

## **Hyperhomocysteinemia in patients with acute ischaemic stroke in Malaysia**

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### **Abstract**

This case-control pilot study aims to determine the prevalence and degree of hyperhomocysteinemia among Malaysian patients with ischaemic strokes, to ascertain whether there were any differences in homocysteine levels between the various ethnic groups and between lacunar and large vessel strokes. The study was based on consecutive first ever ischaemic stroke patients admitted to University Malaya Medical Centre from June to November 2000. Patients with known factors that affected homocysteine levels were excluded. Non-fasting blood samples were collected within 1 week of admission. Eighty subjects with comparable age and gender with no prior history of strokes or other known vascular diseases were recruited as controls. Patients in the top quartile of the control group were classified as hyperhomocysteinemic while those above the 95<sup>th</sup> percentile was classified as moderately hyperhomocysteinemic. Eighty-three stroke patients consisting of 52 males and 31 females were studied. The prevalence of mild hyperhomocysteinemia was 23% (12.0  $\mu\text{mol/l}$  to 15.1  $\mu\text{mol/l}$ ) while moderate hyperhomocysteinemia was 30% (>15.2  $\mu\text{mol/l}$ ). The mean plasma homocysteine level of the stroke patients was 13.5  $\mu\text{mol/l}$  (95% CI 11.82-17.6  $\mu\text{mol/l}$ ), which was significantly higher than that of controls at 10.4  $\mu\text{mol/l}$  (95% CI 9.9-11.1  $\mu\text{mol/l}$ ) ( $p < 0.001$ ). Multivariate analysis showed moderate hyperhomocysteinemia to be an independent risk factor of stroke with odds ratio of 5.3. Homocysteine levels in both lacunar and large vessel atherothrombotic strokes were significantly higher compared with controls. No significant differences were found between various ethnic groups and between lacunar and large vessel strokes.

*Conclusion:* Moderate hyperhomocysteinemia was an independent ischaemic stroke risk factor seen in 30% of a group of Malaysian ischaemic stroke patients. No significant differences were found between various ethnic groups, and between lacunar and large vessel strokes.

### **INTRODUCTION**

Stroke is the second most common cause of death in the world.<sup>1</sup> The most promising strategy to reduce the world-wide burden of stroke is effective stroke prevention. To date, only 70% of all strokes can be attributed to known risk factors. An emerging risk factor for stroke, which is prevalent and modifiable, is plasma homocysteine.

Homocysteine is a sulphur containing amino acid formed during the metabolism of methionine, an essential amino acid derived from dietary protein. It is metabolized with folate as a co-substrate, vitamin B12 as a co-factor and the help of several other enzymes. Elevated plasma homocysteine is a risk factor for atherothrombotic disease and is associated with atherosclerotic vascular diseases. Large epidemiological studies have demonstrated this relationship in coronary artery, peripheral arterial and cerebrovascular diseases.<sup>2-5</sup>

Homocysteine has also been shown in a large multi-centered case control study<sup>3</sup> to have a multiplicative effect on conventional atherosclerotic risk factors. This has important public health implications. However, most studies have been performed in Western populations and to date, no systematic study has been performed in the Malaysian population with atherosclerotic cerebrovascular disease. Malaysia has a multi racial population of 65% Malays and other indigenous races, 26% Chinese and 7.7% Indians with a total of 23.3 million (year 2000 census). The dietary habits and genetic profile, of the population may affect the role of homocysteine in the local population, thus the justification for this pilot study. This is a prospective case-control study performed among consecutive, first-ever ischaemic stroke patients admitted to the University Malaya Medical Centre from June to November 2000.

## METHODS

The study design was that of a prospective case-control study. Subjects with a first-ever ischaemic stroke were enrolled consecutively. The University Malaya Medical Centre Ethics Committee approved this study. Consecutive ischaemic stroke patients presenting to the University of Malaya Medical Centre from June to November 2000 were screened for eligibility for enrolment in the study. Patients were recruited according to the criteria described below.

### *Patients*

Stroke was defined as a clinical syndrome characterized by rapidly developing clinical symptoms and/or signs of focal and at times global loss of brain function with symptoms lasting more than 24 hours or leading to earlier death and with no other apparent cause except that of vascular origin.

