International Journal of Biomedicine 14(2) (2024) 260-264
http://dx.doi.org/10.21103/Article14(2)_OA3

ORIGINAL ARTICLE

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Investigating the Role of Serum Hepcidin and Interleukin-6 in Non-Anemic Women with Acute Ischemic Stroke

Zahir Hussain*

Department of Physiology, Faculty of Medicine, Umm Al-Qura University
Makkah, Saudi Arabia

Abstract

Background: Hepcidin (HP) is an important regulator of iron homeostasis. Iron status and IL-6 have been shown to regulate the expression of HP. Serum iron (SI), HP, and IL-6 have a highly significant role in inflammation since inflammation elevates the levels of HP for expressing the ischemic condition. The present study was carried out to investigate the impact of an interactive association between HP, SI, and IL-6 in non-anemic women with acute ischemic stroke (AIS).

Methods and Results: The present case-control, descriptive study comprised 25 non-anemic women with AIS and 25 healthy non-anemic women controls. The age range of AIS and control subjects was 50 to 54 years. Non-anemic AIS women within 8 hours after AIS onset (AIS<8 hrs) and 72 hours after onset of AIS (AIS-72 hours) were examined. The patients underwent assessment of traditional risk factors, physical examination, CBC, blood biochemistry test, 12-lead ECG, CT scans, MRI, CT or MR angiogram, carotid ultrasound of the arteries, transcranial doppler ultrasound, and EEG. Office blood pressure was measured using a mercury sphygmomanometer. Significantly increased values were obtained for systolic blood pressure and diastolic blood pressure in AIS women, compared to the control subjects: 137.44±12.35 vs. 130.88±7.30 mmHg (P=0.0267) and 86.72± 8.48 vs. 81.80± 5.42 mmHg (P=0.0183), respectively. Serum C-reactive protein and low-density lipoprotein cholesterol levels in AIS were significantly higher than in healthy control women (P<0.0001).

A significant increase in serum HP, SI, and IL-6 for AIS<8 hrs was found, compared to AIS-72 hrs (P<0.001 in all cases). Comparison for AIS<8 hrs vs. controls showed a highly significant increase in serum HP, SI, and IL-6 for AIS<8 hrs (P<0.001 in all cases). On the other hand, AIS-72 hrs vs. controls indicated a significant increase in SI (P=0.0028) and IL-6 (P=0.0065) but a non-significant increase in serum HP (P>0.05) in AIS-72 hrs. Linear regression expressed a high strength of the relationship between serum HP and SI for AIS<8 hrs (R^2=0.82, P<0.0001) and AIS-72 hrs (R^2=0.73, P<0.0001), but a negligible linear association for controls (R^2=0.01, P=0.5990). There was a high relationship between HP and IL-6 for AIS<8 hrs (R^2=0.67, P<0.0001) and AIS-72 hrs (R^2=0.67, P<0.0001), but a small linear association for controls (R^2=0.28, P=0.0063). The R^2 value of 0.47 (P<0.0002) and 0.42 (P<0.0004) was found between SI and IL-6 for AIS<8 hrs and AIS-72 hrs, and a negligible linear association (R^2=0.006, P=0.7250) for controls.

Conclusion: The present study provides evidence of the association of AIS in non-anemic women with increased hepcidin. The interactive pathophysiological role of HP, IL-6, and SI in non-anemic women with AIS has also been shown.**(International Journal of Biomedicine. 2024;14(2):260-264.)**

Keywords: acute ischemic stroke • non-anemic women • hepcidin • serum iron • interleukin-6

For citation: Hussain Z. Investigating the Role of Serum Hepcidin and Interleukin-6 in Non-Anemic Women with Acute Ischemic Stroke. International Journal of Biomedicine. 2024;14(2):260-264. doi:10.21103/Article14(2)_OA3

Abbreviations

AIS, acute ischemic stroke; **BMI**, body mass index; **BP**, blood pressure; **CBC**, complete blood count; **CKD**, chronic kidney disease; **CRP**, C-reactive protein; **CT**, computed tomography; **DBP**, diastolic blood pressure; **ELISA**, enzyme-linked immunosorbent assay **Hb**, hemoglobin; **HDL-C**, high density lipoprotein cholesterol; **HP**, hepcidin; **IL-6**, interleukin-6 **IS**, ischemic stroke; **LDL-C**, low-density lipoprotein cholesterol; **MRI**, magnetic resonance imaging; **SBP**, systolic blood pressure; **SI**, serum iron.

