CLINICAL STUDY

Plasma total homocysteine and retinal vascular disease

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Abstract

Purpose **Hyperhomocysteinaemia has been linked to macrovascular disease. Our aim was to investigate whether there is a relationship between fasting plasma total homocysteine levels and retinal vascular disease.**

Methods **We measured the homocysteine levels in 70 patients with arterial or venous retinal vessel occlusion and compared them with the levels in 85 controls without evidence of ischaemic heart disease. Homocysteine levels were determined by high-performance liquid chromatography with electrochemical detection and compared after logarithmic transformation.**

Results **Homocysteine levels were found by univariate analysis (unpaired two-tailed *t*-test) to be significantly higher in the group with retinal artery occlusion than the group with retinal vein occlusion ($p = 0.045$) and in both groups compared with controls (18.4 and 13.8 vs 9.5 μmol/l; $p = 0.0002$ and < 0.0001, respectively). The controls, however, were significantly younger than the subjects (51.5 ± 15.4 vs 66.2 ± 11.9 years; $p < 0.0001$), but analysis of the results by age revealed significant differences between the groups and controls for the seventh decade (vein occlusions, $p = 0.05$) and for the eighth decade (artery occlusions, $p = 0.037$). Subgroup analysis of the retinal vessel occlusion group revealed significant differences in mean blood pressure between those with branch retinal vein occlusions (175/100 mmHg) and both those with central retinal vein occlusions (155/88 mmHg) and those with retinal artery occlusions (157/86 mmHg). Both vein occlusion subgroups also differed significantly with regard to homocysteine levels, branch < central (12.2 ± 1.3 vs 15.0 ± 1.6 μmol/l, $p = 0.03$). Multiple linear regression analysis revealed significant relationships between homocysteine levels and the presence of retinal vessel occlusion ($p = 0.0002$), serum creatinine ($p = 0.001$) and age ($p = 0.003$), but not gender.**

Conclusions **We conclude that homocysteine may be a risk factor for retinal vascular disease and could be simply and cheaply treated with folate and vitamins B_6 and B_{12}.**

Key words Homocysteine, Microvascular disease, Retinal artery occlusion, Retinal vein occlusion

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Homocysteine (HCySH) is a thiol-containing amino acid which is not incorporated into protein. It is an intermediary metabolite generated by the demethylation of methionine when methyl groups are transferred to DNA or proteins intracellularly. Its fate is either to be remethylated to methionine or to undergo trans-sulphuration to cysteine and further metabolism to CO_2, NH_4, H_2O and SO_4. Small amounts of HCySH leak out into the plasma, however, where it is thought to be atherogenic. The evidence for this comes from homocystinuria, an inherited metabolic disease associated with the development of premature, aggressive arterial and venous disease, where the only biochemical abnormality is very high plasma levels of HCySH. In recent years, macrovascular diseases such as stroke,[1] early-onset peripheral vascular disease[2] and recurrent deep venous thrombosis[3] have also been linked to mildly elevated plasma homocysteine levels (> 15 μmol/l) with about a threefold increased relative risk. The absolute difference in mean HCySH levels between the affected groups and controls in these studies has been small (1–3 μmol/l), suggesting that HCySH is a potent atherogenic agent. There have also been studies of homocysteine levels in patients with myocardial infarction, but the evidence is conflicting,[4,5] and there have only been isolated case reports of elevated HCySH levels in microvascular disease.[6,7] Homocysteine, therefore, may not be a risk factor for all types of atherosclerotic disease.

Arterial or venous retinal vascular occlusive disease (RVOD) is a microvascular disease often presenting as sudden-onset visual loss and has previously been associated with diabetes, hypertension and hyperlipidaemia.[8] Our aim was to investigate whether fasting plasma total HCySH levels are elevated in retinal microvascular disease.

Materials and methods

Subjects

Seventy consecutive patients presenting with RVOD were recruited from the Medical Ophthalmology clinic at Heartlands Hospital in Birmingham. All subjects underwent complete ophthalmological and medical examinations

