Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(2C):476-482 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2016.v04i02.032

Original Research Article

The Role of Hyper Homocysteinemia in Acute Cerebral Stroke Patients in Western Rajasthan

Mukesh Babu[1], Raghuveer Choudhary[2], N. D. Soni[3]
[1]Final year resident, [2] Associate Professor, [3]Head and Sr. Professor, Department of Physiology, Dr. S.N. Medical Collage Jodhpur, India

***Corresponding author**
Mukesh Babu
Email: mukeshbabu05@gmail.com

Abstract: The present study is undertaken to estimate the plasma homocysteine level in cerebrovascular accident cases with the aim to search for the prevalence of elevated homocysteine level in cerebrovascular accident (Stroke) among patient of western Rajasthan. This study was conducted on 50 stroke patient (Case group) and 50 normal subjects (Control group)of both sex and aged between 30 yrs-75 yrs. Both groups were subjected to routine investigations like Blood sugar, Lipid profile, and Plasma Hcy concentration. A detail medical history was obtained. All study groups were subjected to investigation including neurological examination, blood examination, renal function, urine examination, chest X - ray & ECG, and CT/MRI. Plasma Hcy concentration was measured by Chemiluminescent Magnetic Immunoassay (CMIA), using plasma on the ARCHITECT I System from standard laboratory in all the stroke patients of case group. Similar investigations were done for control group except CT Scan/MRI. In results the Plasma homocysteine (PHcy)were found significantly raised in stroke patient as compared to control groups(34.79± 17.76/11.42±2.43) p<.001, elevated plasma Hcy level was found significantly raised in both younger age group (<40) & older age group (>40) as compared to control group subjects. NIHSS score & PHcy level showed a positive co-relation. As NIHSS score rises in stroke patients, Proportionately PHcy level also rises. 40% of stroke patient showed moderate NIHSS score (5-14).In these subjects mean PHcy was found 33.89±16.18. 28% of stroke patient showed NIHSS score between15-24, Have severe stroke, these patient PHcy level was 43.9 ± 18.51. 4% of stroke patient showed NIHSS score >25, having very severe stroke. There mean plasma homocysteine was 57.5± 10.60. As per TOAST classification of stroke, our result showed 24 subjects with large artery infarct with mean Hcy level 39.73 ±16.50 while 9 subjects showed medium size artery infarct with Mean Hcy level 36.24 ± 17.57, 12 Subject were having Territory vessel infarct with PHcy level 36.17±23.26.2 Subject were having Lacunar infarct with mean PHcy level 29.24±2.16. Raised PHcy is significantly associated with ischemic stroke and treating hyper- homocysteine may be an effective way of decreasing the risk of stroke.

Keywords: Hyperhomocysteinemia, Cerebral Stroke, NIHSS.

INTRODUCTION

A stroke is a cerebrovascular accident in which loss of brain function due to the disturbance of blood supply in the brain, World Health Organisation of Stroke is "rapidly developed clinical sign of focal disturbance of cerebral function of presumed vascular origin and of more than 24 hour duration. This definition does not include transient ischemic attack [1]. Stroke is a common cause of mortality and morbidity in developing countries, Stroke is a second leading cause of death after cardiac disease worldwide [2].

In India the estimated prevalence ratio of stoke 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[3] stroke accounts for two percent of hospital registrations and 9-30% of neurological admissions [4, 5].

The broad classification of strokes, primarily is ischemic (85%) and haemorrhagic (15%), of ischemic strokes commonest aetiologies 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 [6]. Cerebral ischemia and infarction are caused by sudden occlusion of an artery supplying the brain or less often

by low flow distal to an already occluded or highly stenosis artery [7].

The modifiable biochemical risk factors are abnormal lipid metabolism [8], hypercholesterolemia, lipoprotein, (a) [Lp (a)] [9] hyper- homocysteinemia [10].

