Plasma Homocysteine Levels in Patients With Deep Venous Thrombosis

Tore Amundsen, Per Magne Ueland, Anders Waage

Abstract Increased risk of arterial and venous thromboembolic disease is well documented in the homocystinuric patient. There is growing evidence that hyperhomocysteinemia is an independent risk factor for premature arteriosclerotic disease, including cerebral, peripheral, and coronary vascular diseases. So far no association has been established between hyperhomocysteinemia and venous thromboembolism.We studied 35 patients, young adults (age less than 56 years) with venographically and/or ultrasonographically verified deep venous thrombosis (DVT). Patients with coexisting diseases were excluded. Plasmahomocysteine levels before and after intake of methionine were measured 3 months or more after the time of diagnosis and compared with 39 control subjects. We found no significant difference in plasmahomocysteine levels between the young adults with deep venous thrombosis and control subjects. This indicates that hyperhomocysteinemia is not a frequent cause of DVT. (*Arterioscler Thromb Vasc Biol.* 1995;15: 1321-1323.)

Key Words • thrombosis, deep venous • adults, young • plasmahomocysteine

Thromboembolism of the veins and arteries and premature arteriosclerosis are common causes of death in patients with the inherited disorder homocystinuria. Vascular episodes may occur in early adolescence and even in childhood.[1,2]

Homocystinuria denotes a class of disease characterized by impaired homocysteine metabolism that leads to marked elevation of the homocysteine level in the blood and massive urinary excretion of homocystine.[1] The fact that early vascular disease is observed in homocystinuria caused by different enzymatic defects suggests that homocysteine itself may be responsible for the vascular lesions.

Moderate hyperhomocysteinemia is common in the general population and is caused by both genetic and acquired conditions, including folate and cobalamin deficiency.[3] Approximately 25 retrospective studies based on approximately 2500 patients and a comparable number of control subjects[4-6] and two recent prospective investigations[7,8] have demonstrated that hyperhomocysteinemia is an independent risk factor for premature arteriosclerotic disease in the coronary, cerebral, and peripheral arteries. The condition can often be corrected with an oral vitamin supplement that is simple and inexpensive.[9]

VTE, including DVT and PE, accounts for at least 50% of the vascular events in homocystinuria.[1,10] However, only three reports on hyperhomocysteinemia and venous thrombosis have been published, and the results are conflicting. Brattström et al[11] found no significant difference in plasma homocysteine concentrations between 42 patients with VTE and healthy control subjects, although the male patients showed a tendency toward higher plasma homocysteine than male control subjects. In contrast, Bienvenu et al[12] demonstrated a significant association between fasting plasma homocysteine and VTE, and Falcon et al[13] recently reported a high prevalence of hyperhomocysteinemia in patients less than 40 years of age who had VTE.

The present study addresses whether hyperhomocysteinemia may predispose to venous thrombosis in addition to arteriosclerotic disease. We measured plasma homocysteine during fasting and after methionine loading in 34 patients presenting with DVT before the age of 56 years.

Methods

Patients

Adults less than 56 years of age at the time of diagnosis who had venographically and/or ultrasonographically verified DVT of the lower extremities and had been hospitalized over a 4-year period, from 1988 through 1991, in the Department of Medicine at University Hospital, Trondheim, were included. Patients hospitalized from 1988 through 1989 were studied retrospectively, and patients hospitalized from 1990 through 1991 were included consecutively.

Exclusion criteria included patients with diabetes mellitus; patients with kidney, liver, or thyroid diseases; patients receiving lipid-lowering agents; patients receiving nitrous oxide within 3 months of onset of DVT; patients with acute or severe illness requiring immobilization in bed; and patients with malignant diseases. Secondary causes of exclusion (1 patient each, unless indicated otherwise) in this study were essential thrombocythemia and cerebrovascular disease, ulcerative colitis, hypertension and cesarean section less than 1 month before the onset of DVT, purulent meningitis and purulent arthritis, ovarian cancer and cerebrovascular disease, oligophrenia and epilepsy, chronic alcoholism, chronic renal failure (3 patients), and recurrent DVT at the time of inclusion (2 patients). In addition, death (4 patients), living too far away from the hospital (2 patients), and lack of consent (18 patients) were causes of exclusion. Altogether, 37 of 72 patients were excluded.

The remaining patients comprised 17 women (mean age, 41.5 years; range, 21 to 56 years) and 18 men (mean age, 46.5 years; range, 20 to 56 years), including postoperative DVT (3 patients), posttraumatic DVT (3 patients), and protein S

Received January 8, 1995; accepted June 2, 1995.

From the University Hospital of Trondheim (T.A., A.W.), Norway, and Haukeland Hospital (P.M.U.), Bergen, Norway.

Correspondence to Tore Amundsen, Department of Medicine, Section of Haematology, University Hospital, N-7006 Trondheim, Norway.

