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Homocysteine as an Independent Risk Factor for Cerebral Ischemic Stroke in South Indian Population in Rural Tertiary Care Centre

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Abstract: Objective: *Cerebral ischemic stroke is a life threatening neurological disorder leads to mortality and long term disability in survival patients. Atherosclerosis is a common cause of ischemic strokes which inturn predisposes thromboembolism and than triggers thrombotic strokes or transient ischemic attacks. The atherogenic nature of homocysteine plays a vital role in the development of cardiac or cerebral ischemic strokes. The purpose of this case - control study was to corelate homocysteine in cerebral ischemic stroke patients.*
Method: *This study was done at the Department of Medicine, DR. PSIMS & RF foundation Chinaoutpally, A.P.India between March 2011 to March 2013. In this study, we recruited patients of ischemic non cardioembolic stroke with history of sudden onset of focal neurological deficit lasted more than 24 hours.*
Results: *The mean homocystein levels in cases were 17.58 ± 10.3 statistically significant than controls 6.34 ± 4.22(p< 0.05). Mean homocystein levels of cerebral ischemic stroke males were 19.21 ± 12.1statistically significant than controls 7.428 ± 4.091 (p 0.05). Mean homocystein levels of cerebral ischemic stroke females were 14.86 ± 5.34 statistically significant than controls 4.536 ± 3.844 (<0.05). Presence of hyperhomocysteinemia in 59cases and 6 controls, statistically significant than controls (p <0.05), among in males, hyperhomocysteinemia in 40 cerebral ischemic stroke patients than control (4) group, statistically significant than controls (p <0.05)*
Conclusions: *Elevated serum homocysteine is a strong and modifiable risk factor of cerebral ischemic strokes. Many studies are showed the significance of development of complications like cardiac and cerebral vascular events. We support the consideration of serum homocysteine as a regular and routine screening marker to protect target organ damage.*
Key words: *stroke, homocysteine, ischemia, thrombosis*

I. Introduction:

A cerebrovascular stroke has become a major health problem and is the most common cause of mortality and morbidity in the entire world. Stroke is defined as sudden occurrence of clinical symptoms and or signs of either focal or global loss of cerebral function with the duration of more than 24 hours or leading to mortality with no other reason than vascular origin[1.] The global burden of disease (GBD) study, in 1990, reported 9.4 million deaths in India of which 61900 were from stroke and disability adjusted life years (DALYs) lost to 28.5 million. Recent epidemiological surveys for cerebrovascular diseases like hemiplegia or paraplegia, showed 320 cases in 145456 persons indicating an overall crude prevalence rate of 220per 100000 persons [2].The broad classification of strokes, primarily in to ischemic (85%) and hemorrhagic (15%) [3], of ischemic strokes commonest etiologies include atherosclerosis with thromboembolism and cardiogenic thromboembolism. The modifiable personal or social risk factors like hypertension, diabetes, obesity and cigarette smoking triggers proportional incidence of cerebrovascular events, like stroke and TIA[3]. The modifiable biochemical risk factors like abnormal lipid metabolism [4], hypercholesterolemia, lipoprotein (a) [Lp(a)][5], hyperhomocysteinemia [6] for increased incidence of atherothrombotic cerebrovascular disease. Hyperhomocysteinemia recently has been recognised as a easily modifiable risk factor for the presence of atherosclerotic cerebro or cardiovascular disease and hypercoagulability states.[7] In humans, less severe genetic mutations associated with enzyme abnormalities particularly methylenetetrahydrofolate reductase (*MTHFR*) in the metabolic pathways involving folate and homocysteine leads to elevated levels of homocysteine [8]. Elevated homocysteine levels play a causal role in the pathogenesis of atherosclerosis, thrombo-embolism and vascular endothelial dysfunction [9] with an increased incidence of ischaemic stroke. An other positive correlation for the observed association between