The most promising strategy to reduce the world-wide burden of stroke is effective stroke prevention. To date, only 70% of all strokes can be attributed to known risk factors. An emerging risk factor for stroke, which is prevalent and modifiable, is plasma homocysteine. Homocysteine is a sulphur containing amino acid formed during the metabolism of methionine, an essential amino acid derived from dietary protein. It is metabolized with folate as a co-substrate, vitamin B12 as a co-factor and the help of several other enzymes. Elevated plasma homocysteine is a risk factor for atherothrombotic disease and is associated with atherosclerotic vascular diseases. Large epidemiological studies have demonstrated this relationship in coronary artery, peripheral arterial and cerebrovascular disease [11-14]. Hyper-homocysteinemia 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 form the west and other part of country may not be applicable to Western Rajasthan population. Homocysteine is newly identified risk factor for stroke and alot 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.

MATERIALS AND METHODS

The study was carried out in the Department of Physiology & Neurology, Dr. S.N. Medical Collage, Jodhpur Rajashan.50 patient between age of 30-75 year(both sex)and 50 healthy subject matched with age and sex were taken for study. They were divided in to case group (n=50)& control group(n=50).

Inclusion criteria used in this study which were:

- Patient admitted with new onset of focal neurological deficit after acute cerebral stroke.
- Patient greater than 30 years and less than 75 years of both sex included.
- Patient with focal neurological deficit and C.T. scan of brain within 24 hours documented by C.T. scan of brain.
- Patient with new onset of stroke with past history of essential hypertension diabetes mellitus, alcohol consumption, dyslipidaemia, heart disease oral contraceptive uses.
- Patient with post-partum corticovenous thrombosis and atrial stroke also included.

Exclusion criteria were

- Patient admitted in neurology ward with features of C.V.A diagnosed as brain infection, subdural hematoma , intra cranial malignancy and other brain lesion.
- Patient admitted with neurological deficit due to old C.V.A following cerebral ischemia, transient ischemic attacks is not included.
- Subject having confounding factor which could raise homocysteine level like use of anti-epileptic drugs, foliate antagonist and subject with lung liver and kidney diseases.

All the patients were subjected to a detailed history taking and clinical evaluation to obtain the information's like age, sex, personal history, drug history, family history and history of Hypertension, Diabetes mellitus, Cardiac Diseases, Cerebrovascular accident.

Central nervous system was examined, Patients functional status was scored according to NIHSS score and TOAST classification (trial of Org 10172 in Acute Stroke Treatment) done for aetiological /anatomical classification. Routine investigations like Haemoglobin, TLC. DLC, urine for routine microscopic, blood sugar, lipid profile CT scan /MRI was done in each patient. One twelve lead ECG was recorded. Special investigation;-total plasma Homocysteine was done by Chemiluminescent Magnetic Immunoassay (CMIA) by ARCHITECT I System from standard laboratory. All subjective history, possible risk factor and investigation were entered in pre designed proforma for this study.

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.

OBSERVATION AND RESULTS

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

Table 1: Age Wise Distribution of Subjects

Age (in yrs.)	No. of Subjects	
	Case Group	Control Group
26-35	8	7
36-45	8	22
46-55	11	9
56-65	15	7
66-75	8	5

Table 1: Is showing age wise distribution of subjects in case & control group. Maximum stroke patients were found in age group 56-65 years of age in case group, while in control group maximum subjects were in age group 36-45 years. In the present study, the youngest patient was in 2nd decade between 26 to 35 years and oldest patient was between 66 to 75 years. In 5th de maximum no. of cases were distributed.

Table 2: Plasma Homocysteine Levels in Case & Controls Group

Gender	Case		Control		p-value
	N	pHcy	N	pHcy	
Male	38	34.84±17.21	36	10.96±2.29	<.001 (HS)
Female	12	36.12±17.78	14	12.62±2.47	<.001 (HS)
Total	50	35.15±17.66	50	11.42±2.43	<.001 (HS)

Table-2 showing plasma homocysteine level in case and control group, both male and female subjects has showed significantly raised homocysteine in stroke patients (case-group) as compared to control group. (p<.001).

