International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319 – 6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 7; July 2017; Page No. 4834-4838
DOI: http://dx.doi.org/10.24327/ijcar.2017.4838.0593

Research Article

A STUDY TO EXPLORE THE CORRELATION BETWEEN PLASMA HOMOCYSTEINE AND ISCHAEMIC STROKE AND MORTALITY OUTCOMES IN A TERTIARY CARE CENTRE IN EASTERN INDIA

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ARTICLE INFO

Article History:

Received 26th April, 2017
Received in revised form 14th May, 2017
Accepted 20th June, 2017
Published online 28th July, 2017

Key words:

Plasma, Homocysteine, Ischaemic, Stroke, Mortality

ABSTRACT

Ischaemic stroke is an important cause of mortality and morbidity. There are many conventional risk factors for ischaemic stroke like hypertension, diabetes mellitus and dyslipedemia. There are some nonconventional risk facters also for ischaemic stroke like plasma homocysteine.In this study we got a positive correlation between plasma homocysteine and ischaemic stroke and its mortality.

INTRODUCTION

Cerebrovascular accident or CVA or stroke is defined as abrupt onset of a neurological deficit that is attributable to a focal vascular cause of >24 hrs duration. Cerebral stroke resulting from the vascular lesion are of two main types-ischemia (with or without infarction) and haemorrhage.[1] Although numerous risk factors for stroke have been identified like age, sex, family history of stroke, hypertension, smoking, diabetes, hyperlipidemia, atrial fibrillation,etc,[2-8], only two third of all stroke can be attributed to known causal risk factor.[9,10] Large clinical trials of LDL-cholesterol lowering therapy reported adverse event up to 19% of patients, despite its powerful intervention. This observation has intensified the search for "new nonlipid" risk factors of Atherosclerotic vascular Disease.[11] During the last decade numerous studies has observed a strong correlation between hyperhomocystenemia and ischemic stroke independent of long recognized risk factors such as dyslipidemia, hypertension, smoking.[12,13] After coronary heart disease and all cancers, stroke is the third common cause of death in the world, causing about 4 million deaths in 1990, and three quarters of them in developing countries. Studies conducted across many regions of India indicate that stroke accounts for two percent of hospital registrations and 9 to 30 percent of neurological admissions [14,15]. India will face an enormous socio-economic burden to meet the costs of rehabilitation of stroke victims because the population is now surviving through peak years (age 55-65) of occurrence of stroke or cerebrovascular disease (CVD). Despite the advent of treatment of selected patients with acute ischemic stroke with tissue plasminogen activator and the promise of other experimental therapies, the best approach to reducing the burden of stroke remains identification of high-risk or stroke-prone individuals and specific interventions. [16,17]

In this study, we have tried to identify the common risk factors as well as homocysteine level and its correlation with the outcome regarding morbidity and mortality within the available resource settings.

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MATERIALS AND METHODS

The study population consisted of 50 cases and 50 controls selected from the patients admitted in the Department of Medicine in R.G.Kar Medical College during the period of one year-June 2015 to May 2016. Patients were selected by

adhering strictly to certain inclusion and exclusion criteria. It was a case-control study in which patients with ischaemic stroke were taken as cases and patients admitted for other diseases as controls after matching in age, sex and other confounding factors**.** Both male and female patients above 18 years of age presenting with clinical features of acute stroke within 3 days of onset of symptoms and confirmed by C.T.SCAN of brain as ischaemic in nature were included in the study. Patients with known ischemic heart disease & peripheral arterial disease, any disabling terminal illness, with history of head injury, known intracranial tumor, intracranial metastasis, or who were later diagnosed as metabolic encephalopathy were excluded from the study.

After, detailed history and clinical examination were performed along with fundoscopy, where needed CT scan of brain was advised and fasting blood samples were collected from the selected patients and analysed for lipid profile and plasma homocysteine. Samples for homocysteine estimation were collected in clot activator vials (containing a gel) and centrifuged within 30 mins to separate the serum. The quantification of Homocysteine was achieved by chemiluminescence immunoassay. All the parameters of the fasting lipid profile estimation was done by the XL 600 systems using the principle of homogenous enzymatic colorimetric method.

