Homocystinemia and stroke in vegetarians

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ABSTRACT

Homocysteine is a sulfurated amino acid with a central role in the metabolism of thiol compounds. Homocystinemia is a recognized independent potentially remediable risk factor for vascular disease. It is associated with both macro and micro vascular ischemic stroke. It can often be normalized by polyvitamin therapy. This inexpensive and well-tolerated treatment is considered effective in decreasing the incidence of stroke. We report 2 young strict vegetarians with no known vascular risk factors. The first suffered a left middle cerebral artery infarct, and the second multiple bilateral small cerebral infarctions. Extensive investigations showed moderately elevated homocysteine and low serum B12 levels, suggesting that these are most probably the underlying etiology. We believe that a high index of suspicion is needed, particularly in younger people with a potential underlying cause for B12 deficiency and no identifiable stroke risk factor.

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Stroke is the second most common cause of death in the world, and it remains a major cause of long-term disability. Arterial thromboembolism constitutes a principal cause of ischemic stroke. The most promising strategy to reduce the global burden of stroke is effective stroke prevention. This includes control of the known modifiable causal risk factors (hypertension, smoking, diabetes and hyperlipidemia), antiplatelet therapy, and revascularization procedures (carotid endarterectomy, angioplasty and stenting). These measures have failed to prevent a substantial proportion of recurrent strokes. This is because of the existence of other unrecognized causal risk factors for atherothrombosis and thromboembolism. However, only two thirds of all attacks of ischemic stroke can be traced to known, well-established genetic and environmental factors. One of these hitherto unrecognized and untreated, but relatively common causal risk factors for atherosclerotic ischemic stroke may be an elevated plasma level of homocysteine.[1] It was first implicated in the pathogenesis of atherosclerosis in the late 1960s. Later, several studies indicated that homocystinemia is associated with an increased risk for atherothrombotic vascular events of the brain, heart, and limbs. It is perceived as an independent causal risk factor. It is arteriosclerotic, atherosclerotic, atherogenic and prothrombotic. On the whole, it remains to be determined whether the association is truly causal or confounded by other concomitant factors such as smoking, renal impairment, atherogenic diet, cysteine deficiency or perhaps even acute vascular events which temporarily increase plasma homocysteine levels by inflicting acute tissue damage.[1,2] Homocysteine is a sulfhydryl containing amino acid that is derived from the metabolism of dietary methionine, which is abundant in animal sources of protein. The total plasma homocysteine consists of free, bound, reduced, and oxidized forms. The normal level, among the fasting individuals ranges from 5-15 umol/l. The elevations could be moderate (16-30 umol/l), intermediate (31-100 umol/l), and severe (>100umol/l). A wealth

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of epidemiologic evidence has accumulated over the past 20 years, and made it evident that even moderate elevations in plasma homocysteine levels, are a common and important risk factor for vascular disease. The metabolism of homocysteine depends on the presence of N5, N10 methylene tetrahydrofolate (derived from dietary folate) and the enzyme N5, N10 methylene tetrahydrofolate reductase. In the process of its remethylation, and trans-sulphuration, folic acid, vitamin B12, and B6 act as essential co-factors.[1-3] Secondary homocystinemia may be caused by diverse conditions that include hypothyroidism, psoriasis, malignancy, renal failure, and deficiency of folate, pyridoxine, or cyanocobalamin. Although a strong nonlinear inverse association between homocystinemia and plasma concentrations of folate, B12 and B6 are established, it was not definitely concluded that lowering plasma homocysteine by increasing vitamin intake will reduce the risk of vascular disease. However, 40% of vegans were later shown to have vitamin B12 deficit manifested by macrocytosis, serum B12 levels of <150 pmol/L, or methylmalonic acid of >376 nmol/L.[3,4] An important observation that homocysteine (along with methylmalonic acid) is a sensitive indicator of subtle vitamin B12 deficiency was previously made.[2,4] Stroke associated homocystinemia was not linked with specific, consistent neuroradiological or clinical findings, but anecdotal reports on diffuse periventricular white matter lesions or multiple small infarctions exist. Further, it has been suggested that it is not related to a certain stroke subtype or to other known risk factors.[1,3] However, a higher rate of lesions typical of cerebral microangiopathy and a trend to multiple infarctions was later proposed.[1-4] Since the elevated level of homocysteine in principle is more readily treatable with vitamin supplements, than other common vascular risk factors which often require pharmacologic intervention, our interest was evoked to search for homocystinemia, particularly in young stroke patients who did not have apparent risk factors.

