Comparison of serum homocysteine level with the risk factors of stroke: A prospective study

Dr. Shailendra Singh, Dr. Chetan Mathur, Dr. Hritu Singh and Dr. Shahid Abbas

Abstract
Background: Stroke is one of the leading causes of mortality and morbidity worldwide. Approximately 20 million people each year suffer from stroke and of these 5 million do not survive.

Aims and Objective: To compare the serum homocysteine level among the groups and compare it with the presence of hypertension, diabetes and smoking status.

Materials and Methods: Ninety subjects were studied in the Department of General Medicine at Sri Aurobindo Medical College and PGI, Indore (M. P.) for one and half year from June 2015 to March 2016 after dividing them into Cases (n=45; patients of ischemic stroke) and Control (n=45; subjects with no documented stroke). After recording general information on smoking/alcohol habit, hypertension and diabetes status, serum homocysteine level was assessed in all the subjects.

Results: Mean homocysteine level (37.75±9.39 μmol/L) was greater in Case group as compared to Control group (11.79±1.64 μmol/L) (p<0.001). Amongst those who had a stroke but had a habit of smoking/tobacco, had hypertension and diabetes diagnosed with high mean Homocysteine level (54.01±6.00 μmol/L, 41.08±1.25 μmol/L and 35.88±8.65 μmol/L) as compared to those without stroke. That patient had mixed diet detected with raised Homocysteine as compared to those patients whose type of diet was vegetarian.

Conclusion: Hyperhomocysteinemia appears to be an important risk factor for cerebrovascular accidents.

Keywords: Cardiovascular diseases, stroke, hyperhomocysteinemia, hypertension, diabetes mellitus

Introduction
Stroke is the second leading cause of death worldwide. It caused an estimated 5.7 million deaths in 2005, and the global number of deaths is projected to rise to 7.8 million in 2030 [1]. Risk factors themselves do not cause stroke directly, but instead promote the development of the underlying pathological process like atherosclerosis, responsible for stroke. A number of factors that may be classified as modifiable & non-modifiable, increase the risk for ischemic stroke. Non modifiable risk factors for stroke are older age, male gender, ethnicity and family history [2].

Modifiable risk factors may be subdivided into lifestyle and behavioral risk factors and non-lifestyle risk factors. The modifiable lifestyle risk factors include cigarette smoking and illicit drug use [3]. Non-lifestyle risk factors include low socioeconomic status, arterial hypertension, dyslipidemia, diabetes mellitus, heart disease, asymptomatic carotid artery disease and hyper-homocysteinemia [4].

Hyperhomocysteinemia is generally acknowledged as a treatable risk factor for atherothrombotic disease, but a causal relationship between both is not yet definitely established. There is a very high sensitivity, specificity, and accuracy with 89% positive predictive value and odds ratio for homocysteine in cardiovascular patients when compared to other risk factors [5, 6].

There is very limited evidence available comparing the serum homocysteine level with the risk factors. Hence in present study we tried to compare the serum homocysteine level with different risk factors of stroke.

Materials and Methods
Present case-control study was performed in the Department of General Medicine at Sri Aurobindo Medical College and PGI, Indore (M. P.)
for one and half year from June-2015 to March-2016. Patients with first ever episodes of ischemic stroke presenting within two weeks of the event having age between 15 years to 45 years and those willing to give informed consent were included in the present study. Patients with non-hemorrhagic stroke, renal, hepatic thyroid dysfunction, collagen vascular diseases, chronic inflammatory diseases like HIV, syphilis, tuberculosis, cancer, patient on steroids and anticonvulsants, pregnancy state and Postpartum period and patients with rheumatic heart disease were excluded from the present study. Forty five cases of ischemic stroke visited/admitted at study center with weakness of limb, and a rise in serum biomarkers of stroke included as subjects in case group while forty five individuals had no documented stroke served as subjects in control group for this study. Controls recruited from hospital staff or individuals who accompany patients referred to the hospital. The patient and controls had explained about the complete study in his/her own language and his/her willingness to participate had recorded in a consent form duly signed by him/her. After recording general information serum Homocysteine level was assessed in cases of ischemic stroke and controls to identify the role of serum Homocysteine as a risk factor.

Method of estimation of serum homocysteine

Serum Homocysteine was estimated by Chemiluminescent Immunoassay method, variant of standard ELISA. The upper limit of the manufacturer and the laboratory was 15 μmol/L. Values above 15 μmol/L were acceptably high.110 Luminescence is described as the emission of light from a substance as it returns from an electronically excited state to ground state. The various forms of luminescence (Bioluminescence, Chemiluminescence, Photoluminescence) differ in the way the excited state is reached. Chemiluminescence is light produced by chemical reaction. “The chemiluminescent substance is excited by the oxidation and catalysis forming intermediates. When the excited intermediates return back to their stable ground state, a photon is released, which is detected by the luminescent signal instrument.” It is believed that luminescent assays, are the most sensitive detection method currently in use due to the ability of signal multiplication and amplification. Luminescent reactions are measured in the relative light units (RLU) that are typically proportionate to the amount of analyte in a sample.

Normal Levels of Homocysteine

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult male</td>
<td>06-15 μmol/L</td>
</tr>
<tr>
<td>Adult female</td>
<td>03-12 μmol/L</td>
</tr>
<tr>
<td>Elderly &gt;65 years</td>
<td>15-2 μmol/L</td>
</tr>
</tbody>
</table>

Statistical analysis

The analysis of the gathered data done by using both descriptive and inferential statistics based on the predetermined objectives of the study. The descriptive statistics had used to identify the features and the characteristic of the subjects while inferential statistics used to test the significance in order to make a comparison of serum Homocysteine levels between patients with ischemic stroke and controls from the gathered data. Results on continuous measurements presented on Mean ± SD (Min-Max) while the results on categorical measurements presented in numbers or percentage. Independent sample t-test was used to identify the significance of differences in serum Homocysteine between groups (case group and control group) that treated as Z-test due to large size of sample (n>30). The Pearson’s Chi-Square test had used to observe the association of habit of smoking/tobacco, hypertension, diabetes mellitus, ischemic heart disease, diet pattern and family history with groups (case and control). The probability value from p<0.05 to p<0.02 was considered as statistically significant while from p<0.01 to p<0.001 was considered as statistically highly/strongly significant.

