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CONTENTS

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- Review articles are subject to the peer review process. They should contain a maximum of 4000 words and 75 references.
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- Brief communications should include brief original studies or reports on one or a small number of cases. They should not exceed 1,000 words; 1-2 illustrations and up to 10 references are permitted.
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ORIGINAL ARTICLES

Association between Chlamydia Pneumonia Seropositivity and Ischemic Stroke: A Case-Control Study

UDDIN MN[1], DEY SK[2], AHMED A[3], ISLAM MR[4], JAHAN M[5], DOLAN MAR[6], HASSAN S[7], RAKUNUZZAMAN M[8], RASHID MB[9]

Abstract:

Background: *Apart from traditional risk factors, infectious agent might contribute to ischemic stroke. The aim of this study was to evaluate the association between* Chlamydia pneumonia *seropositivity and ischemic stroke.* ***Methods:*** *42 ischemic stroke patients diagnosed by history, clinical examination and confirmed by CT scan or MRI of brain selected as case. The same number (42) of age and sex matched subjects having no history or clinical evidence of ischemic stroke were selected as control. Blood samples were collected within 2 to 14 days of ischemic stroke from indoor patients. Controls were collected from both indoor and outdoor patients with neurological disorders other than ischemic stroke. Anti-*C. pneumoniae *antibodies IgG and IgA were detected by ELISA (enzyme-linked immunosorbent assay) in the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU).* ***Results:*** *Among the study population, 66.7% of cases and 45.2 % of control patients were seropositive to* C. Pneumoniae *IgG (OR: 2.42, 95% CI: 1.00 - 5.85, p = 0.048). Whereas IgA were positive in 81% of case and 57.1% of control (OR: 3.19, 95% CI: 1.19 - 8.52, p = 0.018). Seropositivity to IgA showed more significant results than IgG.* ***Conclusion:*** *There was a significant association between* Chlamydia pneumonia *seropositivity both IgG and IgA with ischemic stroke.*

Key words: Chlamydia pneumoniae*, Ischemic stroke, Seropositivity etc.*

Introduction:

Stroke is one of the major global health problems. Its incidence rises steeply with age and in many developing countries, it is rising because of the adoption of less healthy life style. It is caused by atherosclerotic as well as non-atherosclerotic mechanisms[1]. Atherosclerosis is a multifactorial disease. It is a progressive inflammatory disorder of the arterial wall[2]. Infectious agents have been proposed to a contributory factor in the pathogenesis of atherosclerosis[3]. Known risk factors for ischemic stroke fail to account for all cases. Besides the conventional risk factors, organisms that cause chronic infections may contribute to the pathogenesis of ischemic stroke through atherosclerosis[4]. The microorganism most strongly implicated in the initiation and progression of atherosclerosis is the obligate intracellular gram negative bacterium *Chlamydia pneumoniae*, which commonly causes respiratory infections[5].Infectious

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agents that cause chronic infections have been considered as activating factors of chronic inflammation. Additionally, infections may play a direct role in endothelial dysfunction implicated in atherosclerosis and may increase ischemic stroke risk by activation of thrombotic processes[6]. *C. pneumoniae* infection causes an increase in serum triglycerides and a decrease in HDL (high-density lipoproteins), thereby turning the lipid profile toward an atherogenic one[3]. There is an independent association between *C. pneumoniae* seropositivity and raised fibrinogen levels, thereby facilitate platelet aggregation and thrombus formation[8]. Furthermore, a chronic *C. pneumoniae* infection increases the expression of its own 60kDa heat shock proteins (HsP60), especially when they are persistently elevated. The host immune response to microbial HsP60 may gradually lead or contribute to autoimmunity to human HsP60 and consequently, to the development of atherosclerosis[9]. Serology has been the traditional method of diagnosing infection by *chlamydia pneumoniae*[10]. Primary *C. pneumoniae* infection is characterized by a significant immunoglobulin M (IgM) antibody response, a delayed IgG titer and a low IgA level. The presence of elevated IgG antibodies reflects prior infection with *C. pneumonia*e and IgG titres remain elevated for a prolonged period of 3 – 5 years. IgA antibodies last only 3 – 5 days in the circulation and are a marker of recent or persistent infection. The serological pattern of increased IgA and IgG titres has been suggested to indicate chronic persistence of active infection[11]. Seropositivity usually is first detected at school age and rates generally increase by about 10% per decade. About 50% of individuals have detectable antibody at 30 years of age[10]. Evidence for the causal link between *C. pneumoniae* infection and ischemic stroke arise from seroepidemiology, detection of *C. pneumoniae* by PCR in carotid atherosclerotic plug, immunohistochemistry, culturing, and animal models. *C. pneumoniae* is known to cause persistent chronic infection and was found in atherosclerotic plaques in coronary and carotid arteries, and in the aorta by immunohistochemistry and PCR techniques[12]. Few previous studies reported on the association between *C. pneumoniae* infection and stroke risk[1]. Case-control studies revealed that specific anti-*C. pneumoniae* antibody levels were significantly higher in patients with cerebrovascular disease than in control patients. So far our knowledge, limited study has been carried out on association between serum *Chlamydia pneumonia* seropositivity and ischemic stroke patients in Bangladesh. So the aim of the present study was to know seroprevalence of *Chlamydia pneumoniae* infection in Bangladesh and its association with ischemic stroke patients.

Materials and methods:

This was a case-control study conducted in the Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka. Following to a predefined protocol, two groups of subjects were recruited. First group (Stroke patients): Forty two ischemic stroke patients (26 males and 16 females) diagnosed by history, clinical examination and confirmed by CT scan or MRI of brain were selected as case. Inclusion criteria were– age > 18 Years and patient or patient's legal guardian willing to participate. Exclusion criteria were- age ≤18 years, patients with hemorrhagic stroke, transient ischemic attack and venous stroke, patients with atrial fibrillation, valvular heart disease, prosthetic heart valve or recent myocardial infarction (<6 weeks) and patients not willing to take part in this study. All ischemic stroke patients were selected from indoor, Department of Neurology and blood samples were collected within 2 to 14 days of ischemic stroke. Second group (control subjects): The same number (26 males and 16 females) of age and sex matched subjects having no history or clinical evidence of ischemic stroke were selected as control. Control subjects were the patients with neurological disorders other than ischemic stroke. Control patients were selected from both indoor and outdoor, Department of Neurology.

Other risk factors for atherosclerosis have been studied for both groups which include hypertension, diabetes, dyslipidemia, smoking and previous vascular events. Hypertension, diabetes mellitus (DM), and dyslipidemia were diagnosed according to established criteria.

With all aseptic precaution 3 to 4 ml venous blood samples were collected by standard venipuncture in a sterile test tube. After collection, samples were sent immediately to the Department of Virology, BSMMU. Sera were separated by centrifugation and stored in -20^{o}C until analysis for anti- C*hlamydia pneumoniae* antibody IgG and IgA. Sera from both patient and control subjects for anti-*Chlamydia pneumoniae* antibody IgG and IgA were detected by ELISA (enzyme-linked immunosorbent assay) method in ETI max-3000 in the Department of Virology, BSMMU. All other relevant investigations were be done in the respective Department of Bangabandhu Sheikh Mujib Medical University. Continuous variable was expressed as Mean ± SD. Categorical variable was presented by frequency and percentage. Qualitative data were analyzed by chi-square test. Quantitative data was analyzed by unpaired t-test. A p-value of < 0.05 was considered statistically significant. Statistical analysis was done using SPSS (Statistical package for social sciences) win version 22 software programme. Approval from the Institutional Review Board (IRB) of BSMMU was obtained prior to the commencement of this study. The aim and objective of the study along with its procedure, risk and benefits were explained to the respondents in easily understandable local language and informed written consent was taken from each. It was assured that all information and record will be kept confidential.

Ischemic Stroke:

An episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction attributable to ischemia, based on

1. Pathological, imaging, or other objective evidence of cerebral, spinal cord or retinal focal ischemic injury in a defined vascular distribution; or
2. Clinical evidence of cerebral, spinal cord, or retinal focal ischemic injury based on symptoms persisting ≥24 hours or until death, and other etiologies excluded[13].

Results:

A total of 84 patients were assessed in this study. Forty two ischemic stroke patients were enrolled as case and compared with 42 control subjects. In case group, most 15 (33.3%) were belonged to the age group 51-60 years followed by 14 (31%) patients in the age group 61-70 years. In control group, most frequent age groups were 51 – 60 years and 61 – 70 years, each representing 15(35.7%) patients. The mean (± SD) age among cases was 61.74 ± 10.86 and in control 60.29 ± 8.40 years (Table-1).

Male and female were equally distributed between case and control groups. Out of 42, male were 26 (61.9%) and female were 16 (38.1%) in each group with a ratio of 1.6:1.In case group out of 42 patients, most 15 (35.7%) were housewife followed by13 (31%) were businessman. In control group most 16 (38.1%) were businessman (38.1%) followed by 15 (35.7%) werehousewife.

Among the study population, diabetes mellitus was present in 59.5% of case and 33.3% of control patients, hypertension was 83.5% and 47.6% in

Table-I

Distribution of the study subjects according to age in case and control (n=84)

Age (years)	Case (n=42)n (%)	Control (n=42)n (%)	p-value
41 - 50	8 (19.0)	8 (19.0)	
51 - 60	14 (33.3)	15 (35.7)	
61 - 70	13 (31.0)	15 (35.7)	
>70	7 (16.7)	4 (9.5)	
Mean±SD	61.74 ± 10.86	60.29 ± 8.40	0.495

Unpaired t test was done to measure the level of significance and p-value < 0.05 was considered as significant.

case and control respectively. Dyslipidemia was present 81.0% of case and 54.8% of control patients. Hypertension (OR:2.96, 95% CI:1.99-15.13, p=0.001), Diabetes mellitus (OR:2.94, 95% CI: 1.207.15, p=0.016) and dyslipidemia (OR:3.51, 95% CI:1.31-9.36, p=0.010) were statistically significant. Previous vascular event (14.3% of case and 7.1% of control), family history of stroke (16.7% of case and 7.1% of control) and smoking (57.1% of case and 38.1% of control) were more frequent in case than control patients but did not reach the statistically significant level (Table-2).

Chlamydia pneumonia IgG antibodies were detected in 28 (66.7%) case and 19 (45.2%) control patients. The difference between the two groups was statistically significant (OR: 2.42, 95% CI: 1.00-5.85, p=0.048). The seroprevalence of *Chlamydia pneumoniae* IgA were 81% in case compared to 57.1% in control group. This difference was also statistically significant (OR: 3.18, 95% CI: 1.19-8.52, p=0.018). Combined IgG and IgA antibodies were found in 22 (52.38%) among case and only 8 (19.04%) in control, revealed most significant results (OR: 4.67, 95% CI: 1.75-12.45, p=0.0014) (Table-3).

Brain imaging findings among the cases, 36 (85.7%) were MCA territory stroke. PCA territory stroke were 4 (9.5%) and ACA territory stroke and lacunar stroke were only 1 (2.4%) in each (Figure-1). Out of 42 stroke patients, most 36 (85.71%) patients had hemiplegia, followed by speech difficulty in 23(54.71%) patients, facial weakness in 16(38.09%) patients.

Table-II

Risk factor of the study subjects in case and control (n=84)

Risk factors	Case (n=42) n (%)	Control (n=42) n (%)	p-value	OR	95%CI
DM	25 (59.5)	14 (33.3)	0.016	2.94	1.20-7.15
HTN	35 (83.5)	20 (47.6)	0.001	5.50	1.99-15.13
Dyslipidemia	34 (81.0)	23 (54.8)	0.010	3.51	1.31-9.36
Previous vascular event	6 (14.3)	3 (7.1)	0.483	2.16	0.50-9.31
Family history	7 (16.7)	3 (7.1)	0.178	2.60	0.62-10.83
Smoking	24 (57.1)	16 (38.1)	0.081	2.16	0.90-5.18

Chi-square test was done to measure the level of significance and p-value < 0.05 was considered as significant.

Table-III

Chlamydia pneumoniae antibody of the study subjects in case and control (N=84)

Chlamydia pneumoniae antibody	Case (n=42) n (%)	Control (n=42) n (%)	p-value	OR	95%CI
IgG	28 (66.7)	19 (45.2)	0.048	2.42	1.00-5.85
IgA	34 (81.0)	24 (57.1)	0.018	3.18	1.19-8.52
Combined	22 (52.38)	8 (19.04)	0.0014	4.67	1.75-12.45

Chi-square test was done to measure the level of significance and p-value < 0.05 was considered as significant.

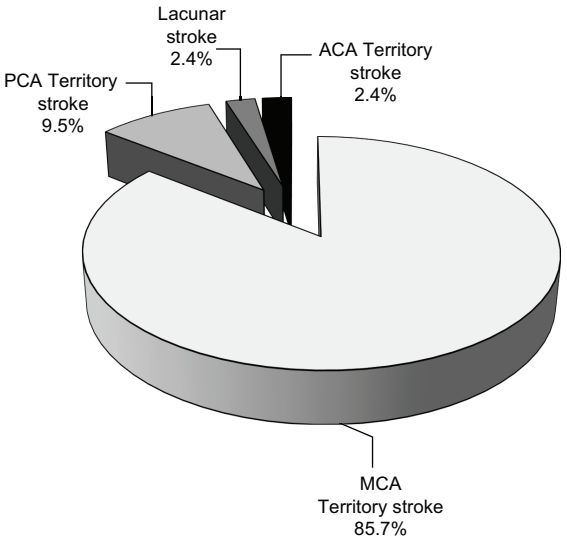

Fig.-1: *Pie chart findings CT scan of head/MRI of brain in cases (n=42)*

Discussion:

This present study investigated to find out the association between *Chlamydia pneumonia* seropositivity and ischemic stroke. In this case-control study, total 42 cases were enrolled and compared with 42 control subjects. In this study some relevant risk factors of ischemic stroke and some demographic profile like age, sex and occupation were also evaluated. The mean (± SD) age among cases was 61.74 ± 10.86 ranging from 40 to 81 years with male female ratio of 1.6:1. The maximum numbers (33.3%) of the patients were in the 51-60 years age group. Kenina et al., (2011)[14] and Alamowitch et al., (2007)[6] found that mean age of 63.19±11.3 and 66±13 years and a male/female ratio of 1.68:1 and 1.48:1 respectively in European population. Study in Indian population, Rai, et al., (2011)[15] found that mean age was 53.6±14.7 years with male female ratio of 2.18. Stroke prevalence is generally more in male that is found in all of these studies. Regarding mean age of patients, it is variable in different countries because of variation of life expectancy and other co-morbidities. In this study, hypertension was found in 83.5% in cases and 47.6% in control. In the Northern Manhattan stroke study, Elkind et al., (2000)[16] was found hypertension in 75.3% in cases and 49.4% in control group. Another study in Latvian population, kenina, et al., (2011)[14] was found 84.3% and 37.5% in case and control group respectively. Those studies showed that hypertension was more frequently present in case than control group which was similar to this study. According to Saha, et al (2016)[17] hypertension was the commonest risk factor among the stroke patients which was found all of these studies. Diabetes mellitus was found 59.5% of case and 33.3% of control patient in this study. In the Northern Manhattan stroke study, Elkind et al., (2000)[16] found diabetes mellitus in 36% of case and 17% of control group. Similarly, three case-control studies done by Bandura et al., (2008)[18]; Rai et al., (2011)[15]; Srivastava et al., (2014)[19] in the Department of Neurology, All India Institute of Medical Sciences found diabetes mellitus in the following frequencies among case and control group: 11.8% and 4.2%; 21% and 11%; 23.4% and 15% respectively which support steady increase in incidence of diabetes mellitus and higher frequency in this study. All of these studies showed diabetes mellitus is more frequent in case group than control group.

In this study, dyslipidemia was found in 81% of cases and 54.8% of control patients. Kenina et al., (2011)[14] found dyslipidemia in the frequency of 47% in cases and 12.5% in control. Srivastava et al., (2014)[18] found hyperchlesterolaemia 29.4% and 18% among case and control group. But no differences was seen (40% in both case and control group) by Hasan, (2011)[20]. In this study, smoking was found in 57.1% of case and 38.1% of control group. A considerable number (16 out of 42) of patients were female who were non-smoker in each group. So that difference in the frequency of smoking between case and control was not statistically significant in this study. Similar difference in the frequency of smoking was found by Alamowitch et al., (2008)[6] and Srivastava et al., (2014)[18], 29.3% in case and 21.1% in control; 20.14% in case and 12.2% in control respectivly.

In this study, anti-*Chlamydia pneumonia* seropositivity was found significantly higher in cases. In cases, out of 42 patients, 28 (66.6%) were positive for IgG and 19 (45.2%) were positive in control group. This difference was statistically

significant (OR: 2.42, 95% CI: 1.00-5.85, p = 0.048). The prevalence of IgA was higher in study population. Seropositivity to IgA was found 81% in case and 57.1% in control group. This difference was statistically more significant (OR: 3.18, 95% CI: 1.19-8.52, p = 0.018) than IgG. However, when combined (both IgG and IgA) status was compared between case and control group, a striking differences was found. In case 22 (52.38%) was positive to both IgG and IgA compared to 8 (19.04%) were in control group. That was much more significant (OR: 4.67, 95% CI: 1.75-12.45, p = 0.0014). Johnsen et al., (2005)[21] found similar result, the combined status differences (OR: 1.77, 95% CI: 1.043.00) compared to IgG (OR: 1.28, 95% CI: 0.83-1.95) and IgA (OR: 1.54, 95% CI: 0.96-2.47). Eini et al., (2014)[22] found that 30% of cases were IgG positive vs 15% of control (p = 0.016) and regarding IgA, 67% were positive in cases compared to 15% in cotrol (p = 0.0001). Similarly Piechowski-Jozwiak, et al., (2007)[23] found IgG in 78.7% of case and 52.5% of control (p = 0.0001) and IgA were 41.1% in cases and 15.6% in control group (p = 0.0001). These studies showed statistically significant results for both IgG and IgA which was found in this study. Furthermore, IgA was more significant than IgG, similar to this study. In indian study, Rai, et al., (2011)[15] and Srivasta, et al., (2014)[18] showed that seropositivity was significantly hihger in stroke patient but difference was less striking for IgG. Only IgA seropositivity yeild statistically significant result (p value were 0.005 and 0.003). Similarly, Alamowitch et al., (2008)[6]; Hasan (2011)[19]; Elkind et al. (2006)[24]; Madre et al., (2002)[25]; Njamnshi et al., (2006)[26]; Wimmer et al., (1996)[11] found statistically significant differences between case and control group with respect to IgA. In a systematic review and meta-analysis done in China by Chen et al., (2013)[27] selected 42 studies performed in case-control and cohort design showed an association between *C. pneumoniae* infection and cerebrovascular disease revealed by serum IgG and IgA as well as PCR technique in peripheral blood cells. (OR: 1.9; 95% CI: 1.17 to 3.07).

