

## THE STUDY OF BIOMARKER HOMOCYSTEINE AS RISK FACTOR IN ACUTE CEREBRAL STROKE PATIENTS OF WESTERN RAJASTHAN

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### ABSTRACT

#### BACKGROUND

Traditional risk factors for stroke include advanced age, male sex, hypertension, diabetes mellitus, cardiovascular diseases, hyperlipidaemia, obesity, smoking, physical inactivity and psychological factors. However, recent studies indicate that a moderately elevated plasma level of the amino acid homocysteine constitutes an additional risk factor for stroke, coronary heart disease and deep vein thrombosis. The present case control study was undertaken to estimate the biomarker plasma homocysteine level in Cerebrovascular Accident (CVA) cases and normal subjects of Western Rajasthan.

#### MATERIALS AND METHODS

This study was conducted on 50 stroke patients (Case group) and 50 normal subjects (Control group) of both sex aged between 30 yrs. - 75 yrs. All study groups were subjected to investigations including neurological examination, blood examination, renal function, urine examination, chest x-ray, ECG and CT/MRI.

#### RESULTS

Plasma homocysteine (PHcy) levels were observed significantly high in patient with stroke group compared to control group ( $p < .001$ ). Observation of present study showed that in stroke patients 40 subjects were showing hyperhomocysteinaemia, while in control group only 2 subjects showed hyperhomocysteinaemia. The Barthel Index and PHcy level showed a negative correlation. It was observed that as the severity of stroke rises in study group the plasma homocysteine level also rises, which was found statistically significant ( $p = < .05$ ). Triglyceride (TG) and HDL were raised significantly in case group as compared to control group ( $p < .05$ ). Mean diastolic blood pressure level and mean blood sugar level was raised significantly in case group. Highest mean plasma homocysteine level was observed in Ischaemic Heart Disease (IHD) subjects in case group.

#### CONCLUSION

Raised plasma homocysteine is a significant cardiovascular risk factor in Western Rajasthan population, commoner than diabetes, smoking and even hypertension and carrying the same risk roughly as each of the 3 above. It is therefore recommended that PHcy estimation should be included as a routine laboratory test for patients with cerebrovascular risk factors and public should be made aware of it.

#### KEYWORDS

Elevated Homocysteine level, Acute Cerebral Ischaemic Stroke, Barthel Index, Western Rajasthan.

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#### BACKGROUND

Stroke is one of the leading cause of death and disability worldwide. The best way to reduce its incidence is prevention of its risk factors. Elevated Homocysteine level is an approved risk factor in stroke and atherosclerosis.<sup>[1,2]</sup>

Homocysteine is produced by metabolism of methionine amino acid in our body. Its level can be raised in kidney diseases, genetic abnormalities by various drugs and deficiency of B12. Homocysteine shows many harmful actions on cardiovascular system including impaired endothelial function, raised oxidants and induction of thrombosis.<sup>[3]</sup>

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Elevated plasma homocysteine ( $> 16$  micromole/L) is significantly associated with ischaemic stroke and treating hyperhomocysteinaemia may be an effective way of decreasing the risk of stroke.<sup>[4]</sup>

Hyperhomocysteinaemia has a multifactorial origin incorporating genetic, nutritional, pharmacological and pathological factors. Western Rajasthan region have altogether different genetic and ethnic factors, and very hot and arid climatic conditions with different dietary habits. So this case control study was selected to observe the plasma homocysteine level in acute stroke patients of Western Rajasthan.

#### MATERIALS AND METHODS

The present study was carried out in the Department of Physiology and Department of Neurology, Dr. S. N. Medical College, Jodhpur, Rajasthan.

Total hundred (100) adult male and female subjects with age range from 30 to 75 years participated in this study. Among them 50 were stroke patients and were considered as

case group and another 50 apparently healthy adults (Male and female) of same age group were designated as control group.

