Ref: Ro Med J. 2024;71(2)
DOI: 10.37897/RMJ.2024.2.10

Biomarkers of hemorrhagic transformation of acute ischemic stroke – A cross-sectional study

Subhashini K., Devipriya Surapaneni, Jagadeesan M.

Department of General Medicine, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India

ABSTRACT

Background. Hemorrhagic transformation (HT) is a serious complication that can arise in individuals experiencing acute ischemic stroke (AIS), whether it occurs spontaneously or following thrombolytic therapy, resulting in considerable morbidity and mortality. Hemorrhagic changes are associated with several risk factors, including excessive blood sugar levels, hypertension, the severity of a stroke, and reperfusion.

Aim. To assess the commonly available blood parameters such as lipid profile, serum magnesium, and serum ferritin as tools to identify patients who are at risk of hemorrhagic transformation after an acute ischemic stroke.

Materials and methods. The study was conducted at the Saveetha Medical College, Chennai, Tamil Nadu, in India, as a cross-sectional study, and a total of 142 patients diagnosed with acute ischemic stroke were enrolled for this study from January 2023 to January 2024.

Results. Among the 32 patients who had a hemorrhagic transformation, all 32 (100%) patients had elevated serum ferritin levels and the remaining 110 patients who had no hemorrhagic transformation were found to have normal serum ferritin levels and the difference was statically significant ($p<0.001$). Total cholesterol, serum LDL, serum HDL, and serum triglycerides had no statistically significant association with hemorrhagic transformation among the patients.

Conclusion. The present study concludes that people with lower serum magnesium on admission and higher serum ferritin were found to be at risk. Serum lipid profile had no statistically significant association with hemorrhagic transformation among the patients.

Keywords: hemorrhagic transformation, acute ischemic stroke

INTRODUCTION

Acute cerebrovascular accident remains a significant global and Indian health concern [1]. Thrombolytic treatments have advanced acute ischemic stroke management significantly. However, hemorrhagic transformation poses a frightening risk associated with various factors such as high blood sugar, hypertension, stroke severity, and reperfusion. Certain radiological findings and high NIHSS scores predict the risk of hemorrhagic transformation [2].

Although strokes in individuals under 45 are rare, they still account for 0% to 15% of all cases [3]. Despite advancements in prevention, diagnostics, and therapy, stroke continues to rank high among causes of mortality and disability.

By 2030, it's projected to be equal to other leading causes of death. Stroke, especially in young adults, contributes significantly to healthcare costs and lost productivity [4]. High NIHSS scores have also been linked to a higher likelihood of hemorrhagic transformation in studies [5].

Therefore, healthcare planning must prioritize stroke prevention due to its cost-effectiveness in mitigating both treatment expenses and economic impacts. Despite advancements in primary prevention, diagnostic techniques, and therapeutic amenities, stroke still ranks as the second or third leading cause of death [6]. By the year 2030, as per mortality records and projections, there will be an equilibrium reached. [7]. Moreover, stroke plays a significant role in causing impairment. Contrary to popular belief, strokes are prevalent among young adults.

Stroke contributes significantly to healthcare costs as a result of the widespread occurrence of the condition and the substantial expenses linked to the

Corresponding authors:
Devipriya Surapaneni
E-mail: devi.priya1998@gmail.com

Article History:
Received: 5 June 2024
Accepted: 28 June 2024

treatment of each patient on an individual basis. Hence, it is imperative to consider stroke and its repercussions when devising healthcare plans [8]. Due to the significant costs associated with treatment and the economic impact of decreased productivity, stroke prevention is poised to be an extremely cost-efficient strategy [9].

Magnesium plays a crucial role as an ionized mineral in numerous enzymatic processes, such as hormone receptor binding, energy metabolism, muscle function, and neuronal activity. [10] It is predominantly found within cells, with storage locations divided among bone (53%), muscle (27%), and soft tissue (19%). The levels of magnesium in the blood are carefully regulated (0.65–1.05 mmol/L), with a balance maintained through absorption in the intestines, storage in bones, and excretion by the kidneys. [11] Due to its ability to inhibit neuronal synapses, magnesium is utilized as an anticonvulsant, particularly in cases of eclamptic seizures.

Serum ferritin contributes to the damage in the ischemic area of the brain through the generation of free radicals. During ischemic conditions, iron is released from storage proteins such as ferritin, leading to the formation of harmful hydroxyl radicals. Additionally, elevated levels of ferritin can promote the liberation of glutamate from brain cells, potentially expediting the progression of a stroke. The cascade of events, including the release of free radicals and other biochemical reactions that culminate in neuronal death, are predominantly influenced by glutamate.