RESULTS AND ANALYSIS

Table 1 Distribution of Plasma Homocysteine Level among Cases and Controls and Their Percentage

Plasma Homocysteine Level (µmol/L)	≤15	15-30	>30
No. of Cases(n=50)	17(34%)	16(32%)	17(34%)
No. of Controls(n=50)	41(82%)	8(16%)	1(2%)

The above table shows that 34% of cases and only 2% of controls had plasma homocysteine level >15 µmol/l which is the cut off value for normals.

Table 2 Comparison of Mean Homocysteine Level in Cases Based on Vascular Territory Involved (small vessel vs large vessel)

Vascular Territory Involved	Mean Homocysteine µmol/L
Small Vessel Stroke	20.28
Large Vessel Stroke	27.07

Table 3 Correlation of Plasma Homocysteine Level in Cases with Their Outcome

Outcome	Plasma Homocysteine Level(µmol/l) ≤15	Plasma Homocysteine Level(µmol/l) 15-30	Plasma Homocysteine Level(µmol/l) >30
Stable At 1 Month(n=27)	17(62.96%)	10(37.04%)	0
Expired In Hospital(n=15)	**0**	1(6.67%)	14(93.33%)
Expired At 15 Days(n=5)	**0**	2(40%)	3(60%)
Expired At 1 Month(n=3)	**0**	2(66.67%)	1(33.33%)
Total(n=50)	17(34%)	15(30%)	18(36%)

This table depicts the fact that increased homocysteine level negatively influenced the outcome of the cases.

Table 4 Correlation of Plasma Homocysteine Level With Serum Cholesterol Level in Cases

Cholesterol level	Plasma Homocysteine Level(µmol/l) <15	Plasma Homocysteine Level(µmol/l) 15-30	Plasma Homocysteine Level(µmol/l) 15-30
< 200 mg/dl(n=18)	6	**7**	**5**
200-239 mg/dl(n=9)	3	**1**	**5**
>239 mg/dl (n=23)	8	**8**	**7**

Correlation of plasma homocysteine level with serum cholesterol level in cases shows out of 18 cases who had serum cholesterol level less than 200 mg/dl, 6, 7 and 5 cases had plasma homocysteine level <15, 15-30, >30 µmol/l respectively. Out of 9 cases that had serum cholesterol level of 200-239 mg/dl, 3, 1 and 5 cases had plasma homocysteine level <15, 15-30 and >30 µmol/l respectively. Out of 23 cases who had serum cholesterol level more than 239 mg/dl, 8, 8 and 7 cases had plasma homocysteine level <15, 15-30, >30 µmol/l respectively.

Table 5 Correlation of Plasma Homocysteine Level With Serum Triglyceride Level in Cases

Triglyceride(mg/dl)	Plasma Homocysteine Level(µmol/l) <15	Plasma Homocysteine Level(µmol/l) 15-30	Plasma Homocysteine Level(µmol/l) >30
<150 (n=5)	1	3	1
150-200 (n=14)	4	5	5
>200 (n=31)	12	8	11
Total(n=50)	17(34%)	16(32%)	17(34%)

Correlation of plasma homocysteine level with serum TG level in cases. It shows out of 5 cases who had serum TG level less than 150 mg/dl, 1, 3 and 1 cases had plasma homocysteine level <15, 15-30, >30 µmol/l respectively. Out of 14 cases who had serum TG level of 150-200 mg/dl, 4, 5 and 5 cases had plasma homocysteine level <15, 15-30, >30 µmol/l respectively. Out of 31 cases who had serum TG level more than 200 mg/dl, 12, 8 and 11 cases had plasma homocysteine level <15, 15-30, >30 µmol/l respectively.

