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Decreased Endothelial Progenitor Cells and Increased Serum Glycated Albumin Are Independently Correlated With Plaque-Forming Carotid Artery Atherosclerosis in Type 2 Diabetes Patients Without Documented Ischemic Disease

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Background: The aim of the present study was to investigate the serum levels of endothelial progenitor cells (EPCs) in type 2 diabetic patients without documented ischemic disease and the association between EPCs and atherosclerotic plaque formation in the carotid artery.

Methods and Results: A clinic-based, prospective study of type 2 diabetic patients was conducted. A total of 73 subjects were enrolled in this study after cardiac magnetic resonance imaging and ankle-brachial index measurements to exclude patients with ischemic disease. Plaque formation in the carotid artery was measured on ultrasonography. Circulating EPCs ($CD34^+/CD133^+/CD309^+$ cells) were counted on flow cytometry. Compared to subjects without carotid artery plaques, patients with plaques were significantly older (P=0.006) and had decreased EPC count (P=0.027). Serum glycated albumin (GA) level and the GA/glycated hemoglobin ratio tended to decrease in patients with plaques (P=0.091 and 0.067, respectively). Other cardiovascular disease risk factors were not significantly different between the 2 groups. On binary logistic regression analysis old age, low EPC count, and high serum GA level were independently correlated with carotid artery plaque formation.

Conclusions: EPC count and serum GA level appear to be a protective and an aggravating factor for endothelial damage, respectively, and therefore, a reduced EPC count or an increased GA level results in atherosclerotic plaque formation in type 2 diabetic patients. (*Circ J* 2012; **76:** 2273–2279)

Key Words: Atherogenesis; Endothelial progenitor cell; Glycated albumin; Plaque formation; Type 2 diabetes mellitus

Cells able to repair the endothelium have been termed endothelial progenitor cells (EPCs), and they contribute to ischemic tissue regeneration via vascular repair and angiogenesis.[1–3] Although numerous investigations on the functions and clinical implications of EPCs have been done, growing controversy remains. EPC count reflects the endogenous capacity to repair damaged vascular endothelium and is influenced by other confounding factors. Conventionally, EPC count was lower in patients with old age, diabetes, obesity, a family history of coronary artery disease (CAD), high C-reactive protein levels, smoking habits, or physical inactivity.[4–9] Even in 45 healthy subjects, EPC count was reported to be inversely associated with the Framingham risk score.[10] In contrast to these findings, a positive correlation between EPC count and smoking,[11] or no correlation between EPC count and certain cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes, a family history of CAD, and obesity were also reported.[12] Furthermore, a large-scale population-based study reported that the number of EPCs increased with cardiovascular risk as estimated by the Framingham risk score, al-

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though that study found an inverse association between EPC count and the extent of carotid atherosclerosis.[13] These previous studies used different definitions for EPCs and various methods to assess EPCs including flow cytometry and colony-forming cell assay. These methodological variations could have contributed to the controversy. These conflicting data also suggest that EPC count might be influenced by the characteristics of the subjects. Furthermore, EPCs are known to increase in response to physiological and pathological stimuli, including myocardial and peripheral ischemia.[14–16] Therefore, to investigate whether EPC count can be used as a biomarker of atherogenesis or cardiovascular disease (CVD) risk, a homogenous subject group is required.

In this study, we investigated EPC count in homogenous type 2 diabetic patients, and the association between EPC level and carotid artery atherosclerosis using ultrasonography. To homogenize the subject group, we excluded patients with documented cardiac or peripheral ischemic disease using cardiac magnetic resonance imaging (MRI) and ankle-brachial index (ABI) measurements.

Methods

Subjects

We conducted a clinic-based, prospective study of type 2 diabetic patients. The Institutional Review Board of Yonsei University College of Medicine approved this study. We recruited patients who (1) were between 40 and 70 years of age; (2) had maintained stable glycated hemoglobin (HbA_{1c}) levels (change in HbA_{1c} <0.5%) for at least 3 months prior to screening; and (3) had no documented history or symptoms and signs of atherosclerotic vascular disease such as angina, myocardial infarction, cerebrovascular accidents, or peripheral vascular disease. Patients were excluded for the following reasons: plasma aspartate transaminase and/or alanine transaminase levels 2-fold higher than the upper normal limit; serum creatinine concentration ≥177 μmol/L; and/or hematological and malignant disease. We performed 24-h urine analysis, fundoscopy, cardiac MRI, and ABI assessments of 90 screened subjects, and excluded 17 patients with overt macroalbuminuria defined as 24-h urinary albumin excretion ≥300 mg/day (n=1), more than moderate non-proliferative diabetic retinopathy (n=2), documented ischemic heart disease (n=13), or ABI <1.0 (n=1).