Choi K H et al. have found that a high ferritin level can be a key predictor of HT, PH, and sHT in patients with acute ischaemic stroke. Lowering ferritin levels through the use of iron-modifying agents or free radical scavengers could potentially help in the prevention of HT in cases of ischaemic stroke. [12]

The male-to-female ratio, stands at approximately 1.7. This means that for every 1.7 males, there is 1 female. Among individuals below the age of 40, approximately 12% have experienced a stroke. This statistic highlights the prevalence of strokes among younger individuals, indicating that strokes are not solely limited to older age groups. It is estimated that strokes account for roughly 1.2% of all fatalities in India. With strokes contributing to a significant portion of deaths, it emphasizes the importance of stroke prevention, early detection, and effective treatment methods.

This study aimed to assess the commonly available blood parameters such as lipid profile, serum magnesium and serum ferritin as tools to identify patients who are at a risk of hemorrhagic transformation after an acute ischemic stroke. The primary objective was to find whether a significant association exists between the occurrence of hemorrhagic transformation after an acute ischemic stroke and low lipid profile, low magnesium and high ferritin.

MATERIALS AND METHODS

Study design and setting

This cross-sectional study was carried out at the Outpatient Department of Saveetha Medical College situated in Chennai, Tamil Nadu, spanning six months from January 2023 to January 2024. Saveetha Medical College is a large multi-specialty hospital with 1600 beds, situated in Kuthambakkam, southwest of Chennai.

Study population

The study included patients admitted with acute ischemic stroke to the Department of General Medicine

Sample size calculation

The sample size calculation, using the prevalence formula to calculate the sample size, using p of 49.7, a confidence interval of 95% and a margin of error of 10% came out to be 142. Therefore, 142 cases admitted to the medical wards of Saveetha Medical College, who provided consent and met the inclusion criteria, were incorporated into the study.

Inclusion criteria comprised patients above 18 years with acute ischemic stroke who provided informed consent.

Exclusion criteria:

1. Patients with acute hemorrhagic stroke.
2. Prior history of acute cerebrovascular accidents, trauma.
3. Patients who underwent thrombolysis for acute cerebrovascular accident.
4. Presence of other brain mass lesions or vascular malformations.
5. Patients diagnosed with cerebral venous thrombosis were excluded.

Method of data collection: After obtaining informed consent, blood samples were collected from acute ischemic stroke patients within 24 hours of admission. The following parameters were assessed:

i) Lipid profile (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol)

ii) Serum magnesium

iii) Serum ferritin

Patients were followed up for one week, and those with declining Glasgow Coma Scale (GCS) underwent repeat CT scans. If hemorrhagic transformation was detected, initial blood parameters were correlated and reassessed.

Ethical considerations

Prior to the study's initiation, ethical clearance (SAV/AP/22/89) was obtained from the Committee on

Human Research Publication and Ethics of Saveetha Medical College, Chennai. Written informed consent was obtained from each participant before administering the standard structured questionnaire and collecting blood samples. Eligible participants were required to sign or thumbprint a consent form before enrollment, ensuring participant confidentiality and anonymizing all data collected.

All study participants received standard treatment according to our routine protocol, with no expenses incurred by either the participants or the hospital for the study's completion. Additionally, there were no personal or professional gains received directly or indirectly from any commercial entity by the study participants.

Data collection

All participants underwent interviews utilizing a pre-tested structured questionnaire to collect demographic information and relevant data. Physical activity levels were evaluated using a validated questionnaire, and smoking and alcohol consumption were also recorded. In addition to the above-mentioned assessments, participants underwent a thorough physical examination to evaluate their overall health status. Vital signs such as blood pressure, heart rate, and respiratory rate were measured, and any abnormalities were noted. The data collected from these assessments were analyzed using statistical software to identify any correlations or associations between the various parameters measured.

This comprehensive approach allowed for a thorough evaluation of the participants' health status and provided valuable insights into potential risk factors for various health conditions. Overall, the rigorous methodology employed in this study ensured that all relevant data were collected and analyzed in a systematic and standardized manner, allowing for reliable and accurate conclusions to be drawn from the findings.

Statistical analysis

Statistical analysis was conducted using SPSS Version 27. Continuous variables were presented as mean (standard deviation), while discrete variables were expressed as number (percentages). Descriptive statistics such as frequency, percentages, and graphs were utilized to address the first objective, while the association between variables and healthcare seeking behavior was examined using the Chi-square test for proportions, with Fisher's exact test applied where applicable.

