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Original Article

Paediatrics Section

Reactive Thrombocytosis and Changes in Platelet Parameters in Children with Iron Deficiency Anaemia: A Cross-sectional Study

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ABSTRACT

Introduction: Iron Deficiency Anaemia (IDA) is the most common type of anaemia in paediatric age group. IDA has reported to be associated with thrombotic complications such as fatal cerebrovascular thrombosis, central retinal vein thrombosis, suggesting the correlation between various platelet parameters and IDA.

Aim: To observe changes in platelet parameters: platelet count, Mean Platelet Volume (MPV), Plateletcrit (PCT), and Platelet Distribution Width (PDW) in children with IDA. Also, to observe the severity of changes in platelet parameters with the increasing severity of IDA.

Materials and Methods: The present cross-sectional study was conducted in SGT Medical College, Hospital and Research Institute, Gurugram, India, over a period of 18 months from October 2022 to March 2024. A total of 141 Children aged between 6 months to 18 years with clinical and laboratory findings suggestive of IDA were enrolled. Children having anaemia other than IDA and any chronic illness were excluded. Platelet parameters {Platelet counts, MPV, PCT, and PDW} of enrolled patients were recorded. Data were statistically analysed by using Pearson correlation, Independent t-test and paired t-test. The p-value <0.05 was considered significant.

Results: Among 141 enrolled IDA patients, 75 (53.1%) belonged to moderate while 33 (23.4%) belonged to each mild and severe IDA category. Almost two-thirds patients 86 (61%), have shown thrombocytosis with nearly 68 (80%) mild and rest moderate thrombocytosis. An inverse correlation (r) ($p<0.05$) was observed between few platelet parameters and iron parameters i.e., haemoglobin (Platelet counts: r=-0.693, MPV: r=-0.759, PDW: r=-0.776), serum ferritin (Platelet counts: r=-0.405, MPV: r=-0.398, PDW: r=-0.572), and serum iron (Platelet counts: r=-0.244, MPV: r=-0.226, PCT: r=-0.166, PDW: r=-0.296). The incidence of thrombocytosis increased with the severity of iron deficiency {mild=06 (18.1%), moderate=47 (62.5%), severe=33 (100%)}.

Conclusion: Nearly two third of IDA children had Reactive Thrombocytosis (RT) with significant inverse correlation between other platelet indices and iron parameters. The severity of changes in platelet parameters increases with the increasing severity of iron deficiency with serum iron being the most reliable indicator.

Keywords: Haemoglobin, Low iron, Mean platelet volume, Platelet count, Plateletcrit

INTRODUCTION

The IDA is the most common type of anaemia in paediatric age group. Its prevalence is 67% among children six months to five years of age in India as per the National Family Health Survey-5 (NFHS-5) (2019-21) [1]. IDA affects children of all age groups, from infancy till adolescents. The causative factors linked are inadequate breast feeding, use of unsupplemented milk diets, food faddism, recurrent infections, parasitic infestation, increased iron requirements during the period of active growth along with blood loss during menstruation in adolescent girls [2].

Thrombocytosis has largely been correlated with IDA in various animal studies and in clinical studies on adult population [3,4]. The overall incidence of thrombocytosis in children varies from 6 to 15% [5]. It can be primary/essential thrombocytosis and secondary RT. Primary thrombocytosis is rare in children and is attributed to haematological and non haematological malignancies. Secondary RT is commonly seen in association with infections, haemoglobinopathies, drug related, post-splenectomy and secondary to iron deficiency [6].

The exact mechanism correlating thrombocytosis and IDA is still not clear. Recent data has demonstrated that low iron preferentially diverts Megakaryocytic-Erythroid Progenitors (MEPs) towards commitment to the megakaryocytic lineage and results in increased production of platelets [7]. Some researchers also observed the role of Erythropoietin (EPO) because of its structural similarity with Thrombopoietin (TPO) [8-10]. The newly formed platelets are metabolically and enzymatically more active and have a great prothrombotic potential [11,12]. Changes in other platelet parameters like MPV, PCT and PDW have also been observed in relation with IDA as well as with increased risk of thrombosis in different studies [12-14]. These parameters can be used as alternative markers for platelet activity [11].