Waist circumference was measured at the midpoint between the lateral iliac crest and the lowest rib. Hip circumference was measured at the maximum protrusion of the greater trochanter. Waist-to-hip ratio (WHR) was calculated as the ratio of waist to hip circumferences. Blood pressure was measured twice with a mercury sphygmomanometer on the right upper arm after resting for at least 10 min.

Laboratory Assessment and Calculation of β-Cell Functional Status

We performed a standardized mixed meal stimulation test in enrolled subjects using commercial liquid nutritional supplements (Ensure, Meiji Dairies Corporation, Tokyo, Japan; total 500 kcal, 17.5 g fat, 68.5 g carbohydrate, and 17.5 g protein) after overnight fasting. Blood samples were collected at 0 and 90 min after intake of the liquid supplement (basal and stimulated levels, respectively) for glucose, triglyceride, insulin, and C-peptide analyses.

Plasma glucose was measured using the glucose oxidase method. Plasma triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Serum glycated albumin (GA) was determined by an enzymatic method using an albumin-specific proteinase, ketoamine oxidase, albumin assay reagents (LUCICA GA-L, Asahi Kasei Pharma, Tokyo, Japan), and a Hitachi 7699 Pmodule autoanalyzer. Serum HbA_{1c} was measured on high-performance liquid chromatography (HPLC) using a Variant II Turbo system (Bio-Rad Laboratories, Hercules, CA, USA). Serum insulin and C-peptide levels were measured in duplicate using immunoradiometric assay (IRMA; Beckman Coulter, Fullerton, CA, USA).

Pancreatic β-cell function and insulin sensitivity were obtained by homeostasis model assessment (HOMA) using HOMA of pancreatic β-cell function (HOMA-β) = basal insulin (μIU/ml) × 20 / [basal glucose (mmol/L) − 3.5] and HOMA of insulin resistance (HOMA-IR) = [basal insulin (μIU/ml) × basal glucose (mmol/L)] / 22.5, respectively.

Cardiac MRI

Cardiac MRI was performed using a 3.0-T MRI unit (Achieva 3.0T TX, Philips Medical Systems, Netherlands) with a 32-channel receiver coil. To evaluate myocardial ischemia, first-pass myocardial perfusion MR images were acquired during adenosine stress and at rest. After 3 min of pharmacological stress with adenosine (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$), perfusion imaging was started. A bolus of 0.1 mmol/kg gadolinium-DTPA (Magnevist, Schering, Berlin, Germany) was injected at the rate of 4 ml/s, and adenosine infusion was continued during the scan. Resting perfusion images were obtained 15 min after the stress assessment. Delayed enhancement MRI was obtained using a 3-D phase-sensitive inversion recovery sequence 10 min after the rest perfusion assessment. Myocardial ischemia was defined as a perfusion deficit at stress perfusion that was reversed at resting perfusion with no evidence of delayed hyper-enhancement. Myocardial infarction was defined as an area with hyper-enhancement at delayed enhancement MRI consistent with a coronary distribution.

Measurement of Intima-Media Thickness (IMT) and ABI

All measurements were conducted over a 4-h period in a quiet room kept at a constant temperature. Ultrasound was performed by 2 sonographers who used an Aloka ProSound ALPHA 10 with a 13-MHz linear probe. All measurements were done with the patient in a supine position, with their head elevated up to 45° and tilted to either side at 30°, depending on the side being examined. We performed B mode examinations at 1.5 cm proximal to the carotid bifurcation on the far wall of the common carotid artery on both sides. IMT was defined as the distance between the media-adventitia interface and the lumen-intima interface. Average IMT was the mean of computer-based points in the region, and maximum IMT was the IMT at a maximum point of the region.[17] Plaque was defined according to the Mannheim consensus,[18] in which a plaque was diagnosed when the vessel wall thickness was >1.5 mm or when the vessel wall appeared to be at least 0.5 mm or 50% thicker than the surrounding wall.

