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Original Article

Hypocholesterolemia in Patients with Polycythemia Vera

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Polycythemia vera (PV) is characterized by low serum total cholesterol despite its association with vascular events such as myocardial and cerebral infarction. Serum cholesterol level has not been used as a diagnostic criterion for PV since the 2008 revision of the WHO classification. Therefore, we revisited the relationship between serum lipid profile, including total cholesterol level, and erythrocytosis. The medical records of 34 erythrocytosis patients (hemoglobin : men, > 18.5 g/dL ; women, > 16.5 g/dL) collected between August 2005 and December 2011 were reviewed for age, gender, and lipid profiles. The diagnoses of PV and non-PV erythrocytosis were confirmed and the *in vitro* efflux of cholesterol into plasma in whole blood examined. The serum levels of total cholesterol, low-density-lipoprotein cholesterol (LDL-Ch), and apolipoproteins A1 and B were lower in PV than in non-PV patients. The *in vitro* release of cholesterol into the plasma was greater in PV patients than in non-PV and non-polycythemic subjects. Serum total cholesterol, LDL-Ch, and apolipoproteins A1 and B levels are lower in patients with PV than in those with non-PV erythrocytosis. The hypocholesterolemia associated with PV may be attributable to the sequestration of circulating cholesterol into the increased number of erythrocytes. 〔*J Clin Exp Hematopathol 52(2) : 85-89, 2012*〕

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INTRODUCTION

Polycythemia vera (PV) is classified as a myeloproliferative neoplasm and usually occurs in people aged 60-79 years. Thrombotic events such as myocardial infarction, cerebral infarction, and deep vein thrombosis are the main clinical complications of PV.[1] The *JAK2* gene mutation has become an important criterion for the diagnosis of PV because *JAK2 V617F* mutations are noted in approximately 95% of PV patients.[2] However, other mutations involving *JAK2* exon 12, the *VHL* gene, and hypoxia-inducible factor-2 are known to be associated with idiopathic erythrocytosis.[3,4] We previously examined the relationship between clotting activity and erythrocyte phosphatidylserine (PS) expression in erythrocytosis patients with (PV) and without the *JAK2 V167F* mutation.[5] The activities of coagulation factors were significantly lower in the patients with PV than in those with erythrocytosis without the *JAK2 V167F* mutation.[5] Although the serum cholesterol level in PV patients has been studied previously,[6] it has not been used as a diagnostic criterion for PV since 2008, when the diagnostic criteria were changed on the basis of the revised WHO classification.[2] Therefore, we first examined the relationship between the serum lipid profile, including total cholesterol (TC) level, and erythrocytosis. Serum levels of TC, low-density-lipoprotein cholesterol (LDL-Ch), and apolipoprotein B were lower in patients with PV than in other (non-PV) erythrocytic patients.

Next, we examined the presence of intra-erythrocytic hemoglobin-binding cholesterol (Hb-Ch), a new form of circulating cholesterol described by Nikolié *et al*.[7] Hb-Ch is the major source of the efflux of cholesterol from human erythrocytes into plasma.[8] Cholesterol erythrocyte membrane (CEM) is known to be a risk factor for coronary artery diseases.[9] A previous study reported that the levels of CEM are similar between patients with PV and healthy subjects.[6] However, there are no published studies on the relationship between Hb-Ch or efflux of cholesterol and PV. Therefore, we first examined the release of erythrocyte cholesterol content into the plasma in whole blood from patients with erythrocytosis. The amount of cholesterol released into the plasma was higher for the patients with PV than for those with non-PV erythrocytosis or the non-polycythemic subjects, although the patients with PV exhibited hypocholesterolemia. We speculated that the hypocholesterolemia associated with PV

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may be due in part to the sequestration of circulating cholesterol within the increased number of erythrocytes.

PATIENTS AND METHODS

Patients

Tokyo Metropolitan Bokutoh Hospital is located in Eastern Tokyo. We retrospectively reviewed the medical records of 34 patients with erythrocytosis (hemoglobin [Hb] : men, > 18.5 g/dL ; women, > 16.5 g/dL) collected between August 2005 and December 2011. The medical records were reviewed for age, gender, and laboratory test results. Patients taking medications such as statins for hypercholesterolemia were excluded from this study. Venous blood was collected into evacuated tubes in order to measure the hematology and biochemistry including the cholesterol profile (TC, high-density lipoprotein cholesterol (HDL-Ch), LDL-Ch, triglycerides (TG), apolipoprotein A1, and apolipoprotein B) in a fasting state for the initial phlebotomy tests in our department. The *JAK2 V617F* mutation statuses of these patients were determined by the SRL Company (Tokyo, Japan) from the venous blood samples, using allele-specific polymerase chain reaction analysis. Serum samples were collected, frozen immediately, and stored at −80℃ until further analysis. The serum lipid profiles were measured by the BML Company (Tokyo, Japan).

