Serum Ferritin Levels in Acute Ischemic Stroke Patients - A Cross Sectional Study from Thiruvananthapuram, Kerala

Rupa Gopinathan[1], Deepa Gopalakrishna[2], Saboora Beegum[3], Thomas Iype[4]

[1, 2] Department of Physiology, Government Medical College, Thiruvananthapuram, Kerala, India.
[3] Department of Biochemistry, Government Medical College, Thiruvananthapuram, Kerala, India.
[4] Department of Neurology, Government Medical College, Thiruvananthapuram, Kerala, India.

ABSTRACT

BACKGROUND

Disturbance of brain iron homeostasis have been linked to acute neuronal injury following cerebral ischemia. Increased body iron stores measured as serum ferritin is an acute-phase reactant involved in cellular defence against oxidative stress and it constitutes the main intracellular iron storage protein. In a healthy population, iron excess may not be a major concern; however, in persons with high oxidative stress and dyslipidaemia, iron excess may place them at greater risk. Hence this study is undertaken to find out the role of iron in acute ischemic stroke and to estimate the iron stores, measured as serum ferritin in acute ischemic stroke patients.

METHODS

A minimum of 180 consecutive patients above 40 years in the acute phase of ischemic stroke within 72 hours of first episode admitted to the neurology department was selected for the study. Data collection was based on Interview method by detailed questionnaire and laboratory investigations. Quantitative determination of serum ferritin was done by immunoenzymatic colorimetric method using ELISA technique. Association of risk factors like age, gender, place of residence, history of hypertension, dyslipidaemia and diabetes between cases with elevated ferritin and normal values were analysed.

RESULTS

In the present study incidence of stroke was more common among patients with the age group of more than 50 years and among the 180 cases, 75 % showed elevated serum ferritin levels. Association of risk factors between cases with elevated ferritin and cases with normal ferritin were studied and it shown that history of hypertension, dyslipidaemia and diabetes were statistically significant. Multiple logistic regression showed history of hypertension and dyslipidaemia that were independent predictors of elevated ferritin levels among stroke patients.

CONCLUSIONS

Serum Ferritin was increased in acute ischemic stroke patients. There was significant association of factors like history of hypertension, dyslipidaemia and diabetes with elevated ferritin levels.

KEYWORDS

Stroke, Ferritin, Oxidative Stress, Free Radicals

Corresponding Author:
Dr. Thomas Iype,
Professor and HOD,
Department of Neurology,
Government Medical College,
Thiruvananthapuram, Kerala, India.
E-mail: drthomasiypeneuro@gmail.com

DOI: 10.18410/jebmh/2021/287

How to Cite This Article:
Gopinathan R, Gopalakrishna D, Beegum S, et al. Serum Ferritin levels in Acute Ischemic Stroke patients - A cross sectional study from Thiruvananthapuram, Kerala. J Evid Based Med Healthc 2021;8(20):1516-1520. DOI: 10.18410/jebmh/2021/287

Submission 26-01-2021,
Peer Review 01-02-2021,
Acceptance 26-03-2021,
Published 17-05-2021.

BACKGROUND

Stroke is now considered as an important health problem and is the third leading cause of death after acute myocardial infarction. Among all neurological diseases, stroke ranks first in frequency and importance. As per World Health Organization, Stroke is defined as a clinical syndrome consisting of rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, with duration lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin. The crude stroke prevalence in various parts of India ranges from 44.29 to 559 / 1,00,000 persons which is significantly higher as compared to western data.[1] Following cerebral ischemia, acute neuronal injury has been linked with the disturbances in brain iron homeostasis. Ferritin, the main intracellular iron storage protein which catalyses the production of radical oxygen species, stores the metal within cells in an available safe manner.[2] It happens to be an acute-phase reactant playing a vital role in cellular defence against oxidative stress and inflammation together with transferrin. As both serum iron and transferrin are not primarily associated with cerebrovascular event occurrences, serum ferritin is considered as the best clinical measure of iron storage in body. Serum ferritin has now caught the attention of researchers as a potential prognostic marker for stroke, though, it started out initially as a stress response.