Limitation:

Our study was done in short period, with a small sample size. Also, the method of sampling was not random rather purposive. Rather than measurement, only detection of antibodies was performed. So correlation with antibodies level cannot be done. Imaging was not done in all control patients, some control patient could have silent infarcts which may underestimate or overestimate the association between *Chlamydia pneumonia* seropositivity and ischemic stroke. Patients of neurological diseases other than ischemic stroke were selected as control rather than healthy control.

Recommendation:

Population based study should be done to find out the prevalence of serological marker of *Chlamydia pneumoniae* infection in Bangladeshi population. Further multi-centered large scale studies should be carried out to consider chronic *Chlamydia pneumoniae* infection as a risk factor of ischemic stroke. Study period should be extended. Sample size should be large. Other diagnostic technique like PCR may be included to support this association.

Conclusion:

In conclusion, this study revealed that there was a significant association between *Chlamydia pneumonia* seropositivity both IgG and IgA with ischemic stroke. This association was stronger for IgA. Moreover, combined seropositivity to IgG and IgA yielded striking significance. So, there may be an increased risk of ischemic stroke in patients seropositive to anti-*Chlamydia pneumonia* IgG and IgA. Anti-Chlamydial therapy may be a potential preventive measures for stroke risk patients.

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Association between Modifiable Risk Factors and Stroke Recurrence in Patients with First Ever Ischemic Stroke

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Abstract:

Background: *Stroke is the third leading cause of death in adult population throughout the world and is the most common cause of severe adult physical disability. It is increasing at an alarming rate in Asia including Bangladesh. The effect of recurrent stroke is devastating on patient as it is the main reason of mortality and morbidity among patients* ***Methods:*** *A longitudinal, observational study was conducted from April 2018 to October 2018 in the department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka. All the patients of first ever ischemic stroke confirmed by neuroimaging (CT scan of head / MRI of brain), meeting the inclusion and exclusion criteria were included in the study. Our study was performed with sixty stroke patients. We followed up patient up to 90 days and observed for stroke recurrence.* ***Results:*** *Present study showed among the 60 stroke patients, only 4 (6.67%) suffered from stroke recurrence within 3 months. In our study, uncontrolled systolic blood pressure (p=0.04), uncontrolled diastolic blood pressure (p=0.027), dyslipidaemia (p=0.001), smoking (p=0.0003) and antiplatelet discontinuation (p=0.0001) were significantly associated with stroke recurrence whereas uncontrolled diabetes mellitus (p=0.46) and presence of atrial fibrillation (p=0.057) had no significant association.* ***Conclusion:*** *Smoking, hypercholesterolemia, uncontrolled systolic &/or diastolic blood pressure and discontinuation of antiplatelet therapy were significantly associated with stroke recurrence in this population. Therefore, early identification and control of these risk factors are essential to prevent recurrent stroke, thereby decrease morbidity and mortality.*

Key words: *Stroke, Cerebral infarct, Risk factors, Recurrence, Complications etc.*

Introduction:

Stroke is a leading cause of death and disability worldwide. There are approximately 795,000 new or recurrent strokes annually in the United States (610,000 being first events and 185,000 being recurrent events)[1]. Some 88% of these strokes are ischemic and 8% to 12% of ischemic strokes result in death within 30 days. By 2020, 19 of 25 million annual stroke deaths will be in developing countries[2].

Stroke recurrence is a major problem around the world, leading to permanent and more severe disability among patients. It has been reported that recurrence rate is approximately between 15-40% within five years after a first episode. The maximum incidence of recurrent stroke is in the first 30 days after initial stroke[3]. Recurrence is frequent and is a major contributor to stroke morbidity and mortality. The immediate period after a stroke carries the greatest risk for recurrence. In the Stroke Data Bank, of 1273 patients with infarcts, 3.3% had an early recurrence within 30 days. Nearly one third of the recurrent strokes in 2 years of follow-up occurred within the first 30 days. Early stroke recurrence increased motor weakness scores, early mortality, and duration of hospital stay. The predictors of first recurrent stroke were advanced age, hemorrhagic index stroke and diabetes mellitus. In the Northern Manhattan Stroke Study stroke recurrence was frequent, with 25% suffering a recurrent stroke by 5 years. Moreover, mortality after a recurrent stroke was greater than after the index stroke[4].

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The prevalence of stroke is increasing at an alarming rate in Asia including Bangladesh. Increasing portion of aging population and escalating risk factors such as hypertension, diabetes, tobacco abuse, unhealthy diet, obesity and physical inactivity contribute to the development of atherosclerosis and ultimately stroke[5]. It is very much important to find out the risk factors for recurrent stroke and to modify these factors as much as possible. Unfortunately, there is scanty data regarding recurrent stroke in Bangladesh. Therefore, this study intends to find out the impact of controlling modifiable risk factors and recurrence rate after first-ever ischemic stroke in a tertiary care hospital in Bangladesh. The findings of this study will help the physicians in this country as well as other countries of the world to predict the risk of recurrence in ischemic stroke patients and take necessary steps to modify the risk factors. It will also help the policy makers to allocate resources in appropriate places, which will help in decreasing the rate of mortality and disability in stroke patients.

Materials and methods:

This was a longitudinal, observational study conducted from April 2018 to October 2018 in the Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka. All the patients of first ever ischemic stroke confirmed by neuroimaging (CT scan of head / MRI of brain), meeting the inclusion and exclusion criteria were included in the study. We followed up the patients up to 90 days and observed for stroke recurrence. Assuming a recurrence rate of 25 % (95% CI, 7.2-9.0), sample size should have been 106[3]. But due to time and resource constraints, data was taken from 60 subjects in our study. Purposive sampling method was followed. Inclusion criteria was- patients of first ever ischemic stroke with age 18 to 80 years, and the patient or patient's legal guardian willing to participate. Exclusion criteria were- age less than 18 years or more than 80 years, ischemic stroke in whom there is evidence of previous stroke, hemorrhagic stroke, silent cerebral infarct, and patient or patient's legal guardian not willing to take part in the study. After ethical clearance from Institutional Review Board (IRB), patients were selected following the mentioned inclusion and exclusion criteria. Patients in whom 15 days have crossed after index stroke were not taken and evaluated as sample. Informed written consent was taken from each patient or his/her attendant. Proper history was taken, physical and neurological examination was done, and all relevant investigations were completed including a CT or MRI of brain. All the biochemical and hematological tests were done within 15 days of index stroke. Samples were collected with aseptic precaution and sent to respective laboratories of BSMMU (i.e Biochemistry, Pathology and Immunology labs). Hypertension, diabetes mellitus (DM), and hyperlipidemia were diagnosed according to established criteria. The included patients were followed up at 4 weekly intervals after index stroke. Modifiable risk factors for stroke were reevaluated at each follow-up upto 90 days. Previously diagnosed hypertension was regarded as controlled when blood pressure was lower than 130/80 mm Hg. DM was regarded as controlled when fasting serum glucose level was lower than 7.2 mmol/L, 2ABF was lower than 10 mmol/ml and HbA1c was less than 7% (American Diabetes Association 2018). Hyperlipidemia was regarded as controlled LDL-C level $\leq$100 mg/dL. Patients with atrial fibrillation (AF) who were on anticoagulation were regarded as adequately anticoagulated when the international normalized ratio (INR) was kept in the range of 2.0–3.0. Patients were considered as being under appropriate antiplatelet therapy when they received aspirin at a daily dose between 75 mg, or clopidogrel at 75 mg, after index stroke. Ischemic stroke was confirmed by CT scan or MRI.

Data collection sheet was filled-up by face to face interview. Patients were followed up every four weekly after the first visit. Blood glucose, lipid profile, ECG, adherence to antiplatelet therapy and smoking habit were followed up and recorded in a semi-structured data collection sheet. Statistical analysis was conducted using a software package, SPSS for Windows, Version 21.0 (SPSS Inc., Chicago, Il, USA). Approval from the Institutional Review Board (IRB) of BSMMU was obtained prior to the commencement of this study. The aim and objective of the study along with its procedure, risk and benefits were explained to the respondents in easily

understandable local language and informed written consent was taken from each. It was assured that all information and record will be kept confidential.

Results:

Total 83 patients were included in our study. Of them 23 patients lost from follow-up. Data was collected from the remaining 60 patients. Of the 60 patients included in the study, most 17 (28.33%) belonged to the age group 51-60 years, followed by 13 (21.67%) patients in the age group 61-70 years. Eleven (18.33%) patients were from 41-50 year age, 8 (13.33%) in 71-80 years, 7 (11.67%) in 31-40 years and only 4 (6.67%) patients were from 18-30 year age group. Mean age of the respondents was 53.04±16.72 years (Figure-1).

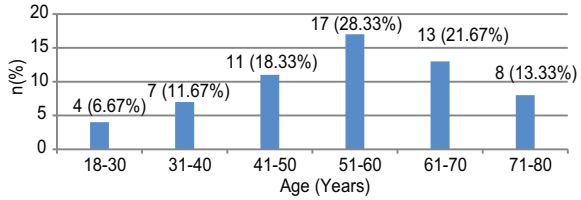

Fig.-1: *Age group distribution of the study subjects (N=60)*

Thirty-eight (63.33%) of the study subjects were male. Only 22 (36.67%) were of female gender.

Most 17 (28.33%) of the respondents were retired, followed by service holder 12 (20.0%) and business 11 (18.33%). Hypertension was the most common risk factor, followed by dyslipidemia, diabetes mellitus, smoking, previous, and ischemic heart disease for ischemic stroke patients.

Table-I

Distribution of study population by risk factors (N=60)*

Risk factors	n (%)
DM	35 (59.5)
HTN	51 (83.5)
Dyslipidaemia	49 (81.0)
Family history	9 (16.7)
Smoking	34 (57.1)
Drug Abuse	1 (1.67%)
Alcoholism	1 (1.67%)
OCP	1 (1.67%)
Heart disease	
• Ischemia/Old MI	39 (65.0%)
• Atrial fibrillation	3 (5.0%)

*Multiple response elicited

Regarding the baseline clinical parameters of the study subjects, mean pulse rate was 81±17.64 b/min, mean systolic and diastolic blood pressures were 157.13±22.06 and 98.35±13.79 mm Hg respectively. Irregular pulse was found in 5 (8.33%) patients, carotid bruit in 7 (11.67%) patients, and cardiac murmur in 4 (6.67%) patients (Table-II).

Table-II

Baseline clinical parameters of the study subjects at presentation (N=60)

Parameter		
Pulse rate(b/min)	(Mean ±SD)	81±17.64
Systolic BP (mm Hg)	(Mean ±SD)	157.13±22.06
Diastolic BP (mm Hg)	(Mean ±SD)	98.35±13.79
Temperature (^{0}F)	(Mean ±SD)	99.8±2.26
Irregular pulse		
• Present	n(%)	5 (8.33%)
• Absent	n(%)	55 (91.67%)
Carotid Bruit		
• Present	n(%)	7 (11.67%)
• Absent	n(%)	53 (88.33%)
Cardiac murmur		
• Present	n(%)	4 (6.67%)
• Absent	n(%)	56 (93.33%)

Table-III

Baseline biochemical and hematological parameters of study subjects (N=60)

Investigation		Mean±SD
Hb% (gm/dL)		12.3±2.27
WBC count (per mm^3)		8340±2533
ESR (mm in 1st hour)		28.2±13.4
Fasting blood glucose (mmol/L)		9.8±3.71
HbA_{1c} (%)		8.44±2.88
Blood urea (mg/dL)		26.4±9.31
Serum creatinine (mg/dL)		0.93±0.17
Serum ALT (u/L)		48.3±18.8
ECG*		
• Ischemia/Old MI(%)		39 (65.0%)
• Atrial fibrillation(%)		3 (5.0%)
Echocardiogram*		
• RWMA	n(%)	16 (26.67%)
• Valvular heart disease	n(%)	2 (3.33%)

*Multiple response elicited

The mean Hb% was 12.3±2.27gm/dL, mean total WBC count was 8340±2533per mm3, ESR 28.2±13.4mm in 1st hour, mean fasting blood

glucose9.8±3.71 mmol/L, HbA1c8.44±2.88%, blood urea26.4±9.31 mg/dL, serum creatinine0.93±0.17 mg/dL, serum ALT 48.3±18.8 u/L (Table-III).

Regarding neurological deficit, 57 (95.0%) patients had hemiplegia, 43(71.67%) had speech difficulty, 37(61.67%) had facial weakness, 21(35.0%) had unconsciousness, 11(18.33%) had swallowing difficulty, 11(18.33%) had vertigo, 8 (13.33%) had ataxia and 3 (5.0%) had nystagmus. Of the 60 study subjects, most (48.33%) had large vessel atherosclerosis. Thirteen (21.67%) had stroke due to undetermined etiologies, 11 (18.33%) suffered from small artery disease, and only 5 (8.33%) had cardioembolic stroke (Table-IV).

Table-IV

Type of stroke at presentation according to TOAST classification (N=60)

Stroke subtype	n(%)
Large vessel atherosclerosis	29 (48.33%)
Cardioembolic	5 (8.33%)
Small artery disease	11 (18.33%)
Other determined etiologies	2 (3.33%)
Undetermined etiologies	13 (21.67%)

Most 54 (90.0%) strokes involved the middle cerebral artery (ACA) territory. Five (8.33%) were in posterior cerebral artery territory, and only 1 (1.67%) in anterior cerebral artery territory (Figure-2).

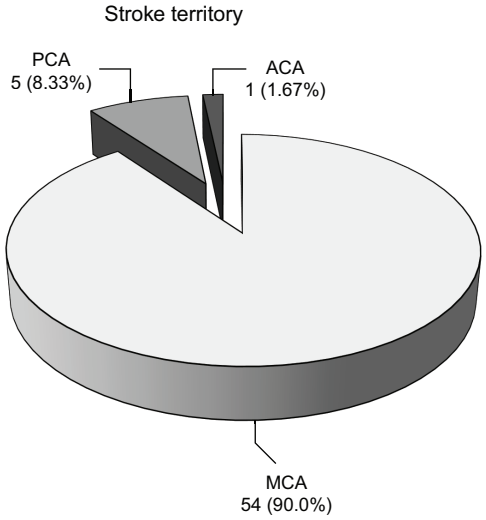

Fig.-2: *Distribution of study subjects by vascular territory (N=60)*

According to NIHSS stroke scale, majority (61.6%) of the study subjects were of moderate severity. Only 3 (5%) were suffering from severe stroke. Among the 60 stroke patients, only (6.67%) suffered from stroke recurrence within 3 months (Figure-3).

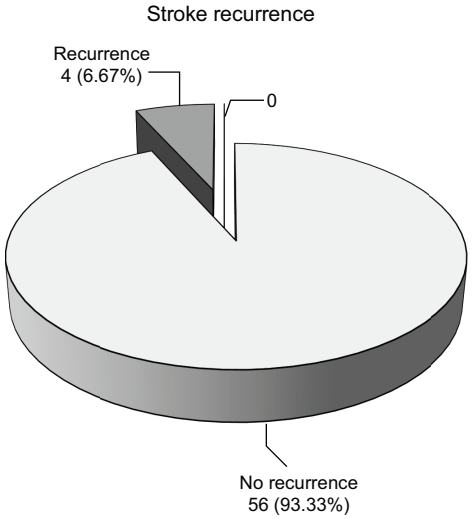

Fig.-3: *Recurrence of stroke within 3 months in the study subjects (N=60)*

Table-V

Association of control of systolic BP with stroke recurrence (N=60)

	Stroke recurrence	No recurrence	P value
SBP controlled	1	41	0.04
SBP uncontrolled	3	15	
Total	4	56	

Derived by χ^2 test.χ^2 value 4.1327.df 1.

Table-XI shows that of the 4 patients with recurrent stroke, 3 had uncontrolled systolic BP, 3 had uncontrolled diastolic BP, 2 had uncontrolled diabetes, 3 had LDL>100 mg/dl, one had atrial fibrillation, 2 patients continued smoking and 3 patients discontinued antiplatelet therapy.