A significance level of $p < 0.05$ was chosen to determine the strength and significance of associations.

Level of significance (Alpha level): The alpha level, set at 0.05, defines the boundaries between high and low probability samples, separating the most unlikely 5% from the most likely 95%.

P-value: A p-value less than 0.05 indicates statistical significance, representing the probability of obtaining the result if the null hypothesis were true, thereby indicating a type I error.

Chi-square Test: A non-parametric test used to evaluate hypotheses about proportions and study population relationships, including testing goodness of fit to theoretical expectations.

RESULTS

The majority of our study participants fell within the age range of 31-40 years (24.6%), with males comprising 76.8% of the total participants. Table 1 shows the distribution of clinical profile of the patients. Among the 142 patients, 41.5% had FP Hemi-motor type, 26.8% had hemi-motor, sensory type, 9.9% had hemi motor, aphasia, 5.6% had monoplegia, 4.9% had only aphasia, 3.5% had only cerebellar type and 3.5% had Hemi motor, FP, HH aphasia. Table 2 shows the distribution of hemorrhagic transformation of the patients. Among the 142 patients, 32 (22.5%) had hemorrhagic transformation.

Table 2 outlines the distribution of comorbidities among the study participants. The most prevalent comorbidities were diabetes mellitus (45.8%) and hypertension (23.2%).

Table 3 shows the distribution of blood parameters among the patients. 40.1% of the patients had elevated total cholesterol, 53.5% had elevated serum LDL, 95.8% had abnormal HDL level and 14.8% had elevated serum triglycerides.

Table 4 shows the association between hemorrhagic transformation of the patients and lipid profile. Total cholesterol, serum LDL, serum HDL and serum triglycerides had no statistically significant association with hemorrhagic transformation among the patients.

Table 5 shows the association between hemorrhagic transformation of the patients and serum ferritin. Among the 32 patients who had hemorrhagic transformation, all 32 (100%) patients had elevated serum ferritin level and the remaining 110 patients who had no hemorrhagic transformation were found to have normal serum ferritin level and the difference was statically significant ($p<0.001$).

Table 6 shows the association between hemorrhagic transformation of the patients and serum magnesium. Among the 32 patients who had hemorrhagic transformation, all 32 (100%) patients had abnormal serum magnesium level and the remaining 110 patients who had no hemorrhagic transformation, 10.9% had abnormal serum magnesium level and the difference was statically significant ($p<0.001$).

TABLE 1. Distribution of study participants based on clinical profile

S. No.	Variables	Frequency (n)	Percentage (%)
1.	Aphasia	7	4.9
2.	Cerebellar	5	3.5
3.	FP motor, sensory	2	1.4
4.	FP hemi-motor	59	41.5
5.	Hemi-motor, sensory	38	26.8
6.	Hemi-motor, aphasia	14	9.9
7.	Hemi-motor, FP, HH, aphasia	5	3.5
8.	Hemianopia, motor, aphasia	2	1.4
9.	Monoplegia	8	5.6
10.	Motor, sensory aphasia	2	1.4

