Platelet Count Is Independently Associated With Insulin Resistance in Non-obese Japanese Type 2 Diabetic Patients

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The aim of the present study was to investigate the relationship between platelet count and insulin resistance in non-obese Japanese type 2 diabetic patients. A total of 163 non-obese Japanese type 2 diabetic patients (112 men and 51 women, aged 36 to 84 years, body mass index [BMI] 16.2 to 26.9 kg/m^2) were studied. In conjunction with BMI, glycosylated hemoglobin (HbA_{1c}), fasting concentrations of plasma glucose and serum lipids (triglycerides, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, and total cholesterol), and hematological parameters (platelets, white blood cell count, red blood cell count, hematocrit, hemoglobin) were measured. LDL cholesterol was calculated using the Friedewald formula. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment (HOMA-IR). Univariate regression analysis showed that HOMA-IR was positively correlated to BMI ($r = 0.465$, $P < .0001$), HbA_{1c} ($r = 0.423$, $P < .0001$), platelet count ($r = 0.310$, $P < .0001$), triglycerides ($r = 0.277$, $P < .0005$), white blood cell count ($r = .222$, $P = .005$), red blood cell count ($r = 0.210$, $P = .008$), hematocrit ($r = 0.156$, $P = .047$), total cholesterol ($r = 0.178$, $P = .023$), and systolic ($r = 0.216$, $P = .011$) and diastolic ($r = 0.263$, $P = .002$) blood pressure, and inversely correlated to HDL cholesterol ($r = -0.312$, $P < .0001$) level in our diabetic patients. Multiple regression analysis showed that HOMA-IR was independently predicted by BMI ($P < .0001$, $F = 22.45$), HbA_{1c} ($P < .0001$, $F = 16.15$), platelet count ($P < .0001$, $F = 10.75$), and serum triglycerides ($P < .0001$, $F = 10.47$) levels, which explained 34% of the variability of HOMA-IR in non-obese Japanese type 2 diabetic patients. These results indicate that not only BMI, HbA_{1c}, and triglycerides levels but also platelet counts are independent predictor of insulin resistance in non-obese Japanese type 2 diabetic patients.

THE MAJOR clinical consequence of type 2 diabetes is mortality and morbidity from atherosclerotic vascular disease. Bierman[1] has previously estimated that typical risk factors, including smoking, cholesterol level, and blood pressure, can account for no more than 25% to 30% of excess cardiovascular risk factor in diabetic patients. This suggests that other factors might play a key role in the progression of atherosclerosis in diabetes.

Insulin resistance is established to be one of the risk factors for the coronary heart disease events.[2] There are some data suggesting that hematologic abnormalities are associated with atherosclerosis in humans.[3-6] High red blood cell count is known to be a strong independent predictor of acute cardiovascular events such as stroke and myocardial infarction. Endothelial injury or plaque rupture with platelet adhesion and aggregation at the site of injury may be the crucial event in producing morbidity and mortality from atherosclerosis.

To the best of our knowledge, the relationship between hematological parameters and the degree of insulin resistance has not been fully clarified in type 2 diabetic patients. One problem is that the degree of weight excess and of hyperglycemia per se effects hematological parameters and insulin resistance in man. To overcome this difficulty, we recruited non-obese well-controlled unique Japanese type 2 diabetic patients who had no evidence of cardiovascular disease, ischemic stroke, or chronic renal failure and investigated the relationships between hematologic parameters and the degree of insulin resistance.

SUBJECTS AND METHODS

One hundred sixty-three non-obese Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. Type 2 diabetes mellitus was diagnosed based on the World Health Organization (WHO) criteria.[7] The subjects had no evidence of current acute illness or infectious process. The duration of diabetes was 11.6 ± 0.7 years (mean ± SEM). One hundred eight of 163 diabetic patients were taking sulfonylureas (gliclazide) and the rest were controlled with diet alone. None had received insulin therapy. All subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease. They did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with glucose oxidase method. Triglycerides, and total and high-density lipoprotein (HDL) cholesterol were measured. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[8] Platelets, white blood cell count, red blood cell count, hematocrit, and hemoglobin were also measured.