Table-VI

Risk factor status of subjects with recurrent stroke (n=4)

Variables	Case-1	Case-2	Case-3	Case-4
Age (Years)	60	74	65	60
Sex	Male	Female	Male	Male
Date of First stroke	05-08-2018	24-06-2018	20-07-2018	22-07-2018
Date of Recurrent stroke	25-08-2018	01-09-2018	28-08-2018	06-08-2018
Hypertension	Yes	Yes	Yes	Yes
Systolic BP	Uncontrolled	Uncontrolled	Uncontrolled	Controlled
Diastolic BP	Controlled	Uncontrolled	Uncontrolled	Uncontrolled
DM	No	Yes	No	Yes
Glycemic status	Controlled	Uncontrolled	Controlled	Uncontrolled
Dyslipidemia (LDL>100 mg/dl)	No	Yes	Yes	Yes
Atrial fibrillation	Yes	No	No	No
Smoking status	Yes	No	No	Yes
Adherence to antiplatelet/ anticoagulant therapy	Anticoagulant	No	No	No

Discussion:

In the present study we intended to find out the frequency of stroke recurrence within 3 months of first ever ischemic stroke and to find out the association of modifiable risk factor control with stroke recurrence. This longitudinal observational study was done on 60 stroke patients presenting to the outpatient and inpatient departments of BSMMU over the period of April to October 2018. Total 60 patients were included in the study after their first ever ischemic stroke. Out of the 60 patients included in the study, most 17 (28.33%) belonged to the age group 51-60 years, followed by 13 (21.67%) patients in the age group 61-70 years. Eleven (18.33%) patients were from 41-50 year age, 8 (13.33%) in 71-80 years, 7 (11.67%) in 31-40 years and only 4 (6.67%) patients were from 18-30 year age group. Mean age of the respondents was 53.04±16.72 years. This result is consistent with a previous study, where mean age was 55.4 (±10.4) in a population of 1155 first ever ischemic stroke patients which was done on an Asian population of Philippines[3]. But in The Northern Manhattan Study the mean age was much higher (69.7 ± 12.7 years)[4]. In a cross sectional study carried out in 100 patients of stroke, most of the patients suffering from stroke were male and most of them were between 51-70 years of age[9]. Uddin et al. (2008)[10] and Idicula et al. (2009)[11] showed that ischemic stroke was more common in male than female. Hannan et al. (2001)[12] showed that male to female ratio was 2.53:1.

This study shows that among the baseline clinical parameters, mean pulse rate was 81±17.64 b/min, mean systolic and diastolic blood pressures were 157.13±22.06 and 98.35±13.79 mm Hg respectively. Irregular pulse was found in 5 (8.33%) patients, carotid bruit in 7 (11.67%) patients, and cardiac murmur in 4 (6.67%) patients. In the Framingham Study 5070 participants were followed up for 34 years. The age adjusted incidence of stroke was more than doubled in the presence of coronary heart disease (p< 0.001) and more than trebled in the presence of hypertension (p<0.001), compared with subjects free of these conditions. There was a more than fourfold excess of stroke in subjects with cardiac failure (p<0.001) and a near fivefold excess when atrial fibrillation was present (p<0.001). Among this biochemical findings it is obvious that mean fasting blood glucose was high (9.8±3.71 mmol/L). It is also being seen in postprandial blood glucose. HbA1c 8.44±2.88% shows that most patients were in the diabetic range .Many land mark studies showed the casual relationship between diabetes and ischemic stroke .In patients with type 2 diabetes the risk of diabetic

complications was strongly associated with previous hyperglycaemia[13]. On initial neurologic deficits of study subjects at presentation. fifty-seven (95.0%) patients had hemiplegia, 43(71.67%) had speech difficulty, 37(61.67%) had facial weakness, 21(35.0%) had unconsciousness, 11(18.33%) had swallowing difficulty, 11(18.33%) had vertigo, 8 (13.33%) had ataxia and 3 (5.0%) had nystagmus. Among the findings hemiplegia and speech disturbance are the most common neurological deficits among patients, which was followed by unconsciousness and other disturbances. These findings are common and readily bring the patient to the attention of physician and medical service. In the study by Siddique et al. (2009), most of the patients with ischemic stroke presented with right sided hemiparesis (51.25%). Dysarthria was present in 48(60%) cases, motor dysphasia in 47(58.75%), sensory dysphasia in 1(1.25%), impaired consciousness in 43(53.75%), headache in 43.75% (35) patients, vomiting in 40% (32) and nystagmus in 3(3.75%) cases of ischemic stroke[14]. Of the 60 study subjects, most (48.33%) had large vessel atherosclerosis. Thirteen (21.67%) had stroke due to undetermined etiologies, 11 (18.33%) suffered from small artery disease, and only 5 (8.33%) had cardioembolic stroke. In the study by Karapanayiotides et al. (2004), 33% were due to large vessel atherosclerosis, 21% cardioembolic, 17% small vessel disease, 16% due to other causes and 13% of undetermined etiology[15]. Nedeltchev et al. (2004) found that most strokes were caused by cardiac embolism and cervical artery dissection (24%),whereas only 9% and 4% resulted from small vessel disease and large artery atherosclerosis, respectively. Thirty percent were due to other determined etiology[16].

In this study, most 54 (90.0%) strokes involved the middle cerebral artery (ACA) territory. Five (8.33%) were in posterior cerebral artery territory, and only 1 (1.67%) in anterior cerebral artery territory. Nedeltchev et al. (2004) found that of the total 203 patients with stroke, 31(15.27%) had total anterior circulation stroke, 80(39.40%) had partialanterior circulation stroke, 38 (18.71%) had lacunar stroke and 34 (16.73%) had posterior circulation stroke[16]. In our study among the 60 stroke patients, only 4 (6.67%) suffered from stroke recurrence after 3 months. Buenaflor et al. (2017) found that among the 1155 first onset ischemic stroke patients, 12.8% had a second ischemic event within the next year, with an average of 8% annual risk for stroke recurrence over three years[3].

A study done by Leoo et al.(2007) in Sweden found that among the 889 patients who had recurrent stroke, the most frequent risk factor was hypertension (75%) followed by hyperlipidemia (56%), 37% had ischemic heart disease, 29% atrial fibrillation and 24% diabetes mellitus. Thirteen percent were current smokers and 11% were classified as obese[17]. The findings of this study is comparable to our study. In a study by Moroney et al. (1998) it was found that among vascular risk factors, there was a higher rate of recurrence among patients with hypertension, consistent cigarette use, and alcohol consumption but those differences failed to reach statistical significance. Among cardiac conditions, there was a trend toward a higher rate of early recurrence in patients with atrial fibrillation, but a significant effect was not found for other cardiac conditions[18]. In a similar hospital based retrospective study by Fu at el. (2016) in China, it was found that recurrent stroke in older men included previous history of myocardial infarction (OR 6.761; 95% CI 1.03-44.371), ischemic stroke or transient ischemic attack (OR, 2.496; 95% CI, 1.567-3.976), diabetes mellitus (OR, 1.986; 95% CI, 1.223-3.227), and coronary atherosclerotic disease (OR, 1.733; 95% CI, 1.010-2.974). In young men, hypertension (OR, 1.709; 95% CI, 1.104-2.645), coronary atherosclerotic heart disease (OR, 1.812; 95% CI, 1.129-2.911), and previous history of ischemic stroke or transient ischemic attack (OR, 2.317; 95% CI, 1.580-3.397) were independent risk factors of recurrent strokes[19]. As we did not evaluate non modifiable risk factors it was similar to our study in comparison.

Limitations:

Every study has some limitations. Our study was done in short period, with a small sample size. Study population were enrolled from only one center hence it may not represent the whole

population of the country. Also, the method of sampling was purposive, i.e. non-random sampling, which may affect the findings.

Recommendations:

We recommend that risk factors for recurrent stroke should be identified early and interventions done for their control to prevent recurrence. Further multi-centered prospective cohort study with large sample size and longer period should be conducted. Stroke patients and caregivers should be made aware of the importance of risk factor modification.

Conclusion:

This longitudinal observational study showed that chance of stroke recurrence within 3 months of first ever ischemic stroke is 6.67% in a tertiary care hospital. Smoking, hypercholesterolemia, uncontrolled systolic &/or diastolic blood pressure and discontinuation of antiplatelet therapy were significantly associated with stroke recurrence in this population, whereas there was no significant association with uncontrolled diabetes and atrial fibrillation. Therefore, early identification and control of these risk factors are essential to prevent recurrent stroke, thereby decrease morbidity and mortality.

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Association of Insulin Resistance with Sodium Valproate Therapy among Epileptic Patient

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Abstract:

Background: *Epilepsy is a common neurological disorder. Sodium valproate is one of the commonest broad spectrum antiepileptic drugs and it is used worldwide. Weight gain is the common side effect which is known to be associated with insulin resistance. The aim of this study was to see the association of sodium valproate therapy with insulin resistance among epileptic patients.* ***Methods:*** *It was a cross-sectional analytical study. Total 102 patients (51 epileptic patients with valproate monotherapy for at least one year and another 51 age and sex matched newly diagnosed epileptic patients without any anti-epileptic drugs) were selected in this study. The study was carried out from March 2016 to April 2017 for one year in the epilepsy clinic and outpatient Department of Neurology at Bangabandhu Sheikh Mujib Medical University, Dhaka. Participants underwent anthropometric evaluations and biochemical tests including fasting blood sugar and fasting insulin level. Insulin resistance (IR) index was calculated.* ***Result:*** *In this study mean duration of valproate treatment was 3.12±1.26 years and mean sodium valproate dose was 1133±440.5 mg/day (17.7±6.65 mg/kg/day). This study revealed serum fasting insulin level in valproate group and non-valproate group was 11.05±4.86 (¡U/ml) and 7.39±2.01 (¡U/ml) respectively. Fasting blood glucose was 4.71±0.79 (mmol/L) in valproate group and 4.41±0.62 (mmol/L) in non- valproate group. Calculated IR index in valproate group and non-valproate group was 2.17±0.55 and 1.46±0.39 respectively. IR index, fasting insulin and blood glucose all were significantly higher in valproate group than non- valproate group. This study also revealed mild positive correlation of IR index with dose and duration of valproate treatment.* ***Conclusion:*** *Sodium valproate treated patient had significantly higher IR index than control group.*

Key words: *Insulin Resistance, Valproate, Epileptic patients etc.*

Introduction:

Valproic acid (N-dipropylacetic acid, or 2-propylpentanoic acid) is a simple branched-chain carboxylic acid[1]. Sodium valproate is the sodium salt of valproic acid. It is one of the commonest 1st line antiepileptic drug used for all kind of seizure[1]. It is also used as a major treatment in bipolar disorder[2] and a prophylactic drug for migraine[3]. The underlying mechanism of action is increment of concentration of GABA in brain, inhibition of neuronal firing inactivation of voltage sensitive sodium channels and t type calcium channels[1]. Continuous use of valproic acid is associated with several side effects. Weight gain is a common side effect of VPA[4]. The increase in body weight is found to be associated with metabolic disorders indicating an increase in

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insulin resistance (IR) during VPA therapy[5]. Sodium valproate causes hyper insulinemia both in obese and lean patient[6]. Insulin resistance is a pathological state characterized by a lack of physiological response of peripheral tissues to insulin action[7]. It is related to obesity, non-insulin dependent diabetes mellitus, hypertension, atherosclerotic cardiovascular disease, dyslipidemia, hyperinsulinemia[8]. Insulin resistance is also related to the development of nonalcoholic fatty liver disease[9]. The exact pathogenesis of valproate induced insulin resistance is still not fully clarified. One hypothesis is valproic acid induced elevation of free fatty acid level which has role in impairment of action of insulin. As valproic acid is branched chain fatty acid and highly protein bound drug, it competes with free fatty acid for protein binding[10]. Beta oxidation of fatty acids may also be inhibited by valproic acid which causes increased level of non-esterified fatty acid in blood[11]. Other hypotheses are valproate induced direct toxicity on pancreatic beta cell[12], defective sympathetic neuronal activity[13], impairment of insulin signal transduction pathway[14]. Insulin resistance can be measured by several methods like homeostasis model assessment (HOMA), quantitative insulin sensitivity check index (QUICKI) etc.[15]. Homeostasis model assessment was first developed by Matthews et al.[16]. It is derived from the use of the insulin glucose product, divided by a constant.

Although studies regarding the association of long term sodium valproate therapy with insulin resistance were done previously in other countries, so far it is known, no such study was found to be done in this country. Therefore, the objective of this study was to determine the insulin resistance (IR) index in patients with epilepsy receiving VPA mono therapy for long time.

Materials and Methods:

This cross sectional analytic study was carried out in the Epilepsy clinic and outpatient Department of Neurology at Bangabandhu Sheikh Mujib Medical University (BSMMU) from March 2016 to April 2017 for a period of one year. Total 102 epileptic patients of both sexes and age >18 years having focal or generalized epilepsy were selected. Among them 51 patients were with sodium valproate therapy for at least 12 months and another 51 patients were newly diagnosed epileptic on arrival without any antiepileptic drug for comparison. Patients were excluded from this study who were diabetic, obese, hypertensive, noncompliant, had neurological disease other than epilepsy, and took antiepileptic other than sodium valproate or any drug causing insulin resistance. Patients and controls were selected by purposive sampling. Detailed history and clinical examination were carried out for each patient and control using especially prepared proforma. Previous records and data were reviewed. Structured questionnaire were used to collect the necessary information. Each participant was undergone measurements of height, weight, waist circumference and blood pressure. BMI was calculated as weight (kg) divided by height (m). After 8 to 12-hours fast, a venous blood sample was withdrawn and analyzed for fasting plasma insulin (FI) and fasting glucose (FG).Test is carried out by automated analyzer: Dimension EXL with LM, Architect Plus ci8200 in the department of Biochemistry, BSMMU. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as proposed by Mathews et al.[16] as follows: Fasting insulin (μU/ml) x Fasting glucose (mmol/L)/22.5.

Data Analysis: Statistical analysis was conducted using SPSS Version 22. The results were expressed as means ± SD for continuous variables and as frequency & percentages for categorical variables. Comparisons of continuous data between patients on VPA and non-valproate group were performed with unpaired student's t-test, and those of categorical data, with the Chi-square test. For comparison of more than two mean group ANOVA test were used. For correlation, Pearson's correlation coefficient test was used. P value <0.05 were taken as statistically significant at 95% confidence interval.

Operational definitions

Long term sodium valproate therapy: Those patients taking sodium valproate for at least 12 months.

Obesity: Defined as BMI ≥30kg/m^2, where BMI is weight in KG/square meters of height [17].

Hypertension: Systolic Blood pressure ≥140 mm of Hg and/ or Diastolic Blood pressure ≥90 mm of Hg after sitting quietly in a chair for at least 5 minutes with feet on the floor[18].

Diabetes mellitus: Defined as

Fasting plasma glucose ≥ 7.0 mmol/L

Plasma glucose in random sample or 2 hrs after 75 mg glucose load ≥11.1 mmol/L[19].

Ethical consideration: Approval was obtained from the Department of Neurology, BSMMU and Institutional Review Board of BSMMU prior to the commencement of this study. The aims and objectives of the study were explained to the patients and/or attendants in easily understandable local language and then informed consent was taken. It was assured that all information and records would be kept confidential and the procedure was helpful for both the physician and the patients in making rational approach of the case management.

Results:

All 102 participants were separated into two groups. First group comprised epileptic patients being treated with VPA(n=51, mean age 23±7.01 years, age range 18-45 years, 32 male, 19 female).. The second group comprised newly diagnosed epileptic patients on arrival who did not take any antiepileptic drug(n=51, mean age 24.8±3.7 years, age range 18-43 years, 34 male, 17 female).

Mean duration of treatment with valproic acid was 3.12±1.26 years and mean drug dose was 1133.12 ± 440.5 mg/day range (400-2000), 17.7± 6.65 mg/kg/day range (7-32). Mean BMI of valproate and non-valproate group were 23.4±1.22(kg/m^2) and 22.63±1.59 (kg/m^2) respectively.

The fasting insulin level in valproate and non - valproate group was 11.05±4.86µU/ml and 7.39±2.01µU/ml with significantly higher in valproate group. The fasting blood glucose in valproate group and non-valproate group was 4.71±0.79mmol/L and 4.41±0.62mmol/L respectively. The blood glucose was significantly higher in valproate group. Calculated insulin resistance index from HOMA-IR formula in valproate and non -valproate group was 2.17± 0.55 and 1.46±0.39 respectively. IR index was significantly higher in valproate group.

Table-I

Participants demographic and clinical characteristics

Characteristics	Valproate group	Non-valproate group	P value
Age (years)	23±7.0 (18-45)	24.8±3.7(18-43)	
Sex			
Male (n)	32	34	
Female(n)	19	17	
Mean duration of treatment (years)	3.12±1.26		
Drug dose (mg/day)	1133.12±440.5		
(mg/kg/day)	17.7± 6.65		
BMI (kg/m^2)	23.4±1.22	22.63±1.59	0.081

Table-II

Comparison of laboratory data between two groups

Variables	Valproate group (n=51) Mean ±SD	Non valproate group (n=51) Mean ±SD	P value
Serum fasting insulin level (µU/ml)	11.05±4.86 (5.10-39.4)	7.39±2.01 (2.80-12.0)	<0.05*
Blood glucose-Fasting (mmol/L)	4.71±0.79 (3.4-6.1)	4.41±0.62 (3.5-5.7)	<0.05*
IR index	2.17±0.55 (1.02-4.70)	1.46±0.39 (0.46-2.75)	<0.05*

Data were analyzed using Student's t-test and were presented as mean ±SD, parenthesis figure indicate range
*significant

There was moderate positive correlation of IR index with duration and dose of valproic acid treatment.