hyperhomocysteinemia and stroke is a additive effect of factors associated with hyperhomocysteinaemia particularly with cigarette smoking, renal dysfunction, an atherogenic diet, cystine or folate deficiency triggers the acute vascular events themselves, whereby the vascular endothelial damage increases total homocysteine levels[9]. Other mechanisms, by which total homocysteine induces vascular changes include high propensity for development of thrombus, impaired thrombolysis, increased production of hydrogen peroxide, and increased oxidation of LDL - cholesterol are responsible for development of vascular endothelial dysfunction [10].
On this background, our study aims at comparing the occurrence of homocysteine levels in patients with cerebral ischemic stroke group, is to determine the role of homocysteine as a marker for ischemic stroke, with regard to Indian population.

II. Material and methods:

A case – control study was undertaken in the Department of General Medicine, Dr.PSIMS&RF, Chinoutpally, Gannavaram, Krishna (Dt), Andhra Pradesh, during the period of two years i.e., March 2011 to March 2013 with the diagnosis of acute cerebral ischemic stroke as per history of sudden onset of focal neurological deficit lasted more than 24 hours. A detailed history and thorough clinical examination was done as per the proforma and were investigated further.
The levels of homocysteine were measured by Chemiluminescent microparticle immunoassay (CMIA) in acute cerebral ischemic stroke patients and age and sex matched controls. The levels were analyzed and compared between different groups.

Inclusion criteria:
Clinical evidence of stroke
Cranial computed tomography (CT) scan or MR imaging consistence with ischemic stroke

Exclusion criteria:
Patients with CT scan or MR imaging showing hemorrhage or mass lesion
Patients with Coronary artery disease
Patients with Vasculitis
Patients with other endocrine, liver and renal diseases

Sample collection and analysis:
Both heparinised and plain blood samples were collected from each case and control. For analysis of FBS, lipid profile, - serum was used. Complete haemogram, Urine Routine, ECG, Serum Homocysteine Levels, Blood urea, serum creatinine , Liver function tests, Serum glucose estimation was done by Trindler's GOD – POD method (commercial kit – ERBA – MANNHEIM), All these estimations were performed by Randox Daytona Autoanalyzer.

Estimation of serum homocysteine: Homocysteine assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of total L-homocysteine in human serum or plasma on the ARCHITECT i System optics.
According to Kang et al. an abnormal homocysteine level is defined by an arbitrary cut off (95th percentile) of the concentration found in normal population. The normal fasting plasma homocysteine levels in adults usually range between 5 – 15 μ mol/L with mean level of about10 μmol/L[11], Kang and co-workers have classified hyperhomocystenemia as > 15μmol/L.

III. Results:

The mean age in cases and controls was 56.26 ± 12.57, among in males was 54.4 ± 12.56 and in females was 59.36 ± 12.06, the percentage of males (62.4%) were higher than females (37.6%). The mean BMI in cases was 23.8 ± 1.01and in controls was 23.6 ± 1.19. Presence of hyperhomocysteinemia in 59 (41.8%) of cerebral ischemic stroke patients than control group 6 (4.25%), among in male cerebral ischemic stroke patients 40 (45.4%) than control group 4 (4.54%), among in female cerebral ischemic stroke patients 19 (35.8%) than control group 2(3.77%) were statistically significant. The mean homocystein levels in cases were 17.58 ± 10.3 statistically significant than controls 6.34 ± 4.22($p< 0.05$). Mean homocystein levels of cerebral ischemic stroke males were 19.21 ± 12.1statistically significant than controls 7.428 ± 4.091 (p 0.05). Mean homocystein levels of cerebral ischemic stroke females were 14.86 ± 5.34 statistically significant than controls 4.536 ± 3.844 (<0.05). Presence of hyperhomocysteinemia in 59cases and 6 controls, statistically significant than controls (p <0.05), among in males, hyperhomocysteinemia in 40 cerebral ischemic stroke patients than control (4) group, statistically significant than controls (p <0.05). Among in females, hyperhomocysteinemia in 19 cerebral ischemic stroke patients than control (2) group, statistically significant than controls (p <0.05). The risk factors

