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RISK FACTORS AND BIOCHEMICAL VARIATIONS IN PATIENTS WITH ISCHEMIC STROKE

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ABSTRACT

There has been much interest in homocysteine (Hcy) as an important risk factor for vascular diseases including stroke, independent of the long-recognized factors like hyperlipidemia, hypertension, diabetes mellitus, and smoking, although its association was described many decades ago. During the last decade, numerous studies observed a strong positive correlation between hyperhomo-cysteinemia and ischemic stroke, while others could not establish the same. The present study was, hence, planned to explore an association between Hcy levels in Pakistani patients with ischemic stroke in view of the limited data available, so that some practical recommendation for screening and treatment of this modifiable risk factor could be provided. The patients were categorized into several groups on the basis of the major risk factors involved. Hypertension , diabetes, high cholesterol, cigarette smoking, atrial fibrillation, and previous stroke or TIAs were the major risk factors in these patients. The biochemical changes studied in several groups of stroke patients were homocysteine (Hcy;µmol/g), albumin(g/dL), hemoglobin (Hb;g/dL), and total cholesterol (mg/dL). No significant change was obtained in most of the comparisons except significant variation of homocysteine level ($p<0.05$) in stroke patients with previous stroke and TIAs. Determination of levels of Hcy in patients with ischemic stroke and age- and sex-matched controls provide us knowledge about whether Hcy vary at different time periods following stroke. This investigation, hence, might be helpful in assessing the association of Hcy with stroke severity, outcome, recurrence, etiology, infarct volume, or risk factors.

Key-words:

INTRODUCTION

Stroke is the third leading cause of death (Donnan *et al*.,2008*)* and the leading cause of adult disability (Stineman *et al*.,1997)**.** The high incidence and high prevalence of stroke have a major impact on society (Stineman *et al*.,1997). Stroke is a sudden disruption in blood flow to the brain caused by a blockage or bleeding of a blood vessel. Areas of the brain that are affected by the blockage or bleeding can become damaged within minutes. This phenomenon can be caused by thrombosis, embolism, or hemorrhage (Robbins and Cotran *et al*.,2005). Stroke is one of the foremost causes of morbidity and mortality throughout the world, posing a major socio-economic challenge in the occupational and neuro-rehabilitational programs for 'stroke-survivors.'

The stroke may be mild or severe and temporary or permanent, depending on which brain cells are damaged, how much of the brain is involved, and how quickly the blood supply is restored to the area. Symptoms of a stroke are usually sudden and may include: Numbness, weakness, or lack of movement (paralysis) in the face, an arm, or a leg, especially on only one side of the body, trouble seeing in one or both eyes, confusion and difficulty speaking, dizziness and a loss of balance or coordination, vomiting, a sudden, severe headache. A person with stroke symptoms needs immediate medical attention to help limit potential damage.

In an ischemic stroke, which is the cause of approximately 80% of strokes, a blood vessel becomes occluded and the blood supply to part of the brain is totally or partially blocked. Ischemic stroke is commonly divided into thrombotic stroke, embolic stroke, systemic hypoperfusion (Watershed or border Zone stroke), or venous thrombosis.

Numerous risk factors for stroke have been identified and modification of these factors is the crux of primary and secondary prevention (Dallas. American Heart Association, 2000.). Despite recent advances, only two-third of all strokes can be attributed to known causal risk factors **(**Whisnat *et al*., 1997).

Large clinical trials of LDL cholesterol-lowering therapy reported adverse events in up to 19% of patients, despite this powerful intervention. This observation has intensified the search for 'new nonlipid' risk factors for atherosclerotic vascular disease (ASVD) (Stein & McBride *et al*.,1998).

