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Plasma total homocysteine and subarachnoid haemorrhage in a co-factor replete population

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Summary. Mild hyperhomocysteinaemia is a postulated risk factor for occlusive vascular disease, including stroke. Subarachnoid haemorrhage (SAH) has an annual incidence of 10–20 per 100,000 and accounts for 5–10% of all strokes. Measurement of plasma total homocysteine (tHcy) in a cohort of vitamin B12 and folate replete patients did not reveal any association between tHcy and the aetiology of SAH.

Keywords: Amino acids – Homocysteine – Subarachnoid haemorrhage – Stroke – Folate – Vitamin B12

Introduction

The high prevalence of vascular complications in severe hyperhomocysteinaemia in homozygotes for cystathionine-β-synthase deficiency, (CBS; or homocystinuria [HCU]) and methylene tetrahydrofolate reductase (MTHFR) deficiency focused attention on homocysteine as an atherogenic and thrombophilic agent (McCully and Wilson, 1975). Additionally, epidemiological studies have revealed a significant association between mild hyperhomocysteinemia and occlusive vascular disease, including stroke (Brattstrom and Lindgren, 1992; Graham et al., 1997). Subarachnoid haemorrhage (SAH) has an annual incidence of 10–20 per 100,000 (Linn et al., 1996) and accounts for 5–10% of all strokes (Bamford et al., 1990). The modifiable risk factors for SAH include those known to correlate with tHcy in other vasculopathies, e.g. smoking, hypertension and alcohol intake (DeRose et al., 2000). It has been suggested that patients who smoke or are hypertensive should be screened for hyperhomocysteinaemia as a possible additional risk factor that may potentiate arteriosclerosis. In the absence of the primary

genetic defects, CBS and MTHFR deficiency, the other main determinants of plasma tHcy are the nutritional co-factors for CBS and MTHFR, i.e. vitamin B6 and, particularly, vitamin B12 and folate. In this study we aim to assess the possible involvement of tHcy in the aetiology of SAH in a B12 and folate replete population.

Materials and methods

This was a prospective, cross-sectional study in which 36 consecutive patients (26 female, 10 male), who presented to the hospital with a SAH, or who were reviewed in the clinic with a previous SAH, had plasma tHcy, vitamin B12 and red cell folate assayed. All patients underwent cerebral angiography. This showed that 24 patients had intracranial aneurysms, 8 had suffered an aneurysm negative SAH and 4 patients had an underlying arteriovenous malformation. One patient with an aneurysm positive SAH was excluded from the study on the basis of poor vitamin B12 status (tHcy 44 umol/L). The remaining patients comprised 25 females mean age 55 years (21–98) and 10 males, mean age 49 years (23–98).

A control population of 32 males, mean age 43 years (17–98) and 32 females, mean age 43 years (17–98) with no co-factor deficiency were also assayed and compared to well established age and sex matched ranges for tHcy (Fermo et al., 1993). These were patients attending for investigation of neurological symptoms, including peripheral neuropathy, ataxia and dystonia and in whom any biochemical pathophysiology, including low B12 and folate, had been excluded as part of their routine investigation.

Blood was taken, without haemolysis and before medical intervention, into lithium heparin and separated within 30 minutes. Plasma was stored at $-20°C$ prior to analysis. Total homocysteine was assayed by automated ion-exchange chromatography (Briddon, 1998) using a Biochrom 20 amino acid analyser (Amersham Pharmacia Biotech, Amersham, UK). Vitamin B12 and erythrocyte folate were measured using the Abbott IMX assay according to the manufacturers protocol (Abbott Diagnostics, Maidenhead, UK). The lower limit for B12 was taken to be 220 ng/L (Patel and Briddon, 2000) and folate 186 μg/L.

Comparison between multiple groups was by analysis of variance (ANOVA) and between groups by two-tailed, unpaired Students' t-test. Significance levels were set at 0.05.

Results

Control data were in excellent agreement with previously reported ranges (Fermo et al., 1993) and confirmed that male values are higher and more variable with age (Fig. 1). Analysis of variance between the 3 SAH groups revealed no significant difference, $F = 1.148$ ($F_{critical} = 3.294$), $P = 0.329$. In particular, no difference was found between aneurysm positive and negative patients ($P = 0.28$). The 3 groups were therefore considered as a single population for comparison against the control group, although age and sex matched. 72% of the SAH patients were female with lower tHcy values than the males (Fig. 1). The mean value for male SAH was 9.8 (SD 3.63) and for female SAH 7.8 (SD 2.56). We found no significant difference between these and sex matched controls, $P_{male} = 0.97$ and $P_{female} = 0.89$ (Table 1).