The *exclusion* criteria were: Patients who were unable to give consent, had cerebral haemorrhage, venous thrombosis, dissection, hypoperfusion, a periprocedural ischaemic event or had confounding factors which could raise homocysteine levels. The latter included: i) subjects taking anti-epileptic drugs, L-dopa, folate antagonists such as methotrexate, cholestyramine, penicillamine, oral contraceptive, hormonal replacement therapy and subjects on regular vitamin supplements particularly B12 and folate; ii) subjects with renal impairment or end stage renal failure on haemodialysis; iii) subjects with malignant disease; iv) patients whom consents for study were not available. Subjects with cardioembolic disease were also excluded from the study. This was defined as: i) ischaemic stroke with a major cardioembolic source such as atrial fibrillation and myocardial infarction; ii) if there is more than one explanation for the stroke and this included cardioembolism.

The patients were categorized into two stroke subtypes with the following criteria:

*Small-artery disease:* Ischaemic stroke with a) consciousness and higher cerebral function maintained plus b) the clinical manifestation fit into one of the classical lacunar syndromes, i.e. pure motor hemiparesis, pure hemisensory loss, pure hemisensorimotor loss or ataxic hemiparesis.

*Large-artery disease:* Ischaemic stroke with a) evidence of extracranial or intracranial occlusive disease on magnetic resonance angiography or ultrasound imaging; b) clinical opinion that the most likely cause of the brain infarction was

atherothrombosis with a major cortical syndrome involving the aortic arch, carotid arteries or its major branches (main stem of middle cerebral artery), vertebral, basilar, posterior cerebral arteries or both; c) no major cardioembolic source with no recent (within 4-6 weeks) evidence of a myocardial infarction, atrial fibrillation, prosthetic heart valve or endocarditis; d) supportive evidence by neuroimaging study, i.e. CT brain and/or MRI; which was repeated if clinically indicated.

All cases were assessed and classified by the principal study neurologist (KST) who had a special interest in stroke neurology. A standard questionnaire was used whereby demographic and conventional stroke risk factors were also determined.

### *Control Subjects*

Control subjects were recruited from the hospital employees, and residents from the nearby areas. Subjects who have overt cardiovascular disease and confounders of homocysteine levels as described earlier were excluded.

### *Collection of Blood Samples and Biochemical Analysis*

Non-fasting blood samples were obtained from cases and controls. For stroke patients, blood samples were collected as soon as possible following admission, usually within 7 days. The samples were transported in ice to the laboratory within one hour and were centrifuged immediately at 3000 rpm for 10 minutes. The separated serum was fraction aspirated and transferred into plastic tubes. The samples were kept at 0°C and subsequent analysis was undertaken in weekly batches. The analysis was performed with a commercially available homocysteine fluorescence polarization immunoassay. The assay was performed in the Clinical Diagnostic Laboratory, University Malaya Medical Centre by two technicians who have been specifically trained.

### *Statistical Methods*

Inferential statistics in the form of independent sample Student's t-tests and one-way analysis of variance to compare subgroups where relevant were used. Multivariate analysis using logistic regression was performed on the common known risk factors for stroke. Statistical significance was taken as p-0.05 and were two tailed

## RESULTS

The mean age of the stroke patients was  $60 \pm 14$  years while the control subjects was  $55 \pm 11$  years with no significant difference between the two groups ( $p = 0.14$ ). The male to female ratio was 1.7 : 1 for the patients and 1.1 : 1 for the controls with no significant difference between the two groups ( $p = 0.55$ ).

The distribution of homocysteine level in the control subjects is shown in Figure 1. The relationship between serum homocysteine and age in the control subjects is as shown in Figure 2. There was a linear relationship between age and homocysteine concentration. The mean homocysteine level among the control subjects was  $10.5 \pm 2.7 \mu\text{mol/l}$ . The *top quarter* was  $12.0 \mu\text{mol/l}$  and *top fifth percentile* was  $15.2 \mu\text{mol/l}$ .

The mean homocysteine levels among the patients were  $13.4 \pm 4.9 \mu\text{mol/l}$ . There was a statistically significant difference in the mean homocysteine level between the patients as compared to the controls ( $p < 0.001$ ). With hyperhomocysteinemia being defined as the top quartile of the homocysteine distribution in the control subjects ( $>12.0 \mu\text{mol/l}$ ), the prevalence of hyperhomocysteinemia among the ischaemic

stroke patients was 53%. With moderate hyperhomocysteinemia being defined as top fifth percentile in the control subjects ( $>15.2 \mu\text{mol/l}$ ), the prevalence of moderate hyperhomocysteinemia was 30%.

