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Original Research Article

Clinico-hematological study of abnormalities of platelet count in children with iron deficiency anemia

Sanghamitra Ray[1]*, Jagdish Chandra[2], Sunita Sharma[3]

[1]Department of Paediatrics, Dr. Baba Saheb Ambedkar Medical College and Hospital, Rohini, New Delhi, India
[2]Department of Paediatrics, [3]Department of Pathology, Kalawati Saran Children's Hospital and Associated Lady Hardinge Medical College, New Delhi, India

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***Correspondence:**
Dr. Sanghamitra Ray,
E-mail: dr.sanghamitra.ray@gmail.com

ABSTRACT

Background: Iron deficiency anemia is a major cause of morbidity in developing countries like India. The aim of the study was to assess abnormalities of platelet count in iron deficiency anemia and to relate the severity of thrombocytosis with severity of anemia and its association with erythropoietin (EPO) level.
Methods: A prospective observational study comprising of 200 children below 18 years confirmed to have IDA. Erythropoietin (EPO) level was done in patients who had thrombocytosis. Degree of thrombocytosis was correlated with EPO and also with ferritin, haematological indices like hemoglobin and MCV (mean corpuscular volume) and blood counts were followed up while on iron therapy for one month.
Results: Thrombocytosis was noted in 24.5%. In 75.5% thrombocytosis was mild. Platelet had negative correlation with Hb (hemoglobin). EPO was elevated in 67.35% of thrombocytosis. EPO showed negative correlation with Hb and Ferritin and positive correlation with platelet however, these were non-significant. All patients were treated with standard preparation of ferrous fumarate (33mg elemental iron every 5 ml) in a dose of 3mg/kg/day of elemental iron along with appropriate dietary advice. On one month follow up 92% of the study population showed normalization of platelet count.
Conclusions: Nearly One-fourth of children had thrombocytosis. Platelet count was inversely related to Hb and ferritin level. EPO was increased in two-third cases of thrombocytosis and showed positive correlation with platelet count. As authors excluded patients with severe IDA requiring blood transfusion, authors did not get any thrombocytopenia in present study.

Keywords: Children, Erythropoietin, Iron deficiency anemia, Thrombocytosis

INTRODUCTION

In a developing country like India where 28.6% of the population is below poverty line, nutritional anemia constitutes a major disease burden with iron deficiency accounting for majority of the cases.[1] Other than anemia, deficiency of iron is incriminated in the causation of wide variety of pathophysiological changes in the body resulting in protean clinical manifestations. Association of iron deficiency with thrombosis is also increasingly being recognized.[2-5] Studies on thrombocytosis in children have shown IDA as an etiological factor for reactive thrombocytosis. As most of the studies on thrombocytosis are from developed countries, where nutritional anemia is less prevalent, these studies do not truly reflect the proportion of cases of thrombocytosis

due to underlying iron deficient state. A recent study from our center has shown IDA constituting second most common cause of secondary thrombocytosis responsible for 41.6% cases with or without infection.[6]

Prevalence of thrombocytosis in IDA is not widely studied. Moreover, mechanism underlying reactive thrombocytosis in IDA and how iron replacement affects thrombocytosis has also not been completely elucidated. Erythropoietin (EPO), the regulator of erythropoiesis has structural similarity with thrombopoietin (TPO)- the stimulator of megakaryopoiesis. Studies on association of EPO with thrombocytosis in IDA have shown conflicting results.[7,8] Present work was an attempt to study the prevalence of thrombocytosis and its correlation with EPO in patients with IDA and the course of thrombocytosis with iron replacement therapy.

METHODS

This prospective study was conducted on patients attending a tertiary care hospital of North India. The study included two hundred patients from Oct. 2008 to March 2010. Informed consent of the parents/guardian was obtained. Sample size was calculated with confidence level of 95% and confidence interval of 7 with taking presumptive prevalence of thrombocytosis in IDA as 40% an observation of a similar study from Turkey.[9]

The study group comprised of patients of both sexes below 18 years of age suspected of having IDA on the basis of microcytic anemia on initial hematological investigation. Microcytosis was defined as MCV <70fl in age group of <2 years, MCV <(70+age in years) among 2-10 year age group and MCV <80 fl in more than 10 year old children.[10] Children who had received blood transfusion or hematinic and those who were critically ill were excluded from the study. All the subjects were evaluated with a detailed history and clinical examination. Tests performed included complete blood count with peripheral smear examination and biochemical studies (serum ferritin and/or serum iron, TIBC) to confirm iron deficiency anemia. Serum erythropoietin (EPO) level was measured only in patients of confirmed IDA having thrombocytosis.

