



## ASSOCIATION OF SERUM FOLIC ACID AND HOMOCYSTEINE WITH ISCHEMIC STROKE

<b>Singh P</b>	M.D.,D.M., Assistant Professor Neurology, Department Of Medicine, Motilal Nehru Medical College, Prayagraj U.P. India
<b>Diwedi N C*</b>	M.D.,D.M., Professor Medicine, Department Of Medicine, Motilal Nehru Medical College, Prayagraj U.P. India *Corresponding Author
<b>Verma A K</b>	MBB, P.G. student, Department Of Medicine, Motilal Nehru Medical College, Prayagraj U.P. India
<b>Mathur M</b>	Associate Professor, Department Of Medicine, Motilal Nehru Medical College, Prayagraj U.P. India
<b>kirti</b>	M S obs & gynae ADMO NCR hospital Prayagraj U.P. India

### ABSTRACT

**Background:** Stroke is one of the commonest cause of death in developed countries and is most common cause for the physical disability of a large population. Role of low serum folic acid and hyperhomocysteinemia as risk factor for stroke is not clear. This study was designed to see the association of serum folic acid and hyperhomocysteinemia with ischemic stroke.

**Aim and objectives:** To evaluate the association of serum folic acid level and homocysteine level with ischemic stroke and to measure and compare the serum folic acid level among the cases and control subjects.

**Material and method:** This study was a case-control study which was conducted in the Department Medicine MNL Medical college Prayagraj Uttar Pradesh, India between march 2015 to may 2016 period of 1 year and 2 month

A total number of 78 enrolled, 8 were excluded, 70 patients ischemic stroke were selected and 70 control persons were enrolled in this study. All patients of both sexes, aged 18 years and above presented with ischemic stroke, from 0 day to 1 month that was confirmed by CT scan of head/MRI of brain. Blood sample was collected from the cases and the controls analyzed for estimation of serum folic acid, serum homocysteine fasting blood sugar and Fasting lipid profile.

**Result:** The mean  $\pm$  SD of age of the cases was  $58.9 \pm 14.47$ , the control population mean age was  $56.6 \pm 15.4$ .

The mean ( $\pm$ SD) serum folate level of case and control group was  $6.34 (\pm 4.42)$  and  $15.69 (\pm 5.9)$  respectively. Statistically significant differences was observed between case and control group in level of Serum folate ( $p < 0.05$ ). The study also showed the mean homocysteine level in cases and control was  $16.77 \pm 2.01$  and  $7.34 \pm 2.53$  respectively. Homocysteine level was raised statically significant ( $p < 0.001$ ) in stroke as compared to controls

**Conclusion:** Low serum folic acid level and hyperhomocysteinemia is significantly associated with increased risk for ischemic stroke.

### KEYWORDS :

#### INTRODUCTION

Stroke is defined by the World Health Organization<sup>1</sup> as 'a clinical syndrome consisting of rapidly developing clinical signs of focal (or global in case of coma) disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than a vascular origin<sup>2</sup>. Stroke is fifth leading causing of mortality and commonest cause of disability. In India annual incidence rate as observed in Kolkata ( $123.15/100000$ )<sup>3</sup>, Mumbai ( $148/100000$ ) and Trivandrum ( $135/100000$ )<sup>4</sup> are higher than that of united states ( $107/100000$ )<sup>5</sup> but similar to that reported from Chinese population<sup>6</sup>. In India a study by Joshi et al by verbal autopsy found that stroke was cause of death in 13% very similar to death due to coronary artery disease 14%<sup>7</sup>. Risk factors for stroke can be classified as non modifiable age, ethnicity, and gender. Modifiable risk can be classified as hypertension diabetes mellitus smoking, hyperhomocysteinemia. supplementation of folic acid decreases homocysteine level some of the study showing beneficial effect homocysteine lowering by vitamin B-12, vitamin B-6, pyridoxine supplementation while other are showing neutral results.

In our study we tried to find out serum homocysteine and folic acid level in stroke patient and comparing with age and gender match controls.

