ORIGINAL ARTICLE

SERUM FERRITIN AND PLASMA FIBRINOGEN LEVEL IN ACUTE ISCHEMIC STROKE

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ABSTRACT

Acute ischemic stroke is the primary pathology of brain vascular system that temporarily or permanently affects brain functions as a result of ischemia. In ischemic stroke events, many biochemical and immunological reactions occur secondarily in response to the reduced cerebral blood flow. Brain tissue damage after acute ischemic stroke is mediated partly by inflammation induced by ischemia-reperfusion injury. It is thought that acute ischemic stroke due to cerebral ischemia triggers acute phase reaction and the blood concentration of ferritin and fibrinogen could rise during brain infarction. This study was carried out in the Department of Biochemistry, Sylhet MAG Osmani Medical College during the period of July 2015 to June 2016 to evaluate the status of ferritin and fibrinogen in acute ischemic stroke patients. For this study serum ferritin and plasma fibrinogen level were assessed in 50 acute ischemic stroke patients and 50 normal individuals. Data were analyzed with the help of SPSS version 19.0. Unpaired Student's 't' test and Mann-Whitney U test were done to see the level of significance. Findings of the study revealed that serum ferritin and plasma fibrinogen levels were 83.1 ng/ml and 190.6 mg/dl in subjects with acute ischemic stroke, while in control subjects these were 41.3 ng/ml and 177.0 mg/dl respectively. Study showed that serum ferritin and plasma fibrinogen levels were significantly higher in patients with ischemic acute stroke compared to that of normal controls ($p < 0.001$). It may be concluded that serum ferritin and plasma fibrinogen levels are significantly increased in acute ischemic stroke.

Key Words: Serum Ferritin, Plasma Fibrinogen, Acute Ischemic Stroke

Introduction

Stroke is defined as rapidly developing clinical signs of focal disturbance of cerebral function lasting for more than 24 hours or leading to death, with no apparent cause other than vascular origin. Three months following a stroke, 15-30% of stroke survivors are permanently disabled and 20% require institutional care[1]. Incidence of ischemic stroke is 61.4% and hemorrhagic stroke is 38.6% in Bangladesh[2]. Stroke is the third leading cause of death in Bangladesh. WHO ranks Bangladesh's mortality rate due to stroke as number 84 in the world. The mortality rate of stroke in Bangladesh is about 5.8%[3].

Pathophysiology of all cerebrovascular diseases has their origin in the vessels supplying or draining the brain. Changes in the vessel wall lead to obstruction of blood flow and by interacting with blood constituents they may cause thrombosis and blockade of blood flow in the vessels[4]. Under ischemic conditions, mitochondrial production of ATP ceases and intracellular ATP stores deplete, resulting cell membrane depolarization leading to a large influx of calcium and sodium and an efflux of potassium. Cells in the infarct core are rapidly and irreversibly destroyed by lipolysis,

proteolysis, and disaggregation of microtubules due to metabolic failure[5].

It is assumed that acute phase response proteins i.e. ferritin, fibrinogen or others play important role in pathogenesis of ischemic stroke because acute cerebral ischemia triggers interleukin-6 release into cerebrospinal fluid and blood, which is a key mediator of acute phase reaction and induces synthesis of acute phase proteins during ischemia[6]. Ferritin is the main iron storage compound in the body. It provides a reserve of the element, which can be drawn upon as the need arises for the synthesis of molecules such as hemoglobin, cytochromes and iron-sulfur compounds[7]. Plasma ferritin is a suitable index of the amount of cellular iron stores and consequently might be related to the availability of iron in the infarcted area. In brain tissue most of the non-heme iron is in the form of ferritin, which is localized in astrocytes and microglia. It is an acute phase response protein and its concentrations increases during inflammation[8].