Selected Abbreviations and Acronyms

DVT = deep venous thrombosis
PCG = cysteinylglycine
PCys = cysteine
PE = pulmonary embolism
PHCy = plasmahomocysteine
VTE = venous thromboembolism

deficiency (1 patient). All patients and control subjects were screened for protein C-, protein S-, and antithrombin III deficiency.

As control subjects 39 healthy blood donors were matched to the patient group for age and sex (19 women [mean age, 40.4 years; range, 25 to 53 years] and 20 men [mean age, 44.8 years; range, 23 to 53 years]). The study design was approved by the ethics committee of the Medical Faculty, University of Trondheim, and informed consent was obtained from all participants.

A methionine loading test was performed by oral administration of methionine (100 mg/kg body wt) in 200 mL of orange juice after overnight fasting. Patients and control subjects had no extra vitamin intake during the week before the loading test. Blood samples were collected immediately before the methionine intake (8 AM) and after 6 hours, centrifuged, and stored at −70°C until they were assayed at the same time. Total PHCy, PCys, and PCG were measured by high-performance liquid chromatography; details of these assays are provided elsewhere.[14]

The Mann-Whitney test was used to compare the PHCy, PCys, and PCG concentrations between patients and control subjects. A value of $P<.05$ was considered significant. The results are expressed as mean±SD.

Results

PHCy preloading values for patients were 10.3±3.5 μmol/L and for control subjects 10.3±2.6 μmol/L (Figure). PHCy postloading values for patients were 39.5±14.2 μmol/L and for control subjects 37.2±14.2 μmol/L (Figure).

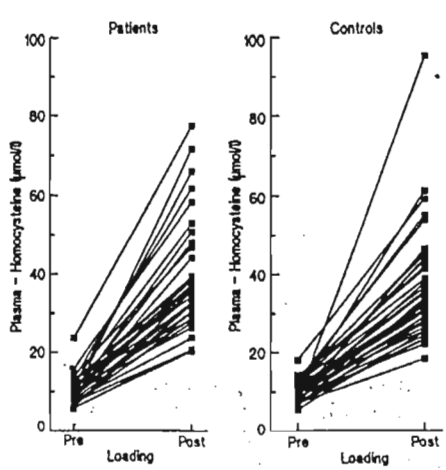
Two (5.7%) of the 35 patients and 1 (2.6%) of the 39 control subjects had PHCy values 2 SD above mean values for both preloading and postloading values. The control subject with a markedly elevated postloading value was retested after 11 months and at that time had normal PHCy levels. Logarithmic PHCy preloading and postloading values in each group were compared and showed no significant difference ($P>.1$).

PCys preloading values for patients were 249.1±30.7 μmol/L and for control subjects were 256.9±32.1 μmol/L. PCys postloading values for patients were 230.7±32.9 μmol/L and for control subjects 242.7±27.8 μmol/L.

PCG preloading values for patients were 29.2±4.8 μmol/L and for control subjects 28.4±4.6 μmol/L. PCG postloading values for patients were 22.8±4.4 μmol/L and for controls 21.1±3.9 μmol/L.

There was no significant difference between patients and control subjects for PHCy, PCys, and PCG concentrations for preloading and postloading values and for the difference between the preloading and postloading values.

Serum B_{12} levels for patients were 289.2±94.7 nmol/L and for control subjects 325.1±103.9 nmol/L ($P=.127$). Serum folate values for patients were 12.8±4.3 nmol/L and for control subjects 14.49±5.4 nmol/L ($P=.144$).



Plasma homocysteine levels in patients and control subjects before and after intake (preloading and postloading, respectively) of methionine.

Discussion

We found no significant difference in preload and postload PHCy between 35 patients with DVT (28 without known cause) occurring before the age of 56 years and 39 sex- and age-matched control subjects. The mean fasting PHCy concentration in both patients and control subjects (≈10.3 μmol/L, the Figure) equaled the levels previously found in 3000 healthy men aged 40 to 42 years.[3]

Although the number of patients is small, our data show that hyperhomocysteinemia and abnormal homocysteine responses to a standard methionine load are not important risk factors for DVT. Our results are in agreement with those of Brattström et al[11] who found no significant difference in fasting and postload plasma homocysteine levels between 42 patients with DVT and 42 healthy control subjects. Most of their patients had no predisposing disease or risk factor for thrombosis.

In contrast, Bienvenu et al[12] reported that 7 of 23 patients with VTE had significantly increased (>2 SD) fasting PHCy levels and concluded that hyperhomocysteinemia is a risk factor for venous and arterial thromboses. However, the subjects with VTE studied by Bienvenu et al included patients with pulmonary embolism, Budd-Chiari syndrome, central vein occlusion, and mesenterial vein thrombosis, in addition to DVT; some patients also had coexisting disease often associated with VTE. The mean age of the patient group (40 years; range, 17 to 57 years) seemed to be higher than that of the control group (33 years; range, 18 to 50 years). Finally, they used the Student's t test to compare PHCy levels, which are known to be not normally distributed.[12,15] Thus, the design of this study makes it difficult to evaluate hyperhomocysteinemia as an isolated risk factor for DVT, and therefore to compare their study to the current study.