The aetiology of diseases like cancer, atherosclerotic cardiovascular disease and aging process is linked with oxidative stress or compromised antioxidant status. The balance between reactive oxygen species and the antioxidant defence system determines the desirable oxidative status.[3] Disturbances in brain iron homeostasis is a chief contributor of reactive oxygen species. Free iron that gets released from intracellular stores like ferritin during cerebral ischemia, catalyses the conversion of superoxide along with hydrogen peroxide into a toxic hydroxyl radical.[4] Intensified excitotoxicity mediated by glutamate was also put forward as one of the mechanisms of iron related injury in cerebral ischemia.[5] Brain is susceptible to oxidative stress primarily due to lower amount of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase and higher amount of polyunsaturated fatty acid side chains in the membrane lipids.[6] Majority of the nonheme iron as ferritin is found in astrocytes and microglia in brain, gets triggered in hypoxic acidosis or oxidative stress.[7] Ferrous iron gets generated from ferritin as a result of superoxide radicals released during ischemia and reperfusion injury. Thus, superoxide radicals are able to convert a trivial subclinical event such as transient ischemic attack into a major clinical episode. During reperfusion after cerebral infarction, progressive tissue destruction and cellular death results from the profound release of iron ions and marked increase in oxygen-radical production.[8] This effect of free radicals in brain ischemia and subsequent reperfusion has knock-on effects on vasodilatation and associated membrane permeability leading to a bad prognosis of stroke.[9] Generation of free radicals and development of atherosclerosis is also chiefly linked with the prooxidant cofactor, mainly iron.

Though, iron excess may not be a major issue in a healthy population, in persons inclined to oxidative stress and dyslipidaemia, excess of iron may heighten the cerebrovascular risk. So far, only a few articles have reported on the association between iron and the risk of stroke in population-based studies. A greater understanding of brain iron metabolism and its correlation with neuronal injury can lead to more therapeutic targets and enhance the current outcome of stroke patients.

Objectives

1. The primary objective was to estimate serum ferritin levels in acute ischemic stroke.
2. The secondary objective was to find out the association of risk factors like age, gender, hypertension, dyslipidaemia and diabetes with cases with normal and elevated serum ferritin levels.

METHODS

The study design was a cross sectional study and its participants were a minimum of 180 consecutive patients in the acute phase of ischemic stroke within 48 hours of first episode admitted to the Neurology department at Government Medical college, Thiruvananthapuram, were selected for the study. The study duration was for two years from January 2015 to January 2017.

Inclusion Criteria

Consecutive patients above 40 years in the acute phase of ischaemic stroke within 48 hours of first attack admitted to Neurology department.

Exclusion Criteria

- Previous disability, trauma.
- Alcohol consumption.
- Inflammatory or infectious disease of liver, renal and haematological disorders.
- Current treatment with iron.
- Repeated blood transfusion.
- Cerebral haemorrhage.

Data collection was based on Interview method by detailed questionnaire and laboratory investigations. The study was approved by the Institutional Research Committee and Human Ethics Committee and was conducted under the State Board of Medical Research at Government Medical College, Thiruvananthapuram. Under aseptic precautions 2 ml of blood was taken in a plain glass bottle and serum was separated by centrifugation. Quantitative determination of serum ferritin was done by immunoenzymatic colorimetric method using enzyme linked immunosorbent assay (ELISA) technique.

Diametric Ferritin ELISA test is based on simultaneous binding of human Ferritin to two monoclonal antibodies, one

immobilized on microwell plates and the other conjugated with the enzyme horseradish peroxidase. After incubation the bound and free separation is performed by a simple solid phase washing. Then the enzyme in the bound fraction reacts with the substrate hydrogen peroxide and develops a blue colour that changes into yellow when the sulphuric acid stop solution is added. The colour intensity is proportional to the Ferritin concentration in the sample. The Ferritin concentration in the sample is calculated based on a standard curve. The normal serum ferritin values in males were in the range of 12 to 300 ng / ml and in females within the range of 12 to 150 ng / ml.

