Serum Ferritin Level - A Predictor of Hemorrhagic Transformation in Acute Ischaemic Stroke

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ABSTRACT

Introduction: Hemorrhagic transformation (HT) in acute ischemic stroke (AIS) is associated with significant morbidity and mortality. High serum ferritin has been claimed to be associated with HT. This study investigated whether AIS patients having high serum ferritin level are at increased risk of hemorrhagic transformation.

Material and Methods: Patients with acute ischemic stroke presenting within 48 hours of onset of symptoms were included in the study. Detailed clinical, laboratory and radiological evaluation was done. All patients were evaluated for hemorrhagic transformation of the infarct over a five days period.

Results: One hundred twenty one patients with AIS were enrolled in the study. CT/MRI showed HT in 10 patients. Serum ferritin level with HT was independent with gender, age, tobacco use. The critical level of serum ferritin was taken as 161.89ng/ml, which was the minimum ferritin level in HT patients in this study. Among patients with hemorrhagic transformation having serum ferritin level above the critical level, the relative risk of HT to male was 2.25 which demonstrated that male patients having serum ferritin above critical value are more prone to develop HT than females. Patients with diabetes mellitus were more at risk than non-DM ones. It was observed that the mean of serum ferritin among patients with diabetics (132.97) was higher than the mean of non diabetic patients (117.95). The relationship between diabetics, non diabetics and serum ferritin was statistically significant (p =0.048).

Conclusion: This study substantiated the observations of previous research, that higher level of serum ferritin (within the normal physiological range) is correlated with higher conversion to HT in AIS patients, and can be used as a predictor, it is not a strong predictor alone. It becomes a strong predictor in combination of T2DM and HTN. The study opened the vista that in cases of AIS, history of T2DM and HTN should be taken into consideration along with measuring serum ferritin, and those cases having all three factors should be observed more diligently and frequently for development of HT.

Keywords: Serum Ferritin, Hemorrhagic Transformation, Acute Ischaemic Stroke

INTRODUCTION

Stroke is a non-traumatic, focal vascular injury of the nervous system and typically results in permanent damage in the form of cerebral infarction or intracerebral hemorrhage (ICH) and/or sub arachnoid hemorrhage (SAH)[1]. Stroke is a principal cause of death and disability worldwide. Ischemic stroke accounts for 80% of all strokes with the remaining 20% being composed of ICH and SAH.

According to the American Heart Association (AHA) and American Stroke Association (ASA), stroke comprises of ten possible scenarios[2]: 1. CNS infarction, 2. Ischemic stroke, 3. Silent CNS infarction, 4. ICH, 5. Stroke caused by ICH, 6. Silent cerebral hemorrhage, 7. SAH, 8. Stroke caused by SAH, 9. Stroke caused by Cerebral Venous Thrombosis (CVT), and 10. Not otherwise specified strokes. Cheung has described all these parameters in detail[1].

As pathological confirmation of stroke is generally lacking and Computed Tomography (CT) is widely available, AHA/ASA emphasized that stroke is a clinical and radiological diagnosis. Symptoms and signs should be interpreted according to our knowledge of neuroanatomy, vascular anatomy and vascular pathology, i.e. the lesion location, affected blood vessel and disease mechanism. Neuroimaging via modalities with Magnetic Resonance Imaging (MRI) or CT permits confirmation of ischemia and hemorrhage in the nervous system, reveals the size and location of lesion, along with exclusion of stroke mimics[3].

An ischemic stroke occurs due to cessation of blood flow due to extracranial or intracranial thrombosis, embolism and hypoperfusion. Neurons stops functioning in absence of oxygen and nutrient. Irreversible neuronal ischemia and injury begins when the blood flow reaches at a rate less than 18 ml/100 g of tissue/min with cell death occurring rapidly at rates below 10 ml/100 g of tissue/min[4,5]. According to Trial of ORG 10172 in Acute Stroke Treatment (TOAST) there are five aetiologies of ischemic stroke[6].

The diagnosis starts with a thorough physical and neurological examination. Examination is further followed by detailed investigations which include CT Scan[7], Magnetic Resonance Imaging (MRI)[8], Carotid Ultrasound, and Cerebral Angiogram.

