Eur J Vasc Endovasc Surg **27**, 75–79 (2004)
doi: 10.1016/j.ejvs.2003.09.001, available online at http://www.sciencedirect.com on SCIENCE DIRECT®

Abdominal Aortic Aneurysm and its Correlation to Plasma Homocysteine, and Vitamins

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Background. *Hyperhomocysteinemia is a recognised independent risk factor in the genesis of atherosclerotic diseases. However, very little is known about the relationship between homocysteine and abdominal aortic aneurysm (AAA). Vitamins, namely B_{12} and folic acid have been implicated in the regulation of plasma homocysteine levels. However, there has been no prospective study that has analysed the relationship of AAA and plasma homocysteine in light of serum vitamin levels.*

Aims. *To study the relationship between plasma homocysteine, serum B_{12} and folic acid levels, and AAA.*

Method. *Case control study including 38 AAA patients and 36 controls. Fasting homocysteine, B_{12} and folic acid were determined in serum separated within 1 h of blood collection using a fluorescence polarisation immunoassay technique (FPIA).*

Results. *Twenty-six (68%) of the AAA patients had elevated levels of homocysteine compared to 2 (6%) in the case control group. The mean homocysteine level in the AAA group was 19.4 μmol/L (SE ± 1.1) (95% CI 17.17–21.65) and in the control group was 10.9 μmol/L (SE ± 1) (95% CI 9.95–11.88) ($p < 0.001$). Mean vitamin B_{12} levels in the AAA and the controls was 332.11 pg/L (SE ± 16.44) and 414.33 pg/L (SE ± 19.72), respectively ($p < 0.004$). Mean folic acid in the AAA was 8.02 (SE ± 0.71) and the control was 9.8 ηgm/L (SE ± 0.69), (ns).*

Conclusion. *This study confirms significantly higher levels of plasma homocysteine in AAA patients but lower levels of B_{12}. Use of supplemental vitamins that should lower plasma homocysteine may modify vascular disease progression. Clinical trials in this direction are warranted.*

Key Words: Abdominal aortic aneurysm; Total plasma homocysteine; Vitamin B_{12} and folic acid.

Introduction

Homocysteine biochemistry is complex, with the plasma total homocysteine (THCY) being the sum of homocyst(e)ine, and its oxidised forms homocystine, and the homocysteine—cystine mixed disulphide, in free and protein bound forms.[1] It is a sulphur containing amino acid, elevated plasma levels of which are referred to as hyperhomocysteinemia. It is an intermediary product in methionine metabolism. The transfer of methyl group from methionine is an important step in the metabolism of nucleic acids, fats, and high-energy bonds. When methionine donates its methyl group, homocysteine is formed. The majority of homocysteine is recycled in a transmethylation reaction involving vitamin B_{12} and folic acid while a smaller amount is metabolised by transulphuration involving vitamin B_6. Hence the vitamin intake and serum vitamin levels play an important part in the regulation of plasma homocysteine levels.

It was the observation that patients presenting with homocystinuria, an autosomal recessive metabolic disorder leading to raised plasma homocystine, also displayed premature vascular disorders that drew attention to the causal relationship between vascular disorder and homocystine.[2–4] It was subsequently shown in experimental models that intravenous infusion of homocysteine caused endothelial vascular injury and atherosclerosis.[5] Further studies demonstrated elevated levels of homocysteine as a risk factor in the causation of coronary artery,[6] cerebrovascular[7] and peripheral vascular diseases,[8] independent of other risk factors such as age, gender, lipids, lipoproteins, cholesterol, hypertension and smoking.[9–11] There is currently very little data that has examined the possible relationship between plasma homocysteine and the development of abdominal aortic aneurysm (AAA)[12] and serum vitamin levels. Therefore, this study was designed to investigate possible

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1078–5884/000075 + 05 $35.00/0

relationship between THCY, levels of serum vitamin B_{12} and folic acid, and AAA.

