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A CASE CONTROL STUDY OF PLASMA HOMOCYSTEINE LEVELS IN ISCHAEMIC STROKE PATIENTS WITH AND WITHOUT HYPERTENSION VERSUS CONTROLS

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ABSTRACT Cerebrovascular disease designates any abnormality of the brain resulting from a pathologic process of the blood vessels. Stroke of all types ranks third as a cause of death, and this is the leading cause of disability in adults. Ischaemic events account for approximately 80% of all strokes. In India, based on a single population based study, the two year prevalence and annual incidence rate are 84 and 13 per 100,000 population, which, when compared with figures quoted from western studies, are much less. A few Indian studies point out that 9.2% to 30% of neurological admissions are patients suffering from a stroke. Increased plasma levels Homocysteine is now considering as individual risk factors for cerebrovascular disease, myocardial infarction, and peripheral arterial occlusive disease. Hyperhomocysteinemia not only accelerates atherosclerosis but also by various mechanisms can increase the incidence of cerebrovascular disease. The present study is to measure the plasma homocysteine levels in patients presenting with ischaemic strokes and correlate with the levels in age and sex-matched controls. The present study included age, sex, and BMI matched individuals in Hypertensive individuals.. There is growing evidence that high homocysteine levels contribute to the pathogenesis of ischaemic stroke. Homocysteine is believed to cause atherogenesis and thrombogenesis via endothelial damage, vascular smooth muscle proliferation, and coagulation abnormalities. High homocysteine levels are associated with increased risk of cardiovascular and cerebrovascular disease. The present study has shown an elevation of Homocysteine levels > 10 µmol/L in 89% of patients aged above 45 years.

1.INTRODUCTION

Cerebrovascular disease designates any abnormality of the brain resulting from a pathologic process of the blood vessels. The pathologic process may result in ischaemic of the blood vessels resulting in infraction of the brain .

Stroke of all types ranks third as a cause of death, and this is the leading cause of disability in adults. Ischaemic events account for approximately 80% of all strokes.

The incidence in the UK is approximately 2 per 1000 population per year, and about 100,000 patients have a first stroke every year, one every 5 minutes.

In the United States every year, there are approximately 500,000 cases of stroke, roughly 400,000 infractions, and 100,000 hemorrhages. In India, based on a single population based study, the two year prevalence and annual incidence rate are 84 and 13 per 100,000 population, which, when compared with figures quoted from western studies, are much less12

HOSPITAL DATA 2

Based on many retrospectives and a few prospective studies, cardiovascular disease accounts for 0.9% to 4.5% of total medical admissions in India. A few Indian studies point out that 9.2% to 30% of neurological admissions are patients suffering from a stroke.

The figures are much lower than the figures of Fisher et al. from the U.S.A. where about 50% of neurological admissions are patients with cerebrovascular diseases. As pointed out by Wad, if Indian figures were to be calculated only for adult Neurological hospital admissions excluding the pediatric age group, there might come closer to those mentioned by Fisher et.al. This contention, however, is not held by Venkataraman et al., who state that Cerebrovascular disease constitutes 18.8% of all admissions to the Neurological services of AIIMS. Socioeconomic factors, dietary and lifestyle behaviors, different patterns of risk factors, and environmental conditions may explain the different incidences of stroke observed in different parts of the world.

Several risk factors that may be classified as modifiable and unmodifiable increase the risk of ischaemic stroke. The modifiable risk factors have very much clinical significance because modification or alteration of these risk factors can decrease the incidence of stroke in the population. The modifiable risk factors of clinical significance are Hypertension, Diabetes mellitus, Dyslipidemia, Cigarette Smoking,

Alchohol consumption, increased fibrinogen, elevated Homocysteine, obesity, etc. Increased plasma levels Homocysteine is now considering as individual risk factors for cerebrovascular disease, myocardial infarction, and peripheral arterial occlusive disease. Hyperhomocysteinemia not only accelerates atherosclerosis but also by various mechanisms can increase the incidence of cerebrovascular disease.