ABI was measured in the supine position after 5 min of rest. Systolic blood pressure of 4 limbs was measured simultaneously using a vascular screening device (VP-1000, Omron Healthcare, Kyoto, Japan). ABI was calculated by dividing the ankle systolic pressure by the brachial systolic pressure.

Fluorescence-Activated Cell Sorting

Immediately after blood sampling, 3-ml aliquots of whole heparinized blood specimens were mixed with 1× fluorescence-activated cell sorting (FACS) lysing solution and incubated for 10 min. Specimens were centrifuged for 10 min (2,000 rpm,

Table 1. Subject Characteristics vs. EPC Count

	EPC Quartiles				P value for trend†
	Low EPC count (n=18)	Medium-low EPC count (n=18)	Medium-high EPC count (n=18)	High EPC count (n=19)	
EPC count (CD 34⁺/133⁺/309⁺)					
Median	122	260	442	754	
Range	67–197	200–332	337–533	555–1,601	
Demographic variables					
Age (years)	58.9±7.2	52.8±7.7	55.2±6.9	56.2±7.7	0.974
Sex (%)					0.011*
Men	77.8	50	44.4	36.8	
Women	22.2	50	55.6	63.2	
Lifestyle and vascular risk variables					
Smoking (%)	31.3	25	11.8	11.1	0.092**
SBP (mm/Hg)	130.5±9.0	132.2±15.2	132.9±15.3	132.8±16.3	0.628
DBP (mm/Hg)	75.9±8.5	78.1±10.7	80.1±10.7	78.3±10.7	0.422
Body mass index (kg/m²)	24.7±3.4	25.1±4.2	25.4±3.0	25.4±1.8	0.491
Waist-to-hip ratio	0.91±0.06	0.89±0.04	0.93±0.05	0.91±0.06	0.402
DM duration (years)	8.6±6.8	9.3±7.8	6.2±6.0	8.2±5.2	0.585
Fasting glucose (mmol/L)	6.80±1.19	6.69±1.38	7.01±1.40	6.94±1.58	0.621
Post-prandial glucose (mmol/L)	12.87±3.12	12.62±3.45	12.05±4.45	12.26±3.47	0.555
HbA_{1c} (%)	7.3±0.7	6.8±1.0	6.8±0.7	7.1±0.7	0.626
GA (%)	17.4±3.0	15.8±3.3	15.5±3.3	16.9±2.5	0.61
GA/HbA_{1c}	2.40±0.32	2.32±0.33	2.26±0.35	2.38±0.28	0.761
TC (mmol/L)	4.14±0.60	4.40±0.89	4.54±1.17	4.36±0.86	0.447
LDL-C (mmol/L)	2.36±0.53	2.53±0.75	2.44±0.95	2.47±0.72	0.776
HDL-C (mmol/L)	1.07±0.24	1.17±0.28	1.21±0.21	1.18±0.25	0.192
Triglyceride (mmol/L)	1.52±0.47	1.31±0.59	1.98±1.15	1.75±0.79	0.119
HOMA-IR	6.64±13.18	5.98±9.46	3.87±3.38	3.62±3.06	0.226
HOMA-β (%)	210.1±425.7	183.0±408.9	75.0±52.8	86.4±91.6	0.148
IMT					
Mean IMT (mm)	0.85±0.12	0.68±0.14	0.74±0.12	0.74±0.14	0.086**
Maximum IMT (mm)	1.04±0.23	0.81±0.13	0.85±0.14	0.90±0.22	0.080**
Plaque formation (%)	100	58.8	64.7	61.1	0.025*

Data given as mean±SD or percentage. †ANOVA test for continuous variables or chi-square test for discrete variables. *P<0.05; **P<0.10. EPC, endothelial progenitor cell; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; HbA_{1c}, glycated hemoglobin; GA, glycated albumin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, HOMA of β-cell function; IMT, intima-media thickness.