Hemorrhagic Transformation (HT) represents the transformation of an insipid infarction into a hemorrhagic

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area and may be due to reperfusion of ischemic tissue, or from re-canalization of an occluded vessel or from collateral blood supply to the ischemic site or disruption of the blood-brain barrier. With disruption of the blood-brain barrier, red blood cells expel from the weak capillaries network, producing petechial hemorrhage or frank intraparenchymal hematoma[9].

Recent experimental studies suggested the effect of iron overload in the ischemic brain and endothelium damage. Iron intake was associated with the larger size of infarction, higher oxidative stress and more inflammatory response[10,11]. Iron depletion or chelation decreases the size of infarction, brain edema and neurological deficits in the cerebral ischemic-reperfusion experimental models[12, 13]. Recent studies revealed that the serum ferritin level could be a novel predictor for HT[14, 15]. Also it was reported that high serum ferritin level can be an important predicting factor for hemorrhagic transformation and the serum ferritin level of greater than 164.1ng/ml could be an independent predicting factor for HT with 85% sensitivity and 75% specificity[11, 14]. Furthermore, high serum ferritin level was also concluded as a predictor for symptomatic hemorrhagic transformation and severe brain edema[15].

The goal of this study was to determine serum ferritin levels in patients presenting with AIS and the patients who progress to develop hemorrhagic transformation so as to establish an association between high serum ferritin level and hemorrhagic transformation in patients with AIS.

MATERIAL AND METHODS

The study was conducted in the Department of General Medicine, Himalayan Institute of Medical Sciences (HIMS), Swami Ram Nagar, Dehradun, over a period of 12 months on the patients with a primary diagnosis of acute ischemic stroke attending medicine/neurology OPD or emergency or admitted in medical/neurology wards of Himalayan Institute Hospital after obtaining written informed consent from the patient or attendant and ethical clearance from the institutional ethics committee.

Study Design

Type of the study: Observational study type

Sample size: The calculated sample size was 113.

The formula used for calculation of sample size was z^2PQ/d^2 where Level of significance is 10%. Absolute error (d) is 05%. Proportion (p) is 11.9%, according to a study done by Choi et al[14]. A total of 121 patients were included in the study.

Inclusion Criteria

- Patients admitted within 48 hours of onset of symptoms to our hospital.
- Patients with acute ischemic stroke confirmed by CT or MRI.
- Patients more than 18 years of age.
- Patients suffering from MCA territory infarcts.

Exclusion Criteria

- Patients having an underlying medical disease that had an affect on the level of ferritin such as anemia, tumors, and recent myocardial infarction within <4 weeks.
- Patients with Arterio Venous Malformations.
- Patients having a history of previous stroke or intracranial hemorrhage.
- Patients with current treatment for iron supplements.
- Patients with chronic kidney disease.
- Patients with chronic liver disease.
- Patients who consumed >40 g/day alcohol, which is a well-known factor for iron loading and elevated ferritin levels.
- Patients with bleeding disorders.
- Patients with Hemorrhagic stroke at the time of presentation.
- Patients with hematological malignancies.
- Patients presenting after 48 hours of onset of symptoms have also been excluded.

Study tool

Structured study instruments i.e. NIHSS score sheet and case reporting form approved by Institutional Ethics Committee were used to generate data.

Study Protocol

Selection of cases: Patients suffering from Acute ischemic stroke on the basis of clinical and radiological diagnostic criteria for Acute ischemic stroke presenting within 48 hours of onset of symptoms were selected and their baseline characteristics were noted which included their demographic characteristics, detailed history and clinical information about stroke risk factors, radiological findings and serological findings.

The following investigations were done:

1. Electrocardiography, Complete blood count, Blood glucose, Electrolytes, Renal Function Tests, Liver Function Tests.
2. Serum Ferritin level will be measured by enzyme linked fluorescent assay using VIDAS immunofluorescence analysers version 1.0
3. CT or MRI on admission, and with any worsening. CT was done using Siemens Somatom Sensation 64 slice machine. MRI was done using 1.5 Tesla Avanto Magnatom machine Hemorrhagic transformation was judged present when any one or more follow up scan(s) showed a region consistent with presence of acute hemorrhage.