The distribution of the homocysteine level in patients is shown in Figure 3. There was no significant skew in the distribution of the homocysteine level for both the patients as well as the control and no log transformation was calculated. The patient and control subjects were analyzed as a group to determine any graded association between the homocysteine level and risk of stroke. For those with homocysteine level at the top quartile of the normal homocysteine distribution ( $>12.0 \mu\text{mol/l}$ ), the odds ratio for risk of stroke was 3.2 (95%CI 1.6-6.5  $P < 0.001$ ) in comparison with those in the lower three quartiles ( $<12.0 \mu\text{mol/l}$ ). For those with homocysteine level at the top five percentile ( $>15.2 \mu\text{mol/l}$ ), the odds ratio was 6.5 (95%CI 2.2-20.1  $P < 0.001$ ) in comparison with those in the lower 95 percentile ( $<15.2 \mu\text{mol/l}$ ).

On multivariate analysis with other known risk factors for stroke, moderate hyperhomocysteinemia remained significant (OR 5.3)

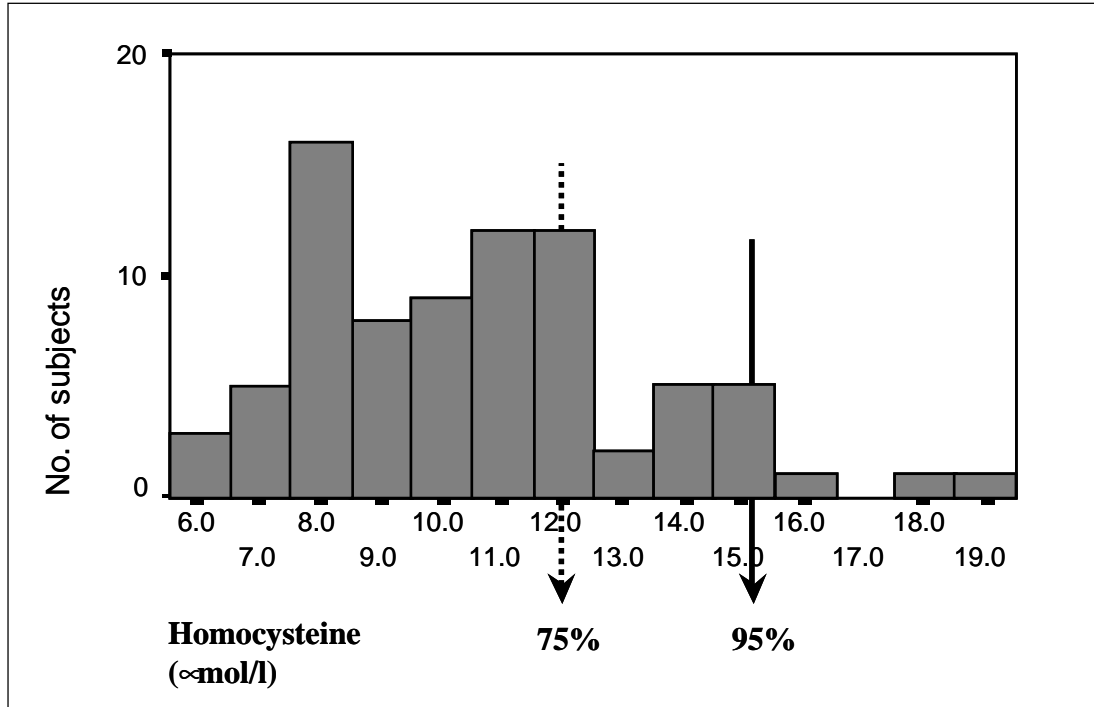


Figure 1: The distribution of homocysteine level in control subjects.

Mean homocysteine level =  $10.5 \mu\text{mol/l}$  (S.D.  $\pm 2.7$ ), 95% CI Mean = 9.9-11.1, 75 percentile value =  $12.0 \mu\text{mol/l}$ , 95 percentile value =  $15.2 \mu\text{mol/l}$ , minimum =  $5.9 \mu\text{mol/l}$ , maximum =  $18.8 \mu\text{mol/l}$ , median =  $10.4 \mu\text{mol/l}$ , skewness = 0.66

