

Original Article



Effect of Folic Acid on Homocysteine Level in Ischemic Stroke Patients in a Tertiary Level Hospital in Rajshahi.

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Abstract

Background: A number of risk factors are responsible for ischemic stroke some are modifiable and some are not modifiable. Homocysteine is a modifiable, independent risk factor of ischemic stroke. **Objective:** To evaluate the effectiveness of folic acid on homocysteine levels in ischemic stroke patients. **Materials and Methods:** This study was an experimental study carried out in the Department of Pharmacology and Therapeutics, Rajshahi Medical College in collaboration with the Department of Neurology, Rajshahi Medical College Hospital from July 2016 to June 2017. A total number of 90 ischemic stroke patients diagnosed by CT scan findings who came for treatment in the Neurology department of Rajshahi Medical College Hospital were enrolled in this study. Simple random sampling is done. The patients were divided into two groups: experimental group (45 patients) who were treated with 5mg folic acid along with other traditional treatment, control group (45 patients) who were treated by only traditional therapy given by the neurology department. Before starting treatment, along with other investigations, serum homocysteine level of both experimental and control groups was estimated. After three months of continuous treatment with folic acid in the experimental group, the serum homocysteine level of both groups was measured again. Data were analyzed using SPSS version 16 for windows. **Results:** The results showed that there was a small numerical difference in homocysteine level before and after treatment in the experimental and control group, yet the change was statistically significant in the experimental group after three months of treatment with 5mg folic acid. **Conclusion:** It is revealed that use of folic acid may play an important role in reducing homocysteine level in ischemic stroke patients.

Key words: Homocysteine, Folic acid, Ischemic stroke, Hyperhomocysteinemia.

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Introduction

Stroke is one of the leading causes of death and disability all over the world. The World Health Organization (WHO) defines stroke as rapidly developed clinical signs of focal disturbance of cerebral function lasting for more than 24 hours leading to death without any apparent cause other than vascular origin.¹ There are two main types of strokes: (i) ischemic stroke (85%) caused by primary cerebral ischemia resulting in infarction and (ii) haemorrhagic stroke (15%) caused by cerebral haemorrhage.² The incidence of stroke increases with age and

affects many people in their golden years. It is the third most common cause of death in developed countries. In Bangladesh, stroke is the third leading cause of death after coronary heart disease and infectious diseases such as influenza and pneumonia. WHO ranks mortality due to stroke in Bangladesh as number 84 in the world. The crude death rate per 1000 people in Bangladesh is reported at 5-8%.³

Risk factors for stroke include irreversible or non-modifiable factors like age, sex and modifiable factors like hypertension, heart disease, diabetes mellitus, hyperlipidemia, smoking,

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excess alcohol and oral contraceptive.⁴ Many studies showed the above mentioned risk factors are responsible for stroke. It is thought that raised concentrations of homocysteine in blood have been suggested to be a modifiable, independent risk factor for coronary artery disease, stroke and deep vein thrombosis.⁵

Homocysteine is a sulfhydryl containing non proteinogenic, non-essential amino acid synthesized by demethylation of a methionine, which is abundant in proteins.⁶ Remethylation of homocysteine is catalyzed by methionine synthase produces methionine by an addition of a methyl group from 5-methyltetrahydrofolate (5-MTHF), which is synthesized by 5,10- methylenetetrahydrofolate reductase (5-MTHFR). In this reaction, vitamin B12 in the form of methylcobalamin acts as a cofactor. Thus the capacity for Hcy metabolism is largely dependent on the supplies of folate and cobalamine. Therefore, folic acid and vitamin B12 deficiency can cause a reduction in methylenetetrahydrofolate reductase (MTHFR) activity, leading to a decrease in methionine synthesis and homocysteine accumulation.⁷⁻⁹

