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ORIGINAL RESEARCH

To investigate the role of Serum Ferritin levels in acute cerebrovascular events(Haemorrhagic and Ischemic stroke)

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Abstract

Background: Cerebrovascular accidents (CVAs), commonly known as strokes, are among the most significant health challenges globally. A stroke occurs when the blood supply to part of the brain is disrupted which prevents brain tissue from receiving oxygen and nutrients. The present study was conducted to evaluate the serum ferritin in both ischemic and hemorrhagic stroke.

Methods: The present study was conducted on 50 individuals with cerebrovascular accident. Diagnosis of stroke was confirmed by CT or MRI scan of brain and examination was done by NIHSS stroke scale and GCS score at the time of admission. Serum ferritin levels are measured by two site immunoenzymatic (sandwich) assay.

Results: With respect to NIHSS and GCS score on presentation, we found that the mean NIHSS score was significantly higher in haemorrhagic stroke subjects as compare to those with ischemic stroke. While GCS score did not show any such significant difference. 62% of all study subjects had survived and 38% of all study subjects had expired.We concluded that, mean serum ferritin levels were increasing significantly as stroke severity increased across the stroke subtypes.

Conclusion: This study emphasizes the positive relationship between acute stroke severity and serum ferritin level. The findings of this investigation further support the role of serum ferritin in the prognostication of acute stroke.

Introduction

Cerebrovascular accidents (CVAs), commonly known as strokes, are among the most significant health challenges globally. A stroke occurs when the blood supply to part of the brain is disrupted which prevents brain tissue from receiving oxygen and nutrients. Strokes are categorized into two main types: ischemic and haemorrhagic.[1]Stroke is the second largest cause of death globally, accounting for 113 million disability-adjusted life years (DALYs) and an estimated 6.5 million deaths in 2013.In India, stroke accounts for 3.5% of disability-adjusted life years (DALY), making it a major non-communicable disease. Computed tomography (CT) is the first line used in the diagnosis of hyperacute stroke.[2] Another technique is magnetic resonance imaging (MRI) and Positron emission tomography (PET) scanwhich is utilized for its capacity to produce accurate and detailed anatomical pictures of the brain. However, the gold standard for diagnosing and measuring cerebral artery

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atherosclerotic stenoses is conventional x-ray cerebral angiography.[3]Biomarkers are objective indicators that are used to predict results, analyze responses to treatment, and evaluate normal or pathological processes. These includes lipids, proteins, metabolites, and ribonucleic acids along with serum ferritin. Thus, the present study was conducted to evaluate the serum ferritin in both ischemic and hemorrhagic stroke.

Materials and methods

The present study was conducted on 50 individuals with cerebrovascular accident presented in Medicine Emergency at Guru Nanak Dev Hospital, Amritsar. The study was carried out after seeking permission from Institutional Ethics Committee, Government Medical College, Amritsar. Written informed consent was obtained from the patients. The patients were divided into two groups.

Group A - Cerebrovascular accidents with ischemic stroke (25 patients).

Group B - Cerebrovascular accidents with Haemorrhagic stroke (25 patients).

Inclusion criteria

- All cases of acute cerebrovascular accident patients from 18 to 80 years
- Diagnosis of CVA confirmed by CT or MRI scan of brain.
- Patients should present within 48hrs of onset of symptoms

Exclusion criteria

- anaemia (Hb<8mg/dl), history of malignancy, liver disease or renal disease, recent myocardial infarction within <4 weeks, history of previous stroke or intracranial haemorrhage.

Appropriate questionnaire was used to collect data of patients. Diagnosis of stroke was confirmed by CT or MRI scan of brain and examination was done by NIHSS stroke scale and GCS score at the time of admission. Venous blood sample was collected for measuring serum ferritin levels, within 48hrs of onset of symptoms. Serum ferritin levels are measured by two site immunoenzymatic (sandwich) assay.

The data was collected systematically and analysed statistically according to the standard statistical methodsusing SPSS 25.0 version.