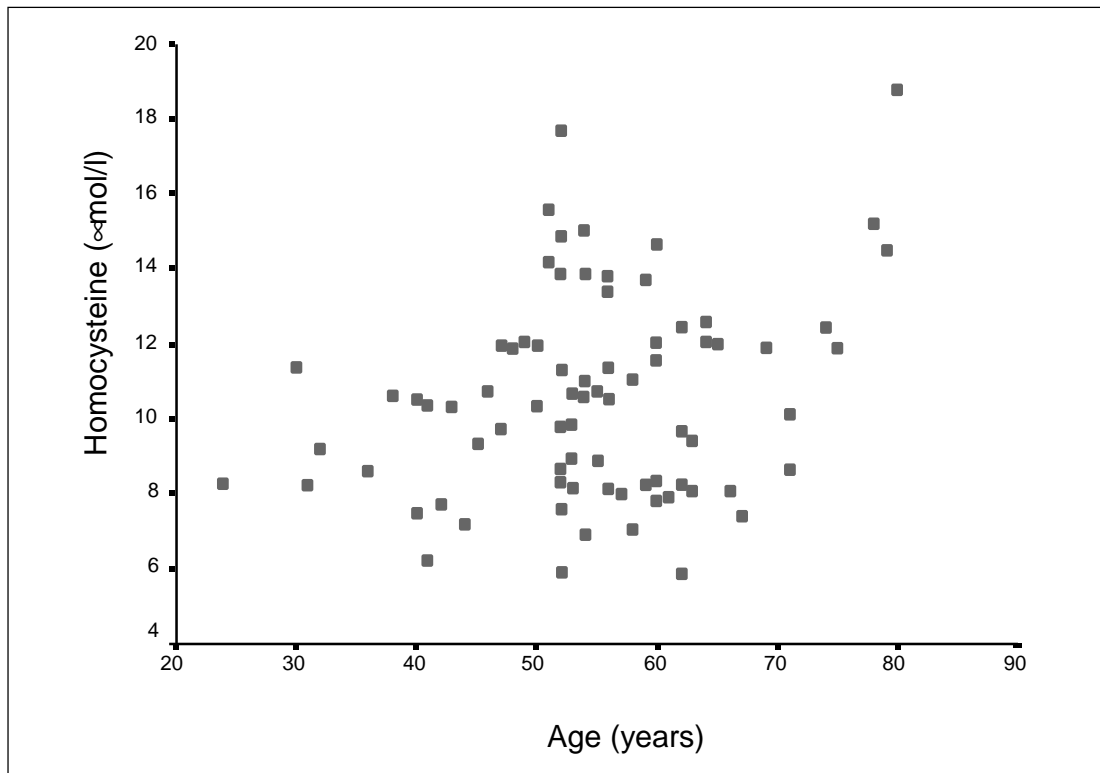


Figure 2: The relationship between homocysteine level and age in control subjects.  
 There was linear regression of homocysteine and age. Homocysteine = 0.05698 Age + 7.377. If age is 55 years; then homocysteine is 10.5 µmol/l, If age is 60 years, then homocysteine is 10.8 µmol/l

