The Incidence of Hyperhomocysteinaemia in Vascular Patients

J. I. Spark, ***** **P. Laws and R. Fitridge**

Department of Surgery, Queen Elizabeth Hospital, University of Adelaide, Woodville 5011, Adelaide, Australia

Introduction. *Hyperhomocysteinaemia has recently been identified as an important risk factor for atherosclerotic vascular disease. Screening for hyperhomocysteinaemia has been recommended, however, the incidence of hyperhomocysteinaemia in vascular patients is not known.*

Aims. *To determine the incidence of hyperhomocysteinaemia in vascular patients, to determine the relation of hyperhomocysteinaemia with folate, vitamin B_{12} levels and lipid profiles in vascular patients. To examine if there is a relationship between the degree of vascular injury and homocysteine concentration.*

Methods. *New vascular patients at The Queen Elizabeth Hospital were recruited and divided into peripheral, and aneurysmal presentations. Patients demographics were recorded, blood samples were taken for fasting lipid profile, and homocysteine concentration. Samples were also taken for vitamin B_{12}, plasma and red cell folate levels. Sixty age and sex matched controls were included for comparison.*

Results. *One hundred and twenty-six patients have been recruited, (95 men and 31 women) with a median age of 68 years (61–74 years). The incidence of elevated homocysteine, and cholesterol levels was 33, 47 and 24%. The levels of vitamin B_{12} and folate were normal in all patients. Homocysteine was elevated in 27% of claudicants, 50% of patients with rest pain and 53% of patients with an aortic aneurysm.*

Conclusion. *There is a high rate of hyperhomocysteinaemia in vascular patients with a higher incidence in patients with rest pain. There was also a high incidence of elevated homocysteine levels in patients with an abdominal aortic aneurysm. The rate of growth of these aneurysms is currently under review. Low folate or B_{12} concentrations is not the cause of raised homocysteine levels.*

Key Words: Homocysteine; Risk factors; Vascular disease.

Introduction

In 1969, McCully made the clinical observation linking elevated plasma homocysteine concentrations and vascular disease.[1] Subsequent investigations have confirmed McCully's hypothesis, though it is only recently that sufficient evidence has mounted to suggest that the association is independent and dose related, it remains to be established whether it is causal and modifiable.[2]

Although severe hyperhomocysteinaemia is rare, mild hyperhomocysteinaemia (>15 μmol/l) occurs in 5–7% of the general population.[2,3] Abundant epidemiologic evidence has demonstrated that the presence of mild hyperhomocysteinaemia is an independent risk factor for coronary, cerebral and peripheral

atherosclerosis.[4] As a result of this, recommendations for screening for hyperhomocysteinaemia have been made although the incidence of the condition in the 'vascular community' is unknown.[5]

Homocysteine is an amino acid intermediate formed during the metabolism of the essential amino acid methionine. It is metabolised by one of two pathways; remethylation back into methionine or transulphuration to cystathionine which is converted into cysteine, and ultimately excreted in the urine (Fig. 1).

Elevations in plasma homocysteine are typically caused by either genetic defects in the enzymes involved in homocysteine metabolism or by nutritional deficiencies in vitamin cofactors. The majority of the genetic defects such as cystathionine (beta)-synthase deficiency and homozygous deficiency of methylenetetrahydrofolate reductase are rare and result in marked hyperhomocysteinaemia.[6,7]

Nutritional deficiencies in the vitamin cofactors (folate, vitamin B_{12} and vitamin B_6) required for
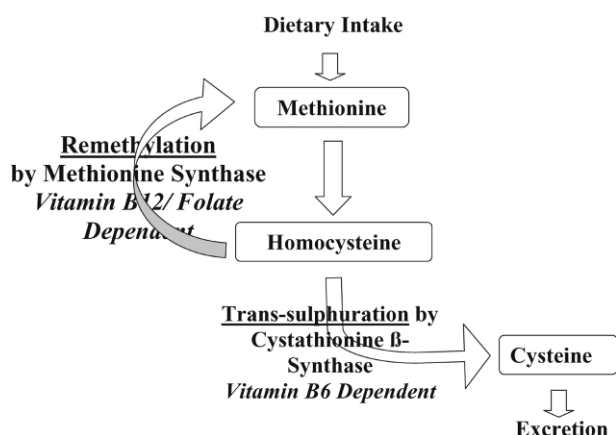
Fig. 1. Homocysteine metabolism.

homocysteine metabolism might promote hyperhomocysteinaemia. Elevated homocysteine concentrations have been observed in patients with nutritional deficiencies of the essential cofactor vitamin B_{12} and the co-substrate folate.[8,9] It has been suggested that inadequate plasma concentrations of one or more of these vitamin cofactors contribute in approximately two thirds of all cases of hyperhomocysteinaemia.[10] Vitamin supplementation can normalise high homocysteine concentrations, however, it remains to be seen whether normalising homocysteine concentrations will improve cardiovascular morbidity and mortality.

