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Serum pepsinogen I/II ratio is correlated with albuminuria in patients with type 2 diabetes

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Abstract. *Helicobacter pylori* infection, which is a common cause of atrophic gastritis, has been reported to represent a causal factor increasing the vascular damage and consequent albuminuria. On the other hand, decreased serum pepsinogen (PG) I/II ratio can be used to assess gastric mucosal atrophy. To the best of our knowledge, there are no studies investigating the correlation between PG I/II ratio and diabetic nephropathy. Therefore, we investigated a relationship between PG I/II ratio and degree of urinary albumin excretion (UAE) in patients with type 2 diabetes. We evaluated relationships between PG I/II ratio and degree of UAE or estimated glomerular filtration rate as well as various factors, including age, body mass index, blood pressure, hemoglobin A1c, serum lipid concentrations, uric acid or C-reactive protein in 333 consecutive patients with type 2 diabetes. PG I/II ratio correlated positively with logarithm of UAE in all patients ($r = 0.174$, $P = 0.0016$) and in patients without *Helicobacter pylori* infection ($r = 0.352$, $P < 0.0001$). Multiple regression analysis identified that PG I/II ratio correlated independently with logarithm of UAE in all patients ($\beta = 0.264$, $P = 0.0005$) and in patients without *Helicobacter pylori* infection ($\beta = 0.295$, $P = 0.0022$). These data suggest that serum PG I/II ratio is correlated with diabetic nephropathy.

Key words: Pepsinogen, Albuminuria, Nephropathy, Type 2 diabetes

CARDIOVASCULAR DISEASE (CVD) is the primary cause of mortality and morbidity in patients with type 2 diabetes, and several metabolic disorders including hypertension and dyslipidemia combined with diabetes accelerate the progression of CVD [1, 2]. *Helicobacter pylori* (Hp) infection also has been reported to be associated with the development and progression of atherosclerosis [3]. Moreover, Hp infection, especially with strains carrying the cytotoxin-associated gene A (CagA), has been reported to represent a causal factor increasing the vascular damage and consequent albuminuria [4].

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Atrophy of the gastric mucosa is the endpoint of chronic processes, such as chronic gastritis associated with Hp infection, other unidentified environmental factors and autoimmunity directed against gastric glandular cells. Pepsinogen (PG) is a precursor of pepsin, and consists of two biochemically and immunologically distinct types, namely, PG I and PG II. Decreased serum PG I levels and PG I/II ratio can be used to assess gastric mucosal atrophy [5]. Certainly, Hp infection is associated with atrophy of the gastric mucosa and low PG I/II ratio.

We hypothesized that PG I/II ratio, as a marker of gastric mucosal atrophy, may be associated with diabetic nephropathy, irrespective of Hp infection. However, to the best of our knowledge, there are no studies investigating the correlation between PG I/II ratio and diabetic nephropathy. Therefore, we investigated a relationship between PG I/II ratio and degree of urinary albumin excretion (UAE) in patients with type 2 diabetes.

Materials and Methods

Patients

Serum PG levels were measured in 333 consecutive patients with type 2 diabetes who were recruited from the outpatient clinic at Kyoto Prefectural University of Medicine. Type 2 diabetes was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [6]. Retinopathy was assessed with a state of mydriasis by ophthalmologists who were unaware of the data, and was graded as follows: no diabetic retinopathy, NDR; simple diabetic retinopathy, SDR; and proliferative, including preproliferative, diabetic retinopathy, PDR. If the finding in the left and right fundi were discordant, the worse side was taken as a representative for the subject. Neuropathy was defined by the diagnostic criteria for diabetic neuropathy proposed by Diagnostic Neuropathy Study Group [7]. In brief, in the absence of peripheral neuropathies, diabetic neuropathy is diagnosed by two or more of the following: neuropathic symptoms such as numbness, paresthesia and neuropathic pain, decreased or absent ankle reflex (bilateral) and decreased distal sensation. Nephropathy was graded as follows: normoalbuminuria, UAE less than 30 mg per gram of creatinine (mg/g Cr); microalbuminuria, 30 to 300 mg/g Cr; or macroalbuminuria, more than 300 mg/g Cr. Sitting blood pressure was measured after a 5-min rest. CVD was defined as a previous myocardial or cerebral infarction based on the clinical history or physical examination. Subjects were classified as nonsmokers, past smokers or current smokers according to a self-administered questionnaire. Patients were excluded if they had a history of gastrectomy and a history of Hp eradication treatment.

