short report
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PLASMA HOMOCYSTEINE LEVELS IN 10 PATIENTS WITH POLYCYTHEMIA

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ABSTRACT

Polycythemia and hyperhomocysteinemia are risk factors for thrombosis. Since red blood cells actively metabolize methionine to homocysteine, we investigated whether or not patients with polycythemia have increased plasma levels of homocysteine, which might contribute to their increased thrombotic risk. In ten patients with polycythemia, the plasma homocysteine levels were measured before phlebotomy, three days after the procedure and 1-2 months later. The baseline mean plasma homocysteine levels in patients (9.7±1.6 µmol/L [± SD]) did not differ significantly from that found in 30 sex- and age-matched healthy controls (12.2±6.9). Despite a fall in the patients' mean [± SD] hematocrit from 0.50±0.02 at baseline to 0.47±0.03 three days after phlebotomy (significant at 95%) and to 0.48±0.02 after 1 to 2 months (not significant), the mean plasma homocysteine levels did not change significantly (9.9±2.3 µmol/L at 3 days and 9.7±2.1 µmol/L at 1-2 months). It is unlikely that high plasma homocysteine levels contribute to the increased thrombotic risk of polycythemic patients.

Key words: homocysteine, polycythemia, hyperhomocysteinemia, thrombosis, erythrocytes

Arterial and venous thromboses are frequent causes of morbidity and mortality in patients with primitive (polycythemia vera), secondary or stress polycythemias.[1,2] Although increased blood viscosity is considered the most important thrombogenic mechanism in these patients, additional, as yet unidentified, factors may be involved. Among these, we considered hyperhomocysteinemia, which is a risk factor for arterial and venous thrombosis.[3-7] Homocysteine is a sulfhydryl amino acid, derived from the transmethylation of dietary methionine. It can be trans-sulfurated to cysteine through a pyridoxine sensitive pathway or remethylated to methionine through pathways that are sensitive to folates, cobalamin and betaine.[8] Since homocysteine is actively synthesized by red blood cells (RBC), we hypothesized that patients with increased RBC mass might have high plasma homocysteine levels that could contribute to their thrombotic risk.

Materials and Methods

Materials

L-cystine, L-homocystine, tri-n-butylphosphine and 7-fluorobenzo-2-oxa-1,2-diazole-4-sulfonamide (ABDF) were obtained from Sigma (St. Louis, MO, USA). All other chemicals were reagent grade.

Subjects

Ten patients with polycythemia were studied during routine visits to our Center for phlebotomy to decrease their red cell mass. Their personal and clinical characteristics are shown in Table 1. Seven had polycythemia vera according to the diagnostic criteria of the Polycythemia Vera Study Group,[1,2] 2 had stress erythrocytosis and 1 hypoxic erythrocytosis.[1,2] Plasma homocysteine and serum folate and cobalamin were measured in 30 age- and sex-matched healthy controls (3 men, 27 women; median age 49 years, range 23-76).

Design of the study

Blood samples, collected in the morning from fasting subjects, were obtained immediately before phlebotomy (300 mL), three days after the procedure and on the day of the following phlebotomy (after a median of 36.5 days, range 23-53).

Plasma homocysteine assay

Blood samples were collected in EDTA, immediately placed on ice and centrifuged within 60 minutes (1200 g for 30 minutes) to obtain platelet-poor plasma, which was frozen at –80°C until assay. Plasma homocysteine was determined by high performance liquid chromatography according to Ubbink *et al.*[9]

Measurement of serum cobalamin and folic acid and intra-erythrocyte folic acid

Blood samples were collected in tubes without anticoagulant (for measurement of serum vitamin levels) or in ones containing EDTA (for intra-erythrocyte folate levels) and protected from light. Vitamin levels were determined by a radioassay (Dualcount, Solid phase no boil assay, Diagnostic Product Corporation, Los Angeles, CA, USA).

Statistical analysis

Data were analyzed with ANOVA for repeated measurements; subsequent multi-comparisons were made using the Scheffè test.

Results

The patients' mean hematocrit fell from 0.50±0.02 at baseline to 0.47±0.03 (significantly different at 95%) at 3 days and to 0.48±0.02 at 1-2 months after phlebotomy (not significant). The

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Table 1. Personal characteristics and clinical features of 10 patients with polycythemia.

M/F	1/9
Median age (y) (range)	49 (25-74)
Type of polycythemia (no. of patients)	
Polycythemia vera	7
Secondary erythrocytosis	1
Stress erythrocytosis	2
Previous thrombotic events (no. of patients)	
Myocardial infarction	1
Stroke	1
Peripheral arterial disease	1
Deep-vein thrombosis	1
Therapy (no. of patients)	
Hydroxyurea	2
Acetylsalicylic acid	3
Phlebotomy	10
Risk factors for thrombosis (no. of patients)	
Smoking	3
Hypertension	2
Hypercholesterolemia	3
Hypertriglyceridemia	1
Overweight	4

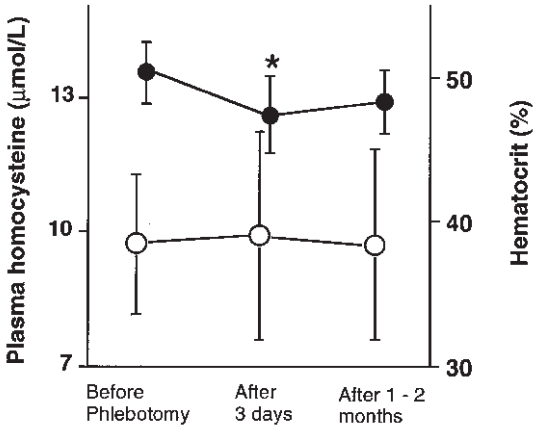

Figure 1. Plasma total homocysteine and hematocrit levels (mean ± standard deviation) in patients before phlebotomy, after three days and after 1-2 months, before next phlebotomy (m = homocysteine; l= hematocrit).
**significantly different at 95% versus before phlebothomy.*

mean hemoglobin level fell from 16.7±0.7 g/dL at baseline to 15.8±0.8 g/dL at 3 days and rose to 16.8±0.8 g/dL 1-2 months later.

Baseline plasma homocysteine levels in patients (9.7±1.6 µmol/L) did not significantly differ from those measured in 30 sex- and age-matched healthy controls (12.2±6.9), nor did they change 3 days (9.9±2.3) or 1-2 months after phlebotomy (9.7±2.1) (Figure 1).

All patients had normal levels of serum and intra-erythrocyte folate and serum cobalamin.

Conclusions

This study shows that the plasma homocysteine levels of 10 patients with polycythemia were not higher than those found in 30 age- and sex-matched healthy controls. Moreover, these values did not decrease after phlebotomy. Although the number of patients studied was relatively small, these results indicate that the contribution of RBC to plasma homocysteine is negligible. Therefore this study does not support our hypothesis that hyperhomocysteinemia contributes to the thrombotic risk of patients with polycythemia.

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