Homocysteine can also be increased as part of the acute phase response, diabetes, chronic renal failure, cancer and hypothyroidism (Table 1).

The aim of this study was to determine the incidence of hyperhomocysteinaemia and lipoprotein (a) concentration in the vascular population, to determine if there is an association between the degree of vascular injury and the level of homocysteine or lipoprotein (b) concentration, and to determine if patients with hyperhomocysteinaemia have deficiencies in the vitamin cofactors (folate and vitamin B_{12}).

Table 1. Causes of hyperhomocysteinaemia.

Diseases
Renal impairment
Pernicious anaemia
Hypothyroidism
Malignancy (acute lymphoblastic leukaemia, carcinoma of the breast, ovary and pancreas
Severe psoriasis

Medications and toxins
Folate antagonists; methotrexate, phenytoin, carbamazepine
Vitamin B_6 antagonists; theophyline, nicotinic acid, colestipol
Thiazide diuretics

Patients and Methods

The study was approved by the local hospital ethics committee.

All new vascular patients attending the Queen Elizabeth Hospital were recruited. Patients were classified into aneurysmal, peripheral and cerebrovascular. The peripheral vascular patients were further subdivided into claudicants and chronic critical ischaemia.[11] Patient's demographic data were recorded including age, risk factor history, past medical history, and drug history.

Sixty age and sex matched control patients without vascular disease were recruited from orthopaedic and general surgery outpatient clinics, as increasing age and the male sex have been associated with mild hyperhomocysteinaemia.[12]

Renal impairment commonly causes hyperhomocysteinaemia because of impaired metabolism of homocysteine by the kidney, the major route by which homocysteine is cleared from the plasma. Plasma homocysteine concentrations can be increased by various drugs and diseases that interfere with folate, vitamin B_6 and B_{12} metabolism (Table 1).[13] Patients with these conditions or medications were excluded.

All blood samples were taken after an overnight fast, the samples placed on ice and plasma separated within 60 min and frozen at $-20\,^{\circ}C$ prior to assay.[14] Total plasma homocysteine was measured using high performance liquid chromatography without methionine loading. Methionine loading stresses the homocysteine metabolic pathways and maybe more sensitive than a fasting sample, however, this method is inconvenient for clinician and patient and the reference values for post load results are uncertain.[15]

Red cell folate, serum folate and vitamin B_{12} were measured using the Abbott AxSYM™ kit.

Plasma cholesterol was measured using the Roche cholesterol kit.

Statistical Analysis

The data were recorded on a proforma and transferred to Microsoft Excel version 7.0. Statistics were performed using the Excel add-in, Astute (University of Leeds).

As the data were skewed, non-parametric analyses were employed. The results are expressed as medians and interquartile ranges. The Mann–Whitney U test of significance was used to examine the difference between results in two groups.

Results

One hundred and twenty-six patients have been analysed, of which 95 men and 31 women, with a median age of 68 years (IQR 61–74 years). The patients were divided into claudicants (39 patients), chronic critical limb ischaemia (CCLI) (40 patients, 26 with rest pain, 14 with tissue loss) and abdominal aortic aneurysm (AAA) (47 patients). Moderate hyperhomocysteinaemia has been classified as a value >16 mmol/l,[16] therefore any patients with a homocysteine level >16 mmol/l were counted as elevated. The incidence of the standard vascular risk factors and the incidence of elevated homocysteine for all patients and subdivided as patients with claudication, CCLI and AAA are shown in Table 2. The control patients had slightly lower risk factors, but not statistically significant.

The actual values of homocysteine compared to age matched controls are shown in Table 3. There is a statistically significant increase in homocysteine in all groups, with the CCLI patients having the greatest increase. There was also a significant difference between patients with CCLI and claudication ($p < 0.05$). There was no significant difference between those patients with rest pain and those with tissue loss.

Fifty-three percent of the AAA patients had moderately raised Hcy levels. All these cases were infra-renal and none were inflammatory in nature. Patients with hyperhomocysteinaemia had similar size aneurysms to those with normal homocysteine levels (3.9 cm (3.5–7.5) vs. 4.2 cm (3.8–10) $p > 0.05$).