Case Report. ***Patient 1.*** A 34-year-old Indian man, left-handed, non-smoker, not known to have any known prior medical problems presented to our Hospital with a history of sudden onset of vomiting, drowsiness, and right sided weakness. There was no familial history of young onset stroke. On examination, he appeared drowsy, irritable, and confused. His blood pressure was 140/80. Pulse was regular and all peripheral pulses were well felt. Central nervous system examination revealed a right supranuclear 7th nerve palsy and right hemiplegia. His speech

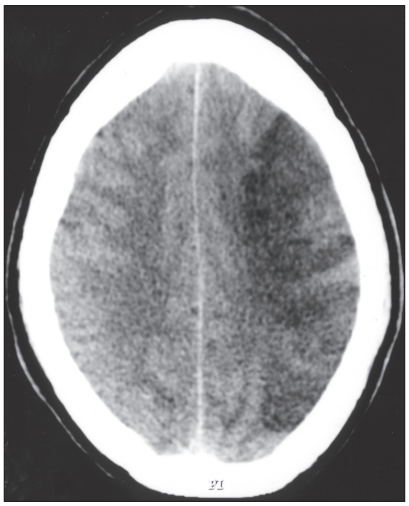

Figure 1 - Brain CT (axial non-contrasted) showing huge left middle cerebral artery stem infarct involving frontotemporoparietal lobe with mass effect.

was normal. No clinical evidence of neglect was noticed. There were no carotid bruits. Cardiovascular, respiratory, and abdominal examinations revealed no abnormality. Brain CT showed huge left middle cerebral artery stem infarct involving the fronto-temporo parietal lobes with mass effect (**Figure 1**). A brain MRI confirmed the previously observed CT findings. However, diffusion weighted sequences were not carried out due to the lack of the optimal software. He was started on low molecular weight heparin for deep venous thrombosis prophylaxis, physiotherapy, and anti-platelet drugs. Investigations disclosed mild hypochromic microcytic anemia (12.9gm/dl) and peripheral blood smear also showed target cells and hypersegmented polymorphs. Sickle cell test was negative, but hemoglobin electrophoresis showed beta thalassemia trait. White blood cell count, platelets, erythrocyte sedimentation rate, blood sugar, lipid profile, kidney and liver function tests, serology, connective tissue screen, bleeding time, clotting time, prothrombin time, activated partial thromboplastin time, protein C, S and anti-thrombin III, chest x-ray, electrocardiogram, transthoracic and transesophageal echocardiography were all normal. Fasting serum homocysteine level was 22 umol/L (normal 5-15 umol/l). Serum B12 was low at 145 ng/L (normal 160-925). Due to the clearly abnormal homocysteine and B12 levels, methyl malonic acid measurement in the serum was not carried out. Serum and red cell folate and urinary excretion of homocysteine were normal. As magnetic resonance angiography (MRA) of the brain and neck vessels and magnetic resonance venography (MRV) of the cerebral venous circulation

were normal, conventional cerebral angiography was not carried out. The patient was also started on vitamin B12 injections and folic acid and showed signs of improvement at the end of 4 weeks with power on the right upper limb and lower limb becoming 2/5 proximally, but still 0/5 distally. He was discharged on aspirin, Persantine, folic acid, vitamin B12, and a physiotherapy program. He was lost from follow up as he returned to his home country after discharge.

Patient 2. This 36-year-old Indian man, right-handed, was admitted to our unit with complaints of recurrent left-sided numbness and weakness since 2 months. However, sudden left hemiparesis developed on the morning of admission. He had no history of hypertension, diabetes mellitus, ischemic or rheumatic heart disease. He is a strict vegetarian and non-smoker. There is no familial history of stroke. On examination, his pulse was regular and all peripheral pulses were felt. He had a normal blood pressure. He was conscious, oriented and higher mental functions were normal. He had a left supranuclear mild facial palsy and left hemiparesis with power of 2-3/5 in the upper limbs and 3-4/5 in the lower limbs. He deteriorated, and his power became 0/5 by evening. Investigations revealed a normal full blood count, urea, electrolytes, liver and thyroid functions, lipid profile, serology, ECG, transthoracic and transesophageal echocardiography. Connective tissue screen, protein C, protein S, antithrombin III, and folate levels were all within normal range. Brain CT revealed multiple lacunar infarcts in the subcortical white matter of both parietal lobes, right more than left (**Figure 2**). Brain MRI confirmed the CT findings. However, diffusion weighted sequences were not carried out due to the lack of the optimal software. Serum fasting homocysteine level was 30 umol/L, and B12 level was low at 130 ng/L. Due to the clearly abnormal homocysteine and B12 levels; methyl malonic acid concentration was not measured in the serum. Carotid Doppler, MRA of the brain and neck vessels, and MRV of the cerebral venous sinuses were normal. He was started initially on intravenous heparin and then on oral anticoagulants. This was later changed to antiplatelet therapy. Vitamin B12, 0.5 mg intramuscularly daily, and folic acid 5 mg orally daily were added later. He showed improvement after 5 weeks and his power became 3/5 in the left upper limb, and 4/5 in the lower limbs. He was discharged on a program of physiotherapy, and followed up for the last 3 years with no recurrence.