Experimental design

We evaluated relationships between PG I/II ratio and degree of UAE or estimated glomerular filtration rate (eGFR) as well as various factors, including age, body mass index, blood pressure, hemoglobin A1c, serum lipid concentrations, uric acid or C-reactive protein. We compared PG I/II ratio according to sex, degree of diabetic complications, smoking status, current treatment for diabetes and presence of CVD or Hp infection. Furthermore, we evaluated if PG I/II ratio correlated independently with log UAE by multivariate linear regression analysis after adjustment for variables, which are known risk factors for diabetic nephropathy. This study was approval by the local Research Ethics Committee and was conducted in accordance with Declaration of Helsinki, and informed consent was obtained from all participants.

Biochemical analysis

Serum PG levels were measured by chemiluminescent enzyme immunoassay (PG I/II Lumispot; Eiken Chemical Tokyo, Japan) at Yamashiro Public Hospital, Kyoto. Serum immunoglobulin G antibodies to Hp were measured using an enzyme immunoassay (E plate; Eiken Chemical Tokyo, Japan); an assay value of at least 10 U/mL was considered as positive.

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and uric acid concentration were assessed using standard enzymatic methods. Hemoglobin A1c was assayed using high-performance liquid chromatography. Urinary albumin and Cr concentration were determined in an early morning spot urine. Albumin excretion rate was measured with an immunoturbidimetric assay. A mean value for UAE was determined from three urine collections. GFR was estimated using the equation of Japanese Society of Nephrology: $eGFR = 194 \times Cr^{-1.094} \times age^{-0.287}$ (mL/min/1.73m^2). For women, eGFR was multiplied by a correction factor of 0.739.

Statistical analysis

Means, medians or frequencies of potential confounding variables were calculated. Skewed variables such as UAE was presented as median (interquartile range), and continuous variables were presented as the mean ± standard deviation. Because UAE showed a skewed distribution, logarithmic (log) transformation was carried out before performing correlation and regression analysis. Unpaired Student's *t*-tests or analyses of variance were conducted as appropriate to assess statistical significance of differences between groups using Stat View software (version 5.0; SAS Institute, Cary, NC, USA). Relationships between PG I/II ratio and log UAE, eGFR or other variables were examined by Pearson's correlation analyses. Multiple regression analysis was performed to assess the combined influence of variables on log UAE. To examine the effects of various factors on log UAE, the following factors were considered as independent variables: sex (male = 0, female = 1), age, duration of diabetes, body mass index, hemoglobin A1c, systolic blood pressure, total cholesterol, triglycerides, uric acid, C-reactive protein, smoking status (none = 0, past = 1, current = 2), usage

of angiotensin-converting enzyme inhibitors (ACE-I) and/or angiotensin II receptor blockers (ARB) (no = 0, yes = 1) and PG I/II ratio. A P-value < 0.05 was considered statistically significant.

Results

Characteristics of the 333 patients with type 2 diabetes enrolled in this study are shown in Table 1. Mean PG I/II ratio was 4.74 ± 2.69 in all patients. Serum PG II levels were significantly higher and PG I/II ratio was significantly lower in patients with Hp infection than in patients without Hp infection. However, no significant differences were found in other clinical variables between with and without Hp infection. Relationships between PG I/II ratio and other variables are shown in Table 2. Systolic blood pressure, C-reactive protein and log UAE correlated positively with PG I/II ratio, while age and eGFR correlated inversely with PG I/II ratio in all patients. Systolic blood pressure, triglycerides, uric acid, C-reactive protein and log UAE (r = 0.352, P < 0.0001) correlated positively with PG I/II ratio, while eGFR (r = -0.262, P = 0.0003) correlated inversely with PG I/II ratio in patients without Hp infection. No significant correlations were found between PG I/II ratio and other variables in patients with Hp infection.