Fibrinogen is an important component of the coagulation cascade, as well as a major determinant of blood viscosity and blood flow. It is a high molecular weight plasma adhesion protein and a biomarker of inflammation. Increased levels of fibrinogen result in changes in blood rheological properties that exacerbate the complications in peripheral blood circulation during stroke[9,10].

Fibrinogen regulates NF-kappa B activation and expression of inflammatory chemokines in endothelial cells, binding to its integrin receptor on the surface of leucocytes, facilitating chemotactic response, increasing phagocytosis, antibody mediated leucocyte toxicity and delay in apoptosis. As acute phase protein, fibrinogen is up-regulated by cytokines like interleukin-6 and by glucocorticoids[11]. Some researchers identified elevated level of serum ferritin and plasma fibrinogen in acute ischemic stroke[9,14,15,17,19]. While others found no such benefits of ferritin and fibrinogen testing in acute ischemic stroke[20,21]. In spite of potential possibility of higher serum ferritin and plasma fibrinogen levels in acute ischemic stroke events and variable results of different studies, this study was carried out to evaluate the levels of ferritin and fibrinogen in acute ischemic stroke patients in our population.

Materials and Methods

This observational analytic study was carried out in the Department of Biochemistry, Sylhet MAG Osmani Medical College during the period from July 2015 to June 2016. Fifty (50) patients with acute ischemic stroke and age-and sex-matched 50 healthy controls were selected. Inclusion criteriae were, subjects with first ever ischemic stroke within 48 hours from onset of symptoms and confirmed by CT-scan of brain. Exclusion criteriae were cerebral hemorrhage, malignant tumor, coagulation disorders, acute or chronic infections, ischemic stroke with other complications, anemia, liver disease and renal failure. Informed written consent was taken from the study subjects and ethical approval was obtained from the Ethical Committee of Sylhet MAG Osmani Medical College. Age, sex and blood pressure were recorded. Serum ferritin was assessed by ELISA (Enzyme-linked Immunosorbent Assay) and Plasma fibrinogen was assessed by Clauss Clotting Method. Data was analyzed with the help of SPSS version 19.0. Unpaired Student's 't' test and Mann-Whitney U test were applied to obtain the level of significance.

Results

Mean($\pm$SD) age of acute ischemic stroke patients was 60.6 ± 9.2 years and that of control group was 58.1 ± 7.8 years ($p=0.1$) who showed no statistically significant differene (Table-I). There was significant difference of both systolic blood

pressure and diastolic blood pressure among acute ischemic stroke patients and healthy controls ($p < 0.001$) (Table-II). The serum ferritin and plasma fibrinogen level were significantly increased in subjects with acute ischemic stroke compared to healthy controls ($p < 0.001$) as shown in Table-III.

Table-I: Distribution of age and sex in the study subjects.

Demographic features	Acute Ischemic Stroke (n=50)	Control (n=50)	p-value
(Mean±SD) Age (years)	60.6±9.2	58.1±7.8	0.1
Sex (male, female)	28 (52%), 22 (48%)	28 (52%), 22 (48%)	1.0

Unpaired student's 't' test were and proportion test done to measure the level of significance; Significant= ($p < 0.05$).

Table-II: Clinical parameters in the study subjects

Clinical parameters	Acute ischemic stroke (n=50)	Control (n=50)	p-value
Mean(±SD) SBP(mm of Hg)	160.6±38.5	127.4±21.1	<0.001
Mean(±SD) DBP (mm of Hg)	92.8±20.2	78.8±11.9	<0.001

Unpaired student's 't' test was done to measure the level of significance; Significant= ($p < 0.05$).

Table-III: Serum ferritin and Plasma fibrinogen in the study subjects

parameters	Acute ischemic stroke (n=50)	Control (n=50)	p-value
	Median (Range)	Median (Range)	
Serum ferritin (ng/ml)	83.1 (26.5-397.1)	41.3 (14.5-158.6)	<0.001
Plasma fibrinogen (mg/dl)	190.6 (175.6-298.1)	177.0 (153.4-199.7)	<0.001

Data were presented as median with range. Mann-Whitney U test was done to measure the level of significance; Significant= ($p < 0.05$).

