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Correlation of Serum S-100 Protein Level with Severity of Ischaemic Stroke

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Abstract

Background: Stroke is currently the second leading cause of death worldwide and the first leading cause of death in Bangladesh. The National Institute of Health Stroke Scale is the most commonly used deficit rating scale to assess stroke severity. S-100 protein is a low molecular weight calcium-binding protein expressed mostly in glial cells like astrocytes, oligodendrocytes, and microglial cells. During the ischaemic process, S-100 protein is secreted from the glial cells into the extracellular space. After secretion, S-100 protein releases initially into the cerebrospinal fluid and then eventually into the bloodstream due to disruption of the blood-brain barrier. Aim of the study: To correlate serum S-100 protein level with the severity of ischaemic stroke. ***Methods:*** This cross-sectional study was conducted at the Department of Laboratory Medicine in collaboration with the Department of Neurology, BSMMU, Dhaka, Bangladesh from September 2018 to August 2019. A total of 70 ischaemic stroke patients were enrolled in this study. After taking proper history and neurological examination, the severity of ischemic stroke was assessed on the basis of NIHSS score. Then, serum S-100 protein levels were measured by the Electrochemiluminescence Immunoassay method. Statistical analysis was done by SPSS version 22.0. ***Results:*** According to the NIHSS scores, 35(50.0%) of the patients had moderate stroke (NIHSS score=5-15), 17(24.3%) had minor stroke (NIHSS=1-4), 12(17.1%) had moderate to severe stroke (NIHSS=16-20) and 4(5.0%) had severe stroke (NIHSS=21-42). The mean S-100 protein level was found 0.283±0.165 µg/L. Mean S-100 protein levels was assessed in different categories of severity of ischaemic stroke. Maximum Mean± SD value of serum S-100 protein was found in case of severe stroke (NIHSS score=21-42; Mean ± SD: 0.739±0.207, range: 0.523-1.019). The significance test was done by ANOVA test which was found statistically significant (p-value <0.001). Pearson's correlation test revealed a significant strong positive correlation (r=+943, p<0.001) between serum S-100 protein level and NIHSS scores of ischaemic stroke patients. ***Conclusion:*** In the present study it was found that serum S-100 protein levels were higher in severe ischaemic stroke in relation to the ischaemic stroke of lower severity. S-100 protein level is rapidly determined by the method used in the present study. Serum S-100 protein level in this regard can be used as an important tool to predict the severity of ischaemic stroke. Further study is needed to confirm the findings of the present study.

Keywords: Ischaemic stroke, Severity, NIHSS score, S-100 protein.

INTRODUCTION

According to the World Health Organization (WHO), stroke is currently the second leading cause of death worldwide and the first leading cause of death in Bangladesh [1]. Bangladesh ranks in 34th position in the World Health Ranking of mortality due to stroke. In 2017, stroke alone was responsible for 128,190 deaths in this country, and the mortality rate due to stroke was 16.27% of all deaths [1]. The WHO definition of stroke (introduced in 1970 and still used) is "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin" [2]. There are multiple causes for ischaemic stroke to occur. These are characterized by the rule of quarters: 25% cardioembolic, 25% arterio-embolic (large artery disease), 25% lacunar (small-vessel disease), and 25%