Patients were evaluated clinically and on the basis of National Institute of Health Stroke Scale (NIHSS) at admission and on fifth day of hospitalization. Patients were evaluated radiologically by CT or MRI at presentation or if any worsening was recorded on NIHSS scale.

STATISTICAL ANALYSIS

Data was analysed by using statistical software SPSS 22. Qualitative variable are represented in form of frequency and percentage. Quantitative data have been represented in form of mean ± standard deviation.

Statistical analysis was carried out with Statistical Package

for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 22.0 software for Windows. Categorical variables have been compared with $\chi 2$ test. Non-categorical variables have been compared using Mann–Whitney U test (non-parametric), where appropriate. P value of $p < 0.05$ was considered as significant.

Relative risk or risk ratio (RR) was calculated to estimate the probability of an event occurring in an exposed group to the probability of the event occurring in a comparison, non-exposed group.

RESULTS

This study was conducted over a period of twelve months and one hundred and twenty one cases of acute ischemic stroke were taken up for this study, out of which ten cases developed hemorrhagic transformation. The proportion of cases developing HT was 0.083, i.e. 8.3% cases.

In the present study, 121 Acute Ischemic Stroke (AIS) patients admitted were observed, out of which 82 were male (67.8%) and 39 were female (32.2%).

Among all AIS patients, 10 (8.3%), had Hemorrhagic Transformation (HT), out of which 9 were male having serum ferritin above critical level. The calculation of relative risk (RR) of HT to male, its value is above 1.0 (2.25) which demonstrated that male patients having serum ferritin above critical value are more prone to develop HT than female

Parameters	Sex	N	Mean	SD	P-value
Age	M	82	60.30	14.04	0.003
	F	39	61.2	12.52	
Pulse (per minute)	M	82	90.66	23.21	0.337
	F	39	91.78	23.14	
SBP (mm(Hg)	M	82	149.23	35.56	0.258
	F	39	150.31	35.34	
DBP (mmHg)	M	82	84.90	19.90	0.246
	F	39	85.13	18.61	
RBS (mg/dl)	M	82	185.47	77.23	0.005
	F	39	172.71	71.16	
FBS (mg/dl)	M	82	128.39	46.94	0.002
	F	39	118.62	42.80	
HbA1c %	M	82	7.11	2.27	0.074
	F	39	6.66	2.02	
S. Ferritin (ng/ml)	M	82	123.29	38.75	0.960
	F	39	117.91	30.86	
Hb (g/dl)	M	82	13.20	2.67	0.171
	F	39	13.20	2.44	
TLC (per mm^3)	M	82	10.09	4.63	0.944
	F	39	10.05	4.45	
Platelet (per mm^3)	M	82	187.89	81.11	0.337
	F	39	196.80	87.65	
S. Creatinine (mg/dl)	M	82	1.39	0.65	0.010
	F	39	1.34	0.72	
S. Sodium (mmol/l)	M	82	134.15	20.89	0.447
	F	39	135.08	18.17	
S. Potassium (mmol/l)	M	82	3.99	0.78	0.234
	F	39	3.98	0.74	
Total Bilirubin (mg/dl)	M	82	1.21	0.64	0.711
	F	39	1.20	0.61	
AST (IU/l)	M	82	47.67	27.19	0.298
	F	39	44.32	22.37	
ALT (IU/l)	M	82	46.15	26.79	0.147
	F	39	42.45	22.27	
PT/INR (min.)	M	82	1.09	0.28	0.976
	F	39	1.10	0.31	
S. Albumin (g/dl)	M	82	3.59	0.85	0.726
	F	39	3.60	0.79	
NIHSS Score (at admission)	M	82	26.74	6.90	0.064
	F	39	25.95	7.11	
NIHSS Score (after 5 days)	M	82	21.90	8.35	0.011
	F	39	20.06	8.08	

Table-1: Difference of baseline characteristics on the basis of Gender. Mann-Whitney U Test was applied to test the mean differences between male and female patients.

