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Association between reduced ADAMTS13 and diabetic nephropathy

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ABSTRACT

Introduction: Deficiency of Von Willebrand factor (VWF)-cleaving protease (ADAMTS13) causes platelet thrombosis in the microcirculation. Intrarenal coagulation is thought to be associated with the development and progression of diabetic nephropathy. Our aim was to clarify the association between plasma ADAMTS13 antigen (ADAMTS13Ag) levels and diabetic nephropathy.

Material and Methods: We measured the plasma levels of VWF antigen (VWFAg) and ADAMTS13Ag, and calculated the VWF/ADAMTS13 ratio in 86 type 2 diabetic patients and 26 healthy volunteers, to investigate the relationship between these levels and renal function. With regard to diabetic macroangiopathy, the relationship between these levels and carotid intima-media thickness (IMT) was also investigated.

Results and Conclusions: A significant positive and negative correlation was noted between ADAMTS13Ag and the estimated glomerular filtration rate (eGFR), vWF/ADAMTS13 ratio and eGFR, respectively. The diabetic patients were divided into normoalbuminuria (n = 50), microalbuminuria (n = 8) and overt nephropathy (n = 28) groups. Compared among these three groups and the 26 healthy volunteers, ADAMTS13Ag was significantly lower only in the overt nephropathy group. The mean carotid IMT was measured in 69 patients and was significantly negatively correlated with ADAMTS13Ag and positively correlated with VWF/ADAMTS13 ratio. When these 69 patients were divided into four groups according to eGFR and ADAMTS13 levels (ADAMTS13/eGFR; low/low: n = 12; high/low: n = 7; low/high: n = 25; high/high: n = 25), the mean carotid IMT was increased in patients with a low ADAMTS13Ag in the same eGFR group. The present study suggests that reduced ADAMTS13 might be associated with diabetic nephropathy.

Introduction

Von Willebrand factor (VWF) is a mediator of platelet adhesion that binds to exposed collagen to form a bridge between platelets and damaged endothelium. It is synthesized in endothelial cells and megakaryocytes, and stored as an unusually large VWF multimer (UL-VWFM) held together by intermolecular disulfide bonds in Weibel-Palade bodies and platelet α-granules [1]. The molecular weight/multimeric composition of VWF is a key determinant of its platelet-anchoring function. The larger the VWF multimer, the higher the number of exposed binding sites and the greater the potential for VWF-ligand interaction and formation of a competent platelet thrombus [2–4]. UL-VWFMs are more active than other VWF multimers under high shear stress. This is because of the fact that the molecular conformation of VWF changes from a globular (functionally cryptic) to an extended (functionally activated) form under increasing shear stress [5]. The VWF multimeric size is modulated by ADAMTS13. ADAMTS13 is produced predominantly by hepatic stellate cells [6,7], which rapidly cleave the bond between tyrosine-842 and methionine-843 in the A2 domain of VWF multimers. This cleavage results in two fragments of 176 and 140 kDa [8–11], reducing the molecular weight and consequently the VWF platelet-anchoring function [8–12]. A deficiency of ADAMTS13 is seen in patients with thrombotic thrombocytopenic purpura (TTP) [8] which is characterized by platelet thrombosis in the systemic microcirculation [12,13].

Diabetes has become the most common single cause of end-stage renal disease in the United States and Europe [14]. Diabetic nephropathy develops in approximately 40% of all type 2 diabetic patients and is mainly characterized by persistent albuminuria and

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with thrombospondin type I domain 13; eGFR, estimated glomerular filtration rate; hsCRP, high sensitive C-reactive protein; IMT, intima-media thickness; MA, microalbuminuria; NA, normoalbuminuria; ON, overt nephropathy; TTP, thrombotic thrombocytopenic purpura; UAE, urinary albumin excretion; UL-VWFM, unusually large Von Willebrand factor multimers; VWF, Von Willebrand factor.

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elevated blood pressure [15]. Once patients with microalbuminuria (MA) progress to macroalbuminuria (overt nephropathy), they are likely to progress to end-stage renal disease [16].

Numerous factors have been implicated in the pathogenesis of diabetic nephropathy including hyperfiltration, hypertension, poor metabolic control of diabetes and growth factors [17]. It has been suggested that platelet activation and hypercoagulability also contribute to the pathogenesis of diabetic microvascular complications [18–20]. However, the relationship between ADAMTS13 and diabetic nephropathy remains unknown.

Recently, a new link has been found between thrombosis and inflammation in relation to ADAMTS13. ADAMTS13 plays an important role in preventing excessive spontaneous Weibel-Palade body secretion, and in the regulation of leukocyte adhesion and extravasation during inflammation [21].

We hypothesize that ADAMTS13 plays a role in diabetic renal microvascular thrombosis and inflammation, and contributes to the development and/or progression of diabetic nephropathy. In the present study, we measured plasma ADAMTS13 and its substrate VWF antigen levels in diabetic patients, in order to study the association between ADAMTS13, the VWF/ADAMTS13 ratio and diabetic nephropathy.

With regard to macroangiopathy, we also investigated the relationship between plasma ADAMTS13Ag level, the VWF/ADAMTS13 ratio and the carotid artery intima-media thickness (IMT).