The IDA has been reported to be associated with thrombotic complications such as fatal cerebrovascular thrombosis, central retinal vein thrombosis, further suggesting a strong relationship between thrombopoiesis and iron metabolism [15]. The correlation between IDA and platelet parameters has largely been studied in developed countries including the adult population with scarcity of paediatric data [12-14]. Few authors studied this correlation in paediatric patients, but either they studied only one parameter i.e., platelet counts or they have not co-related the effect of varying severity of IDA on platelet parameters [16,17]. Hence, the present study was planned to observe the changes in platelet parameters among paediatric population with IDA along with an objective to observe the severity of changes in platelet parameters with the increasing severity of IDA.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Paediatrics, SGT Medical College, Hospital, & Research Institute,

Gurugram, India. This Institute is a tertiary care hospital of Northern India with a bed strength of more than 800 beds and availability of all paediatric healthcare facilities. The study was conducted after taking the ethical clearance from Institutional Ethical Committee (IEC/FMHS/F/30/09/22/84) over a period of 18 months (October 2022 to March 2024). Written informed consent and assent was taken before enrolment.

Inclusion criteria: All children of age six months to 18 years either admitted in the Department of Paediatrics or presented to Outpatient Department (OPD) having clinical findings of anaemia and haemoglobin levels below the age specific World Health Organisation (WHO) cut-off, were included in the study [18]. Any child with anaemia along with acute infection was enrolled once the acute infection is completely cured.

Exclusion criteria: Patients with chronic infections/illness, with known haematological disorders, malignancies, autoimmune diseases, collagen vascular diseases, known cases of splenectomy and on drugs associated with thrombocytosis, were excluded from the study. Patients with anaemia due to causes other than IDA were also excluded from the study.

Sample size calculation: The sample size for the study was based on a study by Ray S et al., who reported the proportion of subjects with RT as 24.5% [16]. The proportion of subjects with RT: p=0.245 (24.5%) with precision: δ=0.075 (7.5%) and type I error: α=0.05 (5%),=1.96. Based on the formula and values given above:

$N=\{1.96^2\times0.245\times(1-0.245)\}/0.075^2=126.32 \approx 127$

Thus, for 95% confidence interval, the proposed sample size for the study is 127.

Study Procedure

All children of age 6 months to 18 years, presenting to paediatric OPD/Inpatient Department (IPD) or emergency, with clinical findings of anaemia (Pallor, poor feeding, easy fatiguability, pica) were followed for the laboratory work-up. Baseline characteristics of patients including demographic details and relevant history and examination findings were recorded. Baseline investigations including complete blood counts, red cell indices {Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and Red Cell Distribution Width (RDW)} and peripheral smear were sent.

WHO criteria based on age-specific haemoglobin levels, was used to diagnose and categorise anaemia in children. The anaemia was defined as haemoglobin level <11 gm/dL (mild: 10-10.9 gm/dL, moderate: 7-9.9 gm/dL, severe: <7gm/dL) in children six months to five years of age, haemoglobin level <11.5 gm/dL (mild: 11-11.4 gm/dL, moderate: 8-10.9 gm/dL, severe: <8 gm/dL) in 5-10 years children and haemoglobin level <12 gm/dL (mild: 11-11.9 gm/dL, moderate: 8-10.9 gm/dL, severe: <8 gm/dL) among adolescents (10-18 years) [18]. In patients having haemoglobin below the age specific cut-off value, iron studies were sent {serum ferritin, serum iron, Total Iron Binding Capacity (TIBC), and serum transferrin saturation}. The cause of anaemia was assessed based on peripheral smear, red cell indices, and iron studies. Serum ferritin levels <12 ng/mL in six months to five years and <15 ng/mL in more than five-year-old child, serum iron <30 mg/dL, TIBC >350 mg/dL and serum transferrin saturation <16% were considered diagnostic of IDA [19,20]. Patients with investigations suggestive of IDA, were enrolled for the study and their platelet parameters including platelet counts, MPV, PCT, and PDW were collected. The normal reference values and cut-offs are shown in [Table/Fig-1] [5,18-23]. The incidence of thrombocytosis and changes in other platelet indices among children with IDA was calculated and the association of degree of thrombocytosis and changes in other platelet indices with varying severity of IDA was established.

