Homocysteine; a Likely Cause of Ischemic Stroke in a Subset of Pakistani Population

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Abstract

Objective: The aim of this work was to investigate, if total homocysteine was an independent hazard issue for ischemic stroke in Pakistani stroke patients.

Materials and Methods: This cross sectional study was carried out on 150 stroke patients included from September 2010 till August 2011, who were diagnosed as having clinical stroke on the basis of history, physical examination and CT (Computerized Tomography) scan of brain. These patients were recruited from neurology and emergency wards of two public sector hospitals of Lahore. Blood samples were collected from the same stroke patients.

Results: There were 150 patients with cerebrovascular event. In 126 victims of ischemic stroke, the geometric mean tHcy was ±SD 19.7± 9.6 while in 24 patients with hemorrhagic stroke, the geometric mean ±SD of tHcy was 20.0 ±15.7. The difference in geometric mean tHcy in ischemic and hemorrhagic events was not found statistically significant (p= 0.346).

Conclusion: We did not find significant association of ischemic stroke with elevated total plasma homocysteine level in our stroke patients.

Key Words: Homocysteine; Ischemic stroke; Haemorrhagic stroke; Risk factor.

Introduction

According to World Health Organization two thirds of stroke deaths occur in developing countries. Several epidemiological studies have coupled hyperhomocysteinemia to increased risk for ischemic stroke. Hyperhomocysteinemia is claimed to be atherogenic and thrombogenic, still it is a modifiable risk factor.

The neurobiology of stroke is one of the most challenging fields in neurology and neuroscience research today. The main determinant of the injury caused by ischemic stroke is the degree and duration of ischemia to the affected brain tissue. Cerebral ischemia can be focal (embolic or thrombotic occlusion of extra-cranial or intracranial blood vessels) or global ischemia (cardiovascular failure or respiratory disease). Ischemic strokes are classified into two main subtypes: strokes where arterial occlusion takes place due to thrombosis and embolism, and small vessel strokes (lacunes) which involve obstruction of small arterioles. The phenomenon of thrombosis and embolism is more common than the latter. Atherosclerosis is the most common phenomenon implicated in stroke and one of the most actively researched areas in neurological disorders.

Diffuse haemorrhagic stroke (bleeding into spaces around brain tissue) is caused by rupture of vessels on or near the surface of the brain or ventricles such as aneurysms and other vascular malformations. Localized haemorrhagic stroke (caused by an intraparenchymal or intra-cerebral bleed) may result from hypertensive hemorrhage or vascular malformations. The commonest variety of stroke is an ischemic stroke.

Homocysteine (Hcy) is a non-protein forming 4-carbon sulphur containing amino acid with a free thiol group. It is an essential amino acid derived from methionine as an intermediate product formed by the conversion of methionine into cysteine. The total homocysteine (tHcy) is determined by acquired and genetic factors. Vitamins B6, B12 and folates are cofactors in Hcy metabolism. Deficiency of these supplements leads to hyperhomocysteinemia (HHcy) and predispose to increased prevalence of stroke.
The tHcy concentration ranges from 5 to 15 µmol/Litre in the fasting state. The molecular mechanism by which HHcy causes atherosclerosis is not clearly defined. There could be many mechanisms by which HHcy is atherogenic, like, increase in oxidative stress, disturbances in endothelial related vasorelaxation, smooth muscle cell multiplication and activation of coagulation. HHcy adversely affects vascular disease and stroke in particular.

Most affects of HHcy have been studied in animal models and later supported by findings in studies based on patients with HHcy. The studies finally concluded that Hcy seems to be the cause of atherosclerosis. Numerous studies have associated HHcy with cardiovascular risk and have established Hcy as a strong graded independent risk factor for vascular disease including cerebral stroke. HHcy has been reported to be associated with two to three fold increase risk for ischemic stroke. On the basis of current statistics, it has been predicted that the burden of stroke would rise in the next decade, if appropriate preventive measures are not taken.

In this study, we have examined the Hcy patterns in local stroke patients, so as to ascertain the Hcy status in one community. We measured total homocysteine in the first 24 hours of stroke event and then compared its levels in ischemic and haemorrhagic stroke. Homocysteine is a risk factor for ischemic as well as haemorrhagic stroke but it has been found to be higher for ischemic stroke. However, there is a controversy regarding the nature of relationship between Hcy and stroke; it is the causative factor or consequence of stroke.

