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## Impact of the Serum Homocysteine Levels in Patients of Vascular Dementia

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### ABSTRACT

**Background:** *Vascular dementia is a degenerative cerebrovascular disorder that results from ischemic or haemorrhagic brain damage. Persons with vascular dementia are at great risk of morbidity and mortality than those with Alzheimer's disease.*

*Elevated serum total homocysteine level is associated with vascular dementia. Metabolism of homocysteine is closely associated with that of folic acid, vitamin B6 and B12. Homocysteine has a neurotoxic effect that could lead to neurologic disturbances. Hence, it is important to explore the status of serum homocysteine in vascular dementia to evolve the treatment strategies for the same.*

**Objectives:** *To determine the levels of serum homocysteine in cases of vascular dementia and to compare it with age and sex matched controls.*

**Methodology:** *In this case control study, 30 vascular dementia cases and 30 age and sex matched controls were selected. Serum homocysteine levels were estimated by enzymatic photometric method.*

**Results:** *Serum homocysteine levels were elevated significantly in cases with  $26.28 \pm 14.22$  (Mean  $\pm$  SD) compared to  $19.44 \pm 7.36$  in controls with  $p = 0.023$ . Pearson correlation of MMSE score with homocysteine levels was found to be significant with  $p$  value of 0.009. This indicates that cases with high serum homocysteine levels had more cognitive deficit.*

**Conclusion:** *Hyperhomocysteinemia is an independent and modifiable risk factor for vascular dementia, hence vitamin B supplementation should be considered as preventive and therapeutic intervention. It is recommended to measure serum homocysteine levels in all high risk individuals who are prone for cerebrovascular accident and dementia.*

**Key words:** *Vascular dementia, homocysteine, enzymatic photometry, MMSE score*

## INTRODUCTION

Dementia is a major health problem worldwide and the number of people affected is expected to rise considerably, especially in developing countries like India. Dementia is defined as an acquired deterioration in cognitive abilities that impairs the successful performance of activities of daily living. Memory is the most common cognitive ability lost with dementia.<sup>1</sup>

Vascular risk factors are involved in causation of both vascular dementia and Alzheimer's disease, which accounts for 90% of all dementias. Vascular dementia is a degenerative cerebrovascular disorder that results from ischemic or haemorrhagic brain damage. Persons with vascular dementia are at great risk for morbidity and mortality than those with Alzheimer's disease.<sup>2,3</sup>

Elevated total homocysteine is significantly associated with vascular dementia (VaD).<sup>4</sup> Metabolism of homocysteine is closely associated with that of folic acid, vitamin B6 and B12.<sup>5</sup> A shortage of B12 or folic acid causes an increase in homocysteine concentrations. Homocysteine is a sensitive marker of vitamin B12 and folate status.<sup>6</sup> Abnormality of methylene tetrahydrofolate reductase enzyme results in hyperhomocysteinemia.<sup>7</sup>

Hyperhomocysteinemia has been related to microangiopathy, endothelial dysfunction, impaired Nitric oxide activity and increased oxidative stress. All factors associated with aging of the brain.<sup>8</sup>

Low vitamin B12 concentration and hyperhomocysteinemia are common in Indian

men, particularly in vegetarians.<sup>9</sup> Hyperhomocysteinemia, now a days is being considered as an independent and modifiable risk factor for cerebrovascular diseases.

Supplementation of vitamin B12 and folic acid is recommended for elderly by many studies, to prevent dementia and associated morbidity. The present study is undertaken to compare the serum levels of Homocysteine, in patients of vascular dementia with controls.

## MATERIALS AND METHODS

A case control study of serum homocysteine levels in patients of vascular dementia was conducted in patients admitted to hospital. The diagnosis of vascular dementia was established by NINDS-AIREN<sup>10</sup> criteria. Each gave informed consent and the study was approved by ethical and research committee of BMC & RI to use human subjects in the research study. Duration of study was from November 2011 to March 2013.

Inclusion criteria for cases- Patients above 18 years of age, fulfilling criteria of probable vascular dementia according to NINDS-AIREN criteria.<sup>10</sup>

Exclusion criteria-Cases of Endocrine abnormalities, Chronic infectious diseases like HIV/Neurosyphilis, Toxin induced cognitive dysfunction, Primary neoplasm/metastasis.

Controls - Normal healthy age and sex matched individuals.

Cognitive deficit in cases was assessed on the basis of MMSE<sup>1</sup> (minimal status examination) score.

Informed consent was taken from patients and control subjects. Selected subject's blood samples were collected with all aseptic precautions. 5 ml of blood was collected from the median cubital vein. It was allowed to clot for 30 minutes in a clean dry test tube and was subjected to centrifugation for 20 minutes to separate the serum. The serum samples were stored at  $-80^{\circ}\text{C}$  till they were analyzed.

