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Change in urinary *N*-acetyl-β-D-glucosaminidase levels relevant to postprandial glycemic control conditions in subjects without diabetes mellitus

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ABSTRACT

Background: To assess the relationship between the serum level of 1,5-anhydroglucitol (1,5-AG), a marker of postprandial hyperglycemia, and the ratio of the urinary activity of *N*-acetyl-β-D-glucosaminidase to creatinine (NAG index) in subjects without diabetes mellitus (DM).
Methods: This was a cross-sectional study with 495 subjects without DM who had an estimated glomerular filtration rate ≥ 30 ml/min/1.73 m^2. Subjects were divided into tertiles based on serum 1,5-AG levels: high (>21.0 μg/ml), middle (14.0–21.0 μg/ml), and low (<14.0 μg/ml). Adjusted odds ratios for an elevated urinary NAG index (>5.8 U/g creatinine) according to the HbA1c (≤5.4%, 5.5%–5.9%, and 6.0%–6.4%) and 1,5-AG tertiles were calculated.
Results: The NAG index was negatively correlated with the serum 1,5-AG level in all subjects. The slopes of the regression lines for these variables did not differ significantly between elderly (≥65 y) and nonelderly subjects. As compared with high 1,5-AG and HbA1c ≤ 5.4%, the odds ratios for an elevated urinary NAG index increased progressively to 7.71 across the categories of low 1,5-AG and HbA1c of 6.0% to 6.4%.
Conclusion: Poor control of postprandial glucose is related to an elevated urinary NAG index in persons without DM.

1. Introduction

Many recent studies have found a relationship between postprandial glucose and the development of complications in patients with diabetes mellitus (DM). Accordingly, controlling postprandial hyperglycemia is considered to play a key role in preventing cardiovascular complications in patients with DM. Even in patients with impaired glucose tolerance (IGT), the risk of cardiovascular disease is reportedly increased by postprandial hyperglycemia but not by fasting hyperglycemia [1]. In 2007, measurement of the serum level of 1,5-anhydroglucitol (1,5-AG) was described as an emerging technology in the International Diabetes Federation's guidelines for managing postprandial glucose [2]. A naturally occurring dietary polyol,1,5-AG has been shown to be a useful marker for postprandial hyperglycemia. Serum levels of 1,5-AG decrease rapidly as glucose is excreted in the urine [3–5]. Previously, a strong negative correlation was found between the serum 1,5-AG level and the plasma glucose level at 120 minutes of the oral glucose tolerance test (OGTT) in subjects with IGT [6].

Whether IGT is independently associated with the traditional microvascular complications of DM, as well as with macrovascular events, has been the subject of considerable debate [7–12]. However, we believe that there is no evidence for renal tubular injury in persons with IGT. A widely distributed lysosomal enzyme with a molecular weight of 100 to 140 kDa, *N*-acetyl-β-D-glucosaminidase (NAG) is present at highest concentrations in the renal proximal tubules [13,14]. Therefore, elevated urinary levels of NAG indicate proximal tubular damage and are present in tubulointerstitial diseases, toxic renal injury, and glomerulonephritis. Furthermore, urinary NAG levels have been used to evaluate and predict subtle degrees of tubular injury [15–18]. To date, only 2 studies have examined the changes in urinary NAG levels in subjects with IGT [19,20].

On the other hand, only 2 studies, including our previous study, have examined the relationship between serum 1,5-AG and urinary NAG levels in subjects with type 2 DM [21,22]. Additionally, no studies have evaluated this relationship in subjects without DM.

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2. Research design and methods

2.1. Study subjects

Among outpatients of our department 495 patients (age, 16 to 95 y) who had never received a diagnosis of DM and had a HbA1c <6.5% and a fasting plasma glucose (FPG) level <7.0 mmol/l were defined as subjects without DM. Subjects who had been brought to out department by ambulance were excluded. All subjects were able to walk without being supported and did not have anorexia or stress conditions possibly affecting glycemic conditions. Other exclusion criteria included severe illness, pregnancy, previous gastrectomy, anemia, renal glucosuria, estimated glomerular filtration rate (eGFR) < 30 (ml/min/1.73 m^2), urine protein test >1+ (equivalent to >0.3 g/l), and a history of proteinuria, kidney disease, liver cirrhosis, or chronic hepatitis. Subjects were also excluded if they used uric acid-reducing drugs, traditional Chinese medicines that affect 1,5-AG metabolism, or drugs that might affect 1,5-AG measurements, such as oral hypoglycemic agents, insulin, and steroids. The glycemic conditions of all subjects were stable from the time of a non-DM diagnosis to the start of the study period. Before the start of the study, each subject was given detailed information regarding the nature of the study, after which informed consent was obtained. The study was designed in compliance with the ethics regulations set out by the Declaration of Helsinki.

2.2. Outcome measures

Urinary levels of NAG were measured with the use of synthetic substrate, 4-hydroxymethyl-2-pyridinyl 2-(acetylamino)-2-deoxy-1-thio-β-D-glucopyranoside (L-Type NAG [4HP-NAG substrate method], Wako Pure Chemical Industries, Ltd.). When saline is assayed, the absorbance change is not more than 0.01 (Δ*E*/min). When a sample is assayed, the coefficient of variation for the results is not more than 5%. Furthermore, when a sample of known activity is assayed, the value falls within ±6% of the known activity.