Table 3: NIHSS Score & Plasma Homocysteine Level

NIHSS Score	No. of cases	Mean Homocysteine Level
<5(Mild)–Stroke	14 (28%)	25.01±12.97
5-14(Moderate)-Stroke	20 (40%)	33.89±16.18
15-24(Severe)-Stroke	14 (28%)	43.9±18.51
>25(very Severe)-Stroke	2 (4%)	57.5±10.60

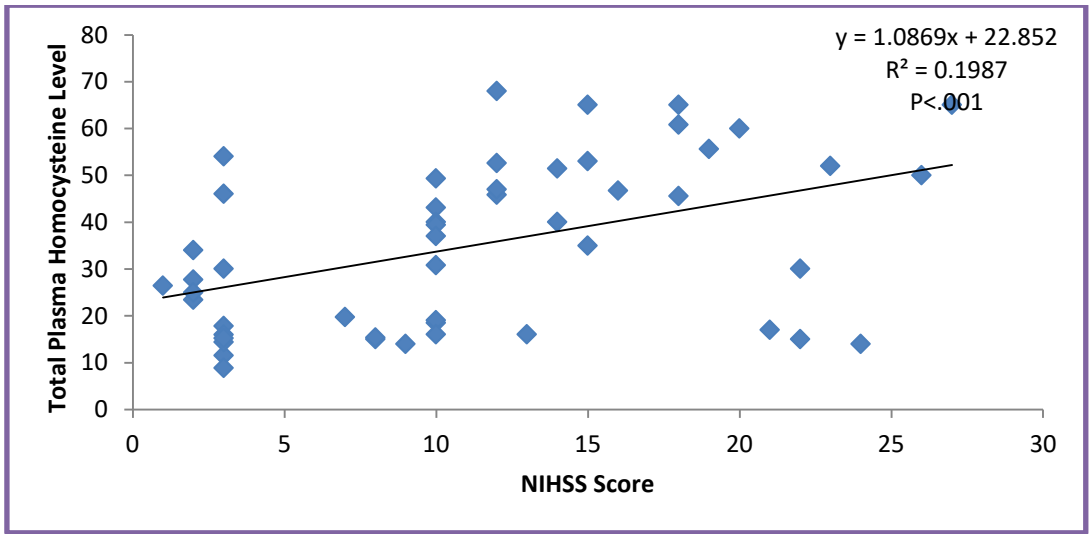

Fig-1: NIHSS Score Vs Plasma Homocysteine Level

Table-3; showing relationship between plasma homocysteine level and National institute of health stroke scale (NIHSS) score. NIHSS score is also a tool to measure severity of stroke. It was observed that as the NIHSS score increase, total plasma homocysteine level also increases, proportionately. Statistically NIHSS have showed significant association with Plasma Homocysteine level. (p<001). 28% of stroke

patients showed mild NIHSS score i.e. <5. These subjects showed mean homocysteine level 25.01±12.97. 40% of stroke patients showed moderate NIHSS score i.e. 5-14. In these subjects mean homocysteine level was found 33.89±16. 28% of stroke patients showed NIHSS score between15-24, they were having severe stroke. This patient's mean homocysteine level was 43.9±18.51. 4% of stroke patients showed NIHSS score >25. They were having very severe stroke. There mean plasma homocysteine was 57.5±10.60. It is observed that as NIHSS score increases plasma homocysteine level also rises proportionately in stroke patients.

Table 4: Prevalence of Other Risk Factors with Homocysteinemia in Case Group

Risk factor	Yes/no	No. of cases	Homocysteine level (≥16µmol/L) (n=40)	Prevalence Rate
Hypertension	Yes	30	26	88.66
	No	20	14	70
Diabetes	Yes	16	15	93.75
	No	34	25	73.52
Ischemic heart disease	Yes	8	7	87.5
	No	42	33	78.57
Smoking	Yes	30	27	90
	No	20	13	65
Alcohol liver disease	Yes	20	19	95
	No	30	21	70
Dyslipidaemia	Yes	10	8	80
	No	40	32	80

Table-4 is showing prevalence of Hyperhomocysteinemia with other risk factor of stroke. In case group subjects having hypertension level showed higher prevalence rate (86.66%) for Hyperhomocysteinemia, as compared to normotensive subjects, who show prevalence rate (70%) for Hyperhomocysteinemia. Likewise diabetes, subjects have shown prevalence rate 93.75% for Hyperhomocysteinemia, IHD subjects showed prevalence rate 87.5%, smoker subjects showed prevalence rate 90%, and alcoholic liver disease 95%