Results

In the study, the majority of individuals (38%) fall into the “51-60” age group.Males (56.0%) outnumber females (44.0%) in the studied population. Equal number of patients were presented in each category i.e. Haemorrhage or an ischemic infarct (50.0%). In ischemic patients GCS score on presentation were mild in 16 percent, moderate in 36 percent and severe in 48% and In Haemorrhage patients GCS scores were mild in 28% cases, moderate in 32% and severe in 60% cases.In ischemia patients NIHSS scores on presentation were minor in 4 percent, moderate in 40%, moderate to severe in 16 percent cases and severe in 40% cases and in Haemorrhage patients NIHSS score on presentation was moderate in 16 percent cases, moderate to severe in 20% cases and severe in 64% cases.

Table 1: Association of GCS and NIHSS on presentation with imaging finding of total study subjects

	Imaging	N	Mean	Std. Deviation	P value
GCS at Presentation	Haemorrhage	25	7.64	3.01	.099
	Ischemic	25	9.12	3.21	.099

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NIHSS on presentation	Haemorrhage	25	26.04	11.04	.025
	Ischemic	25	18.84	11.04	.025

Table 1 showed that GCS and NIHSS on presentation with imaging. It was found that the p-value for GCS between Haemorrhage and ischemic groups is 0.099, indicating no statistically significant difference. With NIHSS on Presentation the p-value between Haemorrhage and ischemic groups is 0.025, suggesting a statistically significant difference.

In the present study, most of the patients 62.0% were discharged and a smaller proportion 38.0% did not survive and passed away during their hospital stay.The GCS (Glasgow Coma Scale) scores with outcomes in total study subjects in discharged patients,19% of the total study subjects had a mild GCS score, 48% of the total study subjects had a moderate GCS score and 32% of the total study subjects had a severe GCS score. In Expired patients 10% of the total study subjects who expired had a moderate GCS score and 90% of the total study subjects who expired had a severe GCS score. In total 62% of all study subjects had survived and 38% of all study subjects had expired.

In Discharged patients 3% of the total study population had a minor NIHSS score, 45% of the total study population had a moderate NIHSS score, 26% of the total study population had a moderate to severe NIHSS score and 26% of the total study population had a severe NIHSS score. While in Expired patients 5% of the total study population who expired had a moderate to severe NIHSS score and 95% of the total study population who expired had a severe NIHSS score. In total, 62% of all study subjects had a minor NIHSS score and 38% of all study subjects had a moderate NIHSS score.For patients who were discharged mean GCS score was 9.94 and for patients who did not survive (death) mean GCS score was 5.84. The p-value (statistical significance) for the difference in GCS scores between discharge and death groups is very low ($p < 0.001$), indicating a significant association. For NIHSS on presentation for patients who were discharged Mean NIHSS score was 15.06 and for patients who did not survive (death) Mean NIHSS score was 34.47 Similar to GCS, the p-value for the difference in NIHSS scores between discharge and death groups is very low ($p < 0.001$), suggesting a strong association. The table represents the association of GCS score with outcome. The mean GCS in discharged patients in haemorrhagic stroke was 9.58 ± 2.5 while in ischemic stroke it was 10.16 ±2.7. However, there was no significant difference between them($p<0.56$). In patients who are expired (Death) the mean GCS score in haemorrhagic stroke was 5.85 ± 2.27 while in ischemic stroke it was 5.83 ± 2.32 with no significant difference($p<0.99$). It was observed in study that mean GCS score was lower in patients who are expired than discharged.The table represents the association of NIHSS score with outcome. The mean NIHSS score in patients which were discharged was 16.33 ± 5.23 in haemorrhagic stroke while 14.26 ± 6.90 in ischemic stroke. In expired patients the mean NIHSS score was 35.00 ± 6.11 in haemorrhagic stroke while 33.33 ± 9.1 in ischemic stroke. There is no significant difference in both the groups. However, it was observed that mean NIHSS score was higher in expired subjects.

Table 2: Association of mean serum ferritin levels with NIHSS score grade and type of stroke

Imaging	**NIHSS at presentation**	**N**	**Mean ferritin levels (ng/ml)**	**Standard deviation**	**P value**
Haemorrhage	Minor (1-4)	0	.	.	
	Moderate (5-15)	5	138.08	124.72	

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	Moderate to severe (16-20)	4	67.77	40.30	
	Severe (21-42)	16	408.39	189.95	
	Total	25	299.83	218.24	0.001
Ischemia	Minor (1-4)	1	120.00	.	
	Moderate (5-15)	10	83.79	73.265	
	Moderate to severe (16-20)	4	248.12	244.81	
	Severe (21-42)	10	230.91	164.39	
	Total	25	170.38	159.38	0.014

Table 2: The table represents that there was significant correlation in both haemorrhagic and ischemic stroke groups with serum ferritin levels as NIHSS increases across the groups. In our study, the total mean serum ferritin of haemorrhagic stroke was higher than ischemic stroke subjects.