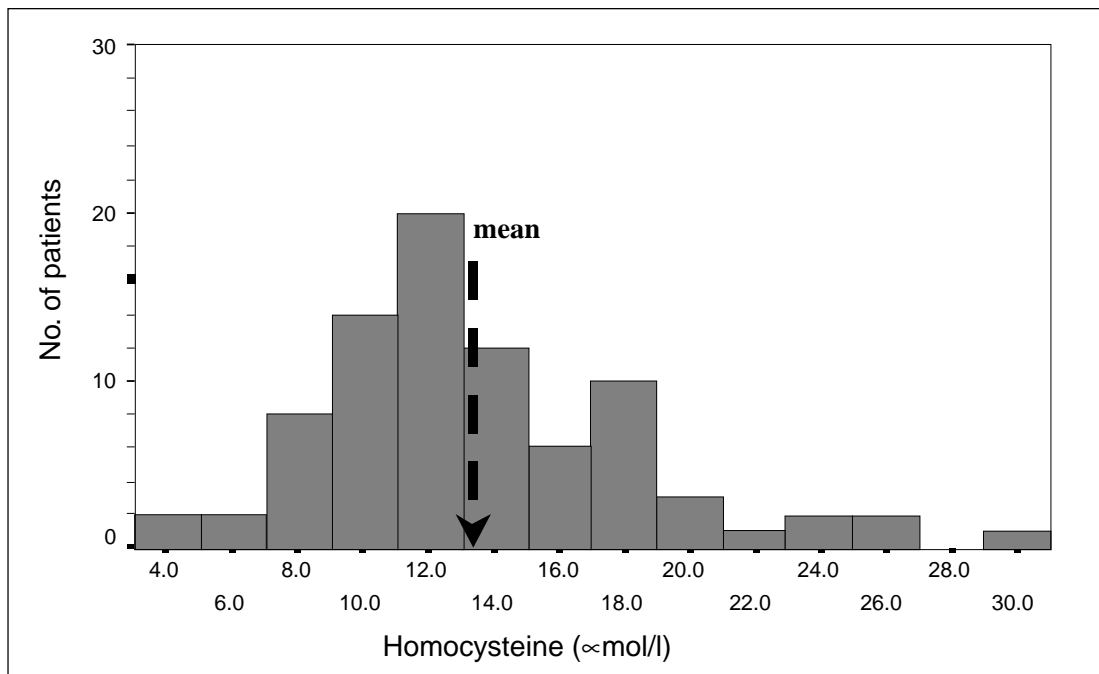


Figure 3: The distribution of homocysteine level in stroke patients. Mean homocysteine level = 13.4 (S.D.±4.9) µmol/l 95% CI for mean = 12.4 – 14.5, maximum = 29.6 µmol/l, minimum = 4.9 µmol/l, median = 12.3 µmol/l, skewness = 0.95

whereas mild hyperhomocysteinemia was not significant. The other significant risk factors were: diabetes mellitus (OD 6.3), hypertension (OD 3.9), and smoking (OD 2.9).

The ethnic composition of the 83 stroke patients was Chinese (34), Malays (25), Indians (23) and others (1). Fifty-nine percent of the Chinese stroke patients had hyperhomocysteinemia of  $>12.0$   $\mu\text{mol/l}$ , and 32% had level  $>15.2$   $\mu\text{mol/l}$ . For the Malays, 36% was  $>12.0$   $\mu\text{mol/l}$  and 25%  $>15.2$   $\mu\text{mol/l}$ . Among the Indians, 65% was  $>12.0$   $\mu\text{mol/l}$  and 35% was  $>15.2$   $\mu\text{mol/l}$ . There was no significant difference between the three ethnic groups in the rate of hyperhomocysteinemia  $>12.0$   $\mu\text{mol/l}$  and  $>15.2$   $\mu\text{mol/l}$ .

The mean plasma homocysteine level of the 33 patients with large vessel infarction was 12.8  $\mu\text{mol/l}$ . The level was 13.8  $\mu\text{mol/l}$  for the 50 patients with small vessel infarction. There were statistically significant differences in the mean homocysteine levels between the large vessel infarct subgroup and controls ( $p<0.001$ ) as well as small vessel infarction subgroup and controls ( $p<0.001$ ). On the other hand, there was no significant difference between large vessel infarct subgroup and small vessel infarct subgroup.

## DISCUSSION

This study had the following strengths. A cohort of patients with ischaemic stroke was assembled prospectively with the assessments undertaken by a single neurologist with special interest in stroke on the basis of strict predefined criteria. The investigator had no knowledge of the initial homocysteine results. Potential confounders of homocysteine levels were noted whereby cases and controls were rigorously excluded. However, our sample size was relatively small.

Although most recent studies have utilized fasting blood samples when determining homocysteine, our study was performed using non-fasting samples of homocysteine levels. Previous studies using non-fasting blood samples for analysis of homocysteine have shown strong associations between raised homocysteine level and strokes.<sup>6,7</sup> On the other hand, there were also negative studies when non-fasting samples were used.<sup>8,9</sup> Non-fasting samples were used in this study mainly for logistic reason.

This pilot study showed that homocysteine levels were considerably higher in stroke patients compared to healthy controls in Malaysia. The higher level of homocysteine at 13.4  $\mu\text{mol/l}$  is consistent with several major European studies<sup>3,4,6</sup> and the results of a recent case-control study

in Taiwan on Chinese ischaemic stroke.<sup>10</sup> The latter study involved 92 patients. The mean homocysteine level for the patients was 14.9  $\mu\text{mol/l}$  as compared to 11.78  $\mu\text{mol/l}$  among the control subjects.