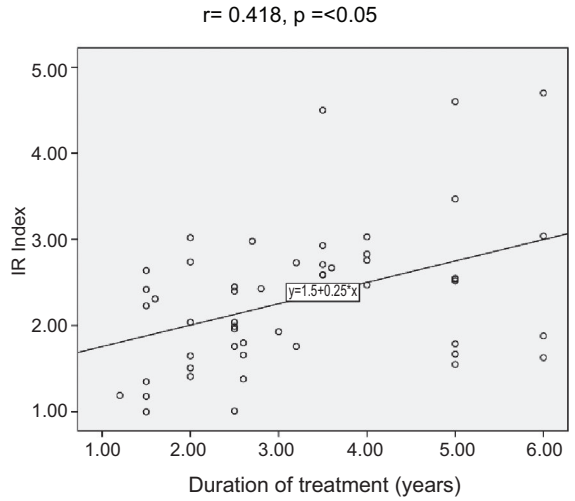

Fig.-1: *Scatter diagram showing moderate positive correlation of IR index with duration of treatment(r=0.418; p = < 0.05).This test was done by Pearson's correlation coefficient test.*

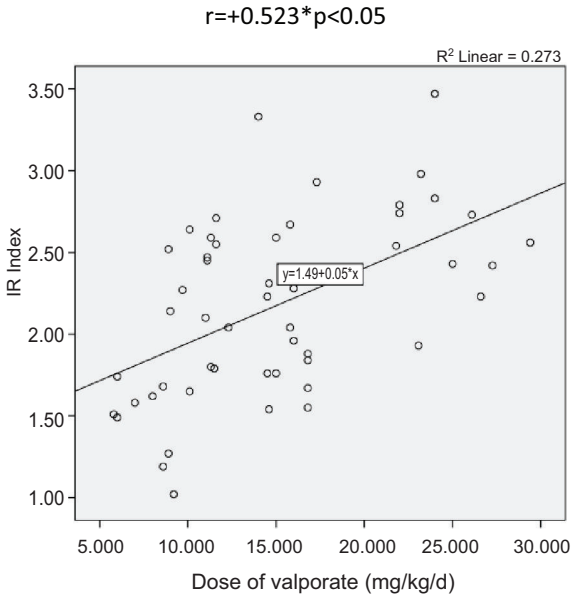

Fig.-2: *Scattered diagram showing moderate positive correlation of IR index with dose of sodium valproate. In this diagram r = +0.523 and p = < 0 .05 which indicates moderately positive. This test was done by Pearson's correlation coefficient test*

Table-III

Comparison of IR index (> 2.6) between valproate group and control group (n=102)

IR index	Valproate group (n=51) No. (%)	Non-Valproate group (n=51) No. (%)	P value
> 2.6	18(35.3)	4(7.8)	<0.05*
<2.6	33(64.7)	47(92.2)	
Total	51(100)	51(100)	

Data were analyzed using Chi-square-test and were presented as frequency and percentage, *significant

35.3% patients in valproate group and 7.8% patients in non-valproate group had IR index > 2.6 which was significantly higher in valproate group.

Discussion:

This cross sectional study was carried out to see the association of insulin resistance in sodium valproate therapy among epileptic patients. In this study it was observed that majority patients were in 19-30 years range. The mean age of patients taking sodium valproate was found to be 23.6 ± 7.01 years. These findings are compatible with studies[20, 21].

In this current study it was observed that male were predominant among the epilepsy patients which was 62.7% of the study population. This male predominance was also observed in studies[20,22]. In this study the mean sodium valproate dose was 1133±440.5 mg/day and mean duration of treatment was 3.12±1.26 years. This present study's dose was compatible to study[6]. In this study the mean BMI of valproate treated patients was 23.4±1.22 kg/m^2 and mean BMI of control group was 22.63± 1.59 kg/m^2. The difference between the two groups was not significant.

In this study IR index, fasting insulin and fasting blood glucose all were significantly higher in valproate group than non-valproate group. Several study shows significantly higher IR index and insulin level in valproate treated patients[6,16].Pylvanen et al.[6] reported hyper insulinemia and higher insulin resistance index both in both obese and non obese patients with equal BMI. Keskin et al.[23] conducted a study with 111 participants (80 epileptic, 31

healthy volunteers) and found that valproate treated patients had higher insulin resistance index than volunteer group with same BMI. The present study showed moderate positive correlation with dose of sodium valproate with fasting insulin level and IR index. Moderate positive correlation of IR index was also seen with duration of treatment. This study is compatible with study[24].

Several study reported valproate induced weight gain [,25,26,27] The causes of increased body weight may be due to valproate induced raised proinsulin and insulin secretion, increased appetite for carbohydrate, reduction of beta oxidation of leptin level due to carnitine deficiency and restricted energy expenditure.[28] In the present study we could not give comment about weight gain because we had no previous records of weight during starting of treatment. There were debate in case of valproate induced weight gain either insulin resistance induced obesity or obesity induced insulin resistance responsible but Pylvanen[6] asserted that obesity was not the cause it was insulin resistance.

Although there was no clean cut reference cut off value in this country for insulin resistance Bhowmik et al.[29] set a cut off value of 2.6 for non-diabetic Bangladeshi population. The present study showed the mean IR index in valproate and non-valproate group were 2.17 and 1.46 respectively. Considering the cut off value of 2.6, in this present study 35.3% patient had IR index> 2.6 in valproate group and 7.8% patient had IR index> 2.6 in control group. The differences between these two groups were significant. It revealed that valproate treated patient were more insulin resistant than non-valproate group.

The optimal method of insulin resistance measurement is hyperinsulinemic-euglycemic clump test. It is costly and time consuming. HOMA-IR calculation is easy to apply and correlates highly with the hyperinsulinemic-euglycemic clump test. That is why HOMA-IR is applied in this study[16].

Limitation

This study was a cross sectional study, the results of this study would have been more reliable if it was a longitudinal study. The study population was selected from one selected hospital in Dhaka city, so that the results of the study may not be reflect the exact picture of the country.

Conclusion:

This study revealed that patients with long term sodium valproate therapy had significantly higher IR index than non-valproate group and there was moderate positive correlation of insulin resistance with dose and duration of valproate treatment. So those patients who are taking sodium valproate both in high dose and long duration are more prone to develop insulin resistance.

Recommendation:

Patients taking sodium valproate for long duration should be warned about the possible weight gain. Adequate dietary advice and exercise advice can be given. Those patients who are taking sodium valproate of long duration and higher dose may be followed up with IR index. Further studies can be undertaken by large number of patients and adequate time.

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Association of Serum Magnesium Concentration with Alzheimer's Disease

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Abstract:

Background: *Alzheimer's disease is a neurodegenerative disease. It is the most common cause of dementia in individuals older than 60 years of age. Age is the most important risk factor for Alzheimer's disease. It is important to identify modifiable risk factors. One such important modifiable risk factor is Magnesium, a trace element. The objective of the study was to see the association of serum Magnesium concentration with Alzheimer's disease patients.* ***Method:*** *It was a case control study carried out in neurology department of BSMMU, Dhaka. Total 68 patients were enrolled as study population after satisfying inclusion and exclusion criteria. Among them, 34 were grouped as case and rest 34 were control. Serum Magnesium concentration was detected.* ***Result:*** *Serum Magnesium concentration was significantly lower in AD patients than that of control group [2.04±0.19 mg/dl vs 2.36±0.21 mg/dl.* ***Conclusion:*** *The results of our study revealed an expression that the trace element, Magnesium concentration has an association with Alzheimer's disease.*

Key Words: Alzheimer's dementia, Magnesium concentration, Risk factor etc.

Introduction:

Alzheimer's disease is a progressive and fatal neurodegenerative disorder manifested by cognitive and memory deterioration, progressive impairment of activities of daily living and a variety of neuropsychiatric symptoms and behavioral disturbances.

Alzheimer's disease is the most common form of dementia, accounting for 50-56% cases at autopsy and in clinical series[2]. More than 35 million people worldwide; 5.5 million in the United States have Alzheimer's disease, a deterioration of memory and other cognitive domains that leads to death within 3-9 years after diagnosis[2].

Alzheimer disease (AD), specifically the late onset form of AD (LOAD) is common. Although mutation in the genes PS1, PS2, and APP cause less common forms of early-onset, autosomal dominant familial AD (FAD), these cases represent <1% of AD[3].

Exact aetiopathogenesis of Alzheimer's disease is still poorly understood. Well-known hypothesis includes amyloid hypothesis by Hardy and Higgins[4]in 1992, by Hardy and Selkoe[5]in 2002.Many risk factors have been defined in the literature and the roles of environmental factors, nutrition, some vitamins and trace elements have been investigated[6,7,8].Magnesium is one of the

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important ions in the central nervous system and dietary deficiency of Mg^{2+} increases the neurotoxicity[9]. Because of its involvement in a number of bioenergetics and biochemical activities, magnesium appears to play an important role in normal neuronal activity[10].

Onset of AD is usually insidious. Family members or even the patient himself or herself cannot mention the time of beginning. Clinically it is characterized by loss of memory, inability to learn new things, loss of language function, a deranged perception of space, unable to calculate, depression and other manifestations.

AD is a progressive disorder. Familial occurrence of Alzheimer's disease is also well established. In less than 1 percent of such cases, there is a dominant inheritance pattern with a high degree of penetrance and appearance of disease at a younger age[11,12]. The annual incidence worldwide increases from 1% between the ages of 60 and 70 years to 6% - 8% at the age of 85 years or older[13].

The most commonly used and widely accepted criteria for Alzheimer's disease is the NINCDS-ADRDAcriteria[14,15].This criteria is very useful. For early and presysmptomatic (prodromal) AD diagnosis, a criteria is proposed by Dubois *et al.* in 2007[16].

There is no definitive investigation to confirm the AD. But some helpful investigations are CT/MRI of brain, PET scanning, PET imaging with the use of amyloid-binding compounds, such as carbon 11-labeled pitsburgh compound B (PIB) and CSF markers.

Magnesium is the fourth most abundant mineral. It is a cofactor for >300 metabolic reactions in the body. Magnesium is the second most abundant divalent cation in serum, exceeded only by calcium[17]. Approximately 1% of total body magnesium is extracellular. Intracellular concentration of Magnesium is higher than that in serum.

The most common test for the evaluation of magnesium levels and magnesium status in patients is serum magnesium concentration[18]. It is most practicable and inexpensive.

Mg has been shown in vitro to gate cation channels opened by glutamate and particularly those on the N-methyl-D-aspartate (NMDA) receptors. Glutamate binding to NMDA receptors was reduced by 75-80% in the hippocampus of Alzheimer disease brains. The hypothesis of a link between this loss of glutamatergic transmission and Mg depletion in the hippocampus in Alzheimer's disease has been proposed. Glick goes further and suggests that Alzheimer's disease involves a defective transport process characterized by both an abnormally low Mg incorporation and an abnormally high Al incorporation into brain neurons[19].

Methods:

It was a case control study. Place of study was Dementia clinic, outpatient and inpatient department of Neurology, BSMMU, Dhaka. Study period was July'16 to March' 18. Study Population includes all adult patients with clinical diagnosis of AD. Patients with AD (as cases) and age and sex matched non-demented patients (as controls) were selected by purposive sampling method.

Inclusion criteria for cases included were patients with diagnosis of Alzheimer's disease (according to NINCDS-ADRDA Criteria), Age- more than 40 years, both male & female. Exclusion Criteria for cases included were congestive heart failure, acute exacerbation of COPD, acute MI, uncontrolled hypertension (SBP $\geq$180 mm Hg), uncontrolled DM, moderate to severe renal or hepatic disease, diseases causing progressive deficit in cognition, Parkinson's disease, subdural hematoma, NPH, Stroke, brain tumor.

Inclusion criteria for control included were non-demented patients (age and sex matched) in inpatient and outpatient department of Neurology, BSMMU. The patients with the diagnosis of tension type headache, migraine, hemifacial spasm, writer's cramp, low back pain, cervical spondylitis, PLID were included as control group.

Exclusion criteria for control included were patients of any type of dementia, patients unwilling to take part in study, drugs causing decreased magnesium level eg. diuretics, ethanol, cisplatin, cyclosporine.

H/O stroke, acute MI, acute or unstable medical conditions, heart failure, malignancy, chronic liver disease, chronic kidney disease were also included in exclusion criteria.

After taking careful history, physical and neurological examination including MMSE was done. The cognitive impairment was assessed by MMSE Score (Mild: 20-24, Moderate: 10-19. Severe: <10). MMSE is a simplified, scored form of the cognitive metal status examination. It includes eleven questions, requires only 5-10 min to administer, and is therefore practical to use serially and routinely[20].

Relevant investigations including MRI of Brain were done to diagnose AD and to rule out other causes of dementia. Diagnosis of AD was established before doing Serum Mg concentration.

A questionnaire was developed in English. Bengali version of MMSE was reproduced for easy communication. According to specific objectives, the questionnaire was developed using the selected variables. A checklist section was also made for data collection. Before starting data collection, the details of the study were explained to each patient and patient's attendants and informed consent of the respondents was obtained.

At the end of data collection, the mean and standard deviation of Serum Mg levels of both cases and controls were calculated. Quantitative data was analyzed by unpaired t test and qualitative data was analyzed by χ^2(Chi Square) test. The level of significance was set at P<0.05.

Results:

Table I shows that the mean age (±SD) was 63.26±8.18 years in case group and 60.23±8.22 year in control group. There was no significant difference in age distribution between case and control (p>0.05).In case, 55.9% were male and 44.1% were female. Statistically no significant difference was observed between the two groups in term of gender (p>0.05).

Table II shows mean serum magnesium concentration in case group was 2.04±0.19, in control group was 2.36±0.21.In case group, serum magnesium concentration is significantly less in comparison to control group.

Table III shows the odds ratio between case and control group is 10.63 (95%CI:3.26-34.65). The odds ratio 10.63 indicated that serum magnesium concentration below 2.15 mg/dl can increase the risk of Alzheimer's disease by 10.63 times.

Table IV shows that most of the patients presented with moderate dementia (58.8%) followed by mild (23.5%) and then severe dementia (17.6%).

Table-I

Distribution of study subjects according to age (n=68)

	Group		p value
	Case (n=34)	Control (n=34)	
Age (years)			
40 – 49	1 (2.9)	3 (8.8)	0.613
50 – 59	8 (23.5)	11 (32.4)	0.416
60 – 69	20 (58.8)	17 (50.0)	0.466
≥70	5 (14.7)	3 (8.8)	0.709
Mean±SD	63.26±8.18	60.23±8.22	0.133
Gender			
Male	19 (55.9)	19 (55.9)	1.000
Female	15 (44.1)	15 (44.1)	

Table-II

Serum magnesium concentration in case and control (n=68)

Serum magnesium	Group		p value
	Case (n=34)	Control (n=34)	
Mean ± SD	2.04 ± 0.19	2.36 ± 0.21	<0.001

Table-III

Distribution of study subject at 2.15mg/dl serum magnesium concentration (cut off value) in case and control (n=68)

	Group	n (%)	OR	95%CI (lower – upper)		p value*
Serum Magnesium <2.15 mg/dl	Case	22 (64.7)	10.63	3.26	34.65	<0.001[s]
	Control	5 (14.7)				

Table-IV

Distribution of AD dementia patient according to severity (n=34)

Dementia	Frequency (n)	Percentage (%)
Mild	8	23.5
Moderate	20	58.8
Severe	6	17.6

Figure 1 shows correlation of serum Magnesium concentration with MMSE score. Here positive correlation coefficient (r=+0.018) was observed which was not statistically significant (p=0.920).

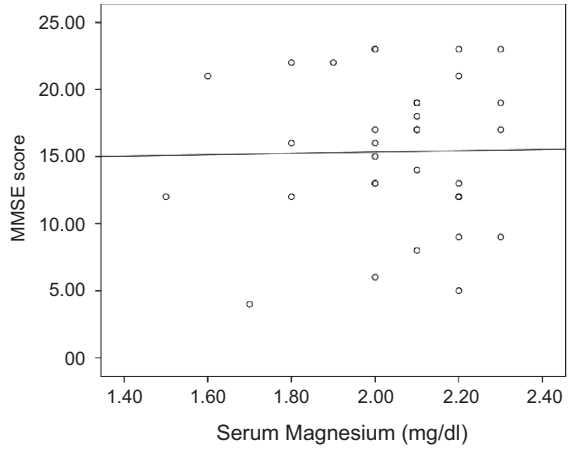

Fig.-1 *(Scatter diagram): Correlation of serum Magnesium concentration with MMSE score.*

Figure 2 shows correlation of serum Magnesium concentration with duration of dementia. Negative correlation coefficient(r=-0.196) was observed which was not statistically significant (p=0.250).

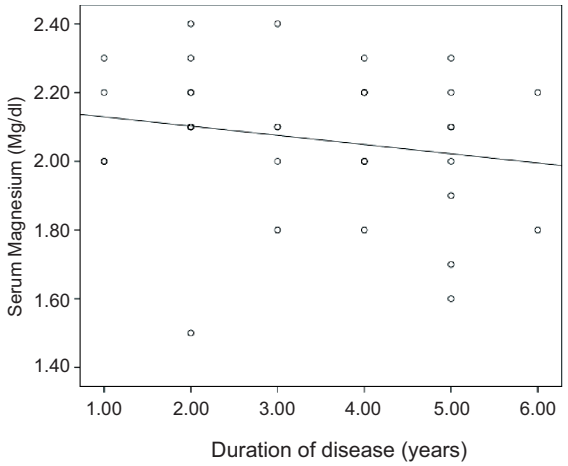

Fig.-2 *(Scatter diagram): Correlation of serum Magnesium concentration with duration of dementia.*

Discussion:

In this study, analysis of age distribution showed that there was no significant difference in mean age between two groups (P>0.05) It is consistent with studies like Veronese *et al*.[21], Lemke *et al*.[22], but these age groups seemed to be higher in comparison to our study. It might be due to lower life expectancy of our population.

Both in case and control group, 55.9% (19) were male and 44.1% (15) were female. There was male preponderance both in case and control groups. Statistically no significant difference was observed between the two groups in terms of gender ($p>0.05$). AD is more common in female patients in many studies of other countries[22,23]. In the context of our country, female patients have less health seeking behavior. That's why female patients were enrolled less. The mean (±SD) value (mg/dl) of Serum Magnesium concentration in AD patients was found less than that of control group [2.04±0.19 Vs 2.36±0.21] which is statistically significant ($p<0.001$). It is consistent with other studies like Vural *et al*. [23], Basheer *et al.*[24].The cut off value of serum Magnesium was set at 2.15 mg/dl. In this study, below cut off value in case group was 64.7% and in control group was 14.7%. It is statistically significant ($p<0.001$). The odds ratio was 10.63 (95% CI : 3.26-34.65). It indicated that serum magnesium concentration lower than 2.15 mg/dl can increase the risk of Alzheimer's by 10.63 times.

Outpatient Department of Neurology, BSMMU runs a weekly dementia clinic where dementia patients are evaluated and treated. This study revealed that most patients presented with moderate dementia (58.8%).The rest had mild (23.5%) and severe dementia (17.6%).

So we can comment that in agreement with previous studies, in our study serum magnesium concentrations were found to be significantly decreased in AD dementia patients when compared to controls.