The separated serum was used to estimate

- A quantitative measurement of serum homocysteine was done in instrument Olympus AU2700 by enzymatic photometric method.<sup>11,12</sup>
- Estimation of serum fasting blood sugar [FBS] and postprandial blood sugar [PPBS] by Hexokinase method, blood urea by Urease method & serum creatinine by Jaffe's method, serum lipid profile by Enzymatic method and HIV test by ELISA method were also performed.
- The chemicals and reagents used for the procedures were of analytical grade.
- Normal reference range of homocysteine:
  - Adult male : 6-15  $\mu\text{mol/L}$
  - Adult female : 3-12  $\mu\text{mol/L}$
  - Elderly > 65 yrs : 15-20  $\mu\text{mol/L}$

## RESULTS

**Statistical software:** The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

**Statistical Methods:** Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean  $\pm$  SD [Min-Max] and results on categorical measurements are presented as Number [%]. Significance is assessed at 5 % level of significance.

Student t test [two tailed, independent] has been used to find the significance of study parameters. Pearson correlation between MMSE and study variables is performed to find the relationship.

### Significant figures

+ Suggestive significance [ p value:  $0.05 < p < 0.10$ ]

\* Moderately significant [ p value:  $0.01 < p \leq 0.05$ ]

\*\* Strongly significant [ p value:  $p \leq 0.01$ ]

Among 30 cases in this study, 3 were in 32 – 40 years age group [10%], 7 were in 41 – 50 years age group [23.3%], 8 were in 51 – 60 years age group [26.7%], 8 were in 61 - 70 years age group [26.7%] and 4 were in 71 – 80 years age group [13.3%].

Among 30 cases, 21 were men [70%] and 9 were women [30%]. Among 30 controls, 21 were men [70%] and 9 were women [30%].

In the present study, 12 [40%] out of 30 cases were vegetarians and 18 [60%] were on mixed diet. Among controls, 8 [26.7%] were vegetarians and 22 [73.3%] were on mixed diet.

Among 30 cases, 8 [26.7%] had diabetes mellitus, 22[73.3%] had hypertension, 15 [50%] were smokers and 7 [23.3%] were alcoholic.

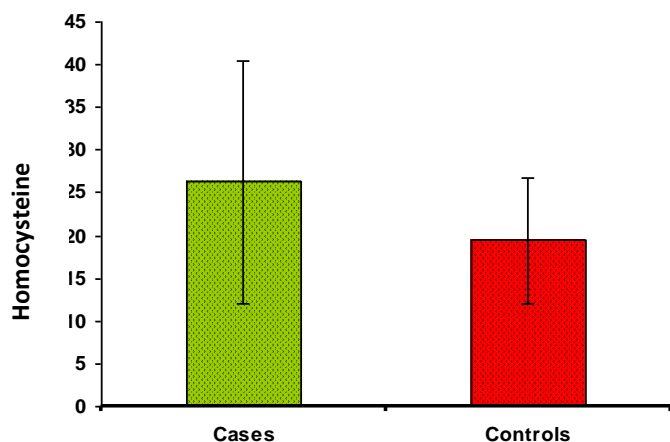
The mean and SD of serum homocysteine in the controls were 19.44  $\mu\text{mol/L}$  and 7.36  $\mu\text{mol/L}$  respectively and the mean and SD in the cases

were 26.28  $\mu\text{mol/L}$  and 14.22  $\mu\text{mol/L}$  respectively with significant p value [0.023]. Mean  $\pm$  SD of total cholesterol [TC] in cases was 201.33  $\pm$  50.05 mg/dL and in controls mean  $\pm$  SD was 177.43  $\pm$  29.76 mg/dL with significant p value [0.028] which is shown in table 1 and graph 1.

**Table 1:** Comparison of levels of Serum Homocysteine in cases and controls

Variables	Cases	Controls	P value
Homocysteine[ $\mu\text{mol/L}$ ]	26.28 $\pm$ 14.22	19.44 $\pm$ 7.36	0.023*
Total cholesterol [mg/dL]	201.33 $\pm$ 50.05	177.43 $\pm$ 29.76	0.028*

**Graph 1:** Comparison of levels of Serum Homocysteine in cases and controls



The mean  $\pm$  SD of homocysteine in vegetarians and mixed diet patients was 33.75  $\pm$  16.50 $\mu\text{mol/L}$  and 21.30  $\pm$  10.16  $\mu\text{mol/L}$  respectively with significant p value 0.016 shown in table 2.