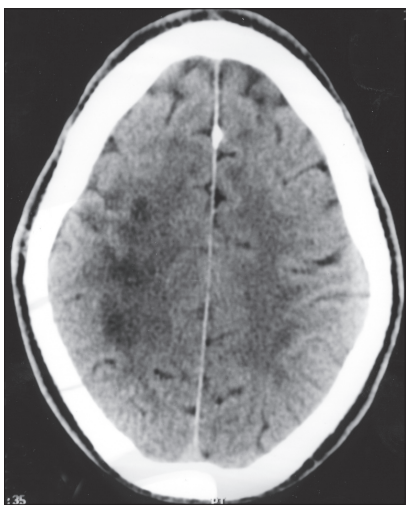

Figure 2 - Axial non-contrasted brain CT scan showing multiple and bilateral lacunar infarcts in the subcortical white matter of both parietal lobes, right more than left.

Discussion. Over the last decade, strong epidemiological evidence has suggested that even moderate elevation in plasma homocysteine levels is a relatively common and important risk factor for vascular disease.[1,4] It is clear that it is a strong and independent risk factor for coronary artery, cerebrovascular, peripheral vascular diseases and venous thrombosis.[1,4,5] Several studies indicated that the prevalence of homocystinemia in ischemic stroke ranges from 19-42%.[3,4,6] The reason for elevated homocysteine levels remains unclear. However, decreased levels of cyanocobalamin, folate, or both were observed in 33% of the patients with homocystinemia enrolled in the previous study. This hypovitaminosis, which was also observed in our 2 patients, might have contributed to elevated homocysteine levels, as there was no family history of homocystinuria. Plasma levels of homocysteine physiologically increase with age and tend to be marginally elevated in males. Our 2 patients were in their mid-30s and have moderate elevations of their homocysteine level. The type of stroke caused by homocystinemia is primarily microangiopathic, and tends to cause multiple infarctions.[1,3-6] However, our first patient had left middle cerebral artery stem infarction, thus indicating macroangiopathy, while the second patient had bilateral multiple small cerebral infarctions in the watershed areas of the anterior and middle cerebral artery territories. These infarcts showed the typical features of recent occurrence. The bilaterality and multiplicity in the second case suggested thrombo-embolism. Several mechanisms have been proposed in the pathogenesis of atherothrombogenesis caused by moderate homocystinemia. These include mitogenic effects on vascular smooth muscle, direct and indirect cytotoxic and thrombophilic effects on vascular endothelium

of the small penetrating arteries, promoting growth of the vascular smooth muscle cells thus leading to thrombosis.[3,4] Although this hypothesis might explain the mechanism of microangiopathy, it cannot explain the macroangiopathy of our first patient. Recent studies,[1,6,7] suggest that there is a strong and graded association between homocystinemia and ischemic stroke caused by large artery (9 folds increased risk) as compared to small artery disease (2 folds increased risk). These studies have demonstrated elegantly the association between carotid atherothrombogenesis and ischemic stroke caused by large as well as small artery diseases.

Our 2 cases demonstrated that moderately elevated homocysteine and low B12 might be the causative factor for the middle cerebral artery infarct and the multiple small infarctions consecutively. Blood levels of B12, folate and to a lesser extent B6 are related inversely to total homocysteine level.[4,8] In both cases, the reason for the high homocysteine level was the low B12 level, which we postulate as being nutritional, as our 2 patients were strict vegetarians. There are no studies that directly relate dietary folic acid or B12 deficiency to occurrence of vascular disease.[2,4] Low dietary intake is the most common cause of compromised folate or B12 status in the tropical and subtropical countries. Other acquired factors that tend to elevate serum homocysteine are unlikely to be operative in our patients. They have normal renal and thyroid functions. They were not taking any drugs that affect this issue, particularly anticonvulsants and antimitotic drugs. Homocysteine levels are relatively stable through the first 4 decades of life. Our patients were in their 30s, and have no genetic or familial evidence to suggest the existence of the rare inborn error of homocysteine metabolism. We measured the homocysteine level in our patients during the first week of admission and in the fasting state, thus eliminating false elevations. Some studies suggested that attaining a high reading of homocysteine measured during the acute phase may be a marker of tissue damage and repair. The mechanism hypothesized is that tissue damage accelerates specific methylation reactions, thus generating S-adenyl homocysteine and releasing homocysteine.[1,4] Other studies suggested that early measurement of homocysteine gives false low readings.[1,4,5] Either way, this does not hold true for our patients, as the cause of their high homocysteine level can be reasonably explained by their low B12 levels.