Parameters	Normal reference range/Cut-off values
Haemoglobin (gm/dL) (WHO) [18]	
6 months-5 years	<11 (mild: 10-10.9, moderate: 7-9.9, severe: <7)
5-10 years	<11.5 (mild: 11-11.4, moderate: 8-10.9, severe: <8)
Adolescents (10-18 years)	<12 (mild: 11-11.9, moderate: 8-10.9, severe: <8)
MCV	72-95 μm^3
MCH	24-32 pg/ cell
MCHC	32-36 gm/ dL
RDW	11.3-15 %
Serum Ferritin (ng/mL) [19]	
6 months-5 years	<12
>5 years	<15
Serum Iron [20]	<30 mg/dL
TIBC [20]	>350 mg/dL
Transferrin [20]	>360 mg/dL
Platelet counts [5]	>450×10^9/L
Mild Thrombocytosis	(450-700) ×10^9/L
Moderate Thrombocytosis	(700-900) ×10^9/L
Severe Thrombocytosis	(900-1000) ×10^9/L
Profound Thrombocytosis	(>1000) ×10^9/L
MPV [21]	7.2-11.7 fL
PDW [22]	10-18%
PCT [23]	0.22–0.24%

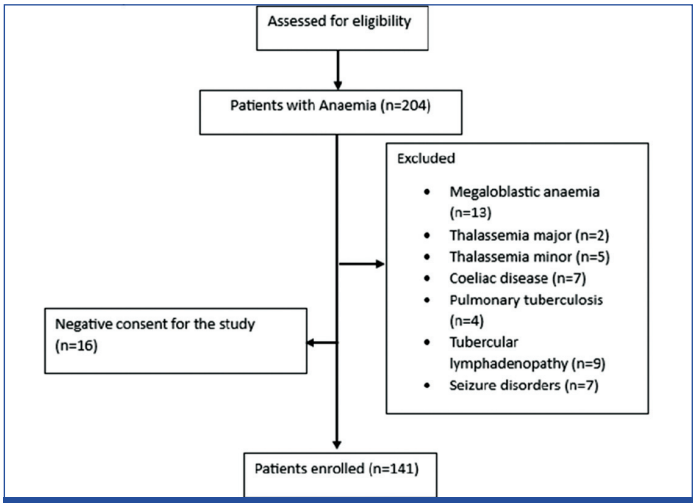
[Table/Fig-1]: Normal reference ranges and cut-off values [5,18-23].
MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; TIBC: Total iron binding capacity; MPV: Mean platelet volume; PDW: Platelet distribution width; PCT: Plateletcrit

STATISTICAL ANALYSIS

Data was coded and recorded in MS Excel spread sheet program. Statistical Package for Social Sciences (SPSS) v23 (IBM Corp.) was used for data analysis. Descriptive statistics were elaborated in the form of means/standard deviations for continuous variables, and frequencies and percentages for categorical variables. Linear correlation between two continuous variables was explored using Pearson's correlation. Independent t-test was applied for independent samples and paired t-test was applied for paired samples. Statistical significance was kept at p<0.05.

RESULTS

A total of 204 patients presented with anaemia. After applying inclusion and exclusion criteria, 141 (69.1%) IDA patients were enrolled in the study, as shown in [Table/Fig-2].



[Table/Fig-2]: Flowchart showing selection process of study population.

The demographic details of study participants collected, which showed almost equal gender distribution with an average age of

3.99±3.34 years. Most of the children {75 (53.19%)} were found to have moderately severe IDA [Table/Fig-3].