Comparisons of PG I/II ratio among various groups are shown in Table 3. PG I/II ratio was significantly lower in patients without neuropathy than in those with neuropathy. PG I/II ratio was significantly lower in patients with normoalbuminuria than that in those with macroalbuminuria. PG I/II ratio was significantly lower in patients with Hp infection than in those without Hp infection.

Multiple regression analysis on log UAE demonstrated that age, hemoglobin A1c, total cholesterol, uric acid and PG I/II ratio correlated independently with log UAE in all patients (Table 4). Age, hemoglobin A1c,

Table 1 Clinical characteristics of patients with type 2 diabetes

	All	H. pylori (-)	H. pylori (+)
n	333	187	146
Sex (male/female)	196/137	103/84	93/53
Age (years)	66.3 ± 8.9	65.8 ± 9.5	66.9 ± 7.9
Age at onset (years)	51.3 ± 12.0	51.2 ± 12.5	51.3 ± 11.3
Duration of diabetes (years)	15.1 ± 10.6	14.8 ± 10.7	15.5 ± 10.4
Body mass index (kg/m^2)	22.8 ± 4.6	22.9 ± 4.9	22.7 ± 4.5
Hemoglobin A1c (%)	7.3 ± 1.1	7.4 ± 1.2	7.3 ± 1.0
Systolic blood pressure (mmHg)	129 ± 16	129 ± 16	129 ± 15
Diastolic blood pressure (mmHg)	69 ± 12	70 ± 13	69 ± 11
Total cholesterol (mg/dL)	189 ± 32	188 ± 31	191 ± 32
Triglycerides (mg/dL)	133 ± 95	126 ± 70	143 ± 119
High-density lipoprotein cholesterol (mg/dL)	54 ± 14	55 ± 15	53 ± 14
Uric acid (mg/dL)	5.3 ± 1.5	5.1 ± 1.5	5.5 ± 1.5
C-reactive protein (mg/dL)	0.10 ± 0.19	0.11 ± 0.20	0.09 ± 0.17
Retinopathy (NDR/SDR/PDR)	211/56/66	104/28/26	80/21/31
Neuropathy (-/+)	191/142	106/81	85/61
Nephropathy (normo-/micro-/macroalbuminuria)	188/107/38	109/56/18	74/49/21
Cardiovascular disease (-/+)	248/85	153/34	125/21
Smoking (none/past/current)	153/132/48	92/68/27	61/27/21
Diabetic treatment (diet/OHA/insulin)	35/191/107	21/102/64	14/89/43
ACE-I and/or ARB (-/+)	177/156	106/81	71/75
Estimated glomerular filtration rate (mL/min/1.73m^2)	75.0 ± 25.1	75.0 ± 24.2	74.4 ± 25.1
Urinary albumin excretion (mg/g creatinine)	24.7 (12.1-76.3)	24.0 (12.1-76.3)	27.0 (12.1-76.3)
Pepsinogen I (ng/mL)	94.1 ± 102.8	97.9 ± 119.4	89.1 ± 76.4
Pepsinogen II (ng/mL)	21.2 ± 16.9	15.8 ± 11.5	28.2 ± 20.0*
Pepsinogen I/II ratio	4.74 ± 2.69	5.95 ± 2.83	3.19 ± 1.46*

Data are number of patients, mean ± SD or median (interquartile range). NDR, no diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; OHA, oral hypoglycemic agents; ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers *P < 0.0001 *vs* H. pylori (-).