TABLE 2. Distribution of study participants based on hemorrhagic transformation

S. No.	Variables	Frequency (n)	Percentage (%)
1.	Yes	32	22.5
2.	No	110	77.5

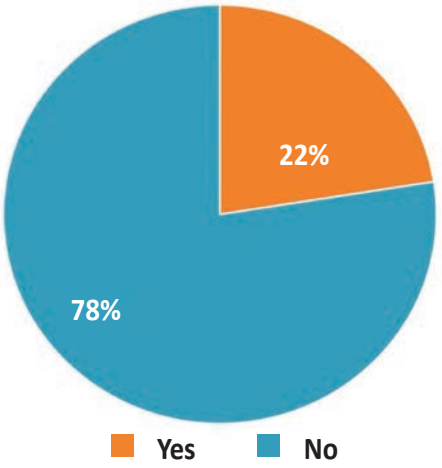

TABLE 3. Distribution of study participants based on Serum Ferritin levels

S. No.	Blood parameters	Normal (%)	Normal (%)
1.	Serum ferritin	110 (77.5)	32 (22.5)

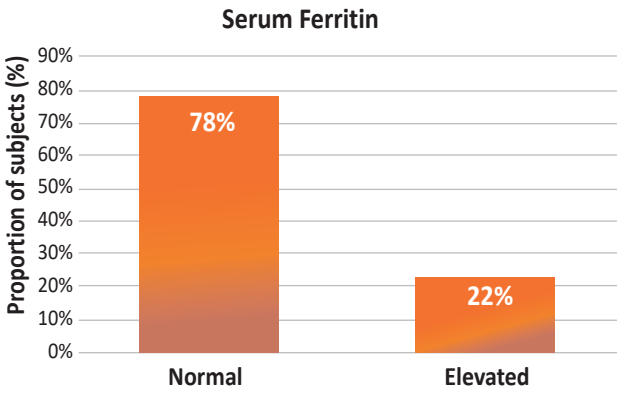

TABLE 4. Distribution of study participants based on lipid profile

S. No.	Blood parameters	Normal (%)	Abnormal (%)
1.	Total Cholesterol	85 (59.9)	57 (40.1)
2.	Serum LDL	66 (46.5)	76 (53.5)
3.	Serum HDL	6 (4.2)	136 (95.8)
4.	Serum Triglyceride	121 (85.2)	21 (14.8)

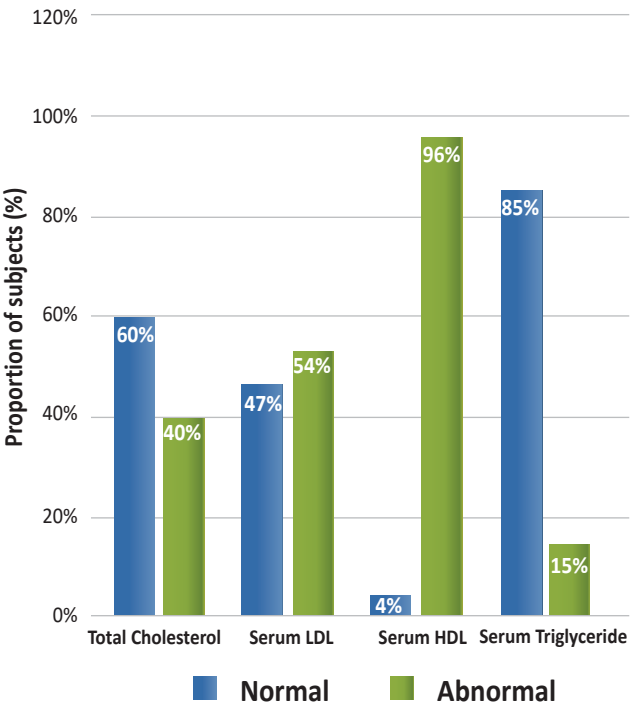

TABLE 5. Distribution of study participants according to Serum Magnesium level

S. No.	Blood parameters	Normal (%)	Normal (%)
1.	Serum Magnesium	98 (69)	44(31)

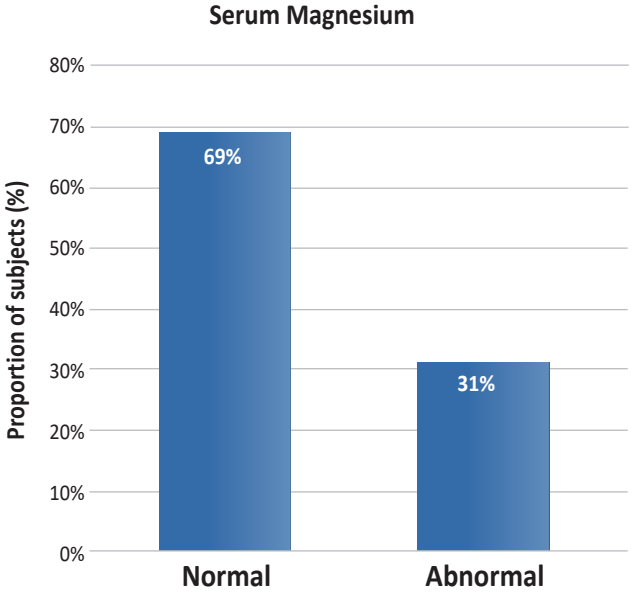

TABLE 6. Association between hemorrhagic transformation and lipid profile

S. No.	Variables	Hemorrhagic transformation		p value
		Yes	No	
1	**Total Cholesterol**			
	Normal	21 (65.6)	64 (58.2)	0.293
	Elevated	11 (34.4)	46 (41.8)	
2	**Serum LDL**			
	Normal	15 (46.9)	51 (46.4)	0.559
	Elevated	17 (53.1)	59 (53.6)	
3	**Serum HDL**			
	Normal	1 (3.1)	5 (4.5)	0.592
	Elevated	5 (96.9)	105 (95.5)	
4	**Serum Triglycerides**			
	Normal	26 (81.3)	95 (86.4)	0.322
	Elevated	6 (18.8)	15 (13.6)	