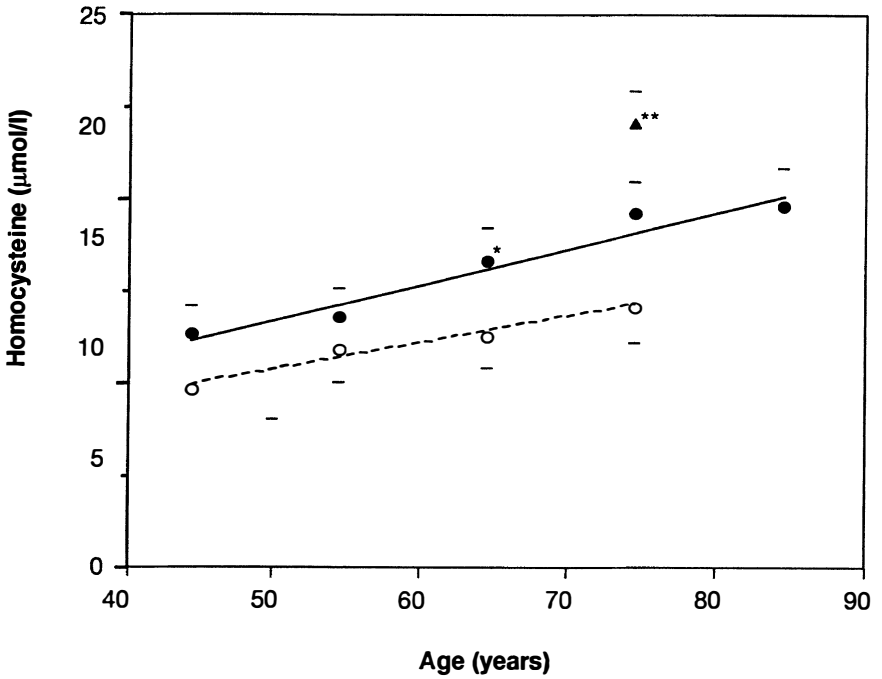

Fig. 1. *The distribution of mean homocysteine values as a function of age in decades for controls (open circles), subjects with retinal vein occlusion (filled circles) and the retinal artery occlusion group (filled triangle). One standard deviation from the mean is shown for each point.* *p = *0.05*; **p = *0.037*.

with particular emphasis on cardiovascular status and drug, smoking and alcohol history. Clinical parameters, including body mass index, blood pressure and the presence or absence of peripheral pulses, were recorded. The diagnosis was confirmed by dilated slit-lamp biomicroscopy and/or fluorescein angiography. Routine biochemical measurements of renal and liver function were performed, together with serum lipids and glucose. Full blood counts and plasma viscosities were determined by the Haematology Department. Thirty-six patients had central retinal vein occlusions (CRVO), 24 had branch retinal vein occlusions (BRVO) and the remaining 10 had retinal artery occlusions (RAO).

Controls

The 85 controls were laboratory staff and patients without known ischaemic heart disease attending other outpatient clinics at Heartlands Hospital.

Measurement of homocysteine

HCySH was measured by high-pressure liquid chromatography (HPLC) with electrochemical detection, as described previously.[9] Basically, plasma samples were reduced with dithiothreitol to liberate homocysteine, the protein was precipitated by sulphosalicylic acid and the supernatant analysed.

Statistics

All univariate analyses were by unpaired two-tailed *t*-test (Astute software) and HCySH values were logarithmically transformed to normalise the data. Multivariate analysis (SPSS software) was by multiple linear regression with log HCySH as the dependent variable, RVOD and gender as dichotomous variables (present/absent, male/female) and age, systolic blood pressure, creatinine and cholesterol levels as continuous variables.

Approval for the study was obtained from the hospital ethics committee.

Results

We measured the fasting plasma total HCySH levels in a group of consecutive patients with RVOD and compared these with the controls. All the groups had equal male/female ratios and there were no significant differences between the RVOD subgroups in age, serum total cholesterol, triglycerides, glucose, cigarettes smoked or ethanol intake. Table 1 shows that the control group, however, was significantly younger than the RVOD group ($p < 0.0001$).

Univariate analysis revealed that HCySH levels were significantly raised in patients compared with controls (RAO 18.4 ± 1.5 and all RVO 13.8± 1.5 vs 9.5 ± 1.5 μmol/l; $p = 0.0002$ and < 0.0001, respectively). As the control group was younger and HCySH levels are known

Table 1. *Mean values and standard deviations for age, plasma total homocysteine and serum creatinine in patients with retinal vascular disease and controls*

	Age (years)	HCySH (μmol/l)	Creatinine (μmol/l)
Controls	51.5 ± 15.4	9.5 ± 1.5	105.8 ± 18.9
RAO	69.8 ± 6.74*	18.4 ± 1.5**	115.1 ± 23.7
RVO	65.6 ± 12.5*	13.8 ± 1.5*	97.7 ± 31.1

*$p < 0.0001$ vs control.
**$p = 0.0002$ vs control.

