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A study to evaluate relationship between hematological indices and iron status with infarct size in pediatric stroke

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ABSTRACT

Objectives: To evaluate the relationship between hematological indices and iron status with the infarct size, number and location in pediatric stroke. **Material and methods:** The current study is an observational cross sectional study conducted in the Pediatric Neurology clinic attached to Department of Pediatrics, Dr S N Medical College, Jodhpur, Rajasthan, India. 16 (8 male and 8 female) pediatric stroke patients presenting in Pediatric Neurology clinic were included in the study. Hemoglobin (Hb) level, Hematocrit (HCT) and RBC indices (MCV, MCH and MCHC), platelet count and Iron status parameters (Serum Iron, TIBC and Serum Ferritin) of these patients were analysed. Area of diffusion restriction on MRI brain was measured as a surrogate marker of the severity of infarction and its correlation with RBC indices, platelet count and Iron status parameters was evaluated. **Results:** Mean age of studied population at presentation was 45.218 ± 47.80 months. Mean Hb, HCT, MCV, MCH, MCHC and platelet count of these patients were 9.229 ± 2.829 gm/dl, 29.531 ± 7.002%, 73.650 ± 7.237 fL, 23.650 ± 3.709 pg, 30.393 ± 3.000 g/dl, 331.667 ± 201.472 $\times 10^9$/L respectively. Mean Serum Ferritin, TIBC and Serum Iron were 89.639 ± 119.788 ng/ml, 286.125 ± 83.858 ug/dl, and 82.375 ± 59.452 ug/dl. No statistically significant correlation was seen between any of the RBC indices, total Platelet Count and serum Iron status indicators with mean total area of diffusion restriction in the whole cohort, in the group of patients having Serum Ferritin levels < 15 and > 15 ng/ml. **Conclusion:** Despite the fact that most of the literature reports association between Iron deficiency anemia with stroke, we did not find any correlation between the various RBC indices, total Platelet count and Iron status indicators with area of diffusion restriction in patients with stroke. Further studies are recommended to study the exact contributors to patho - physiology of stroke associated with Iron deficiency.

Key words: Infarct size, Iron status parameters, Pediatric stroke, RBC Indices

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INTRODUCTION

Pediatric Stroke is an important cause of acute morbidity and mortality in children and can result into severe disability and sequel. This entity is being increasingly recognized due to advancements in stroke radiology and increased awareness.[1] Diagnosis and management still continues to be difficult because of the diversity of underlying risk factors and the absence of a uniform treatment approach.[1] The overall incidence rate of childhood stroke (both ischemic and hemorrhagic) in age group between 30 days to 18 years ranges from 1.3 to 13 per 100,000 population at risk per year.[2,3]

Several risk factors for childhood ischemic stroke have been consistently reported in large cohort studies of children with stroke. These include sickle cell disease, cardiac structural lesions, chronic systemic disease, cerebral arterial disease, coagulation disorders, head trauma, and sub acute varicella zoster infection.[4-8]

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Iron deficiency is very common in developing countries and causes much neurologic and non-neurologic morbidity. Iron deficiency is increasingly being linked with stroke (Arterial ischemic, Cerebral Sinus Venous Thrombosis and hemorrhagic) and several case reports and case series have suggested an association between iron-deficiency anemia (IDA) and ischemic stroke in healthy children.[9-13] However, the correlations between various RBC indices and Iron deficiency parameters with pediatric stroke have not yet been clearly established.

The current study was therefore designed as an observational cross sectional study[14] designed to answer the aforementioned queries and controversies with an objective to evaluate the relationship between hematological indices and iron status with the infarct size, number and location in pediatric stroke.