The aim of this study was to evaluate the association of HHcy as a risk factor for an episode of any stroke type that may be ischemic or haemorrhagic in a sample of successive local stroke patients.

### Material and Methods

This cross sectional study was carried out on 150 stroke patients included from September 2010 till August 2011. A written consent was taken from the patients or their near relatives. Approval of our study was obtained from the research ethic committee, Institution Review Board (IRB), Sheikh Zayed Medical and Dental Complex. Ninety-three were males while fifty-seven were females. The participants’ in the age range from 60-69 were forty-six in number (31%) and above sixty were ninety-five (63%). The average age was 60.2 ± 14.5 years. The stroke patients admitted in Neurology and Emergency wards of Sheikh Zayed Medical and Dental Complex and Jinnah Hospital Lahore, were included in this study. These are government funded hospitals serving the district of Lahore and adjacent localities. Demographic data was collected by a proforma along with history of patients. They were diagnosed on the basis of history, physical examination and CT scan of brain as having stroke, haemorrhagic or ischemic (excluding TIAs). The Glasgow coma scale was employed to assess the conscious state of the stroke patient at the time of admission and subsequently at the time of discharge. The study participants included were the stroke patients who reported within 12 hours of the onset of stroke symptoms and had gone through CT scan of brain and found to be suffering from ischemic or haemorrhagic stroke.

Blood samples were also collected from these stroke patients in the first twenty-four hours of stroke symptoms; blood samples were then centrifuged, plasma was separated and aliquots of plasma were kept frozen at -80°C. The tHcy level was, later, estimated in plasma using commercially available ELISA kit (Axis-Shield Homocysteine ELISA kit UK).

The data was entered and analyzed by using SPSS (Statistical Package of Social Sciences) version 15.0. The qualitative variable, stroke type, was described by using number and percentages. Age, BMI and Hcy level was described by using mean ± S.D. The Hcy level was further categorized as moderate (15 – 30 µmol/ L) intermediate (31- 100 µmol/ L) and severe (> 100 µmol/ L) HHcy. The association of Hcy with stroke type was tested by using Chi-square likelihood ratio test. A comparison was made for age and BMI among the three categories of patients by various Hcy levels.

### Table 1: Age and BMI for different plasma homocysteine levels in the three groups

<table>
<thead>
<tr>
<th>tHcy µmol/L</th>
<th>&lt; 15</th>
<th>15 – 30</th>
<th>30 – 100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Minimum</td>
</tr>
<tr>
<td></td>
<td>57.6</td>
<td>16.1</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>62.4</td>
<td>10.1</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>63.2</td>
<td>15.0</td>
<td>28.0</td>
</tr>
<tr>
<td><strong>BMI (Kg/m²)</strong></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Minimum</td>
</tr>
<tr>
<td></td>
<td>22.7</td>
<td>3.6</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>22.9</td>
<td>3.3</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>22.3</td>
<td>3.9</td>
<td>17.5</td>
</tr>
</tbody>
</table>
The homocysteine level was compared for two categories of stroke type by using Mann Whitney U test. Comparison of Hcy level was made for the stroke patients with ischemic and haemorrhagic stroke type; p value<0.05 was considered highly significant.

**Results**

There were 150 patients with cerebrovascular event. Age and BMI both were insignificantly different among the three categories of moderate, intermediate and severe HHcy, with the p-values 0.240 and 0.561 respectively (Table 1 and 2).

<p>| Table 2: Comparison of age and BMI among three categories of plasma homocysteine levels |</p>
<table>
<thead>
<tr>
<th>tHcy µmol/L</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 – 30</td>
<td>74</td>
<td>0.240</td>
</tr>
<tr>
<td>31 – 100</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

| BMI (Kg/m2) |               |         |
| 15 – 30     | 74            | 0.561   |
| 31 – 100    | 43            |         |
| >100        | 33            |         |
| Total       | 150           |         |

*pvalue < 0.05 is significant*

In 126, the victims of ischemic stroke, the geometric mean tHcy was ±SD 19.7± 9.6 while in 24 patients with hemorrhagic stroke, the geometric mean tHcy was ±SD 20.0 ±15.7. The difference in geometric mean tHcy in ischemic and hemorrhagic events was not statistically significant with the p-value 0.346 (Table 3).