Categorical and quantitative variables were expressed as frequency (percentage) and mean ± standard deviation respectively. chi-square test, Odds ratio with 95 % confident interval was used to find association of abnormal serum ferritin levels among patients with acute ischemic stroke with selected characteristics of patients. Multiple logistic regression was carried out to find independent predictors of abnormal serum ferritin levels.

Statistical Analysis

For all statistical interpretations, P < 0.05 was considered the threshold for statistical significance. Statistical analyses were performed by using IBM - SPSS version 25.0.

RESULTS

Baseline characteristics of the study population showed maximum number of the cases 85 % were above 50 years of age. Majority were males coming from rural side. Dyslipidaemia and hypertension were observed among most of the cases. Family history was reported among 51.7 % of the cases. The percentage distribution of sample according to serum ferritin showed an elevation in 70 % cases (63.3 – 76.7) and the mean value was 334.7 ± 132.

Distribution of serum ferritin based on gender showed elevation in 70.7 % in males and 68.8 % in females with mean and SD were 326.6 ± 121.7 and 349.4 ± 148.8 respectively. The serum ferritin levels among patients with acute ischemic stroke showed median value 363.2 (268.8, 411.6) in males and 377.0 (266.9, 464.3) in females respectively.

Background Characteristics		Count	Percent
Age in years	41 - 50	27	15.0
	51 - 60	64	35.6
	61 - 70	60	33.3
	➢ 70	29	16.1
Gender	Male	116	64.4
	Female	64	35.6
Residence	Urban	12	6.7
	Rural	168	93.3
History	Hypertension	128	71.1
	Dyslipidaemia	133	73.9
	Diabetic mellitus	56	31.1
Family History	Hypertension	93	51.7
	Dyslipidaemia	60	33.3
	Diabetic mellitus	42	23.3

Table 1. Percentage Distribution of the Sample According to Background Characteristics of Patients with Acute Ischemic Stroke

Analysis of association of serum ferritin with age showed that ferritin values increased with age and was statistically significant with P value 0.095. Ferritin levels were elevated in 70.7 % males compared to females 68.8% and were statistically insignificant.

Elevated ferritin levels were mainly found in rural population compared to urban with an odds ratio 3.6 & association was significant. Association with present history of hypertension showed elevation in 84 % cases with odds ratio 9.69 & those with both present and family histories showed 89.7 % elevation with odds ratio 16.15 which were also statistically significant with P value < 0.01. Association with history of dyslipidaemia showed elevation in 70 % of cases with family history & odds ratio 2.21. It showed elevation in 67.5 % of cases with present history with odds ratio 1.97 & cases with both family & present histories showed 88 % elevation with odds ratio 6.95 & was also statistically significant with a P valve 0.003. Association with history of diabetes mellitus showed elevated ferritin in 71.4 % of cases with family history. Cases with present history had 54.8 % ferritin elevation and those with both histories had 57.1 % ferritin elevation & were statistically significant.