MATERIAL AND METHOD

The present study was conducted in the Pediatric Neurology clinic attached to Department of Pediatrics, Dr S N Medical College, Jodhpur, Rajasthan, India. All patients presenting with an acute onset of focal neurologic deficit in a 6 month period were clinically evaluated by a Pediatric Neurologist. In patients having a high suspicion of Arterial Ischemic stroke a complete blood count, blood Iron status parameters including Serum Iron, Serum Total Iron binding capacity and Serum Ferritin were performed within 24 hours of hospitalization. Magnetic resonance imaging (MRI) including standard T1, T2, T2 FLAIR, gradient echo and diffusion weighted imaging was done within 48 hours of stroke. MR angiography was also included in the protocol wherever affordable and feasible. MR Angiography was done in 9 out of total 16 patients. Abnormal MRA finding was seen in 4 patients and rest 5 patients were having normal MRA finding. Out of 4 patients one patient was having complete hypoplastic left vertebral artery with moderate stenosis in M1 (horizontal) segment of left MCA and relatively attenuated left ACA. Second patient was having moderate focal stenosis in supraclenoid right ICA with mild narrowing in supraclenoid left ICA. Complete occlusion of proximal M1(horizontal) segment of left MCA about 8-9 mm distal to its origin was seen in one patient and Moya Moya angiographic pattern secondary to slowly progressive vascular disease was noted in one patient. On confirmation of stroke, children were managed as per standard guidelines and additional investigations and work up were initiated. During the 6 months study period 16 patients were included in study which were diagnosed as having Acute ischemic stroke as per standard definition.[15,16] In all these children location and territory of the stroke (Right/left and anterior/posterior circulation and whether ACA/MCA/PCA/Combined) was recorded. Using computer software maximum transverse and maximum anterior-posterior dimension of the diffusion restricted area (cytotoxic edema due to infarction) in axial view visible on neuroimaging was measured in mm and area of this diffusion restricted area was calculated. In case of multiple infarcts we calculated the area of individual lesions and added up the area of all the individual lesions. The aforementioned method was used to calculate the diffusion restricted area because the MRI machine at our institution does not have dedicated software to calculate area and volume. Correlation between RBC indices (Hb level, HCT, MCV, MCH, MCHC), Total Platelet count, Iron status parameters (Serum Iron, TIBC and Serum Ferritin) and area of diffusion restriction was statistically analyzed using logistic regression.

Ethics

Prior ethical approval for the study methodology was obtained from the Ethics committee of Dr S N Medical College, Jodhpur. Informed parental consent was obtained to be eligible for enrollment in the study.

OBSERVATIONS AND RESULTS

In the present study a total of 16 (mean age 45.218 ± 47.80 months) patients were included out of which 8 (mean age 48.06 ± 50.32 months) were males and 8 (Mean age 42.37 ± 48.43 months) were females. 4 males (mean age 9.125 ± 10.16 months) and 4 females (mean age 9.75 ± 2.62 months) were < 24 months and 4 males (mean age 87 ± 42 months) and 4 females (mean age 75 ± 51.26) were > 24 months in age. Laboratory data of the patients are being presented in Table 1.

Anterior circulation was involved in 10 patients, posterior in 2 patients, both anterior and posterior in 1 patient, Bilateral anterior circulation in 1 patient, border zone areas in 1 patient and bilaterally territory not specific were involved in 1 patient. Single infarct, two infarcts and multiple infarcts were seen in 9 patients, 5 patients and 2 patients respectively.

No statistically significant correlation was seen between Hemoglobin value, Hematocrit, MCV, MCH, MCHC and mean total area of diffusion restriction in all male children, all female children and in the whole cohort (Graph 1a-d).

Similarly no statistically significant correlation was seen between Platelet count and mean total area of diffusion restriction in all male children, all female children and in the whole cohort (Graph 2).

No statistically significant correlation was seen between Serum Iron levels, Serum Ferritin levels & TIBC and mean total area of diffusion restriction in all male children, all female children and in the whole cohort when age and sex were not considered (Graph 3a-d).