Parameters	Results n (%)
Age (Mean±SD)	Total IDA: 3.99±3.34
	Mild IDA: 2.99±1.75
	Moderate IDA: 4.45±3.68
	Severe IDA: 3.94±3.60
Gender	Male: 68 (48.2%)
	Female: 73 (51.7%)
M:F ratio	0.9
Religion	Hindu: 116 (82.2%)
	Muslims: 23 (16.3%)
	Christian: 02 (1.4%)
Severity of IDA	Mild: 33 (23.4%)
	Moderate: 75 (53.19%)
	Severe: 33 (23.4%)

[Table/Fig-3]: Demographic details of enrolled IDA patients.

Among them, 28 (19.8%) patients were admitted with history of fever with raised CRP; they were enrolled after normalisation of CRP value and complete clinical recovery including completion of antibiotic course. Subsequent to complete cure, all relevant samples for assessment of IDA were sent.

The iron and platelet parameters of all enrolled IDA patients were recorded and categorised as per the severity of IDA. All the iron parameters varied significantly (p<0.05) between the different categories of IDA except TIBC, PCT and transferrin levels. Platelet counts, MPV, and PDW also shown statistically significant (p<0.05) variation with the increasing severity of IDA [Table/Fig-4].

parameters while MPV was having significant positive correlation with platelet counts and PDW. PCT was having significant positive correlation only with platelet counts while PDW was having with platelet counts and MPV [Table/Fig-6].

DISCUSSION

The IDA is the most common anaemia in children. It may present with mild symptoms such as lethargy, irritability, and poor feeding, but can also lead to serious complications including cognitive impairment, developmental delay, and congestive heart failure [24]. An additional concern is the risk of thromboembolic events. While well studied in adults, this association is less explored in children. Song AB et al., reported a twofold increase in thrombotic risk in IDA patients with thrombocytosis [25]. Deshmukh AV et al., described six patients (three paediatric) with thromboembolic complications, most with mild to moderate thrombocytosis [26]. Another paediatric case series found thrombocytosis in four of six thromboembolism cases [27]. The present study examines changes in platelet parameters and activity that may contribute to thromboembolic complications in paediatric IDA.

In this study, the incidence of thrombocytosis was approximately 86/ 141 (61%), including 68/86 (79%) of mild thrombocytosis and 18/86 (20.9%) of moderate thrombocytosis. No case with severe or profound thrombocytosis was seen. Among all patients with IDA (n=141), platelet counts were found to have statistically significant inverse correlation with haemoglobin, serum ferritin, and serum iron levels (p<0.05). Although the link between IDA and thrombocytosis is well recognised, the exact mechanism remains unclear. Kadikoylu G et al., highlighted the effect of the duration and severity of IDA on megakaryopoiesis. Proposed mechanisms include increased differentiation of precursor cells into the megakaryocytic lineage, shortened megakaryocyte maturation time, suppression of

Parameters	Total IDA (n=141) Mean±SD	Mild IDA (n=33) Mean±SD	Moderate IDA (n=75) Mean±SD	Severe IDA (n=33) Mean±SD	p-value
Mean Hb (g/dL)	8.42±1.97	10.36±0.23	8.9±0.77	5.38±1.30	<0.01
MCV	65.06±9.15	67.49±6.90	67.19±8.60	57.78±8.60	<0.01
MCH	19.7±3.99	22.19±2.70	20.36±3.36	15.69±3.50	<0.01
MCHC	29.02±3.03	31.18±1.57	29.56±1.61	25.64±3.80	<0.01
RDW	20.7±7.13	16.9±2.39	20.37±7.66	25.64±6.70	<0.01
Ferritin	7.68±2.90	9.70±1.80	8.20±2.33	4.50±2.40	<0.01
Serum Iron	24.37±9.26	29.79±9.50	24.38±7.40	18.9±9.60	<0.01
TIBC	392.9±100.3	377.7±83.50	392.3±81.30	409.71±145.60	0.434
Transferrin	272.1±80.14	251.6±87.20	277.68± 55.02	280.1±113.66	0.063
Platelet counts	4.73±1.56	3.3±1.52	4.66±1.03	6.34±1.02	<0.01
MPV	13.2±2.73	10.9±1.50	12.8±1.95	16.39±2.32	<0.01
PCT	0.32±0.10	0.31±0.11	0.32±0.11	0.339±0.09	0.288
PDW	19.00±3.35	15.57±1.96	18.53±1.76	23.50±2.15	<0.01