The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/L)/22.5.[9] One might argue that the use of sulfonylureas in patients with diabetes might significantly affect the estimate of insulin resistance by HOMA, as these drugs are known to decrease fasting plasma glucose without substantially changing fasting plasma insulin.[10] Bonora et al[11] and Emoto et al,[12] however, confirmed that in the validation studies of HOMA, the

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Table 1. Clinical Characteristics in Insulin-Resistant and Insulin-Sensitive Diabetic Patients

	Insulin-Resistant	Insulin-Sensitive	*P*
No. of subjects	66	97	
Male/female	46/20	66/31	.412
HOMA-IR	3.9 ± 0.2	1.6 ± 0.1	<.001
Age (yr)	60.4 ± 1.2	61.6 ± 1.0	.214
Diabetes duration (yr)	12.1 ± 1.1	11.2 ± 0.8	.299
BMI (kg/m^2)	23.7 ± 0.2	21.8 ± 0.3	<.001
HbA_{1c} (%)	7.6 ± 0.1	6.7 ± 0.1	<.001
Fasting glucose (mg/dL)	162 ± 4	132 ± 3	<.001
Fasting insulin (μU/mL)	9.7 ± 0.5	4.9 ± 0.2	<.001
Triglycerides (mg/dL)	164 ± 28	110 ± 6	.013
Total cholesterol (mg/dL)	210 ± 4	195 ± 4	.005
LDL cholesterol (mg/dL)	129 ± 6	115 ± 3	.013
HDL cholesterol (mg/dL)	48 ± 1	58 ± 2	<.001
Red blood cell count ($\times 10^4/\mu$L)	453 ± 5	438 ± 5	.016
White blood cell count (/μL)	5872 ± 154	5511 ± 140	.047
Platelets ($\times 10^4/\mu$L)	22.3 ± 0.6	18.8 ± 0.5	<.001
Hemoglobin (g/dL)	14.0 ± 0.1	13.8 ± 0.2	.124
Hematocrit (%)	41.5 ± 0.4	40.8 ± 0.4	.134

correlation of insulin sensitivity estimated by such method and that measured by the glucose clamp was not substantially different in diet-treated and sulfonylurea-treated type 2 diabetes.

Statistical Analysis

Data were presented as means ± SEM. Statistical analyses were conducted using the StatView 5 system (Statview, Berkeley, CA). Simple (Spearman's rank) correlation coefficient and stepwise multiple regression analyses were used to examine the relationships between HOMA-IR and body mass index (BMI), triglycerides, or the measures of variables including platelet count. $P < .05$ was considered as significant. In multivariate analysis, an F value ≥ 4 was considered significant.

RESULTS

The subjects studied were all Japanese type 2 diabetic patients (112 men and 51 women) with an age range of 36 to 84 years (61.1 ± 0.8 years) and a BMI of 16.2 to 26.9 kg/m^2 (22.6 ± 0.2 kg.m^2). They all were non-obese.[13] The mean fasting plasma glucose was 144 ± 3 mg/dL and glycosylated hemoglobin (HbA_{1c}) was 7.1% ± 0.1%. Fasting insulin level was 6.8 ± 0.3 μU/mL. Serum triglycerides, and total and HDL cholesterol levels were 132 ± 12 mg/dL, 201 ± 3 mg/dL, and 54 ± 1 mg/dL, respectively. Platelet, white blood cell, and red blood cell counts were 20.2 ± 0.4 × $10^4/\mu$L, 5,658 ± 107/μL, and 444 ± 3 × $10^4/\mu$L, respectively. Hematocrit and hemoglobin concentrations were 41.1% ± 0.3% and 13.9 ± 0.1 g/dL, respectively. There was a wide variation in insulin resistance calculated from HOMA-IR in our diabetic patients (range, 0.54 to 12.74; 2.49 ± 0.13). Sixty-six of 163 (40%) patients had HOMA-IR greater than 2.5, indicating they were insulin-resistant.[14]

Table 1 shows the clinical profile of insulin-resistant and insulin-sensitive type 2 diabetic patients. Compared with insulin-sensitive patients, insulin-resistant patients had significantly higher BMI levels and of HbA_{1c}, triglycerides, total and LDL cholesterol, red blood cell count, white blood cell count, and platelets. HDL cholesterol concentration was significantly lower in insulin-resistant group than in insulin-sensitive group. No significant difference was observed in age, gender, hemoglobin, or hematocrit levels between the 2 groups.

Table 2 illustrates the correlation between HOMA-IR and BMI or the measures of variables including platelet count and serum triglycerides in our diabetic patients. HOMA-IR was positively correlated with BMI ($r = 0.465$, $P < .0001$), hemoglobin A_{1c} ($r = 0.423$, $P < .0001$), platelets ($r = 0.310$, $P < .0001$), triglycerides ($r = 0.277$, $P < .0005$), total ($r = 0.178$, $P = .023$) and LDL ($r = 0.250$, $P = .002$) cholesterol, white blood cell count ($r = 0.222$, $P = .005$), red blood cell count ($r = 0.210$, $P = .008$), hematocrit ($r = 0.156$, $P = .047$), and systolic ($r = 0.216$, $P = .011$) and diastolic ($r = 0.263$, $P = .002$) blood pressure. In contrast, HOMA-IR value was negatively correlated with HDL cholesterol level ($r = -0.312$, $P < .0001$). There was no relationship between HOMA-IR and age, gender, duration of diabetes, or hemoglobin.