Table 1: Laboratory data of stroke patients

Total patients (N=16)	Mean value
Age (months)	45.218±47.80
Hemoglobin (gm/dl)	9.229±2.829
Hematocrit (%)	29.531±7.002
MCV (fL)	73.650±7.237
MCH (pg)	23.650±3.709
MCHC (g/dl)	30.393±3.000
Platelet count	331.667±201.472×10^9/L
Serum Iron (ug/dl)	82.375±59.452
Serum Ferritin (ng/ml)	89.639±119.788
TIBC (ug/dl)	286.125±83.858
Area of diffusion restriction in overall cohort (mmsq)	2448.2612±1859.1180
Area of diffusion restriction in all male patients (mmsq)	2875.648±2265.377
Area of diffusion restriction in all female patients (mmsq)	2020.875±1362.703
Area of diffusion restriction in pt with serum ferritin equal to or below 15 ng/ml (mmsq)	2108.39±1802.338
Area of Diffusion restriction in pt with serum ferritin above 15 ng/ml (mmsq)	2652.184±1957.713

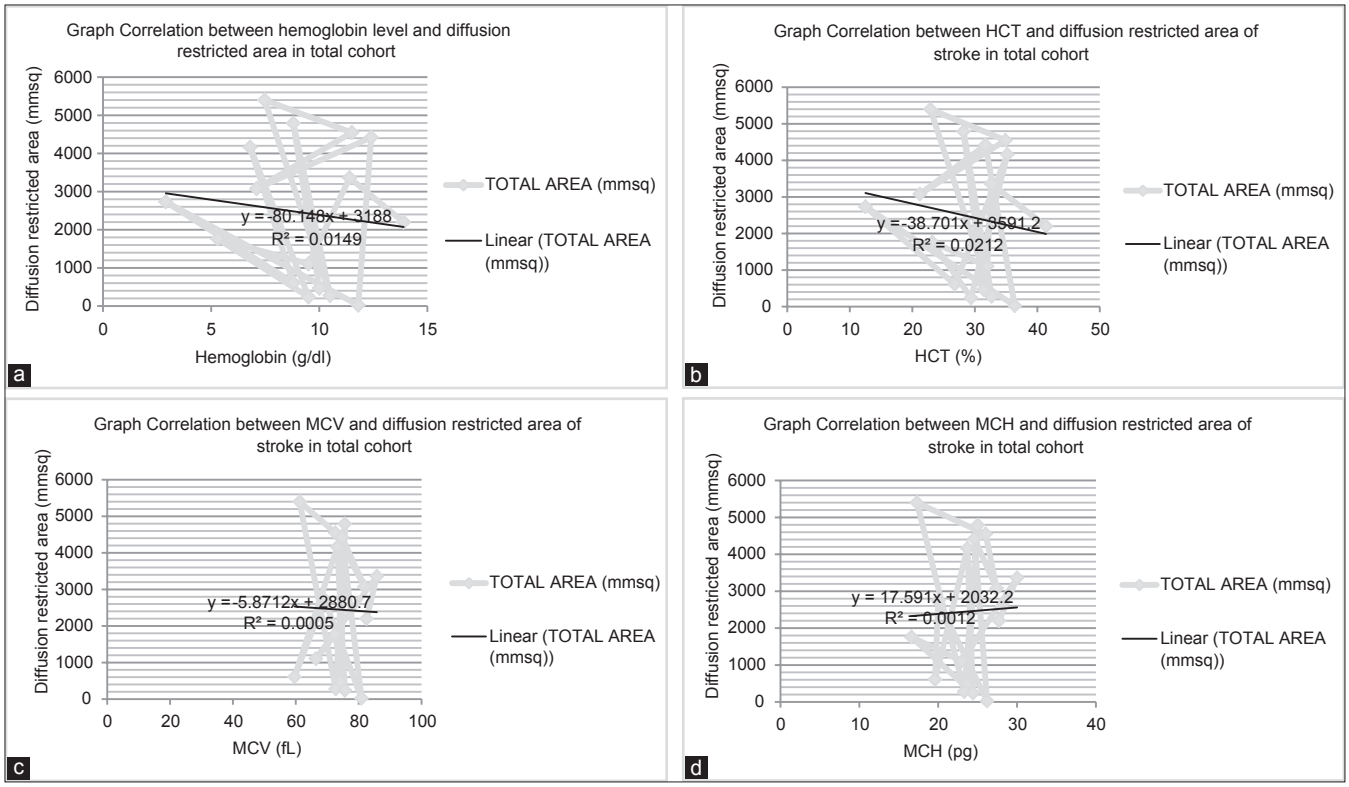
When children with Arterial Ischemic stroke having Ferritin levels below and above 15 ng/ml were analysed separately, no statistically significant difference between the mean total area of diffusion restriction was seen between the two groups.(P value >0.6)