Table 2. *Multiple linear regression analysis with log homocysteine as the dependent variable*

Variable	*B*	95% confidence intervals for *B*	*p*
RVOD	0.119878	0.057825 – 0.181930	0.0002
Creatinine	0.003243	0.001348 – 0.005137	0.0010
Age	0.004605	0.001595 – 0.007614	0.0030
Gender	0.003449	–0.062867 – 0.069766	0.9181

The multiple *R* value was 0.607.
RVOD, retinal vascular occlusive disease.

to increase with age we reanalysed the data grouping patients and controls into decades of age. Fig. 1 shows that HCySH levels tend to increase linearly with age in both the RVO group and controls (7/10 of the RAO group was aged between 70 and 79 years and are shown as a single point). Comparing the means for each group by age yielded significant differences between RVO ($n = 13$) and controls ($n = 22$) for the seventh decade ($p = 0.05$) and between the RAO group ($n = 7$) and controls ($n = 7$) for the eighth decade ($p = 0.037$).

Further analysis of the RVOD group showed that those patients with CRVO ($n = 36$) had significantly higher HCySH levels than BRVO patients ($n = 24$) (15.0 ± 1.6 vs 12.2 ± 1.3 μmol/l, $p = 0.03$) and that the ten RAO patients also had significantly higher HCySH levels than the BRVO group (18.4 ± 1.5 vs 12.2 ± 1.3 μmol/l, $p = 0.008$). There were no differences between the groups with respect to race, age, total cholesterol, triglycerides, cigarette smoking or ethanol consumption; however, both the mean systolic and diastolic blood pressures were significantly elevated in the BRVO group (175.4 ± 25.6/100.8 ± 11.4 mmHg) compared with either the CRVO group (154.7 ± 28.9/88.0 ± 14.7 mmHg) or RAO group (157.4 ± 21.4/86.3 ± 11.7 mmHg) as has previously been reported.[8]

Multiple linear regression analysis of the results from both the subjects and the controls revealed that the presence of retinal vessel occlusion ($p = 0.0002$), serum creatinine ($p = 0.001$) and age ($p = 0.003$) were all significantly linked to HCySH levels, while gender was not (Table 2). In patients with RVOD, age and creatinine level were still significantly related to HCySH levels, while other variables such as systolic blood pressure, total cholesterol and diagnosis (RAO, CRVO or BRVO) were not (not shown). The individual coefficients translate into a mean increase of 32% in the homocysteine levels (95% confidence interval (95% CI) 14–52%) for the RVOD group compared with controls, while HCySH increased by 1.07% (95% CI 0.37–1.77%) for each year of age and by 0.75% (95% CI 0.31–1.19%) for each micromole per litre of serum creatinine.

Discussion

These results demonstrate for the first time that fasting plasma total HCySH levels are significantly elevated in microvascular disease, the differences between the patient and control means (2.7–8.9 μmol/l) being consistent with other studies linking HCySH with macrovascular disease. This difference in means between patients and controls cannot be accounted for by the effects of age or renal function. The HCySH levels seen in our control group were similar to previously reported values,[10] confirming that our control group is representative and that there is an effect of age on HCySH metabolism. One suggestion, based on tissue culture experiments with cystathionine β-synthase, is that there may be a decline in enzyme activity with age[11] which could lead to increased plasma HCySH levels.

Previous reports of retinal vessel occlusion in young adults (defined as < 50 years of age) have found less of an association between RVOD and systemic disease,[12] with the result that some investigators consider it to be a different disease from RVOD in the over-fifties. Our results show that HCySH levels are elevated in RVOD regardless of the age at presentation.

The subgroup analysis of patients showed that the HCySH levels were highest in RAO, and lowest in BRVO. As previously reported, the BRVO group were significantly hypertensive compared with the other groups and this has been proposed as the main cause of BRVO.[8,13] In this study, we found that the HCySH level in BRVO was still significantly higher than in the controls (12.2 vs 9.5 μmol/l; $p = 0.001$) despite being lower than the levels found in CRVO (15.0 μmol/l) or RAO (18.4 μmol/l). The data therefore suggest that HCySH may be an independent risk factor for retinal vascular disease and an additional risk factor to hypertension in BRVO.

Finally, should we treat moderate hyperhomocysteinaemia? The risk of recurrence of RVOD in the fellow eye has been estimated to be 15% over 5 years.[14,15] Moderate hyperhomocysteinaemia has been linked to generalised macrovascular disease in adults and RVOD patients are known to have a high prevalence of macrovascular disease end-points, with excess cardiovascular causes of death on long-term follow-up.[15,16] Treatment to reduce homocysteine is relatively simple with small doses of folate and vitamins B_6 and B_{12}, a combination that is both inexpensive and harmless.[17] Prospective trials of B vitamin supplements are now under way for cardiovascular disease, which, if successful, will suggest that B vitamins should also be used to treat RVOD.

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