Parameters	HT/ NHT	N	Mean±SD	P-value
Age (year)	HT	10	60.30±14.04	0.576
	NHT	111	61.23±12.52	
Pulse (per minute)	HT	10	78.60±16.93	0.048
	NHT	111	93.58±20.63	
SBP (mm(Hg)	HT	10	175.20±20.00	0.011
	NHT	111	152.41±30.64	
DBP (mmHg)	HT	10	99.20±16.87	0.034
	NHT	111	86.25±15.97	
RBS (mg/dl)	HT	10	220.00±57.89	0.011
	NHT	111	173.86±69.33	
FBS (mg/dl)	HT	10	162.90±24.26	<0.001
	NHT	111	119.51±40.65	
HBA_{1C}%	HT	10	9.49±1.63	<0.001
	NHT	111	6.70±1.86	
S. Ferritin (ng/ml)	HT	10	185.62±17.12	<0.001
	NHT	111	118.52±26.14	
Hb (g/dl)	HT	10	12.73±1.71	0.271
	NHT	111	13.43±1.86	
TLC (per mm^3)	HT	10	10.07±3.95	0.936
	NHT	111	10.18±4.41	
Platelet (per mm^3)	HT	10	182.00±84.08	0.395
	NHT	111	199.03±86.91	
S. Creatinine (mg/dl)	HT	10	1.42±0.60	0.162
	NHT	111	1.37±0.72	
S. Sodium (mmol/l)	HT	10	137.74±8.02	0.267
	NHT	111	137.38±5.52	
S. Potassium (mmol/l)	HT	10	3.89±0.50	0.083
	NHT	111	4.04±0.57	
Total Bilirubin (mg/dl)	HT	10	1.21±0.51	0.472
	NHT	111	1.22±0.61	
AST (IU/l)	HT	10	56.30±38.49	0.174
	NHT	111	44.73±22.45	
ALT (IU/l)	HT	10	57.00±41.06	0.131
	NHT	111	42.75±22.39	
PT/INR (min.)	HT	10	1.14±0.15	0.158
	NHT	111	1.12±0.29	
S. Albumin (g/dl)	HT	10	3.80±0.63	0.390
	NHT	111	3.66±0.67	
NIHSS Score (at admission)	HT	10	28.00±5.19	0.204
	NHT	91	26.49±6.37	
NIHSS Score (after 5 days)	HT	10	27.70±8.26	<0.001
	NHT	91	20.44±7.78	

Table-2: Difference of baseline characteristics between HT and NHT patients with AIS. Values are expressed as Mean ± SE.

ones.

Out of 121 patients, 19 (15.70%) patients were in the age group of ≤ 50 years and 102 (84.30%) were in the age group of >50 years. Their mean age was 61.1±12.6 SD years. Mann-Whitney U test does not reveal significant difference in serum ferritin levels between the two age groups (P = 0.631).

The calculation of relative risk (RR) of HT to older age group (> 50 years), its value is much above 1.0 which demonstrated that older patients having serum ferritin above critical value are more prone to HT than those which are < 50 year old ones.

Out of 121 AIS patients observed, 59 (48.76%) were either smokers/reformed smokers or tobacco chewers. There was no significant effect of smoking on serum ferritin level (p = 0.453).

Out of 59 patients who were smokers/tobacco chewers, 51 (86.44%) patients had serum ferritin below critical level and 8 (13.55%) patients had serum ferritin above critical level. Out of 62 patients, who were non-smokers, 55 (88.71%) patients had serum ferritin below critical level and 7 (11.29%) patients had serum ferritin above critical level. The relationship between smokers and non-smokers and serum ferritin level was statistically insignificant (P= 0.7051).

Mean FBS, RBS, HbA1c and serum ferritin levels were significantly higher among AIS patients with HT than in those without HT (p < 0.001 each). A significant association was seen between diabetes and hemorrhagic transformation

AIS cases		Serum Ferritin	HTN	T2DM
With HT	1	212.40		+
	2	210.56	+	+
	3	195.30	+	+
	4	192.67	+	+
	5	183.32	$	+
	6	182.40	+	+
	7	178.93	+	+
	8	170.20	+	+
	9	168.50	$	+
	10	161.89	+	+
Without HT	11	174.86		+
	12	173.78	+	
	13	173.29		
	14	167.90	+	+
	15	167.41		+

Table-3: Patients with AIS having high ferretin level along with HTN and T2DM factors. + indicates higher values according to AHA(American Health Association)

