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Hyperhomocysteinemia and Hyperuricemia are Closely Associated with a Decline in Renal Function in Patients with Type 2 Diabetes

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Abstract

Objective: This study examined the association of homocysteine and uric acid with estimated glomerular filtration rate (eGFR) and explored their pathophysiological significance in patients with type 2 diabetes mellitus.

Methods: We performed a cross-sectional study in 56 Japanese patients with type 2 diabetes. The correlations of serum total homocysteine level with age, diabetes duration, HbA1c, body mass index, systolic blood pressure, eGFR, urinary albumin-to-creatine ratio (ACR), and uric acid were investigated. The correlation between eGFR and uric acid was also investigated. Serum total homocysteine level was compared in men and women, as well as in patients with and without diabetic nephropathy, hypertension, smoking, and alcohol consumption. Stepwise multiple regression analyses were performed to identify explanatory factors for eGFR, total homocysteine, and uric acid.

Results: Serum total homocysteine level showed a significant positive correlation with diabetes duration, ACR, and uric acid, and an inverse correlation with eGFR. Uric acid level and eGFR showed a significant inverse correlation. Patients with diabetic nephropathy and hypertension showed a significantly higher total homocysteine level. Multiple regression analyses demonstrated that age, total homocysteine, and uric acid were independent determinants of eGFR, and that age and eGFR were independent determinants of both total homocysteine and uric acid levels.

Conclusion: Although total homocysteine, uric acid, and eGFR showed correlations, the relationship between uric acid and homocysteine might be weaker than that between eGFR and homocysteine, and between eGFR and uric acid. A relatively weak association between hyperuricemia and hyperhomocysteinemia observed in our study suggests confounding by decreased renal function. Both total homocysteine and uric acid can be used as biomarkers of renal dysfunction in patients with type 2 diabetes.

Introduction

Diabetes mellitus (DM) [1], chronic kidney disease (CKD) [2], and diabetic nephropathy [3] are prominent risk factors for cardiovascular disease (CVD). CVD is one of the most important causes of morbidity and mortality in diabetic patients with nephropathy [4]. Mortality rates are higher in patients with DM and nephropathy and in CKD patients with DM [5]. In diabetic patients, decline in estimated glomerular filtration rate (eGFR) is an independent risk factor for cardiovascular events and cardiovascular mortality [6].

Hypertension, high blood glucose level, and dyslipidemia are classic risk factors for CVD [7]. In addition, non-classic, residual risk factors for CVD have been reported [8]. For example, chronic inflammation [9], oxidative stress [10], advanced glycation end-products [11], homocysteine [12], and uric acid [13] are such risk factors. Serum homocysteine level is increased in patients with CKD [14-16]. However, there are conflicting reports that the homocysteine level is increased [17] or decreased [18] in diabetic patients. Elevated homocysteine level may be a risk factor for metabolic syndrome [19] and deterioration of renal function [16]. Hyperuricemia is common in

diabetic and CKD patients. Elevated serum uric acid level is reported to be a risk factor for metabolic syndrome [20,21] and end-stage kidney disease [22]. Although homocysteine and uric acid possess many similarities, the relationships between homocysteine, uric acid, and eGFR are not clear in diabetic patients; few studies have investigated both homocysteine and uric acid levels in the same diabetic patients while focusing on renal function. Therefore, in order to examine the associations of homocysteine and uric acid with eGFR and to explore their pathophysiological significance in diabetic patients, we conducted this retrospective cross-sectional study.

Methods

Study subjects

We performed a cross-sectional study in 56 consecutive Japanese patients (28 men and 28 women) with type 2 diabetes mellitus (age 38-81 years; mean 63 years) who were admitted to Keio University Hospital for blood glucose control from January to September 2011. We included all inpatients that agreed with this study and did not meet the following exclusion criteria during this period. Patients receiving hemodialysis or peritoneal dialysis, aged <20 years, who were pregnant, or whose hemodynamic condition was unstable were

excluded from this study. The study was approved by the ethics committee of Keio University School of Medicine and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

All patients provided life and medical histories and medication use at the time of admission. We measured systolic/diastolic blood pressure, height, and weight. Blood pressure was measured twice at rest in a seated position, at intervals of 1 min, and the mean of two measurements was adopted. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Diabetic nephropathy was defined as urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g creatinine (Cr) or estimated glomerular filtration rate (eGFR) <30 ml/min/1.73 m^2. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, and/or having received treatment for hypertension. Drinking was defined as prior or current habit of drinking alcohol. Smoking was defined as prior or current tobacco use.

