EVALUATING SERUM VITAMIN B12 LEVEL AS THE RISK FACTORS FOR STROKE: A PROSPECTIVE STUDY

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Article Info: Received 18 March 2020; Accepted 24 April 2020
DOI: https://doi.org/10.32553/ijmbs.v4i4.1100
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Conflict of interest: No conflict of interest.

Abstracts
Background: Hyperhomocysteinemia has been reported to be an independent risk factor for the development of stroke. Vitamin B12 is an important element of homocysteine metabolism.

Aims and Objective: To compare the vitamin B12 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 in to 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 vitamin B12 level was assessed in all the subjects.

Results: Vitamin B12 level (184.98±46.41 picomol/L) was greater in Case group as compared to Control group (639.6±157.43μmol/L) (p<0.001). Amongst those who had a stroke but had a habit of smoking/tobacco, had hypertension and diabetes diagnosed with very lowered vitamin B12 level (199.00±0.00 picomol/L, 176.00±31.21 picomol/L and 152.00±11.31 picomol/L) as compared to those without stroke. Those who had a stroke but had mixed diet had much lowered vitamin B12 level (188.71±54.63 picomol/L) as compared to control (592.69±143.71 picomol/L).

Conclusion: Patients with stroke had lower level of vitamin B12 mainly in those with had a habit of smoking/tobacco, had hypertension, diabetes and had mixed diet as compared to those without stroke.

Keywords: vitamin B12, stroke, hypertension, diabetes, smoking

Introduction
Previous reports have demonstrated an increased vitamin B12 levels with lower plasma homocysteine levels. This combination is an established risk factor for myocardial infarction and stroke. 1,2 However Indian data on this relationship is limited. Prospective observational studies may provide valuable information on this point by throwing some light on the associations between B12 vitamins and stroke risk. Only few prospective studies have investigated the association between vitamin B12 and the risk of stroke; however the results are inconsistent.3–6

Stroke has several risk factors such as presence of diabetes, hypertension, smoking and drinking habits. 7 To best our knowledge there is no study comparing the association between vitamin B12 with the established risk factor. We therefore tried to examining the associations between vitamin B12 levels and the established risk factors stroke in Indian population.

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.

Non-probability sampling technique used to recruit the desired samples from the target population. Samples had chosen by using purposive sampling technique that met inclusion-exclusion criterion.

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.

Patient who had ischemic stroke screened through CT/MRI findings and the normal individuals who had no documented heart disease further met the inclusion
criteria selected as subjects during specified schedule. A maximum of ninety, patients and normal individuals purposively chosen and divided equally into two groups of equal size of 45 from the inpatient department of General Medicine and neurology at Sri Aurobindo Medical College and PGI, Indore were designated as subjects for the present research 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 dually signed by him/her. Observations on selected parameters for two groups recorded.

Informed consent was taken from patients and control subjects. Selected subject's fasting blood samples were collected with all aseptic precautions. 5 ml of blood was collected from a median cubital vein and were send for analysis. Vitamin B12 levels were assessed in cases of ischemic stroke and controls. Clinical information including age and sex was obtained.

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 vitamin B12 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.

A comparison in vitamin B12 level among smokers and non-smokers, hypertensive and non-hypertensive, diabetics and non-diabetics and vegetarian and mixed diet was carried out between cases and controls by using independent sample t-test. The Pearson’s Chi-Square test had used to observe the association of habit of smoking/tobacco, hypertension, diabetes mellitus, ischemic heart disease and diet pattern with groups (case and control).

The probability value, p=0.05 was considered as statistically insignificant. The probability value from p<0.05 to p=0.02 was considered as statistically significant while from p<0.01 to p=0.0001 was considered as statistically highly/strongly significant.

### Results

Out of a 90 subjects, 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 and found within ranges from 15 to 45 years while for controls (n2=45) was 36.27±7.22 years had ranges from 20 to 45 years.

#### Table 1: Comparison of vitamin B12 levels between cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Spread</th>
<th>Mean Diff</th>
<th>Z-value</th>
<th>LOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>Case</td>
<td>184.98±46.41</td>
<td>454.64</td>
<td>18.5</td>
<td>p&lt;0.00</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>639.62±157.4</td>
<td>184.66</td>
<td>8</td>
<td>1 #</td>
</tr>
</tbody>
</table>

# The mean differences between groups are highly significant at the 0.005 and 0.001 levels of significance. * The mean differences between groups are significant at the 0.02 level of significance. ** The mean differences between groups aren’t significant (insignificant) at the 0.05 level of significance. [SD: Standard Deviation; Mean Diff: Mean Difference; LOS: Level of significance]

#### Table 2: Comparison of vitamin B12 level according to habit of smoking between cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>n</th>
<th>Spread</th>
<th>Mean Diff</th>
<th>Z-value</th>
<th>LOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Smoking\Tobacco</td>
<td>Vitamin B12</td>
<td>Case</td>
<td>4</td>
<td>184.66±46.8</td>
<td>454.96</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4</td>
<td>639.62±157.4</td>
<td>9</td>
<td>1 #</td>
<td></td>
</tr>
<tr>
<td>Smoking\Tobacco</td>
<td>Vitamin B12</td>
<td>Case</td>
<td>1</td>
<td>199.0±0.00</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</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]

A comparison on the basis of diagnosis of hypertension between cases and controls was carried out to evaluate the significance of differences in vitamin B12 level.