In children with Arterial Ischemic Stroke having Serum Ferritin levels below 15 ng/ml, no statistically significant correlation was seen between Hb, Hematocrit, MCV, MCH, MCHC, Total Platelet counts, Serum Iron, Ferritin and TIBC levels with mean total area of diffusion restriction.

Similarly in children having Ferritin levels more than 15 ng/ml no correlation was seen between Hb, Hematocrit, MCV, MCH, MCHC, Total Platelet Count, Serum Iron, Ferritin and TIBC levels with mean total area of diffusion restriction.

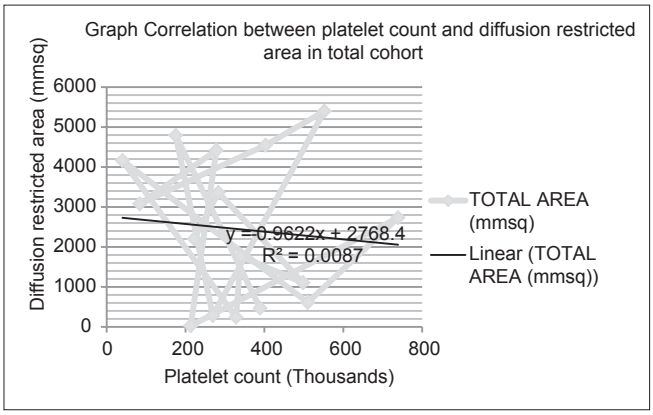
DISCUSSION

Researchers have indicated that Arterial Ischemic Stroke has been associated with Iron deficiency anemia. In a recent case-control study conducted by Seham et al[17] in Egypt it was concluded that IDA was present in 57.1% of stroke cases with no identified cause, as compared to 26% of controls.



Graph 1: Showing correlation between RBC indices and the diffusion restricted area of stroke. (a) Correlation between hemoglobin level and the diffusion restricted area of stroke in total cohort. (b) Correlation between HCT and diffusion restricted area of stroke in total cohort. (c) Correlation between MCV and diffusion restricted area of stroke total cohort