Laboratory data

After an overnight fast, morning venous blood samples were collected for measurement of hemoglobin A1c (HbA1c), Cr, uric acid, and total homocysteine, and urine samples were collected for measurement of albumin and Cr. Serum Cr and uric acid were measured using an enzymatic method [23]. Urinary albumin and Cr were measured with turbidimetric immunoassay [23]. Urinary albumin excretion was shown as ACR and was calculated from the values of albumin and creatinine in spot urine. Serum homocysteine level was measured using high performance liquid chromatography (HPLC) [24]. HbA1c was determined by performing HPLC (Toso, Tokyo, Japan) and presented as the equivalent National Glycohemoglobin Standardization Program value. eGFR was calculated using the following formula established by the working group of the Japanese Chronic Kidney Disease Initiative: eGFR (ml min^{-1} 1.73 m^{-2}) = 194 × (serum creatinine)$^{-1.094}$ × (age)$^{-0.287}$ (if female: × 0.739) [25].

Statistical analysis

The correlations of serum total homocysteine level with age, type 2 diabetes duration, HbA1c, BMI, systolic blood pressure, eGFR, urinary ACR, and serum uric acid were investigated. The correlation between eGFR and uric acid was also investigated. We divided the subject in two groups according to their age. One group consisted of 29 subjects whose age was 38-64 years, and the other group consisted of 27 subjects whose age was 65 years or older. In both groups, correlation of total homocysteine and eGFR were investigated. Correlations between two variables were assessed using Pearson's coefficient.

Serum total homocysteine level was compared in men and women, and in patients with and without a history of diabetic nephropathy, hypertension, smoking, and alcohol consumption. Comparisons between two groups were performed using an unpaired t-test. Stepwise multiple regression analysis was performed to examine the effects of various factors on eGFR, and the following were considered as independent variables: age, sex, duration of diabetes, BMI, total homocysteine, hypertension, and uric acid. Stepwise multiple regression analyses using total homocysteine and uric acid as dependent variables were also performed, and the following were considered independent variables: age, sex, duration of diabetes, BMI, hypertension, eGFR, and either uric acid or total homocysteine.

All statistical analyses were conducted using the SPSS (version 17.0, Chicago, IL, USA). All data are expressed as mean ± SD, and values of p<0.05 (two-sided) were considered significant.

Results

As shown in Table 1, the mean serum total homocysteine level was 8.7 ± 2.9 nmol/mL. Of 56 patients, 22 (39%) had diabetic nephropathy. Serum total homocysteine level showed a significant positive correlation with duration of diabetes (r=0.38, p=0.0051), urinary ACR (r=0.30, p=0.028), and uric acid (r=0.38, p=0.0052), and an inverse correlation with eGFR (r=-0.47, p=0.0002). However, correlations of total homocysteine concentration with HbA1c (r=-0.086, p=0.53), BMI (r=0.20, p=0.14), age (r=0.025, p=0.86), and systolic blood pressure (r=0.23, p=0.098) were not significant. Uric acid concentration and eGFR showed a significant inverse correlation (r=-0.59, p<0.00001). In patients whose age was 38-64 years, total homocysteine showed inverse relationship between eGFR (r=-0.48, p=0.0083) and positive correlation with uric acid (r=0.41, p=0.0314). On the contrary, in patients whose age was 65 years or older, total homocysteine showed inverse correlation with eGFR, but did not show significant correlation with uric acid (r=0.30, p=0.13).