The patients were divided into 2 groups according to the new WHO classification : the PV group and the non-PV erythrocytosis group. The non-PV erythrocytosis group could not be diagnosed with PV despite erythrocytosis (serum erythropoietin within normal limits), and the erythrocytosis was improved due to phlebotomies. We diagnosed non-PV erythrocytosis as a reactive erythrocytosis. Furthermore, patients with secondary polycythemia due to chronic obstructive pulmonary or cyanotic heart diseases and patients with erythropoietin-producing tumor were excluded from this study. The non-polycythemic subjects included both medically healthy workers under 65 years of age and hypertensive patients without hyperlipidemia over 65 years of age (N = 20). All subjects provided informed consent to participate in the study, and the study design was approved by the ethics review board of our institution.

Measurement of the release of erythrocyte cholesterol content into the plasma

In those of the study subjects who provided informed consent for additional analysis, we examined the *in vitro* efflux of cholesterol from the erythrocytes into the plasma. Briefly, venous blood from each patient was collected into 3 evacuated tubes containing ethylenediaminetetraacetic acid (EDTA) in order to measure the plasma levels of total cholesterol 0, 4, and 24 hr after venipuncture. The whole blood (WB) was incubated in EDTA-containing tubes at 37℃ .

The *in vitro* efflux of cholesterol from the erythrocytes into the plasma was also examined in whole blood from patients with non-PV erythrocytosis (N = 8) under conditions similar to those described above. Briefly, WB was collected into bags containing citrate phosphate dextrose adenine (CPDA) (Karmi^{R} CA, Kawasumi Laboratories, Inc., Tokyo, Japan). The bags were divided into 2 groups, 1 in which the buffy coat was retained (WB group) and a second from which the buffy coat was removed by centrifugation (buffy coat-deleted WB group).

The released cholesterol content was calculated as the difference between plasma TC from incubated whole blood and plasma TC from whole blood measured prior to incubation as follows : Released cholesterol content (4 hr or 24 hr) = TC (4 hr or 24 hr) − TC (0 hr).

Statistical analysis

We compared the differences between the survival and non-survival groups by Wilcoxon's analysis. Data are expressed as group means ± standard errors of the mean or medians with interquartile ranges. The rates of hypocholesterolemia were compared by the chi square test with Yates's correction. All statistical calculations were performed using JMP version 8.0 software (SAS Institute, Inc., Cary, NC), and significance was defined as $p < 0.05$.

The correlation between the released cholesterol content and the hematology results was assessed by linear regression analysis. For the linear regression analysis results, significance was defined as $p < 0.0001$.

RESULTS

Serum lipid profiles of erythrocytosis (PV and non-PV) patients

Of the 34 patients with erythrocytosis, 17 were diagnosed with PV. The other 17 patients with erythrocytosis tested negative for the *JAK2 V617F* mutation and were not diagnosed with PV (non-PV erythrocytosis). Patients with PV were significantly older than those with non-PV erythrocytosis ; however, no gender difference was noted between the 2 groups. All patients with non-PV erythrocytosis were male and exhibited higher body mass index (BMI) than those with PV or without polycythemia. No differences in age and gender were noted between the PV and non-polycythemic subjects. Serum albumin levels were similar among the 3 groups.

Laboratory tests performed upon initial presentation revealed that the serum levels of TC, LDL-Ch, and apolipoproteins A1 and B were significantly lower in the PV group than