Introduction

Stroke is one of the leading causes of disability and death in adults[1] suffering from diabetes, heart disease, hypertension, and chronic kidney disease.[2,3] Despite enormous research that has been conducted, an incomplete understanding of the pathophysiology of stroke is the one main barrier limiting the research progress.

The majority of stroke cases (about 90%) are ischemic.[4] Several conditions or risk factors[5] predisposing to ischemic stroke (IS) include high blood pressure (BP), high cholesterol levels, and cardiovascular diseases, among other causes. Patients with acute ischemic stroke (AIS) present with the most common symptoms of the sudden occurrences of abnormal functions and deficits in the nervous system, making it harder to diagnose during the initial 24 hours properly. The AIS is caused by sudden obstruction or blockage of arteries by a thrombus or embolus and leads to almost immediate loss of oxygen and glucose supply to the brain. It causes irreparable neuronal damage within just a few minutes after onset.

The symptoms of IS progress swiftly in just a few hours.[6] A transient ischemic attack is considered at least one risk factor.[7] Several of the risk factors can be managed by changing lifestyle activities, including mainly the control of high BP, high cholesterol levels, heart disease, diabetes, and smoking.[8]

Iron status is suggested as one major factor or cause involved in the occurrence and progression of various diseases.[9-12] Hepcidin (HP), produced in the liver, is a factor associated with iron status. It is the main antimicrobial regulatory hormone/peptide for iron metabolism.[13] In association with IL-6 and other inflammatory cytokines, HP has been recognized as having a significant involvement in the homeostasis of systemic iron, and it serves as a bridge or link between iron regulation and inflammation.[4]

Iron status and IL-6 have been shown to regulate the expression of HP.[14] Serum iron (SI), HP, and IL-6 have a highly significant role in inflammation since inflammation elevates the levels of HP for expressing the ischemic condition.[15]

Several therapeutic products have been introduced in the past decade to reduce the disability and death associated with stroke. Still, only a little progress has been made in understanding the neuronal mechanisms involved.[15,16]

Despite the mentioned reports providing evidence for the role of HP in stroke patients, serum levels of HP in association with IL-6 and SI are still not known. Hence, the present study was carried out to investigate the impact of an interactive association between HP, SI, and IL-6 in non-anemic women with AIS. It is hoped that further research may clarify the role of HP in AIS prognosis and therapeutic approaches.[17,18]

Materials and Methods

The present case-control, descriptive study comprised 25 non-anemic women with AIS and 25 healthy non-anemic women controls. The age range of AIS and control subjects was 50 to 54 years. Baseline characteristics of the subjects are presented in Table 1. A detailed questionnaire was prepared to contain information about clinical, biochemical, and hematological estimations. After obtaining ethical approval, the research was carried out in affiliated hospitals from January 2022 to January 2023. The consent of the women patients and healthy women subjects was obtained before the start of the consultation. The subjective and objective information for AIS patients and control subjects was obtained following the regulations and guidelines of the Biomedical Ethics Committee.

The present work is the next step to our previous study.[19] For the present study, the specialist neurologists and other medical experts helped in the differential diagnosis of patients with AIS, using CT scans, MRI, CT or MR angiogram, carotid ultrasound of the arteries, transcranial doppler (TCD) ultrasound, EEG, and other techniques. The data for the present report was collected from properly diagnosed patients with AIS. Blood tests were CBC, coagulation, blood glucose, blood protein, C-reactive protein (CRP), cholesterol, serum electrolytes, and other lab tests (Table 1).

The subjects (normal control and AIS subjects) selected for the present report had a normal range BMI of 18.5 to <25 kg/m^2 for the healthy Saudi population.[20] BMI was calculated as weight (kg) divided by height (m) squared.[21] Hence, the patients and healthy control subjects with higher or lower BMI values than the mentioned levels were not included in the present work.

Iron status was defined properly,[22] and the AIS and control subjects without anemia were included in the present study, following the definition of anemia as Hb<12 g/dL.[23,24] According to ACC/AHA, SBP/DBP below 120/80 mmHg was considered normal, and SBP/DBP ≥130/80 mmHg was considered hypertension.[25] Office blood pressure was measured using a mercury sphygmomanometer.[26] The subjects with hypotension and hypertension stage 2 were not included in the study.