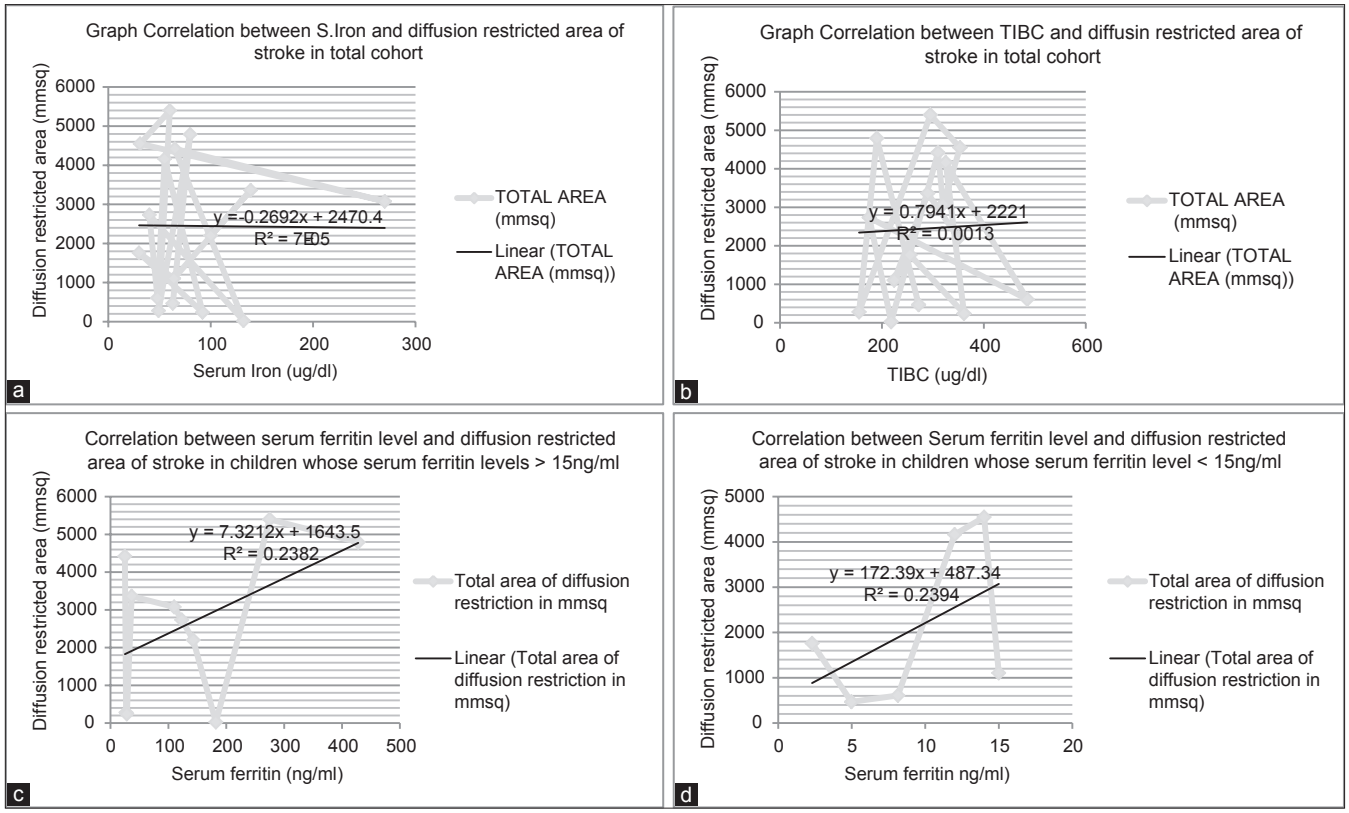
Previously healthy children who developed stroke are 3.8 times more likely to have IDA than healthy children who do not develop stroke (OR, 3.8; 95% CI: 1.3-11.2 *P*=0.005). In addition, there was significant interaction between IDA and thrombocytosis among studied cases (OR, 10.5; 95% CI, 1.0-152 *P*=0.02). There were no significant differences between stroke patients with IDA and those with normal iron parameters regarding stroke subtype (*P*>0.05).



Graph 2: Showing correlation between platelet count and diffusion restricted area of stroke in total cohort

Majority of the studies have concluded that stroke in children with IDA may be due to three mechanisms; a hypercoagulable state directly related to iron deficiency and/or anemia; thrombocytosis secondary to IDA; and anemic hypoxia, whereby a mismatch between oxygen supply and end-artery oxygen demand leads to ischemia and infarction.[18] It is however not clear as to what hematologic parameters and Iron status indicators contribute to the pathophysiology of stroke related to Iron deficiency. Also no studies in the past have however studied the correlation between various RBC indices and Iron status biochemical parameters with the severity of stroke. In the current study we attempted to study this relation using MCV, MCH and MCHC as the RBC morphological indices and Serum Iron, TIBC and Serum Ferritin as Iron status biochemical indicators. Area of diffusion restriction (representing cytotoxic edema) on MRI was taken as a surrogate marker of severity of infarction.

