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Anemia and type 2 diabetes mellitus associated with peripheral arterial disease progression in Chinese male patients

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ABSTRACT

Objective: Peripheral arterial disease (PAD) is a chronic occlusive disease mainly occurred in elderly adults. Arteriosclerosis obliterans (ASO) mainly occurring from small or medium sized arteries of the lower extremities is one of the most common causes of PAD. The gender-related differences of circulating risk factors in diabetic patients with ASO in China remain unknown. The aim of this study is to investigate the gender-related differences in the pattern of several potential risk factors between male and female patients with ASO and type 2 diabetes mellitus (T2D).

Design and methods: Clinical profiles and risk factors were analyzed in 323 Chinese patients with ASO and 112 patients were confirmed with T2D. Severities of limb ischemia were staged according to Fontaine classification.

Results: The significant inverse correlation was seen between the increased age and hemoglobin. The significant positive correlation was seen between the increased age, urea and creatinine both in the non-diabetic and diabetic male patients. The expression levels of hemoglobin significantly correlate with the classification of Fontaine clinical symptoms in Chinese male patients with T2D/ASO.

Conclusion: The study is the first report indicating that the gender-related differences of circulating risk factors are associated with T2D patients with ASO in China. Anemia in Chinese male patients with T2D/ASO may play an important role in peripheral arterial disease progression.

Introduction

Peripheral arterial disease (PAD) affects over 27 million adults in Europe and the US and has long been considered to be an effective predictor of cardiovascular disease and systemic atherosclerosis [1,2]. PAD is a chronic arterial occlusive disease of the lower or upper extremities, mainly resulting from atherosclerosis subsequently reducing limb blood flow. Arteriosclerosis obliterans (ASO) prominently occurring from abdominal aorta and the small or medium sized arteries of the lower extremities, is one of the most common causes of PAD [3,4]. Anemia has been shown to be an independent risk factor in patients with cardiovascular diseases [5]. However, whether the expression levels of hemoglobin are associated with ASO clinical symptoms still remain unclear. Diabetes mellitus has been considered to be an independent risk factor in the development of PAD which leads to clinical consequences of ischemia, claudication and ulceration [6]. PAD risk factors including diabetes, hypertension and smoking are all associated with increased levels of circulating inflammatory biomarkers [7,8]. Several reports have indicated that patients with PAD generally have higher levels of inflammatory proteins, such as C reactive protein and interleukin 6, when compared with people without PAD [9,10]. Furthermore, in early atherosclerosis, inflammatory responses such as monocytes and lymphocytes are recruited into vascular intima, leading to platelet aggregation and thrombus formation [11]. Evidences have shown that the clinical symptoms of PAD patients are associated with gender-related differences in Western countries [12]. However, the gender-related differences of patients with ASO in China remain unknown. Identifying the significance of circulating risk factors in patients with ASO will improve the understanding of the mechanisms of the lower extremity of atherosclerosis. The aim of the present study was therefore to screen the circulating risk factors of gender-related differences in Chinese patients with ASO and diabetes.

Methods

Study population and data collection

The study comprised 247 male and 76 female patients including 73 male and 39 female with type 2 diabetes (T2D) and ASO aged 40–

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92 years at baseline from 2007 to 2012. Patients were diagnosed with T2D/ASO previously and were scheduled for blood sample collection at Fuzhou General Hospital of Nanjing Military Command (southern China) and Wuhan Union Hospital (central China). To certify the consequence of ASO and the significant alterations of circulating factors, ASO patients with T2D were previously diagnosed by hemoglobin A1c and oral glucose tolerance test (OGTT) in an outpatient management in the two hospitals. The patients with known cardiovascular or cerebrovascular disease and dyslipidemia were then excluded in this study. The diagnostic criteria of T2D were according to an international committee composed of experts from the American Diabetes Association and the European Association for the Study of Diabetes [13].

In this study, diagnostic approaches to ASO symptoms such as intermittent claudication, rest pain, tissue loss, foot ulcers, history of risk factors, physical examination, laboratory assessment as well as imaging confirmation by Duplex ultrasound (Philips iU22 xMATRIX ultrasound system) and/or computed tomography angiography (CTA) (SOMATOM Definition 128 Slice, Siemens) were undertaken in each case (supplementary Fig. 1). ASO patients with intermittent claudication have poor walking economy, as their oxygen uptake of pain-free ambulation is higher than age-matched controls. Therefore, to evaluate the severity of symptoms and the levels of claudication in the patients with ASO, patients were assessed on a 10-minute walk test [14,15]. The severity of symptoms was recorded by the classification of Fontaine stages as followed by I: asymptomatic, IIa: mild claudication, IIb: moderate–severe claudication, III: ischemic rest pain and IV: tissue loss or ulceration.