due to other causes [3]. The severity of ischaemic stroke ranges clinically from mild or transient (termed as minor stroke) to severe, but the underlying causes are identical [4]. There are several scales to determine the severity of stroke. Of these, the most commonly used deficit rating scale is the National Institute of Health Stroke Scale (NIHSS). It is most widely used to assess stroke severity, treatment efficacy and to predict outcomes [5]. However, it is well accepted that the NIHSS score is not a substitute for a comprehensive neurological examination. There is a lack of standardization in the way neurologic function is monitored across institutions [6]. Currently, diagnosis of ischaemic stroke relies on neurological examination and is further supplemented with various neuroimaging and vascular imaging techniques. Neuroimaging techniques are considered as the gold standard in the diagnosis of ischaemic stroke. Several supportive investigations are done to monitor patient status, which includes blood glucose, complete blood count, lipid profile, electrolyte panel, arterial blood gas analysis, Prothrombin Time, and International Normalized Ratio (PT/INR), D-dimer, Electrocardiogram, Echocardiogram, etc. These assessments should be performed quickly enough to start treatment earlier [7]. Several strokes associated biomarkers have been the subject of extensive research in the last few decades [8]. Biomarkers specific for glial and neuronal ischaemic injuries provide valuable and timely diagnostic information for ischaemic stroke. S-100 protein is a multigenic family of low molecular weight calcium-binding proteins. The presence of S-100 protein in nervous tissue is restricted to glial cells like astrocytes, oligodendrocytes and microglial cells. Within these cells, the S-100 protein is involved in various intracellular and extracellular functions [9]. There are multiple mechanisms by which S-100 protein is secreted. Intracellular ATP depletion after ischaemia causes a rapid rise in extracellular adenosine and glutamate concentration. This leads to the immediate release of S-100 protein in the extracellular space. Secretion of S-100 protein also occurs by stimulation of astroglial 5-HT1A receptors and by adrenocorticotopic hormone (ACTH) and corticotrophin-like intermediate lobe peptide. This mechanism is relatively slower causing the continuous rise of the S-100 protein level during ischaemic process with a gradual decrease around 8-9 days. The later release of S-100 protein also occurs due to the proliferation of reactive astrocytes [10]. S-100 protein in higher concentration causes apoptotic cell death by interacting with the Receptor for Advanced Glycation End-products (RAGE) leading to elevation of reactive oxygen species, cytochrome C release and activation of the caspase cascade. These mechanisms lead to mitochondrial dysfunction and induction of apoptosis of the surrounding cells. After secretion, S-100 protein releases into the cerebrospinal fluid (CSF) and then eventually into the bloodstream due to disruption of the blood-brain barrier [11]. Elevated S-100 protein level in CSF and serum has been reported earlier in ischaemic stroke. Its level rises immediately after ischaemia and increases according to the size of the lesion (Kumar *et al*., 2015). Serum S-100 protein level may be a promising serum biomarker for the severity assessment of ischaemic stroke. There is a scarcity of studies that explored the possible correlation between serum S-100 protein level and severity of ischaemic stroke. The study aimed to correlate serum S-100 protein level with the severity of ischaemic stroke.

METHODOLOGY AND MATERIALS

This was a cross-sectional study, conducted at the Department of Laboratory Medicine in collaboration with the Department of Neurology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from September 2018 to August 2019. As study subjects, in total 70 diagnosed patients of ischaemic stroke from the Department of Neurology, BSMMU were enrolled in this study. According to the inclusion criteria of this study, on patients aged >18 years diagnosed with ischaemic stroke in the Department of Neurology, BSMMU, within 7 days of onset of symptoms were included. On the other hand, according to the exclusion criteria, neurological diseases patients of traumatic brain injury, haemorrhagic stroke, CNS infection and chronic neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, and multiple sclerosis were excluded. Besides these, patients with malignant melanoma, astrocytoma, schwannoma, glioma, anaplastic glioblastoma, schizophrenia, mood disorder and temporal lobe epilepsy were excluded. The demographic variables of this study were age and gender. The variables related to risk factors of the disease were, history of previous vascular event, family history of stroke, heart disease, hypertension, dyslipidaemia and diabetes mellitus. Ischaemic stroke was the dependent variable whereas Serum S-100 protein level and CT scan/MRI of Brain reports were the investigational variables. The clinical variable of this study was the National Institute of Health Stroke Scale (NIHSS) score. The sampling technique of this study was purposive. Patient information was taken by thorough history taking from the patient and from the patient's attendant if the patient was unable to speak. Then relevant clinical examination was done by an experienced senior resident/medical officer. Then it was evaluated by a Neurologist. Severity assessment of ischaemic stroke was done by NIHSS score. It was conducted by an experienced senior resident/medical officer accompanied by the researcher. Then it was evaluated by a Neurologist. Serum S-100 protein level was estimated using the serum S-100 reagent kit (code no. 03175243 190; lot no. 31100201) for Elecsys and Cobas e411 Immunoassay auto analyzer from Roche Diagnostics, Mannheim, Germany. This assay employs the quantitative Electrochemiluminiscence Immunoassay (ECLIA) method. The expected value of serum S-100 protein level in this study was >0.105 µg/L. (Cut-off value: <0.105 µg/L; Elecsys and Cobas

e411: S-100 datasheet). For estimation of serum S-100 protein level, the Eppendorf tubes were taken out from -22˚C temperature and kept in an upright position at room temperature for thawing. After 30 minutes, the serum samples were thoroughly agitated using a Vortex mixer, each tube at a time. Then the Eppendorf tubes were given into the sample rack of Elecsys and Cobas e411 Immunoassay auto analyzer. 500µl serum sample was aspirated by the analyzer probe. Test results were given after 18minutes. The data collection procedure was initiated by the researcher through a face-to-face interview.