TABLE 7. Association between hemorrhagic transformation and Serum Ferritin

S. No.	Serum Ferritin	Hemorrhagic transformation		p value
		Yes	No	
1	Normal	0 (0%)	110 (100%)	< 0.001
	Elevated	32 (100%)	0 (0%)	

TABLE 8. Association between hemorrhagic transformation and Serum Magnesium

S. No.	Serum Magnesium	Hemorrhagic transformation		p value
		Yes	No	
1	Normal	0 (0%)	98 (89.1%)	< 0.001
	Elevated	32 (100%)	12 (10.9%)	

DISCUSSION

The study included 142 patients who suffered from acute ischemic stroke, between the ages of 18 and 60, who presented at the Outpatient Department in the Department of General Medicine at Saveetha Medical College, Chennai.

Cerebrovascular diseases, such as stroke, are a major public health concern due to their devastating impact and widespread occurrence. Furthermore, they also contribute significantly to the burden of illness, affecting the quality of life for countless individuals.

In India, the prevalence of stroke is alarmingly high, with an estimated 203 cases per 100,000 individuals aged 20 years and older. This translates to approximately 1 million cases of stroke in the country. The sheer magnitude of this problem highlights the urgent need for effective prevention and management strategies.

The impact of cerebrovascular diseases goes beyond mortality and morbidity rates. In 1990, stroke was recognized as the sixth highest cause of disability adjusted life years (DALYs). DALYs represent the number of years of healthy life lost due to a particular condition. This ranking underscores the profound impact that stroke has on individuals, families, and society as a whole.

Disturbingly, projections indicate that the burden of stroke is expected to worsen in the coming years. By 2030, it is predicted that stroke will become the fourth leading cause of DALYs. This projection highlights the urgent need for comprehensive and targeted interventions to address this growing public health crisis.

The study analyzed a range of demographic and clinical variables in individuals diagnosed with acute ischemic stroke. Notably, there was a diverse distribution of patients across different age categories. Specifically, 24.6% of the patients were between the ages of 31-40, 19% were aged 61-70, and 18.3% were aged 41-50. The gender breakdown indicated a significant predominance of male patients, making up 77% of the total cohort, while females represented 23%. Comorbidities were prevalent among the patient group, with 86.6% of individuals presenting with one or more additional health conditions.

Diabetes emerged as the most common comorbidity, affecting 45.2% of patients, followed by hypertension at 23.2%, coronary heart disease at 8.5%, and chronic kidney disease at 7.7%. The clinical profile of patients showcased various manifestations, with the most frequent being FP Hemi-motor type (41.5%), followed by hemi-motor, sensory type (26.8%). Hemorrhagic transformation, a significant complication, was observed in 22.5% of patients. Concerning lipid profile, a substantial proportion of patients displayed abnormalities, with 40.1% exhibiting elevated total cholesterol, 53.5% elevated serum LDL, and 14.8% elevated serum triglycerides. Moreover, 95.8% had abnormal HDL levels.

Examination of serum ferritin and magnesium levels unveiled intriguing connections with hemorrhagic transformation. All patients who experienced hemorrhagic transformation had elevated serum ferritin (100%) and abnormal serum magnesium (100%), with statistically significant variances compared to non-transformed patients ($p<0.001$). Nevertheless, no statistically significant correlations were identified between hemorrhagic transformation and age or gender, nor with common comorbidities such as diabetes, hypertension, coronary artery disease, chronic kidney disease, or bronchial asthma. Similarly, there were no significant associations observed between hemorrhagic transformation and lipid profile parameters, including total cholesterol, serum LDL, serum HDL, and serum triglycerides.

Limitations of the study

1. The study enrolled 142 patients, which, although sufficient, represented a smaller cohort compared to many Western studies.

2. Notably, seriously ill patients who were directly admitted to the intensive care unit were excluded due to difficulties in obtaining consent.
3. This exclusion may have introduced a selection bias that could impact the generalizability of the findings.