**Inclusion Criteria used for Case group were as Following**

1. Patient admitted with new onset of focal neurological deficit after acute cerebral stroke.
2. Patient aged more than 30 years and less than 75 years of both sex were included.
3. Patient with focal neurological deficit and CT scan of brain within 24 hours documented by CT scan of brain.
4. Patient with new onset of stroke with past history of essential hypertension, diabetes mellitus, alcohol consumption, dyslipidaemia, heart disease and oral contraceptive uses.
5. Patient with post-partum corticovenous thrombosis and arterial stroke were also included.

**Exclusion Criteria for Case Group were as Following**

1. Patient admitted in Neurology ward with features of C.V.A diagnosed as brain infection, subdural haematoma, intracranial malignancy and other brain lesion.
2. Patient admitted with neurological deficit due to old C.V.A following cerebral ischaemia, transient ischaemic attacks are not included.
3. Subject having confounding factor which could raise homocysteine level like use of anti-epileptic drugs, folate antagonist and subject with lung, liver and kidney diseases.

The purpose and expected outcome of the study were explained to each subject. They were encouraged for voluntary participation. Written consent was obtained from each subject. Detailed medical and family history was taken and thorough clinical examination was done.

During central nervous system examination, patient's disability status was scored according to Barthel Index.

Routine investigations like haemoglobin, total leukocyte count, differential leukocyte count, urine for routine microscopic examination, blood sugar level, lipid profile and CT scan/MRI was done in each patient. One 12-lead ECG was recorded.

Special investigation of total plasma homocysteine was done by Chemiluminescent Magnetic Immunoassay (CMIA) by Architect I System from Standard Laboratory in case and control group subjects.

All data were expressed as mean ± SD and were statistically analysed by using the Microsoft Excel and Open Epi software (Version 2.31). To find the significant association between two variables Karl Pearson coefficient of correlations was performed. Student 't' test was calculated to find the significant difference between two variables of case and control group.

**RESULTS**

A total of 100 subjects were studied, 50 were in case group and 50 in control group.

Gender	Case Group		Control Group		P-value
	N	PHcy Micromole/L	N	PHcy Micromole/L	
Male	38	34.36±17.18	36	10.83±2.29	< .001 (HS)

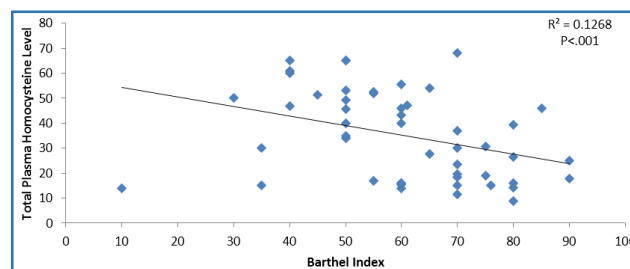
Female	12	36.12±17.78	14	12.62±2.47	< .001 (HS)
<b>Total</b>	<b>50</b>	<b>35.15±17.66</b>	<b>50</b>	<b>11.42±2.43</b>	<b>&lt; .001 (HS)</b>

**Table 1. Plasma Homocysteine Levels in Case and Controls**

Table 1 is showing plasma homocysteine level in case and control group, both male and female subjects have showed significantly raised homocysteine in stroke patients (Case - group) as compared to control group (p < .001).

Barthel Index	No. of Cases	Mean Homocysteine Level (Micromole/L)
< 41 (Severe disability)	8 (16%)	42.68 ± 20.51
41 - 60 (Moderate - Disability)	20 (40%)	40.28 ± 16.67
> 60 (Mild - Disability)	22 (44%)	27.75 ± 15.21

**Table 2. Disability in Stroke Cases as per Barthel Index and Homocysteine Level**



**Graph showing Co-Relation Coefficient between Barthel Index and Plasma Homocysteine Level**

Table 2 and Graph is showing that as the Barthel index is raised in stroke patients, plasma homocysteine level falls. Statistically, this correlation was found significant, (p < .001); 16% of stroke patients showed Barthel index < 41. It means they were having severe disability due to stroke. In these patients mean plasma homocysteine level was 42.68 ± 20.51 micromole/L, while in 40% stroke patients Barthel index was observed between 41 - 60. It shows moderate level of disability due to stroke. In these patients, mean plasma homocysteine level was 40.28 ± 126.67 micromole/L; 44% of stroke patients were recorded Barthel index > 60, which means they were having mild grade disability due to stroke. These patients showed mean plasma homocysteine level 27.75 ± 15.21 micromole/L.