Then, brief history taking along with NIHSS scoring was done by the experienced resident or medical officer accompanied by the researcher. Classification of ischaemic stroke was done on the basis of the TOAST classification system. With proper aseptic precaution, 2.0 ml of whole blood was drawn in a plastic red screw-capped plain tube for estimation of serum S-100 protein level. Analysis of Serum S-100 protein level was done in Elecsys and Cobas e411 Immunoassay autoanalyzer in the Department of Laboratory Medicine, BSMMU by Electrochemiluminiscence Immunoassay (ECLIA) method. About 30-35 minutes was needed to collect data from each patient. After getting the lab reports it was recorded in the datasheet. Association between serum S-100 protein level and severity of ischaemic stroke was done by ANOVA test. Correlation of serum S-100 protein level with NIHSS scores was done by Pearson's correlation coefficient (r) test. All statistical analysis was done by SPSS version 22. P-value <0.05 was considered statistically significant. Data and results were presented in the form of tables, figures and diagrams where applicable.

RESULTS

In this study, in total 70 clinically diagnosed ischaemic stroke patients, aged more than 18 years irrespective of sex were selected for the study as the study subjects. Among all the participants, the maximum number of patients were 23(32.9%) were from the age group of 51-60 years followed by 22(31.4%) patients in the age group of 61-70 years. The mean age of the study group was 61.21± 10.98 years; minimum age 36 and maximum 86 years. Maximum patients were male 41 (58.6%) and the rest 29(41.4%) patients were female out of 70 ischaemic stroke patients. The male-female ratio was 1.4:1. In this study, as risk factors, maximum patients 45(64.3%) had hypertension followed by dyslipidemia 34(48.6%), diabetes mellitus 23(32.9%), heart disease 22 (31.4%), history of previous vascular events 9(12.9%) and family history of stroke 8 (11.4%). In analyzing the severity of ischaemic stroke according to NIHSS score among the participants we observed, a maximum of 35 (50.0%) patients had a moderate stroke (NIHSS score=5-15) followed by 17(24.3%) patients who had a minor stroke (NIHSS score=1-4), 12(17.1%) patients had moderate to severe stroke (NIHSS score=16-20), 4 (5.7%) patients had a severe stroke (NIHSS score=21-42) and 2 (2.9%) patients had no stroke symptoms (NIHSS score=0). Regarding CT scan/MRI of brain findings, maximum patients 36 (51.4%) had cortical and subcortical infarction followed by 18(25.7%) patients had subcortial (<1.5 cm) infarction, 6 (8.6%) patients had brainstem (>1.5 cm) infarction, 5(7.1%) patients had subcortical (>1.5 cm) infarction, 2(2.9%) patients had cerebellar infarction, 2(2.9%) patients had cerebellar (<1.5 cm) infarction and only 1(1.4%) patient had brainstem (<1.5 cm) infarction. Maximum patients 32(45.7%) had a stroke of undetermined etiology followed by 19(27.1%) patients who had cardioembolic stroke, 10(14.2%) patients had small artery occlusion (lacunar stroke), 8(11.4%) patients had large artery atherosclerosis and only (1.4%) patient had a stroke of other determined etiology. In assessing the relationship among severity of ischaemic stroke with Serum S-100 protein level we observed that the mean ± SD S-100 protein level was found 0.283±0.165 µg/L with the range of 0.103-1.019 µg/L. Mean± SD levels of serum S-100 protein were measured in different categories of severity of ischaemic stroke. The maximum Mean ± SD value of serum S-100 protein was found in case of severe stroke (NIHSS score=21-42; Mean ± SD: 0.739±0.207, range: 0.523-1.019). The significance test is done by the ANOVA test which was found statistically significant (p value<0.001). Besides these, Pearson's correlation test was done and that also showed a significant strong positive correlation between serum S-100 protein level with NIHSS stroke score in ischaemic stroke patients (r=+.943, p<0.001).