CONCLUSION

In this study, we investigated the role of serum lipid profile, serum magnesium, and serum ferritin in hemorrhagic transformation post-stroke. Among the 142 patients studied, serum ferritin levels were significantly elevated in all 32 patients (100%) who experienced hemorrhagic transformation compared to those without ($p<0.001$). Similarly, abnormal serum magnesium levels were found in all hemorrhagic transformation cases versus 10.9% in non-hemorrhagic cases ($p<0.001$). Conversely, total cholesterol, serum LDL, serum HDL, and serum triglycerides showed no significant association with hemorrhagic transformation. The severity of stroke at presentation was assessed using the National Institutes of Health Stroke Scale. These findings underscore the potential predictive value of serum magnesium and serum ferritin levels in identifying patients at higher risk of hemorrhagic transformation post-stroke, suggesting their utility in guiding early monitoring and management strategies to mitigate complications and improve clinical outcomes.

The present study concludes that people with lower serum magnesium on admission and higher serum ferritin were found to be at risk. Serum lipid profile had no statistically significant association with hemorrhagic transformation among the patients.

Public health initiatives should prioritize raising awareness about risk factors, such as hypertension, smoking, and poor diet, and promote healthy lifestyle choices. Efforts to combat cerebrovascular diseases must focus on prevention, early detection, and effective treatment.

Additionally, healthcare systems need to ensure access to timely and appropriate medical care, including emergency services, rehabilitation, and long-term support for stroke survivors. Addressing the devastating impact and widespread prevalence of cerebrovascular diseases requires a multi-faceted approach involving governments, healthcare providers, researchers, and communities.

Conflict of interest: none declared

Financial support: none declared

Authors' contributions:
Subhashini K and Devipriya Surapaneni contributed equally to the study.

REFERENCES

1. Chandra A, Li WA, Stone CR, Geng X, Ding Y. The cerebral circulation and cerebrovascular disease I: Anatomy. *Brain Circ.* 2017 Apr-Jun;3(2):45-56. doi: 10.4103/bc.bc_10_17.
2. Elsaid N, Mustafa W, Saied A. Radiological predictors of hemorrhagic transformation after acute ischemic stroke: An evidence-based analysis. *Neuroradiol J.* 2020 Apr;33(2):118-133. doi: 10.1177/1971400919900275.
3. Ntaios G. Embolic Stroke of Undetermined Source: JACC Review Topic of the Week. *J Am Coll Cardiol.* 2020 Jan 28;75(3):333-340. doi: 10.1016/j.jacc.2019.11.024.
4. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation*. 2016 Jan 26;133(4):e38-e360. doi: 10.1161/CIR.0000000000000350.
5. Wen L, Zhang S, Wan K, Zhang H, Zhang X. Risk factors of haemorrhagic transformation for acute ischaemic stroke in Chinese patients receiving Intravenous thrombolysis: A meta-analysis. *Medicine*. 2020 Feb;99(7):e18995. doi: 10.1097/MD.0000000000018995.
6. Chen S, Zeng L, Hu Z. Progressing haemorrhagic stroke: categories, causes, mechanisms and managements. *J Neurol.* 2014 Nov;261(11):2061-2078. doi: 10.1007/s00415-014-7291-1.
7. Pierik R, Algra A, van Dijk E, Erasmus ME, van Gelder IC, Koudstaal PJ, et al. Distribution of Cardioembolic Stroke: A Cohort Study. *Cerebrovasc Dis.* 2020;49(1):97-104. doi: 10.1159/000505616.
8. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation*. 2017 Mar 07;135(10):e146-e603. doi: 10.1161/CIR.0000000000000485.
9. Jickling GC, Liu D, Stamova B, Ander BP, Zhan X, Lu A, Sharp FR. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab.* 2014 Feb;34(2):185-199. doi: 10.1038/jcbfm.2013.203.
10. de Baaij JH, Hoenderop JG, Bindels RJ. Regulation of magnesium balance: lessons learned from human genetic disease. *Clin Kidney J.* 2012;5(Suppl 1):i15-i24. doi: 10.1093/ndtplus/sfr164.
11. Jahnen-Dechent W, Ketteler M. Magnesium basics. *Clin Kidney J.* 2012; 5(Suppl 1):i3-i14. doi: 10.1093/ndtplus/sfr163.
12. Choi KH, Park MS, Kim JT, Nam TS, Choi SM, Kim BC, et al. The serum ferritin level is an important predictor of hemorrhagic transformation in acute ischaemic stroke. *Eur J Neurol.* 2012 Apr;19(4):570-577. doi: 10.1111/j.1468-1331.2011.03564.x.