ASSESSMENT OF HOMOCYSTEINE LEVELS IN PATIENTS WITH STROKE IN YOUNG

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ABSTRACT

Objective: To determine the frequency of raised levels of homocysteine and associated socio-demographic factors among the young patients presenting with stroke at the neurology department of a tertiary care hospital of Pakistan.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Pak Emirates Military Hospital Rawalpindi, from May 2017 to May 2018.

Methodology: The sample population comprised of young patients (18-44 years) presenting with the stroke at the emergency department of Military Hospital Rawalpindi. CT-scan brain was done and a consultant neurologist was consulted to confirm the stroke and its type. Fasting homocysteine level was done with in twenty four hours of the acute presentation at the hospital. Age, gender, body mass index, type of stroke, smoking, family history of stroke and hyperlipidemeia were correlated with the presence of raised homocysteine levels among these patients of stroke in young.

Results: Out of 102 young patients confirmed with acute stroke on CT scan at the neurology department, 57 had presence of hyperhomocysteinemia while 45 patients had normal homocysteine levels. Mean age of the study participants was 30.66 ± 3.445. High body mass index and tobacco smoking had a significant relationship with the presence of raised homocysteine levels among these patients of stroke in young when binary logistic regression was applied.

Conclusion: Raised homocysteine levels should be considered a possibility while evaluating the patients of stroke especially those who are young.

Keywords: Homocysteine levels, Risk factors, Stroke in young.

INTRODUCTION

Developing country like Pakistan bears a lot of burden of non-communicable diseases[1]. Cerebro-vascualr accident commonly known as stroke hits both the developed and developing countries and makes a big chunk of these non-communicable diseases[2,3]. Stroke have been associated with many biochemical abnormalities including hyperlipidemia, hyperglycemia, hyperhomocysteinemia and altered vitamin B-12 levels[4,5].

Stroke in young is another emerging domain of stroke medicine which in many aspects is different from conventional strokes which occur after the age of 50 years[6]. Around 10-15% of all stroke cases are stroke in the young adults and are associated with high mortality and morbidity[6].

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Previous research highlights the presence of hyperhomocysteinemia among the patients suffering from both hemorrhagic and ischemic stroke. Gajbhare *et al* from India concluded that post ischemic stroke hyperhomocysteinemia hits around 76.6% of the young patients[7]. Majority of the patients of intracerebral bleed had raised homocysteine levels after the stroke in a study done in the our neighboring country China[8]. Another large study involving around 3000 patients showed the high prevalence of raised homocysteine levels and its association with increased mortality among the patients of stroke. Cerbrovascular accidents among the young population is a widely discussed phenomenon nowadays. Primary and secondary prevention has a key role in limiting the disability and after effects of this devastating illness among the young population. Recognizing the biochemical abnormalities linked to this illness and targeting the high risk population may save many from the disease and disability[6].

The mechanism by which homocysteine levels are linked to the stroke is complex and multidimensional. Hyperhomocysteinemia linked to error of methionine metabolism may lead to atherosclerotic phenomenon. Homocysteine is also linked with the fibrinolytic and coagulation cascade. It may also cause direct endothelial injury and micro-inflammation. All these factors when combine together may predispose the individual to cerebrovascular injury and raise the change of re-stroke after the first event[9].

Multiple risk factors have been found associated with the raised homocysteine levels among the patients of stroke. Some of these include age, gender, smoking status, diet pattern, active life style, hypertension, hyperlipidemia and vitamin B-12 levels[4,7,10].

This study was planned with the objective to assess the prevalence of hyperhomocysteinemia among the patients of stroke in young and analyze the associated socio-demographic factors linked with the presence of this biochemical abnormality among these patients.