[Table/Fig-4]: Baseline investigations, and platelet parameters of enrolled IDA patients.
*p-value<0.05=significant; IDA: Iron deficiency anaemia; Hb: Haemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; TIBC: Total iron binding capacity; MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet distribution width

Pearson correlation was observed between haemoglobin, serum ferritin, and serum iron with platelet parameters. Haemoglobin and serum ferritin were found to have significant inverse correlation with three platelet parameters including platelet counts, MPV, and PDW except in severe IDA group. Serum iron was found to have significant inverse correlation with all the four platelet parameters including platelet counts, MPV, PCT, and PDW [Table/Fig-5].

On further analysis, association between platelet parameters with red blood cell indices (MCV, MCH, MCHC) and the inter-relationship among different platelet parameters were also observed. Platelet counts, MPV, and PDW were having significant inverse correlation MCV, MCH, MCHC, and RDW while PCT was having significant inverse correlation only with MCV and MCH. Platelet count was having significant positive correlation with all other platelet

Parameters	Total IDA (r)	Mild IDA (r)	Moderate IDA (r)	Severe IDA (r)
Platelet counts	-0.693*	-0.183	-0.477*	-0.302
MPV	-0.759*	0.106	-0.545*	-0.363*
PCT	-0.128	0.078	-0.242*	0.007
PDW	-0.776*	0.360*	-0.282*	-0.105
Platelet counts	-0.405*	-0.122	-0.069	0.423*
MPV	-0.398*	0.499*	-0.034	0.141
PCT	-0.020	0.179	0.102	-0.210
PDW	-0.572*	0.072	-0.285*	0.102
Platelet counts	-0.244*	0.119	-0.013	0.011
MPV	-0.226*	0.092	0.180	-0.084

PCT	-0.166*	0.006	-0.305*	0.012
PDW	-0.296*	-0.161	0.046	0.277

[Table/Fig-5]: Correlation of IDA with platelet parameters.
*p-value <0.05; r=Correlation Coefficient, MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet distribution width

Parameters (n=141)	Platelet counts (r)	MPV (r)	PCT (r)	PDW (r)
MCV	-0.448	-0.452	-0.274	-0.332
MCH	-0.527	-0.513	-0.289	-0.493
MCHC	-0.492	-0.462	-0.152	-0.555
RDW	0.176	0.335	0.030	0.396
Platelet counts	--	0.522	0.277	0.510
MPV	0.522	--	0.033	0.688
PCT	0.277	0.033	--	0.020
PDW	0.510	0.688	0.020	--

[Table/Fig-6]: Correlation of red cell indices with platelet parameters and inter-relationship of different platelet parameters in Total IDA patients.
*p-value<0.05; r=Correlation coefficient, Hb: Haemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; TIBC: Total iron binding capacity; MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet distribution width

have most significant inverse correlation between iron and platelet parameters.

Further, inverse correlation was found between RBC indices (MCV, MCH, MCHC) and platelet parameters. This observation can be useful to predict the risk of changes in platelet parameters based on RBC indices, especially in patients with unknown iron studies. The inter-relationship among different platelet parameters was also highlighted in this study. This finding suggests that change in single platelet parameter is closely linked with other and can act as a proxy marker.

Similar studies and their key findings are shown in [Table/Fig-7] [14,16,26,28].

Limitation(s)

Although, there was significant correlation between IDA and various platelet parameters especially in patients with moderate IDA; lesser correlations were noted in mild and severe IDA patients, likely due to smaller sample size in both categories. An equal sample size in each category would have yielded better results. Further studies on platelet parameters in moderate and severe IDA are needed on a larger cohort.