Sub-Group	Stroke Patients		Controls Patients		P-Value
	N	Mean ± SD PHcy (Micromole/L)	N	Mean ± SD PHcy (Micromole/L)	
Male	38	34.36±17.18	36	10.83±2.63	< .001
Female	12	36.12±19.78	14	12.62±2.46	< .001
Hypertensive	31	32.08±16.02	3	12.76±2.00	< .05
Normotensive	19	39.47±19.47	47	11.34±2.45	< .001
Smoker	30	36.73±16.6	16	11.07±2.51	< .001
Non-Smoker	20	31.99±19.16	34	11.59±2.41	< .001

**Table 3. Mean Homocysteine Levels according to Different Variables in Case and Control Group**

Table 3 is showing mean homocysteine level according to different variables in case and control group.

On comparing all the male subjects of case and control group, it was found that male stroke patients showed mean homocysteine level of 34.36±17.18 micromole/L and control group males showed mean homocysteine level of 10.83 ± 2.63 micromole/L.

Stroke patients have showed significantly higher homocysteine level (p<.001). Like these female patients also showed significantly raised homocysteine level in stroke patients as compared to control group subjects (p < .001).

Other sub-groups like hypertensive subjects, normotensive subjects, smoker and non-smokers all showed statistically significant rise in plasma homocysteine level in case group as compared to control group (P = < .001).

Parameters	Cases Group	Control Group	P-Value
Total Cholesterol (mg/dL)	176.06±49.45	176.07±32.90	> 0.05 (NS)
TG (mg/dL)	152.87±81.24	119.25±33.1	< 0.05 (S)
HDL(mg/dL)	33.02±21.60	23.60±6.05	< 0.05 (S)
LDL(mg/dL)	103.21±31.74	110.82±29.29	> .05(NS)
LDL/HDL	2.27±1.03	2.79±0.82	< 0.05 (S)

**Table 4. Comparison of Lipid Profile in Case and Control Group**

Table 4 showing comparison of lipid profile in case and control. Total cholesterol and LDL level have showed statistically no significant difference between control and case group (p > .05), but Triglyceride (TG) and HDL were raised significantly in case group as compared to control group (p < .05).

Parameters	Case Group	Control Group	P-Value
Systolic BP (mmHg)	148.68±23.30	140.8±9.60	< 0.02 (S)
Diastolic BP (mmHg)	90.28±9.90	83.88±8.79	< 0.001 (HS)
Blood Sugar (mg/dL)	143.52±6.55	109.67±21.15	< 0.001 (HS)

**Table 5. Comparison of Blood Sugar and Blood Pressure Level in Case and Control Group**

Table 5 is showing comparison of blood sugar and blood pressure in cases and control group. Mean diastolic blood pressure level was around 90.25 ± 9.90 in case group and 83.88 ± 8.79 in control group. It was raised significantly in case group (p < .001). Mean blood sugar level was around 143.52 ± 56.55 in case group and 109.67 ± 21.15 in control group. It was raised significantly in case group (p < .001).

Sl. No.	Various Diseases	No. of Cases	Plasma Homocysteine (Micromole/L) (Mean ± SD)
1	Hypertension	31	34.36±17.18
2	Diabetes Mellitus	16	37.75±16.60
3	IHD	8	43.69±19.16
4	Smoking	30	36.73±16.6

**Table 6. Plasma Homocysteine with Past History of Various Diseases**

Table 6 is showing plasma homocysteine level in stroke patient with history of various diseases. Highest mean homocysteine level (43.69±19.16 micromole/L) was observed in IHD subjects in case group. Subjects having diabetes mellitus has showed mean homocysteine level 37.75±16.60 micromole/L, smoking 36.73±16.6 and hypertension 34.36±17.18 micromole/L.