METHODOLOGY

After ethical approval from concerned ethical review committee and written consent from all potential participants this cross-sectional analytical study was planned from 10th May 2017 to 10th May 2018. Sample size was calculated by using the WHO calculator and non-probability purposive sampling technique was used. All subjects were between the age of 18 and 45 years and of both the genders. They all presented with acute stroke at emergency of Pak Emirates Military Hospital Rawalpindi. Stroke was confirmed by a neurologist on the CT scan within 24 hours of the acute presentation. All individuals who did not give consent and those with age less than 18 or more than 45 were excluded from the study. Subjects with the history of trauma, diabetes mellitus, renal and/or liver insufficiency, diseases of the thyroid gland, valvular heart disease, dyslipidemia, malignant tumors, psoriasis or thrombophilia, previous stroke, pregnancy or recent child birth or those who were unable to understand/ complete the required questionnaire were also excluded. Sub-jects with recent (up to 3 months back) intake of: folic acid, vitamin B6, vitamin B12 or medications influencing their metabolism, chemotherapy, oral contraceptives, methotrexate, theophylline, met-formin, niacin or steroids were also excluded from the study. After the application of inclusion and exclusion criteria, 60 subjects were included in the analyses.

Table-I: General characteristics of the study participants.

Socio Demo-Graphic factors Total	Patients with raised homocysteine levels n (%) 57 (55.9)	Patients with normal homocysteine levels n (%) 45 (44.1)	*p*-value
Age			
<35	46 (80.7)	36 (80)	0.929
≥35	11 (19.3)	9 (20)	
Gender			
Male	31 (54.3)	24 (53.3)	0.916
Female	26 (45.7)	21 (46.7)	
Type of Stroke			
Hemorrhagic	19 (33.3)	15 (33.3)	1.000
Ischemic	38 (66.7)	30 (66.7)	
Family History of Stroke			
No	46 (80.7)	32 (71.1)	0.258
Yes	11 (19.3)	13 (28.9)	
Body Mass Index			
<25	21(36.8)	30 (66.7)	0.003
≥25	36 (63.2)	15 (33.3)	
Smoking			
No	26 (45.6)	33 (73.3)	0.005
Yes	31 (54.4)	12 (26.7)	
Hyperlipidemia			
No	39 (68.4)	29 (64.4)	0.672
Yes	18 (31.6)	16 (35.6)	

The consenting subjects were provided with a detailed description of the study. Confounding variables were taken care of by detailed history taking about any current or previous physical or neurological illness and any current or previous evidence of significant head trauma or drug intake. Those subjects with confounding variables were excluded from the study. Patients who

fulfilled inclusion criteria underwent CT-scan and detailed evaluation by the neurologist to confirm the presence and type of stroke. Patients of stroke in young also underwent procoagulant workup including protein C, protein S, antithrombin III deficiency, anti-phospholipid antibody (APLA) and vasculitic workup for rheumatoid arthritis (RA) factor, anti-nuclear antibody (ANA), and Antineutrophil cytoplasmic antibodies (ANCA) was performed for patients with young stroke. Lipid profile was also carried out. The risk factors which were to be related with the presence of hyperhomocysteinemia and socio demographic data of the full sample of patients participating in the research were entered in a structured proforma.

Detailed history of smoking was taken in order to class the patients as smokers or non smokers. Body mass index was calculated and interpreted according to the recent world health organization (WHO) guidelines.

Plasma homocysteine levels estimation was done. Fasting levels were measured within the 24 hours after the acute episode of stroke. Five milliliters of venous blood was drawn and collected in a tube containing the anticoagulant ethylene diamine tetra acetate (EDTA). The sample was immediately kept in ice pack and later centrifuged within 30 min to avoid false elevation of homocysteine levels due to its release from red blood cells. Plasma samples were then refrigerated and stored at -70°C till the analysis was done. Total plasma homocysteine was determined by high-performance liquid chromatography (HPLC).

The assay used, measured the total homocysteine (tHcy), in both the reduced and oxidized forms. Homocysteine was dissociated from non-relevant proteins and other disulfides by reduction with sodium borohydride (NaBH4). The proteins were then precipitated by using 0.6 M perchloric acid. The sulfhydryl amino acids so obtained were made to react with fluorescent reagent 7-fluro-benzo-2-oxa-1, 3 diazole-4 sulfonic acid (SBDF) (Sigma laboratories). Homocysteine was quantified by using reverse phase HPLC and fluorescence detection, as per predefined method[15].