Table 6 Correlations of Plasma Homocysteine Level in Cases and Controls (t-Test: Two-Sample Assuming Equal Variances)

	Cases	Control
Mean	25.7146	12.5606
Variance	130.7309	5.012108
Standard Deviation	11.43	2.24
Observations	50	50
Df	98	
P(T<=t) two-tail	**2.75×10^{-7} ***	
* = Statistically significant(p < .001)		

The mean plasma homocysteine level among cases was 25.71±11.43µmol/l whereas among controls the mean homocysteine level was 12.56±2.24 µmol/l, which is statistically significant (p value < 0.001).

DISCUSSION

Potential new risk factors for stroke include genetic factors (like Factor V Leiden, Phosphodiesterase 4D); inflammatory markers e.g, Interleukins (IL-6, IL-18), vascular and cellular adhesion molecules; biomarkers of haemostasis/

thrombosis/impaired fibrinolysis (viz. Fibrinogen, Factors V, VII, and VIII, D-dimer, Antiphospholipid antibodies.); platelet parameters like size and volume, activity and aggregability; lipid-related factors like small, dense LDL, lipoprotein (a), remnant lipoproteins, HDL subtypes, lipoprotein-associated phospholipase A2, and adiponectin; and certain other factors like Homocysteine, metabolic syndrome, Oxidative stress by oxidized LDL and functional markers like B-type natriuretic peptide, microalbuminuria, cystatin C. Of these, there has been much interest in Homocysteine, as an important risk factor of vascular disease including stroke, independent of long recognized factors such as dyslipidemia, hypertension, smoking.[12]

Homocysteine is the demethylated product of the dietary amino acid methionine. It readily oxidized in plasma to the disulfide homocysteine and to homocysteine-cysteine mixed disulfide. All three chemicals occur normally in plasma in both the free and protein-bound forms and are collectively referred to as homocysteine. Brain infarction, carotid artery occlusion, and premature arteriosclerosis are well-recognized complications of marked hyperhomocystenemia. Several effects of homocysteine may contribute to its role in vascular disease. The reference levels of fasting levels are13-18 µmol/l for serum and 10-15µmol/l for plasma. [18]. Homocysteine promotes endothelial dysfunction (19), endothelial cell injury (20, 21), and proliferation of smooth-muscle cells (22). In addition, it enhances thromboxane α2 formation and platelet aggregation (23), reduces the protective effect of endothelium-derived relaxing factor (24), increases binding of lipoprotein (a) to fibrin (25), and has procoagulant effects. (26) The main cause of morbidity and mortality in homocysteinemic patients is progressive premature arteriosclerosis and associated thromboembolic complications. (27) Numerous studies support an association between elevated homocysteine levels and atherosclerotic disease.

In 1969, McCully, in a paper in the American Journal of Pathology, describing vascular pathology in patients suffering from homocystinuria, first proposed that Homocysteine causes atherosclerosis on the basis of the finding of atherosclerotic plaque at autopsies of young patients of homocystinuria.[28] This hypothesis was later modified to include a broader population, based on the surmise that mild hyperhomocystenemia caused by dietary deficiencies of the vitamin cofactors required for the metabolism of homocysteine, like folic acid, vitamin B_{12}, and vitamin B_6 is a risk factor for atherothrombosis.[29] In developed countries, these vitamins are partially removed from foods during processing and the staple diet is mainly animal proteins which are rich in the precursor amino acid methionine. These conditions result in elevated Homocysteine concentrations.