N (men/women)	56 (28/28)
Age (year-old)	63.0 ± 12.6
Duration of diabetes (years)	15.1 ± 9.7
BMI (kg/m^2)	25.5 ± 5.8
HbA1c (%)	9.1 ± 1.9
Total homocysteine (nmol/mL)	8.7 ± 2.9
Diabetic nephropathy, n (%)	22 (39)
Systolic blood pressure (mmHg)	126.6 ± 16.2
eGFR (mL/min/m^2)	69.0 ± 24.5
ACRmg/g Cr	276.5 ± 838.2
Uric acid (mg/dL)	5.3 ± 1.8
Hypertension, n (%)	20 (36)
Smoking, n (%)	27 (48)
Alcohol drinking, n (%)	23 (41)

Data are presented as mean ± SD or n (%) of patients

BMI: body mass index; HbA1c: hemoglobin A1c; eGFR: estimated glomerular filtration rate; ACR: albumin-to-creatinine ratio

Table 1: Clinical characteristics of patients in this study.

Patients with diabetic nephropathy showed a significantly higher serum total homocysteine concentration than those without nephropathy (9.9 ± 3.8 vs. 8.0 ± 1.7 nmol/mL, p=0.033). In addition, patients with hypertension showed a significantly higher total homocysteine concentration than those without hypertension (9.5 ± 3.2 vs. 7.4 ± 1.5 nmol/mL, p=0.0098). Sex (men 9.0 ± 2.4, women 8.5 ± 3.3 nmol/mL, p=0.48), smoking (absence 8.4 ± 3.4, presence 9.0 ± 2.4 nmol/mL, p=0.43), and alcohol consumption (absence 8.6 ± 3.2, presence 8.9 ± 2.4 nmol/mL, p=0.67) did not show a significant effect on serum total homocysteine level.

variables	β	F	P
Total homocysteine	-0.4	19.5	<0.001
HbA1c	0.07	0.61	0.44
Duration of diabetes	0.035	0.12	0.72
BMI	-0.11	1.37	0.25
Age	-0.50	25.1	<0.001
Male	0.052	0.35	0.56
Hypertension	-0.013	0.018	0.89
Uric acid	-0.48	26.6	<0.001
HbA1c: Hemoglobin A1c; BMI: Body mass index			

Table 2: Stepwise multiple regression analysis for assessment of independent determinants of estimated glomerular filtration rate.

variables	β	F	P
HbA1c	-0.099	0.58	0.45
Duration of diabetes	0.18	1.91	0.17
BMI	-0.063	0.24	0.62
Age	-0.48	9.3	0.0039
Male	0.13	1.22	0.27
eGFR	-0.77	19.5	<0.001
Hypertension	0.15	1.15	0.29
Uric acid	-0.26	2.77	0.10
HbA1c: Hemoglobin A1c; BMI: Body mass index; eGFR: Estimated glomerular filtration rate			

Table 3: Stepwise multiple regression analysis for assessment of independent determinants of total homocysteine concentration.

variables	β	F	P
Total homocysteine	-0.23	2.77	0.10
HbA1c	-0.08	0.45	0.51
Duration of diabetes	-0.0058	0.0021	0.96
BMI	0.016	0.019	0.89
Age	-0.50	12.2	0.0011
Male	0.21	3.64	0.063
eGFR	-0.79	26.6	<0.001
Hypertension	0.18	2.11	0.15
HbA1c: Hemoglobin A1c; BMI: Body mass index; eGFR: Estimated glomerular filtration rate			

Table 4: Stepwise multiple regression analysis for assessment of independent determinants of uric acid concentration.

As shown in Table 2, stepwise multiple regression analysis demonstrated that age (β=-0.50, F=25.1, p<0.001), total homocysteine (β=-0.40, F=19.5, p<0.001), and uric acid (β=-0.48, F=26.6, p<0.001) were independent determinants of eGFR. As shown in Table 3, age (β=-0.48, F=9.3, p=0.0039) and eGFR (β=-0.77, F=19.5, p<0.001) were independent determinants of total homocysteine. As shown in Table 4, age (β =-0.50, F=12.2, p=0.0011) and eGFR (β=-0.79, F=26.6, p<0.001) were independent determinants of uric acid concentration.