There was no difference in vitamin B_{12}, serum folate and red cell folate between vascular patients (medians 400 pmol/l, 28, 625 nmol/l, respectively) and the control group (medians 356 pmol/l, 24, 576 nmol/l, respectively, $p > 0.05$).

Discussion

There is a large body of epidemiological evidence that links increasing homocysteine (Hcy) with increasing risk of atherothrombotic vascular disease.[17] There is not complete consistency, and a contrary view has been proposed that hyperhomocysteinaemia may represent an acute phase reactant that is a marker of atherogenesis.[18] Plasma homocysteine concentrations are known to increase after tissue damage, which could explain the increase noted in the patients with CCLI compared with claudicants, however, there was no significant difference between patients with rest pain or tissue loss.

A striking observation in this study was that 53% of the AAA patients had elevated Hcy concentrations. Mild hyperhomocysteinaemia occurs in approximately 5–7% of the general population[3] and according to this study 27–50% of patients with symptomatic atherosclerotic vascular disease. None of the aneurysm patients had symptoms of claudication to account for this increase. The day-to-day variation in fasting Hcy is small (coefficient of variation 7%),[19] so could not account for the raised levels.

Specimens were placed on ice immediately after venopuncture until the plasma was separated to prevent a time and temperature dependent release of homocysteine from blood cells. Homocysteine also increases with age and is higher in men, however, the control group (age and sex matched) had a significantly lower homocysteine level than the aneurysm population and an incidence of hyperhomocysteinaemia similar to that previously published.[3] The question then arises, whether Hcy has a role in aneurysm formation and/or in aneurysm expansion, or again, is Hcy simply a marker of the condition? The rate of expansion of small aneurysms and homocysteine levels is currently under investigation.

There is one other report in the literature,[20] showing a similar result in the incidence of hyperhomocysteinaemia in aneurysm patients. They also noted that patients with hyperhomocysteinaemia had larger aneurysms than those with normal homocysteine levels which is different to our own findings.

Nutritional deficiencies in the vitamin cofactors

Table 2. Incidence of risk factors in vascular patients and controls.

	All vascular patients	Claudicants	CCLI	AAA	Controls
N	126	39	40	47	60
Diabetes	43 (34%)	11 (29%)	15 (38%)	11 (23%)	15 (26%)
Smoking	79 (63%)	21 (53%)	27 (68%)	24 (52%)	24 (40%)
Hypertension	78 (62%)	22 (56%)	27 (68%)	22 (47%)	31 (52%)
Cholesterol (reported on treatment)	46 (46%)	16 (40%)	20 (50%)	18 (39%)	22 (37%)
Cholesterol >5.5 mmol/l	39 (31%)	15 (38%)	11 (29%)	14 (29%)	15 (25%)
Homocysteine >16 μmol/l	46 (37%)	10 (27%)	20 (50%)	25 (53%)	4 (7%)

CCLI, chronic critical limb ischaemia.

Table 3. Homocysteine in vascular patients compared to age matched controls. Results are expressed as medians and interquartile ranges.

	Claudicants	CCLI (rest pain)	CCLI (tissue loss)	AAA	Controls
Cholesterol median (IQR) mmol/l	5.1 (4.7–6.0)	4.9 (3.5–5.5)	4.6 (3.8–5.3)	4.8 (4.5–5.1)	4.5 (4.0–5.9)
Homocysteine median (IQR) μmol/l	12.5 (8.1–18.1)	13.7 (8.3–24.6)	14.1 (9.8–21.2)	17.9 (14.8–20.8)	8.5 (7.0–11.8)

CCLI, chronic critical limb ischaemia; AAA, abdominal aortic aneurysm.

(folate, vitamin B_{12} and vitamin B_6) required for homocysteine metabolism may promote hyperhomocysteinaemia. Markedly elevated homocysteine concentrations have been observed in patients with nutritional deficiencies of the essential cofactor B_{12} and the co-substrate folate.[21,22] Selhub and colleagues have suggested that inadequate plasma concentrations of one or more B vitamins are contributing factors in approximately two thirds of all cases of hyperhomocysteinaemia[23] and that vitamin supplementation can normalise high homocysteine concentrations. This was not seen in our study as all folate and vitamin levels were all within normal limits. It remains to be determined whether normalising homocysteine concentrations will improve cardiovascular morbidity and mortality or influence the rate of aneurysm expansion.

