

Original Research

Evaluation Of Strong Correlation Between Hyperhomocysteinemia As A Potential Riskfactor In Ischemic Stroke

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ABSTRACT

Background:Stroke was the second most frequent cause of death worldwide in 2012,accounting for 7.1 million deaths. as data availablefrom Indian subcontinent is scarce in relation to Hyper homocysteinemia as a potential risk factor in ischemic stroke The present study is designed to measure theplasma homocysteine levels in patients presenting with ischemic strokes and correlate with the levels in age and sex matched controls.

Materials and Methods: This study is a case - control prospective study. The present study was done on 80 patients with ischemic stroke admitted in local area hospital 30 – 35 age and sex matched controls were recruited for the study. Plasma fasting total homocysteine (tHcy) levels were measured by chemiluminiscence immunoassay. Students‘t’ test and chi-square test were used to analyse data.

Results: The total homocysteine (tHcy) were significantly higher in patients with stroke compared to controls (19.94 ± 4.73 Vs 7.85 ± 4.56 , p-value is <0.001). The tHcy levels were significantly high in smokers compared to non-smokers (31.11 ± 2.44 Vs 12.22 ± 3.63 , p-value is <0.05), patients with hypertension compared to normotensive patients (32.09 ± 1.04 Vs 15 ± 3.21 , p-value is <0.02), patients with diabetes mellitus compared to patients not having diabetesmellitus (23.22 ± 4.39 Vs 12.87 ± 2.99 , p-value is <0.05), patients with dyslipidemia compared to patients not having dyslipidemia (31.12 ± 3.99 Vs 12.93 ± 2.23 , p-value is <0.05). Patients with no risk factors compared to controls (14.11 ± 4.38 Vs 8.76 ± 1.25).

Conclusion: Hyper homocysteinemia emerged as an important risk factor for ischemic stroke. A strong positive correlation was also observed between hypertension, diabetes mellitus, smoking dyslipidemia and tHcy levelsin thepresent study.

Keywords: Hyperhomocysteinemia, Ischemic stroke, Case control study, tHcy (total homocysteine), Chemiluminiscence immunoassay.

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INTRODUCTION

Causes of Hyperhomocysteinemia:

Plasma total homocysteine (tHcy) is used to define combined blood pool of homocysteine, homocystine, other mixed disulphides and homocysteine thiolactone.^[1] Hyperhomocysteinemia may result from abnormalities in function of any of the enzymes involved in homocysteine metabolism or from deficiencies of enzyme cofactors or cosubstrates.^[2]

Genetic Factors:

Yakub et al, concluded that most common polymorphism MTHFR C667T and MTR 2756G.^[3]

The most common form of genetic hyperhomocysteinemia results from production of a thermolabile variant of 5, 10 - methylene tetrahydrofolate reductase (MTHFR) with decreased activity. The common mutation includes point mutation of nucleotide, 677 cytosine to thymidine (677 C → T) of the MTHFR gene.^[4] Some studies found a high prevalence of hyperhomocysteinemia and the 677 C → T polymorphism in children with stroke.^[5]

Homozygous cystathionine β-synthase (CPS) deficiency causes homocystinuria, a rare disorder characterised by mental retardation, arterial and venous thrombosis and premature atherosclerosis.^[6,7] Affected individuals may have plasma total homocysteine levels (tHcy) as high as 300 to 500 μmol/L. Although homocystinuria is a rare disorder heterozygous deficiency of this enzyme is prevalent in the United States a frequency of 1 per 300 and may cause moderately elevated tHcy levels.^[8]

MATERIALS & METHODS

The present study was done on 80 patients admitted to local area govt hospital along with size and age matched asymptomatic control population. The study was carried out during the period from November 2018 to November 2019. All Patients in this study satisfied the following inclusion criteria.

Inclusion criteria:

All patients first ever diagnosed of having Ischemic stroke were taken into the present study.^[9,10]

Exclusion criteria:

Patients with Ischemic stroke presenting after 4 days from onset were excluded from the study.^[11]

- Patients with hemorrhagic stroke
- Patients who were on drugs that were likely to modify the results of Homocysteine were excluded. These included patients who were using Cholestyramine, Methotrexate, L-Dopa, Niacin, Theophylline, Androgens, Cyclosporins, Fibrin Acid Derivatives, Phenytoin, Carbamazepine.
- Patients with prior history of Renal failure, Hypothyroidism, SLE, Hepatic disease, Psoriasis were also excluded from the study.
- Patients with cardioembolic stroke

Controls:

The control group were selected from amongst age and sex-matched healthy volunteers. After informed consent, 80 controls in same age group were evaluated for homocysteine levels. Healthy volunteer was defined as asymptomatic individuals without any similar illness and was absolutely normal on detailed physical examination.