Serum ferritin estimation was done by microplate immunoenzymometric assay using BIOMEDA USA Kit.[11] Serum iron were measured by Ferrozine calorimetric assay using Labkit (Ref 30270), Chemelex S.A. Barcelona.[12] TIBC was measured using saturation-Precipitation method using LABKIT (Ref 30340), Chemelex, S.A. Barcelona.[13] Serum EPO level was measured using DRG EPO- ELISA Kit (EIA-3646).[14]

IDA was diagnosed by presence of microcytic hypochromic red cell morphology on peripheral smear along with either serum ferritin <12 ng/ml or S. ferritin >12 ng/ml with serum iron <51 µg/dl and total iron binding capacity (TIBC) >390 µg/dl (cut-offs were taken based on controls established in hospital laboratory). Anemia was graded according to definition of WHO.[15] Thrombocytosis was graded as mild-(500-700)×109/L; moderate- (700-900)×109/L; severe- (900-1000)×109/L and profound-(>1000)×109/L. Normal value of erythropoietin was taken as (4.3-32.9) mU/ml as per the reference of the standard kit (2.5-97.5 percentile).[16]

All patients were treated with standard preparation of ferrous fumarate (33 mg elemental iron every 5 ml) in a dose of 3 mg/kg/day of elemental iron along with appropriate dietary advice.

All the patients were followed up at one month of oral iron supplementation. A repeat complete hemogram was performed to assess changes in hemoglobin level, RBC, MCV and platelet count.

The results were analyzed using windows SPSS software. For comparisons of means between different groups and means of two sets of readings within the same group, unpaired and paired student's t-test respectively were used. For comparisons of proportions chi-square test was applied. For correlation studies carl-Pearson correlation coefficient (r) was used.

The study was approved by institutional ethical committee.

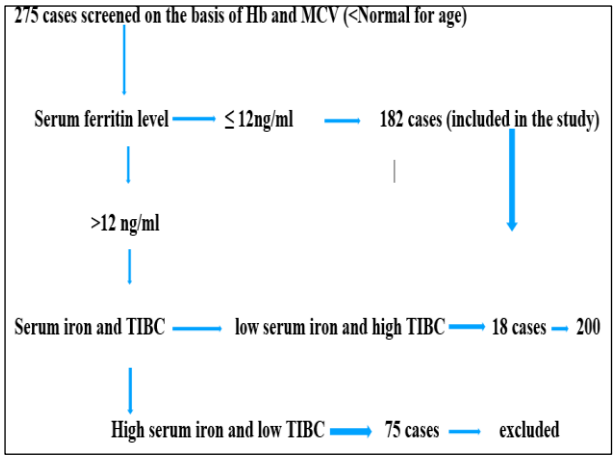

Figure 1: Patient allocation based on hematological parameters.

As the Figure 1 shows, out of 200 cases,182 had serum ferritin less than 12 ng/ml. Eighteen patients were diagnosed to have iron deficiency on the basis of low serum iron and increased TIBC.

RESULTS

Male female ratio of study subjects was 2:1. Children under 5 years accounted for 85 % of subjects. 67.5% had

moderate anemia while 27.5% children had severe anemia.

Mean platelet count of study subjects was (3.76±1.68)x $10^3/mm^3$. However, 49 patients (24.5%) were found to have thrombocytosis. Most of these (75.5%) had mild thrombocytosis while moderate thrombocytosis was documented in 18.4%. Only 3 cases had thrombocytosis of severe grade (6.1%). No case of profound thrombocytosis was documented. Platelet count had statistically significant negative correlation with Hb (p value 0.042, r value-0.157) and ferritin level (p value 0.048, r value-0.137). Platelet count had negative correlation with MCV as well, but this correlation was statistically not significant (p value 0.197). Mean serum erythropoietin level in cases with thrombocytosis, were 65.53±39.65 mU/ ml which were considerably higher than normal (normal range 4.3-32.9 mU/ml). However, elevated erythropoietin levels were found in 67.35% of cases. Highest mean erythropoietin levels were noted among the cases with severe thrombocytosis and lowest in the group with mild thrombocytosis. These differences however were statistically not significant (Table 1).

Table 1. Erythropoietin level in different grade of thrombocytosis.