Table 1. Clinical profiles and lipid profiles of patients with erythrocytosis

Clinical data	*PV* group (N = 17)	*Non-PV* group (N = 17)	Non-polycythemic group (N = 20)
Age	69 (2)*	56 (3)	61 (3)
Gender (M/F)	6/8	14/0	6/8
Height (cm)	158 (2)	165 (2)	160 (2)
Weight (kg)	57 (4)*	70 (4)***	59 (4)
Body mass index	22.6 (1.0)*	25.8 (0.9)***	22.8 (1.2)
Serum albumin (g/dL)	4.20 (0.08)	4.40 (0.07)	4.24 (0.07)
Hematology			
Hemoglobin (g/dL)	18.8 (0.4)	19.1 (0.3)	13.4 (0.3)
Hematocrit (%)	57.8 (0.9)	56.1 (0.9)	40.0 (0.8)
Red blood cells ($\times 10^9$/L)	6997.0 (220)*, ***	5887.1 (83.2)***	4165.2 (91.8)
Platelets ($\times 10^9$/L)	401.9 (43.1)*, ***	194.4 (12.1)	197.2 (9.8)
Leukocytes ($\times 10^9$/L)	16.3 (2.3)*, ***	6.4 (0.3)	4.8 (0.4)
Lipid profile			
Total cholesterol (mg/dL)	157 (7)*	196 (5)	190 (7)**
HDL cholesterol (mg/dL)	44 (3)	53 (4)	61 (3)**
LDL cholesterol (mg/dL)	90 (6)*	120 (5)***	105 (6)
Triglycerides (mg/dL)	129 (16)	178 (40)***	84 (6)**
Apolipoprotein A1 (mg/dL)	124 (4)*	137 (4)	151 (5)**
Apolipoprotein B (mg/dL)	84 (5)*	108 (4)	88 (5)
Apolipoprotein B/A1 ratio	0.71 (0.05)	0.80 (0.04)***	0.59 (0.04)
TC/HDL-cholesterol ratio	3.9 (0.3)	4.0 (0.3)***	3.2 (0.1)
LDL Cholesterol/ HDL-cholesterol ratio	2.2 (0.2)	2.5 (0.2)***	1.8 (0.1)
TC < 120 mg/dL (n, %)	1 (5.9%)	0 (0%)	0 (0%)
LDL-C < 50 mg/dL (n, %)	2 (11.8%)	0 (0%)	0 (0%)
LDL-C < 80 mg/dL (n, %)	6 (35.3%)	0 (0%)*	3 (15%)

Data represent the means with the standard errors in parentheses.
* : $p < 0.05$ vs. non-PV, ** : $p < 0.05$ vs. PV, *** : $p < 0.05$ vs. non-polycythemic subjects

in the non-PV group (Table 1). The HDL-Ch, TG, and the 3 commonly calculated ratios (B/A1, T/C, and L/C) did not differ significantly between the groups.

The rate of low LDL-C (< 80 mg/dL) was significantly higher in PV patients (35.3% ; 6/17) than in non-PV patients (0%, 0/17), as shown in Table 1. There was no difference of laboratory findings in terms of lipid profile between the homozygous state (N = 7) and the heterozygous state (N = 10) in the PV group (TC : homozygous state, 154 ± 12 mg/dL ; heterozygous state, 160 ± 8 mg/dL, not significant ; HDL-C : homozygous state, 42 ± 4 mg/dL ; heterozygous state, 44 ± 4 mg/dL, not significant ; LDL-C : homozygous state, 85 ± 12 mg/dL ; heterozygous state, 93 ± 6 mg/dL, not significant ; apolipoprotein A1 : homozygous state, 125 ± 7 mg/dL ; heterozygous state, 124 ± 6 mg/dL, not significant ; apolipoprotein B : homozygous state, 84 ± 8 mg/dL ; heterozygous state, 84 ± 6 mg/dL, not significant).

Release of cholesterol content into the plasma in blood from erythrocytosis (PV and non-PV) patients

As shown in Table 2, the release of cholesterol content (4 hr) in WB was significantly higher in PV patients than in those with non-PV erythrocytosis or non-polycythemic subjects. The plasma levels of TC after 24 hr did not differ among the 3 groups (Table 2). The amounts of cholesterol content released (24 hr) were significantly higher in WB from PV and non-PV erythrocytosis patients than in that from non-polycythemic subjects (Table 2). We next examined the correlation between release of cholesterol content (24 hr) and hematology results among all subjects (N = 36). Among the hematology parameters, the red blood cell count (RBC), Hb, and hematocrit (Hct) showed significant positive correlations with the released cholesterol content (RBC : $r^2 = 0.6000703$, $p < 0.0001$; Hb : $r^2 = 0.489283$, $p < 0.0001$; Hct : $r^2 = 0.605707$, $p < 0.0001$; leukocyte count : $r^2 = 0.173084$, $p = 0.0179$; platelet count : $r^2 = 0.376283$, $p = 0.0002$).