Method of detection of homocysteine:

For the measurement of homocysteine, 5ml of blood sample was drawn from the ante cubital vein and collected in the blood collecting tube. All the specimens were transported to the laboratory within 30 minutes of collection. Thereafter, specimens were centrifuged for 5-7 minutes at 3000 rpm. Then clear serum was transfused in a plastic vial and stored in refrigeration until analysis. Samples were stored at 2-8°C (stable upto 14 days when refrigerated).^[12,13]

Chemiluminescence immunoassay was used for determining total homocysteine levels in the blood. The system used was an automated, random access, direct CLIA analyzer. This diagnostic test was designed to quantitatively measure Homocysteine in serum or EDTA plasma.

Statistical analysis:

Chi-square test (X²), Students 't' paired test was used to analyse data

RESULTS

A total of 80 stroke patients and 80 age and sex matched controls were included in the present study.

Age:

Mean age of stroke patients was 45.9 years and that of controls was 47.6 which is capable in both groups Age wise distribution of cases is given in [Table 1]

Table 1: Age wise distribution of cases and controls

Age (years)	Cases		Controls	
	No of cases	Percentage	No of cases	Percentage
20-30	10	5.7	15	7.5
31-40	36	66.5	29	54.0
41-50	25	24.5	19	25.2
>50	9	3.3	17	13.3
Total	80	100	80	100

X² 2.57, p = < 0.05 significant

Sex:

Replicate number of male and female individuals are included in present study. As given in [Table 2] indicates sex wise distribution of cases.

Table 2: Sex wise distribution of cases and controls

Sex	Cases		Controls	
	Noof cases	Percentage	Noof cases	Percentage
Male	60	80.7	60	80.7
Female	20	19.3	20	19.3
Total	80	100	80	100
$X^2 = 0.00$, $p=1.00$ No difference				

Smoking

Distribution of cases in smokers in relation to stroke is shown in [Table 3].

Table 3: Smokers in relation to stroke.

Relation	No. of cases	Percentage
Smokers	62	82.3
Non-smokers	18	17.7
Total	80	100

Hypertension in relation to stroke

In present study 52 patients had Hypertension and 28 patients were non- hypertensive and distribution of cases in hypertensives in relation to stroke is shown in [Table 4].

Table 4: Hypertension in relation to stroke

Relation	No. of cases	Percentage
Hypertensive	52	78.3
Non hypertensive	28	21.7
Total	80	100

Diabetes mellitus and stroke

In this present study 44 patients had Type 2 Diabetes and rest of the 36 patients were non-Diabetics. Distribution of cases in diabetes in relation to stroke is shown in [Table 5]

Table 5: Diabetes mellitus and stroke

Relation	No. of cases	Percentage
Diabetic	44	60.8
Non diabetic	36	39.2
Total	80	100

Stroke and Increased LDL levels:

In the present study 63 patients were having LDL levels > 190mg/dl and rest of 17 patients were having their lipid profile within the normal range. Distribution of cases in smokers in relation to stroke is shown in [Table 6]

Table 6: Stroke and Increased LDL levels.

Disease	No. of cases	Percentage
Increased LDL	63	83.3
Normal LDL	17	16.7
Total	80	100

Homocysteine Levels in Different Subsets

Mean homocysteine levels were studied in different subgroups, divided on the basis of different risk factors like smoking, hypertension, diabetes, dyslipidemia and their levels were compared. The present study comprised 80 patients of Ischaemic stroke and 80 asymptomatic controls. The mean homocysteine values of all 80 patients was 19.94 ± 4.73 where as in controls the mean values was 7.85 ± 4.56 the p value is < 0.001 . there is statistical significance between the two values. Distribution of various subset of risk factors in relation to stroke is shown in following tables Relation between homocysteine levels to stroke is shown in [Table 7]

Table 7: homocysteine in ischaemic stroke patients and controls

	Homocysteine level mean \pm SD	Range	Patients V/S controls	
			't' value	p-value
Patients	19.94 ± 4.73	7.26 - 26.02	10.19	< 0.001 HS
Controls	7.85 ± 4.56	6.17-18.86		