Table 3 Association of mean serum ferritin levels with stroke severity on the basis of GCS score

Imaging	GCS at presentation	N	Mean ferritin levels (ng/ml)	Standard deviation	P value
Haemorrhage	Mild (13-15)	2	126.00	98.99	0.041
	Moderate (9-12)	7	189.47	176.45	
	Severe (3-8)	16	369.84	219.45	
	Total	25	299.83	218.24	
Ischemia	Mild (13-15)	4	114.67	88.56	0.045
	Moderate (9-12)	9	100.21	89.36	
	Severe (3-8)	12	241.57	191.80	
	Total	25	170.38	159.38	

The table 3 represents that there was significant correlation in both haemorrhagic and ischemic stroke groups with serum ferritin levels across the groups as GCS increases across the groups. In our study, total mean serum ferritin levels of haemorrhagic stroke were higher than ischemic stroke subjects.

Table 4: Serum ferritin in discharged subjects

	Haemorrhagic stroke	Ischemic stroke
Mean ferritin(ng/ml)	96.97	100.35
Median ferritin(ng/ml)	55.8	72.4
Standard deviation	88.57	88.11
Range	306.2	277.9
Minimum	25.8	14
Max	332	291.9
P value	0.83	

The table 4 represents the mean ferritin levels of discharged patients in both haemorrhagic and ischemic subjects are 96.97ng/ml and 100.35 and difference were not statistically significant.

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Table 5: Serum ferritin in deceased study subjects

	Haemorrhagic stroke	Ischemic stroke
Mean ferritin (ng/ml)	487.08	392.13
Median ferritin (ng/ml)	494.6	384.7
Standard deviation	94.49	127.80
Range	328	325.9
Minimum	340.8	250.8
Max	668.8	576.7
P value	0.08	

The table 5 shows the mean ferritin levels were more in haemorrhagic study subjects, but the difference was not statistically significant.

Table 6 Association of other hemodynamic parameters with imaging finding of study subjects

	Imaging	N	Mean	Std. Deviation	P value
AGE	Haemorrhage	25	61.20	9.35	0.187
	Ischemic	25	57.00	12.59	
SBP	Haemorrhage	25	177.60	25.05	0.006
	Ischemic	25	154.80	31.24	
DSP	Haemorrhage	25	99.20	14.70	0.020
	Ischemic	25	89.20	14.70	
PR	Haemorrhage	25	70.52	12.32	0.001
	Ischemic	25	83.48	13.41	
RR	Haemorrhage	25	18.08	3.63	0.528
	Ischemic	25	17.44	3.49	
Spo2	Haemorrhage	25	92.16	5.20	0.027
	Ischemic	25	95.20	4.18	
Temp	Haemorrhage	25	99.06	1.06	0.660
	Ischemic	25	99.19	.99	

The table 6 represents association of hemodynamic parameters with imaging findings. It was found that Diastolic BP ($p = 0.020$), pulse rate ($p = 0.001$) and mean SpO2 ($p = 0.02$) was statistically significant.

Discussion

Serum ferritin serves as an indicator of cellular iron reserves and is potentially linked to the iron availability in the infarcted region. Experimental evidence suggests that iron overload contributes to ischemic brain and endothelial damage. In contrast, iron depletion or chelation has been shown to reduce infarct size, brain edema, and metabolic failure in ischemia/reperfusion stroke models.

