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Increased plasma dipeptidyl peptidase-4 activities are associated with high prevalence of subclinical atherosclerosis in Chinese patients with newly diagnosed type 2 diabetes: A cross-sectional study

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ABSTRACT

Objective: Hyperglycemia, insulin resistance, dislipidemia, oxidative stress and inflammation are well-documented risk factors for subclinical atherosclerosis. Dipeptidyl peptidase-4(DPP4) is a newly identified adipokine related to these risk factors. Hence, we aimed to investigate the association between plasma DPP4 activities and subclinical atherosclerosis in type 2 diabetes.

Methods: A total of 985 newly diagnosed type 2 diabetic subjects were studied. Plasma DPP4 activity, mannose 6-phosphate receptor (M6P-R), oxidative stress parameters, inflammatory markers and common carotid artery Intima-Media Thickness (c-IMT) were measured in all participants.

Results: Participants in the highest quartile of DPP4 activity had higher HbA1c, homeostatic model assessment of insulin resistance(HOMA-IR), triglyceride, low-density lipoprotein cholesterol(LDL-C), oxidized LDL, nitrotyrosine, 8-iso-PGF2a, interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), M6P-R, c-IMT compared with participants in the lowest quartile (all $P < 0.001$). DPP4 activities were associated positively with HbA1c, HOMA-IR, triglyceride, LDL-C, oxidized LDL, nitrotyrosine, 8-iso-PGF2a, IL-6, hs-CRP, M6P-R and c-IMT (all $P < 0.05$). The ORs for insulin resistance, dislipidemia, oxidative stress and inflammation were higher with increasing DPP4 quartiles ($P < 0.001$ for trend). In the highest DPP4 quartile, subclinical atherosclerosis risk was significantly higher (OR 4.97; 95% CI 3.03–8.17) than in the lowest quartile. This association remained strong (2.17; 1.21–3.89) after further controlling for HbA1c, HOMA-IR, triglyceride, oxidized LDL, nitrotyrosine, and IL-6.

Conclusions: This study shows that increased DPP4 activities are positively and independently associated with subclinical atherosclerosis in type 2 diabetes. Our findings suggest of potential role of DPP4 in the pathogenesis of subclinical atherosclerosis and in the prevention and management of this disease.

1. Introduction

The accumulated clinical and epidemiological evidence suggests that type 2 diabetes is a chronic multisystem disease associated with a high risk of cardiovascular disease [1]. In clinical practice, subclinical atherosclerosis is one of the most frequently observed complication in type 2 diabetes and is related to an increased risk of cardiovascular death or disability [2], therefore, identifying novel risk factors and therapeutic targets to prevent or reverse diabetes-related subclinical atherosclerosis is of critical importance.

Dipeptidyl peptidase 4 (DPP4), is a serine protease found on the apical surface of diverse cells, with a catalytic activity that cleave X-proline dipeptides from the N terminus of polypeptides such as chemokines and peptide hormones. In addition to its membrane form, DPP4 exists in plasma as a soluble form (sCD26), which is the extracellular domain of the molecule thought to be cleaved from the cell surface [3].

Recent studies have demonstrated that DPP4 is also a newly

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identified adipokine that has been shown to contribute to hyperglycemia, insulin resistance, dislipidemia, oxidative stress and inflammation [4–10], all which have been suggested to be involved in the pathogenesis of subclinical atherosclerosis [11,12]. More importantly, a recent in vitro study proved that soluble DPP4 directly activated the MAPK and NF-κB signaling cascade involving PAR2 and resulting in the induction of inflammation and proliferation of human vascular smooth muscle cells (hVSMC), all direct effects of sDPP4 on signaling, proliferation and inflammation could completely be prevented by DPP4 inhibition [13]. . Furthermore, some DPP4 inhibitors have been shown to reduce subclinical atherosclerosis in experimental models, these studies all demonstrated a reduction in foam cell formation consistently [14,15]. Therefore, it is reasonable to speculate that plasma DPP4 activity may be positively correlated with subclinical atherosclerosis in type 2 diabetes, however, no study has evaluated whether DPP4 may serve as a risk marker for subclinical atherosclerosis in type 2 diabetes and to what extent it is associated with subclinical atherosclerosis.

Consequently, in this study, we aimed to evaluate the association between plasma DPP4 activities and subclinical atherosclerosis in a cross-sectional population study of 985 Chinese patients with newly diagnosed type 2 diabetes. The relationship between DPP4 activities and novel risk factors for subclinical atherosclerosis were also evaluated in type 2 diabetes.

2. Material and methods

2.1. Study population

The study population consisted of males and females, aged 18–74 years, who participated in the Guangxi Diabetes and Metabolic Disorders study, a cohort study that aimed to evaluate the cross-sectional and prospective association between psychosocioeconomic factors, biomarkers, and the incidence of type 2 diabetes, diabetic complications and metabolic disorder. The subjects were volunteers with newly diagnosed type 2 diabetes recruited from the medical examination center of affiliated hospital of Guilin medical university in Guangxi province between 2013 and 2014. All enrolled subjects in this study visited the medical examination center spontaneously for routine health examinations consisting of extensive screening tests for the early detection of diabetes, hypertension, metabolic syndrome, osteoporosis, malignancy, and other age-related diseases. The study was approved by the Drugs/Medical Apparatus & Instruments Ethics Committee at Affiliated Hospital of Guilin Medical University, and consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used. This study was registered on the Chinese clinical trial registry (ChiCTR-EPC-14005273).