The study of Falcon et al[13] on the other hand shows that the postload increment of homocysteine but not the fasting level was significantly higher in patients with VTE before the age of 40 years compared with age- and sex-matched controls. They studied patients with VTE referred to a center for juvenile venous thrombosis (mean age, 33 years). More than 50% of the patients had two or more episodes of VTE, and 48% of the episodes occurred in pregnant women or in women taking oral

contraceptives. All the hyperhomocysteinemic patients had two or more episodes of VTE. Therefore, the study of Falcon et al represents a more selected group of patients (very young patients with recurrent VTE) compared with those in our study. Nearly 50% of the hyperhomocysteinemic patients (7 of 15 patients) had a positive family history of VTE, and 50% of these showed inherited abnormality.

The prevalence of hyperhomocysteinemia in the general population is estimated to be approximately 1%, although it may vary from area to area.[1] In a nonselected DVT population, hyperhomocysteinemia as an independent risk factor probably would be diluted, and the association may not reach significant levels. Twenty-eight of the 35 patients in our study had DVT without known cause, and the remaining 7 patients had postoperative and posttraumatic DVT (3 patients each) and protein S deficiency (1 patient). Despite this selection, hyperhomocysteinemia as a possible risk factor may be underestimated in our study because of the small number of patients (type II error).

In conclusion, only three clinical studies, which included a limited number of patients, have analyzed hyperhomocysteinemia as a possible risk factor for VTE. The current study and one Swedish study[11] failed to demonstrate such a relation, whereas two other studies[12,13] suggest a high prevalence of hyperhomocysteinemia in young patients with VTE. However, the studies are not directly comparable, and the problem is still unresolved. Hyperhomocysteinemia possibly predisposes to early-onset VTE, but its association with VTE is weaker than with premature arteriosclerosis. The combination of hyperhomocysteinemia and VTE may reflect an inherited abnormality,[13] and this relation may be more pronounced in certain ethnic groups and in younger patients with recurrent DVT. Further studies are recommended before the clinical value of plasmahomocysteine screening can be determined in patients with VTE.

Note added in proof. In a newly published article by den Heijer et al, they concluded that hyperhomocysteinemia is a common risk factor for recurrent venous thrombosis. This is in agreement with our final suggestions.[16]

Acknowledgments

We thank Chief Bioengineer Mona Undeland and her staff at the Hematological Laboratorium for help with the sampling procedure, Dr Erik Juel and his staff at the Department of Blood Donors for the selection of blood donors, and Dr Harald Johnsen at the Department of Clinical Chemistry for help with the statistical calculations.

References

1. Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet WS, Sly S, Valle D, eds. *The Metabolic Basis of Inherited Disease*. New York, NY: McGraw-Hill; 1989:693-734.
2. Favre JP, Becker F, Lorcerie B, Dumas R, David M. Vascular manifestations in homocysteinuria. *Ann Vasc Surg*. 1992;6:294-297.
3. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem*. 1993;39:1764-1779.
4. Ueland PM, Refsum H, Brattström L. Plasma homocysteine and cardiovascular disease. In: Francis RBJ, ed. *Atherosclerotic Cardiovascular Disease, Hemostasis, and Endothelial Function*. New York, NY: Marcel Dekker; 1992:183-236.
5. Kang SS, Wong PWK, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. *Annu Rev Nutr*. 1992;12:279-298.
6. Malinow MR, Stampfer MJ. Role of plasma homocyst(e)ine in arterial occlusive disease. *Clin Chem*. 1994;40:857-858.
7. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV, Hennekens CH. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA*. 1992;268:877-881.
8. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE. The Tromsø study: a population based prospective study of serum total homocysteine and coronary heart disease. *Int J Epidemiol*. accepted, 1994.
9. Ueland PM, Refsum H. Plasmahomocysteine, a riskfactor for vascular disease: plasma levels in health, disease and drug therapy. *J Lab Clin Med*. 1989;114:473-501.
10. Wilcken DEL, Dudman NPB. Homocystinuria and atherosclerosis. *Monogr Hum Genet*. 1992;14:311-324.
11. Brattström L, Tengborn L, Israelsson B, Hultberg B. Plasma homocysteine in venous thromboembolism. *Haemostasis*. 1991;21:51-57.
12. Bienvenu T, Ankri A, Chadefaux B, Montalescot G, Kamoun P. Elevated total plasma homocysteine, a risk factor for thrombosis: relation to coagulation and fibrinolytic parameters. *Thromb Res*. 1993;70:123-129.
13. Falcon CR, Cattaneo M, Panzeri D, Martinelli I, Mannucci PM. High prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis. *Arterioscler Thromb*. 1994;14:1080-1083.
14. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. *Clin Chem*. 1993;39:263-271.
15. Ueland PM, Refsum H, Stabler S, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem*. 1993;39:1764-1779.
16. den Heijer M, et al. Is hyperhomocysteinemia a risk factor for recurrent venous thrombosis? *Lancet*. 1995;345.