Hyperhomocysteinaemia is Associated with the Rate of Abdominal Aortic Aneurysm Expansion

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Objectives. Previous literature has suggested an association between AAA and the presence of elevated plasma homocysteine levels (HCY). Homocysteine can stimulate elastolysis in the arterial media via activation of elastase and matrix metalloproteinases. No evidence in the literature exists correlating aneurysm expansion and HCY. The study objective is to identify whether the rate of AAA expansion is related to HCY.

Methods. 108 patients undergoing surveillance for AAA were identified at our vascular surgical unit. AAA size and growth rate were assessed by serial ultrasonographic measurements. Fasting total HCY levels were measured using fluorescence polarisation immunoassays. Demographic details and atherosclerotic risk factors were noted all AAA patients. A multivariate analysis was performed for growth rate vs. HCY, hypertension and hypercholesterolaemia. The correlation between AAA growth rate, AAA size and HCY levels were calculated.

Results. 60% of patients with AAA had some degree of hyperhomocysteinaemia (>15 µmol/l). Multivariate analysis showed HCY to be the only significant factor affecting AAA growth rate. A positive correlation was demonstrated between HCY levels and AAA growth rate using a linear regression model (R = 0.28, p = 0.003). Median growth rate among patients with hyperHCY was double that of patients with normal HCY (0.5 mm/month vs. 0.25 mm/month, p = 0.003). A growth rate of >10 mm/year was seen in 25% of hyper HCY patients and in only 2% of patients with normal HCY. In addition patients with hyper HCY and larger AAAs (>4 cm) had a growth rate twice as fast as patients with hyper HCY and AAAs <4 cm.

Conclusions. A correlation between HCY and growth rate exists, although this is weak due to the multifactorial aetiology of AAAs. HyperHCY patients have faster expansion rates than patients with normal HCY, with significant numbers demonstrating rapid expansion (>10 mm/year) and therefore an increased risk of rupture.

Keywords: Aorta; Aneurysm; Risk factors; Homocysteine.

Introduction

Homocysteine is a sulphur containing amino acid that is formed during the metabolism of the essential amino-acid methionine (Fig. 1). High levels of homocysteine (Hcy) in the blood (hyperhomocysteinaemia) have been associated with increased risks of developing vascular occlusive disease.2 Plasma Hcy is now regarded as an independent risk factor for atherosclerotic disease, with raised levels present in significant numbers of patients with peripheral, cerebrovascular and coronary heart disease (CHD).3–6 High values have also been proven to predict the failure of vascular intervention,7 and more rapid progression of CHD and peripheral vascular disease.8–10

Mild hyperhomocysteinaemia (Table 1) is present in 5–7% of the general population, due to either inherited or acquired dietary deficiencies of vitamin B12, and folic acid (co-enzymes which regulate pathways catalysed by methylenetetrahydrofolate reductase) or vitamin B6 (a cofactor for cystathionine β-synthase).11,12 Other causes of acquired elevated homocysteine include, renal failure, malignancy, hypothyroidism and use of folate and vitamin B6 antagonists.11

The prevalence of raised homocysteine levels in vascular patients has been demonstrated in a number of recent studies, with up to 50% of patients with aortic aneurysms having raised homocysteine levels but data is still relatively limited.13–15 The literature remains conflicting with respect to the possible role...
of Hcy in AAA expansion, with recent studies finding an association between Hcy levels and larger aneurysms, while others have found no connection between Hcy levels and AAA expansion. The aim of this study is to test the hypothesis that the level of plasma Hcy predicts the rate of expansion of AAAs.

Methods

The study was approved by the local research ethics committee, and informed consent was obtained from all participants. All patients with an AAA under surveillance from January 2004 to January 2006 at St James’ University Hospital were identified. Ultrasound scan reports of the aneurysms were collected, as were the patient demographics and risk factors. Inclusion criteria included the availability of at least two consecutive ultrasound scans, availability of clinical notes, and the adequate sampling of homocysteine levels as described.

Homocysteine levels were measured prospectively at the time of diagnosis of the AAA. Patients were fasted for 6 hours prior to venesection, and samples were concurrently taken for cholesterol, CRP and creatinine analysis. Homocysteine samples were collected in Vacutainer© tubes containing ethylenediamine tetraacetic acid (EDTA) and the plasma was separated by centrifuge within 30 minutes of venesection. Samples were stored at −80 °C. Total plasma homocysteine was the measured using a fluorescence polarisation immunoassay on an Abbott IMX analyser.

Statistical Methods

Data was collected using Microsoft Excel version X, GraphPad Prism version 4.00 for Mac OSX was used to analyse statistics.

