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Association of angiotensin-II levels with albuminuria in subjects with normal glucose metabolism, prediabetes, and type 2 diabetes mellitus

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ABSTRACT

Objectives: The relationship between the renin–angiotensin system (RAS) and diabetes has been studied for many years. However, studies that assessed RAS components comprehensively were limited. We hypothesized that serum RAS components, especially the effector peptide angiotensin-II, might be closely associated with glucose metabolism status and diabetic complications.
Methods: We investigated the association of individual RAS component with albuminuria in 407 subjects with normal glucose metabolism (NGM), prediabetes, or type 2 diabetes mellitus (T2DM). Anthropometric and biochemical parameters, including glucose homeostasis, albuminuria, and RAS-related parameters such as plasma renin activity (PRA), aldosterone, angiotensin-converting enzyme (ACE), and angiotensin-II levels, were measured.
Results: The mean ± standard deviation (SD) age and body mass index were 57.1 ± 11.1 years and 24.7 ± 3.3 kg/m^2, respectively. There were 54 subjects with NGM, 102 with prediabetes, and 251 with T2DM. The mean ± SD angiotensin-II levels in these groups were 9.32 ± 6.89, 12.89 ± 10.39, and 17.00 ± 15.28 pg/mL, and the respective urinary albumin-to-creatinine ratios (ACRs) were 8.1 ± 5.3, 13.3 ± 17.3, and 30.7 ± 51.9 mg/g, which were significantly different among the groups. The serum angiotensin-II levels were correlated with levels of PRA, insulin resistance, C-reactive protein, and urinary ACR. Among RAS-related parameters, only the angiotensin-II level was significantly associated with urinary ACR after adjusting for relevant risk factors.
Conclusions: Angiotensin-II may play an important role in the development of albuminuria, particularly in subjects with impaired glucose metabolism.

1. Introduction

The global burden of diabetes mellitus (DM) is rising continuously because of urbanization, unhealthy dietary habits, and physical inactivity. According to the estimates of the prevalence of DM published by the International Diabetes Federation in 2015,[1] 415 million adults were estimated to have DM in 2015, and this number is expected to increase to 642 million by 2040 (2015). DM is a chronic, progressive disease characterized by long-term vascular complications resulting in microvascular and macrovascular types of damage to several organs, including the eyes, kidney, heart, and peripheral nerves, which represent the main causes of increased morbidity and mortality.

Several potential mechanisms for the causes of vascular complications and tissue damage in subjects with DM have been proposed. The increased generation of reactive oxygen species (ROS) causes increased flux of the polyol pathway, protein kinase-C activation, increased formation of advanced glycation end-products, and an overactive hexosamine pathway.[2] In addition, the role of the renin–angiotensin system (RAS) in diabetic complications has been suggested since 1970.[3–5] Dr. Luetscher published many papers regarding renin, prorenin, and its association with various complications of DM.[6,7] The RAS is a crucial neuroendocrine system in maintaining body fluid and electrolyte balance.[8] Short-term activated RAS acutely regulates the homeostasis of extracellular volume and blood pressure; however, its chronic activation leads to adverse effects not only in the onset of DM, but also in the progression of diabetic complications, including microvascular and macrovascular ones.[9]

Abbreviations: ACE, angiotensin-converting enzyme; ALT, alanine aminotransferase.
Conflicts of interest: The authors declare that they have no conflict of interest.

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Several clinical trials have demonstrated the benefit of using RAS blocking agents such as angiotensin converting enzyme (ACE) inhibitors or angiotensin-II receptor blockers (ARB) in lowering the risk of progression of diabetic complications.[10–12] Among the components of RAS, angiotensin-II, an effector peptide of the activated RAS system, is considered to play an important pathogenic role in the progression of diabetic complications.[9] Therefore, angiotensin-II levels might be closely associated with glucose metabolism status and the degree of diabetic complications. However, the association of individual RAS components with glucose metabolism status was not sufficiently investigated in humans. Moreover, its associations with diabetic complications have not been clearly elucidated. Here, we investigated the relationships between RAS components including plasma renin activity (PRA), ACE, and angiotensin-II levels and albuminuria in various subjects with normal glucose metabolism, prediabetes, or type 2 DM (T2DM).

2. Materials and methods

2.1. Study population

This was a single-center, cross-sectional study that included individuals who visited the Endocrinology Clinic of the Seoul National University Bundang Hospital (SNUBH), Seongnam, Korea, from January 2014 to June 2016 for evaluation of their glucose metabolism. Because few studies investigated RAS parameters comprehensively in relation with diabetic complications, particularly with urinary albumin excretion, we arbitrarily planned to recruit 500 participants to enhance the power of the study, targeting a 1:2:4 allocation for normal glucose metabolism (NGM), prediabetes, and T2DM, respectively. We screened 623 individuals aged ≥20 years who underwent a 75-g oral glucose tolerance test (OGTT) and a test for measuring RAS components, including angiotensin-II levels. Among them, those with current usage of diuretics, β-blockers, or RAS blockers (such as ACE inhibitors or ARBs) were excluded from the study, as were patients who had type 1 diabetes (T1DM) (C-peptide <0.3 pg/mL) or who were receiving insulin therapy. Also excluded were subjects who were suspected of having primary aldosteronism, based on an aldosterone-to-plasma renin activity (PRA) ratio of >30 and the presence of adrenal adenomas shown by imaging tests, and those with active malignancies and medical histories of severe liver or renal disease. Finally, 407 subjects were enrolled: 54 were classified as having NGM, 102 as having prediabetes, and 251 as having T2DM. This study was approved by the institutional review board of the SNUBH (IRB-B-1601-332-307).