		Normal		Elevated		χ^2	p	Odds (95% CI) *
		Count	%	Count	%			
Age in years	41 - 50	9	33.3	18	66.7	6.38	0.095	-
	51 - 60	22	34.4	42	65.6			-
	61 - 70	20	33.3	40	66.7			-
	> 70	3	10.3	26	89.7			-
Gender	Male	34	29.3	82	70.7	0.07	0.786	-
	Female	20	31.3	44	68.8			-
Residence	Urban	7	58.3	5	41.7	4.9 +	0.027	1
	Rural	47	28.0	121	72.0			3.60 (1.09 – 11.92)
History of Hypertension	No History	24	64.9	13	35.1	69.21	P < 0.01	1
	Family history only	14	93.3	1	6.7			0.13 (0.12 – 1.12)
	Present history only	8	16.0	42	84.0			9.69 (3.52 – 26.71)
	Both family and present history	8	10.3	70	89.7			16.15 (5.97 – 43.70)
History of Dyslipidaemia	No History	18	48.6	19	51.4	14.09 ++	0.003	1
	Family history only	3	30.0	7	70.0			2.21 (0.49 – 9.89)
	Present History only	27	32.5	56	67.5			1.97 (0.89 – 4.34)
	Both Family and Present history	6	12.0	44	88.0			6.95 (2.39 – 20.24)
History of Diabetic mellitus	No History	21	21.9	75	78.1	8.79 +	0.032	1
	Family history only	8	28.6	20	71.4			0.7 (0.27 – 1.81)
	Present History only	19	45.2	23	54.8			0.339 (0.16 – 0.74)
	Both Family and present history	6	42.9	8	57.1			0..37 (0.117 – 1.19)

Table 2. Factors Associated with Serum Ferritin Levels among Patients with Acute Ischemic Stroke

*: Confidence Interval, + Significant at 0.05 level, ++: Significant at 0.01 level

Multiple logistic regression analysis was carried out to find independent predictors of abnormal serum ferritin levels among patients with acute ischemic stroke. Abnormal serum ferritin levels of patients were taken as dependent variable. Age, gender, place of residence, histories of hypertension, diabetes and dyslipidaemia were takes as independent variables. Backwards logistic regression analysis was carried out to find independent predictors of abnormal serum ferritin levels among patients with acute ischemic stroke. Histories of Hypertension and dyslipidaemia were extracted as significant independent predictors of abnormal serum ferritin. Patients with present history of hypertension were prone to develop abnormal serum ferritin with odds of 21.6 whereas patients with both family and present histories had more chance to develop abnormal serum ferritin with an odds ratio of 27.27. Similarly, the odds ratio was 4.64 and 2.46 in patients with family and present histories of dyslipidaemia whereas it was 7.56 in patients who showed both family and present histories. The R^2 (coefficient of determination) of the regression was found as 0.376, it means that 37.6 percent of variation on abnormal serum ferritin levels among patients with acute ischemic stroke can be explained by history of Hypertension and history of dyslipidaemia.

DISCUSSION

Acute Stroke is a major adverse outcome in patients with cardiovascular risk factors. Serum ferritin levels (mean and standard deviation 334.7 ± 132) were found to be elevated in 70 % (95 % confidence interval 63.3 to 76.7 %) of the study subjects. A cross sectional study conducted by (Garg et al.)[10] demonstrated similar ferritin levels (mean and standard deviation 336.86 ± 57.28) in stroke patients.

In the present study ferritin levels showed statistically significant elevation in 108 out of 180 cases. Note that majority of these patients were more than 50 years of age (84.8 %). Elevation of serum ferritin can occur as a part of a low-grade inflammation with ageing, which is known to alter iron metabolism. Age related variation was not significant in other similar studies.[11] Since majority of our study population were above 50 years, a separate cut off values for ferritin based on gender were not taken. Also, the value of ferritin after 40 years was within the range for male subjects. The 25th percentile in males was 268.83 and 75th percentile was 411.60 with median value 363.29. In females 25th percentile was 266.98 and 75th percentile was 464.30 with median value 377.02. Hence there was no statistically significant gender difference in ferritin values in our study in line with observations by other studies. It was worth noting that the incidence of ischemic vascular disease was high in postmenopausal women owing to the elevation in iron stores with oxidative imbalance as the central biologic mechanism.[12] The loss of endogenous oestrogen production puts postmenopausal females at increased risk of developing atherosclerotic diseases from dyslipidaemia, oxidative stress, elevated iron stores and insulin resistance.[13]