The present study is to measure the plasma homocysteine levels in patients presenting with ischaemic strokes and correlate with the levels in age and sex-matched controls.

1.AIM AND OBJECTIVES OF STUDY : The present study aims to determine the levels of Homocysteine in patients with Ischaemic stroke aged above 45 years with hypertension and diabetes or both and compare it with controls.

2.RISK FACTORS [3,7,8] **:** Several risk factors that are classified as modifiable and non-modifiable for ischaemic stroke are :

NON – MODIFIABLE RISK FACTORS FOR ISCHAEMIC STROKE;
Age
Race/ethnicity
Gender
Family history
Genetics

MODIFIABLE RISK FACTORS FOR ISCHAEMIC STROKE ;
Hypertension
Transient ischaemic attacks
Cardiac disease / atrial fibrillation
Aortic arch atherosclerosis
Diabetes mellitus
Dyslipidemia

Hyperhomocysteinaemia
Cigarette smoking
Alcohol consumption
Obesity
Oral contraceptive and postmenopausal estrogens.
Above all, the Hyperhomocystenaemia is the parameter of interest taken in this study in ischaemic stroke patients among risk factors of hypertensive versus non hypertensive among cases and controls.

HYPERTENSION ; a review literature:
Either systolic or diastolic, hypertension is the most important and

highly prevalent risk factor. According to the Framingham study, there is no threshold value above which the incidence of stroke begins to rise. The rates of later stroke are higher for those with high normal than those with low normal blood pressure.

The veterans' administration co-operative studies showed that the treatment of moderate and high levels of diastolic pressure substantially reduced the incidence of both fatal and non-fatal stroke. The effect of treatment of mild hypertension is more controversial. The MRC trial showed no reduction in mortality from all causes, however, there was a significant reduction in the risk of stroke, a reduction that was more in those patients taking thiazide diuretic. Propranolol was not considered relevant. The question is whether treatment of hypertension in the elderly is worthwhile, bearing in mind the presumably long duration of vascular disease.

The European working party on HTN in elderly recruited patients over the age of 60 with diastolic pressures between 90 and 119 mm Hg. Cardiovascular events were reduced by about a quarter and non-fatal strokes by a half. There was no difference in fatal stroke rate. The important point is that benefit was independent of entry blood pressure and the presence of previous cardiovascular events. No benefit could be observed in treating those over the age of 80 years. There is anecdotal evidence that abrupt reduction in B.P and circulating blood volume may precipitate cerebral infarction in the elderly.

HYPER HOMOCYSTEINEMIA : The interest in Homocysteine has burgeoned during the last few years. Homocysteine is now considered a risk factor for several diseases particularly in Cerebrovascular stroke, cardiovascular diseases, and peripheral athero-occlusive diseases.

Homocysteine is a sulfur-containing amino acid that is closely related to the essential amino acid methionine and cysteine. Butz and Duvigneaud first described Homocysteine in 1932. During the last 15 years, it has been documented that moderately elevated homocysteine levels in serum or plasma are a strong and independent risk factor for occlusive arterial disease and venous thrombosis. As many as 50% of patients with stroke and other atherothrombotic diseases have high homocysteine levels.