Materials and Methods

Study design

This was a case-control study in which patients were selected into two groups. Group 1 (AAA group) consisted of patients with AAA (AP diameter ≥ 3 cm) diagnosed on ultrasound scan (USS) (using Gray-scale B-mode images) of the abdomen. Group 2 (Control group) consisted of individuals free from any known AAA (excluded using an ultrasound abdominal scan), and free of obvious peripheral vascular disease, assessed clinically and on the basis of ankle brachial ratio >0.9. These individuals were randomly selected from the open access aneurysm USS screening programme.

Exclusion criteria

Patients who had not fasted for 8 h prior to blood sample withdrawal.
Patients who were taking any vitamin supplementation—prescribed or self medicated.
Patients whose blood sample could not be centrifuged for separation of serum within 1 h of collection.

Collection of samples

Approximately 4 mL of venous blood were taken from the antecubital vein into a vacutainer tube containing EDTA (Beckton-Dickinson). The sample was immediately transferred to an ice bag and taken to the biochemistry laboratory. Within 60 min of collection, the blood samples were centrifuged at 3000 revolutions per min (rpm) for 5 min, to separate the supernatant serum from the cells. This serum was stored at $-80\,^{\circ}C$ prior to the analysis of plasma homocysteine concentration.

For vitamin B_{12} and folate analysis, serum was obtained from a second EDTA tube using identical methodology.

Biochemical analysis

Homocysteine
Fasting homocysteine plasma levels were analysed using a fluorescence polarisation immunoassay (FPIA) technique for the quantitative measurement of total homocysteine (Imx system, Axis Biochemicals, ASA Ulvenvein, Norway). Plasma homocysteine levels >15 μmol/L were taken as abnormally elevated.

Vitamins
The vitamins B_{12} and folic acid were measured with an automated immunoassay analyser, using chemiluminescence detection technique (Bayer ACS 180, Newbury, Berkshire, UK). The normal range of vitamin B_{12} was taken as 160–350 pg/L and that of folic acid was 3.5–15 ηg/L.

In addition, the urine specific gravity and the haematocrit were ascertained to facilitate correction for concentration factor difference between the two groups that could have been induced due to overnight fasting.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to test the significance of differences in plasma total homocysteine, vitamin B_{12} and folic acid between the AAA and control groups. Data are presented as mean ± standard errors (SE) of the mean. Differences were considered significant at the 95% level ($P < 0.05$). Difference in the characteristics or risk factors in the AAA and control groups were analysed using Chi-square and Fisher's exact test. All statistical tests were performed using SPSS computer software package.

Results

There were a total of 74 patients, 38 in the AAA group with a mean age of 70 years (range 53–79), and 36 in the control group with a mean age of 66 years (range 48–79).

The characteristics of patients in the AAA group and the control group are summarised in Table 1.

Twenty-six (68%) of the AAA patients had elevated

Table 1. Characteristics of patients in the AAA group and the control group.

Characteristics	AAA	Control	'*p*'
Hypertension	13	12	0.955
Hyperlipidaemia	4	3	0.769
Diabetes	3	4	0.677
COAD	3	0	0.097
Cardiac disease	24	9	0.038
Stroke	5	1	0.130
Family history of AAA	4	0	0.057
Constipation	8	2	0.087
Cancer	3	3	0.949
Benign hypertrophy of prostate	8	6	0.690
Smoking	13	12	0.955
Alcohol	7	9	0.581

levels of homocysteine compared to 2 (6%) in the case control group (Fig. 1). The mean THCY concentration for patients in the AAA group was 19.4 μmol/L (95% CI 17.17–21.65) and for patients in the control group 10.9 μmol/L (95% CI 9.95–11.88) (Table 2). The difference between the two groups was highly significant ($p < 0.001$). However, there was no correlation between the size of the AAA and the plasma THCY concentration (Fig. 2). In the AAA group the mean homocysteine levels for patients ≥70 years compared to those <70 years was 19.2 and 19.6 μmol/L, respectively (p = ns). For the control group these values were 11.3 vs. 10.3 μmol/L (ns).