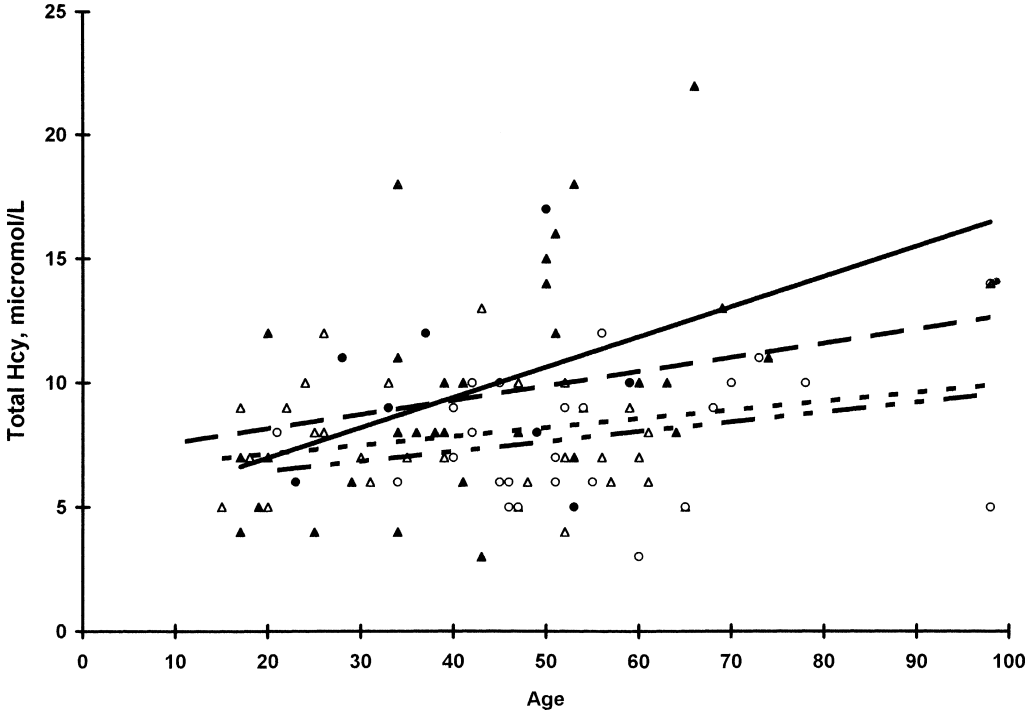

Fig. 1. Age and sex versus total homocysteine, showing trend lines for control male (———▲), SAH male (— —●), control female (– – – -▽) and SAH female patients (— - -○)

Table 1. Comparison between plasma tHcy in sex matched controls and SAH patients

	Female				Male			
	Control		SAH		Control		SAH	
	age	tHcy	age	tHcy	age	tHcy	age	tHcy
n	32		25		32		10	
mean	43	7.94	55	7.84	43	9.75	49	9.80
range	17–98	5–14	21–98	3–14	17–98	3–22	23–98	5–17
SD		2.54		2.56		4.48		3.63
		$P_{FEMALES} = 0.89$				$P_{MALES} = 0.97$		

Discussion

The control data used in this study are in excellent agreement with previously reported ranges (Fermo et al., 1993) and confirm the higher concentrations found in males, especially with increasing age. Paradoxically, and in agreement with previous findings (Linn et al., 1996), we also found the prevalence of SAH to be higher in females. In order to ensure independence from other physiological risk factors only patients who were folate and B12 replete were included and this may be one explanation for our failure to demonstrate an association between tHcy and SAH.

Homocysteine, formed from methionine as a consequence of essential transmethylation reactions, can be remethylated to maintain the folate cycle and methionine supply and also converted to cystathionine and cysteine. Homozygous defects in these pathways lead to overt homocystinuria, i.e. the presence in plasma of free oxidised homocysteine, (homocystine), in which there is a strikingly high prevalence of occlusive vascular disease if not effectively treated by lowering the circulating free homocystine concentration (Wilcken and Wilcken, 1997). In addition to these primary genetic causes plasma homocysteine is largely determined by the nutritional cofactors for CBS and MTHFR, especially B12 and folate, poor availability of which lead to mildly increased concentrations of, primarily, protein bound homocysteine. Several putative mechanisms for vascular and endothelial damage have been proposed (Bellamy and McDowell, 1997). However, in many of the experimental systems used the results can be attributed to the presence of high concentrations of free homocystine (Harpel, 1996) and this may well underlie the causes of vascular damage in overt HCU. However, despite the continued presence of high protein bound homocysteine concentrations in HCU patients the risk of vascular disease can be near normalised by treatment resulting in reduction of free homocystine concentrations (Wilcken and Wilcken, 1997). It is therefore unclear whether the postulated mechanisms of vascular damage can be translated to the mild hyperhomocysteinaemic state where tHcy exists almost totally in the protein bound form. Additionally, folate has frequently been used to modify homocysteine values in experimental and animal models (Bellamy and McDowell, 1997; Harpel et al., 1996) and together with the possibility that relative deficiencies of co-factors in the population may go unrecognised (Donnelly and Isotalo, 2000; Patel and Briddon, 2000) makes it difficult to dissect out purely homocysteine related effects.

Therefore, although the epidemiological evidence is consistent with an association between mild hyperhomocysteinaemia and occlusive vascular disease, in our view the biochemical evidence is less compelling. Our failure to demonstrate a relationship between tHcy and SAH in a co-factor replete population is, perhaps, not surprising.

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