Table-1: Distribution of risk factors of the study population (N=70)

Risk factors	n	%
Hypertension	45	64.3
Dyslipidemia	34	48.6
Diabetes mellitus	23	32.9
Heart disease	22	31.4
History of previous vascular events	9	12.9
Family history of stroke	8	11.4

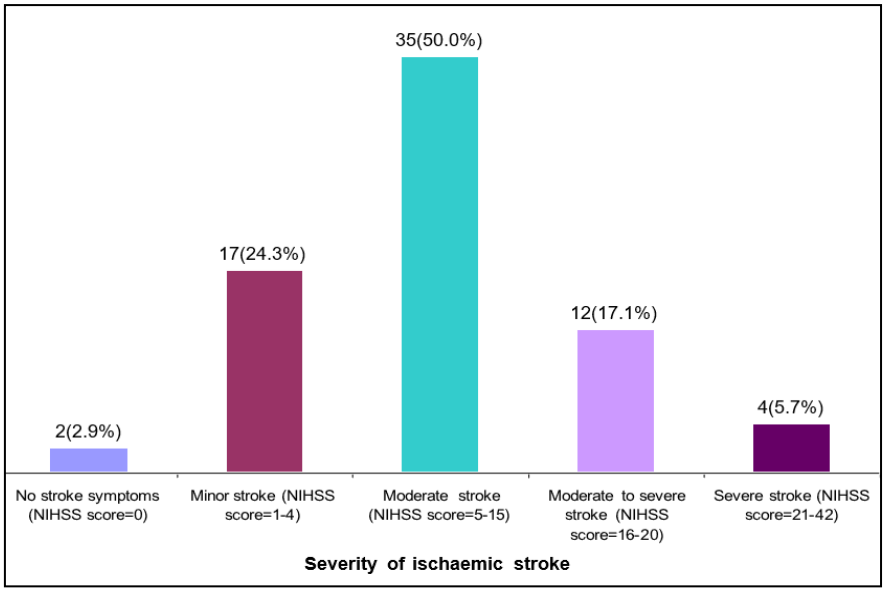

Fig-I: Severity status of ischaemic stroke in study patients as per NIHSS score (N=70)

Table-2: Distribution of the study patients by CT scan/MRI of brain findings (N=70)

CT scan/MRI of brain findings	n	%
Cortical + Subcortical	36	51.4
Subcortical (<1.5 cm)	18	25.7
Brainstem (>1.5 cm)	6	8.6
Subcortical (>1.5 cm)	5	7.1
Cerebellum (<1.5 cm)	2	2.9
Cerebellum (>1.5 cm)	2	2.9
Brainstem (<1.5 cm)	1	1.4

Table-3: Types of ischaemic stroke in patients as per TOAST classification (N=70)

Type of ischaemic stroke	n	%
Stroke of undetermined etiology	32	45.7
Cardio-embolism	19	27.3
Small artery occlusion (Lacunar)	10	14.2
Large artery atherosclerosis	8	11.4
Stroke of other determined etiology	1	1.4

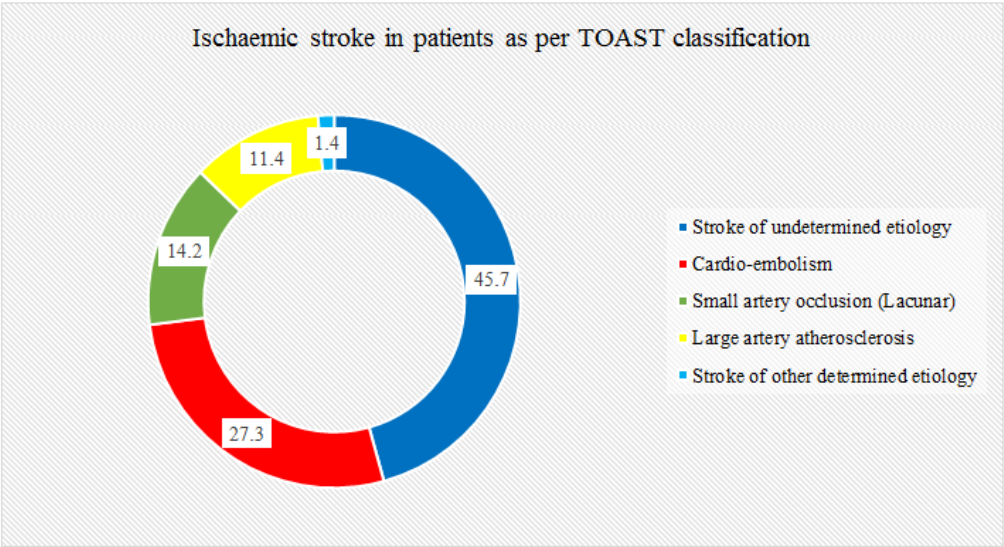

Fig-II: Ischaemic stroke in patients as per TOAST classification (N=70)

