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Research Article

SERUM HOMOCYSTEINE AS A RISK FACTOR FOR STROKE: A PROSPECTIVE STUDY FROM A RURAL TERTIARY CARE CENTRE

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ABSTRACT

Objective: Stroke is one of the leading causes of mortality and long-term disability in both developed and developing countries. Serum homocysteine level is one of the emerging modifiable risk factors for atherosclerosis which may result into a cerebrovascular accident. This study was designed to study the association of Serum Homocysteine level with the development of acute stroke at a rural tertiary care centre in North India.

Methods: The present study was a prospective cross-sectional study conducted in the Department of Medicine, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala. The study population included 100 patients presenting with Stroke (either ischemic or hemorrhagic) in the indoor and outdoor facilities in the Department of Medicine. 50 age and sex-matched healthy individuals were taken as controls. Serum total Homocysteine level was measured in all the cases and controls.

Results: Majority of the patients suffered from ischemic stroke (78%), while only 22% patients had hemorrhagic stroke. The mean Serum Homocysteine level in stroke patients (19.88±8.78 µmol/l) was significantly higher than in controls (10.48±4.39 µmol/l) ($p<0.01$). In a subgroup analysis, stroke patients with a positive history of smoking had significantly higher homocysteine level as compared to non-smokers ($p<0.05$).

Conclusion: Increased level of Serum Homocysteine is significantly associated with risk of cerebrovascular accident, which is independent of the risk attributed to traditional risk factors.

Keywords: Homocysteine, Stroke, Ischemic, Hemorrhagic, Risk factors.

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INTRODUCTION

Vascular disease of the brain remains a very important cause of neurologic morbidity and mortality. Stroke is a cerebrovascular disorder resulting in the rapidly developing loss of brain functions. This can be due to ischemia or hemorrhage. Stroke is one of the leading causes of mortality and long-term disability in both developed and developing countries. There are various non-modifiable and modifiable risk factors for stroke [1]. Plasma homocysteine concentration is one of the emerging modifiable risk factors for atherosclerosis which may manifest as a cerebrovascular accident [2,3].

Homocysteine is a sulfhydryl amino acid that is readily oxidized to homocysteine and homocysteine-cysteine mixed disulfide in the plasma. Mechanisms by which hyperhomocysteinemia increases risk of cerebrovascular accidents are not clear, but several possible mechanisms have been proposed [4]. Hyperhomocysteinemia is associated with premature atherosclerosis. Experimental studies both *in vivo* and *in vitro* show that homocysteine causes endothelial injury and cell detachment. Endothelial cell injury, platelet activation, deleterious effect on thrombomodulin expression, protein C activation, and an increased oxidizability of low-density lipoprotein have been described as a few possible mechanisms by which homocysteine provokes arteriosclerosis and thrombosis [5,6]. Animal studies have also shown that raised Homocysteine levels may induce marked remodeling of the extracellular matrix in the arterial wall [7]. An ideal homocysteine level is <9 µmol/L. Hyperhomocysteinemia is the term used for elevated levels of homocysteine in plasma, i.e., >15 µmol/L.

India, with more than 1 billion inhabitants, is undergoing remarkable economic and demographic changes in recent years. This has resulted in a rapid increase in lifestyle related noncommunicable disorders including cardiovascular and cerebrovascular diseases. Given the anticipated increase in the burden of stroke in coming years and limited availability of organized stroke care services to the majority of people in India, it is important to place greater emphasis on population-based stroke prevention strategies. The present study is, therefore, undertaken to look for the association between Serum Homocysteine and cerebrovascular accidents.

METHODS

The present study was a prospective cross-sectional study conducted in the Department of Medicine, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala. The study population included 100 patients presenting with Stroke (either ischemic or hemorrhagic) in the indoor and outdoor facilities in the Department of Medicine. 50 age and sex-matched healthy individuals were taken as controls. The study was done in 2012–2013 after approval from the Ethical Committee of the institute. The total duration of the study was 1 year.

Inclusion criteria

The following criteria were included in this study:

1. Patient diagnosed with stroke
2. Age >18 years.

Stroke was defined as rapidly developing signs of a focal or global neurological deficit with no apparent cause other than vascular, documented by computed tomography (CT) or magnetic resonance imaging done within 24 h of admission.

Table 1: Comparison of baseline characteristics between cases and control groups

Parameter	Cases 100 (%)	Control 50 (%)	p value
Age (in years) (Mean±SD)	59.02±10.26	56.44±13.43	0.193
Gender (n, %)			
Males	68 (68)	32 (64)	0.624
Females	32 (32)	18 (36)	
BMI (in kg/m^2) (Mean±SD)	22.19±2.51	20.04±1.83	<0.05*
Stroke risk factors (n, %)			
Smoking	26 (26)	12 (24)	0.791
DM	29 (29)	6 (12)	0.022*
HT	48 (48)	8 (16)	<0.01**
Dyslipidemia	11 (11)	6 (12)	0.855
Obesity	18 (18)	5 (10)	0.067
Positive family history	8 (8)	2 (4)	0.422
SBP (mm Hg) (Mean±SD)	150.41±21.83	128.52±17.06	<0.01**
DBP (mm Hg) (Mean±SD)	89.22±11.60	79.6±9.45	<0.01**
Type of stroke (n, %)			
Ischemic	78 (78)		
Hemorrhagic	22 (22)		