Stroke patients who hadn’t hypertension diagnosed with had much lowered vitamin B12 level (185.85±47.82 picomol/L) as compared to vitamin B12 level (639.62±157.43 picomol/L) of subjects included as controls. Result of present study showed that the differences in mean vitamin B12 (453.77 picomol/L) levels.
between case group and control group that hadn’t hypertension were confirmed statistically highly significant (p<0.001). Amongst those who had an ischemic stroke but had hypertension had much lowered vitamin B₁₂ level (176.00±31.21 picomol/L). Hypertension wasn’t noted in any of the controls of the control group and hence no differences in vitamin B₁₂ levels were applicable to examine in order to test the significance between case and control. Moreover, the statistical agreement projected that vitamin B₁₂ level were much lowered in stroke patients had hypertension as compared to normal individuals.

A comparison on the basis of diagnosis of diabetes mellitus between cases and controls was conducted to rule out the significance of differences in vitamin B₁₂ level.

Amongst those cases who hadn’t diabetes mellitus had much lowered vitamin B₁₂ level (186.51±46.89 picomol/L) as compared to vitamin B₁₂ level (639.62±157.43 picomol/L) among normal individual of control group. Analysis revealed that the differences in mean vitamin B₁₂ (453.11 picomol/L) levels between subjects of case and control groups that hadn’t diabetes mellitus were confirmed statistically highly significant (p<0.001). Amongst those who had an ischemic stroke with diabetes mellitus had much lowered vitamin B₁₂ level (152.00±11.31 picomol/L). Diabetes mellitus wasn’t noted in any of the normal of the control group and hence no differences in vitamin B₁₂ levels were applicable to examine in order to test the significance between case and control.

**Table 3**: Comparison between cases and controls according to type of diet to evaluate vitamin B₁₂ levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>N</th>
<th>Spread</th>
<th>Mean Diff</th>
<th>Z- value</th>
<th>LOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₁₂ (picomol/L)</td>
<td>Vegetarian</td>
<td>Case</td>
<td>24</td>
<td>181.7±138.74</td>
<td>476.98</td>
<td>14.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>32</td>
<td>658.69±160.89</td>
<td>##</td>
<td>****</td>
</tr>
<tr>
<td>Vitamin B₁₂ (picomol/L)</td>
<td>Mixed diet</td>
<td>Case</td>
<td>21</td>
<td>188.71±54.63</td>
<td>403.98</td>
<td>11.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>13</td>
<td>592.69±143.71</td>
<td>##</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]

**Discussion**

Cardiovascular disease is the leading threat to human health as well as the major cause of death worldwide, especially in developed countries. And studies found that heredity, nutrition, lifestyle and environmental factors affect total homocysteine concentration. Homocysteine has shown an inverse correlation with the vitamin B₁₂ levels in many previous studies.

In present study vitamin B₁₂ level (184.98±46.41 picomol/L) was greater in those with stroke as compared to those without stroke (11.79±1.64 μmol/L) (p<0.001). A study from Germany involving among 25 770 participants having age 35 to 65 years reported that low vitamin B₁₂ levels were associated with an increased risk of cerebral ischemia which is in line with the present study where patients with stroke has significantly lower level of vitamin B₁₂ as compared to those without stroke. In the same study it was found that the risk increase associated with low vitamin B₁₂ and folate became weaker and nonsignificant after adjustment for homocysteine plasma levels, suggesting that the effect of these B vitamins may be mediated in part by their influence on homocysteine metabolism.

Several studies have proved that smoking as the major and independent risk factor for the development of cardiovascular diseases (CVD) and could increase the incidence of myocardial infarction and fatal coronary artery diseases. In present study patients with stroke who had a habit of smoking/tobacco had lower level of vitamin B₁₂ as compared to those without stroke. Chen et al in a studied 576 elderly patients with CVD and found that the serum vitamin B₁₂ level was significantly lower in current smokers. In same study it was found that serum Vitamin B₁₂ level in former smokers and current smokers were both lower than that in the never smokers, and the lowest level was of current smokers. The possible mechanism of this relationship as derived by the previous study might be due to that smoking might be account for the blocked pathway of homocysteine methylation into methionine.

In present study patients with stroke who had had hypertension had lower level of vitamin B₁₂ as compared to those without stroke. In line with present study findings study done by Zacharia et al and Madonna et al reported similar findings. They reported that in patients with hypertension who had stroke had significantly lower vitamin b₁₂ levels as revealed by the high level of homocysteine levels. They also highlighted the importance of vitamin B₁₂ supplementation in stroke patients.

In present study patients with stroke who had diabetes had lower level of vitamin B₁₂ as compared to those without stroke. In a similar study from Jaipur by Kumawat et al including 80 cases of f ischemic stroke reported that adjusted odds ratio of serum vitamin B₁₂ level for the development of ischemic stroke was 3.22 which was even higher than the risk for stroke due to the presence of diabetes which was 1.34 in this study. This means vitamin B₁₂ deficiency emerged as significant risk factors for occurrence of stroke in the present study which is also advocated by the previous studies.
In present study those who had a stroke but had mixed diet had much lowered vitamin B12 level as compared to those without stroke. Previous studies have also shown that the mean serum B12 was 154 pg/ml, even though 62% of participants were nonvegetarians.\textsuperscript{15} Yajnik et al and Refsum et al both the authors did a detailed analysis of dietary habits of their participants and concluded that though B12 deficiency was more commonly associated with vegetarianism, the food habits could only partly explain the high prevalence of B12 deficiency.\textsuperscript{15, 16}

Conclusion
Based on the findings of present study it can be concluded that patients with stroke had significantly low level of vitamin B12 as compared to normal person. Vitamin B12 was significantly low among those stroke patients who had a habit of smoking/tobacco, had hypertension and diabetes. Those who had a stroke but had mixed diet had much lowered vitamin B12 level as compared to those without stroke. That’s means low vitamin B12 levels can be an important risk factors for the development of stroke.

References