Table 2 Correlation between pepsinogen I/II ratio and other variables

	All		H. pylori (-)		H. pylori (+)	
	r	*P*	r	*P*	r	*P*
Age	-0.134	0.0142	-0.123	0.0926	-0.123	0.1380
Duration of diabetes	-0.006	0.9082	0.019	0.8043	0.001	0.9990
Body mass index	0.076	0.1691	0.075	0.3112	0.085	0.3096
HemoglobinA1c	0.061	0.2661	0.060	0.4176	-0.006	0.9390
Systolic blood pressure	0.131	0.0169	0.170	0.0196	0.105	0.2112
Diastolic blood pressure	0.051	0.3520	0.025	0.7352	0.109	0.1941
Total cholesterol	0.019	0.7251	0.010	0.8929	0.157	0.0585
Triglycerides	0.064	0.2408	0.174	0.0168	0.125	0.1330
High-density lipoprotein cholesterol	-0.028	0.6168	-0.102	0.1655	-0.031	0.7091
Uric acid	0.087	0.1182	0.197	0.0075	0.096	0.2622
C-reactive protein	0.167	0.0023	0.218	0.0026	0.018	0.8281
Estimated glomerular filtration rate	-0.158	0.0060	-0.222	0.0030	-0.153	0.0670
Log (urinary albumin excretion)	0.174	0.0016	0.352	< 0.0001	0.018	0.8334

Table 3 Comparisons of the pepsinogen I/II ratio among various groups

	mean ± SD
Sex (male/female)	4.69 ± 2.49/4.80 ± 3.00
Retinopathy (NDR/SDR/PDR)	4.51 ± 2.30/4.83 ± 2.53/4.78 ± 3.28
Neuropathy (-/+)	4.28 ± 2.07/5.38 ± 3.76*
Nephropathy (normo-/micro-/macroalbuminuria)	4.45 ± 2.14/4.66 ± 2.36/5.68 ± 4.02†
Cardiovascular disease (-/+)	4.49 ± 2.67/5.22 ± 3.05
Smoking (none/past/current)	4.77 ± 3.16/4.38 ± 2.45/4.85 ± 2.37
Diabetic treatment (diet/OHA/insulin)	4.95 ± 2.97/4.50 ± 2.19/5.05 ± 3.36
Helicobacter pylori infection (-/+)	5.95 ± 2.84/3.19 ± 1.46‡

Data are mean ± SD. NDR, no diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; OHA, oral hypoglycemic agents
$^{*}P < 0.01$, $^{\dagger}P < 0.01$ *vs*. normoalbuminuria, $^{\ddagger}P < 0.001$.

Table 4 Multiple regression analysis on log (urinary albumin excretion)

	All		H. pylori (-)		H. pylori (+)	
	β	*P*	β	*P*	β	*P*
Sex	-0.137	0.0854	-0.026	0.7856	-0.285	0.0754
Age	0.316	0.0002	0.407	< 0.0001	0.200	0.1952
Duration of diabetes	-0.130	0.6865	-0.092	0.3519	-0.036	0.7977
Body mass index	-0.021	0.7913	0.007	0.9392	-0.016	0.9063
Hemoglobin A1c	0.170	0.0287	0.286	0.0046	0.048	0.7565
Systolic blood pressure	0.114	0.1233	0.200	0.0232	-0.037	0.7851
Total cholesterol	-0.151	0.0480	-0.158	0.1033	-0.214	0.1442
Triglycerides	0.134	0.0848	0.088	0.3546	0.192	0.2031
Uric acid	0.357	< 0.0001	0.389	0.0001	0.314	0.0731
C-reactive protein	0.025	0.7536	0.017	0.8559	0.111	0.4556
Smoking	0.007	0.9200	0.064	0.4546	-0.117	0.4293
ACE-I and/or ARB	0.078	0.2988	0.147	0.0952	-0.042	0.7982
Pepsinogen I/II ratio	0.264	0.0005	0.295	0.0022	0.032	0.8121

ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers.

systolic blood pressure, uric acid and PG I/II ratio correlated independently with log UAE in patients without Hp infection, whereas no variables correlated significantly with log UAE in patients with Hp infection.

Discussion

PG I/II ratio correlated positively with degree of UAE and correlated negatively with eGFR in patients with type 2 diabetes, suggesting that serum PG I/II ratio might be a risk factor for diabetic nephropathy. Multiple regression analysis also identified that PG I/II ratio correlated independently with log UAE.