Disturbance of brain iron homeostasis and enhanced glutamate mediated excitotoxicity releases free iron from ferritin, inducing generation of toxic hydroxyl free radical during cerebral ischemia. These free radicals play an important role in acute stoke. Such oxidative stress is reported to increase blood brain barrier permeability. Blood brain barrier limits the delivery of plasma iron to brain cells and thus, conversely to peripheral organs, brain doesn't seem to accumulate iron when body iron stores are increased. Brain microvascular endothelial cells possess tight junctions forcing ferritin to be trafficked transcellularly normally as they lack fenestrations. Thus, the circulating free iron can enter the cerebral ischemic areas through the disrupted blood brain barrier and potentially worsen the infarction processes.[14] Elevated accumulation of iron within the brain may be due to blood brain barrier failure resulting from altered vascularization associated with normal or pathological ageing.[15] Disproportionally elevated body iron creates oxidative stress that can generate hydroxyl radicals producing damage to the cellular membrane, lipids, proteins and DNA.[16] Iron through free radicals causes oxidation of lipids and proteins by promoting formation of oxidized low-density lipoprotein and isoprostanes. Hence oxidative stress can produce systemic inflammatory response leading to endothelial dysfunction, an early event in atherosclerosis by accelerating lipid peroxidation. Thus, oxidative metabolism during ischemic stroke together with high iron content in the brain synergize to increase the oxidative damage. Hence the molecular basis for a greater brain injury secondary to iron overload includes generation of hydroxyl radicals from oxygen during reperfusion, increased excitotoxic damage mainly glutamate, blood-brain barrier disruption and endothelial injury.[15] This could explain the elevated ferritin values observed in ischemic stroke patients in the present study.

Association of risk factors like history of hypertension, diabetes mellitus and dyslipidaemia between cases with elevated ferritin and cases with normal ferritin were studied as shown in Table 2. Dysregulation of iron metabolism is an important risk factor for the development of hypertension. The generation of superoxide radicals in hypertension has been shown to affect the production and activity of endogenous vascular nitric oxide, which may result in impaired endothelium dependent vasorelaxation.[17] Thus, iron overload contributes to the generation of reactive oxygen species, increasing oxidative stress and inflammation which have a deleterious effect on endothelial dysfunction resulting in hypertension. Our study results also show a statistically significant association of history of hypertension and ischemic stroke.

Being a strong transition prooxidant, iron can induce oxidative stress and interrupt mitochondrial beta oxidation of long chained fatty acids resulting in hypertriglyceridemia and increased triglyceride accumulation in muscle and liver tissue. Elevated cholesterol levels may reduce integrity of blood brain barrier or can disrupt iron metabolism making the brain susceptible to cholesterol related cellular stress.[15] High lipid peroxidation and raised proinflammatory cytokines triggered by high circulating ferritin mediates the association with dyslipidaemia as observed in our study. The raised levels of triglycerides induce elevated levels of free fatty acids which may leads to insulin resistance and beta cell

dysfunction. Hence, serum ferritin may indirectly affect glucose metabolism through lipid peroxidation.[18]

Overload of iron indicates abnormality in glucose metabolism which triggers beta cell oxidative stress and decreased insulin secretory capacity. The pathophysiological mechanisms underlying diabetes is linked with inflammation as it plays a role in regulating ferritin mRNA and protein levels. Ferritin level correlation with hepatic, adipocyte and muscular insulin resistance reveals its relationship with diabetes.[19] In our study, history of diabetes had a statistically significant association with elevated levels of ferritin.

CONCLUSIONS

Serum ferritin levels were significantly elevated in 75 % of the cases. The incidence of stroke was higher among patients above 50 years of age. In patients with transient ischemic stroke, elevated ferritin levels were also found to be associated with other risk factors of stroke like hypertension, dyslipidaemia and diabetes.