Parameters	No. of patients	% of patients
HTN	49	44.1
T2DM	38	34.2
High serum ferritin	5	4.5
T2DM $ Ferritin	2	1.8
T2DM $ HTN	19	17.1
T2DM $ High serum ferritin $ HTN	1	0.9

Table-4: Patients with AIS without HT having T2DM, HTN and high serum ferritin.

in patients with AIS ($p < 0.001$). Diabetes showed a significant association with serum ferritin level ($p < 0.001$) while a significant association was present between serum ferritin level and hemorrhagic transformation ($p < 0.001$) in patients with acute ischemic stroke. Patients with AIS having serum ferritin above critical level (>161.89 ng/ml) were more prone to HT (Relative Risk 22.2, $p < 0.0001$). Diabetic patients with AIS having serum ferritin above critical level had higher but statistically insignificant risk of HT than non-diabetic patients (Relative Risk 4.5, $p < 0.247$).

All HT patients[10] were diabetic, hence the calculation of relative risk (RR) of HT to DM, its value approaches infinity, which demonstrated that diabetic patients having serum ferritin above critical value are extremely prone to HT than those which are not diabetic.

Among the patients having HTN, 47 (85.46%) had serum ferritin below critical level and 8 (14.54%) had serum ferritin above critical level. Out of 66 patients who had no HTN, 59 (89.39%) had serum ferritin below critical level and 7 (10.61%) had serum ferritin above critical level. The relationship between HTN and non-HTN and serum ferritin level was statistically insignificant (P= 0.513).

Among the patients having HTN+DM, 18 (72.00%) had serum ferritin below critical level and 7 (28.00%) had serum ferritin above critical level. Out of 30 patients, who had HTN alone, 29 (96.67%) had serum ferritin below critical level and only 1 (3.33%) had serum ferritin above critical level. Among 66 patients, who are diabetic (DM) alone, 61 (92.42%) had serum ferritin below critical level and 5 (7.58%) had serum ferritin above critical level. The two-way ANOVA shows that level of serum ferritin and HTN, DM or both HTN+DM were statistically insignificant (P = 0.298, between serum ferritin level and P = 0.255 between HTN+DM, HTN and DM alone).

Out of 121 AIS patients observed, 114 (94.21%) had NIHSS more ≥ 15. The serum ferritin level of AIS patients having NIHSS ≥ 15 was not significantly higher than AIS patients having NIHSS <15.

Among the patients having NIHSS ≥ 15, 99 (86.84%) had serum ferritin below critical level and 15 (13.16%) had serum ferritin above critical level. Out of 7 patients, who had NIHSS <15, all had serum ferritin below critical level and none had serum ferritin above critical level. The relationship between NIHSS ≥ 15 and NIHSS < 15 and serum ferritin level was statistically insignificant (P= 0.305). $\chi 2$ test was used.

The calculation of relative risk (RR) of NIHSS ≥ 15 to NIHSS < 15, its value approaches infinity, which demonstrated that NIHSS ≥ 15 patients having serum ferritin above critical value are extremely prone to HT than those which have NIHSS < 15.

Out of 121 AIS patients observed, 88 (72.73%) had NIHSS more ≥ 15. Table 23 shows that the serum ferritin level of AIS patients having NIHSS ≥ 15 was not significantly higher than AIS patients having NIHSS <15.

Among the patients having NIHSS ≥ 15, 74 (84.09%) had serum ferritin below critical level and 14 (15.91%) had serum ferritin above critical level. Out of 33 patients, who had NIHSS <15, 32 (96.97%) had serum ferritin below critical level and only 1 (2.03%) had serum ferritin above critical level. The relationship between NIHSS ≥ 15 and NIHSS $<$ 15 and serum ferritin level was statistically insignificant (P= 0.056).

The cut-off value of the ferritin level that optimally predicted the occurrence of HT was 161.89 ng/ml, and this resulted in a sensitivity of 70%. However, there were no cut-off values of T2DM (RBS, FBS, HbA1c) and HTN (SBP, DBP) parameters that could optimally predict the occurrence of HT.

Table 4 displays the percentage of patients that had AIS but NHT and had HTN, DM or higher serum ferritin level. It demonstrated that only one of the patients had all these parameters. Very less proportion of diabetic patients had high serum ferritin level (1.8%) and HTN (17.1%).