(*p<0.05-significant, P<0.01 highly significant). BMI: Body mass index, DM: Diabetes mellitus, HT: Hypertension, SBP: Systolic blood pressure, DPB: Diastolic blood pressure

Table 2: Comparison of serum homocysteine level between cases and controls

Gender	Serum homocysteine level in µmol/l (Mean±SD)		p value
	Cases	Controls	
Overall	19.88±8.78	10.48±4.39	<0.01**
Males	19.94±8.91	10.56±4.41	<0.01**
Females	19.75±8.65	10.33±4.54	<0.01**

(*p<0.05-significant, P<0.01 highly significant)

Exclusion criteria

The following criteria were excluded from this study:

1. Previous history of ischemic or valvular heart disease
2. Peripheral vascular disease
3. Epilepsy
4. Renal impairment
5. Drugs that affect homocysteine, Vitamin B_{12}, and Folate metabolism, i.e., fibrates, statins and niacin, methotrexate, sulfasalazine, anticonvulsant drugs, levodopa, oral contraceptives, anticoagulant therapy, and
6. Pregnancy.

After fulfilling the inclusion and exclusion criteria, all the study subjects were explained about the study design in detail, and a written informed consent was taken from all cases as well as controls. Detailed clinical history (including present and past medical illness) and treatment history were noted. Baseline demographic data (age and sex) and conventional cardiovascular risk factors, including diabetes mellitus (DM), hypertension (HT), hyperlipidemia, smoking, and previous coronary diseases, were recorded for the patients and controls. Complete clinical examination including a detailed neurological assessment was done in all patients. All patients underwent routine laboratory investigations (Hemoglobin, random blood sugar, renal and liver function tests, and electrocardiogram) and also radiological investigations including CT scan as required.

Measurement of serum homocysteine level

Serum total homocysteine level was measured in all the study subjects using the standard technique. 2 ml of fasting blood sample were collected to obtain serum. Fasting total serum total homocysteine level was estimated by auto pure automated analyzer (marketed by Accurex) with a normal range of 5–15 µmol/l.

Statistical analysis

The statistical analysis was carried out using SPSS version 13-computer software. The data for continuous variables were expressed as Mean±SD., and categorical data were expressed as frequencies and percentages. p<0.05 was considered significant.

RESULTS AND OBSERVATIONS

A total of 100 cases of Stroke and 50 controls were evaluated in this study. The baseline demographic and clinical characteristics of cases and controls are shown in Table 1. There was no statistically significant difference in the two groups with regard to age and sex. The mean age in stroke patients and controls was 59.02 years and 56.44 years, respectively (p>0.05). Maximum number of cases of stroke were in the age group of 61–70 years (36%) followed by 51–60 years age group (23%). Majority of stroke patients were males (68%). Body mass index (BMI), systolic blood pressure, and diastolic blood pressure were significantly higher in stroke patients as compared to controls (p<0.05). Majority of the patients suffered from ischemic stroke (78%), while only 22% patients had a hemorrhagic stroke.

Table 2 shows the comparison of serum homocysteine level between cases and controls. The mean serum homocysteine level in stroke patients (19.88±8.78 µmol/l) was significantly higher than in controls (10.48±4.39 µmol/l) (p<0.01).

Table 3 shows the Serum Homocysteine level in stroke patients sub-grouped according to the presence of established risk factors of stroke. Serum Homocysteine level was significantly higher in stroke patients with a history of smoking as compared to non-smokers (p<0.05). No significant difference was seen in Serum Homocysteine levels with relation to other risk factors (HT, DM, BMI, and family history).

The comparison of Serum Homocysteine level in patients with ischemic and Hemorrhagic Stroke is shown in Table 4. Mean homocysteine levels in ischemic and hemorrhagic stroke were 20.27 and 19.76 µmol/l, respectively, which was statistically non-significant (p>0.05).

DISCUSSION

Stroke is a major health problem worldwide and is one of the leading causes of mortality and long-term disability. Ischemic stroke accounts for more than 80% patients of stroke worldwide [8]. In the present study, 78 patients had ischemic stroke while only 22 patients presented with Hemorrhagic Stroke. Primary and secondary prevention by managing the various modifiable risk factors for stroke is the key to reducing the incidence of stroke and its impact on health-care resources [9]. Hyperhomocysteinemia is one of the recently recognized modifiable factors that increase the risk of cardiovascular and cerebrovascular disease [4].