Elevated plasma Homocysteine levels in Indians;
Elevated plasma homocysteine levels seem to be a feature of South Asian populations. In the SHARE and the UK study[13], the levels of homocysteine in the South Asians /Indians were higher than those found in the other ethnic groups. For example, in the UK study, fasting homocysteine concentrations were 6% higher in the Indian controls than in the Europeans. Several explanations have been put forward to account for this reduced intake of vitamin B_{12} in Indians and the prolonged cooking of vegetables, which has been observed in some Indian households in the UK. It is believed that this later practice may destroy up to 90% of their folate content. However, it is not known whether lowering plasma homocysteine levels, through B vitamin and folate supplementation, helps in the long run. Subclinical renal dysfunction can be another cause of this elevation. Plasma homocysteine levels rise in parallel to serum creatinine as the glomerular filtration[17] rate falls. Diabetes and resultant renal impairment are known to be much more common among Indians[18]. A recent study found that the most common genetic defect responsible for elevated homocysteine levels in Caucasians, the methylenetetrahydrofolate reductase (MTHFR) C677T mutation, does not significantly influence homocysteine concentrations in Indians in the UK[19]. This is partly because the frequency of this mutation in Indians is less than one-third of that in European Whites. Homocysteinemia, an autosomal disease with considerable genetic heterogeneity, is the second most common inborn error to amino acid metabolism. While the full genetic disorder affects only 1:200,00 live births, abnormal homocysteine metabolism – due to enzyme dysfunction or nutritional deficiencies – appears to be surprisingly common.

Homocysteine is an Amino acid formed during the metabolism of methionine. The first step in the synthesis of homocysteine is the formation of S-adenosylmethionine (Adeno-meth), an important methyl donor, from methionine. Adeno-meth is then converted to S-adenosyl homocysteine (Adeno-Hcy), which is further hydrolyzed to yield homocysteine and adenosine, depending on whether there is a relative excess or a deficiency of methionine, homocysteine may then enter either transsulfuration or remethylation pathways.

3. MATERIALS AND METHODS:
STUDY DESIGN: Observational study (case-control)

STUDY PERIOD: From Nov 2017-2020
The present study was carried out on 50 patients (cases) admitted in the Medicine department, King George Hospital, Visakhapatnam. About 50 asymptomatic controls were taken. The patients in this study satisfied the following inclusion criteria.

Inclusion criteria;
*   All patients should be aged more than 45 years.
*   All patients first-ever diagnosed with having Ischaemic stroke were taken into the present study.
*   Patients who are presented with hypertension..

Exclusion criteria:
Patients with
*   Ischaemic stroke of less than 45 yrs of age was excluded
*   Presenting with more than 48 hrs of duration from the onset of ischaemic stroke were excluded
*   Patients who are on drugs that modify the result of homocysteine were excluded ….
Cholestyramine, Methotrexate, L-dopa, Niacin, Theophylline, Androgens, Cyclosporines, Fibricacid derivatives, Phenytoin, Carbamazepine.
*   Prior history of Renalfailure, Hypothyroidism, SLE, Psoriasis excluded.

Data collection:
*   Demographic data like gender and age were collected, and the patients (both cases and controls) were interviewed for the relevant history of risk factor of hypertension.
*   A thorough general physical examination was conducted, followed by a systemic examination, and the findings were noted.

Investigations;
*   Hypertension monitoring for hypertensive individuals.
*   Plasma Homocysteine levels.
*   Homocysteine values more than 10 micromol/l were considered as Hyperhomocysteinemia.

Plasma homocysteine levels: Venous blood (10 ml) collected in EDTA tubes after an overnight fast (14h), using standardized protocol and equipment. Plasma was immediately separated and stored at – 20 C. Plasma samples were analyzed for total homocysteine by the HPCL system.

4 . RESULTS:
Table 1. Comparison of mean homocysteine levels between cases & controls

HOMOCYSTEINE	N	MEAN	STANDARD DEVIATION	P value
CASES	50	19.44	5.84	0.001
CONTROLS	50	9.24	1.93	

Figure 1: Comparison of mean homocysteine levels between cases & controls
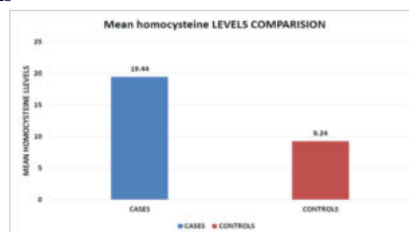
The mean homocysteine value among 50 cases is 19.44± 5.84, whereas, in controls, the mean values were 9.24 ± 1.93, the P-value is < 0.001.