The SI, IL-6, HP, HDL-C, and LDL-C levels were measured. The ELISA method was used to determine serum IL-6, SI, and HP, and a fully automated method was employed to measure serum HDL-C and LDL-C. [27,28]

A comprehensive analysis was conducted using GraphPad Prism (version 6.0) software, San Diego, CA, USA. For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. The one-way analysis of variance (ANOVA) and related Tukey-Kramer post-hoc test was carried out. The coefficient of determination R^2 was estimated to measure the strength of the linear relationship. A probability value of $P<0.05$ was considered statistically significant. The results were compared and analyzed statistically following general statistical principles/concepts.[29]

Results

The age, Hb, and HDL-C in the women patients with AIS did not differ significantly from the healthy control subjects (Table 1). However, significantly increased values were obtained for SBP ($P=0.0267$) and DBP ($P=0.0183$) in

AIS women, compared to the control subjects. Serum CRP and LDL-C levels in AIS were significantly higher than in healthy control women (P<0.0001).

Table 1.

Baseline characteristics of the study participants

Baseline characteristics	Serum levels (mean ± SD)		t-value	*P*-value
	AIS (n = 25)	C (n = 25)		
Age (years)	52.72±1.07	52.34±1.44	1.08	0.2847
SBP (mmHg)	137.44±12.35	130.88±7.30	2.29	0.0267
DBP (mmHg)	86.72±8.48	81.80±5.42	2.44	0.0183
Hb (g/dL)	13.67±1.15	13.31±1.09	1.13	0.2621
CRP (mg/dL)	9.58±1.26	3.49± 0.97	19.15	<0.0001
HDL-C (mg/dL)	55.71±7.08	57.42±5.88	0.93	0.360
LDL-C (mg/dL)	111.85±17.17	95.47±6.40	4.47	<0.0001

A significant increase in serum HP, SI, and IL-6 for AIS<8 hrs was found, compared to AIS-72 hrs (P<0.001 in all cases) (Table 2) Comparison for AIS<8 hrs vs. controls showed a highly significant increase in serum HP, SI, and IL-6 for AIS<8 hrs (P<0.001 in all cases). On the other hand, AIS-72 hrs vs. controls indicated a significant increase in SI (P=0.0028) and IL-6 (P=0.0065) but a non-significant increase in serum HP (P>0.05) in AIS-72 hrs (Table 2).

One-way analysis of variance (ANOVA) for serum HP, IL-6, and iron in women with AIS (AIS<8 hrs and AIS-72 hrs) and healthy women controls showed a highly significant difference (P<0.0001, Table 3).

The coefficient of determination (R^2) was estimated to measure the strength of the linear relationship (Table 4). The strength of the relationship between serum HP and SI for AIS<8 hrs (R^2=0.82, P<0.0001) and AIS-72 hrs (R^2=0.73, P<0.0001) was high, but a negligible linear association for controls (R^2=0.01, P=0.5990). There was a high relationship between HP and IL-6 for AIS<8 hrs (R^2=0.67, P<0.0001) and AIS-72 hrs (R^2=0.67, P<0.0001), but a small linear association for controls (R^2=0.28, P=0.0063). The R^2 value of 0.47 (P<0.0002) and 0.42 (P<0.0004) was found between SI and IL-6 for AIS<8 hrs and AIS-72 hrs, and a negligible linear association (R^2=0.006, P=0.7250) for controls.

Table 3.

One-way ANOVA for serum hepcidin, IL-6, and iron in women with AIS (AIS < 8 hrs and AIS-72 hrs) and control women.

Variables	One-way ANOVA*	
	F	*P*
Serum HP (ng/ml)	11.028	<0.0001
Serum iron (µg/dL)	19.629	<0.0001
Serum IL-6 (pg/ml)	23.556	<0.0001

** - Tukey-Kramer post-hoc test indicates a highly significant difference for the comparisons for AIS< 8 hrs and AIS-72 hrs.*

Table 4.

The strength of the relationship between serum hepcidin, IL-6 and iron in women with AIS and control.

Variables			R^2	*P*-value
HP and SI	AIS	AIS < 8 hrs	0.8242	<0.0001
		AIS-72 hrs	0.7341	<0.0001
	C		0.0122	0.5990
HP and IL-6	AIS	AIS < 8 hrs	0.6692	<0.0001
		AIS-72 hrs	0.6778	<0.0001
	C		0.2820	0.0063
SI and IL-6	AIS	AIS < 8 hrs	0.4680	<0.0002
		AIS-72 hrs	0.4230	>0.0004
	C		0.0055	0.7250

C - control; R^2 - coefficient of determination.

Table 2.

Serum hepcidin, IL-6, and iron in women with acute ischemic stroke.