Table 5: Type of Stroke with Relation of Hyper Homocysteine Level

Type of Stroke	Mean Hyper homocysteine level (≥16 µmol/L) [n=40]
Haemorrhagic (n=8)	35.2±19.39
Ischemic (n=32)	41.78±14.68*

***P-0.29**

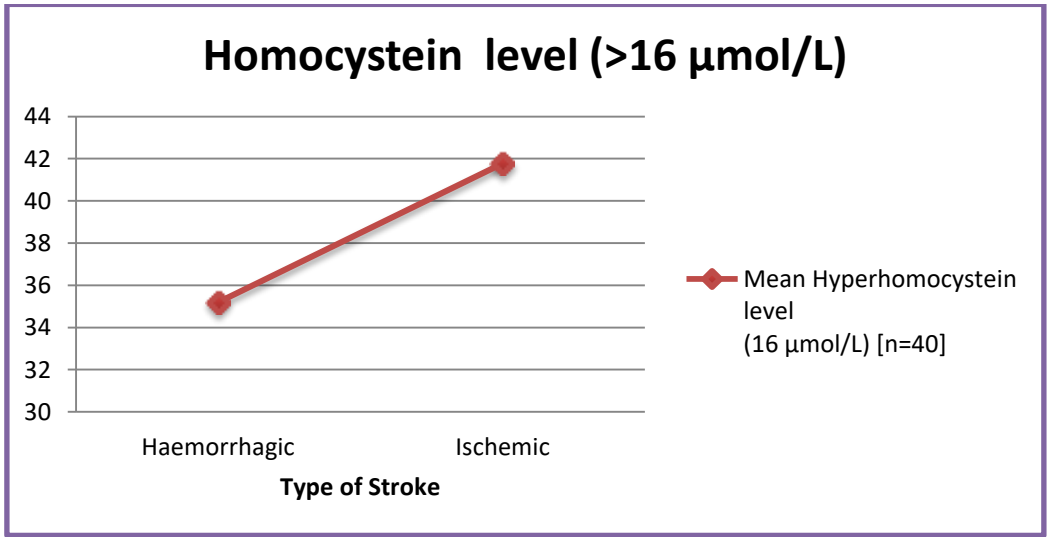

Fig-2: Type of Stroke with Relation of Hyper Homocysteine Level

Table-5:& its Fig-2- showing mean homocysteine level (>16µmol/L) in haemorrhage and ischemic stroke. It was observed that in 8 cases of haemorrhage stroke mean homocysteine level was found 35.2±19.39and in 32 cases of ischemic stroke. Mean homocysteine level was found 41.78±14.68, but statistically this rise in ischemic stroke was not significant (p=>.05).

Table-6:- Plasma Homocystein Level According To Toast Classifcation in Case Group

Toast Classification	No. of cases	pHcy level
Large size artery	24	39.73±16.50
Medium size artery	9	36.24±17.57
Territory vessels	12	26.83±20.16
Lacunar	2	36.17±23.26
Thromboembolic	2	29.24±2.16
Undetermined	1	0
Determine	0	0

Table-6 showing mean plasma homocysteine level according to Toast classification in stroke subjects. Result of our study showed that large artery infarct were present in 24 subjects showing mean homocysteine level 39.73±16.50, while 9 subjects showed medium size artery infarct with mean homocysteine level 36.24±17.57. 12 subjects were having Territory vessels infarct with mean homocysteine level 36.17±23.26 and 2 subjects were having lacunar infarct with mean homocysteine level 29.24±2.16.

DISCUSSION

Stroke is a major cause of death and disability worldwide. Each year about 4.4 million people die of stroke globally of whom almost 3 million are from developing countries.[15] Common causes for ischemic stroke are atherosclerosis with thromboembolisms while less common cause include hypercoagulable disorders, homocysteinemia, collagen vascular disease, fibro muscular dysplasia, oral contraceptive, 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 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 B_6 and B_{12}. Physiologic Hcy levels are determined primarily by dietary intake and vitamin status. Elevated plasma levels of Hcy (eHcy) can be caused by deficiency of either vitamin B_{12} or folate, or a combination there of. Certain genetic factors also cause eHcy, such as C667T substitution of the gene encoding methylene tetrahydro folate reductase. eHcy has been observed in several medical conditions, such as cardiovascular disorders, atherosclerosis, myocardial infarction, stroke, minimal cognitive impairment, dementia, Parkinson's disease, multiple sclerosis, epilepsy, and eclampsia.

