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Immature platelet fraction in diabetes mellitus and metabolic syndrome

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ABSTRACT

Introduction: Dysregulated platelet-endothelial interaction plays a pivotal role in atherothrombotic events in patients with diabetes mellitus (DM). Immature platelet fraction (IPF) is a hematologic parameter of automated hematologic analyzer and is related to platelet size and cytoplasmic RNA contents. It reflects thrombopoiesis and also is often used as the marker of platelet activity.
Material and Methods: We compared peripheral blood IPF, IPF count (IPC), and mean platelet volume (MPV) of DM and metabolic syndrome (MetS) patients with those of healthy controls. The IPF, IPC, MPV, and other blood cell indices were measured.
Results: The DM group had significantly higher IPF (2.20 vs. 1.70%, $P = .020$), IPC (4.80 vs. 4.60 $\times 10^9$/L, $P = .043$), and MPV (10.35 vs. 10.00 fL, $P = .012$) than the control group. Those markers were also increased in MetS patients, but the differences were not statistically significant. Interestingly, when DM patients were stratified according to glycemic control status ($\leq$6.5% HbA1c vs. 6.6–7.9% HbA1c vs. $\geq$8% HbA1c), both IPF and IPC were significantly increased in poor glycemic control group ($P = .014$ and .003). Including various diabetic complications in the analysis, IPF was higher in DM patients complicated by cardiovascular disease than the DM group without cardiovascular disease.
Conclusion: IPF is elevated in patients with diabetes and associated with poor glycemic control and cardiovascular complication.

Introduction

Atherothrombosis is a common complication of diabetes mellitus (DM). Patients with type 2 DM are at two- to four-fold higher risk of coronary artery disease and stroke [1]. Metabolic derangement in DM makes arteries susceptible to atherosclerosis by causing altered functional properties of platelets [2,3]. One mechanism is hyperglycemia and/or insulin resistance enhancing thromboxane biosynthesis and calcium mobilization resulting in platelet hyperactivity [4–6]. Mean platelet volume (MPV), a laboratory marker of platelet activity was shown to be increased in DM patients [7,8]. Higher efficacy of antiplatelet therapy in preventing cardiovascular diseases (CVD) can reasonably be anticipated for patients with DM because their overall platelet activities are higher compared to non-DM population. However, on the contrary, these patients show diminished effect of antiplatelet therapy, and the residual platelet activity of DM patients with coronary heart disease on antiplatelet therapy is higher than that of the non-DM patients [9,10]. The blunted effect of antiplatelet therapy in DM patients can be related to platelet turnover [11]. Hyperactive platelets of DM patients were found to respond to sub-threshold stimuli and thus consumed easily, stimulating thrombopoiesis [12]. Thrombopoiesis or platelet turnover can directly be examined by measuring reticulated platelet count using flow cytometry [13]. Immature platelet fraction (IPF) measured by automated hematology analyzer is now popular in use as a marker of thrombopoiesis as well as platelet activity. In this first correlation study, we compared IPF and MPV of DM patients with those of healthy controls. We also examined associations of IPF with various complications of DM and glycemic control status. Additionally, we evaluated IPF of metabolic syndrome (MetS) patients, with increased risk of CVD and diabetes.

Materials and Methods

Patients

A total of 396 patients, who were diagnosed with DM (n = 366) or MetS (n = 30), were included in this study. The control subjects (n = 54) were selected among those who visited Health Promotion Center for routine physical check-ups and had no laboratory abnormality related to DM or MetS. Diagnosis of DM was established by one of following criteria: random glucose >200 mg/dl; fasting blood glucose

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≥126 mg/dl; HbA1c ≥6.5%; and, presence of treatment with insulin or oral hypoglycemic agents. MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III [14] except for the waist circumference. Criterion for waist circumference complied with International Diabetes Federation [15] considering ethnicity: waist circumference >90 cm in men and >80 cm in women; triglycerides ≥150 mg/dl; HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women; blood pressure ≥130/85 mmHg; and, fasting glucose ≥110 mg/dl. MetS patients with DM were excluded in MetS group. The DM patients were separated into three groups according to the degree of glycemic control: strict glycemic control group (HbA1c ≤6.5%); intermediate glycemic control group (6.6-7.9%); and, poor glycemic control group (≥8.0%). The presence of diabetic complication was reviewed from medical records. This study was approved by the Institutional Review Board of Severance hospital, Seoul, Korea, and waiver of informed consent was requested and provided.

Measurement of IPF and MPV

K_2EDTA whole blood was used for measurement of IPF and MPV. They were tested within 2 hours after collection. The IPF, MPV, and other blood cell indices were measured using Sysmex XE-2100 (Sysmex, Kobe, Japan). The result of IPF measurement was displayed as IPF and IPF count (IPC). IPF is proportion of immature platelets within the total number of platelets, and IPC is actual number of immature platelets per unit volume (IPF x platelet count). All tests were performed according to the standardized protocols recommended by the manufacturers.