**DISCUSSION**

Stroke is a major cause of death and disability worldwide. In India, the estimated prevalence ratio of stroke range in 84 - 262/100000 in rural and 334 - 424/100000 in urban area. The incidence ratio is 119 - 145/100000 based on the recent population based studies.[5]

Stroke accounts for two percent of hospital registrations and 9 - 30% of neurological admissions.[6,7]

Common causes for ischaemic stroke are atherosclerosis with thromboembolism, while less common cause include hypercoagulable disorders, homocysteinaemia, collagen vascular disease, fibrovascular dysplasia, oral contraceptive and eclampsia.

Traditional risk factors for stroke include advanced age, male sex, hypertension, diabetes mellitus, cardiovascular disease, hyperlipidaemia, obesity, cigarette smoking, alcohol and oral contraceptives.

Homocysteine is a newly identified risk factor for stroke and a lot of work has been done in the last decade in this respect. Present study is an effort to observe role of homocysteine level in acute stroke patients of Western Rajasthan.

Homocysteine (Hcy) is a sulphur-containing amino acid that is generated during methionine metabolism. It has a physiologic role in DNA metabolism via methylation, a process governed by the presentation of folate and vitamins B6 and B12.

Hyperhomocysteinaemia has a multi-factorial origin incorporating genetic, nutritional, pharmacological and pathological factors. Considering the differences in dietary, genetic and ethnic factors and extreme hot and dry arid climatic conditions, the data published from the West and other part of the country may not be applicable to Western Rajasthan population.

Therefore, present study was planned to observe the role of homocysteine in stroke subjects.

Our study has shown that Plasma Homocysteine levels were significantly high in stroke group when compared to control group (34.79±17.765 vs 11.42±2.43 micromole/L, p<0.001).

This study has shown that as the disability rises due to stroke (Observed as per Barthel Index), the Plasma Homocysteine level rises significantly (p<.01).

Our results exhibited that serum cholesterol was not significantly raised in stroke patients, while blood pressure and blood sugar was significantly raised (p<.01).

Those patients who were having past history of Diabetes, Hypertension, Smoking have also shown significantly raised level of PHcy as compared to control group (p < 0.01).

Our findings are in agreement with most of the previous studies.[8-10]

There is evidence from laboratory and clinical studies that elevated Homocysteine (eHcy), exerts direct toxic effects on both the vascular and nervous systems.

The physiologic levels of PHcy in healthy populations are determined primarily by the dietary intakes of methionine,<sup>[11]</sup> folate<sup>[11]</sup> and B12.<sup>[12]</sup> It is thought that lifestyle conditions such as excessive coffee or alcohol consumption, cigarette smoking and physical inactivity may play a role in modulating the PHcy,<sup>[13]</sup> although the evidence remains controversial.<sup>[14]</sup> Diets abundant in vegetables, fruits and bread can result in reduction in the PHcy.<sup>[15]</sup>

Elevated levels of Homocysteine (eHcy or hyperhomocysteinaemia) occur with aging and decreased renal function.<sup>[14-17]</sup>

Our study has observed a strong link between hyperhomocysteinaemia and ischaemic stroke. The findings are supported by recent study, which showed that hyperhomocysteinaemia was found in 48% of ischaemic stroke patients.<sup>[9]</sup> However, some researchers have failed to determine any link between hyperhomocysteinaemia and stroke.<sup>[18]</sup>

In our study both in case and control group, female subjects had shown raised plasma homocysteine levels, but statistically, it was found to be insignificant in stroke group subjects both male and female ( $p > 0.05$ ).

APS N et al (2009) has also observed difference of homocysteine levels between males and females and this was statistically insignificant.<sup>[19]</sup>

Some of the workers could not find association between homocysteine and lipid profile, while others reported a declined correlation. In present study positive correlation between total cholesterol, LDL - cholesterol, TG with homocysteine levels were found, which was statistically insignificant in stroke patients.