In the present study, out of 50 subjects, majority of patients were in the age group 51-60 years (38%). A study done byMytherini et al. shows similar age distribution in study with majority are in 51-60 years.[4] A Similar study was conducted by Kapoor et al which was a prospective cohort study shows that the majority of the subjects were in the age group of 51-60 years.[5] Rajendra et al. also found similar results.[6]Male predominance was seen in our study with

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56% males and 44% females. Khan et al., also observed male dominance over females.[7]Similar to these findings, other studies also showed a male predominance.[4, 8, 9]

The basic hemodynamic parameters were measured which shows a significant relation of serum ferritin with blood pressure, pulse rate and oxygen saturation were observed. Rajendra et al., revealed that higher levels of BP indices such as systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), and mean arterial pressure (MAP) were present in the adverse prognostic groups, but the elevation were not significant. However, subjects with significantly higher mean age had bad prognosis or died within a week of hospitalization.[6]

In present study, the majority of patients 62.0% were discharged. A smaller proportion 38.0% did not survive. For patients who were discharged mean GCS score was 9.94 and for patients who did not survive (death) mean GCS score was 5.84. The p-value for the difference in GCS scores between discharge and death groups is very low ($p < 0.001$), indicating a significant association. This was in concordance with study by Mosisa et al in which mean GCS score in deceased was <8 were 7.7 times more likely to die as compared to GCS score 13-15.[10]

For NIHSS on presentation the scores had significant difference in discharge and death groups ($p < 0.001$). This is in concordance to study by Kavitha et al, who stated that the NIHSS score on day 1 on average non survivors were 28.21 and almost half of this were scores in survivors.[11]

Serum ferritin is primarily used in clinical medicine to evaluate the body's iron reserves. It was suggested that an elevation of body iron reserves before the stroke may have contributed to the exacerbation of brain ischemic cytotoxicity. Furthermore, increased iron levels may increase the risk of ischemia events due to an increase in atherogenesis [Kaol et al].[12]

In current study, stroke severity was assessed by NIHSS and GCS scores. It was observed in study that serum ferritin levels were increasing significantly as stroke severity increases. The mean serum ferritin in haemorrhagic stroke was 299.83ng/ml which was higher than ischemic stroke (170.38ng/ml). The mean serum ferritin in discharged study subjects in haemorrhagic and ischemic was not statistically significant. In deceased study subjects' serum ferritin levels in haemorrhagic and ischemic groups were not statistically significant. The study done by Khan et al shows mean serum ferritin in severe ischemic stroke patients was 408.48ng/ml.[7] In similar study done by Ranjan et al also showed that there is significant correlation between serum ferritin levels and stroke severity based on NIHSS score in ischemic strokes with mean serum ferritin in mild, moderate and severe NIHSS score was 197.91ng/ml, 285.56ng/ml, 337.41ng/ml respectively.[13] A study done by Ghadge et al in acute ischemic strokes shows the total mean in serum ferritin level of 227ng/ml, observed that lower serum ferritin had better outcome.[14] According to Thanikachalam et al, the mean serum ferritin levels at admission in ischemic study subjects with severe NIHSS score, moderate NIHSS score, and minor NIHSS score were 337.41±58.76ng/ml, 285.56 ±49.37ng/ml, and 197.91 ±111.01ng/mL, respectively.[15] With respect to haemorrhagic stroke a study by Xiao et al stated that the mean serum ferritin in mild, moderate, severe NIHSS score was 150.2ng/ml, 250.8ng/ml, 350.6mg/ml which increased significantly with stroke severity.[16] A similar study done by Mythreini et al shows mean serum ferritin levels in haemorrhagic strokes was 250ng/ml, 308mg/ml and 415mg/ml respectively, which is statistically significant.[4]

Other scale used in study to assess stroke severity was GCS score. Similar results were there that serum ferritin levels increasing significantly with stroke severity. We found that as stroke severity increases serum ferritin was also increases.A study done by Giri et al, in this study mean serum ferritin levels were compared with GCS score in ischemic stroke study subjects, show that mean serum ferritin in subjects with GCS score >8 was 265.24ng/ml and in subjects with GCS score <8 was 437.36 and study also shows significant correlation of serum ferritin with GCS score with increased stroke severity.[17] Similar study done by Rizk et al

