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Risk Factors and Serum S-100 Protein Level Analysis of Ischaemic Stroke Patients: A Tertiary Care Hospital Study in Bangladesh

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Abstract **Original Research Article**

Background: Information regarding the associated risk factors is very important in treating patients with ischaemic stroke besides severity measurement. The National Institute of Health Stroke Scale (NIHSS) is the most commonly used deficit rating scale to assess stroke severity. Serum S-100 protein is a low molecular weight calcium-binding protein expressed mostly in glial cells like astrocytes, oligodendrocytes, and microglial cells. During 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:*** The aim of this study was to assess risk factors and Serum S-100 protein level of ischaemic stroke patients. ***Methods:*** This cross-sectional study was 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. A total of 70 patients with ischaemic stroke from the Department of Neurology, BSMMU 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 Electrochemiluminiscence Immunoassay (ECLIA) method. Statistical analysis was done by SPSS version 22.0. Results: 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 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). ***Conclusion:*** In this study, hypertension, dyslipidemia, diabetes mellitus, and heart disease was found as more frequent risk factors among ischaemic stroke patients. Serum S-100 protein levels were higher in severe ischaemic stroke in relation to the stroke of lower severity. There is a significant positive correlation found between serum S-100 protein level and severity of ischaemic stroke.

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

1. INTRODUCTION

Besides severity measurement, information regarding the associated risk factors is very important in treating patients with ischaemic stroke. The National Institute of Health Stroke Scale (NIHSS) is the most commonly used deficit rating scale to assess stroke severity. Serum S-100 protein is a low molecular weight calcium-binding protein expressed mostly in glial cells like astrocytes, oligodendrocytes, and microglial cells. During ischaemic process, S-100 protein is secreted from the glial cells into the extracellular space. In 2013, American Heart

Association/American Stroke Association(AHA/ASA) have defined ischemic strokes as, "episodes of neurological dysfunction caused by focal cerebral, spinal or retinal infarction". This definition suggests CNS infarction as brain, spinal cord, or retinal cell death due to ischaemia, based on pathological imaging (CT scan/MRI of the brain) or other objective evidence of focal ischaemic injury in a defined vascular distribution or clinical evidence of focal ischaemic injury based on symptoms persisting ≥24 hours or until death, other etiologies excluded. Ischaemic stroke occurs due to interruption of blood supply by blockage or narrowing of arteries in the brain. The resultant neurologic syndrome corresponds to a portion of the brain that is supplied by one or more cerebral vessels [1]. The aetiopathogenesis of stroke is multifactorial, with multiple modifiable and non-modifiable risk factors being associated. Non-modifiable risk factors for stroke include older age, male gender, ethnicity, family history, and prior history of stroke. Modifiable risk factors include arterial hypertension, Diabetes Mellitus, dyslipidemia and heart diseases. Lifestyle factors include lack of physical activity, cigarette consumption, alcohol abuse, and illicit drug use [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 most commonly used deficit rating scale is the NIHSS. It is most widely used to assess stroke severity, treatment efficacy and to predict outcomes [4]. 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 [5]. Serum S-100 protein is a multigenic family of low molecular weight (approximately 9-13 Kda) calcium-binding proteins. It is present in tissues of different origins but found in much abundance in the cells of the nervous system [6]. 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 [7]. 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 [8]. 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 [9]. 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.

2. METHODOLOGY AND MATERIALS

This cross-sectional study was 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. A total 70 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, 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 a 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 Electrochemiluminescence 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 in 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 face-to-face interviews. 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.

3. RESULT

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 population. Among all the participants, the maximum number of patients was 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). 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 the 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).