#### MATERIAL AND METHODS:

All the patient admitted in medicine department of SRN hospital Prayagraj those meeting inclusion and exclusion criteria between march 2015 to may 2016

**Inclusion criteria:** All patient of ischemic stroke conformed by CT and/or MRI from onset upto 1 month of acute event, having age  $\geq 18$  years.

**Exclusion criteria:** Patient of intracerebral hemorrhage, severely ill patient, those patient taking medicine such as antiepileptics, antimetabolites, multivitamins, folic acid.

After admission detailed history regarding temporal profile of stroke, risk factors like hypertension, diabetes mellitus smoking, past history of stroke, heart disease and other comorbid condition taken. Detailed general examination and system wise examination especially neurological and cardiac examination done. Laboratory test of all patients CBC, RBS, BUN, Creatinine, ECG, lipid profile of all patients done. CT scan of all patients done to establish a diagnosis of stroke. if it is not clear then MRI brain obtained. ECG has done on the day of admission and these findings were noted.

Statistical analysis was done as appropriate, mean in continuous data, nominal data as numbers, percentage and standard deviation. t test for independent variables, Chi-square was used to look statistical significance.

#### OBSERVATIONS AND RESULTS

Total 78 patient were enrolled 8 were excluded 2 patient having previous history of stroke, 1 patient taking sodium phenytoin, 2 patients were taking multivitamin, 2 patient expired with in 2 hour, 1 patient of cardioembolic stroke. Total 70 patient were included in the study.

In cases majority of patient were in 40-80 age group ( $n=60$ ). Most of the cases were above 60 years of age ( $n=42$ ). In control majority of population were in 40-70 age groups ( $n=55$ )

The mean age of cases was  $58.9 \pm 1.6$  while that of control was

56.6±15.4 (P=0.38) stastically non significant Table 1 age wise distribution of cases and control

**Table 1 : Age distribution of cases and control**

Age	Cases	Control	
20-29	3	2	
30-39	4	6	
40-49	10	13	
50-59	11	16	
60-69	19	15	
70-79	20	10	
80-89	3	8	
Mean ±SD	58.9±14.6	56.6±15.4	P=0.38

In cases 50.7%(n=40) were females and 30 were males. In control 50% (n =36 were female) and 34 were male, stastically non significant (P=0.31) table 2.

**Table 2 gender distribution of case and control**

Gender	Case	Control	
Male	30	34	
Female	40	36	
Total	70	70	P=0.31

On comparing mean of folic acid level in cases it was 6.34±4.42, lower than that of control it was 15.7±5.49 (P<0.001). The homocysteine level of the cases was high 16.7±2.02 when compared to control 7.3±2.54 (P <0.001). Among cases the mean homocysteine level among female 16.73±1.94 and among male 16.83±2.135 (P=0.28). Similarly among control the mean homocysteine among female 7.18±2.34 and male 7.50±2.73 (P=0.67) table 3.

**Table 3: comparison of folic acid and homocysteine level in cases and control**

Laboratory finding	Cases	Control	P value
Serum Folic acid level	6.34±4.42	15.59±5.49	P<0.001
Serum homocysteine level	16.77±2.02	7.34±3.54	P<0.001

## DISCUSSION:

One the important observation as age increases stroke incidence increased. The most common age group of cases was 70-79 (28.6%) followed by 60-69 (27.1%), followed by 50-59(15.7%) maximum patient in this study were above 40 years of age. Age is one of most important risk factors. Incidence of stroke increases with age the incidence doubling for each decade after 55 years of age<sup>8</sup>. The overall women has higher lifetime risk for stroke than men, it has been calculated to be 1 in 5 for women and 1 in 6 for men,<sup>9</sup> men having higher rates in younger years and women in older ages. Study done in 8 different European countries found that the risk of stroke increased by 9% per year in men and 10% per year in women<sup>10</sup>. This study also shows that incidence of stroke increases with stroke.