In this case control study, we observed a strong correlation of Hyperhomocysteinemia with ischemic stroke in both younger and older age group. These results are consistent with many case control and prospective study. 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, p<0.001).

In our study association between elevated homocysteine and raised NIHSS scores in stroke subjects have been postulated which may be due to acute vascular events themselves. Those patients who were having past history of Diabetes, Hypertension, Smoking have also shown significantly raised level of plasma homocysteine as compared to control group (p<0.01).

Our study observed a strong link between Hyperhomocysteinemia and ischemic stroke. The findings are supported by recent study, which showed that Hyperhomocysteinemia was found in 48% of ischemic stroke patients [16]. However, some researchers have failed to determine any link between Hyperhomocysteinemia and stroke [24]. Similarly inconsistent results have been found as far as correlation between homocysteine and diabetes mellitus is concerned [18-19].

Some of the workers could not find association between homocysteine and lipid profile while others reported a decline correlation. In present study positive correlation between total cholesterol, LDL- Cholesterol, TG with homocysteine levels were found, which was statistically non- significant in stroke patients. In contrast to the west Indian studies examining the

prevalence of Hyperhomocysteinemia in the community have reported a much higher incidence of 52-84% [20-22]. The mean homocysteine levels too are quiet high, varying from 19.5% to 23.2 µmol/^{L} [20-21]. Our study on Western Rajasthan subjects has also revealed very high mean homocysteine 34.79 micromole/litre in case group

In view of these high levels it is felt that Hyperhomocysteinemia can be considered to be important cardiovascular risk factors in Western Rajasthan population. Hyperhomocysteinemia is now recognized as an independent risk factor for atherosclerosis [23]. Homocysteine is an unstable amino acid, which undergoes auto oxidation to produce free oxygen radicals [24]. Hyperhomocysteinemia thus causes increased production of free oxygen radicals and oxidative stress.

Increased homocysteine level is an important risk factor for the development of ischemic stroke in all the populations especially in younger age group. Hypertension and smoking per se are important contributory factor for Hyperhomocysteinemia. Absolute values of high homocysteine level cannot be established on the basis of this study. A large normal Indian population needs to be screened to establish a definite "High" level of Homocysteine. It will be worthwhile to consider homocysteine level above 15µmol/L as significant in patients with stroke or secondary prevention and supplementation with folate and vitamin B_{12}.

CONCLUSIONS

Raised plasma homocysteine is significantly associated with ischemic stroke and treating hyper homocysteinemia may be an effective way of decreasing the risk of stroke. Physicians should remember raised homocysteine is a very common and important cardio-vascular 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.

Hyper homocysteine is also a risk factor for arterial and venous occlusion. Moreover it is earliest of the risk factor to modify. It is therefore recommended that Hcy estimation should be included as a routine laboratory test for persons with cerebro vascular risk factors and public should make aware of it.

As per guidelines of American stroke Association good dietary intake of foods rich in folic acid, vitaminB_6, vitamin B_{12} are recommended for primary prevention of stroke and supplemented multivitamins (folic acid 400 µgm to 1mg daily, B_{12} vitamin 400 µgm to 600 µgm daily and B_6 vitamin 2mg to 10 mg daily) are recommended for individuals with known cerebrovascular disease and elevated level of plasma homocysteine (ePhcy).