Homocysteine appears as a nerve and vessel toxin, promoting mortality, cardiovascular disease (CVD), stroke, Alzheimer's Disease, birth defects, recurrent pregnancy loss, and eye disorders. Homocysteine (Hcy) is an amino acid that is produced by the body, usually as a byproduct of consuming meat. It is a thiol aminoacid synthesized during the metabolism of methionine. Increased plasma levels of Hcy can be the result of mutations in the enzymes responsible for Hcy metabolism, particularly cystathionine-beta synthase (CBS) and 5,10-methylenetetrahydrofolate reductase (MTHFR). Furthermore, nutritional deficiencies in B vitamin cofactors required for Hcy metabolism,

including folic acid, vitamin B6 (pyridoxal phosphate), and/or vitamin B12 (methylcobalamin), can induce hyperhomocysteinemia (Pezzini *et al*., 2007).

Over the last decade, following in vitro and in vivo observations of a Hcy-associated vascular pathology, convincing epidemiological evidence has been gathered on the relation between moderate elevation of plasma Hcy and vascular disease, including cerebral ischemia. However, causality has yet to be established. The association between Hcy and ischemic stroke might be a spurious epidemiological finding because of confounding or it might reflect reverse causality. If this is the case, elevated levels of plasma Hcy should be interpreted as an epiphenomenon secondary to the vascular disease itself. Thus, whether lowering Hcy concentration prevents cerebral ischemia remains to be determined (Pezzini *et al*., 2007).

The only method to answer the question of the causal relation between Hcy and ischemic stroke is by intervention trials in which patients at high vascular risk, such as those who have had a recent cerebral ischemic event are randomly allocated. Some of these randomized controlled trials are currently ongoing (Pezzini *et al*., 2007). Their results should hopefully resolve the issue in the next future (Pezzini *et al*., 2007).

High concentrations of Hcy have been linked to increased risk of coronary heart disease, arteriosclerosis of blood vessels in the neck and legs, and to stroke, and it has been proposed that stroke risk might be reduced by lowering Hcy levels (Hankey *et al*., 2006).

Wang *et al*. **(**2006) studied correlation between plasma level of Hcy and cerebral large-artery atherosclerosis and found that the effect of Hcy is more pronounced in the presence of other risk factors of ischemic stroke. The Hcy elevation is associated with a two- to threefold fold increased risk of ischemic stroke. Although most commonly associated with large-artery atherosclerosis and venous thrombosis, hyperhomocysteinemia may contribute to stroke by other mechanisms as well. Levels of Hcy are determined by genetic regulation of the enzymes involved in Hcy metabolism associated with those reactions (Furie & Kelly *et al*.,2006).

Emerging evidence suggests that genetic variation within this pathway, such as the methyleneterahydrofolate reductase and cystathionine beta-synthase and nicotinamide N-methyltransferase genes, increase the risk of ischemic stroke (Furie & Kelly *et al*.,2006).

There has been much interest in Hcy as an important risk factor for vascular diseases including stroke, independent of the long-recognized factors like hyperlipidemia, hypertension, diabetes mellitus, and smoking, (Arrastia *et al*.,2000) although its association was described many decades ago (Mc Kully *et al*.,1969).

During the last decade, numerous studies observed a strong positive correlation between hyperhomo-cysteinemia and ischemic stroke **(**Graham *et al*.,1997 ; Perry *et al*.,1995) while others could not establish the same**(**Verhoef *et al*.,1994 ; Alfthan *et al*.,1994**)**. The present study , hence, explores an association between Hcy levels in Pakistani patients with ischemic stroke , and provides an evidence that some practical recommendation for screening and treatment of this modifiable risk factor could be provided.

METHODS AND MATERIALS

It was planned to estimate plasma homocysteine levels. But the grant was not enough to see the role of homocysteine in various groups of patients with stroke compared with the normal subjects. However, only specific groups of patients were only studied for Hcy. Total plasma Hcy was determined by enzyme linked immunosorbent assay (ELISA), and the involvement of homocysteine in patients with stroke was studied in other groups.