Thus in this study it can be conclusively inferred that patients who had HT had serum ferritin level of 161.89 ng/mL or more. Thus the criticality of serum ferritin level with hemorrhagic transformation at a level of 161.89 ng/mL is established.

In this study it was also found that five cases out of one hundred and eleven, which did not develop hemorrhagic transformation had serum ferritin level above the critical value (161.89 ng/mL). Hence the universality of criticality at

serum ferritin level of 161.89 ng/mL or more to hemorrhagic transformation could not be established.

DISCUSSION

Several studies have already identified the predictors of HT in patients with ischemic stroke. The following factors are known to be associated with HT: Systolic blood pressure[16], the history of T2DM[17], the time to perfusion[18], thrombolysis[19], old age[20], the symptom severity, use of aspirin and other anticoagulant drugs[18], cardiac embolism[9], and serum ferritin level[11]. Ferritin is the cellular storage protein for iron and is essentially located within cells and constitutes the main intracellular iron storage protein[21].

Recent studies demonstrated that the serum ferritin level could be a novel predictor of HT in patients with acute ischemic stroke[11, 22]. Cairo et al. demonstrated ferritin synthesis is induced by oxidative stress[23] and Orino et al. reported that high ferritin levels cause oxidative stress in cells[24]. Such oxidative stress was reported to increase blood-brain-barrier (BBB) permeability[25]. Indeed, the cell types that compose the BBB include brain microvascular endothelial cells (BMEC), astrocytes, and pericytes[26]. In the periphery, vascular endothelial cells are often fenestrated and lack tight junctions allowing for the paracellular flux of polar molecules into the surrounding tissue[26]. Brain microvascular endothelial cells lack fenestrations and possess tight junctions, forcing most molecules to be trafficked transcellulary via receptor-mediated transcytosis (insulin, ferritin), adsorptive transcytosis (albumin), or transport proteins (glucose, amino acids)[27], and thus increased BBB permeability may cause HT. The role of increased iron in the stress oxidative reactions, inflammatory responses and vascular endothelium damage in the ischemic brain has already been established[10]. Castellanos et al. demonstrated that the increase in the iron intake was associated with the increase of extent of infarction after obstructing the middle cerebral artery of rats[10].

Shimizu et al. reported that Advanced Glycation End Products (AGEs) reduces the expression of claudin-5 in Brain Microvascular Endothilial Cells (BMECs) by increasing the autocrine signaling through Vascular Endothelial Growth Factor (VEGF) and Matrix Metalloproteinase-2 (MMP-2) secreted by the BMECs themselves[28]. Furthermore, advanced glycation end products increase the amount of fibronectin in the pericytes through a similar up-regulation of the autocrine transforming growth factor (TGF)-β released by pericytes, thus damaging the integrity of BBB and making it prone to disrupt under acute stress. Some clinical studies illustrated that the high serum ferritin level in the patients with ischemic stroke was related to the severity of stroke, increase of size of the lesion and poor prognosis[29].

In this study, 121 patients with AIS were included and evaluated for all the risk factors at the baseline data gathered from the patients. Choi et al. and Mehrpour and Mehrpour suggested 164.1 ng/ml as a cut off for serum ferritin level[52,68]. The outcome of this study was almost consistent with this suggestion (161.89ng/ml). The study of Choi et al[52] demonstrated 11.96% incidence of HT among study AIS patients. This study demonstrated 8.26% incidence, almost similar to that of Choi et al.

High ferritin levels had been related to poor outcome and large lesion size in patients with acute ischemic stroke. High ferritin levels had been related to a higher risk of HT and edema development in patients treated with tissue plasminogen activator after ischemic stroke[15]. Likewise, high ferritin levels at admission were independently associated with poor outcome in patients with intracerebral hemorrhage[30]. These findings suggest a neurotoxic effect of increased body iron stores on the ischaemic brain in patients with stroke[14]. Brabec et al. reported high ferritin level was always associated with several haemolytic disorders[31].