Table-1: Age distribution of the study patients (N=70)

Age (Year)	n	%
31-40 yrs.	2	2.9
41-50 yrs.	7	10.0
51-60 yrs.	23	32.9
61-70 yrs.	22	31.4
71-80 yrs.	12	17.1
>80 yrs.	4	5.7
Mean ± SD	61.21±10.98	

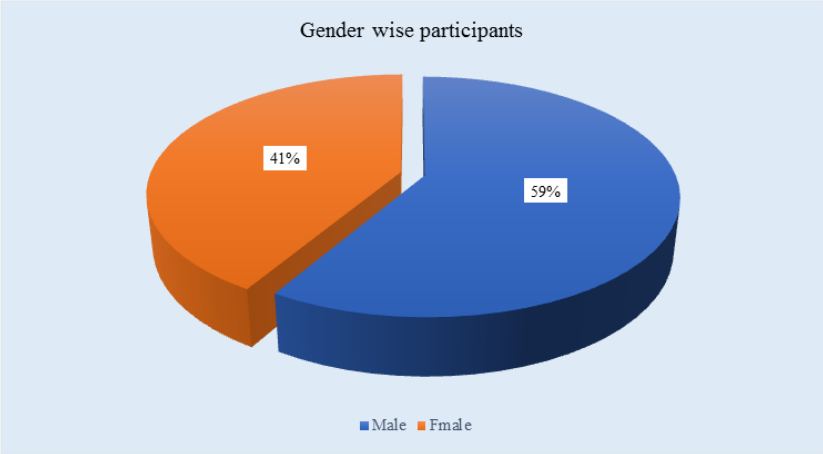

Fig-I: Gender distribution of the participants

Table-2: Distribution of risk factors of the study participants (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

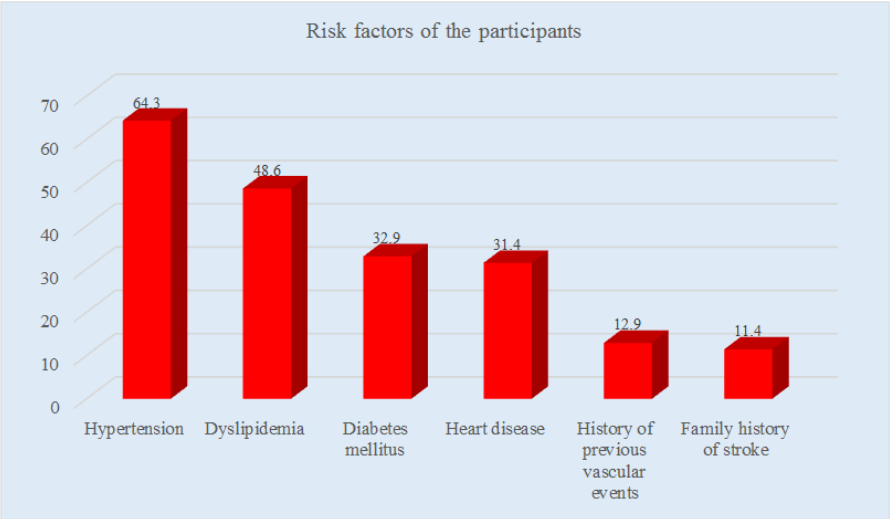

Fig-II: Risk factors of the participants

Table-3: Stroke severity as per NIHSS score among the study patients (N=70)

Stroke severity as per NIHSS score	n	%
No stroke symptoms (NIHSS score=0)	2	2.86
Minor stroke (NIHSS score=1-4)	17	24.29
Moderate stroke (NIHSS score=5-15)	35	50.0
Moderate-severe stroke (NIHSS score=16-20)	12	17.14
Severe stroke (NIHSS score=21-42)	4	5.71

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

Stroke severity as per NIHSS score	Mean ± SD	Range	P Value
No stroke symptoms (NIHSS score=0)	0.112±0.004	0.109-0.114	<0.001
Minor stroke (NIHSS score=1-4)	0.143±0.029	0.103-0.196	
Moderate stroke (NIHSS score=5-15)	0.257±0.071	0.109-0.411	
Moderate-severe stroke (NIHSS score=16-20)	0.430±0.076	0.311-0.532	
Severe stroke (NIHSS score=21-42)	0.739±0.207	0.523-1.019	
Total	0.283±0.165	0.103-1.019	

4. DISCUSSION

The aim of this study was to assess risk factors and Serum S-100 protein level of ischaemic stroke patients. This cross-sectional study was 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. In this study, among all the participants, maximum number of patients 23(32.9%) were from the age group of 51-60 years followed by 22(31.4%) patients in 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%). Acharya *et al*. (2016) [10] have found that hypertension was the most commonly appearing (64.0%) risk factor in their study. Branco *et al*. (2018) [11] have found hypertension in 69.5% of their study population.