Variables	Serum levels and statistical analysis								
	AIS				C	AIS vs. C			
	AIS < 8 hrs	AIS-72 hrs	AIS < 8 hrs vs. AIS-72 hrs			AIS < 8 hrs vs. C		AIS-72 hrs vs. C	
			t*	*P*		t**	*P*	t**	*P*
Serum HP (ng/ml)	28.01±13.78	18.63±10.53	8.88	<0.0001	13.92±7.00	4.56	<0.0001	1.86	0.0684
SI (µg/dL)	122.12± 31.88	96.21± 25.46	12.64	<0.0001	76.20±19.00	6.19	<0.0001	3.15	0.0028
Serum IL-6 (pg/ml)	16.21± 7.07	9.66± 3.98	9.14	<0.0001	7.10± 2.13	6.17	<0.0001	2.85	0.0065

**- paired t-test; ** - unpaired t-test; C - control.*

Discussion

Inflammation increases the HP level and may express the ischemic condition [14,30] that has been confirmed in the present study. Elevated levels of free SI have also been documented in IS, though its mechanism is unclear.[31] The present study confirms the increased levels of SI in non-anemic women with AIS.

Another important factor related to inflammation is IL-6. It is quite well established that IL-6 is the main important cytokine involved in the inflammatory responses that control the HP levels, and it is elevated in brain tissues in ischemia and IS.[30]

The present study shows significantly increased levels of serum HP in non-anemic women with AIS, especially during the first 8 hours, compared to controls.[17,18] This corresponds to other studies reporting similar changes and emphasizes the role of HP and SI in causing brain ischemia. It seems quite interesting that serum HP level might serve as a strong factor for predicting the status of an IS and future therapeutic approaches.[17] It is also necessary to pay attention to iron transport proteins.

Higher levels of SI in AIS patients compared to controls in the present study are like those found in a study by Chi et al.[32] Moreover, Rouault and Cooperman consider the possibility that iron accumulation contributes to the formation of free radicals and oxidative damage to brain tissue.[33] According to Adibhatla and Hatcher,[34] free iron can react with H_2O_2 via the Fenton reaction, a primary cause of lipid peroxidation, and may be particularly important for these brain injuries.

The present study found a significant positive correlation between SI and HP, SI and IL-6, and HP and IL-6 in AIS. Several aspects of these findings are in accordance with the published studies [35,36] that help explore the HP role in iron-dependent damage and neurotoxicity and the role of SI in hypoxia via HP and concomitant changes occurring in IS.[30,37] Further studies may explore ways to develop novel therapeutics for controlling iron toxicity in IS and to understand the pathophysiological mechanisms whereby iron causes neurotoxicity in patients with AIS.[4]

A major limitation of the present report is that the patients consulted have a quite limited age range. Future studies need extensive research work in both male and female subjects/patients of a wider age range and a larger and multicentre-based sample size.

Conclusion

The present study provides evidence of the association of acute ischemic stroke in non-anemic women with increased hepcidin. The interactive pathophysiological role of hepcidin, IL-6, and serum iron in non-anemic women with acute ischemic stroke has also been shown. Furthermore, the present study discusses the introduction of better treatment modalities for modulating the signaling pathways for hepcidin expression and modifying neuronal iron homeostasis in patients with acute ischemic stroke.

Competing Interests

The author declares that there are no competing interests.

Acknowledgments

The author acknowledges the honorary help of neurologists, other medical experts, and laboratory personnel for their kind help and assistance.