Folic acid is vital for the formation of red blood cells. The form of folic acid occurring naturally in food is folate. Folic acid 800 mcg may be a useful dietary supplement for individuals wishing to supplement their diet with this essential vitamin. Fasting total plasma homocysteine level may vary somewhat depending on laboratory methods but levels of 5-15 $\mu\text{mol/L}$ are usually considered normal.¹⁰ It is reported that men to have higher levels than women, and postmenopausal women have higher homocysteine values than premenopausal women. Homocysteine values will normally increase with age, giving a reference range among the elderly (>60 years) of 5-20 $\mu\text{mol/L}$.¹¹ The term "hyperhomocysteinemia" does not define a pathological condition. It is rather used to describe a biochemical abnormality that can be a direct consequence of various pathological conditions. Plasma homocysteine concentration could represent a gradual indicator of the risk for cardiovascular disease. Thus, every increase of 2.5 $\mu\text{mol/L}$ homocysteine in plasma can be associated with an increase of stroke risk of about 20%.¹² Multiple prospective and case-control studies suggested that moderately elevated serum homocysteine level is associated with atherothrombotic vascular disease. Homocysteine concentration is consistently higher in patients with peripheral, cerebrovascular and coronary artery disease than those without such diseases. Elevated plasma total homocysteine is associated with ischemic stroke risk.¹³

Homocysteine may cause vascular disease including propensity for thrombosis, impaired thrombolysis, increased production of hydrogen peroxide, endothelial dysfunction and increased oxidation of low density lipoprotein.¹⁴ Elevated homocysteine is involved in the pathogenesis of atherosclerosis, thromboembolism and vascular endothelial dysfunction and plays a role in the increased incidence of ischemic stroke.¹⁵⁻¹⁶ It is found that reducing total homocysteine by 3 $\mu\text{mol/L}$ is associated with 24% reduced risk of stroke (95% CI, 15%-35%) and 16% reduced risk of ischemic heart disease (95% CI, 11%-20%).¹⁷

Folic acid and vit-B12 are important regulators in the homocysteine metabolism and studies have shown inverse relationship between folic acid intake and homocysteine level. Folic acid, pyridoxine and cobalamin reduce plasma homocysteine levels and may help to reverse endothelial injury associated with elevated total homocysteine. Folic acid supplementation has also been associated with a reduction in carotid atherosclerosis progress.¹⁸

Raised concentrations of homocysteine in blood are a modifiable, independent risk factor for stroke. All these data raised the possibility that stroke is a disease end-point that could be particularly benefited from folic acid supplement. Stroke is one of the major causes of morbidity and mortality and these patients are increasing in our hospital day by day. It is a socio-economic challenge also. The prognosis of these patients is poor. Developing countries like Bangladesh, where the health support system including the rehabilitation system is not within the reach of ordinary people. In a developing country like ours, the best policy for combating stroke is primary prevention. Hyperhomocysteinemia is such a modifiable, independent risk factor of ischemic stroke. It is found that there is a correlation between stroke and serum homocysteine level. So it is thought, worthwhile to supplement folic acid which is very potential along with other traditional therapy in ischemic stroke by lowering serum homocysteine level. This study was intended for this purpose.

Materials and Methods

This study was an experimental study carried out in the Department of Pharmacology and Therapeutics, Rajshahi Medical College in collaboration with the Department of Neurology, Rajshahi Medical College Hospital from July 2016 to June 2017. A total number of 90 ischemic stroke patients diagnosed by CT scan findings who came for treatment in the Neurology department of Rajshahi Medical College Hospital were enrolled in this study. Sampling technique was simple random sampling. Permission was taken from the concerned departments and authorities. All patients were informed about the study and they were also informed that there was no chance of significant harm by inclusion in this study. Then written consent was taken before data collection. Ethical permission was taken from the Ethical Review Committee (ERC) of Rajshahi Medical College.

The patients were divided into two groups: experimental group (45 patients) who were treated by 5mg folic acid along with other traditional treatment, control group (45 patients) treated by only traditional therapy given by the neurology department. Ischemic stroke patients who fulfilled the inclusion criteria were included in this study. After taking informed consent information were collected by taking medical history and physical examination and subsequent laboratory investigations. Data was collected by preformed data sheet and by face to face interview from the patients or relatives of patients in the neurology ward of Rajshahi Medical College Hospital. Then a 4 ml venous blood sample was obtained into a test tube. The sample was immediately kept in an ice pack and later centrifuged within 30 minutes. Serum samples were then refrigerated and stored at -80°C till the analysis was done.