Despite the fact that this was a prospective and controlled study, limitations did exist. The nature of the control group was restricted to people attending hospital, although free of symptomatic vascular disease, it remains a select group.

Another potential criticism of our method is that methionine loading was not used in the evaluation of homocysteine metabolism. However, the development of sensitive assays by use of high performance liquid chromatography have led to accurate determinations of total homocysteine levels without the need for methionine loading.

Although the data demonstrates a high incidence of homocysteinaemia in vascular patients, not all patients in the study group had elevated levels.

Finally, while homocysteinaemia can be reduced with folate, clinical trial data regarding the potential benefits of homocysteine lowering therapy for the prevention of vascular disease are not yet available.

References

1 McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of atherosclerosis. *Am J Pathol* 1969; **56**: 111–128.

2 Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy. *J Lab Clin Med* 1989; **114**: 473–501.

3 McCully KS. Homocysteine and vascular disease. *Nat Med* 1996; **2**: 386–389.

4 Kang SS, Wong PW, Manilow MR. Hyperhomocysteinaemia as a risk factor for occlusive vascular disease. *Ann Rev Nutr* 1992; **12**: 279–298.

5 Stein JH, McBride PE. Hyperhomocysteinaemia and atherosclerotic vascular disease: pathophysiology, screening and treatment. *Arch Int Med* 1998; **158**: 1301–1306.

6 Gallagher PM, Meleady R, Shields DC *et al.* Homocysteine and risk of premature coronary disease: evidence for a common gene mutation. *Circulation* 1996; **94**: 2154–2158.

7 Van Bockxmeer FM, Mamotte CD, Vasikaran SD, Taylor RR. Methylenetetrahydrofolate reductase gene and coronary artery disease. *Circulation* 1997; **95**: 21–23.

8 Brattstrom L, Isrealsson B, Lindegarde F, Hultberg B. Higher total plasma homocysteine in vitamin B_{12} deficiency than in heterozygosity for homocystinuria due to cystathionine (beta)-synthase deficiency. *Metabolism* 1988; **37**: 175–178.

9 Kang SS, Wong PWK, Norusis M. Homocysteinaemia due to folate deficiency. *Metabolism* 1987; **36**: 458–462.

10 Selhub J, Jaques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinaemia in an elderly population. *JAMA* 1993; **270**: 2693–2698.

11 Second European consensus document on chronic critical leg ischaemia. *Eur J Vasc Surg*, 1992; **6**(Suppl.): 1–32.

12 Nygard O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. *Am J Clin Nutr* 1998; **67**: 263–270.

13 Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999; **354**: 407–413.

14 Guttormsen AB, Scheede J, Fiskerstrand T, Ueland PM, Refsum HM. Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. *J Nutr* 1994; **124**: 1934–1941.

15 Graham IM, Daly LE, Refusum HM *et al.* Plasma homocysteine as a risk factor for vascular disease: the European concerted action plan. *JAMA* 1997; **277**: 1775–1781.

16 Manilow MR. Prevalence of hyperhomocysteinaemia in patients with peripheral arterial occlusive disease. *Circulation* 1989; **79**: 1180–1188.

17 Boers GH. Heterozygosity for homocysteinaemia in premature peripheral and cerebral occlusive arterial disease. *N Engl J Med* 1985; **313**: 709–715.

18 Brattstrom L, Wilcken DE. Homocysteinaemia and cardiovascular disease: cause or effect? *Am J Clin Nutr* 2000; **72**: 315–323.

19 Garg UC, Zheng ZJ, Folsom AR. Short-term and long term variability of plasma homocysteine measurement. *Clin Chem* 1997; **43**: 141–145.

20 Brunelli T, Prisco D, Fedi S *et al.* High prevalence of mild hyperhomocysteinemia in patients with abdominal aortic aneurysm. *J Vasc Surg* 2000; **32**: 531–536.

21 Brattstrom L, Israelsson B, Lindegarde F, Hultberg B. Higher total plasma homocysteine in vitamin B_{12} deficiency than in heterozygosity for homocysteinuria due to beta-synthase deficiency. *Metabolism* 1988; **37**: 175–178.

22 Kang SS, Wong PWK, Norusis M. Homocysteinaemia due to folate deficiency. *Metabolism* 1987; **36**: 458–462.

23 Selhub J, Jacques PF, Wilson PW, Rush D, Rosenburg IH. Vitamin status and intake as primary determinants of homocysteinaemia in an elderly population. *JAMA* 1993; **270**: 2693–2698.