TABLE 2: COMPARISION OF HTN BETWEEN CASES AND CONTROLS

Hypertension	Cases	Controls	Total	P value
Yes	26(52%)	41(82%)	67(67%0	0.01
No	24(48%)	9(15%)	33(33%)	
Total	50(100%)	50(100%)	100(100%)	

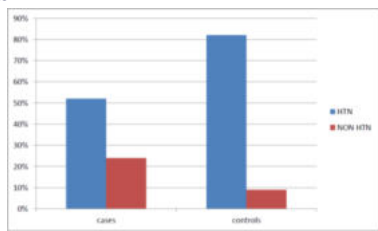**FIG 2; COMPARISION OF HTN BETWEEN CASES AND CONTROLS**

TABLE 3: MEAN HOMOCYSTEINE LEVELS COMPARISON BETWEEN HYPERTENSIVE AND NON HYPERTENSIVES

HOMOCYSTEINE	N	MEAN	STANDARD DEVIATION	P VALUE
HYPERTENSIVE	24	19.95	6.10	0.055
NON-HYPERTENSIVE	26	18.97	5.68	

Fig: 3: Mean homocysteine LEVELS COMPARISION
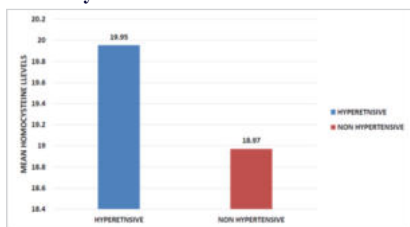
TABLE 4: MEAN BMI COMPARISON BETWEEN CASES AND CONTROLS

BMI	N	MEAN	STANDARD DEVIATION	P VALUE
CASES	50	24.2436	3.20484	0.33
CONTROLS	50	23.3370	5.73724	

Fig 4: Mean BMI comparision
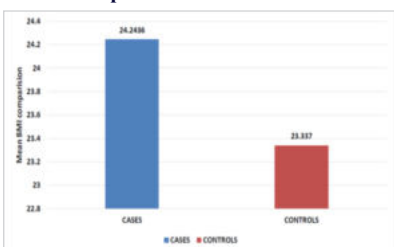
TABLE 5: RISK FACTORS ANALYSIS AMONG CONTROLS

RISK FACTOR	SUB CATEGORY	FREQUENCY	PERCENTAGE
HYPERTENSION	YES	41	82%
	NO	9	18%

Table 6: ODD"S RATIO

	CASES	CONTROLS	Total
Homocysteine >10	45(a)	12(b)	69
Homocysteine <10	5(c)	38(d)	31
Total	50	50	100

P value= 0.001
Exposure rate among cases = a/a+c= 45/45+5=45/50=0.9
Exposure rate among controls = b/b+d= 12/12+38=12/50=0.24
Odd's ratio= ad/bc= 45×38†5×12=28.5

5. DISCUSSION :

The present study comprised 50 patients of Ischaemic stroke and 50 asymptomatic controls. The mean Homocysteine values of all 50 patients were 19.44± 5.84, whereas, in controls, the mean values were 9.24 ± 1.93, the P-value is < 0.001. There is a statistical significance between the two values.

FIG-5 : MEAN HOMOCYSTEINE VALUES IN ISCHAEMIC STROKE PATIENTS AND CONTROLS
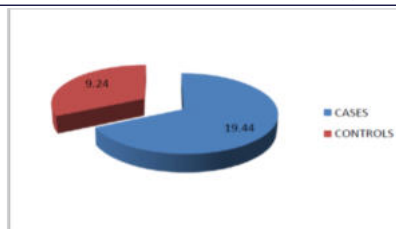
COMPARISON OF PATIENT GROUP AND CONTROL GROUP:

Inpatient group, the mean age is 60.68 and in that of the control group is 63.44, and the mean of the BMI in patients and control groups is 24.24 and 23.33, respectively. So the present study included age, sex, and BMI matched individuals.