2.2. Definition of glucose metabolism status

According to the[13] guidelines (2016), DM was defined by a fasting plasma glucose (FPG) concentration ≥126 mg/dL, a 2 h plasma glucose concentration ≥200 mg/dL after a 75 g OGTT, or a level of glycated hemoglobin (HbA1c) ≥6.5%. Prediabetes was defined as having impaired fasting glucose (IFG) (FPG 100–125 mg/dL) and/or impaired glucose tolerance (IGT) (2 h plasma glucose concentration 140–199 mg/dL). NGM was defined by not meeting the criteria for either diabetes or prediabetes.

2.3. Measurement of anthropometric and biochemical parameters

Height and body weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, while only wearing light clothing according to standard procedures. Body mass index (BMI) was calculated by dividing weight by height squared (expressed in kg/m^2). Blood pressure measurements were taken using an automated blood pressure monitor at the time the patients visited the clinic. Smoking status was divided into the following three categories: current smokers; ex-smokers; and never-smokers. Alcohol intake was assessed by asking about the amount and frequency of beer, wine, or spirits intake during the previous 12 months. Physical activity was assessed with questions about the number of days of exercise in the past week. Regular exercise was defined as three or more times of exercising for at least 20 min in the last week. Evaluation of dietary habits was made by means of simple questions and classified into two categories: regular or irregular diet. The subjects were asked about the presence of a family history of diabetes, and participants' comorbidities such as hypertension and dyslipidemia with any history of respective medications were identified through a comprehensive review of medical records.

Because salt intake may affect RAS system, the study subjects were advised to keep low salt diet for 3 days before test. After 12 h fasting, blood samples for the determination of RAS-related hormones were drawn in resting state for 30 min. For PRA, samples were collected into prechilled tubes containing EDTA and immediately centrifuged (4000 rpm, 15 min, 4 °C).[14] The PRA was measured using a PRA radioimmunoassay (RIA) kit (TFB Inc., Tokyo, Japan) immediately, and plasma aldosterone concentration was measured using the SPAC-S aldosterone RIA kit (TFB Inc.). ACE was assayed kinetically by direct quantitation with the Buhlmann ACE kinetic test kit (Buhlmann Laboratories AG, Schonembuch, Switzerland), and serum levels of angiotensin-II were measured using ELISA kits (Phoenix Pharmaceuticals, Burlingame, CA, USA).

Fasting plasma concentrations of glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL)-cholesterol, and serum creatinine were measured using standard automated laboratory methods (Hitachi 747; Hitachi, Tokyo, Japan). Estimated glomerular filtration rate (eGFR) was calculated from the serum creatinine using the Modification of Diet in Renal Disease study equation.[15] HbA1c was determined using an HPLC Bio-Rad variant II Turbo analyzer (Bio-Rad Laboratories, Hercules, CA, USA) in the National Glycohemoglobin Standardization Program level II certified laboratory at SNUBH. Plasma C-peptide and insulin concentrations were measured in fasting status by RIA (Linco, St. Louis, MO, USA). Serum aspartate aminotransferase and alanine aminotransferase were measured with an autoanalyzer (TBA-200FR, Toshiba, Tokyo, Japan). Serum levels of high sensitivity C-reactive protein (hsCRP) were measured with an automated latex turbidimetric immunoassay method using CRP Latex X2 (Denka Seiken, Tokyo, Japan).

Insulin resistance (IR) index and pancreatic β-cell functions assessed by the homeostasis model assessment (HOMA) were calculated from the following formula: HOMA-IR = fasting plasma insulin (μU/mL) × FPG (mg/dL) / 405; HOMA-β = 360 × fasting plasma insulin (μU/mL) / [FPG (mg/dL) − 63].[16]

Urinary albumin levels were measured using a turbitimer assay (A&T 502X, A&T, Tokyo, Japan), and urinary creatinine levels were measured using the Jaffe method (Hitachi 7170, Hitachi). The albuminuria was assessed with the urinary albumin-to-creatinine ratio (ACR) (mg/g). Participants collected first-morning urine samples and the mean value of the ratios which were determined on three different occasions within 6 months was used to define albuminuria.

2.4. Statistical analysis

Continuous variable results are presented as the mean and standard deviation (SD) or interquartile ranges and categorical variables as counts and percentages. Variables with non-normal distributions such as angiotensin-II, urinary ACR, HOMA-IR, HOMA-β, triglycerides, and hsCRP were analyzed using the Mann–Whitney *U* test or log-transformed to achieve normal distribution for the same statistical analysis. Differences in anthropometric and biochemical variables among participants with NGM, prediabetes, and T2DM were evaluated using an analysis of variance followed by Tukey's post-hoc

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