The patients were selected from those attending the Department of Neurology, Jinnah Postgraduate Medical Centre (JPMC), Karachi, and Department of Neurology, KMDC Abbasi Shaheed Hospital, Karachi. The patients of ischemic stroke with multiple established risk factors such as diabetes, hypertension, and smoking in combination were excluded in the present study. The controls were selected from amongst age- and sex-matched healthy volunteers, patients with unrelated complaints and their relatives after informed consent. The cases and controls were subdivided into the groups according to age and sex.

In the present study, ischemic stroke is defined as a stroke with either a normal CT brain scan or with an evidence of a recent infarct in the clinically relevant area of the brain on a CT or MRI brain scan performed within 3 weeks of the event. On the basis of clinical evaluation imaging and other investigations, cases were included in the present study, as per predefined criteria. All patients with recent (3 months) major systemic illness, including myocardial infarction, hepatic disease, renal disease, thyroid disease, cardiomyopathy, pregnancy, patients on drugs causing rise in Hcy levels such as anticonvulsant medication, evidence of nonatherothrombotic vascular disease, namely vasculitides, fibromuscular dysplasia, or dissection were excluded from the present study.

Detailed history, including history of hypertension, diabetes, TIAs, coronary artery disease, smoking, alcohol intake, and drug history were recorded in all individuals. Respective patients got CT scan/MRI of head, Doppler analysis of neck vessels, transthoracic echocardiography, detailed lipid profile along with routine biochemistry, and

hemogram. Transoesophageal echocardiography was done in select group of patients of young stroke.. Five milliliters of blood was drawn and collected in a tube. The sample was immediately kept in ice pack and later centrifuged within 30 min to avoid false elevation of Hcy levels due to its release from RBC. Plasma samples was then refrigerated and stored at -80°C till the analysis done.

Regarding ethical consideration, venepuncture is a minimally invasive procedure used frequently as part of the investigation procedure in patients with stroke. The patients was informed about the study and the benefit thereof. After taking consent, 5 ml of blood was withdrawn. Descriptive values were expressed as mean ± SD or SEM. Further analysis was done using students ' *t* ' test. ' *p* ' value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The patients were categorized into several groups on the basis of the major risk factors involved (Table 1). Hypertension , diabetes, high cholesterol, cigarette smoking, atrial fibrillation, and previous stroke or TIAs were the major risk factors in these patients.

Table 1. Categorization of the stroke patients with major risk factors

	Normal	Hypertension	Diabetes	High Cholesterol	Cigarette Smoking	Atrial Fibrillation	Previous stroke or TIAs
Age Range(Y)	40-70	45-68	42-68	45-69	43-67	41-65	40-68
Male	7(50%)	7(54%)	6(43%)	7(50%)	8(50%)	9(56%)	7(50%)
Female	7(50%)	6(46%)	8(57%)	7(50%)	8(50%)	7(44%)	7(50%)

The biochemical changes studied in several groups of stroke patients were homocysteine (Hcy;µmol/g), albumin(g/dL), hemoglobin (Hb;g/dL), and total cholesterol (mg/dL). No significant change was obtained in most of the comparisons except significant variation of homocysteine level ($p<0.05$) in stroke patients with previous stroke and TIAs (Table 2).

It still remains to know whether lowering Hcy concentration prevents cerebral ischemia remains to be determined (Pezzini *et al*., 2007).

Table 2. Biochemical variations in stroke patients with major risk factors

	Normal (14)	Hypertension (13)	Diabetes (14)	High Cholesterol (14)	Cigarette Smoking (16)	Atrial Fibrillation (16)	Previous Stroke or TIAs (14)
Hcy(µmol/g)	9.63±3.2	10.32 ±2.88	9.88±2.91	10.22±2.92	9.87±3.1	9.99±2.87	14.59±3.12
Albumin(g/dL)	4.2 ±0.6	4.4±0.7	4.1±0.5	4.3±0.6	4.2±0.7	4.1 ±0.6	4.2±0.7
Hb(g/dL)	13.23±1.9	14.93±2.3	15.14±2.5	14.93±1.9	15.29±2.1	15.11±2.2	14.95±1.89
Total Cholesterol (mg/dL)	180±43	212±58	209±53	230±59	213±52	199±48	213±49