Hossain *et al*. (2011) [12] and Basu *et al*. (2007) [13] showed that 63.0%, 68.38% and 66.0% of their study population were known hypertensive respectively. So, the findings of the current study were found consistent with the previous studies. In the present study, dyslipidemia was found in 34(48.6%) patients. Branco *et al*. (2018) [11] found dyslipidemia in 45.0% of their study population. So, the current study was consistent with the results of the previous studies. In the present study, it was observed that 23(32.9%) patients had diabetes mellitus. Acharya *et al*. (2016) [10] have found diabetes mellitus in 32.0% of their study population. Hasan *et al*. (2018) [14]. These findings are nearly consistent with the present study. Heart disease was found in 22(31.4%) patients in the present study. A similar finding was shown by Aquil *et al*. (2011) [15] where they have found the history of heart disease as a risk factor in 30.0% of their study population. This finding is nearly consistent with the findings of the current study. In this study, a history of the previous vascular events was found in 9(12.9%) patients and family history of stroke was present in case of 8(11.4%) patients. Acharya *et al*. (2016) [10] have found a history of previous vascular events in 14.0% of their study population. Fischer *et al*. (2005) have found the history of previous vascular events in 11.0% of their study population. These findings are similar to the findings of the current study. However, Aquil *et al*. (2011) [15] have found the history of the previous stroke in 29.0% of their study population and Srivastava *et al*. (2014) [16] have shown a family history of stroke in 20.14% of patients, the study populations of which are different from that of the current study. 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) [10] have found moderate stroke in 40.0% of their study population. Gajurel *et al*. (2015) [17] have obtained moderate and minor stroke among 47.0% and 19.0% of their study population respectively. In the present study, 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) [11] have found mean± SD of S-100 protein level was 439.76±562.03. Kumar *et al*. (2015) [9]. Üstündağ *et al*. (2011) [18] have shown that patients with low, moderate, and high severity strokes had significantly increasing patterns of serum S-100 protein level ($p<0.001$). These findings are nearly consistent with the findings of the current study. 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. Moreover, in this study, hypertension, dyslipidemia, diabetes mellitus, and heart disease were found as more frequent risk factors among ischaemic stroke patients.

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.

5. CONCLUSION & RECOMMENDATION

In this study, hypertension, dyslipidemia, diabetes mellitus, and heart disease were found as more frequent risk factors among ischaemic stroke patients. Serum S-100 protein levels were higher in severe ischaemic stroke in relation to the stroke of lower severity. There is a significant positive correlation found between serum S-100 protein level and severity of ischaemic stroke. So, serum S-100 protein level in this regard can be used as an important tool to predict the severity of ischaemic stroke. Therefore, it will be greatly beneficial for clinicians to assess the severity of ischaemic stroke to start treatment earlier. For getting more specific information regarding this issue we would like to recommend conducting more studies in several places with large sample size.

REFERENCES

1. Sacco, R. L., Kasner, S. E., Broderick, J. P., Caplan, L. R., Connors, J. J., Culebras, A., ... & Vinters, H. V. (2013). An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*, *44*(7), 2064-2089.
2. O'donnell, M. J., Xavier, D., Liu, L., Zhang, H., Chin, S. L., Rao-Melacini, P., ... & Yusuf, S. (2010). Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. *The Lancet*, *376*(9735), 112-123.
3. Furie, B., & Furie, B. C. (2005). Thrombus formation in vivo. *The Journal of clinical investigation*, *115*(12), 3355-3362.
4. Kwah, L. K., & Diong, J. (2014). National institutes of health stroke scale (NIHSS). *Journal of physiotherapy*.
5. Marsh, E. B., Lawrence, E., Gottesman, R. F., & Llinas, R. H. (2016). The NIH Stroke Scale has limited utility in accurate daily monitoring of neurologic status. *The Neurohospitalist*, *6*(3), 97-101.
6. Abraha, H. D., Butterworth, R. J., Bath, P. M., Wassif, W. S., Garthwaite, J., & Sherwood, R. A. (1997). Serum S-100 protein, relationship to clinical outcome in acute stroke. *Annals of clinical biochemistry*, *34*(4), 366-370.