Patients were divided into two groups, those with hyperhomocysteaemia (Hcy ≥ 15 μmol/l) and those within the normal range (Table 1). As the distribution of the patients age and Hcy levels was skewed toward higher values, analysis was based on medians and interquartile ranges. Subsequent analysis of the data began with calculation of the change in size of the infrarenal aortic diameter and the time in months between the scan performed at the time of the Hcy assay and latest scan for all patients who attended at least 2 follow-ups after venesection. The two patient groups and rate of expansion of aneurysms with elevated homocysteine were compared with those with normal levels using the Mann-Whitney U test.

Rapid expanders were compared with slow expanders across a range of independent factors that we hypothesized may be associated with rates of expansion. These factors were age (in 5-year strata), initial aortic diameter, smoking status (categorized into lifelong nonsmokers, ex-smokers, and current smokers), hypertension (treatment for and measured), presence of hypercholesterolaemia, history of coronary heart disease or stroke, presence of peripheral arterial disease and level of Hcy. All these factors were initially included in a multivariate logistic regression, with the use of SPSS software.

At the beginning of the study 10 subjects were selected at random for assessment of intra-observer and inter-observer agreement of aortic diameter measurements. Each patient was scanned three times by each operator as well as by a senior vascular sonographer. All scans were performed with the operators blinded to previous aortic diameter measurements. Nonparametric tests and SPSS software were used to compare mean intra-observer and inter-observer differences in aortic diameter measurements. No significant differences were found between observers, with 95% of differences in each of anteroposterior and transverse diameters being <3 mm.

Results

Over the study period, 202 patients were under surveillance at the vascular unit. Of these 108 were included in this study and 94 were excluded. Twenty
two patients were excluded due to lack of 2 ultrasound scan results, sixty two patients did not have homocysteine levels tested at the time of the study, six had renal failure prior to Hcy testing, and four patients did not have adequate clinical notes available.

A total of 26 women and 82 men were studied. Mean age was 76.07 (SD 6.93, range 59.51–94.42) years. The distribution of risk factors among the patients is summarised in Table 2 with no statistical difference between the two groups. Mean follow up time was 19.5 months (range 6–56 months).

Hyperhomocysteinaemia (Hcy ≥ 15 μmol/l) was found in 65 patients (60%) with AAA. There was no significant difference in size of the AAA at presentation between the patients with normal Hcy and those with elevated Hcy (4.1 cm vs. 4.2 cm, Mann-Whitney U test p = 0.28). There was, however, a significant difference in the rate of expansion of AAA between the two groups. Median monthly expansion among the patients with normal Hcy was 0.25 mm/month (range 0–1.5, yearly expansion rate 3 mm/year), whereas among the Hcy patients, the expansion rate was double (0.5 mm/month, yearly expansion rate 6 mm/year, Mann-Whitney U test p = 0.003). More significantly 25% of aneurysm patients with an elevated Hcy had an annual growth rate of >10 mm/year compared to 2% of patients with normal Hcy. There was no difference in growth rate between AAA <4 cm and >4 cm in patients with normal Hcy. Patients with elevated Hcy and aneurysms >4 cm had growth rates which were double those of patients with normal HCY (0.6 mm/month vs. 0.3 mm/month, Mann-Whitney U test p = 0.04) (Table 3).

Multivariate analysis of growth rate vs. Hcy, hypertension, smoking and hypercholesterolaemia showed that Hcy was the sole significant factor affecting AAA expansion (p = 0.004, B = 0.03, 95% CI for B 0.013–0.065). In addition, a significant correlation between levels of homocysteine and growth rate was found (r = 0.34, p = 0.003). A significant correlation was also found between homocysteine levels and growth rate and AAA size. The correlation was weaker and less significant in the case of the correlation between homocysteine and AAA size (r = 0.28, p = 0.003). This analysis confirms reports from other studies that the rates of expansion of small screen–detected AAA are low.19,20 The median annual increase in aortic diameter was 3 mm for patients with normal homocysteine levels, rising to 6.0 mm for patients with hyperhomocysteinaemia (HyperHcy). For most individual patients, these increments are within measurement error for ultrasound scanning.