It has been postulated that the anemic hypoxia due to low Hb is responsible for stroke.[18] In this context we attempted to study the correlation between Hb and Hematocrit with the mean total area of diffusion restriction in Pediatric



Graph 3: Showing correlation between iron parameters and diffusion restricted area of stroke. (a) Correlation between serum iron and diffusion restricted area of stroke in total cohort. (b) Correlation between TIBC and diffusion restricted area of stroke total cohort. (c) Correlation between serum ferritin levels and diffusion restricted area of stroke in children whose serum ferritin levels above 15ng/ml. (d) Correlation between serum ferritin levels and diffusion restricted area of stroke in children whose serum ferritin levels below 15ng/ml

Arterial Ischemic Stroke. No statistically significant correlation was seen between Hb and Hematocrit levels with the area of diffusion restriction at all ages and in both sexes individually. This indicates that severity of anemia does not influence the severity of infarction. This relationship has not been studied by other researchers and therefore it is difficult to postulate the exact reason for this observation. It is proposed that an alteration of RBC size, shape and hemoglobin content changes viscosity and blood flow dynamics leading to a hypercoagulable state resulting into higher chance of causing stroke.[10,19] We therefore hypothesized that MCV, MCH and MCHC may have a correlation with the area of diffusion restriction because of change in blood flow dynamics and viscosity. In the current study, no statistically significant correlation was seen between Hemoglobin levels, MCV, MCH, MCHC and mean total area of diffusion restriction in all male children, all female children and in the whole cohort when age and sex were not considered. Even an extensive review of the literature did not reveal studies focusing on the relationship between various RBC indices including MCV, MCH and MCHC with the infarct size. Further clinical and physiologic studies in both humans and appropriate animal models with a larger cohort are proposed to evaluate the impact of RBC shape, size and hemoglobin concentration on blood viscosity, flow patterns and pathologic severity of infarcted area. Different platelet indices especially MPV and MPC are considered as good indices of hemostasis and thrombosis.[20,21] In a study by Numminen et al it was observed that increased MPC will increase the chance of arterial thrombosis.[22] While O'Brien et al,[23] Butterworth et al[24] and McCabe et al[25] found no correlation between MPC and stroke. In the current study also, no statistically significant correlation was seen between mean platelet count and mean total area of diffusion restriction in both genders and in the whole cohort. It is therefore possible that the increased platelet count merely predisposes to a prothrombotic state and does not have any relationship with the severity of stroke area in the brain.

In a study conducted on Mice submitted to permanent (by ligature and by in situ thromboembolic models) or transient focal ischemia (by ligature for 1 or 3 h) the effect of stroke on Serum Ferritin and the contribution of iron overload to ischemic damage was studied. Swiss mice were fed with a standard diet or with a diet supplemented with 2.5% carbonyl iron to produce iron overload. Treatment with iron diet produced an increase in the basal levels of Ferritin in all the groups. However, Serum Ferritin did not change after ischemia. Animals submitted to permanent ischemia had the same infarct volume in the groups studied. However, in mice submitted to transient ischemia followed by early (1 h) but not late reperfusion (3 h), iron overload increased ischemic damage and hemorrhagic transformation. The authors of this study concluded that Iron worsens ischemic damage induced by transient ischemia and early reperfusion and that Ferritin is a good indicator of body iron levels but not an acute phase protein after ischemia.[26]

In the current study, no statistically significant correlation was seen between Serum Iron levels, Serum Ferritin levels & TIBC and mean total area of diffusion restriction in both genders, in the whole cohort and also in children having Ferritin levels below 15 ng/ml and above 15 ng/ml when analysed separately. In children with Arterial Ischemic Stroke having Serum Ferritin levels below 15 ng/ml no statistically significant correlation was seen between Hb, Hematocrit, MCV, MCH, MCHC, Platelet counts, Serum Iron, Ferritin and TIBC levels with mean total area of diffusion restriction. Serum Ferritin is also an acute phase reactant and therefore high serum Ferritin may be related to severity of inflammation in stroke. However, Iron deficiency is associated with low Ferritin levels and even on an extensive review of the literature we did not find any other study on human stroke patients or in animal models which evaluated the relationship of low serum Ferritin with stroke. More studies are therefore recommended to study the exact correlation between low serum Ferritin and profile of stroke area in the brain including its severity.