The final sample size for the present analysis was 985 participants (580 men and 405 women) with newly diagnosed type 2 diabetes. The diagnostic criteria of type 2 diabetes were based on the criteria recommended by the World Health Organization [16]. The subclinical atherosclerosis was defined as c-IMT > 0.9 mm and/or presence of AS plaques in the carotid artery, the presence of carotid plaques was defined as focal echo structures encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding c-IMT value, or when c-IMT was >1.5 mm as measured from the media-adventitia interface to the intima–lumen interface [17]. Inclusion criteria: (1) Newly diagnosed type 2 diabetes without using any antidiabetic drug, age between 18 and 74 years old. (2) Long-term residing (≥5 years) in China's Guangxi province. (3) Being able to give informed consent. Exclusion criteria: (1)Patients with prediabetes diagnosed by an oral glucose tolerance test, gestational diabetes, type 1 diabetes, or diabetes induced by steroid use or other endocrinological diseases. (2) Using varieties of drugs to control blood glucose, blood pressure, blood lipid and other drugs used in preventing the natural process of subclinical atherosclerosis. (3) Subjects deprived of personal safety and presence of any of the diseases including nonalcoholic fatty liver disease (NAFLD), pancreatic cancer, acute inflammatory diseases, stroke, myocardial infarction, other heart, liver and respiratory dysfunction were excluded as progression of these in any stage may hinder our study. (4) Subjects with malignancy and pregnant subjects. (5) Subjects with incomplete data (study population data which were not completely determined mainly refer to the study population who failed to complete the questionnaire or the absence of any physical indicators).

2.2. Measurements

A standard questionnaire was administered by trained staff to the participants to record demographic characteristics and life style risk factors [18]. Measurements of body weight and height, waist and hip circumference, body mass index (BMI), waist/hip ratio (WHR) and blood pressure have been described previously [19]. Subjects were instructed to maintain their usual physical activity and diet for at least three days before undergoing an oral glucose tolerance test (OGTT). After an overnight fast of ≥10 h, venous blood samples were collected to measure HbA1c, fasting plasma glucose (FPG), fasting insulin, blood lipids (including total cholesterol [TC], triglycerides [TG], low-density lipoprotein cholesterol [LDL-C] and high-density lipoprotein cholesterol [HDL-C]), oxidized LDL(ox-LDL), IL-6, hs-CRP, M6P-R, nitrotyrosine, 8-iso-PGF2a and DPP4 activity. Blood samples were also drawn at 30 and 120 min after a 75 g glucose load to measure glucose and insulin concentrations.

Plasma glucose levels, insulin, TC, TG, HDL-C, LDL-C, IL-6, hs-CRP, M6P-R, nitrotyrosine, and 8-iso-PGF2a were measured as previously described [5,6,9,20]. B-mode ultrasonography of the common carotid artery (CCA) was performed using an ultrasound machine (Toshiba; Toshiba Medical Systems, Tokyo, Japan).Each subject was examined in the supine position, with the head elevated ≤45° and a side tilt of 30° to the right and then the same to the left. The CCA segment was defined as the distal 1 cm of the CCA, immediately proximal to the onset of increased spatial separation of the walls of the CCA. The CCA-IMT was measured in a longitudinal view at a site free of plaques as the distance between two parallel echogenic lines corresponding to the blood–intima and media–adventitia interface on the posterior wall of the artery. Composite right and left c–IMT were calculated as the average of the three readings in each artery segment, and the mean of the left and right c–IMT measurements was used in the analysis. Both near and far walls of these arterial segments were scanned longitudinally and transversely to assess the presence of plaques. Primary measurement of ox-LDL was performed using a commercially available sandwich enzyme-linked immunosorbent assays (ELISA, Mercodia, Uppsala, Sweden). Plasma DPP4 activity was determined as the rate of cleavage of 7-amino-4-methylcoumarin (AMC) from the synthetic substrate H-glycyl-prolyl-AMC (H-Gly-Pro- AMC; Biovision, San Francisco, CA, USA). DPP4 activity is expressed as the amount of cleaved AMC per minute per ml (nmol/min/ml). DPP4 activity was measured in the absence or the presence of sitagliptin, a specific DPP4 inhibitor, to test the specificity of the enzymatic assay. In our samples, sitagliptin inhibited the assayed DPP4 activity by >95%. The intra- and inter-assay coefficients of variation were 4.58% and 9.85%, respectively. Blood samples were stored at −80 °C and subsequently all parameters were measured within 6 months of sample collection. All samples were analyzed in duplicate, random order, blinded to the clinical status of the participants. The