirty two of the 80 controls and 43 of the 105 LEPAD patients presented with T2DM. There were significant differences in sex, SBP, HOMA-IR, HDL, previous smoking, creatinine, hsCRP and ABI between LEPAD and control patients ($P < 0.05$). AGF levels were significantly higher in LEPAD patients as compared with control patients (103.70 ± 64.69 vs. 53.83 ± 37.87 ng/mL, $P < 0.001$) (Table 1). Moreover, the characteristics of the subgroups further stratified into nondiabetic and diabetic subjects are summarized in Table 2. In nondiabetic subjects, serum AGF levels were significantly higher in LEPAD patients (93.28 ± 65.66 ng/mL) as compared with control subjects (49.56 ± 40.77 ng/mL) ($P < 0.01$). Furthermore, AGF serum levels were significantly higher in LEPAD patients (118.7 ± 60.90 ng/mL) than that in control patients (60.23 ± 32.62 ng/mL) ($P < 0.01$) in diabetic subjects.

3.2. Correlation between serum AGF and clinical parameter

When all patients were studied, serum AGF levels positively correlated with SBP, HbA1c, T2DM, insulin, HOMA-IR, ADP and were negatively associated with hsCRP, ABI ($P < 0.05$) (Table 3). In controls, circulating AGF was positively associated with ADP ($P < 0.05$). However, AGF had a significant negative correlation with DBP ($P < 0.05$). In LEPAD

Please cite this article as: Nie, H., et al., Angiopoietin-related growth factor is independently associated with lower extremity peripheral arterial disease, *Journal of Diabetes and Its Complications* (2016), http://dx.doi.org/10.1016/j.jdiacomp.2016.10.019