The current study therefore clearly infers that despite the fact that most of the world literature speaks about an association between Iron deficiency and stroke; there is no correlation between various hematologic parameters and Iron status indicators with severity of stroke. We propose that since the incidence of Iron deficiency in the population is very high, it may be a mere coincidence to see both higher frequency of Iron deficiency in patients with stroke and there may be no contribution to the etiology or severity of stroke due to Iron deficiency. The current study had a few limitations in the fact that we did not have facilities to perform perfusion imaging and also could not eliminate all other prothrombotic conditions which may have been present in these patients. Perfusion imaging would have definitely added to the knowledge of our understanding of the severity of stroke in these patients and we recommend more research using perfusion imaging to understand the relationship of stroke with various hematologic parameters and Iron status indicators. Even if other prothrombotic states existed in the patients evaluated in the current study, it can be concluded that at least the parameters studied (Hematologic indices and Iron status indicators) do not

have a significant correlation with severity of stroke as assessed by mean area of diffusion restriction.It is recommended that more research be conducted in this area to evaluate the exact relationship between various hematologic parameters and Iron status parameters with stroke pathogenesis and severity of stroke in order to develop specific and targeted preventive and management strategies.

CONCLUSION

In the current study no correlation was observed between the various RBC indices, total Platelet count and Iron status indicators with area of diffusion restriction in patients with stroke.Though most of the literature reports association between iron deficiency anemia and stroke, it may be mere coincidence of association between pediatric stroke and iron deficiency anemia as prevalence of iron deficiency anemia in pediatric population is very high. It is therefore highly probable that other pathophysiological determinants may be responsible for the stroke size, extent and its clinical implications in children with Iron deficiency anemia. Further research with a larger sample size is recommended to evaluate and study the correlation between hematological parameters and stroke area in pediatric stroke.

ABBREVIATIONS

RBC – Red Blood Cells
IDA – Iron Deficiency Anemia
Hb- Hemoglobin
HCT- Hematocrit
MCV – Mean Corpuscular Volume
MCH- Mean Corpuscular Hemoglobin
MCHC- Mean Corpuscular Hemoglobin Concentration
TIBC – Total Iron Binding Capacity
MPV- Mean Platelet Volume
MPC –Mean Platelet Count
ACA- Anterior Cerebral Artery
MCA- Middle Cerebral Artery
ICA- Internal Carotid Artery
PCA- Posterior Cerebral Artery