References

1. Krishnamurthi RV, Ikeda T, Feigin VL. Global, Regional and Country-Specific Burden of Ischaemic Stroke, Intracerebral Haemorrhage and Subarachnoid Haemorrhage: A Systematic Analysis of the Global Burden of Disease Study 2017. Neuroepidemiology. 2020;54(2):171-179. doi: 10.1159/000506396.
2. Holmqvist L, Boström KB, Kahan T, Schiöler L, Hasselström J, Hjerpe P, et al. Cardiovascular outcome in treatment-resistant hypertension: results from the Swedish Primary Care Cardiovascular Database (SPCCD). J Hypertens. 2018 Feb;36(2):402-409. doi: 10.1097/HJH.0000000000001561.
3. Patlolla SH, Lee HC, Noseworthy PA, Wysokinski WE, Hodge DO, Greene EL, et al. Impact of Diabetes Mellitus on Stroke and Survival in Patients With Atrial Fibrillation. Am J Cardiol. 2020 Sep 15;131:33-39. doi: 10.1016/j.amjcard.2020.06.049. Epub 2020 Jun 30. PMID: 32723556.
4. Almutairi MMA, Xu G, Shi H. Iron Pathophysiology in Stroke. Adv Exp Med Biol. 2019;1173:105-123. doi: 10.1007/978-981-13-9589-5_6.
5. Hajar R. Risk Factors for Coronary Artery Disease: Historical Perspectives. Heart Views. 2017 Jul-Sep;18(3):109-114. doi: 10.4103/HEARTVIEWS.HEARTVIEWS_106_17.
6. Musuka TD, Wilton SB, Traboulsi M, Hill MD. Diagnosis and management of acute ischemic stroke: speed is critical. CMAJ. 2015 Sep 8;187(12):887-93. doi: 10.1503/cmaj.140355. Epub 2015 Aug 4.
7. Simmatis LER, Scott SH, Jin AY. The Impact of Transient Ischemic Attack (TIA) on Brain and Behavior. Front Behav Neurosci. 2019 Mar 11;13:44. doi: 10.3389/fnbeh.2019.00044.
8. Boehme AK, Esenwa C, Elkind MS. Stroke Risk Factors, Genetics, and Prevention. Circ Res. 2017 Feb 3;120(3):472-495. doi: 10.1161/CIRCRESAHA.116.308398.
9. Chopra VK, Anker SD. Anaemia, iron deficiency and heart failure in 2020: facts and numbers. ESC Heart Fail. 2020 Oct;7(5):2007-2011. doi: 10.1002/ehf2.12797.
10. Barandiarán Aizpurua A, Sanders-van Wijk S, Brunner-La Rocca HP, Henkens MTHM, Weerts J, Spanjers MHA, et al. Iron deficiency impacts prognosis but less exercise capacity in heart failure with preserved ejection fraction. ESC Heart Fail. 2021 Apr;8(2):1304-1313. doi: 10.1002/ehf2.13204.
11. Guedes M, Muenz D, Zee J, Lopes MB, Waechter S, Stengel B, et al. Serum biomarkers of iron stores are associated with worse physical health-related quality of life in nondialysis-dependent chronic kidney disease patients with or without anemia. Nephrol Dial Transplant. 2021 Aug 27;36(9):1694-1703. doi: 10.1093/ndt/gfab050.