Some of the randomized controlled trials are currently ongoing (Pezzini *et al*., 2007) and present similar results as presented here. Their results should hopefully resolve the issue in the next future (Pezzini *et al*., 2007). High concentrations of Hcy have been linked to increased risk of coronary heart disease, arteriosclerosis of blood vessels in the neck and legs, and to stroke, and it has been proposed that stroke risk might be reduced by lowering Hcy levels (Hankey *et al*., 2006) . Similar suggestions can be given considering the current report of study.

Our results resemble to the findings of Wang *et al* **(**Wang *et al*.,2006) who studied correlation between plasma level of Hcy and cerebral large-artery atherosclerosis and found that the effect of Hcy is more pronounced in the presence of other risk factors of ischemic stroke. The Hcy elevation is associated with a two- to threefold fold increased risk of ischemic stroke. Although most commonly associated with large-artery atherosclerosis and venous thrombosis, hyperhomocysteinemia may contribute to stroke by other mechanisms as well. Levels of Hcy are determined by genetic regulation of the enzymes involved in Hcy metabolism associated with those reactions (Furie & Kelly *et al*.,2006).

Emerging evidence suggests that genetic variation within this pathway, such as the methyleneterahydrofolate reductase and cystathionine beta-synthase and nicotinamide N-methyltransferase genes, increase the risk of ischemic stroke (Furie & Kelly *et al*.,2006).

It is possible that free homocysteine is more harmful than protein-bound homocysteine (Chambers *et al*.,2000). At this time, few studies on homocysteine and disease have distinguished between the two. Eventually, the research may focus on free homocysteine, although the treatment will probably be the same.

There has been much interest in Hcy as an important risk factor for vascular diseases including stroke, independent of the long-recognized factors like hyperlipidemia, hypertension, diabetes mellitus, and smoking **(**Arrastia *et al*.,2000) although its association was described many decades ago (Mc Kully *et al*.,1969**).** During the last decade, numerous studies observed a strong positive correlation between hyperhomo-cysteinemia and ischemic stroke**(**Graham *et al*.,1997 ; Perry *et al*.,1995). While others could not establish the same**(**Verhoef *et al*.,1994 ; Alfthan *et al*.,1994). The present study hence, explores an association between Hcy levels in Pakistani patients with ischemic stroke in view of the limited data available, so that some practical recommendation for screening and treatment of this modifiable risk factor could be provided.

Determination of levels of Hcy in patients with ischemic stroke may provide us knowledge about whether Hcy vary at different time periods following stroke. This investigation might be helpful in assessing the association of Hcy with stroke severity, outcome, recurrence, etiology, infarct volume, or risk factors. On the basis of the present work, future studies can be carried out to understand whether Hcy levels are correlated with hemostatic factors or C-reactive protein values.

If the effect of Hcy is more pronounced in the presence of specific risk factors of ischemic stroke, it may provide us clues about the association of Hcy and that specific risk factor/ factors. This evidence of the involvement of Hcy with stroke in specific conditions may leads to a better way to manage the specific patients with cerebral ischemia. Although the normal range for Hcy level is already known, a large number of studies have documented increased risk of vascular disease within this range. Hence, the present report explains the sensitivity levels of Hcy according to underlying discomfort and risk factors.

Hyperhomocysteinemia has a multifactorial origin incorporating genetic, nutritional, pharmacological, and pathological factors. Considering the differences in dietary, genetic, and ethnic factors, the data published from the West may not be applicable to our population. It is difficult to propose a definite cut off value for Hcy levels to be taken as significant and would require a larger population analysis. Hence, the results of present investigation provides newer and potential information for further insights.

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