4°C), and the supernatant was removed carefully. The cells were washed 3 times with PBS and 1.5 mmol/L EDTA, and then resuspended in 1 ml PBS and 1.5 mmol/L EDTA. A total of 2×10^6 cells was added to the respective FACS tubes and the tubes were incubated for 10 min at 4°C in the dark with blocking reagent (MACS, Berlin, Germany). Subsequently, the cells were stained with appropriate antibodies: anti-CD133 antibody conjugated with fluorescein isothiocyanate (Ancell, Bayport, MN, USA); anti-CD34 antibody conjugated with peridinin-chlorophyll proteins (BD Biosciences, San Jose, CA, USA); anti-CD309 antibody conjugated with phycoerythrin (BD Biosciences) or corresponding isotype controls, for 30 or 45 min at 4°C in the dark. After washing the cells with PBS containing 1.5 mmol/L EDTA, flow cytometry was carried out on a FACSCalibur (BD Biosciences). The acquisition goal was 1×10^6 events. If fewer events were detectable, results were processed statistically only if at least 5×10^5 events could be obtained. EPCs were expressed in absolute numbers per 10^6 white blood cells for comparison.

Statistical Analysis

All statistical analysis was performed with PASW (version 18.0; SPSS, Chicago, IL, USA). Continuous variables with a normal distribution are expressed as mean±SD, and variables with non-normal distributions ware expressed as median (interquartile range). Discrete variables are expressed as percentages. Statistical comparisons among groups according to EPC level were performed using the ANOVA or chi-square test. The Spearman correlation coefficient was used to determine the relationships between EPC level and the continuous variables. Statistical comparisons between groups with and without carotid artery plaque formation were performed using the independent t-test, Mann-Whitney U test, or chi-square test. Binary logistic regression analysis was used to estimate multiple correlations between carotid artery plaque formation and clinical and laboratory risk factors. Chi-square test was used in a comparison of carotid plaque formation according to the EPC and GA level. Data with P<0.05 were considered significant.

Table 2. Subject Characteristics vs. Presence of Carotid Artery Plaque

	Carotid artery plaque formation		P value†
	Yes (n=51)	No (n=22)	
Demographic variables			
Age (years)	57.8±7.1	52.3±8.0	0.006*
Sex (%)			0.301
Men	59.2	45	
Women	40.8	55	
Life-style and vascular risk variables			
Smoking (%)	20	19.1	0.995
SBP (mm/Hg)	132.1±13.9	131.5±12.6	0.859
DBP (mm/Hg)	77.5±10.1	79.7±10.1	0.408
Body mass index (kg/m^2)	24.9±3.3	25.7±2.7	0.361
Waist-to-hip ratio	0.91±0.05	0.90±0.05	0.787
DM duration (years)	7.9±5.8	8.6±7.9	0.697
Fasting glucose (mmol/L)	6.86±1.38	6.86±1.37	0.995
Post-prandial glucose (mmol/L)	12.32±3.57	12.75±3.73	0.658
HbA_{1c} (%)	7.0±0.8	6.9±0.6	0.409
GA (%)	16.8±3.2	15.5±2.7	0.091**
GA/HbA_{1c}	2.38±0.34	2.24±0.25	0.067**
TC (mmol/L)	4.41±0.80	4.26±1.12	0.565
LDL-C (mmol/L)	2.47±0.64	2.41±0.97	0.798
HDL-C (mmol/L)	1.16±0.24	1.16±0.26	0.943
Triglyceride (mmol/L)	1.60±0.82	1.74±0.83	0.132
EPC count			
CD 34$^+$/133$^+$/309$^+$	279 (354)	412 (349)	0.027*
IMT			
Mean IMT (mm)	0.79±0.13	0.64±0.12	<0.001*
Maximum IMT (mm)	0.95±0.20	0.76±0.10	<0.001*

Data given as mean±SD, median (interquartile range), or percentage.
†Independent t-test for continuous variables except for EPC count, Mann-Whitney U-test for EPC count, or chi-square test for discrete variables. *P<0.05; **P<0.10.
Abbreviations as in Table 1.

Results

Subject Characteristics

A total of 73 subjects (38 men and 35 women; mean age, 56.2±7.7 years) were finally enrolled in this study. The mean duration of diabetes was 8.1±6.5 years. The levels of serum HbA_{1c} and GA were 7.0±0.8% and 16.4±3.1%, respectively. The mean body mass index (BMI) was 25.2±3.1 kg/m^2.