Before starting treatment patients were divided into two groups one was the experimental group and other was control group. Before treatment serum homocysteine was determined of all included patients. Then 5 mg folic acid was given only to the experimental group for three months and no folic acid was added to the control group. After three months serum homocysteine level was measured again of both the groups. After collection data were checked for inadequacy, irrelevancy and inconsistency. Irrelevant and inconsistent data were discarded. All data was recorded systematically in preformed data collection form. Values were given in terms of mean ± SD. The data was analyzed using SPSS version 16 for windows. Unpaired t-test was used to compare laboratory values between experimental group and control group. The changes in laboratory values after 3 months were analysed using paired t-test with the baseline values of their respective groups. Probability values less than 5% were considered significant.

Results

Table-I: Age distribution of the patients (n=90).

Age (years)	Group		P-value
	Experimental [n(%)]	Control [n(%)]	
≤30	5(11.11)	2(4.44)	
31-50	25(55.55)	17(37.78)	
>50	15(33.33)	26(57.78)	
Total	45(100)	45(100)	
Mean±SD	47.07±12.84	55.36±12.98	0.056

Unpaired t test was done to measure the level of significance.

Table I shows distribution of patients according to age. In the experimental group, two-third patients were ≤50 years old but in control group, more than half of the patients were >50 years old. Mean age of the experimental group and control group was 47.07±12.84 and 55.36±12.98 respectively. There was no statistically significant difference between the experimental and the control group (P >0.05).

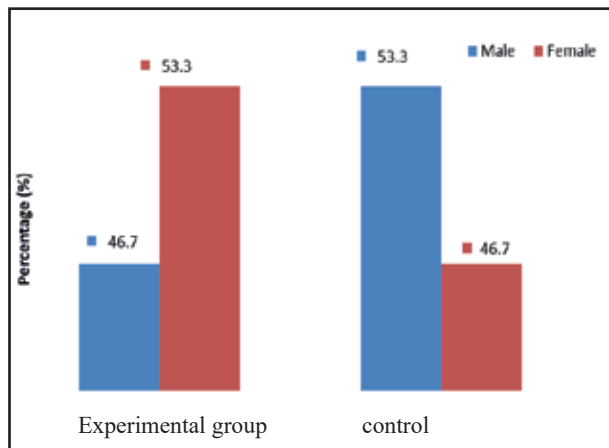


Figure 1: Bar diagram showing gender of the patients.

The above figure 1 shows the distribution of patients according to gender. In the experimental group, females (53.3%) were more than males (46.7%) but in the control group, males (53.3%) were more than females (46.7%) but there was not statistically significant difference between the two groups (P>0.05). Chi-square test was done to measure the level of significance.

Table-II: Homocysteine level before and after intervention in experimental and control group (n=90).

	Homocysteine (µmol/L)		p value
	Group		
	experimental (n=45) [mean±SD]	Control (n=45) [mean±SD]	
Before intervention	13.29 ± 7.62	12.96 ± 7.43	0.837
After intervention	12.18 ± 7.35	12.95 ± 7.35	0.658
P value (within groups)	<0.001	0.028	

Unpaired t test was done between groups and paired t test was done within groups to measure the level of significance.

Table II shows a comparison of homocysteine levels between experimental and control groups before and after the intervention. The change of homocysteine level was significantly reduced in the experimental group after intervention.

Table III: Distribution of risk factors of the patients (n=90).

Risk factors	Group		p value
	Experimental [n(%)]	Control [n(%)]	
History of smoking	12 (26.7)	13 (28.9)	0.814
History of diabetes mellitus	9 (20.0)	10 (22.2)	0.796
History of hypertension	34 (75.6)	35(77.8)	0.803

Chi-square test was done to measure the level of significance.