Sex-wise distribution of Homocysteine levels

In the present study of the 80 people in the patient group 60 were male patients and 20 were female patients and the mean homocysteine values in male patients was 20.45 ± 5.81 . The mean Homocysteine values in the female patients was 12.18 ± 3.47 In the control group out of the 80 people studied 60 were males and 20 were females and the mean values of homocysteine levels were 10.12 ± 3.42 and 8.46 ± 2.64 respectively. The difference of homocysteine levels in males and females in both stroke patients and controls was statistically significant as shown in [Table 8]

Table 8: Sex-wise distribution of Homocysteine levels

	Mean \pm SD (nmol/l) cases	Significance	Mean \pm SD (nmol/l)control
Males	20.45 ± 5.81	yes	10.12 ± 3.42
Females	12.18 ± 3.47		8.46 ± 2.64
	t= 0.81		t = 2.01
	P = <0.05 , S		P= <0.05 ,S

Homocysteine Levels inHypertensives:

In present study 52 patients had Hypertension and 28 patients were non- hypertensive. Among 52 patients the mean homocysteine values were 32.09 ± 1.04 and the mean homocysteine values of non-hypertensives was 15 ± 3.21 . The difference of homocysteine

levels in both hypertensives and non-hypertensive was statistically significant as observed from [Table 9]

Table 9: Homocysteine Levels in Hypertensives

Disease	Total	Homocysteine Mean+SD	t-value	p-value
Hypertensives	52	32.09±1.04	1.99	< 0.02 Significant
Non-Hypertensive	28	15+3.21		

Homocysteine And Diabetes:

In this present study 44 patients had Type 2 Diabetes and rest of the 36 patients were non-Diabetics. The mean homocysteine values in diabetics was 23.22±4.39 and that in non-diabetics was 12.87±2.99 which is significant statistically shown in [Table 10]

Table 10: Homocysteine and Diabetes

Disease	Total	Homocysteine	t- value	p-value
Diabetics	44	23.22±4.39	2.00	< 0.05 significant
Non-Diabetic	36	12.87±2.99		

Homocystiene and LDL levels:

In the present study 63 patients were having LDL levels > 210mg/dl with meanLDL value was 31.12±3.99 and rest of the 17 patients were having their lipid profile within the normal range of < 100mg/dl with mean LDL value of 12.93±2.23 is establishing statistically significant correlation as shown in [Table 11]

Table 11: Homocystiene and LDL levels

Disease	Total	Homocysteine Mean ± S.D	t- value	P-value
Increased LDL	63	31.12±3.99	2.03	< 0.05 significant
Normal LDL	17	12.93±2.23		

Homocystiene and smoking:

Among the 80 patients 18 patients were non-smokers and 62 patients were smokers. The mean Homocysteine value in smoker group was 31.11±2.44 and that in the non-smoker group was 12.22±3.63 indicating strong statistical significance among smoking vs elevated levels of homocysteinemia as mentioned in [Table 12]

Table 12: Homocystiene and smoking

Disease	Total	Homocystiene mean±SD	t-value	p-value
Smoker	62	31.11±2.44	1.89	< 0.05
Non smoker	18	12.22±3.63		

Homocysteine and multiple risk factors:

Among the 80 patients; 14 patients had no risk factors. The remaining 66 patients had one (or) more risk factors namely Hypertension, Diabetes mellitus, smoking, dyslipidemia. [Table 13] Single risk factor was noted in 4 subjects of whom 1 was hypertensive, 1 was diabetic, 1 was smoker and 1 subject had dyslipidemia.

About 10 patients out of the 80 patients in the study group had 2 major risk factors and 35 patients had 3 risk factors and 68 patients had all the 4 major risk factors. The mean value of Homocysteine levels in patients with 3 or > 3 risk factors was 26.96 ± 3.39 which is significantly higher than patients with no risk factors who has mean homocysteine values of 12.27 ± 3.21 (p value <.001)

Similarly controls with no risk factors had a Homocysteine level of 6.82 ± 2.24 when compared with patients with no risk factors had a homocysteine level of 13.12 ± 5.61 which is statistically significant (p value <.001)

Table 13: ?