We observed that there was a graded increase in the risk of stroke with higher level of homocysteine. Whereas the odd ratio of stroke in the top quarter compared to the lower three-quarters of the homocysteine distribution was 3.2, it was 6.5 for top five percentile of the homocysteine distribution as compared to the lower 95 percentile. This graded association was in keeping with published literature in Western populations with ischaemic stroke.<sup>6</sup>

This study also showed that on multivariate analysis, moderate hyperhomocysteinemia remained a significant risk factor for ischaemic stroke, with odd ratio of 5.3, seen in 30% of the patients. Hyperhomocysteinemia is thus an important risk factor in Malaysian ischaemic stroke, with its strong association with stroke and involving large proportion of patients. Homocysteine assays were performed in the first 7 days after the acute stroke event in our study during which time homocysteine may be lower than usual.<sup>11</sup> This study may thus underestimate the importance of homocysteine in the development of stroke.

The high prevalence rate of moderate hyperhomocysteinemia (30%) noted among the stroke patients may reflect the high frequency of genes predisposing to hyperhomocysteinemia in Malaysia. The C677T mutation in the methylene-tetra-hydrofolate reductase (MTHFR) gene<sup>12</sup> have been identified in Western populations to correlate with raised homocysteine level.<sup>13</sup> The prevalence of the MTHFR gene and the nutritional status of folic acid in the local population is unknown.

Malaysia has a multi-ethnic population consisting mainly of Malays, Chinese and Indians. This study showed that there was no significant difference in the rate of hyperhomocysteinemia among the stroke patients between the three ethnic groups. A cross-sectional study was done in neighboring Singapore<sup>14</sup> on 726 normal subjects also showed no significant difference in the fasting homocysteine level between the same three races, Malays, Chinese and Indians. There is no data to suggest a predilection of stroke among any of the main races in Malaysia. However, the rate of cardiovascular event has been reported to be higher among Singapore Indians. As the homocysteine level is not significantly different among the races, it could not account for the

higher rate of cardiovascular disease seen among the Indians.<sup>14</sup>

The mean homocysteine levels in our control subjects was 10.5  $\mu\text{mol/l}$ . This was in the range of several reported studies where the levels ranged from 9.7<sup>3</sup> to 10.6<sup>4,6,15</sup>. On the other hand, homocysteine levels in our study was considerably lower in comparison to a Singapore study with a mean level of 16.3  $\mu\text{mol/l}$  among healthy subjects.<sup>14</sup> As Singapore's ethnic composition and lifestyle is fairly similar to that of Kuala Lumpur, the difference is probably mainly technical. Variations among laboratories in homocysteine measurement is well known.<sup>16</sup> The Singapore study is based on fasting samples which had been noted to be significantly higher in a recent Norwegian study.<sup>17</sup>

Our study has shown strong association between raised homocysteine level and ischaemic stroke patients, in both large artery and small artery disease when compared with control subjects. No significant difference in the homocysteine level was noted between large vessel infarctions and lacunar or small vessel infarctions. The association between raised homocysteine level and the extent of atherosclerotic large artery disease particularly in the extracranial carotid circulation<sup>15,18,19</sup> and in large vessel atherothrombotic strokes has been previously noted.<sup>20</sup> However, in lacunar or small vessel infarctions, the pathophysiology is less clear but appears to involve microatheroma formation and lipohyalinosis.<sup>21</sup> Homocysteine was noted by Eikelboom et al.<sup>20</sup> to be less important in small vessel or lacunar infarctions compared with large artery ischaemic infarctions in a predominantly Caucasian population in Perth. On the other hand, the Taiwanese study described earlier<sup>10</sup>, reported the association of lacunar (small vessel) infarction but not large vessel atherothrombotic strokes and raised homocysteine levels.

There is higher prevalence of intracranial large artery atherosclerotic disease in Asian stroke patients as compared to the Caucasian population. In one study, 33% of acute Chinese stroke patients were found to have intracranial large vessel atherosclerotic disease.<sup>22</sup> Significant topographic diversity of atherosclerotic middle cerebral artery disease with the common occurrence of small cortical and sub-cortical infarcts was found in a Korean study.<sup>23</sup> Thus, atherosclerotic intracranial large artery disease may contribute significantly to lacunar infarctions among Asians. As such, the association between raised homocysteine levels

and small vessel infarctions in Asians may not necessarily mean that it contributes directly to microatheroma and lipohyalinosis formation in lacunar infarction.

In conclusion, hyperhomocysteinemia is a potentially modifiable risk factor associated with a significant percentage of ischaemic stroke in Malaysian population.

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