There were 35 males and 3 females in the AAA group and 21 males and 15 females in the control group. The mean homocysteine for males in AAA group was 19 μmol/L and for females was 23.4 μmol/L. The mean homocysteine for males and females in the control group was 11.5 vs. 10.2 μmol/L, respectively. These differences were not significant. Thus there was no correlation between homocysteine levels and age for either the AAA or control groups nor were there any relationship between gender for either group.

The mean vitamin B_{12} in the AAA group was 332.11 pg/mL (SE ± 16.44) and in the control group was 414.33 pg/mL (SE ± 19.72). The difference between the two groups was highly significant ($p < 0.004$). The mean folic acid in the AAA group was 8.02 ηg/mL (SE ± 0.71) and control was 9.8 ηg/mL (SE ± 0.69). However, there was no statistical difference in the folic acid levels between the two groups.

The urinary specific gravity and the haematocrit were similar in the two groups (1015 in AAA group and 1018 in control group), indicating the comparability of the two groups in terms of hydration status.

RESULTS

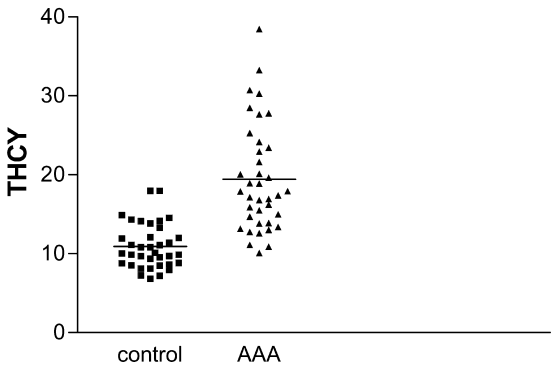

Fig. 1. Plasma homocysteine (THCY) (μmol/L) in the controls, and patients with AAA. The transverse line in the scatterplot represents the median value.

Discussion

The primary finding of this study is that THCY levels of patients with AAA are significantly higher than those of the control group. The importance of homocysteine in coronary artery disease, cerebrovascular disease and peripheral vascular disease is well documented.[6–8] However, current literature on the role of homocysteine in the aetiopathogenesis of AAA is limited.[12–15] Moreover, 26 of the 38 (68%) patients in the AAA group had raised plasma homocysteine compared to only two of the 34 (6%) patients in the control group. Authors have found a prevalence of hyperhomocysteinemia in 40% patients with peripheral vascular disease[9] and 48% in patients suffering from AAA.[12] Our data suggests that there may be a higher prevalence of hyperhomocysteinemia in AAA than previously believed. Our data of a 6% prevalence of hyperhomocysteinemia in control population is very similar to 5 and 7% reported in previous studies.[16,17] In the AAA group, we found no correlation between the size of the AAA and the levels of homocysteine. Though there is evidence that homocysteine is associated with aortic atherosclerosis[18] and AAA,[12] it is possible that hyperhomocysteinemia initiates the atherosclerosis, but once an aneurysm is established any subsequent dilatation is more of a mechanical event in relation to the blood pressure in the AAA, and with time the AAA progressively dilates.

Homocysteine causes elastolysis and damage to tunica media by activation of matrix metalloproteinases (MMP-2) and serine elastase; damage to the endothelium by impaired production of nitric oxide leading to atherosclerosis and aneurysm formation.[19–22]

Since elevated plasma homocysteine levels bear an inverse relation to the serum vitamins, plasma homocysteine levels may be lowered by supplemental vitamins thus preventing AAA formation—'a risk factor reduction'.

Four patients in the AAA group had familial history of AAA against none in the control group. This raises the question whether the familial nature of AAA is due to higher plasma homocysteine, as hyperhomocysteinemia can itself be familial. It is well known that patients with homocysteinuria, an autosomal metabolic disorder leading to elevated plasma homocysteine, develop premature vascular disorder.[2,3]

Plasma homocysteine levels, with reference to vascular disease, have been found to increase with age in some[23,24] but not all the previous studies.[25,26] We found no such association when we analysed the plasma homocysteine levels in relation to age, in either the AAA or the control group. There may have been

Table 2. Plasma homocysteine and vitamins—B_{12} and folic acid, in AAA and control.