Discussion

In the present study, total homocysteine concentration correlated with diabetes duration, but not with HbA1c. As the mean diabetes duration of the subjects in this study was as long as 15.1 years, this suggests that high homocysteine concentration was associated with long-term exposure to hyperglycemia. This is supported by our finding that total homocysteine concentration was higher in patients with nephropathy and was correlated with urinary ACR. Similarly, homocysteine level might be associated with long-term exposure to high blood pressure. This is supported by our finding that homocysteine level was higher in patients with hypertension and that it did not correlate with blood pressure values measured during hospitalization. Hyperglycemia, high blood pressure, and hyperhomocysteinemia [15,26,27] all accelerate atherosclerosis. Thus, we must assume that subjects with hyperhomocysteinemia have a long history of diabetes or hypertension and very high risk for atherosclerotic diseases.

In multiple regression analyses, when homocysteine was used as a dependent variable, uric acid was not selected as an independent variable. Similarly, when uric acid was used as a dependent variable, homocysteine was not selected as an independent variable. However, when eGFR was used as a dependent variable, both homocysteine and uric acid were selected as independent variables. Although total homocysteine, uric acid, and eGFR were correlated with each other, the relationship between uric acid and homocysteine might be weaker than that between eGFR and homocysteine, and between eGFR and uric acid. This possibility might be supported by our observation that in the patients whose age was 65 years or older, total homocysteine showed significant correlation with eGFR but not with uric acid.

Hyperhomocysteinemia is an independent risk factor for both CVD [12,28,29] and CKD [30,31]. Serum homocysteine possesses atherogenic and prothrombotic properties [32,33], triggers the production of reactive oxygen species, and causes endothelial dysfunction [28]. Hyperhomocysteinemia might cause renal damage through these mechanisms. Conversely, renal dysfunction elevates homocysteine concentration, probably due to decreasing renal metabolic extraction [15]. Thus, there exist bidirectional, functional relationships between hyperhomocysteinemia and decreased renal function. Similar interactions exist between hyperuricemia and decreased kidney function. Hyperuricemia, as well as activated xanthine oxidase, might cause renal dysfunction through oxidative stress, inflammation, and endoplasmic reticulum stress [34,35]. Conversely, renal dysfunction might increase serum uric acid level and xanthine oxidase activity through decreased renal clearance or tissue ischemia [36].

To the authors' knowledge, there are no reports of such bidirectional and functioning interactions between homocysteine and uric acid. A relatively weak association between hyperuricemia and hyperhomocysteinemia observed in our study might suggest that they

are confounded by decreased renal function. That is, the concentrations of both homocysteine and uric acid might increase due to decreased clearance by the kidney. The stronger association of homocysteine with eGFR than with uric acid shown in this study is consistent with previous reports showing that elevated serum homocysteine level was not correlated with uric acid level, but instead with decreased kidney function in patients with gout [37].

Our study has several limitations. First, this is a retrospective, cross-sectional study in a small number of the patients. Therefore, cause and effect relationships cannot be clarified, and the possibility of bias cannot be excluded. Second, we included only individuals who were admitted to a university hospital as study subjects. Therefore, these were selected patients, and the results of our study may not be applicable to the general population or to diabetic patients in a primary care setting. Third, control subjects without diabetes were not included in our study. Finally, we did not measure plasma levels of vitamin B and folate, both of which influence the plasma level of homocysteine.

Conclusions

In conclusion, our study shows that both total homocysteine and uric acid levels are closely related with eGFR in type 2 diabetic patients. Both of these can be used as biomarkers of renal dysfunction, although further evaluation is warranted. As eGFR is a most reliable marker of renal function, we need to compare the usefulness of total homocysteine with that of eGFR. In addition, it remains to be clarified whether treatment of hyperhomocysteinemia and hyperuricemia can prevent the onset or progression of chronic kidney disease. Further prospective studies will be necessary to address these issues.

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