In contrast to the West, Indian studies examining the prevalence of hyperhomocysteinaemia in the community have reported a much higher incidence of 52 - 84%.<sup>[20-22]</sup> The mean homocysteine levels too are quite high, varying from 19.5 to 23.2  $\mu\text{mol/L}$ .<sup>[20,21]</sup> Our study on Western Rajasthan subjects has also revealed a very high mean homocysteine of 34.79 micromole/litre in case group.

In view of these high levels, it is felt that hyperhomocysteinaemia can be considered to be important cardiovascular risk factors in Western Rajasthan population.

Hyperhomocysteinaemia is now recognised as an independent risk factor for atherosclerosis.<sup>[23]</sup> Homocysteine is an unstable amino acid, which undergoes auto oxidation to produce free oxygen radicals.<sup>[19]</sup> Hyperhomocysteinaemia, thus causes increased production of free oxygen radicals and oxidative stress. The free oxygen radicals convert LDL-c deposited in the sub-endothelial tissue to oxidised LDLc (oxLDL-c). Ox LDL-c then acts as the key mediator of the inflammatory process in atherosclerosis.<sup>[24]</sup> Ox LDL-c causes the release of Vascular Cell Adhesion Molecule (VCAM) and monocyte chemoattractant protein (MCP-1), which in turn causes monocyte adhesion penetration respectively. The monocytes then get converted to macrophages, which take up OxLDLs to get converted to foam cells. The foam cells get deposited below the endothelium to form a fatty streak, the first lesion in atherosclerosis. The free oxygen radicals also combine with Nitric Oxide (NO), inactivating it to peroxynitrite. The resulting endothelial dysfunction also contributes significantly to atherosclerosis.

It has been reported that risk confirmed by homocysteine, add to or multiplies the risk confirmed by other risk factors

such as smoking, hypertension, diabetes and lipid disorders, all of which are common amongst Indians.<sup>[25-27]</sup> PHcy of greater than 14.0  $\mu\text{mol/L}$  is significantly associated with the progression of aortic arch atheroma, which is an independent risk factor for recurrent vascular events in transient ischaemic attack and stroke patients.<sup>[28]</sup> These findings suggest that eHcy serves as a mediator of aortic plaque progression.

It was reported that patients with both elevated homocysteine and LDL-c levels were at the highest risk.<sup>[29]</sup> There is strong evidence from laboratory and clinical studies that eHcy is an independent risk factor for cardiovascular disease, although some recent studies appear to refute this claim.<sup>[30]</sup> The findings of these laboratory studies and clinical observations suggest that eHcy exerts toxic effects on endothelial cells, the vascular wall structure and the blood coagulation system.<sup>[31]</sup> The actions of eHcy on vascular endothelial cells lead to the proliferation of smooth-muscle cells, promote the oxidation of low-density lipoprotein and increase collagen synthesis and procoagulant activity with all of these actions accounting for the development of atherosclerosis.<sup>[32]</sup>

In our study hyperhomocysteinaemia has been found to be a common risk factor than hypertensive, diabetes mellitus, smoking or alcoholism. Furthermore, its effect is synergistic rather than additive when associated with other risk factors. There is growing evidence that elevated levels of homocysteine play an important role in atherosclerotic disease.<sup>[33]</sup> Hyperhomocysteinaemia promotes oxidative damage by Reactive Oxygen Species<sup>[34]</sup> and cause smooth muscle proliferation.

## CONCLUSION

People with elevated plasma homocysteine are more likely to have a stroke in Western Rajasthan area. Homocysteine is a well-established biomarker for risk of stroke. Homocysteine is an amino acid with a well-established relationship with vascular diseases linked with atherosclerosis, oxidative damage and vascular smooth muscle proliferation. With this in mind, it is not surprising that in Western Rajasthan region higher plasma homocysteine level can distinguish ischaemic stroke patients from healthy control subjects. It is therefore recommended that PHcy estimation should be included as a routine laboratory test for persons with cerebrovascular risk factors and public should be made aware of it.

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