Table 3: Comparison in serum homocysteine levels in the patient group (n=100) with or without different stroke risk factors

Parameter	Number of patients "n"	Serum homocysteine level (in µmol/l) Mean±SD	p value
Age			
<60 years	41	19.23±8.25	0.383
>60 years	59	20.80±9.53	
HT			
Present	48	20.17±9.24	0.730
Absent	52	19.56±8.36	
Diabetes			
Present	29	19.27±8.58	0.476
Absent	71	20.64±8.76	
Smoking			
Present	26	23.23±9.91	0.023*
Absent	74	18.71±8.11	
BMI			
<25 kg/m^2	82	19.98±8.89	0.795
>25 kg/m^2	18	19.39±8.47	
Family history of stroke			
Present	8	19.75±8.68	0.968
Absent	92	19.62±8.94	

(*p<0.05-significant, *P*<0.01 highly significant). HT: Hypertension, BMI: Body mass index

Table 4: Comparison of serum homocysteine level between patients of ischemic and hemorrhagic stroke

Type of stroke	Number of patients "n"	Serum homocysteine level (in µmol/l) (Mean±SD)	p value
Ischemic stroke	78	20.47±9.74	0.235
Hemorrhagic stroke	22	17.36±7.44	

(*p<0.05-significant, P<0.01 highly significant)

In the present study, we evaluated the association of Serum total Homocysteine level with Stroke. Hyperhomocysteinemia is defined as serum homocysteine level >15 µmol/l. In our study, the proportion of stroke patients with hyperhomocysteinemia (44%) was significantly higher than that seen in controls (8%). The mean fasting Serum Homocysteine level in stroke patients (19.88±8.78 µmol/l) was significantly higher than the controls (10.48±4.39 µmol/l) (p<0.01). Furthermore, on subgroup analysis based on gender, significantly higher homocysteine levels were seen in male stroke patients as compared to control males (19.94±8.91 vs. 10.56±4.41, p<0.01) as well as in female stroke patients as compared to control females (19.75±8.65 vs. 10.33±4.54, p<0.01). Our findings are in agreement with results of previous studies done to evaluate the role of Homocysteine as a risk factor for stroke in different demographic populations [10-14]. In a nested case–control study done prospectively within the British Regional Heart Study cohort, Perry *et al*. evaluated the relation between serum total homocysteine level (tHcy) and risk of stroke in the middle-aged British men. Serum homocysteine was measured in 107 cases and 118 control men. tHcy concentrations were significantly higher in cases than controls (geometric mean 13.7 vs. 11.9; p=0.004). This association was found to be present even after adjustment for factors such as age group, town, social class, body mass index, hypertensive status, cigarette smoking, and diabetes. Their findings showed that Homocysteine is an independent predictor of cerebrovascular disease [10].

In a similar prospective case–control study in Japanese subjects 40–85 years of age, serum Homocysteine levels were compared between 150 patients of incident strokes and controls. Compared with control subjects, total (n=150), hemorrhagic (n=52), and ischemic (n=98) strokes had higher geometric mean values of total homocysteine. They concluded that high total Homocysteine levels were associated with the increased risk of stroke, more specifically ischemic stroke and lacunar infarction, among Japanese men and women [11]. In an Indian study, Narang *et al*. evaluated the role of homocysteine as a risk factor for ischemic stroke. Serum Homocysteine level was measured in 117 patients of ischemic stroke and 101 controls. The mean homocysteine levels in patients with ischemic stroke were 16.80 micromol/l while in controls it was 12.30 micromol/l which was statistically significant (p<0.01). The increased homocysteine levels in patients with ischemic stroke were found to be independent of factors such as presence of DM, age, and sex [12].

In a subgroup analysis of our data, we compared the Serum Homocysteine level between subgroups of stroke patients based on the presence or absence of Stroke risk factors. Our results showed that stroke patients with a positive history of smoking had significantly higher homocysteine level as compared to non-smokers (p<0.05). There was no significant difference in homocysteine level in stroke patients divided on the basis of other risk factors such as age, HT, DM, BMI, and Family History. Similar results were seen in a study done on Iranian stroke patients where homocysteine levels were shown to be significantly higher in smokers than the non-smokers [14].

In our study, 78 patients had ischemic stroke whereas 22 patients had a hemorrhagic stroke. The mean homocysteine level in both the subtypes was significantly higher than that in controls (p<0.05). On comparing the two subtypes of stroke, it was found that mean serum homocysteine level in ischemic stroke patients (20.47±9.74) was higher than that of hemorrhagic stroke patients (17.36±7.44), although this difference was not found to be statistically significant (p>0.05). Although the role of homocysteine in ischemic stroke has been established by many studies in the past, its role in Hemorrhagic Stroke is still uncertain. In a meta-analysis done to evaluate the association between elevated plasma homocysteine levels and the risk of different types of strokes, He *et al*. showed that although elevated Homocysteine levels are associated with an increased risk for ischemic strokes and recurrent strokes they showed no distinct association with hemorrhagic strokes [15]. On the contrary, in a recent systematic review and meta-analysis, Zhou *et al*. showed that Hcy level in patients of intracerebral bleed was significantly higher than that in healthy controls. They suggested that high homocysteine level may act by aggravating the process of atherosclerosis, which is positively associated with a high risk of intracranial hemorrhage [16].

CONCLUSION

Increased level of serum Homocysteine is significantly associated with risk of cerebrovascular accident, which is independent of the risk attributed to traditional risk factors.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally in design, data collection, manuscript preparation, and editing for this article.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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