7. Sedaghat, F., & Notopoulos, A. (2008). S100 protein family and its application in clinical practice. *Hippokratia*, *12*(4), 198.
8. Yardan, T., Erenler, A. K., Baydin, A., Aydin, K., & Cokluk, C. (2011). Usefulness of S100B protein in neurological disorders. *JPMA-Journal of the Pakistan Medical Association*, *61*(3), 276.
9. Kumar, H., Lakhotia, M., Pahadiya, H., & Singh, J. (2015). To study the correlation of serum S-100 protein level with the severity of stroke and its prognostic implication. *Journal of Neurosciences in Rural Practice*, *6*(03), 326-330.
10. Acharya, H., Kelaiya, A., Singel, V., & Makwana, P. (2016). A study of association between the national institute of health stroke scale score and arteriographic findings in acute ischaemic stroke. *International Journal of Advances in Medicine*, *3*(2), 225- 228.
11. Branco, J.P., Oliveira, S., Sargento-Freitas, J., Costa, J.S., Cordeiro, G., Cunha, L., Gonçalves, A.F., & Pinheiro, J. (2018). S100β protein as a predictor of poststroke functional outcome: a prospective study. *Journal of Stroke and Cerebrovascular Diseases*, *27*(7), 1890-1896.
12. Hossain, A.M., Ahmed, N.U., Rahman, M., Islam, M.R., Sadhya, G., & Fatema, K. (2011). Analysis of sociodemographic and clinical factors associated with hospitalized stroke patients of Bangladesh. *Faridpur Medical College Journal*, *6*(1), 19-23.
13. Basu, S., Sanyal, D., Roy, K., & Bhattacharya, K.B. (2007). Is post-stroke hyperglycemia a marker of stroke severity and prognosis: A pilot study. *Neurology Asia*, *12*(1), 13-19.
14. Hasan, T. H., Habib, K. R., Nuzhat, E., Gomes, L. C., Hossain, M. M., Miah, M. T., ... & Alam, M. B. (2018). Association of National Institute Health Stroke Scale (NIHSS) Score on Admission with the Outcome of Acute Ischemic Stroke at Discharge from Hospital. *Journal of Medicine*, *19*(2), 91-94.
15. Aquil, N., Begum, I., Ahmed, A., Vohra, E. A., & Soomro, B. A. (2011). Risk factors in various subtypes of ischemic stroke according to TOAST criteria. *J Coll Physicians Surg Pak*, *21*(5), 280-283.
16. Srivastava, M. P., Bhasin, A., Chaudhry, R., Sharma, S., Subbaiah, V., Bhatia, R., & Tripathi, M. (2014). Novel inflammatory biomarkers and their correlation to Chlamydia pneumoniae titres in acute ischemic stroke. *Journal of Stroke and Cerebrovascular Diseases*, *23*(9), 2391-2396.
17. Gajurel, B. P., Dhungana, K., Parajuli, P., Karn, R., Rajbhandari, R., Kafle, D., & Oli, K. K. (2014). The National Institute of Health Stroke Scale Score and Outcome in Acute Ischemic Stroke. *Journal of Institute of Medicine*, *36*(3).
18. Üstündağ, M., Orak, M., Güloğlu, C., Tamam, Y., Sayhan, M. B., & Kale, E. (2011). The role of serum osteoprotegerin and S-100 protein levels in patients with acute ischaemic stroke: determination of stroke subtype, severity and mortality. *Journal of International Medical Research*, *39*(3), 780-789.