The National Health and Nutrition Examination Survey(NHANES 1) cohort, there was a small and no significant elevation in the risk of stroke for those with folate serum levels ≤ 9.2 nmol/L compared with those with >9.2 nmol/L(RR1.37; 95% CI: 0.82 to 2.29)<sup>11</sup>. Some studies have shown increased folate intake decreases risk of stroke<sup>12</sup>. Similarly, higher folate intake associated with reduced IS risk in the study of US health professional men<sup>13</sup> and Finnish male smokers<sup>14</sup> but not in those of U.S. nurses<sup>15</sup> and Swedish<sup>16</sup>. The Bronx aging cohort study found no association between serum folate levels and incidence of stroke<sup>12</sup>. In our study we found significantly decreased level of folate in stroke patient as compared to control population.

Homocysteine level partly depend on folate status lower folate level may increase homocysteine level<sup>17</sup>. Data from Framingham study indicate that approximately two third of elevated homocysteine level secondary to low or moderate level of folic acid<sup>18</sup>. The proportion of elevated homocysteine that may be due to low folic acid alone is not clear. However there is limited data to suggest administration of folic acid is associated with the reduction of homocysteine level<sup>19</sup>.

McCully in 1960s first suggested link between homocysteine and atherosclerosis and proposed homocysteine theory of atherosclerosis<sup>20,21</sup>. Several mechanism have been proposed for the association between homocysteine and stroke. The oxidation of homocysteine may result information of free radicals and H2O2 daamaging<sup>22</sup> the endothelial lining<sup>23</sup> and promoting oxidation of LDL

cholesterol<sup>24</sup>. Both of these process may lead to atherosclerosis. Homocysteine and its metabolites can affect coagulation by increasing factor V<sup>25</sup>, platelet thromboxane production<sup>26</sup> and platelet aggregation<sup>27</sup> and by decreasing the activation of protein c<sup>28</sup>. Thus increased level of homocysteine increases both risk of atherosclerosis and thrombosis. In 1995 Boushey et<sup>29</sup> al done Meta analysis of 27 observational studies of hyperhomocysteinemia and atherosclerotic vascular disease of which 11 studies addressed the association of hyperhomocysteinemia and risk of stroke. Nine case control study provide support the hypothesis that hyperhomocysteinemia increases the risk of stroke, while two prospective studies showed negative results. In our study we found the mean level of homocysteine level of the cases was higher 16.7±2.02 when compared to control 7.3±2.54 (P <0.001) statistically significant.

## CONCLUSION:

In our study we found that in cases of ischemic stroke folic acid is significantly low and homocysteine level was significantly increased as compared to stroke. Further studies are required to establish these association in our ethnic population. This study is done in single centre Prayagraj U.P. India with small size, to establish low serum folic acid and hyperhomocysteinemia as risk factor more study is required.