Conclusion:

The present study revealed that serum magnesium concentration was significantly lower in AD patients in comparison to control group. So, this biomarker has association with AD. However, there was no significant relationship between serum magnesium level and severity of disease. Also there was no significant correlation between serum magnesium concentration and duration of AD.

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Association of Serum Homocysteine Level with Migraine in Adults

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Abstract:

Background: *Migraine is the second most common primary headache disorder that has close link to the neurovascular system. The exact pathogenesis of migraine is still not fully understood but several possible theories have been proposed. Hyperhomocysteinemia is one of the coincidental factors whose association with migraine is yet in obscure.* ***Methods:*** *This case control study was conducted in the department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka. A total of 65 patients, who were diagnosed as migraine (with aura or without aura) according to ICHD-3 criteria, were considered as case group and another 65 patients (age and sex matched) with headache other than migraine were considered as control group. Serum homocysteine levels were estimated for both groups and other relevant investigations were done in selective cases. Comparison of serum homocysteine levels between two groups were done to see association of serum homocysteine level with migraine in adults.* ***Results:*** *A total of 50 women and 15 men with mean age of 31 (±10.41) years and 50 women and 15 men with mean age of 33 (±10.91) years constituted case and control groups, respectively. The mean (±SD) serum homocysteine level in case group 10.71 (±4.16) ìmol/L was significantly higher than control group 7.62 (±2.26) ìmol/L, (P <0.001).The mean value of serum homocysteine level in migraine without aura (MWOA) patients 11.87 (±4.18) ìmol/L was found significantly higher than migraine with aura (MWA) patients 8.23 (±1.51) ìmol/L, (p<0.05). There was no significant correlation between severity of migraine headache and frequency of migraine attack with serum homocysteine level.* ***Conclusion:*** *Serum homocysteine level was found significantly higher in migraineurs than non-migraineurs.*

Key words: *Homocysteine, Migraine, Hyperhomocysteinemia, Aura etc.*

Introduction:

Headache is the most common symptom in neurology and very common symptom in other systemic diseases. Headache is defined as pain or any kind of discomfort in the head excluding the lower part of the face and including the upper part of the neck. The pain arises from the pain sensitive structure in the head e.g. from blood vessels, venous sinuses, meninges mostly from basal dura, nerve roots, scalp, orbital contents, Para nasal sinuses, teeth, gum etc. as all have pain receptors, These receptors when stimulated either by

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distension, traction, compression or irritation the pain sensation starts & then goes to thalamus when we perceive only pain not the nature but when goes to sensory cortex then we can identify the nature of pain[1].Most headaches are due to primary headache disorders like tension type headache, migraine and cluster headache. Migraine is the second most common cause of headache and headache related disability in the world[2]. Epidemiological studies have documented its high prevalence and high socio-economic and personal impacts. In the Global Burden of Disease Survey 2010, it was ranked as the third most prevalent disorder and seventh-highest specific cause of disability worldwide[3].

Migraine is an episodic primary headache disorder characterized by recurrent attacks of various combinations of headache with neurological, gastrointestinal and autonomic symptoms such as mood change, fatigue, yawning, neck stiffness, polyuria, gastrointestinal disturbance, and a variety of visual, somatic sensory and cognitive phenomena [4]. Migraine is a complex neurological disorder characterized by debilitating headaches and a variety of additional symptoms such as nausea, vomiting, photophobia, phonophobia, occasional sensory disturbances, and other conditions caused by neurological dysfunction in varying combinations[5]. It is a highly prevalent disorder characterized by periodic, commonly unilateral, often pulsatile headaches that begin in childhood, adolescence or early adult life and recur with diminishing frequency during advancing years[6]. The lifetime prevalence of migraine is about 33% in women and 13% in men[7]. The tension type headache was the commonest type (71.13%) followed by migraine (26.05%) and there was a significant preponderance of females (M:F=1:2) of all the subtypes of migraine found with the study done in Bangladeshi populations[8]. However, the presentation diversity of migraine is complex; two common clinical syndromes associated with migrainous headache can be identified easily that are migraine with aura and migraine without aura. Migraine with aura is called classical or neurologic migraine and migraine without aura is known as common migraine. The ratio of classical to common migraine is 1:5[6].

The exact pathogenesis of migraine is still not fully understood but several possible theories have been proposed, “The trigemino-vascular theory of Moskowitz”[9] is one of those. According to the trigemino-vascular theory, the activation of the trigemino-vascular system (TGVS) leads to release of vasoactive neuropeptides contained in their peripheral nerve endings, especially the calcitonin gene-related peptide (CGRP), production of reactive oxygen species (ROS) and homocysteine. Homocysteine causes migraine headache attack through inhibition of GABA-A receptor, activation of NMDA receptor, neurogenic inflammation and oxidative stress[10].

Materials & Methods:

Patients with migraine headache (with Aura and without Aura) according to ICHD-3 criteria, age more than 18 years were enrolled as case group. Age and sex matched headache (other than migraine) patients, age more than 18 years were selected as control group. Patients who were younger than 18 years, pregnant and breast feeding mother, on vitamin B6, B9 and B12 supplementation, taking drugs which interfere with serum homocysteine level such as Anticoagulant, NSAIDs, Antiplatelet agents, Alcohol, Diuretics, Steroids, Estrogen, Methotrexate and other anti-Folate drugs. A semi-structured questionnaire was developed in English. Approval from the Institutional Review Board (IRB) of BSMMU was obtained prior to the commencement of this study. The aims and objectives of the study along with its procedure, risks and benefits were explained to the respondents in easily understandable local language and informed written consent was taken from each. After taking proper history, physical and neurological examination, serum homocysteine level and other relevant investigations were done. Proper diagnosis and treatment were ensured for each person of both groups. For serum homocysteine level, 3cc of whole blood sample was collected from antecubital vein after asepsis. The serum homocysteine level was estimated using photo-spectrometry technique by Architect plus ci 4100 auto analyzer system in the Department of Biochemistry, BSMMU, Dhaka, Bangladesh.

Statistical analysis

At the end of data collection, the frequency of homocysteine were determined for both case and control groups. Then, the mean and standard deviation of serum levels of homocysteine of both case and control groups were calculated. Quantitative data were analyzed by unpaired sample t-test, comparison of two percentages (proportion) between two groups were done by Z-test of proportion and qualitative data were analyzed by χ^2 test. To see the correlation of severity and frequency of migraine with homocysteine, Spearman's rank correlation coefficient test and box plot diagram were used. At 95% Confidence Interval P value < 0.05 was considered as significant. All statistical analyses were done by SPSS software windows version 22.

Results and Observations:

This case control study was carried out in the Headache Clinic, Inpatient and Outpatient Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka. Patients, who were diagnosed as migraine (with aura or without aura) according to ICHD-3 criteria, were considered as case group and patients with headache other than migraine were considered as control group. A total 130 patients were recruited as study population, of them 65 patients were grouped as case and another 65 patients (age and sex match) were as control.

Table-I

Distribution of the study groups by gender (n=130)

Gender	Groups		P-Value
	Case (%)	Control (%)	
Male	15 (23.1)	15 (23.1)	1.000*ns
Female	50 (76.9)	50 (76.9)	
Total	65 (100)	65 (100)	

ns = non-significant,* P- value was derived from Chi square test

Gender distribution showed that both in case and control group, 76.9% were female and 23.1% were male.

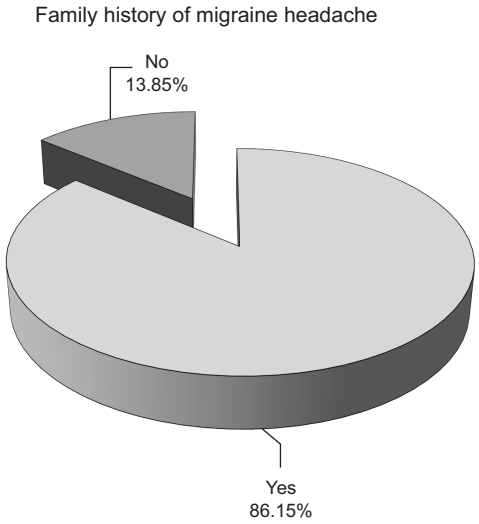

Fig.-1: *Pie charts showing family history of migraine in patients*

This figure exploded pie chart representing the percentage of patient having family history of migraine (86.15%) and 13.85% having no family history of migraine.

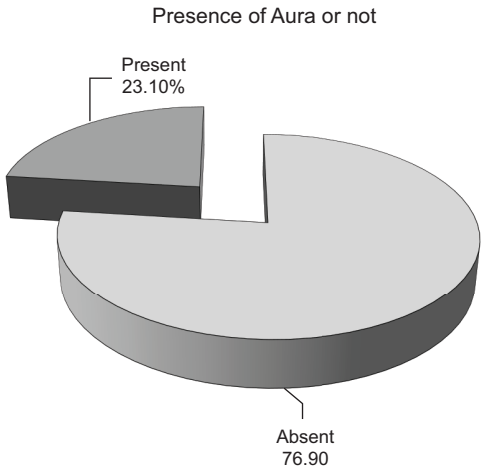

Fig.-2: *Pie chart showing distribution of migraine patients according to aura*

Piechart shows that a major portion of migraine patients were without aura(76.90%). Only 23.10% migraine patients gave history of experiencing aura.

Table-II

The frequency of serum homocysteine level in both case and control groups

Serum homocysteine level (μmol/L)	Groups		P-Value
	Case(%)n=65	Control (%)n=65	
0 – 5	0 (0.0)	6 (9.2)	0.0148* s
5 – 10	30 (46.2)	48 (73.8)	0.0020* s
10 – 15	30 (46.2)	11 (16.9)	0.0006* s
>15	5 (7.7)	0 (0.0)	0.0258* s
Mean ± SD	10.71 ± 4.16	7.62 ± 2.26	0.001* * s

s = significant,* p- value was derived from proportion test,* * p- value was derived from independent sample t test.

Table II shows case versus control serum homocysteine level, in between 10–15 μmol/L 30 (46.2)%vs 11(16.9%); in between 5–10 μmol/L 30 (46.2%) vs 48(73.8%) and only 5 (7.7%) cases had hyperhomocysteinemia. The mean (±SD) value of serum homocysteine level in migraine patients 10.71 (±4.16) was found increased than in control group 7.62 (±2.26) which was statistically significant ($p<0.05$).

This figure 3 shows correlation between headache types and serum homocysteine level both in case and control groups. As headache type is ordinal variable and serum homocysteine level is quantitative variable, so Spearman's rank Correlation coefficient test was done. Here we found positive correlation co-efficient ($r_s = 0.464$)

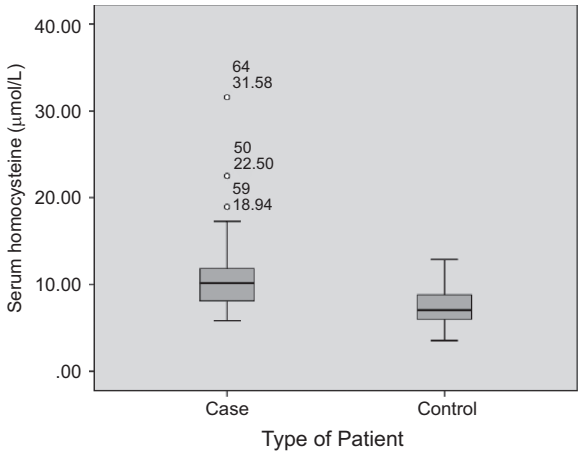

Fig.-3: *Box plot diagram showing correlation between headache types and serum homocysteine level (in both groups).*

Table-III

Mean of serum homocysteine levels by gender both case & control group

Gender	Serum homocysteine level (μmol/L)	Case	Control	P value
Female (n=50)	Mean ± SD	9.57 ± 2.73	6.87 ± 1.54	<0.001ð s
Male (n=15)	Mean ± SD	14.51 ± 5.74	10.14 ± 2.49	0.017 ð s

s =significant, * p- value was derived from independent sample t test.

Table-IV

Mean of serum homocysteine levels among the patients with aura and patients without aura (in case group).

Serum homocysteine Level (μmol/L)	Migraine with aura (n = 15)	Migraine without aura (n = 50)	P valueð
Mean ± SD	8.23 ± 1.51	11.87 ± 4.18	0.0018s

s = significant, * p- value was derived from independent sample t test.

which was statistically significant (p<0.05). Therefore, we can conclude that headache is significantly correlated with serum homocysteine level.

Table III shows the mean value of serum homocysteine level in female migraine patients 9.57 (±2.73) μmol/L was found increased than control group6.87 (±1.54) μmol/L which was statistically significant (p<0.05). The mean value of serum homocysteine level in male migraine patients14.51 (±5.74) μmol/L was found increased than control group 10.14 (±2.49) μmol/L which was statistically significant (p<0.05).

Table IV shows the mean value of serum homocysteine level in migraine patients without aura 11.87 (±4.18) was found increased than in migraine patients with aura (8.23 ± 1.51) which was statistically significant (p<0.05).

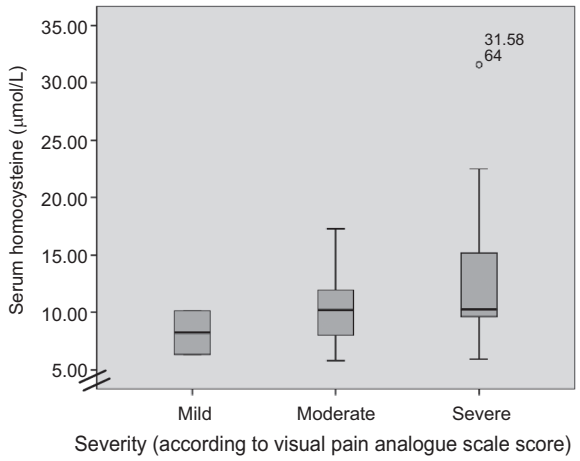

Fig.-4: *Box plot diagram showing correlation between severity of migraine headache (according to VAS score) and serum homocysteine level of migraine patients.*

This figure 4 shows correlation between migraine headache severity (according to VAS score) and serum homocysteine level of migraine patients. Spearman's rank Correlation coefficient test was done. Here we found positive correlation co-efficient (r_s= 0.144)which is not statistically significant (p>0.05).

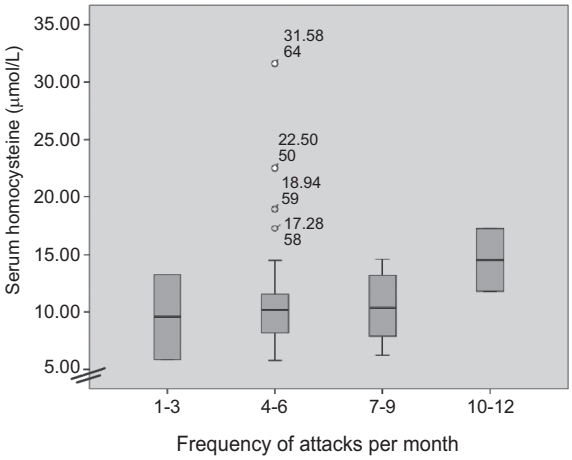

Fig.-5: *Box plot diagram showing correlation between frequency of migraine headache attack per month and serum homocysteine level of migraine patients*

This figure 5 shows correlation between frequency of migraine headache attack per month and serum homocysteine level of migraine patients. Spearman' srank correlation coefficient test was done. Here we found positive correlation co-efficient (r_s= 0.124) which is not statistically significant p>0.05).

Discussion:

Homocysteine, as a highly reactive amino acid, is known to produce endothelial injury via impaired nitric oxide (NO) release that leads to important alterations in vascular function and the coagulant features of the blood. Therefore, homocysteine-related endothelial dysfunction may be included in the initiation and maintenance of a migraine episode. In our study, the mean (±SD) serum homocysteine level in migraine patients was found significantly increased than control group [10.71 (±4.16) μmol/L vs. 7.62 (±2.26) μmol/L, (p<0.05)]. Gavganiand Hoseninian[11] found significantly higher mean serum homocysteine level in migraine patients than control group [14.49 (±5.03) μmol/ Lvs 10.92 (±4.68) μmol/L, p< 0.001] which was similar to our study. We divided the both case and control group into male and female subgroup for separate statistical analysis. The mean value of serum homocysteine level in female migraine patients 9.57 (±2.73) μmol/L was found significantly

increased than female control group 6.87 (±1.54) μmol/L. Gavgani and Hoseninian [11] found significantly higher mean serum homocysteine level in female migraine patients than female control group [14.50 (±5.51) vs 11.16 (±4.46) μmol/L] which coincides with our study.The mean value of serum homocysteine level in male migraine patients 14.51 (±5.74) μmol/L was found significantly increased than male control group 10.14 (±2.49) μmol/L. Gavgani and Hoseninian [11] found statistically significantly higher mean serum homocysteine level in male migraine patients than male control group [14.48 (±4.26) vs 10.59 (±5.45) μmol/L which coincides with our study. These results may show the increasing importance of homocysteinein migraine etiopathology and it is possible that the different levels of homocysteine are due to ethnic differences and also dietary habit.

Hyperhomocysteinemia is an independent and graded cardiovascular risk factor. Homocysteine did not affect the expression of endothelial nitric oxide synthase (eNOS), but it stimulated formation of superoxide anions leading to neuronal inflammation and vascular damage[12]. About 20-40% of strokes in women with migraine seem to develop directly from a migraine attack. The prevalence of hyperhomocysteinemia was 7.69% in patients with migraine. Pizza [13] conducted a study and found the prevalence of hyperhomocysteinemia 29.16% in patient with migraine which was not similar to our study, may be due to ethnic difference. These incidences of stroke may be due to high level of serum homocysteine which causes both the events.

The elevated serum homocysteine level causes primarily endothelial cell injury, trigeminal cell firing, and alterations in the coagulant features of blood. Inflammation in the meninges and dilatation of cerebral vessels are thought to cause the pain associated with migraine, which leads to spontaneous trigeminal cell firing. Thus, homocysteine dysfunction can increase the tendency to develop migraine.