Materials and Methods

Participants and specimens

Eighty-six type 2 diabetic patients (57 males and 29 females of mean age 57.5 ± 13.6 years) who were admitted to Heiwadai Hospital for the purpose of treating diabetes from January 2006 to December 2008 were included in this study. Of the 108 patients who were initially evaluated, only 86 were enrolled as study subjects. Eight patients who had type 1 diabetes were excluded from the study. Additionally, 14 patients were excluded for the following reasons: inflammatory disease (pneumonia, phlegmon, moderate temperature burn and gangrene); thrombotic disease (acute cerebral infarction, acute cardiovascular event); hepatic disease (liver cirrhosis); other renal disease (hypertensive glomerulosclerosis) by medical history, physical examination and routine laboratory tests. None of the patients had collagen disease or disseminated intravascular disease. Before admission, patients who had hypertension and/or hyperlipidemia were using anti-hypertensives and/or hypolipidaemic agents for blood pressure and lipid control. The patients gave their written informed consent to participate in the study, which was approved by the Heiwadai Hospital Clinical Research Ethics Committee. The patients fasted after 7:00 p.m. on the day of admission. At 6:00 a.m. the following day blood samples were collected into 3.8% citric acid-containing plastic tubes using a 21-gauge needle. The samples were promptly centrifuged at 3,000 rpm for 15 min and the plasma was collected and stored at -80 °C until analysis. The healthy volunteers fasted overnight (after 9:00 p.m.) and blood samples were collected and stored as described above. The samples were analyzed within three months of storage.

Clinical and biochemical studies

The height and weight of patients were measured using standardized techniques. The body mass index (BMI) was calculated using the following formula: weight (kg)/height^2 (m^2). Blood pressure was recorded with a mercury sphygmomanometer secured to the right arm of patients while in a sitting position. Plasma glucose was assayed using the enzymatic colorimetric method for measuring glucose oxidase. Total cholesterol, LDL-cholesterol and HDL-cholesterol were measured using a cholesterol oxidase phenol aminoantipyrine method. Triglycerides were assessed using a glycerol-3 phosphate oxidase phenol aminoantipyrine method. Creatinine levels were determined by a spectrophotometric assay using the creatinine-peroxidase-antiperoxidase method. High sensitive C-reactive protein (hsCRP) was analyzed using an ultrasensitive assay of particle-enhanced immunoturbidimetric latex agglutination with an auto-analyzer (7170 S; Hitachi High-Technologies Corp., Tokyo, Japan). HbA1c was measured using the high-performance liquid chromatography method (HLC-723G7; TOSOH, Tokyo, Japan). In consecutive 24 h urine samples, albumin was determined with a commercially available latex agglutination assay (SRL Inc., Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula: $186 \times [\text{plasma creatinine (mg/dl)}^{-1.154} \times \text{age (years)}^{-0.203} \times (0.742 \text{ if female})]$.

Normoalbuminuria, microalbuminuria and overt nephropathy

According to the rate of urinary albumin excretion (UAE) on collection at 24 h, normoalbuminuria (NA) was defined in diabetic patients as UAE <30 mg/24 h, microalbuminuria (MA) as UAE 30–299 mg/24 h and overt nephropathy (ON) as UAE >300 mg/24 h.

Measurement of ADAMTS13 and VWF antigen

The human ADAMTS13 cDNA used in this study was described previously [22]. Human ADAMTS13 was expressed in human embryo kidney 293 cells, stably transfected with pCAG-ADAMTS13 Neo and purified. Murine monoclonal antibodies to human ADAMTS13 were generated by the standard method [23] after the immunization of BALB/c mice with recombinant human ADAMTS13. Two murine monoclonal antibodies, WH10 and WH2-22-1A, were selected for ELISA, and they were shown to bind to the third thrombospondin-1 motif and to the disintegrin domain of ADAMTS13 in binding studies involving recombinant ADAMTS13 mutants, respectively [22,24,25]. WH10 (2 μg/mL) was used for microtiter plate coating (Maxisorp plate; Nalge Nunc International, Rochester, NY, USA). After blocking with 1% casein, plasma samples from healthy subjects and patients were diluted in phosphate-buffered saline, pH 7.2/0.1% casein, and then incubated in WH10-coated plates. ADAMTS13 that was bound to the microtiter plates was detected by peroxidase-conjugated WH2-22-1A. Purified recombinant ADAMTS13 was used as the standard to determine ADAMTS13 antigen (ADAMTS13Ag) levels. The ADAMTS13Ag level in each patient's plasma was expressed as the percentage of the ADAMTS13Ag level in normal pooled plasma.

The level of VWF antigen (VWF Ag) was determined by adding STA LIA VWF reagent to plasma samples. The reagent consisted of latex particles coated with VWF-specific polyclonal antibodies that agglutinate in the presence of VWF. Agglutination results in an increase in the optical density, which is directly proportional to the VWF concentration, and can be measured using an STA analyzer (Boehringer Mannheim Diagnostics, Germany).

Measurement of carotid artery intima-media thickness

The common carotid arteries were evaluated with high-resolution B-mode ultrasonography using a 7.5-MHz linear-array transducer (SSA-790A; Toshiba Medical Systems Corp., Tokyo, Japan) to determine the IMT. The carotid IMT was defined as the distance from the leading edge of the first to the leading edge of the second echogenic line [26], with the first line representing the lumen–intima interface and the second line the collagen-containing upper layer of the adventitia. Three measurements of the IMT were conducted at the site of greatest thickness and at 2 other points, 1 cm upstream and 1 cm downstream from the thickest site. The average value of the three points was calculated for each side, and the average overall value (mean IMT) was used for analysis.