## REFERENCES

- Hatano S. Experience from a multicentre stroke register: a preliminary report. Bulletin of the World Health Organisation. 1976;54(5):541-553.
- Das SK, Banerjee TK, Biswas A, Roy T, Raut DK, Mukherjee CS, et al. A prospective community-based study of stroke in Kolkata, India. Stroke. 2007;38:906-10.
- Dalal PM, Malik S, Bhattacharjee M, Trivedi ND, Vairale J, Bhat P, et al. Population-based stroke survey in Mumbai, India: Incidence and 28-day case fatality. Neuroepidemiology. 2008;31:254-61
- Sridharan SE, Unnikrishnan JP, Sukumaran S, Sylaia PN, Nayak SD, Sarma PS, et al. Incidence, types, risk factors, and outcome of stroke in a developing country: The Trivandrum Stroke Registry. Stroke. 2009;40:1212-8.
- Brown RD, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Stroke incidence, prevalence, and survival: Secular trends in Rochester, Minnesota, through 1989. Stroke. 1996;27:373-80.
- Jiang B, Wang WZ, Chen H, Hong Z, Yang QD, Wu SP, et al. Incidence and trends of stroke and its subtypes in China: Results from three large cities. Stroke. 2006;37:63-8.
- Joshi R, Cardona M, Iyengar S, Sukumar A, Raju CR, Raju KR, et al. Chronic diseases now a leading cause of death in rural India — mortality data from the Andhra Pradesh Rural Health Initiative. Int J Epidemiol. 2006;35:1522-9.
- Roger VL, Go AS, Lloyd-Jones DM, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Executive summary: heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation. 2012; 125:188-197.
- Seshadri S, Beiser A, Kelly-Hayes M, et al. The lifetime risk of stroke: estimates from the Framingham Study. Stroke. 2006;37:345-350.
- Giles WH, Kittner SJ, Anda RF, Croft JB, Casper ML. Serum folate and risk for ischemic stroke. First National Health and Nutrition Examination Survey epidemiologic follow-up study. Stroke. 1995;26:1166-1170.
- Asplund K, Karvanen J, Giampaoli S, et al.; MORGAM Project. Relative risks for stroke by age, sex, and population based on follow-up of 18 European populations in the MORGAM Project. Stroke. 2009; 40:2319-2326.
- Zeitlin A, Frisshman WH, Chang CJ. The association of vitamin B12 and folate blood levels with mortality and cardiovascular morbidity incidence
- He K, Merchant A, Rimm EB, Rosner BA, Stampfer MJ, et al. Folate, Vitamin B6, and B12 Intakes in Relation to Risk of Stroke Among Men. Stroke. 2004;35:169-74.
- Larsson SC, Mannisto S, Virtanen MJ, Kontto J, Albanes D, Virtamo J. Folate, vitamin B6, vitamin B12, and methionine intakes and risk of stroke subtypes in male smokers. Am J Epidemiol.
- Al-Delaimy WK, Rexrode KM, Hu FB, Albert CM, Stampfer MJ, Willett WC, Manson JE. Folate intake and risk of stroke among women. Stroke. 2004;35:1259-1263
- Van Guelpen B, Hultdin J, Johansson I, Stegmayr B, Hallmans G, Nilsson TK, Weinheall L, Wuthoff C, Palmqvist R, Winkvist A. Folate, vitamin B12, and risk of ischemic and hemorrhagic stroke: A prospective, nested case-referent study of plasma concentrations and dietary intake. Stroke. 2005;36:1426-1431.
- Kang SS, Wong PW, Norusis M. Homocystinemia due to folate deficiency. Metabolism. 1987;36:458-62.
- Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocyst(e)ine in an elderly population. JAMA. 1993; 270:2693-2698
- Brattstrom L, Israelsson B, Norving B, Bergqvist D, Thorne J, Hultberg B, Hamfelt A. Impaired homocysteine metabolism in early-onset cerebral and peripheral occlusive arterial disease: effects of pyridoxine and folic acid treatment. Atherosclerosis. 1990; 81:51-60.
- McCully KS. Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. Am J Pathol. 1969;56:111-128.
- McCully KS, Wilson RB. Homocysteine theory of arteriosclerosis. Ath-erosclerosis. 1975;22:215-227
- Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest. 1986; 77:1370-1376.
- Parthasarathy S. Oxidation of low density lipoproteins by thiol compounds leads to its recognition by the acetyl LDL receptor. Biochim Biophys Acta. 1987; 917:337-340.
- Heinecke JW, Rosen H, Suzuki LA, Chait A. The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. J Biol Chem. 1987; 262:10098-10103
- Rodgers GM, Kane WH. Activation of endogenous factor V by homocysteine-induced vascular endothelial cell activator. J Clin Invest. 1986; 77:1909-1916.
- Graeber JE, Slott JH, Ulane RE, Schulman JD, Stuart MJ. Effect of homocysteine and homocystine on platelet and vascular arachidonic acid metabolism. Pediatr Res. 1982; 16:490-493.
- McCully KS, Carvalho AC. Homocysteine thiolactone, N-homocysteine thiolactonyl retinamide, and platelet aggregation. Res Commun Chem Pathol Pharmacol. 1987; 56:349-360.
- Rodgers GM, Conn MT. Homocysteine, an atherogenic stimulus, reduces protein C

- activation by arterial and venous endothelial cells *Blood*. 1990; 75:895-901
- 29 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease Probable benefits of increasing folic acid intakes. *JAMA*. 1995;274:1049-57.