Results

Out of a population of ninety, more than half (56, 62.2%) of the subject was male while rest (34, 37.8%) was female. The age of all cases and controls found to be in the ranges from 15 to 45 years. The mean age (mean ± SD) of all samples (N=90) was 36.53±7.53 years. The scatter of mean age for the case group (n1=45) was 36.80±7.90 years while for controls (n2=45) was 36.27±7.22 years. The scatter of mean age for the subject was male while rest (34, 37.8%) was female. The age of all cases and controls found to be in the ranges from 15 to 45 years. The mean age (mean ± SD) of all samples (N=90) was 36.53±7.53 years. The scatter of mean age for the case group (n1=45) was 36.80±7.90 years while for controls (n2=45) was 36.27±7.22 years had ranges from 20 to 45 years.

Table 1: Comparison of serum homocysteine levels between cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean±SD</th>
<th>Mean diff</th>
<th>Z value</th>
<th>LOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Homocysteine (μmol/L)</td>
<td>Case</td>
<td>37.75±9.39</td>
<td>29.96 μmol/L</td>
<td>18.27</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11.79±1.64</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The mean differences between groups are highly significant at the 0.005 and 0.001 levels of significance. [SD: Standard Deviation; Mean Diff: Mean Difference; LOS: Level of significance]

Table 2: Comparison of serum homocysteine level (μmol/L) according to habit of smoking between cases and controls

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Mean Diff</th>
<th>Z-value</th>
<th>LOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Case</td>
<td>44</td>
<td>37.38±9.16</td>
<td>25.59</td>
<td>18.44</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>45</td>
<td>11.79±1.64</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>Case</td>
<td>1</td>
<td>54.01±0.00</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*The mean differences between groups are highly significant at the 0.001 level of significance. [SD: Standard Deviation; Mean Diff: Mean Difference; LOS: Level of significance]
The differences in mean serum homocysteine (25.63 μmol/L) between subjects of case group and control group that hadn’t diabetes mellitus were confirmed statistically highly significant (p<0.001). Blood pressure is an important risk factor for stroke. However when statistical analysis was done for serum homocysteine levels in subjects with relation to their hypertension status, we found out that there is no significant correlation between serum homocysteine and blood pressure and subsequent hypertension risks. This finding of us was similar to Wayne et al who did not find definite evidence of an increased homocysteine in hypertensive patients [15]. But findings of Li et al. [16] and Cai et al. [17] differ with such co relation and say that definite relation between serum homocysteine level and risk of hypertension.

Amongst those cases who hadn’t diabetes mellitus diagnosed with increased mean homocysteine level (37.84±9.51 μmol/L) as compared to mean Homocysteine level (11.79±1.64 μmol/L) among normal individual of control group. Analysis of table revealed that the differences in mean serum Homocysteine (25.63 μmol/L) levels between subjects of case and control groups that hadn’t diabetes mellitus were confirmed statistically highly significant (p<0.001). Our findings were consistent with study of Sundstrom et al and Bowman et al. [18, 19] Amongst those who had a stroke but had mixed diet reported with greater mean homocysteine level (38.51±8.49 μmol/L) as compared to mean Homocysteine level (11.90±1.78 μmol/L) among normal individual of control group. Analysis of table revealed that the differences in mean serum Homocysteine (26.61 μmol/L) levels between subjects of case and control groups that hadn’t diabetes mellitus were confirmed statistically highly significant (p<0.001). Our findings were consistent with study of Sundstrom et al and Bowman et al. [18, 19].
According to Jayanti Kalita et al. In vegetarians S. Homocysteine levels were higher than non-vegetarians [21]. In cases, the mean Homocysteine level (37.75±9.39 µmol/L) was greater as compared to mean Homocysteine level (11.79±1.64 µmol/L) among normal individuals of control group. Differences in mean serum Homocysteine (29.96 µmol/L) levels between cases and controls were statistically highly significant (p<0.001). Moreover, the statistical agreement were to that serum Homocysteine level were higher in stroke patients as compared to normal.

Hyperhomocysteinaemia defined an elevated homocysteine concentration as one that exceeds 15.8 µmol/L (95th percentile for healthy control subjects) [22]. In current study, serum fasting total plasma homocysteine level in case group was (30.10±14.8 µmol/L) which was significantly higher than the controls (13±5.3 µmol/L), (p=0.001). Our findings were similar to findings of [23] and Brattstrom et al. [24] who concluded that hyperhomocysteinaemia as an important risk factor for ischemic stroke.

This study conducted on a limited sample. Large scale study is required to observe the more significant changes. Folic acid levels was not estimated in this study which could find the relationship between homocysteine with vitamin B12 and folic acid. Deficiency of these vitamins leads to hyperhomocysteinaemia which is a risk factor for atherosclerosis and stroke in young. This study considered only cases having ischemic stroke, we have not included hemorrhagic strokes.

**Conclusion**

Our study revealed that hyperhomocysteinaemia appears to be an important risk factor for cerebrovascular accidents. It is therefore important to use serum homocysteine level as an important tool to investigate all cases of young cerebrovascular accidents and also in those who are at risk of developing it. Significant correlation has been found between homocysteine concentration and ischaemic stroke.

**References**