Association of EPC Level With Cardiovascular Risk Factors and Other Variables

We classified the subjects into 4 groups based on quartiles of the EPC count: the low EPC group, the medium-low EPC group, the medium-high EPC group, and the high EPC group. We compared clinical and laboratory characteristics among these groups focusing on glucose metabolism and cardiovascular risk variables (Table 1). In the groups with higher EPC count, the higher proportion of female patients and the lower proportion of patients with smoking history were found (P for trends=0.011 and 0.091, respectively). The mean and maximum IMT in the carotid artery tended to increase in the groups with lower EPC count (P for trend=0.086 and 0.080, respectively), and carotid artery plaque formation significantly increased in the groups with lower EPC count (P for trend=0.025).

Clinical and Laboratory Data According to Carotid Artery Plaque Formation

We compared clinical and laboratory data including EPC count according to carotid artery plaque formation as measured on ultrasonography (Table 2). Compared to subjects without carotid artery plaques, subjects with plaques were significantly older (P=0.006) and had decreased EPC count (P=0.027). Sex, smoking, BMI, WHR, blood pressure, plasma lipid profile, and medication (data not shown) were not significantly different between the 2 groups. Interestingly, although hyperglycemia parameters including fasting and post-prandial plasma glucose level, and serum HbA_{1c} level were not significantly different between the 2 groups, serum GA level and the GA/HbA_{1c} ratio tended to be higher in the patients with carotid artery plaque (P=0.091 and 0.067, respectively).

Factors Associated With Carotid Artery Plaque Formation

For binary logistic regression analysis, carotid artery plaque formation was used as a dependent variable, and age, duration of diabetes, systolic blood pressure, plasma LDL-C level, EPC count, and glycemic indices were entered as independent variables (Table 3). In this analysis, we used 3 statistical models with different independent variables. We adopted serum HbA_{1c} or GA as the glycemic index in models 1 and 2, respectively. To avoid confounding factors and to investigate the effects of serum GA and HbA_{1c} on plaque formation using a single statistical model, we included the GA/HbA_{1c} ratio and HbA_{1c} as

Table 3. Logistic Regression for Factors Associated With Carotid Artery Plaque Formation

	Model 1			Model 2			Model 3		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Age	1.135	1.036–1.244	0.006*	1.141	1.036–1.258	0.008*	1.147	1.018–1.207	0.008*
DM duration	0.949	0.860–1.047	0.293	0.934	0.847–1.029	0.167	0.939	0.848–1.040	0.224
SBP	1.011	0.965–1.060	0.641	1.008	0.959–1.059	0.766	1.007	0.956–1.059	0.802
LDL-C	0.782	0.340–1.796	0.562	0.667	0.283–1.570	0.354	0.675	0.284–1.604	0.373
EPC count	0.998	0.997–1.000	0.032*	0.998	0.997–1.000	0.041*	0.998	0.996–1.000	0.047*
HbA_{1c}	1.515	0.621–3.694	0.361				1.373	0.536–3.522	0.509
GA				1.283	1.004–1.640	0.046*			
GA/HbA_{1c}							1.024	1.002–1.048	0.040*

*P<0.05. The dependent variable was the formation of carotid artery plaques measured on ultrasonography in all statistical models. Vascular risk factors including the EPC count were entered as independent variables in all models. HbA_{1c} was included as an independent variable in models 1 and 3. GA was included as an independent variable in model 2. GA/HbA_{1c} was included as an independent variable in model 3. OR, odds ratio; CI, confidence interval. Other abbreviations as in Table 1.

independent variables in model 3. Old age and a low EPC count were independently correlated with plaque formation in all models (age, P=0.006, 0.008, and 0.008, models 1, 2, and 3, respectively; EPC count, P=0.032, 0.041, and 0.047, respectively). Of the glycemic indices, a high GA level and a high GA/HbA_{1c} ratio were correlated with carotid plaque presence after multivariate adjustment for other risk factors in models 2 and 3 (GA, P=0.046, model 2; GA/HbA_{1c}, P=0.040, model 3). **Figure** shows the distribution of subjects with and without plaque formation according to the GA/HbA_{1c} ratio and the EPC count. Most subjects with a lower EPC count and a higher GA/HbA_{1c} ratio than the medians of the respective parameters had carotid artery plaques (percentage of patients with plaque, high GA/HbA_{1c} and low EPCs vs. low GA/HbA_{1c} and high EPCs, 46.0% vs. 18.8%, P=0.077).