are present in cerebral ischemic stroke patients, hypertension 31(21.9%), diabetes 26 (18.4%), dyslipidemia 22 (15.6%), smoking 14 (15.9%) and no risk factors in 48 (34.04%), among the risk factors and non risk factors group, the incidence of hyperhomocysteinemia in hypertension12(38.7%), diabetes 8 (30.7%), dyslipidemia 7 (31.8%), smoking 5 (35.7%) and no risk factors in 27 (56.25%) were statistically significant. The mean CRP levels in cases were 3.74 ± 2.44statistically significant than controls 2.09 ± 0.905 (p: 0.05). Mean CRP levels of cerebral ischemic stroke males were 4.13 ± 2.46 statistically significant than controls 2.06 ± 0.89 (p 0.05). Mean CRP levels of cerebral ischemic stroke females were 3.11 ± 2.28statistically significant than controls 2.13 ± 0.93 (<0.001). The mean cholesterol in cases was 196.46 ±32.72and in controls was 177.27 ±21.82 (p<0.05) which showed cholesterol is more significant elevation in cerebral ischemic stroke patients, The mean triglyceride level in cases was 195.54 ±76.78 and in controls was 127.61 ± 56.52 (p <0.05) which showed mean triglyceride level is more significant elevation in cerebral ischemic stroke patients, The mean LDL – cholesterol level in cases was 146.23 ± 37.8and in controls was 104.57 ± 21.52 (p <0.05) which showed mean LDL – cholesterol level is more significant elevation in cerebral ischemic stroke patients, The mean HDL – cholesterol level in cases was 33.12 ± 7.36and in controls was 37.65 ± 8.38 (p <0.05) which showed mean HDL – cholesterol level is more significant reduction in cerebral ischemic stroke patients.

Table 1

Presence of Hyperhomocysteinemia in cases and controls

	Cases	Controls	Chi square value	P value
Total	141	141	54.061	<0.0001
Hyperhomocystenemia	59	6		

Chi squared equals 54.061 with 1 degrees of freedom. The two-tailed P value is less than 0.0001 The association between rows (groups) and columns (outcomes) is considered to be extremely statistically significant.

Table 2

Mean homocystein levels in cases and controls

	Cases	Controls	P value
Male	19.21 ± 12.1	7.428 ± 4.091	< 0.05
Female	14.86 ± 5.34	4.536 ± 3.844	< 0.05
Total mean	17.58 ± 10.3	6.34 ± 4.22	< 0.05

Table 3

Presence of Hyperhomocysteinemia in cases and controls in males

	Cases	Controls	Chi square value	P value
Total	88	88	37.121	<0.0001
Males	40	4		

Chi squared equals 37.121 with 1 degrees offreedom. The two-tailed P value is less than 0.0001 The association between rows (groups) and columns (outcomes) is considered to be extremely statistically significant

Table 4

Presence of Hyperhomocysteinemia in cases and controls in females

	Cases	Controls	Chi square value	P value
Total	53	53	15.202	<0.0001
Females	19	2		

Chi squared equals 15.202 with 1 degrees of freedom. The two-tailed P value is less than 0.0001The association between rows (groups) and columns (outcomes) is considered to be extremely statistically significant.

Table 5
Risk factors in cases

Risk factors	No of cases	Percentage (%)
Hypertension	31	21.9 %
Diabetes	26	18.4%
Dyslipidemia	22	15.6%
Smoking	14	15.9%
No risk factors	48	34.04%

Table 6
Presence of Hyperhomocystenemia in cases with or without risk factors

Risk factors	No of cases	Hyperhomocysteinemia (%)	Males Hcy	Females Hcy
Hypertension	31	12 (38.7%)	7	5
Diabetes	26	8 (30.7%)	5	3
Dyslipidemia	22	7 (31.8%)	3	4
Smoking	14	5 (35.7%)	5	0
No risk factors	48	27 (56.25%)	20	7
Total	141	59 (41.8%)	40	19