There has been intense interest in the use of polyvitamin therapy to lower homocysteine in the public, and in those at risk for vascular disease. Meta-analysis of 12 randomized trials[4] concluded that daily intake of multivitamins containing 0.5-5 mg folic acid and 0.5 mg B12 would lower homocysteine levels by nearly one third. More than 90% of patients respond to multivitamin treatment within 2-6 weeks, irrespective of the cause.[1,4,7] These findings encouraged physicians to prescribe this inexpensive and well-tolerated therapy. The arguments claiming that isolated folic acid supplementation may mask hematological signs of subclinical B12 deficiency and potentially delay therapy for other serious complications such as subacute combined degeneration of the cord are still valid. In that sense, B12 and folic acid therapy should be combined. Studies comparing the effect of polyvitamin therapy in patients with premature atherothrombotic disease have been associated with a decrease in fasting homocysteine levels.[1,8] Other trials tried also to assess the effects of lowering homocysteine levels by vitamin supplementation on hard clinical outcomes such as myocardial infarctions and stroke.[1,9] Vitamin Intervention for Stroke Prevention (VISP),[1,10] was the first large-scale randomized interventional study that investigated the lowering of homocysteine level with the B vitamins in ischemic stroke. There was an association between baseline homocysteine and vascular risk. Plasma concentrations of homocysteine were only modestly reduced by high dose versus low dose formulations and there was no treatment effect on recurrent stroke, coronary events or death. Limitations of VISP were that only patients with mild increases in baseline homocysteine concentrations were studied, only modest reductions of homocysteine concentrations were achieved and follow up was short. In addition, fortification of food with folate and treatment of low vitamin B12 concentrations may have masked the effect of treatment on stroke risk.[10] VITATOPS[9] is a major stroke trial that aims to recruit and follow up 8000 patients from 2000-2004, and provide a reliable estimate of the safety and effectiveness of dietary supplementation with folic acid, B12 and B6 vitamins in reducing recurrent vascular events among a wide range of patients with transient ischemic attack (TIA) and stroke. The results of this study are not yet released. However, we decided to supplement our patients with B12 and folic acid, which would reduce the level of homocysteine and decrease its detrimental effect on thrombogenesis, rate of atherosclerosis and thus incidence of further ischemic stroke. Vitamin B6 has not been found to have a significant additional effect on its own.[1,7,8] However, until it is shown by the aforementioned large randomized trials that polyvitamin therapy reduces the rate of recurrent stroke and other serious vascular events in patients with prior stroke or TIA, widespread screening for, or

treatment of homocystinemia remains experimental and cannot be universally recommended.[1,5-7,10]

An interesting question that arises is why our patients did not show any diagnostic evidence of atherosclerosis. Both carotid Doppler, MRA of the neck vessels (including arch of the aorta) and brain, and echocardiographic studies were normal. We postulate here that the cause of the stroke was possibly a thromboembolic phenomenon in which the embolus has disintegrated, thus explaining the above normal vascular studies. However, if we assume that the risk factor in our patient is the elevated homocysteine level, then our hypothesis does not explain the lack of clinical evidence of atherosclerosis elsewhere in the body. This led us to hypothesize that the elevated level of homocysteine is possibly a marker of thrombogenesis, or a consequence of other factors more closely linked to cerebrovascular disease. This is particularly relevant if we remember the relatively high percentage of young stroke patients with no known etiological factor or exact pathogenesis for their stroke. In addition, it was concluded that B12 and folate depleted diets may be prothrombotic. A positive association between raised total homocysteine level and increased risk of thrombosis on the arterial and venous sides of circulation was confirmed by meta analysis in patients with fasting hyperhomocystinemia.[1,3-5] Large and small vessels were involved.[1,5,6] This may explain the mechanism of thrombogenesis in both of our patients. However, large scale studies would have to be conducted to substantiate this.

In conclusion, epidemiological studies suggest that moderately elevated levels of homocysteine act as an independent risk factor for ischemic stroke and not as an epiphenomenon. As we were unable to find any other risk factor, homocystinemia secondary to low B12 levels was presumed to be the cause for both macro and micro vascular infarctions. Although lowering the levels of homocysteine has yet to be proven to reduce the incidence of stroke, we supplemented our patients with B12 and folic acid in the hope of reducing any further incidence of stroke. It seems reasonable to consider measurement of homocysteine levels in patients presenting with cerebral infarction, whether macro or micro vascular and particularly if below 45 years of age, or when other cerebrovascular risk factors are not prominent. Further prospective studies into this issue are highly recommended, taking into consideration the low cost of vitamin polytherapy in comparison with the high cost of other approaches in secondary stroke prevention.

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