Table-4: Relation of serum S-100 protein level with severity of ischaemic stroke (N=70)

Stroke severity as per NIHSS score	n	Serum S-100 protein level (Cut off value: 0.105 µg/L) Mean ± SD	Range (Min-max)	P-Value
No stroke symptoms (NIHSS score=0)	2	0.112±0.004	0.109-0.114	<0.001
Minor stroke (NIHSS score=1-4)	17	0.143±0.029	0.103-0.196	
Moderate stroke (NIHSS score=5-15)	35	0.257±0.071	0.109-0.411	
Moderate-severe stroke (NIHSS score=16-20)	12	0.430±0.076	0.311-0.532	
Severe stroke (NIHSS score=21-42)	4	0.739±0.207	0.523-1.019	
Total	70	0.283±0.165	0.103-1.019	

DISCUSSION

It is difficult to measure the severity of ischaemic stroke. Several strokes associated biomarkers have been the subject of extensive research in the last few decades. Biomarkers specific for glial and neuronal ischaemic injuries provide valuable and timely diagnostic information for ischaemic stroke. S-100 protein is present in tissues of different origins but found in much abundance in the cells of the nervous system. During ischaemic process, S-100 protein releases initially in the cerebrospinal fluid and then in the bloodstream. The aim of this study was to correlate the serum S-100 protein level with the severity of ischaemic stroke. Some relevant risk factors that may affect the outcome along with some demographic profiles like age and sex were also evaluated. In this study, in total 70 clinically diagnosed ischaemic stroke patients, aged more than 18 years irrespective of sex were selected for the study as the study subjects. Among all the participants, a maximum number of patients 23(32.9%) were from the age group of 51-60 years followed by 22(31.4%) patients in the age group of 61-70 years. The mean age of the study group was 61.21± 10.98 years; minimum age 36 and maximum 86 years. Maximum patients were male 41 (58.6%) and the rest 29(41.4%) patients were female out of 70 ischaemic stroke patients. The male-female ratio was 1.4:1. In this study, as risk factors, maximum patients 45(64.3%) had hypertension followed by dyslipidemia 34 (48.6%), diabetes mellitus 23(32.9%), heart disease 22(31.4%), history of previous vascular events 9(12.9%) and family history of stroke 8(11.4%). According to the NIHSS score observed in this study, maximum 35(50.0%) patients were found in moderate stroke (NIHSS score=5-15) followed by 17(24.3%) patients had minor stroke (NIHSS score=1-4), 12(17.1%) patients had moderate to severe stroke (NIHSS score=16-20) and 4(5.7%) patients had severe stroke (NIHSS score=21- 42). Acharya *et al*. (2016) [12] have found moderate stroke in 40.0% of their study population. Gajurel *et al*. (2015) [13] have obtained moderate and minor stroke among 47.0% and 19.0% of their study population respectively. A similar finding was observed by Kumar *et al*. (2015) [14] where they have found moderate stroke in 41.54% and minor stroke in 23.23% of their study population. Regarding CT scan/MRI of brain findings of the study population, a maximum of 36(51.4%) patients had cortical and subcortical lesions followed by 18(25.7%) patients who had subcortical (<1.5 cm) infarction. Janssens *et al*. (1995) [15] have shown that 27.2% of their study population had smaller subcortical (<1.5 cm) infarction. These findings are nearly consistent with the findings of the current study. In our study, the majority of the patients 32 (45.7%) had ischaemic stroke of undetermined etiology followed by 19 (27.1%) patients who had cardio-embolism. Saber *et al.* (2017) [16] have found a stroke of undetermined etiology in 43.9% of their study population which is similar to the finding of the current study. González-García *et al*. (2012) [17] have found cardio-embolism in 30.4% of their study population. In the current study, small artery occlusion (lacunar stroke), large artery atherosclerosis, and stroke of other determined etiology were found in 14.2%, 11.4%, and 1.4% of the study patients respectively. Kumar *et al*. (2015) [14] have found lacunar stroke in 13.6% of their study population. Ihle-Hansen *et al*. (2012) [18] found large artery atherosclerosis in 11.4% of their study population. These findings are closely consistent with the findings of the current study. In the present study, the mean ± SD of serum S-100 protein level was found 0.283 ± 0.165 µg/L with the range of 0.103-1.019 µg/L. Branco *et al*. (2018) [19] have found mean ± SD of the S-100 protein level was 439.76 ± 562.03. Kumar *et al*. (2015) [14] have shown that serum S-100 protein concentrations were significantly increased (p<0.001) above the normal value for their study (0.1782 ± 0.1622 ng/ml) in case of ischaemic stroke compared to haemorrhagic stroke and transient ischaemic attack. This finding is nearly consistent with the current study. In this study, the Mean ± SD levels of serum S-100 protein were measured in different categories of severity of ischaemic stroke. In patients having no stroke symptoms (NIHSS score=0), the mean ± SD of serum S-100 protein level was found 0.112±0.004 µg/L with the range of 0.109-0.114 µg/L. In patients having a minor stroke (NIHSS score=1- 4), mean ± SD of S-100 protein level was found 0.143±0.029 µg/L with the range of 0.103-0.196 µg/L. The level of S-100 protein level was found to gradually increase in moderate (NIHSS score=5-15), moderate to severe (NIHSS score=16-20), and severe stroke (NIHSS=21-42). The highest increased value of serum S-100 protein was recorded in severe stroke which was 0.739±0.207 µg/L with the range of 0.523-1.019 µg/L. The significance of this increasing trend of serum S-100