Oxidative damage to the vascular endothelium by way of formation of superoxide anions may also increase the probability of migraine and other vascular disorders such as stroke, cardiovascular diseases, vasculitis[5]. The mean value of serum homocysteine level patients migraine without aura 11.87 (±4.18) μmol/L was found increased than patients migraine with aura 8.23 (±1.51) μmol/L which was statistically significant. Bokharietal (14)found plasma homocysteine level was significantly associated with migraine without aura (MWOA) and plasma homocysteine level was lower inpatients migraine with aura (MWA) than in patients migraine without aura (MWOA) which was similar with our study.

As we found significantly high level of serum homocysteine level in migraine patients, so we also observed the correlation between serum homocysteine level with migraine headache, headache severity and frequency of migraine attack per month. Where, we found significant positive correlation between for serum homocysteine level and migraine headache but statistically non-significant positive correlation between for serum homocysteine level with migraine headache severity and frequency of migraine attack per month, ($p>0.05$). Significant relationship between serum levels of homocysteine and characteristics of migraine attacks such as severity, frequency and duration were not found in studies conducted by Sadeghiet al.,[15] and Kharb et al.,[16] which were similar to our study.

Homocysteine and migraine: A narrative review conducted by Lippi et al.,[10] found that in 5 of the 14 cross-sectional studies that measured plasma homocysteine levels, the concentration of plasma homocysteine was found to be significantly higher in migraine patients than in controls andthe concentration of plasma homocysteine was not found to be significantly higher in migraine patients than in controls in remaining 9 studies. In all four trials the treatment of hyperhomocysteinemia with folic acid (Vitamin B9) or vitamin B supplementation was effective to lower the plasma values of this biomarker, and was also associated with a decreased frequency and/or severity of migraine headache attacks. This association remains largely controversial, however, since it could not be clearly demonstrated that these two biological effects were directly linked. It is also noteworthy that vitamin B and folic acid may have some beneficial effect on

migraine on the basis of serum homocysteine having a role of migraine pathogenesis. In our study we found serum homocysteine level is significantly higher in migraine patients than in controls. Hence, Vitamin B and Folic acid supplementation may be a possible prophylactic and therapeutic approach.

Conclusion:

The study suggests that the mean serum homocysteine level in migraineurs was significantly higher than that of non-migraineurs. The mean value of serum homocysteine level in female migraine patients was found increased than female control group which was statistically significant. The mean serum homocysteine level in male migraine was significantly higher than that of male control group. The mean serum homocysteine level in migraine patients without aura was significantly higher than in migraine patients with aura. Migraine headache severity and frequency of migraine attack were not affected by serum homocysteine level.

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Association of Serum Uric Acid level in Patients with Alzheimer's Disease

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Abstract:

Background: *Alzheimer's disease is the most common cause of dementia. Uric acid is the end product of purine metabolism in humans and acts as a natural antioxidant, accounting up to 60% of the free radical scavenging activity in human blood to prevent free radicals induced oxidative cell injury. This study aimed to explore the association between serum uric acid level and cognitive impairment of Alzheimer's disease patients compared to those of the non-demented age and sex matched controls.* ***Methods:*** *This case control study was carried out in the department of neurology, BSMMU, Dhaka. Total 116 patients were enrolled as study population after satisfying inclusion and exclusion criteria. Among them, 58 were grouped as case and rest 58 were control. All blood samples for serum uric acid were measured in the Biochemistry lab, Department of Biochemistry, BSMMU, Dhaka.* ***Results:*** *A signiûcant reduction of serum uric acid levels in the AD group was found compared to those of the control group (4.35±1.59 Vs 6.89±1.68) which was statistically significant (p<0.001). We also found a positive correlation between serum uric acid levels with severity of Alzheimer's disease (rp = 0.633, P<0.001). Among demographic variables educational qualification was statistically significant (p=0.006) in AD patients.* ***Conclusion:*** *This study showed that oxidative injuries have an important role in the pathogenesis of AD. Higher levels of uric acid are associated with a decreased risk of dementia and better cognitive function later in life.*

Key words: *Antioxidants, Alzheimer's disease, Uric acid, Oxidative Injuries etc.*

Introduction:

According to WHO (2015), the number of people living with dementia worldwide in 2015 was estimated at 47.47 million, will reach 75.63 million in 2030 and 135.46 million in 2050[1]. In Bangladesh 4,60,000 people was estimated with dementia in 2015 , will reach 8,34,000 in 2030 and 21,93,000 people will live with dementia in 2050 respectively[2]. Elderly people over 65 years of age Alzheimer's disease (AD) is the most common form of dementia (70%). Pathological hallmarks of Alzheimer's disease in the brains are neuritic plaques

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composed mainly by extracellular fibrillary β-amyloid deposition, with subsequent intra neuronal hyper phosphorylated tau protein aggregation[3]. Increasing evidence suggests that oxidative stress has a key role in late-onset sporadic forms, which are the majority of Alzheimer's disease cases. Abnormal levels of oxidative stress have been reported in Alzheimer's disease in both the brain and blood stream[4]. Changes in Alzheimer's disease that produce a pro oxidative imbalance have been attributed to decrease in antioxidant defenses, toxicity related to amyloid-β, and/or altered metal metabolism in the brain and peripheral tissues[4] .

The free radical hypothesis of aging suggests that the age-related accumulation of reactive oxygen species (ROS) may result in damage to major cell components in specific brain region[5-8]. Central nervous system (CNS) is vulnerable to free radical damage owing to the high brain oxygen consumption, low content of antioxidants enzymes, compared to other tissues[7].

Uric acid is the final product of purine metabolism in humans[8].Uric acid is a natural antioxidant, accounting for up to 60% of the free radical scavenging activity in human blood (Ames et al., 1981). Uric acid can scavenge superoxide, the hydroxyl radical, and singlet oxygen[9]. Uric acid is also very effective in preventing peroxynitrite from nitrating the tyrosine residues of proteins, thereby preventing the inactivation of cellular enzymes and modification of the cytoskeleton[10]. Uric acid also has the ability to bind with iron and inhibit iron-dependent ascorbate oxidation, preventing an increased production of free radicals that can further contribute to oxidative damage[11]. Thus, a reduced Uric acid concentration may decrease the ability of the body to prevent peroxynitrite and other free radicals induced oxidative cell injury[12]. The protective effect of Uric acid on neuro degeneration has been widely studied, which has revealed elevated serum levels of Uric acid to be associated with slower disease progression in patients with Alzheimer's disease and other neurodegenerative diseases[13]. Some recent study showed Uric acid levels are significantly (p= 0.033) lower in Alzheimer's disease patients in comparison to control subjects. Uric acid may have a protective role against Alzheimer's disease[12].

A significant reduction in the albumin, bilirubin, and uric acid levels in the Alzheimer's disease group was found compared to those of the control group[14]. Higher levels of uric acid are associated with a decreased risk of dementia and better cognitive function later in life[15]. But another report found no difference[16]. So the exact role of uric acid in Alzheimer's disease and even whether the low serum uric acid level is a cause or a consequence of disease is not clear. More studies are required to clarify the meaning and possible therapeutic implications of uric acid changes in Alzheimer's disease. With this aim we tried to evaluate the relationship between serum uric acid level and cognitive impairment of Alzheimer's patients and compared them with a group of nondementic age and sex matched control considering other possible markers of age-related cognitive decline.

Methods:

In this case control study was carried out in the department of neurology, Bangabandhu Sheikh Mujib Medical University. After ethical clearance from institutional review board (IRB-BSMMU), total 58 AD patients were grouped as case and same number of non-demented age and sex matched patients were grouped as control. All AD patients were diagnosed by NINCDS-ADRDA (2007) criteria[17]. The bangla version of Mini mental state examination was used to evaluate the cognitive functions of all patients[18]. It tests five areas of cognitive functions: orientation, registration, attention/calculation, recall and language. Folstein (1975) interpretation of MMSE scores was used for severity assessment where score 21-25 means mild dementia, score 10-20 means moderate dementia and score <10 indicates severe dementia[19]. Non-demented was defined as a score of >25 orbetter. Both groups of patients had no major medical illnesses such as hypercholesterolemia, hypertension, renal disease, diabetes. Informed written consent was obtained from all subjects or legal guardians.From each patient, 3 cc of whole blood sample was taken by standard venipuncture from antecubital vein after

proper asepsis. All blood samples for serum uric acid were measured in the Department of Biochemistry, BSMMU as soon as possible by using photometric technology in Beckman Coulter AU680 auto analyzer[20]. The medical records, demographics, clinical and laboratory records of all patients were examined. All data were checked. Data processing and statistical analysis was done by SPSS (statistical package for social science) version 22 programme.

Results:

No significant difference were observed between case and control groups for age (p=0.062), gender (p=0.0851), occupation (p=0.689) and smoking (p=0.440), respectively (Table 1). Educational qualification showed significant difference between two study groups (p=0.006) (Table 1). 12.1% AD patients had positive family history (Figure 1). Mean (±SD) age of dementia duration in case group was 3.0 (± 1.4) years. Most of the AD patients were presented with moderate dementia (69%) followed my mild dementia (22.4%) then severe dementia (8.9%).

A significant reduction of serum uric acid levels in the AD group was found compared to those of the control group (4.35±1.59 Vs 6.89±1.68) which was statistically significant (p<0.001) (Table 2). The scorers of MMSE showed a positive correlation with serum uric acid levels (rp = 0.633, P<0.001) but duration of dementia didn't find no positive correlation with serum uric acid level (Figure 2).

Table-I

Demographic characteristic of study groups with case versus control

Variables	Groups		P- values
	Case (n=58)	Control (n=58)	
Age (years)	65 ± 9.32	61 ± 11.09	0.062
Gender (%)			
Male	34 (58.6)	33 (56.9)	0.851
Female	24 (41.4)	25 (43.1)	
Occupational status (%)			
Housewife	23 (39.7)	18 (31.0)	0.689
Service holder	12 (20.7)	11 (19)	
Others	23 (39.7)	29 (44.7)	
Educational qualifications (%)			
Illiterate	9 (15.5)	7 (12.1)	0.006
Less then 10 class	30 (51.7)	31(53.5)	
More then 10 class	19 (32.8)	20 (34.4)	
Smoking (%)			
Smoker	23 (39.7)	19 (32.8)	0.440
Non smoker	35 (60.3)	39 (67.2)	

Table-II

Serum uric acid values of study groups with case versus control

	Groups		Total (Case & Control)	P value
Uric acid (mg/dl)	Case (n=58)	Control(n=58)	n = 116	
Mean± (SD)	4.35±1.59	6.89±1.68	5.62±2.07	<0.001

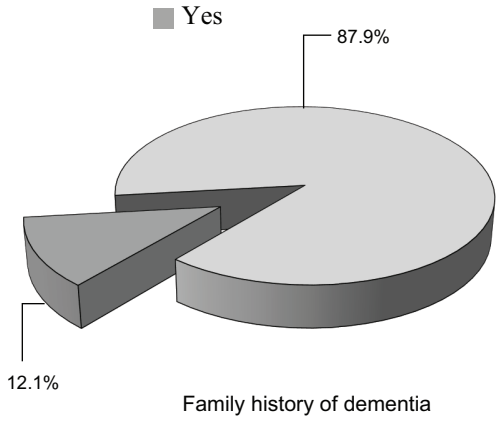

Fig.-1: *Pie chart of Alzheimer's disease patients (case group) according to family history of dementia (n=58)*

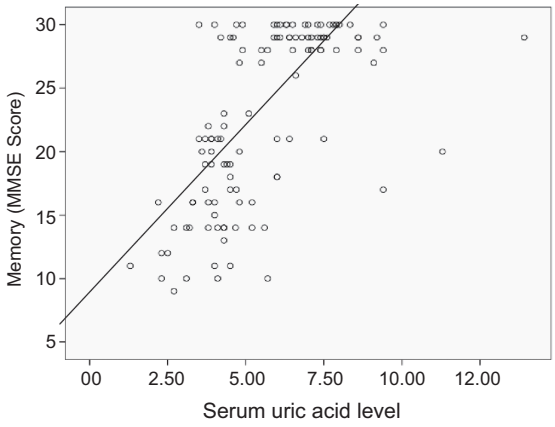

Fig.-2: *Scatter diagram showing positive correlation of Serum uric acid level and MMSE scores in in both case and control groups (n=116)*

Table-III

Unadjusted Odds Ratios of being Alzheimer's disease

Parameter	DF	Standard error	P-value	Odds ratio	CI (95%)	
					Min	Max
Serum uric acid	1	0.1834	**<.0001**	**0.356**	0.249	0.511
Age	1	0.0187	0.0642	1.035	0.998	1.074
Sex	1	0.3760	0.8509	0.932	0.446	1.947
Education	1					
No education		0.6951	0.2939	0.482	0.123	1.883
Primary		0.6700	0.5892	0.696	0.187	2.589
Secondary		0.5742	**0.0209**	0.266	0.086	0.818
Higher secondary		0.7962	**0.0015**	0.080	0.017	0.383
Occupation	1					
House wife		0.4777	0.7054	1.198	0.470	3.055
Others		0.4710	0.4717	0.713	0.283	1.793
Smoking	1	0.3877	0.4402	1.349	0.631	2.884

Table-IV

Adjusted Odds Ratios of being Alzheimer's disease

Parameter	DF	Standard error	P-value	Odds ratio	CI (95%)	
					Min	Max
Serum uric acid	1	0.2221	**<0.001**	**0.321**	0.208	0.497
Education	1					
No education		1.0655	0.9815	1.025	0.127	8.273
Primary		1.1043	0.4334	2.375	0.273	20.689
Secondary		0.8672	0.2729	0.386	0.071	2.114
High secondary		1.0489	0.2262	0.281	0.036	2.196

Discussion:

This current study where mean (± SD) value of serum uric acid level was reduced in AD patients as compared to those of control group (4.35±1.59 Vs 6.89±1.68). Which was statistically significant ($p<0.001$). It was consistent with the other studies like Ames et al., (1981)[9], Khateeb*et al*. (2014)[12], Kim *et al*. (2006)[15], Cervellati et al.,(2012)[21]. All this studies including this one supports the hypothesis that oxidative injuries could play an important role in the pathogenesis of Alzheimer's disease. Reduced serum uric acid level may decrease the ability of the body to prevent free radicals induced cellular injury. Low serum uric acid was not a constant finding in every AD patients. There are possible two hypotheses regarding this finding of uric acid. It is possible that persons with already low serum uric acid levels, who were unable to combat against free radical toxicity leading to the development of inûammation followed by generalized cell destruction, resulting cortical atrophy. Ultimately there will be development of Alzheimer's disease and other neurodegenerative disorders. However, it is also possible that, low serum uric acid level was a consequence of excess consumption or utilization of uric acid for body defense against free radical induced inflammation. So, low serum uric acid levels were a cause or a consequence of Alzheimer's disease is debatable.

This study was also found that MMSE score showed a positive correlation with serum uric acid levels in this study which was analyzed by spearman rank correlation coefficient test. Positive correlation coefficient was observed ($rp=0.633$) which was statistically significant ($p<0.001$). This finding also coincides with previous studies[12,15,22]. Therefore it may be stated that severity of Alzheimer's disease was affected by serum uric acid level. Although this study was found a negative correlation between serum uric acid level with duration of dementia ($rp = -0.142$ with $p=0.287$).

We used a logistic regression analysis to get the effect of serum uric acid on Alzheimer's disease. The adjusted odds ratio was 0.327. That means the odds of developing Alzheimer disease is 0.327 for a participant if a one unit increase of uric acid after eliminating the confounding effect of education. That is, participants have 67.3% lower odds of being developing subsequent Alzheimer disease for a one-unit increase of uric acid.

High serum uric acid levels are associated with a number of disease like Gout, hypertension, cardiovascular disease and kidney disease. So, the use of uric acid in the treatment of neuro-degenerative diseases including Alzheimer's disease is controversial. Although Settle et al. (2014)[23] was suggested that combining uric acid precursors such as inosine with ascorbic acid may have therapeutic benefits for AD patients, but dose and duration of treatment have not been yet determined.

Limitation:

The present case control study was a single centered study, involving only one clinical and laboratory evaluation of the serum uric acid of the individuals without any further follow-up. The changes in serum uric acid levels over time could not be taken into consideration, and therefore, it is not possible to discriminate whether there were differences in serum uric acid levels that preceded the onset of the dementia or whether they developed during the course of the diseases.

Recommendations:

Elderly people should take adequate amount of protein (0.8gm/kg/day). Further multi-centered prospective cohort study with larger sample size could be carried out to see the association of serum uric acid level with the clinical course of Alzheimer's disease. Another Large population based multicenter double blinded studies are needed to find out any treatment response of serum uric acid level against Alzheimer's disease.Comprehensive steps should be taken by government to address this health hazard.

Conclusion:

The present study revealed that serum uric acid level was significantly lower in AD patients in comparison to control group. So, this biomarker might be considered as a risk factor for Alzheimer's disease. In addition this study suggests that there was significant relationship between serum uric acid level and severity of disease although there was no correlation between serum uric acid level and duration of AD.