Statistical Analysis

All statistical analyses were performed using PASW statistics 18.0 (formerly SPSS Statistics, SPSS Inc., Chicago, IL, USA) or Analyse-it software Method Evaluation Edition version 2.22 (Analyse-it Software Ltd., City West Business Park, Leeds, UK). Clinical characteristics between the study groups were compared using the Kruskal-Wallis test with Bonferroni correction for continuous variables and Chi-square test for categorical variables. The accordance of IPF/IPC and MPV were evaluated by Spearman correlation analysis. The IPF, IPC, and MPV of DM or MetS were compared with the control group using the Mann–Whitney U test. Logistic regression was performed with the presence of DM or MetS as the binary dependent variables, and meaningful subjects' characteristics (age of patients) and each markers as the predictor variables. The differences of IPF, IPC, and MPV according to the presence of diabetic complications were assessed via the Mann–Whitney U test. The Kruskal-Wallis test with Bonferroni correction was used for comparison of group of DM patients with different HbA1c levels. A *P*-value of less than 0.05 was regarded as statistically significant.

Results

IPF, IPC, and MPV in DM and MetS

Among the 450 individuals included in this study, 366 were DM patients, 30 were MetS patients, and 54 were healthy controls. Table 1 summarizes demographic and clinical characteristics of patients (DM and MetS) and controls. Clinical parameters excluding age were not significantly different between the groups. IPF correlated well with MPV ($r = 0.83$, $P < .001$). IPC also correlated MPV ($r = 0.69$, $P < .001$), but the correlation coefficient were lower (Fig. 1). DM group showed significantly higher IPF ($P = .007$) and MPV ($P = <.001$) than controls (Table 2). Among other patient factors, only age was meaningful (odds ratio = 1.2, $P < .001$) and included in logistic regression analysis along with IPF, IPC, and MPV. IPF, IPC, and MPV were all still associated with the presence of DM by logistic regression analysis (odds ratio = 1.9, 1.2 and 2.1, $P = .020$, .043 and .012, respectively). Patients with MetS also showed higher IPF ($P = .015$) and MPV ($P = .007$) compared with control group (Table 3), but the increases were not significant by logistic regression analysis with adjusted age. The differences in IPF, IPC, and MPV between DM and MetS groups were not significant ($P > .05$).

IPF, IPC, and MPV According to the Glycemic Controls and/or Complications

Poor glycemic control group based on HbA1c value showed significantly higher values of IPF (2.20 vs. 2.10 vs. 2.55, $P = .014$) and IPC (4.60 vs. 4.60 vs. 6.10, $P = .003$) (Table 4). MPV was also higher in poor glycemic control group but the difference did not reach statistical significance (10.40 vs. 10.30 vs. 10.50, $P = .163$). There was no difference in IPF and IPC between the patients with strict glycemic control and patients with intermediate glycemic control. IPF, IPC, and MPV in the presence or absence of several diabetic complications are summarized in Table 5. There were small but significant difference in IPF between DM patients with and without CVD ($P = .038$). The median IPF in DM patients with or without a history of CVD was 2.30% (1st and 3rd quartiles: 1.62 and 3.67%) and 2.10% (1st and 3rd quartiles: 1.40 and 3.00%), respectively. However, IPC and MPV were not different between these groups. As for other complications including neuropathy, retinopathy, and nephropathy, no significant differences were found among the three parameters.

Discussion

Platelet hyperactivity in DM is an important contributor to the development of cardiovascular complications. Several mechanisms have been found to be involved in platelet hyperactivity pertaining to DM.

Table 1
Summary of clinical characteristics according to the study groups.

	DM	(n = 366)	MetS	(n = 30)	Control	(n = 54)	*P* value
Age, mean (SD) (years)	63.0	(12.2)	60.7	(9.9)	32.4	(10.5)	<.001[a]
Male, n (%)	201	(54.9)	10	(33.3)	27	(50.0)	.063[b]
Platelet count, mean (SD) ($\times 10^9$/L)	235.9	(68.0)	224.7	(62.7)	253.4	(51.4)	.085[c]
Complication							
CVD, n (%)	103	(28.1)					
Neuropathy, n (%)	44	(12.0)					
Retinopathy, n (%)	43	(11.7)					
Nephropathy, n (%)	38	(10.4)					

Abbreviations: CVD, cardiovascular disease; DM, diabetes mellitus; MetS, metabolic syndrome.

[a] Calculated by Kruskal-Wallis tests with Bonferroni correction, $P = <.001$ is value for comparison among all subject groups, $P = <.001$ for DM versus control, $P = <.001$ for MetS versus control, and $P = .525$ for DM versus MetS.

[b] Calculated by Chi-square test, $P = <.063$ is value for comparison among all subject groups, $P = .498$ for DM versus control, $P = .140$ for MetS versus control, and $P = .023$ for DM versus MetS.

[c] Calculated by Kruskal-Wallis tests with Bonferroni correction, $P = .853$ is value for comparison among all subject groups, $P = .095$ for DM versus control, $P = .290$ for MetS versus control, and $P = 1.000$ for DM versus MetS.