Grade of thrombocytosis	Mean EPO level (mU/ml)±2SD
Mild (N-37)	61.0±35.84
Moderate (N-9)	62.7±39.31
Severe (N-3)	126.0±36.47

EPO level showed a positive correlation with platelet count (r-0.236, p-0.103) which was statistically not significant. Similarly, negative correlation of erythropoietin with Hb (r-0.092, p-0.530) and serum ferritin (r -0.054, p 0.714) was not statistically significant.

Hematological indices at one month of follow-up showed increase in the Hb level with a mean of 1.59±0.57 gm%. Among total 49 cases with thrombocytosis, platelet count returned to normal after one month of iron therapy in 45 cases, while rest all 4 cases had mild degree of thrombocytosis.

DISCUSSION

In this study, on pediatric patients with IDA, authors observed thrombocytosis in 49 (24.5%) cases, a finding similar to a study from Turkey who studied 102 children with IDA and observed thrombocytosis in 40 (39.2%) cases.[9] Although association of thrombocytosis with IDA is well recognized, how much IDA contributes to thrombocytosis and how common is thrombocytosis in IDA is only scarcely reported. This is probably on account of the facts that till late, most of these studies on thrombocytosis were from developed countries, where IDA is not that much prevalent. In most of these studies, IDA has accounted for less than 10 % cases of thrombocytosis in children.[6,7] On the contrary, a study from our hospital found IDA (alone or with infection) as the second most common cause of reactive thrombocytosis accounting for almost 41.6% cases of reactive thrombocytosis in children.[6]

Reactive thrombocytosis found in IDA is mostly of mild to moderate degree. Platelet count rarely exceeds 700x $10^3/\mu l$.[17,19,20] In present patients also, moderate and severe thrombocytosis was observed in 18.4% and 6.1% cases and no patient had thrombocytosis of profound degree.

Though thrombotic complications in IDA are not very common, there are reports of thrombotic complications in association with IDA with or without thrombocytosis which has been linked to various factors. Increased level of erythropoietin in IDA has been incriminated to have a possible role in stimulating megakaryopoiesis. Besides this, there is increase in adhesive reticulocytes in blood resulting increased viscosity. There are some reports on cerebral Sino venous thrombosis, carotid artery thrombus and stroke in patients of IDA with or without thrombocytosis.[3-5,21] In present study, no thrombotic complications were noted.

Increased EPO and its structural analogy to TPO (Thrombopoietin) have been incriminated as a possible mechanism for thrombocytosis in IDA. One study assayed serum levels of TPO, EPO, leukemia inhibiting factors, IL-6, IL-11 but none of these cytokines except for EPO showed any effect on reactive thrombocytosis in IDA.[22] Animal studies have also reported thrombogenic effect of EPO.[23]

Experimental studies demonstrated iron replacement causing a decline in EPO platelet counts simultaneously.[24] Another study also found the stimulatory effect of EPO on megakaryopoiesis.[23] Rat and mouse megakaryocytes have been shown to express high-affinity binding sites for EPO. In vitro studies in man as well as in mice have demonstrated that EPO promoted megakaryocytic colony formation and increased the size, ploidy and number of megakaryocytes, cytoplasmic process formation and also stimulated DNA and protein synthesis in megakaryocytes.

Authors observed mean EPO level which was nearly double of upper limit of reference range. Further the level of erythropoietin was plotted according to the severity of thrombocytosis which showed highest mean value among the cases with severe thrombocytosis and lowest mean was observed in the group with mild thrombocytosis. However, these associations were statistically not significant. Other studies have also showed that in patients with iron deficiency, EPO is directly related to the severity of anemia similar to these findings.[18,25] Reactive thrombocytosis is usually a benign condition and platelet count mostly normalizes rapidly with the treatment of underlying aetiology without causing any thrombotic events.

Authors observed that of our 49 cases with thrombocytosis, 44 cases had normalization of platelet count within one month of treatment with oral iron supplement. Data on the follow up of such patients is scare in the literature. However, few authors reported normalization of platelet count after approximately one month of iron therapy.[21,26]

CONCLUSION

One-fourth of children of IDA had thrombocytosis and none had thrombocytopenia. Platelet count was inversely related to haemoglobin and ferritin. Erythropoietin was increased in two-third cases of thrombocytosis and showed positive correlation with platelet count. No thrombotic complications were observed in the study subjects. Platelet count normalised in over ninety percent patients after four weeks of iron replacement therapy.

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