Two samples of 20 mL fasting venous blood were collected without taking any medication into vacuum tubes containing EDTA or sodium citrate for hematologic and biochemical analysis respectively. Plasma was separated by double centrifugation at 800 *g* for 10 min; 1600 *g* for 10 min and the aliquots were immediately frozen at −70 °C. Plasma biochemical analysis was undertaken by Olympus AU5400 Chemistry System. Fibrinogen, prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) were measured by STA-R Evolution®. A complete blood count (CBC) was analyzed by Sysmex XT-1800*i* Automated Hematology Analyzer. All patients involved in this study gave informed consent and the procedures followed were approved by institutional ethics committee.

Statistical analysis

All data are presented as mean values ± standard deviation. Statistical analysis into the differences between male and female potential risk factors was conducted by covariance (ANCOVA) with age as a covariate using SPSS v20 software (IBM, USA). As the continuous data were not normally distributed the correlation between age, sex and expression of risk factors was analyzed using Spearman's correlation statistics. A further analysis of the association between risk factors and Fontaine stages [16] was grouped the samples into tertiles according to the reference values and used chi-square test for ordinal categorical groupings into the following as: lower than normal value range, normal value range, and higher than normal value range. The reference values were following the manufacturer's criteria from each company. According to the World Health Organization criteria, anemia defined as hemoglobin concentration of <120 g/L in women and <130 g/L in men [17]. Data were considered statistically significant at the level of $p < 0.05$.

Results

Subject demographics

A total of 323 subjects with ASO of 247 male and 76 female patients including 174 male and 37 female without type 2 diabetes (T2D) and 73 male and 39 female with T2D, were recruited because blood test data was available for them. None of the subjects had received surgery before blood test. All subjects were included. Patients without T2D had a mean age for the male and female groups being 68.3 ± 12.0 and 75.0 ± 8.5 years respectively. T2D patients had the mean age for the male and female groups being 67.4 ± 10.3 and 72.6 ± 7.8 years respectively. Compared to the female patients, the male patients were significantly younger. Therefore, all data was then adjusted using age as a factor in the analysis (Table 1).

Gender differences in the relationship between circulating factors and patients with ASO

The mean values of systolic blood pressure (BP), diastolic BP, albumin, total protein, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), uric acid (UA), γ-glutamyltransferase (γ-GT), total bilirubin, direct bilirubin, PT, APTT, TT, fibrinogen, white blood cell (WBC) count, red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, neutrophil, lymphocyte and monocyte count are not significantly different between the male and female patients with ASO after age adjustment in the non-diabetic group (supplementary Table 1). The mean values for creatinine ($p = 0.006$), hemoglobin ($p = 0.012$) and hematocrit ($p = 0.05$) are significantly higher in the male than the female patients whereas the mean value of urea ($p = 0.004$) is significantly higher in the female than the male patients (Table 1). In the diabetic group, the male patients are also younger than female patients ($p = 0.007$). Therefore, all data was then adjusted by age as a factor in the analysis. The mean values of plasma level of UA ($p = 0.044$) showed a tendency to be higher in women than men. However, the diastolic BP ($p = 0.008$) and hematocrit ($p = 0.011$) are significantly higher in the male than the female patients with T2D/ASO (Table 1).

The circulating factors are age-related in patients with diabetes and ASO

To contextualize the assessment of the risk factors including urea, creatinine, UA and hemoglobin on the age impacts between diabetic and non-diabetic patients, a further analysis of the gender differences divided the samples into male and female groups and the correlation between the distribution of patients' ages and risk factors was assessed. Spearman's rho analysis revealed a significant inverse correlation between the increased age and hemoglobin both in the non-diabetic male patients ($p = 0.001$) and diabetic male patients ($p = 0.009$, Table 2). However, there were positive significant differences seen between the increased age and urea and creatinine both in the non-diabetic male patients (urea, $p < 0.001$; creatinine, $p < 0.001$) and diabetic male patients (urea, $p = 0.022$; creatinine, $p = 0.001$, Table 2). In contrast there were no significant correlations seen between the increased age and UA in non-diabetic male patients, whereas a significant positive correlation between the increased age and UA ($p = 0.008$) was found in diabetic male patients with ASO. Surprisingly, no significant correlations between age and the risk factors including urea, creatinine, UA and hemoglobin were found in the ASO female patients with and without T2D (Table 2 and supplementary Figs. 2–5).

Hemoglobin significantly correlates with the distribution of Fontaine stages in the male ASO patients with diabetes

To comprehend whether these potential risk factors are correlated with clinical symptoms of ASO patients with or without T2D, the data were grouped into tertiles by urea, creatinine, UA and hemoglobin expression levels in terms of lower than normal value, normal value and higher than normal value and the relationships between these variables were further investigated. There were no significant associations with disease progression (Fontaine stages), when grouped by the levels of urea, creatinine, UA and hemoglobin in the female patients with T2D/ASO the male and female patients without T2D/ASO (supplementary