Authors name (ref no.)	Place/year of the study	Patient population	Incidence and severity of thrombocytosis	Correlation between iron and platelet parameters
Ray S et al., [16]	New Delhi, India from October 2008 to March 2010.	Paediatric (n=200) M:F=2:1	I=24.5% Mild: 75.5%, Moderate: 18.4% Severe: 6.1% No profound	• Platelet count was inversely related to haemoglobin and ferritin • Erythropoietin was increased in 2/3rd cases of thrombocytosis and showed positive correlation with platelet count
Park MJ et al., [14]	Incheon, Korea from September 2008 and August 2010	Adult (n=41) All females	100% (patients having both IDA and thrombocytosis were enrolled) Mild to moderate No severe or profound	• Inverse correlations between Platelet count and serum iron, MCH, and MCHC (p<0.05) • Inverse correlation between PCT and serum iron(p<0.001), Hb, MCH, MCHC(p<0.05) • Inverse correlation between MPV and Hb, MCHC (p<0.05), whereas linear correlation between MPV and PCT (p<0.001) • PCT and MPV were significantly high in severe IDA (p<0.05, for both)
Chalise S et al., [28]	Kathmandu, Nepal between December 2018 to May 2019.	Adult (n=81) Male: 17 Female: 64	I=76.5% Differentiation not given	• Platelet count showed negative correlation with serum iron, PDW, MPV (p<0.05) while positive correlation between platelet count and PCT (p<0.05) • PCT showed negative correlation with serum iron and PDW (p<0.05) whereas positive correlation with MPV (p<0.05) • Negative correlation between MPV and platelet count (p<0.001) where as positive correlation between MPV and PCT (p<0.05) • PDW showed negative correlation with platelet (p<0.001), and PCT (p<0.05)
Deshmukh AV et al., [26]	Sevagram, Maharashtra, India over the period of 24 months	Adult (n=85) Male: 20 Females: 65	I=41% Mild: 36.5% Moderate: 4.7% No severe or profound	• All platelet parameters (Platelet count, MPV, and PDW) except Pct showed significant difference in study and control group. • Platelet count and PDW were significantly increased in IDA while MPV showed a remarkable decrease

[Table/Fig-7]: Similar studies along with their key findings [14,16,26,28].

erythropoiesis with stem cell shunting to other lineages, reduced inhibitory effects of serum iron on thrombopoiesis, and the stimulatory role of transferrin in megakaryocyte maturation [12]. Elevated EPO levels and its structural similarity to TPO have also been implicated [16].

MPV and PDW showed inverse correlation with haemoglobin, serum ferritin, and serum iron (p<0.05). PCT showed a significant inverse correlation with serum iron (p<0.05) and an inverse, though non significant, association with haemoglobin and ferritin. The inverse relationship between MPV, PDW, PCT, and iron parameters can be explained by increased platelet production in IDA, leading to the release of larger, immature platelets. Shortened maturation time and increased polyploidy contribute to elevated MPV, PDW, and PCT. Larger platelets are more adhesive and prone to aggregation, which may increase thrombotic risk, though the exact mechanism remains unclear [12,14].

This study also evaluated changes in platelet parameters with increasing IDA severity. Thrombocytosis was seen in 6 (18.1%) patients with mild IDA (five mild, one moderate), 47 (62.6%) with moderate IDA (41 mild, six moderate), and in all (33; 100%) patients with severe IDA (22 mild, 11 moderate). The incidence was highest in patients with severe IDA. In this study, moderate IDA was found to

CONCLUSION(S)

The RT is found to be closely correlated with IDA in paediatric population. Changes in various other platelet parameters like MPV, PCT, and PDW have also been found to be significantly correlated with IDA specifically with moderately severe IDA. Serum iron was the most affecting factor and platelet counts and MPV were the most sensitive platelet parameters in this study. Although, the present study observed significant changes in platelet parameters in IDA patients, further studies with larger sample size are required to improve the generalisability of the findings.

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