The molecular bases for the HT secondary to a high ferritin concentration are, generation of hydroxyl radicals, endothelial injury, and disruption of the blood brain barrier32. Ferritin is the main intracellular iron storage protein and it keeps iron in a soluble and non-toxic form, and it might be related to the availability of iron in the infarcted area[33]. However, in patients with cerebrovascular diseases, oxygen superoxide radicals increase the amount of iron in the cytosole during neuronal injury under hypoxic-ischemic conditions by enabling the release of iron from ferritin as a source of iron for oxidative damage[34]. The ability of the superoxide radicals generated during ischaemic injury to release ferrous iron from ferritin is assumed to play an important role in acute stroke[35]. Iron is a pro-oxidant cofactor that is associated with increased production of free radicals and increased progression of atherosclerosis[36]. Furthermore, redox-active iron can both initiate and propagate lipid per-oxidation by catalyzing oxidative damage to lipids, and this eventually leads to the formation of edema and membrane disruption[19]. The damage is considered to depend on the concentration of tissue iron[37]. Therefore, a high ferritin concentration may be a biologic deleterious predictor of HT in patients with acute ischemic stroke.

The results of this study need further substantiation with larger multi-centric studies because of the limited sample size. As the thresholds for predicting HT was within the normal ferritin range, the findings should prompt larger, prospective, multicenter, and confirmatory studies. This study lacked long-term data, so hemorrhage rates may have been underestimated. Further, the relationship between the serum ferritin levels and HT might have been confounded by inflammation. Ferritin is also reflected by inflammation as well as the CRP level, and especially amongst subjects with general medical complications of stroke such as aspiration pneumonia and urinary tract infection. Although we excluded those subjects with infectious disease on admission, residual confounding by such factors cannot be absolutely ruled out[14].

In this study it can be conclusively inferred that patients who developed HT had serum ferritin level ≥ 161.89ng/mL. Thus the criticality of serum ferritin with hemorrhagic transformation at a level of 161.89ng/mL is established. In this study it was also found that out of 111 cases which did not develop HT, five cases had serum ferritin more than

the critical value of 161.89ng/mL. Hence the universality of criticality at serum ferritin ≥ 161.89ng/mL co-related to hemorrhagic transformation could not be established.

In the present study, though the sample size is small an important message is conveyed on close perusal of the result. The role of Serum ferritin above a cut-off value has been amply cited by various authors, and has been likewise substantiated in this study also. Literature also says that T2DM and Hypertension also play role to damage BBB. In this study, an important finding was that 9 subjects out of 10 (90%) with HT were having all the three factors combined, viz. serum ferritin above the critical value of 161.89 ng/mL, T2DM and HTN, while those having NHT and serum ferritin above 161.89 ng/ml, only one subject had T2DM and HTN. Thus it can be inferred that high serum ferritin, when gets the support of T2DM and HTN caused more oxidative stress and damage to the already weakened BBB. Despite the acknowledged limitations of our study, these findings warrant further investigations to assess whether lowering the ferritin level with iron-modifying agents and phlebotomy and use of free radical scavengers could also be of therapeutic value.

As the data collection period for this study was only one year and was unicentre, the data sample was not that robust as used in studies done by other authors; a multicentre long term study is further needed. Study is also needed with respect to use of iron chelating agents and its effect on HT in subjects having high serum ferritin.

CONCLUSION

The present study confirmed the findings of previous studies, which found that serum ferritin level was a predicting factor for HT in patients with acute ischemic stroke. Consequently, the serum ferritin level greater than 161.89 ng/ml in the first 24 hours after admission of the patients with AIS can be considered as a red flag to gain more attention when managing these patients, particularly during anticoagulant and thrombolytic therapy. Despite the acknowledged limitations of our study, these findings warrant further investigations to assess ferritin level as a guide in various management modalities such as strict management of blood pressure, thrombolytic therapy, and anticoagulation therapy after acute ischemic stroke in patients with higher ferritin level could be helpful in preventing HT. The effect of interventions on reducing the serum ferritin level, such as iron chelating medications, can be the subject of additional studies in the future.

Small sample size and short time of the follow up were weak points of this study. Furthermore, because ferritin is an acute phase reactant, so many confounding conditions influence its serum level. Hence, impossibility of eliminating all factors affecting serum ferritin level in spite of maximum efforts in this study, limits the interpretations of these findings. Multi-centric studies with long-term follow-ups and larger sample size are warranted in future.

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