12. Neidlein S, Wirth R, Pourhassan M. Iron deficiency, fatigue and muscle strength and function in older hospitalized patients. Eur J Clin Nutr. 2021 Mar;75(3):456-463. doi: 10.1038/s41430-020-00742-z.
13. Kemna EH, Tjalsma H, Willems HL, Swinkels DW. Hepcidin: from discovery to differential diagnosis. Haematologica. 2008 Jan;93(1):90-7. doi: 10.3324/haematol.11705.
14. Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, et al. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell. 2003 Apr;3(4):363-75. doi: 10.1016/s1535-6108(03)00077-1.
15. Chamorro Á. Neuroprotectants in the Era of Reperfusion Therapy. J Stroke. 2018 May;20(2):197-207. doi: 10.5853/jos.2017.02901.
16. Wilkinson DA, Pandey AS, Thompson BG, Keep RF, Hua Y, Xi G. Injury mechanisms in acute intracerebral hemorrhage. Neuropharmacology. 2018 May 15;134(Pt B):240-248. doi: 10.1016/j.neuropharm.2017.09.033.
17. Petrova JJ, Manolov VE, Hadjidekova SP, Hadjiev EA, Bogov BI, Marinov BM, et al. Is There a Link Between Changes in Levels of Hepcidin and Stroke? Clin Lab. 2015;61(12):1935-9. doi: 10.7754/clin.lab.2015.150522.
18. Słomka A, Świtońska M, Żekanowska E. Hepcidin Levels Are Increased in Patients with Acute Ischemic Stroke: Preliminary Report. J Stroke Cerebrovasc Dis. 2015 Jul;24(7):1570-6. doi: 10.1016/j.jstrokecerebrovasdis.2015.03.031.
19. Khan NI, Naz L, Mushtaq S, Rukh L, Ali S, Hussain Z. Ischemic stroke: prevalence of modifiable risk factors in male and female patients in Pakistan. Pak J Pharm Sci. 2009 Jan;22(1):62-7.
20. Harakeh S, Kalamegam G, Pushparaj PN, Al-Hejin A, Alfadul SM, Al Amri T, et al. Chemokines and their association with body mass index among healthy Saudis. Saudi J Biol Sci. 2020 Jan;27(1):6-11. doi: 10.1016/j.sjbs.2019.03.006.
21. Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. Nutr Today. 2015 May;50(3):117-128. doi: 10.1097/NT.0000000000000092.
22. Moliner P, Jankowska EA, van Veldhuisen DJ, Farre N, Rozentryt P, Enjuanes C, et al. Clinical correlates and prognostic impact of impaired iron storage versus impaired iron transport in an international cohort of 1821 patients with chronic heart failure. Int J Cardiol. 2017 Sep 15;243:360-366. doi: 10.1016/j.ijcard.2017.04.110.
23. Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood. 2006 Mar 1;107(5):1747-50. doi: 10.1182/blood-2005-07-3046.
24. von Haehling S, Ebner N, Evertz R, Ponikowski P, Anker SD. Iron Deficiency in Heart Failure: An Overview. JACC Heart Fail. 2019 Jan;7(1):36-46. doi: 10.1016/j.jchf.2018.07.015.
25. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2018 May 15;71(19):e127-e248. doi: 10.1016/j.jacc.2017.11.006. Epub 2017 Nov 13. Erratum in: J Am Coll Cardiol. 2018 May 15;71(19):2275-2279. PMID: 29146535.
26. Oh SY, Ryue J, Hsieh CH, Bell DE. Eggs enriched in omega-3 fatty acids and alterations in lipid concentrations in plasma and lipoproteins and in blood pressure. Am J Clin Nutr. 1991 Oct;54(4):689-95. doi: 10.1093/ajcn/54.4.689.
27. Warnick GR, Nguyen T, Albers AA. Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol. Clin Chem. 1985 Feb;31(2):217-22.
28. Tremblay AJ, Morrissette H, Gagné JM, Bergeron J, Gagné C, Couture P. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. Clin Biochem. 2004 Sep;37(9):785-90. doi: 10.1016/j.clinbiochem.2004.03.008.
29. Zahir H, Javaid A, Rehman R, Hussain Z. Statistical concepts in biology and health sciences. J Ayub Med Coll Abbottabad. 2014 Jan-Mar;26(1):95-7. PMID: 25358229.
30. Ding H, Yan CZ, Shi H, Zhao YS, Chang SY, Yu P, et al. Hepcidin is involved in iron regulation in the ischemic brain. PLoS One. 2011;6(9):e25324. doi: 10.1371/journal.pone.0025324.
31. Chen Z, Gao C, Hua Y, Keep RF, Muraszko K, Xi G. Role of iron in brain injury after intraventricular hemorrhage. Stroke. 2011 Feb;42(2):465-70. doi: 10.1161/STROKEAHA.110.602755.
32. Chi SI, Wang CK, Chen JJ, Chau LY, Lin TN. Differential regulation of H- and L-ferritin messenger RNA subunits, ferritin protein and iron following focal cerebral ischemia-reperfusion. Neuroscience. 2000;100(3):475-84. doi: 10.1016/s0306-4522(00)00317-1.
33. Rouault TA, Cooperman S. Brain iron metabolism. Semin Pediatr Neurol. 2006 Sep;13(3):142-8. doi: 10.1016/j.spen.2006.08.002. PMID: 17101452.
34. Adibhatla RM, Hatcher JF. Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2010 Jan;12(1):125-69. doi: 10.1089/ars.2009.2668.
35. Rehu M, Punnonen K, Ostland V, Heinonen S, Westerman M, Pulkki K, et al. Maternal serum hepcidin is low at term and independent of cord blood iron status. Eur J Haematol. 2010 Oct;85(4):345-52. doi: 10.1111/j.1600-0609.2010.01479.x.
36. Choi HS, Song SH, Lee JH, Kim HJ, Yang HR. Serum hepcidin levels and iron parameters in children with iron deficiency. Korean J Hematol. 2012 Dec;47(4):286-92. doi: 10.5045/kjh.2012.47.4.286.
37. Tan G, Liu L, He Z, Sun J, Xing W, Sun X. Role of hepcidin and its downstream proteins in early brain injury after experimental subarachnoid hemorrhage in rats. Mol Cell Biochem.2016 Jul;418(1-2):31-8.doi: 10.1007/s11010-016-2730-1

***Correspondence:** Prof. Dr. Zahir Hussain, Department of Physiology, Faculty of Medicine, Umm Al-Qura University, Makkah 21955, Saudi Arabia. E-mail: zahirhussa@gmail.com*