Limitations

The main limitation of this study was that serum ferritin levels were measured after the occurrence of stroke. So, it was not ascertained whether ferritin levels were already elevated prior to stroke or higher ferritin levels resulted from the stroke itself. Similarly, there exists strong correlation between the lesion size or the neurologic deficit and the systemic inflammation resulting from stroke. Hence it needs to be studied further as to whether a systemic inflammatory response to a stroke is indeed harmful and not just an epiphenomenon.[14] We recommend studies that further investigates ferritin levels in relation to the timeline of acute ischemic stroke.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: This study was supported by grant from the state board of medical research.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

REFERENCES

[1] Sureshkumar K, Gudlavalleti ASV, Gudlavalleti VSM, et al. Incidence & prevalence of stroke in India: a systematic review. Indian Journal of Medical Research 2017;146(2):175-185.
[2] Cairo G, Tacchini L, Pogliaghi G, et al. Induction of ferritin synthesis by oxidative stress. Transcriptional and post-transcriptional regulation by expansion of the "free" iron pool. J Biol Chem 1995;270(2):700-703.
[3] Cracowski JL, Devillier P, Durand T, et al. Vascular biology of the isoprostanes. J Vasc Res 2001;38(2):93-103.
[4] Pankaj P, Das M, Singh MK. Association between level of serum ferritin and outcome of patients of stroke. Journal of Evolution of Medical and Dental Sciences 2015;4(12):2023-2037.
[5] Boisvert DP. In vivo generation of hydroxyl radicals during glutamate exposure. The Role of Neurotransmitters in Brain Injury 1992: p. 361-366.
[6] Halliwell B. Reactive oxygen species and the central nervous system. J Neurochem 1992;59(5):1609-1623.
[7] Connor JR, Menzies SL, St Martin SM, et al. Cellular distribution of transferrin, ferritin and iron in normal and aged human brains. J Neurosci Res 1990;27(4):595-611.
[8] White BC, Sullivan JM, DeGracia DJ, et al. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. J Neurol Sci 2000;179(Suppl 1-2):1-33.
[9] Nelson CW, Wei EP, Povlishock JT, et al. Oxygen radicals in cerebral ischemia. Am J Physiol 1992;263(5 Pt 2):H1356-H1362.
[10] Garg R, Aravind S, Kaur S, et al. Role of serum ferritin as a prognostic marker in acute ischemic stroke: a preliminary observation. Annals of African Medicine 2020;19(2):95-102.
[11] Milman N. Serum ferritin in Danes: studies of iron status from infancy to old age, during blood donation and pregnancy. Int J Hematology 1996;63(2):103-135.
[12] Van der AD, Grobbee DE, Roest M, et al. Serum ferritin is a risk factor for stroke in postmenopausal women. Stroke 2005;36(8):1637-1641.
[13] Crist BL, Alekel DL, Ritland LM, et al. Association of oxidative stress, iron and centralized fat mass in healthy postmenopausal women. J Women's Health (Larchmt) 2009;18(6):795-801.
[14] Millerot E, Prigent-Tessier AS, Bertrand NM, et al. Serum ferritin in stroke: a marker of increased body iron stores or stroke severity? J Cereb Blood Flow Metab 2005;25(10):1386-1393.
[15] Del C Valdés HM, Allan J, Glatz A, et al. Exploratory analysis of dietary intake and brain iron accumulation detected using magnetic resonance imaging in older individuals: The Lothian Birth Cohort 1936. J Nutr Health Aging 2015;19(1):64-69.
[16] Fearon IM, Faux SP. Oxidative stress and cardiovascular disease: novel tools give (free) radical insight. J Mol Cell Cardiol 2009;47(3):372-381.
[17] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circulation Research 2000;87(10):840-844.
[18] Parhofer KG. Interaction between glucose and lipid metabolism: more than diabetic dyslipidemia. Diabetes Metab J 2015;39(5):353-362.
[19] Wlazlo N, Van Greevenbroek MMJ, Ferreira I, et al. Iron metabolism is prospectively associated with insulin resistance and glucose intolerance over a 7-year follow-up period: the CODAM study. Acta Diabetologica 2015;52(2):337-348.