Table 2. Summary of results comparing growth rate and AAA size

<table>
<thead>
<tr>
<th>Growth Rate</th>
<th>Growth Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy &lt;15</td>
<td>Hcy ≥15</td>
</tr>
<tr>
<td>(mm/month)</td>
<td>(mm/month)</td>
</tr>
<tr>
<td>AAA &lt;4 cm</td>
<td>AAA ≥4 cm</td>
</tr>
<tr>
<td>0.25</td>
<td>0.3</td>
</tr>
<tr>
<td>AAA &gt;4 cm</td>
<td></td>
</tr>
<tr>
<td>0.3 (n = 23)</td>
<td>0.6 (n = 42)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.57</td>
</tr>
<tr>
<td>(Mann-Whitney)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Summary of risk factors and demographics for patients with and without hyperhomocysteinaemia

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Homocysteine &lt;15</th>
<th>Homocysteine ≥15</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients</td>
<td>108</td>
<td>43 (40%)</td>
<td>65 (60%)</td>
</tr>
<tr>
<td>Males</td>
<td>82 (76%)</td>
<td>52 (80%)</td>
<td>30 (70%)</td>
</tr>
<tr>
<td>Mean Age</td>
<td>76.1 ± 9.3</td>
<td>75.4</td>
<td>76.5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>15 (14%)</td>
<td>7 (16%)</td>
<td>8 (12%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60 (54%)</td>
<td>23 (53%)</td>
<td>37 (57%)</td>
</tr>
<tr>
<td>Hypercholesterolaemia/ Statin</td>
<td>56 (52%)</td>
<td>24 (56%)</td>
<td>32 (49%)</td>
</tr>
<tr>
<td>PVD</td>
<td>29 (27%)</td>
<td>12 (28%)</td>
<td>17 (26%)</td>
</tr>
<tr>
<td>IHD</td>
<td>49 (45%)</td>
<td>20 (47%)</td>
<td>29 (45%)</td>
</tr>
<tr>
<td>Smokers</td>
<td>34 (31%)</td>
<td>14 (33%)</td>
<td>20 (31%)</td>
</tr>
<tr>
<td>CVA</td>
<td>11 (10%)</td>
<td>4 (9%)</td>
<td>7 (11%)</td>
</tr>
</tbody>
</table>

Using Spearman Rank Correlation (Spearman’s Rho = 0.28, two tailed p = 0.003) was found.

Discussion

Inflammation is now considered to be of fundamental importance in the pathogenesis of aortic aneurysms. Recent studies have demonstrated that Hcy induces the synthesis of serine elastase in arterial smooth muscle cells, causing elastolysis by degradation of the extracellular matrix and release of elastin peptides chemotactic to smooth muscle cells.21,22 In addition increased binding and activation of matrix metalloproteinases 2 and 9 (MMP-2, MMP-9) occurs in hyperhomocysteinaemia, leading to further elastin/fibrillar collagen degradation in the tunica media of AAAs.23,24

Multivariate analysis of the data comparing growth rate and Hcy, hypertension and high cholesterol showed that Hcy levels were the sole factor that significantly contributed to growth rate (p = 0.004). No independent association with smoking was found, as has been reported elsewhere.18,25 This may be due to the relatively small proportion of current smokers in the study. Hypertension has been documented as a risk factor for AAA in several studies, it is surprising, therefore, that hypertension is not a risk factor for expansion of small aneurysms.26 In the case of this study, it may be because the majority of patients with hypertension are adequately treated, thereby minimizing the impact of this risk factor.

In contrast to previous studies, this work has demonstrated an association between hyperHcy and AAA expansion.18 The correlation is weak and most likely to be more complicated than a linear fit. This is to be expected given the multi-factorial nature of the condition and the need for further research into the subject. Median AAA growth rates in hyperHcy

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were, however, significantly greater ($p = 0.003$) than median growth rates in patients with normal homocysteine levels. The results also showed that a significant proportion of patients with hyperHcy (25%) had rapid growth rates of above 10 mm/year, a rate associated with higher risk of rupture. Those with larger aneurysms (>4 cm) are at greater risk as they were found to have the highest median growth rate. As already mentioned, previous studies have shown a correlation between Hcy levels and AAA size, however, no previous link has been made between size, Hcy level and rate of expansion.17 

The detection of elevated levels of Hcy in patients with large, symptomatic, or ruptured AAA is unlikely to change the treatment of such patients because they need repair of the AAA. However, it is in the management of small (30 to 54 mm in diameter) AAAs that Hcy levels may have a role. Although surgery is not indicated for these AAAs, a proportion will expand until they are large enough to warrant elective intervention before rupture occurs.27 Current practice is to monitor the diameter of AAAs with periodic ultrasound or CT scanning, but our understanding of the natural history of these aneurysms remains incomplete. The identification of risk factor such as Hcy in this study, that are associated with greater rates of expansion may help in the planning of surveillance of AAAs, the identification of high-risk patients who may benefit from early intervention, and possibly the development of strategies to prevent expansion.

References
18 Lindholt JS, Johansen B, Sei GP, Hennekens EW. Relationships between activators and inhibitors of plasminogen, and the levels of homocysteine, lipoprotein (a) and plasminogen activator inhibitor-1 are present in patients with abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 2003 Jun;25(6):546–551.

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