The urinary NAG index, which is the ratio of the urinary activity of NAG to creatinine (Cr), was calculated with spot urine specimens. Determining the NAG index in random urine specimens is a useful and convenient means of assessing daily NAG excretion and avoids many of the problems of 24-hour collection. The correlation coefficient of the NAG index in random early morning urine specimens to 24-hour NAG excretion in children with DM is 0.80 [23]. The normal range for the urinary NAG index in Japanese subjects has been reported to be 1.6 to 5.8 U/g Cr [13], and is reported in the manufacturer's instructions for the L-Type NAG assay substrate [24] to be 1.0 to 6.3 U/g Cr. Therefore, we considered the urinary NAG index to be elevated when greater than 5.8 U/g Cr.

2.3. Classification of postprandial glycemic control

Postprandial glycemic control conditions were determined on the basis of the serum level of 1,5-AG. Although 99% to 100% of 1,5-AG is reabsorbed in normoglycemia, the reabsorption rate decreases in proportion to the degree of hyperglycemia above the renal threshold for glucosuria [25,26]. When glycemic control is good, the 1,5-AG level increases [27,28], and when glycemic control is poor, the 1,5-AG level decreases [29]. The effects of sex and age on serum 1,5-AG levels were evaluated in our previous study [30]. Serum 1,5-AG levels were measured with an enzymatic method using 1,5-AG Auto Liquid reagent (Nippon Kayaku Co) and an automatic clinical analyzer (model 7150, Hitachi High-Technologies Corp.). The within-run precision and day-to-day CVs were 0.52% to 1.29% and 1.17% to 4.48%, respectively [31].

On the basis of a study performed in Japan, a serum 1,5-AG level of 14.0 μg/ml has been proposed as the cut-off value for diagnosing DM [32]. Accordingly, several other studies have adopted this level as a lower cut-off value [22,33,34]. With this in mind, we divided our subjects into tertiles on the basis of serum 1,5-AG levels: subjects with high levels (>21.0 μg/ml, $n = 217$), subjects with middle levels (≥14.0 μg/ml and ≤21.0 μg/ml, $n = 197$), and subjects with low levels (<14.0 μg/ml, $n = 81$).

2.4. Analysis of biochemical variables

Plasma glucose levels were measured with the glucose oxidase method in samples of venous blood. Levels of HbA1c (normal range, 4.1% to 5.9%) were measured with a high-performance liquid chromatograph (Auto A1C analyzer; Arkray, Inc.) and the method recommended by the Japan Diabetes Society (JDS). To convert the HbA1c (JDS) (%) to the internationally used HbA1c (%) defined by the National Glycohemoglobin Standardization Program (NGSP), i.e., HbA1c (NGSP) (%), 0.3% was added when HbA1c (JDS) was ≤4.9%, and 0.4% was added when HbA1c (JDS) was ≥5.0% and ≤6.0% [35]. White blood cell counts were determined with an automated hematology analyzer (XE-5000™, Sysmex Co., Ltd.). Serum levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, albumin, uric acid, and Cr were measured with an automatic analyzer.

Serum Cr was measured with an enzymatic assay. To determine the eGFR, the following 3-variable equation modified for Japanese subjects, as recently proposed by the Japanese Society of Nephrology [36], was used: eGFR (ml/min 1.73 m^{-2}) = 194 × serum Cr (mg/dl)$^{-1.094}$ × age (years)$^{-0.287}$ × 0.739 (if the subject was a woman). The urinary albumin level was measured with a radioimmunoassay, and the urinary albumin-to-Cr ratio was calculated. Specimens of blood and urine were obtained while the subjects were fasting. The urinary NAG index and the albumin-to-Cr ratio were determined in samples of second morning urine specimens.

2.5. Statistical analysis

The χ^2 and the Kruskal–Wallis *H*-test were used to compare characteristics among 1,5-AG tertiles. The Spearman correlation coefficient was used to analyze the relationship between the serum 1,5-AG level and the urinary NAG index. The regression equation and analysis of covariance (ANCOVA) were used to compare elderly and nonelderly subjects. Binary logistic regression analysis was performed to evaluate the relationship between the urinary NAG index and subjects' characteristics, including age, sex, white blood cell count, mean blood pressure, total cholesterol, serum uric acid, eGFR, and urinary pH. As with 1,5-AG levels, the subjects were divided into tertiles on the basis of HbA1c levels: ≤5.4%, 5.5% to 5.9%, and 6.0% to 6.4%. A dummy variable is a variable that takes on the values 1 and 0:1 represents an elevated urinary NAG index (>5.8 U/g Cr), and 0 represents a nonelevated urinary NAG index. Data are presented as means ± SD or odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significance was indicated by $p < 0.05$. All analyses were performed with a statistical software program (IBM SPSS Statistics, version 12, IBM Corp., Armonk, NY).

3. Results

The characteristics of subjects, divided into 1,5-AG tertiles, are shown in Table 1. Characteristics that differed between 1,5-AG tertiles were age, the percentage of female subjects, BMI, white blood cells, hypertension, diastolic blood pressure, serum albumin, uric acid, serum Cr, and urine pH, whereas characteristics that did not differ between 1,5-AG tertiles were systolic blood pressure, mean blood pressure, statin use, total cholesterol, low-density lipoprotein cholesterol, triglycerides, and eGFR. Furthermore, the FPG and HbA1c levels did not differ between the 1,5-AG tertiles (Table 2). The relationship between serum 1,5-AG and urinary NAG index, shown as a scatter plot in Fig. 1, was a weak negative correlation ($r = -0.219$, $p < 0.001$). Furthermore, the lack of a difference in the regression line slopes between elderly and nonelderly subjects supports the null hypothesis (ANCOVA). Table 3