protein level was tested by ANOVA test which was statistically significant (p<0.001). Branco *et al*. (2018) [19] have shown that NIHSS scores on admission were significantly correlated with S-100 protein concentration (P=0.002). Üstündağ *et al*. (2011) [20] have shown that patients with low, moderate, and high severity strokes had significantly increasing patterns of serum S-100 protein levels (p<0.001). These findings are nearly consistent with the findings of the current study. Pearson's correlation coefficient (r) test was done in order to assess the correlation of serum S-100 protein level with NIHSS score of ischaemic stroke patients. In the present study, the r-value showed +0.943 (p<0.001) which indicates a significant strong positive correlation between serum S-100 protein level with NIHSS score. Several studies have found similar results which are consistent with the results of the current study. Elting *et al*. (2000) [10] have compared serum S-100 protein levels on day 1 and day 10 following stroke. They have shown that serum S-100 protein level correlated well with the NIHSS score on day 1(r=+0.58, p=0.014). The serum S-100 levels also correlated with the change in NIHSS score between day 10 and day 1(r=+0.65, p=0.007). Brouns *et al*. (2010) have shown that the S-100 protein level positively correlated with NIHSS scores on admission (S100B: R=0.29, P=0.006). These findings are closely consistent with the findings of the present study. In the current study, the observed serum S-100 protein level was well correlated according to the severity of ischaemic stroke. The findings of the present study suggest that estimation of serum S-100 protein level can be used as a biochemical marker in the acute stage to predict the severity of ischaemic stroke.

Limitation of the study

The sample was taken purposively, so there may be a chance of bias which can influence the result. The study population was selected from one tertiary level hospital in Dhaka city; therefore, the sample may not be representative of the selected population of the country. Patients with exclusion criteria were excluded on the basis of history and clinical features. No confirmatory tests were carried out to exclude these patients due to lack of financial sources and time constraints.

CONCLUSION & RECOMMENDATION

Ischaemic stroke is a major public health issue worldwide because of its increased mortality and morbidity rates. In the present study, serum S-100 protein levels were higher in severe ischaemic stroke in relation to the stroke of lower severity. There is significant positive correlation found between serum S-100 protein level and severity of ischaemic stroke. Serum S-100 protein level is rapidly determined by the method used in the present study. So, serum S-100 protein level in this regard can be used as an important tool to predict severity of ischaemic stroke. Therefore, it will be greatly beneficial for clinicians to assess severity of ischaemic stroke to start treatment earlier. To confirm the findings of present study, further study is needed.

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