Sl. No.	No.of risk factors	No.of patients	Homocysteine mean \pm SD
I	No risk factors	14	14.11 \pm 4.38
II	Single risk factor		
	Hypertension	1	17.23 \pm 2.35
	DM	1	10.39 \pm 2.54
	Smoker	1	20.12 \pm 6.09
	Dyslipidemias	1	19.22 \pm 3.40
III	Two risk factors		
	HTN+DM	2	22.35 \pm 6.20
	HTN + \uparrow LDL	2	20.46 \pm 2.60
	HTN + smoking	3	20.99 \pm 3.72
	\uparrow LDL + smoking	1	18.95 \pm 3.04
	DM + smoking	1	21.72 \pm 2.22
	DM + \uparrow LDL	1	29.68 \pm 2.72
IV	3 or > 3 Risk factors	35	22.80 \pm 2.28
V	4 risk factors	68	29.99 \pm 8.39

Odds ratio:

The strength of the association between a risk factor and stroke is given by ODDs ratio. In the present study out of 80 patients 64 had homocysteine levels > 10 μ mol/L whereas 16 had homocysteine levels < 10 μ mol/L and out of the 80 controls 72 had homocysteine levels < 10 μ mol/L and 8 had homocysteine levels > 10 μ mol/L.

Table 14: ?

	With ischaemic Stroke	Without ischaemic Stroke
Homocysteine levels > 10 μ mol/L	64	8
Homocysteine levels < 10 μ mol/L	16	72

DISCUSSION

There have not been many large-scale studies to find out the prevalence of hyperhomocysteinemia in Indians in particular. The present study was devised to establish the contribution of increased homocysteine level as a high risk factor for stroke in Indian population. In the present study the mean homocysteine levels among the 80 patients were 19.94 ± 4.73 in contrast to controls who had mean value of 7.85 ± 4.56 . There is a significant difference between the patients and controls. The p value is < 0.001 that is statistically significant. The results of the present study are consistent with many case control studies involving patients in all age groups. Especially in younger age group increased homocysteine level is an important risk factor for the development of ischemic stroke in all populations. Even larger Indian population needs to be screened to establish a definite strong correlation between high level of homocysteine and stroke. It will be worthwhile to consider homocysteine levels of >10 mmols as significant in patients with stroke for secondary prevention and supplementation of folate and vitamin B. It is worthwhile to consider proper dietary intake and foods rich in folic acid, vitamin B6 and B12 for primary prevention and sufficient multivitamin supplements (folic acid, B6, B12) for individuals with known cerebrovascular disease and hyperhomocysteinemia.

Table 15: Homocysteine and vascular disease prospective cohort and case control studies

	Study	Vascular disease	Sex	tHcy ($\mu\text{mol/L}$)		p- value
				Case	Controls	
1	Meiklejohn et al, ^[2]	CVA	M/F	10.1	8.2	S
2	Brattstrom et al ^[3]	CVA	M/F	12.5	11.0	S
3	Giles et al, ^[4]	CVA	M/F	11.9	10.2	S
4	Verhoeve et al, ^[13]	CVA	M	11.1	10.6	NS
5	Perry et al, ^[1]	CVA	M	13.7	11.9	S
6	Graham et al, ^[5]	CVA	M/F	11.11	9.73	S
7	Kristensen et al, ^[6]	CVA	M/F	12.88	13.23	NS
8	Eikelboom et al, ^[7]	CVA	M/F	12.4	10.5	S
9	P. J. Kelly et al, ^[9]	CVA	M / F	10.1	10.33	NS
10	Chambers et al ^[8]	CAD	M	12.0	10.8	S
11	Nigel Choon Kiat Tan et al, ^[11]	CVA	M/F	13.7	10.8	S
12	M.Modi et al, ^[10]	CVA	M/F	9.91	8.00	S
13	Mili Gupta et al, ^[12]	CAD	M/F	16.57	11.47	S
14	Present Study	CVA	M/F	22.98	4.89	Highly significant.

The relative risk of the patient developing stroke in my study was 4.89 which is comparable with other studies.

CONCLUSION

The present study was done to assess the role of homocysteine as a risk factor for ischemic stroke:

1. The mean homocysteine values in patients with Ischemic stroke was significantly elevated than in asymptomatic control (17.94 ± 4.77 Vs 8.85 ± 2.56).
2. Increased homocysteine level is an important risk factor for the development of ischemic stroke in all populations.
3. Presence of risk factors like hypertension, diabetes, smoking, dyslipidemia can also cause elevated homocysteine levels.
4. Even in patients without diabetes, hypertension, smoking and dyslipidemia homocysteine is an important cause of stroke.
5. Hence homocysteine levels should be routinely assessed in people who are at risk for stroke.

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