The release of cholesterol content was not lower in buffy coat-deleted WB (24 hr) than in WB (released cholesterol content (24 hr) : buffy coat-deleted WB, 17 ± 1 mg/dL, N = 5, WB : 13 ± 1 mg/dL, N = 3).

Table 2. Release of cholesterol content into plasma in whole blood from erythrocytosis patients

Plasma TC/Released cholesterol content	*PV* group (N = 10)	*Non-PV* group (N = 12)	Non-polycythemic group (N = 14)
Plasma TC (mg/dL)			
0 hr	165 (8)	184 (6)	193 (11)**
4 hr	177 (8)	192 (6)	200 (12)**
24 hr	213 (10)	223 (7)	220 (12)
Released cholesterol content (mg/dL)			
4 hr	12 (1.0)*	8.1 (0.8)***	4.4 (0.7)**
24 hr	45 (5)	39 (2)***	24 (2)**

Data represent means with standard errors in parentheses.
*: $p < 0.05$ vs. non-PV, **: $p < 0.05$ vs. PV, ***: $p < 0.05$ vs. non-polycythemic subjects

DISCUSSION

The natural history of PV includes thrombosis,[10] and erythrocytosis has been reported to be a poor prognostic indicator in ST-segment elevation myocardial infarction.[11] The mechanism by which PV leads to chronic hypocholesterolemia and the role of hypocholesterolemia in the pathogenesis of thrombosis remain uncertain.[6] In this study, patients with PV exhibited hypocholesterolemia in comparison with patients with non-PV erythrocytosis and non-polycythemic subjects. All of the patients with erythrocytosis with hypocholesterolemia (< 140 mg/dL) were diagnosed with PV. Elevated serum apolipoprotein A1 in association with PV has been linked to *JAK2 V617F* homozygosity in France.[12] We did not observe elevated serum apolipoprotein A1 in our PV patients, possibly because only 7 were homozygous for the JAK2 V617F mutation allele while 10 were heterozygous. Lipoprotein is involved in rheological effects, and higher lipoprotein levels may increase the viscosity of the blood.[13] We therefore speculated that lower levels of apolipoproteins A1 and B in PV patients might represent a negative feedback response to the thrombogenic tendencies of erythrocytosis, as thrombotic events are rarely observed in the early stages of PV soon after diagnosis.[10] We previously reported that the serum level of granulocyte colony stimulating factor (G-CSF) and the activities of certain clotting factors are lower in patients with PV than in those with non-PV erythrocytosis.[5,14] We speculate that hypocholesterolemia and low apolipoproteins A1 and B might be negative feedback responses similar to the changes in G-CSF and clotting activities.

PV is classified as a myeloproliferative neoplasm. Hematological malignancy also produces hypocholesterolemia due to the extreme demand for cholesterol for the cell membranes of the malignant cells as well as poor nutrition.[15,16] However, Gilbert *et al*. reported that the CEM of erythrocytes from PV patients did not differ from that of healthy subjects.[6] The mechanism by which PV produces chronic hypocholesterolemia thus remained uncertain. We then noticed the presence of Hb-Ch within erythrocytes, a new form of circulating cholesterol previously described by Nikolié *et al*.[7] This Hb-Ch is the major source of the efflux of cholesterol from human RBCs into plasma.[8] We next examined the *in vitro* efflux of cholesterol from erythrocytes into plasma in WB. After incubation for 24 hr, plasma TC in samples from PV patients reached levels similar to those of non-polycythemic subjects (Table 2). The released cholesterol may be derived from the erythrocytes in WB, as depletion of the buffy coat did not affect plasma TC. Moreover, there was a positive correlation between the released cholesterol content and the RBC count, Hb, and Hct. We speculate that the hypocholesterolemia associated with PV may be due to the sequestration of circulating cholesterol within the increased number of erythrocytes.

In conclusion, we report for the first time that serum levels of TC, LDL-Ch, and apolipoproteins A and B are lower in PV patients than in those with non-PV erythrocytosis. The release of cholesterol content into the plasma *in vitro* was greater in WB from PV patients than in that from non-PV patients and non-polycythemic subjects. We hypothesize that the hypocholesterolemia associated with PV may be due to the sequestration of circulating cholesterol within the erythrocytes.

This study is limited by its nature as a clinical study with a small sample size, as the number of patients with PV in a single metropolitan hospital is necessarily small. We are also unable to measure Hb-binding Ch by chromatography in this environment.

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