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Digital Subtraction Angiographic Pattern of Extracranial and Intracranial Atherosclerotic Arterial Stenosis among Ischemic Stroke Patients

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Abstract:

***Background**: Stroke is the second leading cause of death in adult population throughout the world and is the most common cause of severe adult physical disability. Atherosclerotic stenosis is one of the predominant cause of ischemic stroke. The aim of this study was to evaluate the type, number and severity of intracranial and extracranial atherosclerotic stenosis and its association with different risk factors. **Methods:** This prospective observational study was conducted in the Department of Neurology, BSMMU, Dhaka, from July 2017 to August 2018. Only patients having significant (≥50%) symptomatic stenosis were included in this study. **Results**: In total 42 cases, 25 patients had extracranial stenosis, 13 patients had intracranial stenosis and 4 patients had both intracranial and extracranial stenosis. Overall 17 (40.47%) patients have intracranial involvement and 29 (69.04%) patients had extracranial involvement. The most commonly involved intracranial stenotic segment was MCA, present in 8 (32%) out of 25 intracranial segments followed by ICA 7 (28%) and intracranial vertebral artery 4(16%). Most commonly involved extracranial stenotic segment was ICA, present in 37 (77.08%) out of 48 extracranial segments. Diabetes was found to be the most common risk factor of intracranial stenosis (p value 0.022) while hypercholesterolemia was the major risk factor for severe (≥70%) stenosis. **Conclusion**: Extracranial arterial stenosis is more common than intracranial arterial stenosis. Anterior circulation stenosis is more common than posterior circulation stenosis. Intracranial stenosis is more prevalent in diabetic patients. Hypercholesterolemiaismore commonly seen in severe (e"70%) stenosis.*

***Key Words**: Atherosclerotic Stenosis, Digital Subtraction Angiography, Stroke etc.*

Introduction:

The World Health Organization definition of stroke is "Rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than that of vascular origin"[1]. Stroke is the second leading cause of death in adult population throughout the world and is the most common cause of severe adult physical disability. It is also ranked as the sixth leading cause of disability-adjusted life years

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(DALY; one DALY is one lost year of healthy life) in 1990 and is projected to become the fourth leading cause by the year 2020[2].

Strokes are broadly categorized as ischemic or hemorrhagic. Ischemia causes about 80% of stroke cases and 20% are caused by hemorrhage. Ischemic stroke is due to occlusion of a cerebral blood vessel and causes cerebral infarction. The resultant neurologic syndrome corresponds to a portion of the brain that is supplied by one or more cerebral vessels[2].The principle cause of cerebral infarction is atherosclerosis and its sequelae. Atherosclerotic plaques are eccentric focal fibro-fatty intimal thickening and affect large, medium and small arteries[2].

A growing body of data suggest that there are important differences in the distribution of occlusive vascular disease among different ethnic origins [3]. Data from Northern Manhattan Stroke study have shown that intracranial stenosis to be the cause of ischemic stroke in 1% of Caucasian, 6% of African Americans and 11% of Hispanics [3]. In Europeans, intracranial stenosis appears to be the cause of ischemic stroke in 2-7% of the cases in Germany [4,5],10% in Greece and 12% in Spain [6].

For decades, it is well described that patients of Asian, African & Hispanic ancestry were at higher risk of intracranial atherosclerosis [7]. Different studies showed that intracranial stenosis is the most common vascular lesion among ischemic stroke patients from India, Thailand, Singapore, Korea, Japan, China.

For patients with major stroke, the mortality rate in a subsequent stroke is 40%. Prospective Study of Symptomatic Atherothrombotic Intracranial Stenosis (The GESICA Study) found that despite medical treatment, the 2 years recurrence risk rate of ischemic event in the territory of the stenotic artery was 38.2%. Patients with 70% to 99% intracranial stenosis have a > 2-fold risk of stroke in the territory of the stenotic artery than do patients with <70% stenosis [8]. Moreover, most recurrent strokes occurred in same arterial territory, were non lacunar, and nearly half of them were disabling. On the other hand, the stroke recurrence rate is 7% to 13% in patients with symptomatic extracranial stenosis who are treated with appropriate medication[9]. Hence stroke prevention is an important concept. Ability to accurately assess the site of stenosis has become important to identify the patient who would benefit from surgical/ radiological intervention.

Objectives of the study were to evaluate the pattern and distribution of stenosis by Digital Subtraction Angiography though it is invasive, relatively costly and its association with risk factors among ischemic stroke patients in the Bangladeshi population and in other countries for comparison.

Material and Method:

A cross-sectional study was conducted in Dept. of Neurology, BSMMU, Dhaka. An ethical approval was obtained from the Institutional Review Board(IRB). Purposive sampling was performed for 42 known cases of all age groups having the clinical diagnosis of stroke and ischemic stroke on imaging and presence of significant symptomatic stenosis in DSA. Written consent was obtained from all the participants. Patients with stroke of cardioembolic origin and stroke from non-atherosclerotic vasculopathy were excluded from the study. Proper history was taken, physical and neurological examination keeping in mind of the demographic and clinical variables, was done and all relevant investigations were completed. Fitness of the patient for DSA was assessed. DSA was done in the Paediatric Catheter Laboratory of Bangabandhu Sheikh Mujib Medical University (BSMMU). From DSA, information regarding location and degree of atherosclerotic stenosis, number of stenotic segments were obtained according to the specified objectives. Total 145 patients underwent DSA during the study period, among them 82 patients were ischemic stroke. Patients with normal DSA findings, stenosis due to non-atherosclerotic vasculopathy and significant stenosis in asymptomatic side were excluded from the study. Only patients with significant symptomatic stenosis (42 in number) were considered as cases.

The detail history regarding potential risk factors associated with atherosclerotic ischemic stroke

was obtained from each patient and from the medical records. Patients were labeled as hypertensive if their blood pressure surpassed 140 (systolic) and/or 90 (diastolic) mmHg on repeated measurements during hospitalization or when taking anti-hypertensive medications. A diagnosis of diabetes mellitus was based on the American Diabetic Association criteria for diagnosing DM. A person who uses to smoke tobacco in any form (cigarette, tamak, jorda, gul etc) for at least 1 year, he/she was considered as positive for cigarette smoking. Hypercholesterolemia is defined as patients receiving cholesterol-reducing agents or overnight fasting cholesterol level ≥200 mg/dL, or low- density lipoprotein ≥130 mg/dL.

Locations of significant stenosis were categorized as intracranial or extracranial and further in the anterior or posterior circulation. A stenotic lesion which is at or above the petrous part of ICA for carotid system and distal to the point where the vertebral artery pierced the dura at the level of foramen magnum for vertebra-basilar system, is considered as intracranial stenosis Lesions were described as single or multiple based on the number. The degree of stenosis was measured according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET). Data were entered into a database (Microsoft Office Excel 2010). Statistical analyses were performed using statistical software SPSS 24, SPSS Inc., Chicago,USA. The results were analyzed by descriptive statistics and chi-square test.

Results:

Out of 42 cases, there were 31 males and 9 females. The mean age (SD) were 61.55 ± 8.85 years. There was no association between stenosis and age (p>0.05). Out of 42 patients, 25(59.5%) had extracranial stenosis, 13(31%) patients had intracranial stenosis and 4(9.5%) patients had both extracranial and intracranial stenosis. Overall 17(40.47%) patients had intracranial involvement and 29 (69.04%) had extracranial involvement. Out of 73 stenotic segment, 54(73%) stenosis located in anterior circulation and 19(27%) in posterior circulation. 21(50%) patients had single stenosis and 21(50%) had multiple stenosis. Out of 42 patients, 17(40.5%) patients had severe stenosis, 14(33.3%) patients had total occlusion and 11 (26.2%) patients had moderate stenosis.

Total 48 extracranial stenotic segments were present, among which the most commonly involved segment was the internal carotid artery (ICA) in its proximal part after bifurcation 37 (77.08%) followed by the extra cranial segments of the vertebral artery 10(20.83%), common carotid artery (CCA) 1(2.08%). Total 25 intracranial stenotic segments were present in 17 patients (isolated and combined). The most commonly involved segment was the middle cerebral artery (MCA) 8(32%) in its stem. It was followed by the internal carotid artery (ICA) 7 (28%); intracranial segments of the vertebral artery (VA), posterior cerebral artery (PCA), anterior cerebral artery (ACA), basilar artery (BA), and superior cerebellar artery, with the following number of stenotic segments: 4 (16%), 2 (8%), 2 (8%), 1 (4%), 1(4%) respectively. Out of 13 patients with intracranial stenosis 10 (76.9%) had single lesion, 3 (23.1%) had multiple lesions. Out of 25 patients with extracranial stenosis, 11

Table-I

Association between number and type of stenosis

Number VS Type of Stenosis					
Number of stenosis	Type of Stenosis			Total	P-Value
	Intra Cranial Stenosis	Extra Cranial Stenosis	Both Intra and Extra Cranial Stenosis		
	N (%)	N (%)	N (%)	N (%)	
Single	10 (76.9)	11 (44)	0 (0)	21 (50)	0.017*s
Multiple	3 (23.1)	14 (56)	4 (100)	21 (50)	
Total	13 (100)	25 (100)	4 (100)	42 (100)	

Table-II

Association of risk factors with type of stenosis

Risk Factor VS Type of Stenosis Risk Factors	Type of Stenosis			Total	P-Value
	Intracranial Stenosis N (Column%)	Extracranial Stenosis N (Column%)	Both Intra and Extracranial Stenosis N (Column%)	N (Column%)	
Hypertension	12 (92.3)	21 (84)	4 (100)	37 (88.1)	0.560 ns
DM	10 (76.9)	7 (28)	2 (50)	19 (45.2)	0.016*s
Smoking	5 (38.5)	13 (52)	1 (25)	19 (45.2)	0.506 ns
Alcoholism	0 (0)	1 (4)	0 (0)	1 (2.4)	0.706 ns
Hypercholesterolemia	6 (46.2)	12 (48)	2 (50)	20 (47.6)	0.989 ns
Previous Vascular Event	5 (38.5)	11 (44)	3 (75)	19 (45.2)	0.430 ns

Table-III

Association of risk factors with severity of stenosis

Risk Factors VS Severity of stenosis Risk factors	Severity		Total	P-Value
	<70% N (Column%)	≥70% N (Column%)	N (Column%)	
Hypertension	10 (90.9)	27 (87.1)	37 (88.1)	0.737 ns
DM	6 (54.5)	13 (41.9)	19 (45.2)	0.470 ns
Smoking	3 (27.3)	16 (51.6)	19 (45.2)	0.163 ns
Alcoholism	0 (0)	1 (3.2)	1 (2.4)	0.547 ns
Hypercholesterolemia	2 (18.2)	18 (58.1)	20 (47.6)	0.023*s
Previous Vascular Event	3 (27.3)	16 (51.6)	19 (45.2)	0.163 ns

Table-IV

Frequency of extracranialstenotic segments in study population

Extracranial segments	Number of stenosis	%
ICA	37	77.08
VA(extracranial)	10	20.83
CCA	1	2.08
Total	48	100

Table-V

Frequency of intracranial stenotic segments in study population

Intracranial segments	Number of stenosis	%
MCA	8	32
ICA	7	28
VA(Intracranial)	4	16
PCA	2	8
ACA	2	8
BA	1	4
SCA	1	4
Total	25	100

(44%) had single lesion and 14 (56%) had multiple lesions. Single stenosis was found statistically significant for intracranial location (p value 0 .017). Diabetes mellitus was significantly associated with intracranial stenosis (pvalue 0.016) and Hypercholesterolemia was significantly associated with severe (≥70%) stenosis (p value 0.023).

Discussion:

This descriptive observational study was carried out with an aim to evaluate the Digital Subtraction Angiographic pattern of intracranial and extracranial atherosclerotic stenosis among ischemic stroke patients selected from Inpatient, Outpatient and Stroke & Neuro-Intervention clinic of BSMMU. Only the patients with single or multiple significant symptomatic stenosis (≥50% stenosis) were included in this study.In our study out of 42 patients, 25(59.5%) had extracranial stenosis, 13 (31%) patients had intracranial stenosis and 4 (9.5%) patients had both extracranial and intracranial stenosis. Overall 17 (40.47%) patients had intracranial involvement and 29 (69.04%) had extracranial involvement. Wong[10] found 33% to 50% intracranial stenosis in Chinese population with total 345 occlusive arterial segments. Suwanwela[11] found 47% intracranial stenosis in Thailand in 100 patients with significant stenosis, Chang [12]found 47.9% intracranial stenosis in Singapore with 200 cases in total. Shrivastava[2] in a CTA based study found 56% intracranial stenosis in Indian population although only 32 of their 60 cases had significant stenosis.

In contrary, extracranial stenosis is predominant type of lesion in Europe and North America. Sacco RL at al. (1995) found intracranial stenosis in 1%, 6% and 11% among US Whites, US Blacks and US Hispanics respectively. Analyzing the above mentioned study suggest that intracranial stenosis is much more common condition in Asian population than European and US Whites. In our study we found 40.47% intracranial stenosis which to close to the result found by Wong[10] in Chinese population. But a bit away from other studies conducted in our Asian counterpart like Thailand, Singapore, India. This discrepancy of results can be explained by different imaging tools used in other studies (DuplexUSG, TCD, CTA, MRA rather DSA we used), different cut off value for significant stenosis (30% in some study's 50% we used), larger sample size, different inclusion and exclusion criteria, ethnic and geographical difference.

There are several explanation of intracranial atherosclerotic disease (ICAD) being more prevalent in Asians including Bangladesh than in Westerners. It is postulated that during human population evolution and diversification, those who settled in Europe had acquired a stroke-suppressor genotype increases their resistance against atherogenesis, but with protection confined to the intracranial large arteries. The contemporary affluent lifestyle accelerates the development of atherosclerosis. In the whites, it involved the whole arterial bed except the intracranial vessels. People living in non-Western countries used to have a healthier way of living. They did not develop significant atherosclerotic disease until recently when a westernized life style was adopted[13].Unlike the whites, their intracranial arteries are not spared. Predisposition of Asian populations toward hypoadiponectinemia may represent another explanation of increased ICAD[14].

In our study, out of 7 stenotic segments, 54 (73%) were located in anterior circulation and 19 (27%) were located in posterior circulation. study in Iran conducted by Borhani-Haghighi[15] found anterior circulation involvement in 301(88%) patients and posterior circulation involvement in 128(37.4%) patients in their total 342 patients underwent DSA.

There is statistically significant association found between single stenosis and intracranial location ($p < 0.05$). So single stenosis more likely to be located in intracranial site. In a study conducted by Dae[16] evaluated about pattern of atherosclerotic carotid stenosis in Korean patients with stroke, found prevalence of intracranial stenosis was significantly higher in the single-stenosis group than in the multiple stenosis group ($P < .05$).

We found that out of 48 extracranial stenotic segments, the most commonly involved segment was the internal carotid artery (ICA) in its proximal part after bifurcation: 37 (77.08%) followed by the extracranial segments of the vertebral artery-

10(20.83%). 25 intracranial stenotic segments were present in 17 patients (isolated and combined). The most commonly involved segment was the middle cerebral artery (MCA)-8(32%) in its stem. It was followed by the internal carotid artery (ICA) 7 (28%) and intracranial segments of the vertebral artery (VA) 4 (16%).

In a CTA based study conducted by Shrivastava[2] found MCA as the most commonly involved intracranial stenosis segment, present in 10 (41.6%) out of 24 intracranial segments and ICA as the most commonly involved extracranial stenosis segment, present in 14 (66.6%) out of 21 extracranial segments. These results were supported by Dae[16], Borhani-Haghighi[15]and Mazighi[17].

Only DM but not age, sex, hypertension, dyslipidemia, smoking, previous vascular event was significantly associated with intracranial stenosis. This result coincides with findings of a study conducted by Borhani-Haghighi[15] in Iran. In another study Sung [9] in Taiwanese patients found that DM was the most important determinant of IICS and an independent risk factor for both Isolated ICS and Combined EIS but not for isolated ECS. These results were also supported by Mendes [18] and Hossein[19].

In our study we found association of hypercholesterolemia with severity of stenosis. hypercholesterolemia is significantly associated with severe stenotic lesion (≥70% stenosis). In a study conducted by Turan[8] using data on patients enrolled in the Warfarin-Aspirin Symptomatic Intracranial Disease(WASID) trial found that history of a lipid disorder was the only independent predictor of severe intracranial stenosis (odds ratio1.62; 95% CI, 1.09 to 2.42; P 0.02).

A different interventional procedure can be planned on the basis of the results of our study. The effects of antiplatelet, anticoagulant, and lipid-lowering drugs can be evaluated in the treatment of extracranial and intracranial stenosis with the help of the present study. Limitation of the present study was that the sample size was small and it was a hospital rather than a community based study. Further studies can be conducted by performing a large multicentric study in a different regions of Bangladesh.

Conclusion:

Extracranial arterial stenosis is more common than intracranial arterial stenosis. Frequency of intracranial atherosclerotic stenosis is almost similar to other Aasian populations. Anterior circulation stenosis is more common than posterior circulation stenosis. Single stenosis is more commonly associated with intracranial location. Intracranial stenosis is more prevalent in diabetic patients. Hypercholesterolemia is more commonly seen in severe (≥70%) stenosis.

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Utility of Serum Copper Level Estimation in Patients Suffering from Alzheimer's Disease

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Absract:

Background*: Alzheimer's disease is the most common cause of dementia. Metals such as zinc , copper, iron are likely involved in the neurodegeneration of Alzheimer's disease . Copper can catalyze a flux of reactive oxygen species that can damage functional and structural macromolecules in brain. Most studies found association of high serum copper level with Alzheimer's disease but also some studies did not.* ***Methods****: Total 48 patients of Alzheimer's disease who were diagnosed according to NIA-AA (National institute of Aging – Alzheimer's Association) recommendation (revised NINCDS-ADRDA) criteria were taken as study population purposively and 42 age and sex matched control were selected. Fasting serum copper level were done for both groups. Comparison of serum copper level of Alzheimer's patients with that of the control group were done to see association.* ***Results :*** *A total of 28 male and 20 female with mean age of 66.20 ± 9.42 (mean±SD) years, 22 male and 20 female with mean age of 63.54 ± 9.74 (mean±SD) years constituted as case and control groups, respectively. The mean of serum copper in case and control groups were 0.95 ± 0.37 versus 0.92 ± 0.25 mg/L (P > 0.05). The present study found that serum copper levels are non-significantly higher in patients with AD than control group, however it did not show a significant relationship with severity of dementia.* ***Conclusion****: So our suggestion was to perform a study work including total serum copper level , serum ceruloplasmin level and free serum copper level comparing between a large Alzheimer's Disease patients group and age , sex matched apparently healthy control group to understand the copper dyshomeostasis in Alzheimer' Disease.*

Key words: *Alzheimer's disease, Dementia, Serum Copper etc.*

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of unknown etiology characterized by irreversible cognitive deterioration. It has become more common not only in developed nations but also in developing countries as now the population includes more and more old persons. Though exact cause for the disease is not known, it is closely related to the formation of protein deposits (amyloid plaques) and tangled bundles of fibres (neurofibrillary tangles) within the cortex [1].