Multiple regression analyses were performed using the stepwise procedure in all diabetic patients. The analysis included HOMA-IR as dependent variable and candidate risk factors (BMI, HbA_{1c}, plateleta, triglycerides, total, LDL, and HDL cholesterol, white blood cell count, red blood cell count, hematocrit, systolic and diastolic blood pressure) as independent variables. HOMA-IR was statistically predicted by BMI ($P < .0001$, $F = 22.45$), HbA_{1c} ($P < 0.0001$, $F = 16.15$), platelet count ($P < .0001$, $F = 10.75$), and serum triglycerides ($P < .0001$, $F = 10.47$) levels, which explained 34% of the variability of HOMA-IR in our diabetic patients. Other variables, including red blood cell count, were not associated with HOMA-IR in our non-obese Japanese type 2 diabetic patients (Table 2).

DISCUSSION

Our study demonstrated that platelet counts are a statistically predictor of insulin resistance in non-obese Japanese type 2 diabetic patients. Our patients studied were unique in that they

Table 2. Correlation of Insulin Resistance to Measures of Variables in Diabetic Patients

	Univariate		
	r	*P*	Multivariate *F*
BMI	0.465	<.0001	22.45
HbA_{1c}	0.423	<.0001	16.15
Platelets	0.310	<.0001	10.75
Triglycerides	0.277	<.0005	10.47
Total cholesterol	0.178	.023	—
LDL cholesterol	0.250	.002	—
White blood cell count	0.222	.005	—
Red blood cell count	0.210	.008	—
Hematocrit	0.156	.047	—
Systolic blood pressure	0.216	.011	—
Diastolic blood pressure	0.263	.002	—
HDL cholesterol	−0.312	<.0001	—
Age	−0.137	.082	—
Gender	−0.006	.940	—
Diabetes duration	0.049	.583	—
Hemoglobin	0.144	.066	—

were non-obese well-controlled in terms of glucose tolerance (mean HbA_{1c}, 7.1%) and blood pressure (mean, 129/75 mm Hg). They all have received neither insulin therapy nor any medications affecting platelet function such as aspirin and dipyridamole.

When compared with Caucasian populations, non-obese Japanese type 2 diabetic patients are unique in that they are divided into 2 variants: one with insulin resistance and the other with normal insulin sensitivity.[15,16] In the present study, only 40% of the patients had insulin resistance by homeostasis model assessment, consistent with our previous study.[17] The patients with insulin resistance are characterized by higher concentrations of C-reactive protein (CRP), triglycerides, and lower concentrations of HDL cholesterol as compared to the non–insulin-resistant group.[14,17,18] Both CRP and dyslipidemia are postulated to be atherogenic. Thus, insulin resistance seems to be associated with atherosclerosis in non-obese Japanese type 2 diabetic patients.

There are some data suggesting that hematologic parameters are associated with atherosclerosis in humans.[3-6] High red blood cell counts are known to be a strong independent predictor of acute cardiovascular events such as stroke and myocardial infarction. Endothelial injury or plaque rupture with platelet adhesion and aggregation at the site of injury may be the crucial event in producing morbidity and mortality from atherosclerosis. To the best of our knowledge, however, the relationship between hematologic parameters and insulin resistance has not yet been fully investigated in type 2 diabetic patients.

By univariate analysis, we found a positive correlation among platelet, red blood cell, and white blood cell counts and insulin resistance; by multivariate analysis, we found that both red blood cell and white blood cell counts are no longer independent factors of insulin resistance. Thus, platelet count per se seems to be associated with insulin resistance in non-obese Japanese type 2 diabetic patients. This finding is not in agreement with the data shown by Barbieri et al[4] that increased red blood cell count is an aspect of insulin resistance syndrome in nondiabetic subjects. The reason for the discrepancy is currently unknown, but it may be due to the different clinical characteristics studied. Our present finding is very interesting since platelets retain a functional insulin receptor capable of insulin binding and autophosphorylation and since insulin is thought to reduce platelet sensitivity to aggregating agents such as adenosine diphosphate (ADP).[19-21]

The mechanism by which increased platelet count is associated with insulin resistance in non-obese Japanese type 2 diabetic patients is not known at present. This relationship has not been reported in nondiabetic subjects.[4] Compared with nondiabetic subjects, diabetic patients are known to have both endothelial damage and platelet dysfunction.[6]

In conclusion, we first showed in vivo a relationship between platelet count and insulin resistance in non-obese Japanese type 2 diabetic patients. Further study should determine whether improvement in insulin resistance in this population is associated with a decreased platelet count.

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