Mean values	AAA	Control	'*p*' value
Mean homocysteine (μmol/L)	19.4 (SE 1.1)	10.9 (SE 0.47)	<0.001
Mean vitamin B_{12} (pg/mL)	332.11 (SE 16.44)	414.33 (SE 19.72)	<0.001
Mean folic acid (ηg/mL)	8.02 (SE 0.71)	9.8 (SE 0.69)	= 0.126 (ns)

other factors at play such as nutrition status, in terms of methionine-rich diet, in the younger groups of patient.

Similarly, the homocysteine levels have been shown to be greater in male than female subjects with vascular diseases by most authors[27–29] but some have shown no difference or marginally higher homocysteine levels in females than in males.[30,31] Interestingly, in the AAA group in our study, the mean homocysteine levels in males were lower in comparison to females but this failed to reach statistical significance. Moreover, any meaningful conclusion about gender differences in homocysteine levels cannot be derived from our data due to the small sample size of the female population.

It is known that enzymatic reactions involving folic acid and vitamin B_{12} helps in the formation of methionine by remethylation of excess homocysteine, thus preventing hyperhomocysteinemia. Vitamin B_6 assists in the breakdown of excess homocysteine into cysteine in an irreversible trans-sulphuration reaction and thus maintains normal homocysteine levels. Previous studies have examined the relationship between folic acid, B_{12} and their influence on the homocysteine levels.[28,32–34] It is well documented that hyperhomocysteinemia is associated with deficiencies of vitamin B_{12} and folate levels. The strongest inverse correlate for patients with coronary disease being between homocysteine and folate.[35–37]

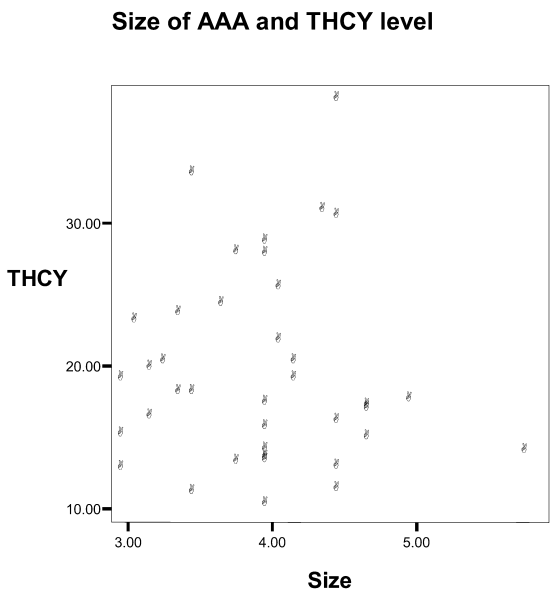

Fig. 2. Plasma homocysteine (THCY) (μmol/L) on the *Y* axis and size of the AAA (cm) in individual patients in this group on the *X* axis. There is no relationship.

We found that the mean vitamin B_{12} levels were significantly higher in the AAA group than the control group, although there was no such relationship for folic acid. It is difficult to explain as to why vitamin B_{12} were generally high. The dietary habits of the subjects that could have influenced the serum vitamin levels, were not known and this we think are one drawback of this study. Perhaps the baseline calibration needed to be re-assessed or the normal range re-defined. Despite this, the vitamin B_{12} levels were significantly higher in the control group vs. the AAA group.

The folic acid levels were reduced in the AAA group vs. the control group but failed short of reaching the statistical significance. Perhaps, a larger sample size may have made this more apparent.

Our data suggests that the homocysteine levels are more susceptible to changes in vitamin B_{12} levels than folic acid levels although, previous studies have shown folate deficiency may be more important than vitamin B_{12} deficiency in influencing the plasma homocysteine levels and vascular diseases.[35,36]

In conclusion, we have demonstrated a positive correlation between the presence of AAA and elevated plasma homocysteine. In order to confirm the relationship, long-term population studies will be required to establish whether patients at high risk of AAA can be prevented from developing aneurysm by selected vitamin supplements.

Acknowledgements

The authors would like to thank Ms S. White for doing the statistical analysis.

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Accepted 9 September 2003