Homocysteine levels of >15µmol/L were interpreted as high levels. Below this were considered as normal[16].

Descriptive statistics were used to describe the risk factors and the frequency of hyper-homocysteinemia. Between-group variances in categorical correlates were determined using chi-square. Binary logistic regression analysis was done to evaluate factors related to the presence of hyperhomocysteinemia. SPSS-23 was used to carry out all the statistical analysis.

Table-II: The correlated factors relating to the homocysteine levels among the patients of stroke in young: The binary logistic regression.

	β	*p*-value	Odds ratio	Confidence interval	
				Lower	upper
Age (ref. is >35 years)	-0.155	0.772	0.856	0.299	2.453
Gender (ref. is male)	-0.093	0.812	0.911	0.424	2.958
Type of stroke (ref. ishemorrhagic)	-0.398	0.311	0.672	0.311	1.451
Family history of stoke (ref. is negative history)	-0.495	0.313	0.610	0.233	1.593
BMI (ref. is <25)	0.959	0.020	2.610	1.162	5.864
Smoking (ref. is no smoking)	1.032	0.023	2.807	1.154	6.832
Hyperlipidemia (ref. is normal lipid profile	-0.383	0.383	0.682	0.288	1.613

RESULTS

A total of 107 young patients reported with stroke in Pak Emirates Military Hospital Rawalpindi in the study time period. Out of these two had history of trauma, one was already diagnosed as a case of cerebral venous sinus thrombosis, one was pregnant and one had valvular heart disease. One hundred and two patients were included in the final analysis which underwent the relevant laboratory investigations. Total 82

(80.4%) were of the age 32 or more and 55 (54%) were males. Fifty seven patients had presence of hyperhomocysteinemia while 45 had normal homocysteine levels. Table-I showed that high body mass index and tobacco smoking were related with the presence of raised homocysteine levels. Both these variables had also significant association with the presence of hyperhomocysteinemia when regression analysis was done (table-II).

DISCUSSION

Many biochemical abnormalities can occur as a cause or consequence of acute stroke in the patient. Raised homocysteine level is one of the understudied but common biochemical abnormality that can occur among the patients of stroke[7,11]. A recent review published by Zhou *et al* concluded that raised homocysteine levels may accelerate the process of atherosclerosis and may predispose the individuals to stroke[8]. Fifty seven out of 102 young patients with stroke included in our analysis showed the presence of hyperhomocysteinemia. Previously this phenomenon has been reported with similar incidence rates in our neighboring country India in two different studies[10,11].

High body mass index was strongly associated with raised homocysteine levels in our study. Both chi-square and regression analysis confirmed this association. Similar findings have been reported earlier too in various studies[14,17]. Causal and temporal relationship of increased body mass index and raised homocysteine levels need to be established in large studies with more sophisticated study design[12-14].

Tobacco smoking has been linked with the raised homocysteine levels among patients of stroke in young in the past[6,11,15]. Similar findings were generated in our study where smoking emerged as a factor associated with the raised homocysteine levels among the patients of stroke in young. As both smoking and raised homocysteine levels have been associated with multiple health risks including the cardiac and neurological problems so this high risk group may require special attention.

Family history of stroke and hyperlipidemias were not associated with raised homocysteine levels in our study population. Variable results have been reported by the studies done in the past[6,14,18]. Though genetic basis of homocysteine metabolism and raised levels have been studied in the past[19] but still it's a complex phenomenon and all the cases could not be attributed to genetic cause of metabolic error. Similarly a large study done in china established a relationship with abnormal lipid profile and raised homocysteine levels[20] but we could not establish this relationship may be due to acute presentation.

LIMITATION OF STUDY

The findings cannot be generalized as our study population was not selected from a randomized sample of all the young people of stroke presenting at various hospitals of Pakistan. Lack of control group in our study was also a limitation which could be addressed in further studies with a large sample size on this biochemical aspect of stroke among the young population.

CONCLUSION

Raised homocysteine levels should be considered a possibility while evaluating the patients of stroke especially those who are young. Special attention should be given to the individuals with high body mass index and those who smoke tobacco.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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