Table III shows distribution of patients according to risk factors. There were no significant difference between two groups in history of smoking, diabetes mellitus and hypertension.

Discussion

Stroke is a serious neurological disorder. It is one of the main causes of morbidity and mortality around the world. About 85% of strokes are ischemic strokes. Hyperhomocysteinemia is one of the modifiable risk factors that can easily, safely,

effectively and affordably be reduced.¹⁹ Many case control and cohort studies have reported that there is a strong, independent and dose related relationship between homocysteine level and atherosclerotic vascular disease, including ischemic stroke.²⁰⁻²³

The present study allowed us to get important information about the role of folic acid on homocysteine level in ischemic stroke patients. In the present study 90 ischemic stroke patients were enrolled. Of them 45 patients were in the experimental group who were given folic acid and 45 patients were in the control group without supplementation of folic acid. Baseline serum homocysteine level of both the groups was measured before starting treatment. Then 5mg folic acid was given to the experimental group for three months and serum homocysteine level was measured again after the treatment. Serum homocysteine level of the control group was also measured after 3 months. Baseline serum homocysteine level was 13.29 ± 7.62 and 12.96 ± 7.43 $\mu\text{mol/L}$ in experimental and control group respectively. After 3 months serum homocysteine level was 12.18 ± 7.35 and 12.95 ± 7.35 in the experimental and control group respectively. The changes of homocysteine level was statistically significant in the experimental group before and after treatment than the control ($p < 0.05$). This study showed that folic acid supplementation reduced plasma homocysteine level and suggested its role in remethylation process thus reducing homocysteine level in ischemic stroke. The results of the present study coincides with the study result of Mohapatra and Sarany (2010) in which 67 ischemic stroke patients were given 5mg folic acid daily for 9 weeks.²⁴ The reduction of plasma homocysteine when compared with their respective baseline values were found to be statistically significant ($p < 0.05$).

In a meta analysis (Homocysteine Studies Collaboration, 2000) shows that by folic acid supplementation a 25% lower homocysteine level was associated with 11% lower ischemic heart disease risk and 19% lower stroke risk.²⁵ Joacob et al (1994) showed an inverse relationship between folate intake and homocysteine level.²⁶ Till et al (2005) suggested that folic acid supplementation has also been associated with a reduction in carotid atherosclerosis progression.¹⁷ Homocysteine has an important role in atherosclerosis. These observations might suggest that folic acid supplementation holds promise as a potential therapy for stroke prevention.

Homocysteine Lowering Trialists' Collaboration (1998) in a meta-analysis showed that daily supplementation with 0.5-5mg folic acid was associated with reductions in plasma homocysteine concentrations of 25%, but the effects of lower daily doses of folic acid could not be investigated.²⁷ Brattstrom et al (1994) found that daily 10mg folic acid supplementation reduced mean serum homocysteine concentration by 4.5 ± 3.5 $\mu\text{mol/L}$.²⁸

The findings of a meta-analysis from the Homocysteine Lowering Trialist' Collaboration (1998) suggest that in North America additional folic acid supplementation (0.8 mg/day) would likely to lower plasma homocysteine concentrations by considerably less than 25% reduction observed in populations in which the baseline homocysteine concentrations are higher.

The overall results and discussion came to a conclusion that supplementation of folic acid have an important beneficial role on serum homocysteine level and thereby reducing risk factor of ischemic stroke.

As folic acid therapy is an inexpensive, safe, simple and widely applicable intervention, it will be helpful for our country on lowering homocysteine level in ischemic stroke patients.

Conclusion

In vascular diseases like ischemic stroke hyperhomocysteinemia is considered as one of the risk factors. It is modifiable and it was found that plasma homocysteine level can be reduced by supplementation of folic acid. A significant fall in plasma homocysteine level was seen in our experimental group of study population. Folic acid supplementation will be important therapy in ischemic stroke patients because it lowers the homocysteine level.

To efficiently assess the efficacy and causal relationship of folic acid supplementation on ischemic stroke, future clinical trials should be done in regions, without grain fortification, with a longer period of follow up (4 years or longer).

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