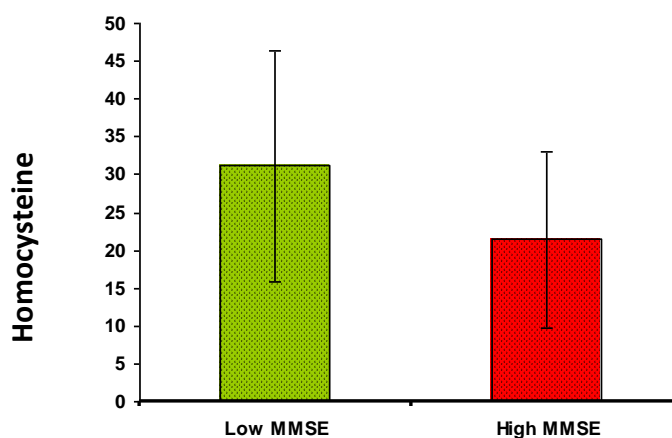
**Table 2:** Mean Levels of Homocysteine according to Diet in cases

Variables	Diet		P value
	Veg	Mixed	
Homocysteine[ $\mu\text{mol/L}$ ]	33.75 $\pm$ 16.50	21.30 $\pm$ 10.16	0.016*
	0	6	

Comparison of Mean Levels of Homocysteine with MMSE score in cases

For low MMSE group, mean  $\pm$  SD of homocysteine was 31.17  $\pm$  15.26  $\mu\text{mol/L}$  and for high MMSE group, mean  $\pm$  SD of homocysteine was 21.40  $\pm$  11.61  $\mu\text{mol/L}$  with p value 0.059 depicted in graph 2.

**Graph 2:** Comparison of Mean Levels of Homocysteine with MMSE score in cases



Pearson correlation between MMSE score and levels of Homocysteine in cases showed  $r = -0.468$  and  $p = 0.009$  indicating highly significant negative correlation. This is shown in table 3.

**Table 3:** Pearson correlation between MMSE score and levels of Homocysteine in cases

Pair	Cases [n=30]	
	r value	p value
MMSE vs Homocysteine	-0.468	0.009**

## DISCUSSION

In this study serum homocysteine level was estimated in all the 30 patients of vascular dementia and compared with controls. Serum homocysteine levels were significantly increased in patients of vascular dementia when compared to controls with a p value of 0.023. Similar results were found in study done by Malaguarnera M et al.<sup>13</sup>

Present study is in accordance with findings of a case control study done by Nafija Serdarevic et al<sup>14</sup> where they found significant high concentration of homocysteine in VaD group with p value being <0.05. Homocysteine concentration rises significantly in acute and post-acute phase of ischemic brain stroke which is a predictor for further development of VaD or a new ischemic brain stroke. Similar study by Pierluigi Quadri et al<sup>15</sup> shows higher mean homocysteine concentration in VaD patients as compared to elderly controls [p<0.05].

High homocysteine levels are associated with increased risk of cardiovascular and cerebrovascular disease, although there are studies that show no increase in risk and there is still debate as to the strength and validity of the association.<sup>16</sup>

In our study, 15 patients [50%] had MMSE score < 20 with mean homocysteine levels 31.17±15.26

μmol/L, which was suggestive of significant association between high homocysteine and low cognitive score. These findings are in accordance with a study done by Pierluigi Quadri et al.<sup>15</sup>

Many studies found significant association between vitamin B12, folate levels and dementia. A study conducted by Merrill F.Elias et al among 812 participants concluded that metabolism of homocysteine is closely associated with that of folic acid, vitamin B6 and B12. Homocysteine is a sensitive marker of vitamin B12 and folate status. Higher folate, vitamin B6 and B12 concentrations have been positively associated with cognitive performances.<sup>5</sup>

Hyperhomocysteinemia has also been reported to have a neurotoxic action independent of its vascular effects by overstimulation of N- methyl D-aspartate receptors or by an increasing hippocampal neuron vulnerability to excitotoxic insults and amyloid-β peptide toxicity.<sup>17,18</sup>

A prospective population-based study done by Giovanni Ravalgia showed that hyperhomocysteinemia doubles the risk of developing dementia and AD independently of several major confounders.<sup>19</sup>

High Hcy levels induced oxidative stress may occur as a result of decreased expression and/or activities of key antioxidant enzymes such as catalase and glutathione peroxidase results in decreased bioavailability of the endothelium-derived signaling molecule nitric oxide via oxidative inactivation.<sup>20</sup>

Hence we conclude that hyperhomocysteinemia is an independent and modifiable risk factor for vascular dementia. This indicates the importance

of this marker in early diagnosis for therapeutic interventions.

A regular supplementation or fortification of food with vitamin B12 and folate which will reduce the homocysteine levels could be a worthwhile preventive measure to decrease the morbidity associated with vascular dementia.

As the sample size of present study was less, further large group studies are needed to assess the association of serum homocysteine and vitamin B status in patients with vascular dementia.

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