The Incidence of Hyperhomocysteinaemia in Vascular Patients

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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. 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.

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

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.

Nutritional deficiencies in the vitamin cofactors (folate, vitamin B_{12} and vitamin B_{6}) required for...
homocysteine metabolism might promote hyperhomo-
cysteinaemia. Elevated homocysteine concentra-
tions have been observed in patients with
nutritional deficiencies of the essential cofactor vita-
m B12 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 hyperhomo-
cysteinaemia.10 Vitamin supplementation can normal-
ise 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 B12).

Table 1. Causes of hyperhomocysteinaemia.

<table>
<thead>
<tr>
<th>Diseases</th>
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<tr>
<td>Renal impairment</td>
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<tr>
<td>Perinicious anaemia</td>
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<tr>
<td>Hypothyroidism</td>
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<tr>
<td>Malignancy (acute lymphoblastic leukaemia, carcinoma of the breast, ovary and pancreas</td>
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<td>Severe psoriasis</td>
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<th>Medications and toxins</th>
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<td>Folate antagonists; methotrexate, phenytoin, carbamazepine</td>
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<tr>
<td>Vitamin B6 antagonists; theophylline, nicotinic acid, colestipol</td>
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<td>Thiazide diuretics</td>
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Fig. 1. Homocysteine metabolism.

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 cerebrovas-
cular. 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 hyperhomo-
cysteinaemia 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 B6 and B12 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°C prior to assay.14

Total plasma homocysteine was measured using high
performance liquid chromatography without meth-
ionine loading. Methionine loading stresses the homo-
cysteine 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 B12 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 per-
formed 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, 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 B12, 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. 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. 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 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%), 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. 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, 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  | Claudicants    | CCLI           | AAA            |
| N             | 126           | 39             | 40             | 47             |
| Diabetes      | 43 (34%)      | 11 (29%)       | 15 (38%)       | 11 (23%)       |
| Smoking       | 79 (63%)      | 21 (53%)       | 27 (68%)       | 24 (52%)       |
| Hypertension  | 78 (62%)      | 22 (56%)       | 27 (68%)       | 22 (47%)       |
| Cholesterol (reported on treatment) | 46 (46%) | 16 (40%) | 20 (50%) | 18 (39%) |
| Cholesterol >5.5 mmol/l | 39 (31%) | 15 (38%) | 11 (29%) | 14 (29%) |
| Homocysteine >16 μmol/l | 46 (37%) | 10 (27%) | 20 (50%) | 25 (53%) |

CCLI, chronic critical limb ischaemia.
(folate, vitamin B<sub>12</sub> and vitamin B<sub>6</sub>) required for homocysteine metabolism may promote hyperhomocysteinaemia. Markedly elevated homocysteine concentrations have been observed in patients with nutritional deficiencies of the essential cofactor B<sub>12</sub> and the co-substrate folate. 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 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