REFERENCES

1. Roach ES, Golomb MR, Adams R, Biller J, Daniels S, deVeber G, et al. Management of stroke in infants and children. American Heart Association scientific statement. Stroke 2008; 39: 2644-2691.
2. Mallick AA and O'callaghan FJK. The epidemiology of childhood stroke. European Journal of Paediatric Neurology 2010; 14(3): 197-205.
3. Lynch JK. Cerebrovascular disorders in children. Current Neurology and Neuroscience Reports 2004; 4:129-138.
4. Lynch JK, Hirtz DG, deVeber G and Nelson KB. Report of the national institute of neurological disorders and stroke workshop on perinatal and childhood stroke. Pediatrics 2002; 109: 116-123.
5. Lanthier S, Carmant L, David M, Larbrisseau A and deVeber G. Stroke in children: the coexistence of multiple risk factors predicts poor outcome. Neurology 2000; 54: 371-378.
6. Sebire G, Tabarki B, Saunders DE, Saunders DE, Leroy I, Liesner R, Saint-Martin C, et al. Cerebral venous sinus thrombosis in children: Risk factors, presentation, diagnosis and outcome. Brain 2005; 128: 477-489.
7. Ganesan V, Prengler M, McShane M, Wade AM and Kirkham FJ. Investigation of risk factors in children with arterial ischaemic stroke. Annals of Neurology 2003; 53:167-173.
8. Askalan R, Laughlin S, Mayank S, Chan A, MacGregor D, Andrew M, et al. Chickenpox and stroke in childhood: a study of frequency and causation. Stroke 2001; 32: 1257-1262.
9. Belman AL, Roque CT, Ancona R, Anand AK and Davis RP. Cerebral venous thrombosis in a child with iron deficiency anemia and thrombocytosis. Stroke 1990; 21: 488-493.
10. Swann IL and Kendra JR. Severe iron deficiency anemia and stroke. Clinical and Laboratory Haematology 2000; 22:221-223.
11. Baptist EC and Castillo SF. Cow's milk-induced iron deficiency anemia as a cause of childhood stroke. Clinical Pediatrics 2002; 41: 533-541.
12. Saxena K, Ranalli M, Khan N, Blanchong C and Kahwash SB. Fatal stroke in a child with severe iron deficiency anemia and multiple hereditary risk factors for thrombosis. Clinical Pediatrics 2005; 44: 175-180.
13. Hartfield DS, Lowry NJ, Keene DL and Yager JY. Iron deficiency: A cause of stroke in infants and children. Pediatric Neurology1997; 16: 50-53.
14. Rohrig B, Du Prel JB, Wachtlin D and Blettner M. Types of study in medical research. Deutsches Arzteblatt International 2009; 106(15): 262-268.
15. Cerebrovascular Disorders: A Clinical and Research Classification. Geneva, Switzerland: World Health Organisation1978;WHO Offset Publication Number 43. ISBN- 92-4-170043-2.OCLC4757533.
16. Kidwell CS and Warach S. Acute ischemic cerebrovascular syndrome: diagnostic criteria. Stroke 2003; 34 (12): 2995-2998.
17. Azab SF, Abdelsalam SM, Saleh SH, Elbehedy RM, Lotfy SM, Esh AM, et al. Iron deficiency anemia as a risk factor for cerebrovascular events in early childhood: a case-control study. Annals of Hematology 2014; 93(4): 571-576.
18. Maguire JL, deVeber G and Parkin PC. Association between iron-deficiency anemia and stroke in young children. Pediatrics 2007; 120 (5):1053-1057.
19. Tillmann W and Schroter W. Deformability of erythrocytes in iron deficiency anemia. Blut 1980; 40: 179-186.
20. Jagroop IA, Tsiara S and Mikhailidis DP. Mean platelet volume as an indicator of platelet activation: methodological issues. Platelets 2003; 14(5): 335-336.
21. Tsiara S, Elisaf M, Jagroop IA and Mikhailidis DP. Platelets as predictors of vascular risk: Is there a practical index of platelet activity? Clinical and Applied Thrombosis/Hemostasis 2003; 9:177-190.

22. Numminen H, Hillbom M and Juvela S. Platelets, alcohol consumption, and onset of brain infarction. Journal of Neurology Neurosurgery and Psychiatry1996; 61:376-380.
23. O'Brien JR and Jamieson S. A relationship between platelet volume and platelet number. Thrombosis et Diathesis Haemorrhagica 1974; 31:363-364.
24. Butterworth RJ and Bath PM. The relationship between mean platelet volume, stroke subtype and clinical outcome. Platelets1998; 9: 359-364.
25. McCabe DJ, Harrison P, Sidhu PS, Brown MM and Machin SJ. Circulating reticulated platelets in the early and late phases after ischemic stroke and transient ischemic stroke. British Journal of Haematology 2004; 126: 861-869.
26. García-Yébenes I, Sobrado M, Moraga A, Zarruk JG, Romera VG, Pradillo JM, et al. Iron overload, measured as serum ferritin, increases brain damage induced by focal ischemia and early reperfusion. Neurochemistry International 2012; 61(8): 1364-1369.

Authors Contribution:
PS – Contributed to the original idea, Designed the study, enrolled the patients, collected the data and analysed, prepared the manuscript and reviewed the manuscript**; MP –** Concieved hypothesis, Designed study, Patient Enrolment, Data collection, Data analysis, preparing of manuscript and reviewing the manuscript**; VK –** Contributed to the study design, Data analysis, preparing of manuscript and reviewing the manuscript; **PM –** Contributed to the Data collection, Data analysis and preparing of manuscript; **VA –** Contributed to Patient Enrolment and Data collection; **PP –** Contributed to Data analysis, preparing of manuscript and reviewing the manuscript.

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