Table 7
Presence of Hyperhomocysteinemia in cases with or without risk factors

	With risk factors	without risk factors	Chi square value	P value
Total	93	48	5.341	<0.0208
Hyperhomocystenemia	32 (34.4%)	27 (56.25%)		

Chi squared equals 5.341 with 1 degrees of freedom. The two-tailed P value equals 0.0208 The association between rows (groups) and columns (outcomes) is considered to be statistically significant.
Table 8
Mean CRP levels in cases and controls

	Cases	Controls	P value
Male	4.13 ± 2.46	2.06 ± 0.89	< 0.05
Female	3.11 ± 2.28	2.13 ± 0.93	< 0.05
Total mean	3.74 ± 2.44	2.09 ± 0.905	< 0.05

IV. Discussion:

In this study, serum hyperhomocystenemia was associated with an increased risk of cerebral ischemic strokes, patients without associated risk factors. Although many studies have previously shown the association between hyperhomocystenemia and stroke patients with associated risk factors. The present study was focused on patients without associated risk factors (34.04%), had developed hyperhomocystenemia in 56.25% of patients. The mean age in cerebral ischemic stroke patients was 56.26 $\pm$ 12.57, and 71.7% cerebral ischemic stroke patients were noticed more between the age group of 41 – 60 years. This study shows the mean serum homocystene was more in cerebral ischemic stroke group 17.58 ± 10.3 than compared to normal subjects 6.34 ± 4.22,when compared with L. Parnetti et al study,[12] the results were similar that is more in patients with large vessel disease group. Presence of hyperhomocysteinemia in 59 (41.8%) of cerebral ischemic stroke patients than control group 6 (4.25%), supported by Carod-Artal FJ et al[13] study. Males showed increase in mean serum homocystene levels and increased incidence compared to females in cerebral ischemic stroke group, supported by Carod-Artal FJet al[13] study. The risk factors like hypertension, diabetes, dyslipidemia and smoking are association of incidence of hyperhomocystenemia in 34.4% of cerebral ischemic stroke patients, the risk increases significantly if associated with smoking in males[13]. Mean CRP levels were more in cerebral ischemic stroke patients 3.74 ± 2.44 compared to controls 2.09 ± 0.905. CRP levels were found to be significant elevation of 54.2% in cerebral ischemic stroke patients with hyperhomocystenemia than elevated CRP levels were found in 35.4% of cerebral ischemic stroke patients without hyperhomocystenemia [14]. Males showed increase in mean

serum CRP levels and homocystene levels compared to females in cerebral ischemic stroke patients. Serum total cholesterol, triglycerides and LDL were elevated in cerebral ischemic stroke patients compared to controls and HDL levels were decreased in cerebral ischemic stroke patients group compared to controls[15].Among 141 patients with cerebral ischemic stroke 59 patients (41.8%) have hyperhomocystenemia and 50 patients(35.4%) showed high CRP levels, out of which in 59 patients with hyperhomocystenemia in cerebral ischemic stroke patients 32 had high CRP levels with a p value of 0.0002.Among 141 controls , 6 people(4.25%) have hyperhomocystenemia and out of which in 3 showed high CRP levels with a p value of <0.05 [16].

V. Conclusions:

Homocystene levels were found to be significantly higher in cerebral ischemic stroke patients by 41.8%. 71.7% of cerebral ischemic stroke patients belong to 41 – 60 years age group. Male to female ratio were found to be 1.66: 1.high incidence of hyperhomocystenemia in 56.25% of cerebral ischemic stroke patients without risk factors than patients with hypertension, diabetes, dyslipidemia and smoking associated incidence of hyperhomocystenemia in 34.4% of cerebral ischemic stroke patients. Elevated serum homocysteine is a strong and modifiable risk factor of cerebral ischemic strokes. Many studies are showed the significance of development of complications like cardiac and cerebral vascular events. We support the consideration of serum homocysteine as a regular and routine screening marker to protect target organ damage.

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