The salient points that emerge from the statistical analysis of this study are summarized below. Firstly, 34% of cases and only 2% of controls had plasma homocysteine level >30 µmol/l; i.e., greater number of patients of stroke had a higher plasma homocysteine level. It may also be noted here that the mean plasma homocysteine level among cases was higher (25.71±11.43µmol/l) than that among controls (12.56±2.24 µmol/l), which is statistically significant (p value < 0.001). Similar observations have also been found in other studies.[30]Secondly,a correlation analysis of the plasma homocysteine level in cases with their outcome, have depicted that increased homocysteine level negatively influenced the outcome of the cases. It was seen that those patients who were stable after one month of admission, 62.96% had plasma homocysteine level <15 µmol/l and 37.04% had plasma homocysteine level 15-30 µmol/l and none had plasma homocysteine level >30 µmol/l. 15 cases expired at hospital out of which 93.33% had plasma homocysteine level >30 µmol/l and only 6.67% had plasma homocysteine level 15-30 µmol/l and none <15 µmol/l. 5 cases expired at 15 days follow up, out of which 60% had plasma homocysteine level >30 µmol/l, 40% had plasma homocysteine level 15-30 µmol/l and none <15 µmol/l. 3 cases expired at one month follow up out of them 33.33% had plasma homocysteine level >30 µmol/l and 66.67% had had plasma homocysteine level 15-30 µmol/l and none had plasma homocysteine level <15 µmol/l. To the best of our knowledge, this is the first study dealing with a correlation analysis of mortality outcomes in CVA with plasma homocysteine levels in this part of the country.

In the Framingham Study, the relative risk for stroke, comparing the lowest quartile with the highest, was 1.82 (95% CI 1.14 to 2.91).[31] Although the association between plasma homocysteine and cerebrovascular risk is biologically plausible, it is more consistently present in case-control studies than in prospective studies, so further confirmatory evidence is required.(32,33) Folic acid and vit B_{12} has been shown to be effective in reducing elevated plasma homocysteine levels,[34] but randomized control trials have not been completed to determine whether reduction of homocysteine levels will eventually reduce the incidence of stroke. Secondary prevential trials are in progress.[35].

During the last 15 years it has also been thoroughly documented that moderately elevated homocysteine levels in serum or plasma is a strong and independent risk factor for occlusive arterial disease, venous thrombosis, and also predicts vascular and all-cause mortality. Almost 50% of patients with stroke and other atherothrombotic diseases have been documented high homocysteine levels, i.e., ≥15µ mol/litre. Apart from the rare inborn errors of metabolism of homocysteine caused by genetic defects in the enzymes involved in homocysteine metabolism, plasma homocysteine concentration also increases with elevations in creatinine and are typically elevated in chronic renal failure, often approaching concentrations that are up to four times the normal value. Although plasma homocysteine concentrations often decrease after dialysis,[36] it is unclear whether the elevation in homocysteine observed in end-stage renal disease is due to impaired metabolism or to reduced excretion. The presence of elevated plasma homocysteine concentrations may partially explain the observed acceleration of atherosclerosis in end-stage renal disease. Elevated homocysteine concentrations have been reported in association with several types of carcinoma, including breast, ovarian, and pancreatic cancer, acute lymphoblastic leukaemia .This is probably because, proliferating tumour cells may also be incapable of metabolizing endogenous homocysteine. Several drugs and toxins like methotrexate, phenytoin, theophylline may also increase plasma homocysteine concentrations.

CONCLUSION

To conclude, our findings suggest that moderately elevated fasting homocysteine level should be added to the established independent risk factors (hypertension, diabetes, smoking, history of previous vascular disease) for ischemic stroke and, more so, ischemic stroke caused by large-artery atherosclerotic vascular disease in particular. In our study, the plasma homocysteine level was significantly elevated among cases than in controls, which is statistically significant. All the cases with plasma homocysteine level >30 µmol/l had expired either in hospital or at follow up. Among the cases, the mean plasma homocysteine level in small vessel stroke was lower than large artery stroke. Prospective, randomized clinical trials in this part of the country, however, will be necessary to determine the efficacy of vitamin supplementation (namely, vitamin B_6 and b_{12}, and folic acid) on cerebrovascular morbidity and mortality.

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How to cite this article:

Pritam Kumar Chatterjee *et al* (2017) 'A Study to Explore the Correlation Between Plasma Homocysteine and Ischaemic Stroke and Mortality Outcomes in a Tertiary Care Centre in Eastern India', *International Journal of Current Advanced Research*, 06(07), pp. 4834-4838. DOI: http://dx.doi.org/10.24327/ijcar.2017.4838.0593

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