Discussion

Ischemic stroke is an acute vascular event that hinders blood supply to the brain and leads to an ischemic process that affects neurons, glial cells and vessels. It is thought that after ischemia, cytoplasmic Ca^{2+} levels rise that can trigger overproduction of free radicals, dysfunction of mitochondria, cell membrane disruption and DNA fragmentation, which acting synergistically cause neuron death. After cerebral ischemia and particularly after reperfusion, robust oxidants are generated including superoxide and hydroxyl radicals, which overwhelm endogenous scavenging mechanisms and are directly involved in the damage to cellular macromolecules[12,13].

Inflammation is an important part of stroke pathophysiology, especially in the context of reperfusion. During early onset of ischemia ferritin, fibrinogen and other inflammatory acute phase reactants play important role in the pathogenesis of ischemic stroke. Iron released from ferritin during ischemia is more available to catalyze the generation of hydroxyl radical, the more malignant free-radical species, which can begin lipid peroxidation process and causes endothelial cell damage. Fibrinogen exacerbates the complications in peripheral blood circulation by changing blood rheological properties during stroke. Fibrinogen forms blood clots made of thinner and tightly packed fibers, more resistant to fibrinolysis resulting with increased mortality in stroke.

Due to increase number of acute ischemic stroke cases in Bangladeshi people and to assess the level of acute phase reactants in acute ischemic stroke, the present study was undertaken to evaluate the pattern of changes of ferritin and fibrinogen levels in acute ischemic stroke patients. There was no significant difference of age among two groups. The findings of this study agree with other study findings[14,15].

In our study systolic blood pressure and diastolic blood pressure significantly differed between two groups. The systolic blood pressure and diastolic blood pressure were significantly higher in subjects with acute ischemic stroke as compared to normal individuals which is in agreement with a case-control study[16]. As hypertension is one of the major risk factors for developing stroke, it is expected to differ significantly with acute ischemic stroke patients and normal individual.

This study showed increased level of serum ferritin in acute ischemic stroke patients compared to normal individuals which is in agreement with several studies conducted in their population[14,17].

Several mechanisms may explain the crucial role of ferritin in ischemic stroke. Free radicals are generated in increased amounts under ischemic conditions that react with and damage proteins, nucleic acids, and membrane lipids, disrupting cellular integrity. This oxygen radical activity is especially intense during reperfusion after sustained ischemia. The generation of hydroxyl radical, the most toxic and reactive of the free radicals, is catalyzed by ferrous iron released from ferritin during ischemia[18].

In our study, plasma fibrinogen level was increased in acute ischemic stroke compared to normal individual and the difference was statistically significant which is similar to some studies conducted in India, Egypt and Nepal population[9,15,19].

As acute phase reactant, plasma fibrinogen levels are strongly associated with the complications of ischemic stroke. During ischemia, fibrinogen plays a vital role in inflammation, atherogenesis and thrombogenesis. Increased fibrinogen level in ischemic stroke causes excessive clumping of blood cells, resulting in formation of abnormal clots in the artery and may lead to development of hemorrhagic stroke.

Elevated levels of serum ferritin and fibrinogen after ischemia can severely deteriorate the condition of acute ischemic stroke to hemorrhagic one. Maintaining an optimal level of serum ferritin and plasma fibrinogen level in early onset of ischemic stroke, seems very beneficial not only for prevention of hemorrhagic stroke but also for good prognosis of the disease. Sample size was small due to limitation of time and fund. It may be concluded that serum ferritin and plasma fibrinogen level is significantly increased in acute ischemic stroke. Further studies are needed to investigate the levels of serum ferritin and plasma fibrinogen in acute ischemic stroke with larger sample size which could give us more information about the changes of acute phase reactants in acute ischemic stroke thus helping the clinicians to manage those patients in better way.

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