A possible explanation of this result is that low PG I/II ratio might be inversely correlated with several metabolic parameters, which have been reported to be related to albuminuria, such as blood pressure, serum lipid, uric acid and glucose concentrations *via* suppression of overeating in patients with type 2 diabetes. Indeed, our study demonstrated that PG I/II ratio correlated positively with systolic blood pressure, serum triglyceride and uric acid concentrations in patients without Hp infection. Plasma ghrelin levels have been reported to be correlated positively with serum PG I levels and PG I/II ratio [8, 9], and plasma ghrelin levels decrease in accordance with the extent of atrophic changes in gastric mucosa irrespective of Hp infection [10]. In addition to its ability to stimulate growth hormone secretion and gastric motility, ghrelin stimulates appetite and induces a positive energy balance leading to body weight gain [11, 12]. These findings support our hypothesis that low PG I/II ratio may have favorable effects on several metabolic parameters *via* suppression of overeating in patients with type 2 diabetes. Serum PG levels have been shown to reflect the morphological and functional status of the gastric mucosa. As the fundic gland mucosa reduces, PG I levels gradually decrease, whereas PG II levels remain constant [13]. Thus, serum PG I levels and PG I/II ratio correlated positively with gastric acid secretion [14]. Gastric acid converts PG into pepsin, which digests orally ingested protein. We speculate that another possible explanation of this result is that low serum PG I levels and PG I/II ratio might be associated with reduced digestion of protein, which means restriction of dietary protein, that may have protective role for diabetic nephropathy.

Our study has shown a positive correlation between PG I/II ratio and log UAE in patients without Hp infection. In addition, we included the presence of Hp infection in multiple regression analysis on log UAE in all patients, so as to investigate the contribution of Hp infection on albuminuria. There was no significant relationship between Hp infection and albuminuria ($\beta = 0.054$, $P = 0.5371$). PG I/II ratio remained correlated independently with log UAE ($\beta = 0.291$, $P = 0.0009$). Interestingly, however, no significant correlation between PG I/II ratio and log UAE was found in patients with Hp infection. Epidemiologic studies have shown a positive association between Hp infection and CVD [15, 16]. Certainly, Hp infection is associated with atrophy of the gastric mucosa and low PG I/II ratio, as is seen in any study. The underlying hypothetical mechanisms include chronic low-grade activation of the coagulation cascade and acceleration of atherosclerosis due to the vascular endothelial damage through the induction of inflammatory response [17, 18]. Pietroiusti *et al*. [4] reported that Hp infection, especially with strains carrying the CagA, represented a causal factor increasing the vascular damage and consequent albuminuria, and some previous reports suggested that both vascular endothelial dysfunction and chronic low-grade inflammation were key features of the pathophysiology of albuminuria as well as atherosclerosis [19, 20]. Ito *et al*. reported that the strain CagA was detected in almost all of Hp positive samples in Japanese individual [21]. The favorable effects of low PG I/II ratio on albuminuria *via* suppression of overeating may be offset by the unfavorable effects of persistent infection with Hp on albuminuria *via* vascular endothelial dysfunction and chronic low-grade inflammation.

An additional finding is that patients without neuropathy had the lower PG I/II ratio than those with neuropathy. This result may be also explained by our speculation that low PG I/II ratio might be inversely correlated with several metabolic parameters *via* suppression of overeating. Furthermore, we investigated the relationship between clinical indicator of autonomic neuropathy and PG I/II ratio. However, no significant difference was found in PG I/II ratio between patients with and without constipation, defined as pharmacological treatment for constipation, which is related to delaying gastric emptying is one of the symptoms of autonomic neuropathy (4.10 ± 2.25 *vs* 4.81 ± 2.75, $P = 0.1320$).

Limitations of our study include a cross-sectional design. In addition, we did not measure ghrelin, which has been reported to be related to appetite, and strains of Hp infection such as CagA. To the best of our knowledge, however, this is the first report of the relationship

between PG I/II ratio and albuminuria in patients with type 2 diabetes and suggests new avenues for research into the pathogenesis of diabetic nephropathy. This study revealed that PG I/II ratio correlated positively with albuminuria in patients with type 2 diabetes, especially in those without Hp infection. Large prospective trials are needed to better assess the effects of gastric mucosal atrophy on diabetic nephropathy.

In conclusion, PG I/II ratio correlated positively with albuminuria in patients with type 2 diabetes, suggesting that serum PG I/II ratio is correlated with diabetic nephropathy.

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Conflict of interests

The authors declare that they have no conflict of interest.

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