REFERENCES

1. Durward BR, Salisburg LG, Rowe PJ; the development of quantifiable function outcome measures for use in the evaluation of physiotherapy in stroke patients, Physiotherapy, 1988; 85(7): 370.
2. Schunkert H, Samani NJ; Elvated C Reactive Protein in Atherosclerosis – Chicken or Egg? N Eng. J Med 2008;Prevention of Stroke in the Chinese Population: Journal of Stroke aCerebrovascular Diseases 2011;20(5): 395–400
3. Pandian JD, Sudhan P; Stroke Epidemiology and Stroke Care Services in India. Journal ofStroke, 2013; 15(3) :128-134
4. Banerjee AK; Pathology of cerebrovascular disease. Neurology India, 2000; 48(4): 305–307.
5. Singh RB, Suh IL, Singh VP; Hypertension and stroke in Asia, prevalence, control and strategies in developing countries for prevention. J Human Hyper, 2010; 14(10/11): 749–763. 5.
6. Itrat A, Ahmed B, Khan M, Muhammad M; Risk factor profiles of South Asians with cerebrovascular disease Int J Stroke. 2011; 6(4):346-8.10.1111/j.1747-4949.2011.00622.
7. Walton J; Brain disease of nervous system, 10th edition, Oxford university press, New York, 1993; 216.
8. Qian Jia, MD, Liping Liu, MD, PhD, Yongjun Wang; Risk Factors and Prevention of Stroke in the Chinese Population: Journal of Stroke and Cerebrovascular Diseases 2011; 20(5): 395–400.
9. Baidarbhi Chakraborty, Gaurav Vishnoi; BinitaGoswami, Lipoprotein (a), Ferritin, and Albumin in Acute Phase Reaction Predicts Severity and Mortality of Acute Ischemic Stroke in North Indian Patients: Journal of Stroke and Cerebrovascular Diseases 2012.10.013, 10.1016.
10. Bertsch T, Mielke O, Höly S, Zimmer W, Casarin W; Homocysteine in cerebrovascular disease: an independent risk factor for subcortical vascular encephalopathy: ClinChem LabMed.2001; 39(8): 721-4
11. Clarke R, Dal L, Robinson K, Naughten E, Cahalane S, Fowler B, *et.al.;* Hyperhomocysteinemia: an independent risk factor for vascular disease. N Eng J Med 1991; 324(17):1149-55
12. Graham IM, Daly L, Refsum HM, *et.al.;* Plasmahomocysteine as a risk factor for vascular disease JAMA 1997;277:1775-81

13. Homocysteine and short term risk of myocardial infarction and stroke in the elderly: The Rotterdam study Arch Intern Med 1999; 159:38-44.
14. Welch G, Loscalzo J; Homocysteine and athero thrombotic Engl J Med 1998; 338:1042-50.
15. Murray DJ, Lopez AD; Mortality by cause for eight regions of the world: global burden of disease study. Lancet, 2012; 349(9061): 1269-1276.
16. Hassan A, Hunt B J, Michael O, D'souza RJSB, John, M, Hugh M. *et.al.;* Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. Brain: 2004: 127(1): 212-9.
17. Ho GY, Eikelboom JW, Hankey GJ, Wong CR, Tan SL, Chan JB *et.al.;* Methylene tetra hydro folate reductase polymorphisms and homocysteine lowering effect of vitamin therapy in Singaporean stroke patients. Stroke. 2006; 37(2):456-60.
18. Adunsky A, Weitzman A, Fleissig Y; The relation of plasma total homocysteine levels to prevent cardiovascular disease in older patients with ischemic stroke. Ageing (Milano) 2000; 12: 48–52.
19. Munishi MN, Stone A, Fink L; Hyperhomocysteinemia following a methionine load in patients with non-insulin dependent diabetes mellitus and macrovascular disease. Metabolism 1996; 45: 133–135.
20. Wadia RS, Edul NC, Bhagat S, Bandishti S, Kulkarni R, Sontakke S, *et.al.;* Hyperhomocysteinemia and Vitamin B12 Deficiency in Ischaemic Strokes in India. Ann Ind Acad Neurol 2004; 7(2): 387-92.
21. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Örning L *et.al.;*. Hyperhomocysteinemia and elevated methyl malonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr 2001; 74(2):233-41
22. Misra A, Vikram NK, Pandey RM, Dwivedi M, Ahmad FU, Luthra K *et.al.;* Hyperhomocysteinemia and low intakes of folic acid and vitamin B12 in urban North India. Eur J Nutr 2002; 41(2):68-77.
23. Welch G, Loscalzo J; Homocysteine and Atherothrombosis. NEJM 1998; 338:1042-50.
24. Aps N, Verma I, Kaur S, Narang A, Gupta S, Avasthi G; Homocysteine - risk factor for ischaemic stroke. Indian J Physiol Pharmacol 2009; 53 : 34-8