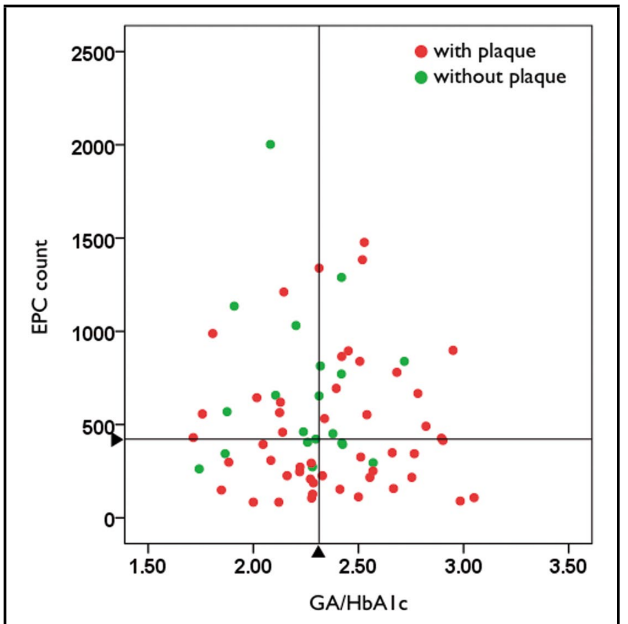

Figure. Presence of carotid artery plaque formation vs. serum glycated albumin (GA)/glycated hemoglobin (HbA_{1c}) and endothelial progenitor cell (EPC) level. Carotid artery plaque formation was assessed on ultrasonography. ▶, median.

Discussion

Fadini et al suggested that EPC level in diabetic subjects had biphasic trends in the development of atherosclerosis.[19] Reduced number and dysfunctional EPCs, including impaired proliferation, adhesion, and attachment, have been shown to occur in insulin resistance[20] and at an early stage in the pathogenesis of atherosclerosis.[21] When plaque complications occur, the EPC level is known to increase because of its mobilization to overcome the obstruction.[19] Furthermore, an abrupt surge in circulating EPCs was reported in myocardial infarction, unstable angina and direct vascular injury.[16] Recently, we found that significantly increased EPC count was associated with revascularization in asymptomatic patients with type 2 diabetes.[22] Based on previous research, we hypothesized that decreased EPC count would be associated with early-stage atherosclerosis and investigated the factors predicting atherosclerotic change using carotid artery ultrasonography in asymptomatic type 2 diabetic patients without documented diabetic vascular complications. To do this, we ensured that the group of asymptomatic type 2 diabetic subjects was homogeneous by excluding diabetic microvascular disease and cardiac or peripheral ischemic disease using cardiac MRI and ABI measurements.

In the present subjects, type 2 diabetic patients without documented ischemic disease, EPC count was associated with sex, and tended to be associated with smoking history. A previous study with middle-aged adults (between the ages of 45 and 65 years) reported no sex difference in EPC number.[23] In another study with fertile (mean age, 41.2±0.7 years) and postmenopausal (mean age, 57.2±1.0 years) women, EPCs were mobilized cyclically and EPC level was higher than in men only in fertile women.[24] In the present study, female subjects were between the ages of 42 and 70 years and the mean age was 57.7±7.9 years. In addition, all of the patients with smoking history were male. In this regard, there is a possibility that the sex difference of EPC number in the present subjects is due to the association between smoking and EPC count. The opposite interpretation, however, is also possible because the association between smoking and EPC count is still controversial,[6,11] and was not statistically significant in the present study. Other CVD risk factors such as age, duration of diabetes, blood pressure, plasma lipid profile and serum HbA_{1c} or GA showed no association with EPCs. Although the associations between EPC level and other known CVD risk factors are controversial,[4–13] the present results suggest that EPC level is not a byproduct of established risk factors. We analyzed the medications taken by

the subjects. Although the dipeptidyl peptidase-4 (DPP4) inhibitor, sitagliptin, has been reported to increase the EPC count,[25] and HMG-CoA reductase inhibitors have been reported to increase the EPC count and improve EPC function,[26,27] DPP4 inhibitor or statin use were not associated with EPC count in this study (data not shown).