Homocysteine and Hypertension :

In the present study, 24 patients had Hypertension, and 26 patients were non-hypertensive. Among 24 non hypertensive patients the mean Homocysteine values were 19.95 ± 6.10, and the mean Homocysteine values of hypertensives were 18.97 ± 5.68

ODDS RATIO ;

The ODDs ratio gives the strength of the association between a risk factor and stroke. In the present study out of 50 patients 45 had homocysteine levels >10 μmol/L whereas 5 had homocysteine levels < 10 μmol/L and out of the 50 controls 12 had homocysteine levels >10 μmol/L, and 38 had homocysteine levels < 10 μmol/L.

Calculation of relative risk :

1. Relative Risk :

Risk of stroke in those with risk factors
--- = 6.14
Risk of stroke in those without risk factor

Exposure rate among cases = a/a+c= 45/45+5=45/50=0.9
Exposure rate among controls = b/b+d= 12/12+38=12/50=0.24

Odd□s ratio= ad/bc= 45×38†5×12=28.5

2. ODD□s ratio =
ODD□s of stroke with risk factor
-------------------------------------- = 28.5
ODD□s of stroke without a risk factor

6.SUMMARY

The present study has shown an elevation of Homocysteine levels > 10 μmol/L in 89% of patients aged above 45 years. Dr. Nigel Tan et al. had found an elevation of homocysteine > 10 μmol/L in 78% of patients of Ischemic stroke aged above 45 years.

The mean homocysteine levels among the 50 patients in the present study were 19.44 ± 5.84 in contrast to controls who had a mean value of 9.24 ± 1.93. There is a significant difference between the patients and the controls. The p-value is < 0.001 that is statistically significant.

A similar study was conducted by Dr. Nigel Tan, Dr. N. Venkata Subramanian et al. in which they had selected 109 cases and 88 controls. The mean Homocysteine values in cases were 15.7, and that of in controls was 9.8, with a p-value of < 0.001.

Hence plasma homocysteine levels are to be measured regularly among patients with Ischaemic stroke.

The British regional heart study, the Rotterdam study of the elderly, and the Framingham studies, which were cohort studies, have shown elevated plasma homocysteine levels in Ischaemic strokes.

TABLE -7 :MEAN HOMOCYSTEINE LEVELS IN CASES AND CONTROLS

	Present study	Dr.Nigel Tan et al
Cases	19.44 ± 5.84	15.7
Controls	9.24 ± 1.93	9.8
p Value	< 0.001	< 0.001

HOMOCYSTEINE AND HYPERTENSION :

In the present study number of patients having hypertension was 24 (48%). The mean and standard deviation of homocysteine levels in the hypertensive patient's group was 19.95 ± 6.10. The mean homocysteine levels of the non hypertensives patient group was 18.97 ± 5.68, p-value is less than 0.055, which is statistically significant.

RELATIVE RISK OF CEREBROVASCULAR DISEASE ASSOCIATED WITH AN INCREASE OF HOMOCYSTEINE LEVELS

Table8:

STUDY	RELATIVE RISK
Verhoel Study	2.8
Alfthan Study	3.6
Brattstrom Study	4.5
Araki, stroke Study	5
Coul, stroke Study	5.5
Present Study	6.14

7. CONCLUSION:

The present study was done to assess the role of homocysteine as an independent risk factor for ischemic stroke in patients greater than 45 years of age in risk factor of hypertension in cases and controls.

1. The mean Homocysteine values in patients above the age of 45 years were significantly elevated (19.44 ±5.84 Vs. 9.24± 1.93) than asymptomatic control (19.44 ±5.84 Vs. 9.24± 1.93). Hence Homocysteine should be assessed routinely in all patients with ischemic stroke.

2. The present study addresses the fact that hypertension is an important risk factors for ischemic stroke. The mean homocysteine levels were more in patients with risk factor group than in the non-risk factor group. This infers that patients with risk factors should be assessed for homocysteine levels and homocysteine is a predominant independent risk factor for ischaemic stroke.

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