<p>| Table 3: Comparison of plasma homocysteine level in different stroke subtypes |</p>
<table>
<thead>
<tr>
<th>Stroke Type</th>
<th>N</th>
<th>Mean ± SD (Range)</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic</td>
<td>126</td>
<td>19.7 ± 9.6 (2.0 – 38.7)</td>
<td>18.3</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>24</td>
<td>20.0 ± 15.7 (1.6 – 47.9)</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Mann Whitney U test p-value = 0.346

**Discussion**

We failed to ascertain a significant relation between tHcy and ischemic stroke. Elevated plasma tHcy levels have been associated with an increased risk of clinical stroke. Several studies suggest that elevated tHcy is a strong graded dose-related risk factor of stroke. HHcy has been reported to be associated with two to three fold increased risk of ischemic stroke particularly in younger age group.

A number of studies show that a mild rise in tHcy escalate the risk of stroke especially of ischemic type in elderly, young adults and children. In a case control study, tHcy was found to be high in ischemic stroke patients as compared to controls after excluding major thrombophillic factors. They reported that moderate HHcy is the only variable that helps recognize the cause of ischemic stroke in younger age group. They have also claimed that a single genetic abnormality is rarely the sole cause of stroke. The association existed significantly even after adjustment of possible confounders such as hypertension, diabetes mellitus and heavy smoking. An Asian study has found a similar relation between HHcy and ischemic stroke in young Asian adults. A Multicentre study in China revealed that plasma tHcy was correlated with both ischemic and hemorrhagic stroke. Serum level of tHcy has also been reported to correlate with carotid artery atherosclerosis in Chinese with ischemic stroke. On the other hand, a lack of association has been reported between plasma total homocysteine concentrations and extra-cranial carotid stenosis in Iranians. Furthermore, in a case-control, prospective study from India, high Hcy emerged as an important risk factor for ischemic stroke in all populations in India, especially in younger age group.

In a Japanese study, it was highlighted, that a moderate elevation of tHcy is strongly associated with increased incidence of total stroke, ischemic stroke and more specifically lacunar infarction. The alliance between tHcy and haemorrhagic stroke was not distinct. A meta-analysis concluded that there was an insignificant relation between Hcy and haemorrhagic stroke while an enhanced association existed with ischemic and recurrent stroke.

Ischemic stroke is a leading cause of death in developing countries. Its prevalence in young adults (15-45) ranges from 3-5%. HHcy is probably the only offender. A prospective cohort, Northern Manhattan Study, carried out in lower socioeconomic group, tHcy above 15µ mol/L was implicated as an independent risk factor for ischemic stroke. They also concluded that vascular effects of elevated tHcy are greater among whites and Hispanics as compared to blacks.

All studies do not substantiate the connection between tHcy and stroke risk and some contradictory results.
have led to a proposal that Hcy could be the consequence and not the cause of ischemic stroke.\textsuperscript{23} However, large prospective studies like Framingham and Rotterdam support the causal relation between tHcy and risk of stroke.\textsuperscript{18,20,24} They debated; it seems very unlikely that Hcy would be only a marker of disease. We did not attain the same conclusion because of some confines. It was a cross-sectional study with a smaller sample size. It did not define age specific quartiles of tHcy. The patients’ age range from 60-69 were 46 in number (30.6%) and above 60 were 95 (63.3%). The average age was 60.2 ±14.5 years. This age group would have an inherent greater prevalence of cardiovascular risk factors that has led to stroke events. We used a single baseline measure and not repeated measures of tHcy to assess the outcomes.

**Conclusion**

Higher Hcy levels are related to increase number of strokes. However, we did not observe any significant difference between the two groups, ischemic and haemorrhagic, with a p-value 0.346.

**Recommendations**

Randomized controlled trials have shown that vitamin supplements can effectively reduce plasma tHcy levels and the risk of stroke by 18\% provided the treatment duration is for more than thirty-six months.\textsuperscript{162} Large scale trials are needed to determine whether lowering of tHcy levels would reduce the risk of stroke and other cardiovascular diseases.

**Acknowledgments**

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**Conflict of interest**

This study has no conflict of interest to declare by any author.

**References**


19. He Y, Li Y, Chen Y, Feng L, Nie Z. Homocysteine level and risk of different stroke types: A meta-analysis of