We compared EPCs and other CVD risk factors according to carotid artery plaque formation. The patients with plaques were older and had lower EPC count; interestingly, serum GA level and the GA/HbA_{1c} ratio tended to increase in subjects with plaques. This association between serum GA level and carotid artery plaque formation was maintained after multivariate adjustment. Binary logistic regression analysis for the factors that affect carotid artery plaque formation showed that old age, a reduced EPC count, and an increased serum GA level or GA/HbA_{1c} ratio were independently correlated with plaque formation. When we analyzed the data using serum HbA_{1c} as a glycemic parameter rather than serum GA, HbA_{1c} did not have an independent correlation with plaque formation. Serum GA has been reported to be a useful and rapid hyperglycemic indicator for glucose fluctuation and excursion because the turnover of serum albumin is much shorter than that of hemoglobin and the glycation reaction of albumin occurs more rapidly than the glycation of hemoglobin.[28,29] Recently, GA was shown to be a precursor of advanced glycation end-products, and a number of studies have demonstrated that GA is associated with enhanced oxidative stress and endothelial injury.[30–33] These previous studies and the present results suggest that GA might be a parameter of oxidative stress or endothelial injury beyond glycemic index of glucose excursion. Altogether, in the present subjects, old age, a reduced EPC count indicating decreased endothelial repair capacity, and an increased serum GA level indicating increased endothelial injury independently affected plaque formation in the carotid artery. These results are consistent with the pathophysiology of atherogenesis resulting from vascular endothelial damage that is not repaired by protective mechanisms.[34] The lack of association between other CVD risk factors and carotid plaque formation could be due to the subject characteristics. In the present subjects, blood pressure and serum LDL-C level were not high enough to affect atherosclerotic plaque formation. We studied type 2 diabetic patients and excluded those with documented ischemic disease using cardiac MRI and ABI measurements to avoid the influence of ischemia-induced neo-vascularization on EPC count. In the relatively homogeneous, well-controlled (mean HbA_{1c}, 7.0±0.8%) and early stage (mean duration of diabetes, 8.1±6.5 years) diabetic patients, more direct parameters of vascular endothelial status, such as EPC or GA level might be more useful parameters to predict plaque formation than other indirect risk factors such as blood pressure or plasma lipid profile.

This study has some important clinical implications. Considering the importance of primary prevention of CVD in type 2 diabetes, type 2 diabetic patients without documented ischemic disease, should be the main target of efforts to predict CVD in T2DM. In these patients, we showed that EPC level is a highly reliable biologic marker of atherosclerotic plaque formation. In addition, the present results suggest that serum GA level can be used as a marker of vascular damage. Moreover, the measurement of both GA and EPC level could help predict CVD more precisely; new parameters or equations to predict CVD using EPC level and serum GA should be developed in future studies with a larger subject group.

The present study had some challenging limitations. First, even though we conducted a prospective study with a homogeneous subject group, the subjects consisted of only 90 patients, and the study was performed at a single center. To obtain statistical significance, we analyzed the male and female patients together. Future studies including a large subject group and analysis for the gender difference are needed. Second, we measured circulating EPCs only once on flow cytometry. Although there was no inter-observer variability because EPC count was measured by 1 person, we could not demonstrate intra-observer variability for the analysis of EPC count. Previous studies that investigated EPC level generally used the absolute EPC count as measured on flow cytometry or the number of EPC colony-forming units (CFU) as measured on colony-forming cell assay.[10–12,35,36] We did not evaluate EPC-CFU, which reflects the functional capacity of circulating EPCs. EPC count in age-matched, non-diabetic controls without documented vascular complications was not evaluated in this study. In addition, although we used the most widely used definition for EPC (cells co-expressing the surface antigens CD34, CD133, and vascular endothelial growth factor 2 (VEGFR-2 or CD309)[16,37–40]), conflict still remains as to the accurate definition of EPCs.

In conclusion, old age, a decreased EPC count, and an increased serum GA level were correlated with carotid artery plaque formation after multivariate adjustment for other CVD risk factors in type 2 diabetic patients without documented ischemic disease. On the basis of these results, we postulate that EPC count and serum GA level can be biologic markers indicating endothelial protection and damage to predict atherosclerosis and CVD in the early stage in diabetic patients. In addition, the present results suggest that increased serum GA level rather than HbA_{1c} might be used as a marker of vascular damage beyond glucotoxicity itself on vascular cells. New parameters or equations to predict CVD adopting EPC level and serum GA should be developed in future studies with larger subject groups.

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