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shows that serum ferritin on presentation <250ng/ml had better GCS score while >250ng/ml have bad score and was statistically significant.[18] A study done by Singh et al, compare mean serum ferritin levels on admission with GCS score in haemorrhagic stroke, study shows that mean serum ferritin levels with mild, moderate, severe GCS score was 76ng/ml, 94ng/ml, 149ng/ml respectively while in deceased study subjects was 161ng/ml.[19]It was observed in study that serum ferritin levels were increases with stroke severity and differences were statistically significant. The difference in ferritin levels were there that is haemorrhagic stroke had higher levels than ischemic stroke, and it was just outside the limits of statistical significance with p value of 0.08. A study done by Chauhan et al, show that mean ferritin levels in haemorrhagic groups were higher than ischemic group and study show that haemorrhagic stroke had poor prognosis than ischemic stroke.[20] A similar study done by Mythreini et al, observed that patients with haemorrhagic stroke had significant higher level of serum ferritin than ischemic stroke. It also shows that haemorrhagic stroke had higher mortality rate than ischemic stroke.[4]One possible explanation is that high levels of ferritin in the serum indicate higher levels of iron storage in the brain. Cerebrovascular ischemia causes more iron to be discharged from injured brain cells during a CVA. More iron leads to more oxidative stress. When more iron is discharged into the area surrounding the damaged tissue, more free hydroxy radicals are produced. This increases the risk of further tissue damage during cerebral ischemia. Another possible explanation is that damaged brain cells released more glutamate during ischemia, which further damages the tissue. Both of these mechanisms lead to further tissue injury during ischemia [Cote et al.; Reif et al.].[21,22]

In our study, it was observed in study that serum ferritin levels in haemorrhagic stroke was much higher than ischemic stroke in deceased subjects and it is just outside the limits of statistical significance (p-0.08). A study by Mohan et al. stated that the level of serum ferritin was comparatively very much lower in the subjects who were discharged, and it was found to be highly statistically significant.[8]Mehdiratta et al. reported a positive correlation between the serum ferritin levels and the perihematomal edema in patients of spontaneous cerebral hematoma.[23]

Similarly, Pankaj et al. showed the mean serum ferritin (458.7ng/mL) of the clinically deteriorated patients' group on admission day was significantly higher (87.01 ng/mL) than the clinically improved group.[24] Narayan et al. reported the mean serum ferritin of deteriorated patients was significantly higher (463.91 ng/mL) than the recovered patients (96.44 ng/mL).[25] In another study, a significant increase ($p<0.01$) in serum ferritin concentration was observed in patients with large-sized lesions [Erdemoglu et al].[26] This finding aligns with a similar study by Demerdash et al. where they also reported significantly higher serum ferritin levels in patients with larger-sized lesions, and these patients experienced worsened neurological conditions during follow-up.[27]

There are several other studies conducted across India shows positive correlation with severity of stroke and serum ferritin. Koul et al., study conducted in Srinagar using Modified Rankin scale and NIHSS, another study by Thanikachalam et al.,conducted in Tamil Nadu using NIHSS scale for severity of stroke. All the above studies found that serum ferritin levels can be used as a prognostic marker in acute stroke.[12, 15]While in another study, by Üstünda et al, ferritin and pro BNP levels were determined in acute ischemic stroke patients. It was observed that serum pro-BNP levels were clinically useful in predicting stroke subtype severity and outcome while serum ferritin levels were not found to be indicative of these in a significant manner.[28]

Limitations: The current study was single centred and had a limited sample size. Hence the data is not applicable to general population. Hence, more studies with a larger sample size are required, so that a stronger relationship between serum ferritin and stroke can be

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elucidated.The baseline serum ferritin levels before stroke were not available in the study subjects, which could affect the final results.

Conclusion

The present study was done on subjects with cerebrovascular accidents and the imaging outcome was compared to their serum ferritin levels. In the present study it was found that the subjects with increased serum ferritin concentrations have of poor clinical outcomes than patients with low ferritin values. Elevated serum ferritin level may help in predicting early neurological deterioration and this may in turn helps in early decision-making and interventions. The serum ferritin can act as a prognostic marker and indicator of severity in acute stroke. NIHSS and GCS scores were found to have a significant correlation with serum ferritin levels. The subjects who deceased, had significantly higher ferritin levels than those who were discharged. The function of serum ferritin as a prognostic marker in acute stroke has been the subject of very few Indian studies. This study emphasizes the positive relationship between acute stroke severity and serum ferritin level. The findings of this investigation further support the role of serum ferritin in the prognostication of acute stroke. Although larger studies are required to find the association of serum ferritin with acute stroke.

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