Via the portal blood copper goes to liver being bound to albumin, transcuprein, amino acids, small peptides. In hepatocytes, copper binds to one of the copper chaperones [metallothioneins (MTs), reduced glutathione (GSH), etc] regulating the

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traffic of intracellular copper towards ceruloplasmin and other necessary sites .Then in the liver copper is incorporated in ceruloplasmin for secretion into circulation . In blood, about 65%–90% of the copper is bound to ceruloplasmin. The remaining 10–35 percent participate in exchanges with albumin, transcuprein, alpha 2 macroglobulin, and low-molecular-weight compounds .The organs with the high concentrations are the liver, brain, kidney and heart .Copper is excreted from the body either in a non-absorbed form or via the bile mainly[3].

The group of transmembrane copper transporters includes CTR1, ATP7A and ATP7B. ATP7A : expressed in the placenta, gut and nervous system. ATP7B : expressed in the hepatocytes, where it exports copper into the bile and provides copper to nascent ceruloplasmin, and also expressed in the nervous system .ATP7A is critical to deliver copper from endothelial cells across the blood brain barrier(BBB) in the direction of the brain .CTR1is also a key-regulator of copper influx whole body . CTR1 is particularly expressed in the intestinal cells, in the endothelial cells of brain capillaries, in choroid plexus and brain parenchyma .CTR1, divalent metal transporter 1 (DMT1) and ATP7A transport copper also from CSF to blood

Squitti et al[4] in 2006 in their article provided information on the two opposite fluxes of copper supposed to influence copper content in the brain : 1) A blood to brain inward flux, corresponding to the labile fraction of serum copper, that can diffuse or even be transported across the BBB by copper transporter 1-CTR1 and by ATP7A[5]. 2) A brain to blood APP driven outward flux, via APP β-amyloid mediated mechanism across BBB copper comes to circulation , leading to reduction in β-amyloid concentrations in the CSF[6]. Under physiological conditions, activation of NMDA receptor results in increased levels of Aβ and ATP7A mediated copper release into the synaptic cleft of glutamatergic neurons . Both copper and beta-amyloid have been reported separately to again depress long-term potentiation by inactivating NMDA-R activities[7]. However in the AD brain prolonged NMDA-receptor- stimulated Aβ production and ATP7A-mediated copper release into the synaptic cleft can initiate a vicious cycle resulting in altered copper homeostasis . (7).

As AβPP is demonstrated to be a copper detoxification/efflux neuronal transporter in vivo and hypothetised to remove excess Cu from brain tissues and results in increase in serum copper[8]. Although in circulation copper bound to low molecular weight compounds represents only a small fraction of serum copper in AD , excessive levels over time could determine an increase supply of brain soluble copper[9].

Indeed, it is known that the day-to-day and week to week variation in serum copper is insignificant. Thus in AD, the inward copper flux could be considered a chronic condition resulting in a continuous supply of the copper brain reservoir. (Levenson et al 1998).

Aβ possess copper and zinc binding sites. Aβ1-42 has high affinity for copper which could trap excess extracellular copper[10]. At a high peptide to metal ion stoichiometry, Aβ removes the copper into the circulation and is protective. This may explain that serum copper is selectively and markedly elevated in individuals with AD[10]. At high metal- ion- to-peptide stoichiometry, Aβ aggregates and becomes catalytically pro- oxidant. These changes appear at high serum copper concentration as reported by Ashley et al[2].

However, at high metal- ion- to peptide stoichiometry, Aβ, on being catalytically pro-oxidant, leads to simultaneous generation of H2O2(hydrogen peroxide), making the peptide vulnerable to OH• (hydroxyl) attack[11] that can damage functional and structural macromolecules and neuronal degeneration occurs.

Materials and Methods:

This is a cross sectional comparative study which was carried out in Neurology Department of Bangahandhu Sheikh Mujib Medical University (BSMMU), Dhaka and Atomic Energy Commission (AEC), Dhaka during March'2016 to June'2017, 48 Alzheimer's Disease patients and 42 age and sex matched non-demented patients were selected as controls from Neurology Out and in Patient Department of BSMMU, Dhaka andblood samples for trace elements were analyzed at AEC, Dhaka.

Patients having features of AD according to Revised NINCDS-ADRDA criteria (12) were selected. Informed written consent was taken from each patient or his/her attendants. After taking proper history, physical, neurological examination including MMSE were done. The cognitive impairment was assessed by MMSE score (Mild 20-24, Moderate 10-19, Severe <10). Relevant investigations including MRI of brain were done to diagnose AD and rule out other causes of dementia. Diagnosis of AD was established before doing serum copper level. 4 ml venous blood was collected for serum copper level in fasting condition from cases and controls and centrifuged immediately, serum was stored at -20°c in the Department of Microbiology of BSMMU for analysis. All blood samples were measured by Atomic Absorption Spectrophotometry (model: AA240FS, origin: Australia, manufacturer: Varian) technology in the Department of Analytical Chemistry Laboratory , Atomic Energy Centre , Dhaka-1000. The normal ranges of serum copper level is 0.7-1.4mg/L or 10-22 µmol/L (Mayo-clinic , 2018)[13].

Statistical analysis:

At the end of data collection, the mean and standard deviation of serum levels of copper of both case and control group were calculated. Quantitative data were analyzed by unpaired t test and qualitative data were analyzed by χ^2 test. P value <0.05 was considered as significant.

In the AD group, the correlation among serum copper levels, MMSE score, duration of the disease, age were measured by the pearson's correlation coefficient test and the correlation between serum copper levels and the severity of dementia was measured by the spearman rank correlation coefficient test . All statistical analysis were done by SPSS software windows version 22.

Results and Observations:

The mean age (±SD) was 69.20 (±9.42) years in case group and 63.54 (±9.74) year in control group. There is no significant difference in age distribution between case and control (P>0.05).

In case and control group, there were respectively 58.3% and 52.4% male and 41.7% and 47.6% female. Statistically no significant difference was observed between the two groups in terms of gender (P>0.05).

Among 48 AD patients family history of dementia was present in 15% patients. Most of the patients presented with moderate dementia (50%) followed by severe dementia (27.1%) then mild dementia (22.9%).

Table-I

Distribution of the study population according to serum copper level (N-90).

Serum copper level (mg/L)	Case (n=48)	Control (n=42)	p-value*
Mean ± SD	0.95±0.37	0.92±0.25	.765^{NS}

NS=non-significant

* p- value was derived from independent sample t test.

Table I shows mean serum copper level in case group was 0.95mg/L with standard deviation ± 0.37, in control group was 0.92 with standard deviation ±0.25.Serum copper level in case group was elevated than control but it was not statistically significant.

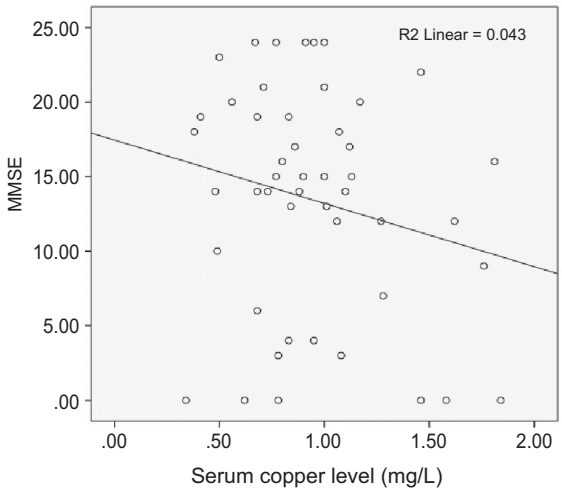

Fig.-1: *Scatter diagram showing correlation between serum copper level and MMSE score in Alzheimer's disease patients (N-48).*

This figure shows correlation between MMSE score and serum copper level in AD patients. As both MMSE score and serum copper level are

quantitative variables, so Pearson's correlation coefficient test was done. Here we found negative correlation co-efficient (r= -0.207) which is not statistically significant (p>0.05). To show the correlation between severity of dementia and serum copper level in AD patients spearman's rank correlation coefficient test was done. Here we found positive correlation co-efficient (r_s=0.139) which is not statistically significant (p>0.05). To find the correlation between serum copper level and disease duration in AD patients Pearson's correlation coefficient test was done. Here we found negative correlation co-efficient (r= -0.017) which is not statistically significant (p>0.05).

For finding the correlation between serum copper level and age in AD patients we did Pearson's correlation coefficient test. Here we found positive correlation co-efficient (r= 0.013) which is also not statistically significant (p>0.05).

Discussion:

In this study analysis of age distribution showed that, the mean age of Alzheimer's disease patients and control group was [66.20 (± 9.42) vs 63.54 (± 9.74)] years. But there was no significant difference in mean age between two groups (P>0.05). In concordance with our study Haqet al[14] found a mean age of 66.84 years among patients of dementia in a tertiary care center of Bangladesh. Our study also coincides with studies like Talebi et al (15), Koseoglu and Karaman[16], Quadriet al[17], and Leblhuberet al[18] but age group seemed to be higher in comparison to this study. It might be due to lower life expectancy of peoples in our country.

In case and control group, there were respectively 58.3% and 52.4% male and 41.7% and 47.6% female. There was male preponderance both in case and control groups. Statistically no significant difference was observed between the two groups in terms of gender (P>0.05). It was consistent with studies like Karimiet al[19]),Talebiet al[16] but does not coincide with studies like Chen et al[20], Koseoglu and Karaman[17],Quadriet al[18], Clarke et al[21]. In context of our country, lower proportion of female patients were enrolled in this study may be due to less preference for females for seeking medical attention. Among all the patients, a major portion of study population had the primary education accounting 31.9%, which is closely followed by illiteracy 27.7% and secondary education 17% in case group. Among all AD patients, 76.6% patients belongs to lower educational level (Illiterate upto SSC). It coincides with studies like Letenneuret al[22] and Ott et al[23] where they found an association between low educational level and higher risk of developing AD. Family history of dementia was present in 15% in the AD patients. The MIRAGE study confirmed that family history of dementia is an important risk factor for Alzheimer's and concluded that by 80 children of conjugal AD couples had a cumulative risk of 54%, 1.5 times greater than the sum of the risks to children having affected mothers or fathers, and nearly 5 times greater than the risk to children having normal parents[24]. Most of the patients presented with moderate dementia (50%), followed in decreasing order by severe dementia (27.1%) and mild dementia (22.9%).

The mean (±SD) value (mg/L) of serum copper level in AD patients was found increased than control group [0.95 (±0.37) vs. .92 (±0.25)], although, this was not statistically significant (p =.765^{NS}). It coincides with studies like Sedighi, Shafa and Shariati[25], Agarwalet al[26], Singh et al[27], Wang et al[28], Pagliaet al[29], Siottoet al[30], and Li et al[31] except that most of the study found a statistically significant association of dementia with serum copper level. Similar to the present study, Sedighi, Shafa and Shariati, (2006) found a tiny increase in serum copper levels between the study and the control populations but which was also not statistically significant (137.8 + 19.8 mg/dl versus 132.5 + 15.7 mg/dl, p=0.14). On the other hand, Agarwalet al(26) in their study found serum copper level was 156.2±30.3 µg/dL in AD patients and 134.46±31.57µg/dL in healthy control and the difference was statistically significant (p=0.002). Singh et al(27) found serum copper level in AD and healthy controls respectively 116.20±3.23µg/dL and 94.71±1.68µg/dL and the difference was statistically significant (p=<0.001). Siottoet al (30) found that increases of one µmol/L unit of free Cu levels significantly raised the adjusted odds of

having AD by 60% (adjusted OR= 1.60, 95% CI = 1.13–2.26; p = 0.008). Li and his team conducted a meta-analysis of studies assessing the Serum Copper, Zinc, and Iron Levels in Patients with Alzheimer's Disease[31]. They found that 26 studies reported an increase of serum Cu levels in AD patients, 9 studies reported a decrease of serum Cu levels in AD patients and 2 studies reported a tiny increase. Combined analysis of the relationship between the serum Cu level and AD was done in their study. Their meta-analysis showed that Cu levels were significantly higher in AD patients than controls . Previously published meta-analysis results from Bucossi et al[32], Schrag et al[33] and Wang et al[28] also came to the same conclusion .

It coincides with studies like Lee and Park[34]. They found a significant correlation between baseline serum copper level and MMSE score from second year onwards in AD patients (r=-0.692, p=0.004). As both serum copper level and disease duration are quantitative variables, so Pearson's correlation coefficient test was done. Here we found negative correlation co-efficient (r= -0.017) which is not statistically significant (p>0.05).

We found positive correlation co-efficient between serum copper level and age (r= .013). This was not statistically significant (p>0.05). Fu, Jiang and Zheng, (35) showed an age related increase in brain copper levels in normal people. On the other hand age is an important risk factor AD itself (30) Therefore, the slightly higher level of serum copper with increasing age should be investigated further in context of Alzheimer's disease.

Our study along with the studies discussed above suggests a positive correlation of serum copper level with Alzheimer's disease. Some other studies have found an association between defective ceruloplasminlevel in the blood with AD (36). Therefore, it may be more beneficial to consider S. copper level together with ceruloplasmin or free S. copper when investigating this topic.

Conclusion:

The present study showed that the serum copper level was not correlated in AD patients in comparison to control group. However there is also no significant relationship between serum copper level and severity of disease or relationship with disease duration. However the sample size was small , study population were enrolled from only one center hence it may not represent the whole population of the country. All the investigations meeting the need for exclusion criteria could not be done. As many of them were decided clinically but still it remains as a limitation, such as full hepatic evaluation , HIV serology etc. Further multi-centered prospective cohort study with larger sample could be carried out. Again an study should be carried out with total serum copper , free copper and serum ceruloplasmin in AD patients and age sex matched healthy control.

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CASE REPORT

Guillain Barré Syndrome after Thrombolysis With Streptokinase for Acute Myocardial Infarction: A Case Report

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Abstract:

We would like to report on a patient, a 52-year-old man with acute neurologic disorder, Guillain Barré Syndrome. He was successfully treated by intravenous immunoglobulin. The patient suffered from acute extensive anterior MI. 2 weeks after thrombolytic therapy with streptokinase, he developed GBS.

Key Words: *Guillain Barré Syndrome, Myocardial Infarction, Streptokinase etc.*

Introduction:

Guillain Barré Syndrome is an acute immune mediated peripheral neuropathy. It is a rapidly evolving polyradiculoneuropathy preceded by a triggering event, most often respiratory or gastrointestinal illness. There are reports of some cases of GBS after intravenous streptokinase administration[1-4]. In 1983, journal of the American Medical Association published an article "Possible association of Guillain Barré Syndrome with thrombolytic therapy". In 1992, British Medical Journal also reported "Guillain Barré syndrome after treatment with streptokinase". International Journal of Cardiology reported a case titled "Guillain Barré Syndrome after myocardial infarction" in the year 2003. Here we will describe about a patient of GBS who was admitted in BSMMU, Dhaka.

Case Report:

The patient was a 52 year old man. On 19th January, 2016, he had severe central chest pain for 1 hour, sweating and nausea but no vomiting. He was taken to a tertiary level hospital where ECG was done and it revealed evidence of extensive anterior myocardial infarction. After initial resuscitation, he was sent to a specialized hospital, National Institute of Cardiovascular Disease (NICVD) for better management. Troponin I was positive and CK-MB level was significantly elevated. On that day (19th January), the patient received streptokinase, gradually clinical and biochemical and other investigations revealed signs of improvement.

On 24th January, 2016, he was discharged from NICVD and advised for coronary angiogram. On 29th January, 2016 he was admitted in Mirpur Heart Foundation Hospital. Two days later, coronary angiogram was done and it revealed Triple Vessel Disease (TVD). He was advised for reperfusion therapy in the form of PCI or CABG. But on 2nd February, he experienced a new problem; distal paresthesia in his arms and legs. There was progressive weakness of both of his lower limbs

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and later in an ascending manner, it involved his upper limbs also. Clinically muscle power was MRC grade 0, both in upper and lower limbs, proximal and distal. There was areflexia and bilateral facial palsy. Sensory function was intact and no sphincter disturbance was present. Nerve conduction study was done. It revealed demyelinating sensory motor polyneuropathy compatible with AIDP. At the same time, there was difficulty in breathing. He was shifted to ICU. After resuscitation, he was improved.

There was no history of drug or toxin exposure. There was no upper respiratory or gastrointestinal infection within the last 2 months. No evidence of arterial or venous embolism, vasculitis and connective tissue disease. He was a diagnosed case of DM and was on medication.

The patient received 5 doses of IVIg (30 gm). His condition gradually improved.

Discussion:

The precise cause of GBS is not yet known, but it has been reported to be associated with history of gastrointestinal or upper respiratory infection. Underlying mechanism is probably immunological. Clinical symptoms are thought to result from streptokinase antibody complex mediated damage to the local blood nerve barrier

Streptoknase is a single chain polypeptide derived from group C beta hemolytic streptococci. The protein nature of this drug makes it antigenic in the body. It stimulates immunologic reactions[6]. This is probably the pathophysiologic basis.

There are also reports of GBS after myocardial infarction in patients without thrombolytic therapy[7]. High creatine kinase from significant muscle injury might be a possible immunological precipitant. Therefore it is not yet determined whether process of MI itself is the promoter of polyneuropathy or thrombolytic agents are the initiators of this conditions[8].

In developing conuntries and in some developed conuntries, there are institutions who lack interventional capability (ie PCI & CABG). For acute ST-elevation MI, they use fibrinolytic therapy. GBS after acute MI treated with reteplase has also been reported[9].

GBS can be seen in the late course of acute MI by the process of triggered autoimmune mechanism. A patient developing paresthesia and progressive muscular weakness after 10-30 days of myocardial infarction, especially if thrombolysed with streptokinase, high degree of suspicion of GBS could be considered. At the same time, other likely causes must be excluded. To be epidemiologically significant, many other reports around the globe should be analyzed.

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