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Can Different Genetic Mutations be the Possible Cause for Thrombocytosis in Children with Beta Thalassemia Major?

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Abstract

Objective: To investigate the primary cause of thrombocytosis observed in children suffering from Thalassemia major in Pakistan.

Methodology: A cohort study was carried out at the Armed Forces Institute of Transfusion (AFIT) Rawalpindi from June to December 2020 after obtaining permission from its Ethical Review Committee. A total of 41 children with Thalassemia major, presenting persistent platelet counts ≥ 1000 x 10^9/l for three months, underwent a revised transfusion protocol and iron overload chelation. After six months of observation and chelation, patients with persistent thrombocytosis were further analyzed for BCR ABL1, JAK2 V617F, CAL-R, and MPN-1 mutations. Data analysis was conducted using IBM SPSS 21.

Results: Following transfusion revision and chelation, 73% of patients achieved normal platelet counts, accompanied by significant improvements in Hb levels, WBC count, ferritin levels, and platelet count. However, 27% of patients with persistent thrombocytosis showed no mutations in BCR ABL1, JAK2 V617F, CAL-R, and MPN-1 genes.

Discussion: The study suggests a potential correlation between thrombocytosis in Thalassemia major and iron deficiency, with 73% of patients responding positively to transfusion revision and chelation. However, for the remaining 27%, the cause of thrombocytosis remains elusive, necessitating further in-depth investigations with a larger sample size.

Conclusion: The research highlights the need for comprehensive studies to elucidate the underlying causes of thrombocytosis in children with Thalassemia major, especially in cases where conventional interventions such as transfusion revision and chelation do not yield conclusive results.

Keywords: Thalassemia major, Thrombocytosis, Ferritin, Chelation, BCR ABL1, JAK2 V617F, CAL-R, MPN-1, iron deficiency.

Introduction

Pediatric thrombocytosis is an increase of platelet count beyond the upper limit of normal in children. In healthy pediatric populations, the normal count of platelets is between 250x10^9/l and 400x10^9/l. A higher platelet count over 2 standard deviations defines a condition of thrombocytosis. In the vast majority of children unlike the adult population thrombocytosis is secondary or reactive in nature and hence requires no active treatment. [1]

With a persistent platelet count of > 1000x10^9/l for a period of three months the underlying pathology in most children is clonal in nature.[2] The common reactive cause leading to thrombocytosis in children include infections that might either be bacterial, viral, parasitic or fungal. Inflammatory causes come next which may include diseases like rheumatoid arthritis, inflammatory bowel disease, Kawasaki syndrome, vasculitis and collagen vascular disease. Surgical causes include post-splenectomy, post-surgical recovery, trauma, burns and blood loss.[3] Hemolytic anaemia, iron deficiency hypersplenism and congenital or idiopathic nephrotic syndrome may also lead to thrombocytosis.[4]

Primary thrombocytosis in children may be investigated e.g., mutational analysis for BCR-ABL1, JAK 2 V617F, CAL-R and MPL1 analysis, if no obvious cause of secondary thrombocytosis is found. It should be suspected in children with hepatosplenomegaly, bleeding or thrombotic disorders and elevated platelet count beyond three months or if a family history of thrombotic

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disorders is present. Primary thrombocytosis however is extremely rare in childhood with an incidence of one per million while secondary or reactive thrombocytosis is quite common in pediatric age occurring in 3-13% of hospitalized children.[4]

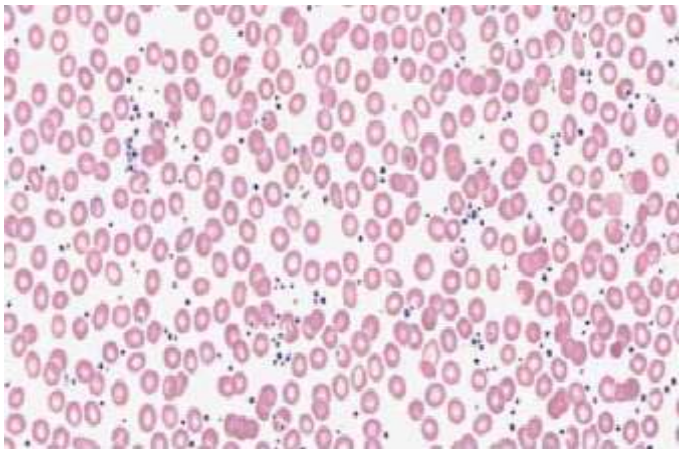

Figure 1. Thrombocytosis in alpha Thalassemia trait.

Methodology

A cohort study was carried out at the Armed Forces Institute of Transfusion (AFIT) Rawalpindi from June to December 2020 after obtaining permission from its Ethical Review Committee. All experimental protocols were approved by the Ethics Committee of the Armed Forces of Transfusion Rawalpindi Pakistan. All methods were performed in accordance with the ethical standards as laid down in the Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants or if participants are under 16, from a parent and/or legal guardian.

Inclusion criteria had children diagnosed as Thalassemia major by DNA mutation analysis at the Armed Forces Institute of Pathology (AFIP) Rawalpindi. These children (n= 41) had a platelet count of > $1000x10^9$/l for a period of three months. Children were between three to five years of age and on prescribed transfusion regimens for more than a year. The maximum interval from diagnosis to the first transfusion was three months. The initial baseline platelet counts at the start of the study were done on Sysmex™ XN 3600 and platelet counts were confirmed manually by an expert hematologist on peripheral blood film. At the start thorough workup for ruling out secondary thrombosis was carried out which included a complete physical and systemic examination by an expert paediatrician, followed by lab investigations including CBC, peripheral blood examination, DAT, IAT, serum ferritin, serum iron, soluble transferrin receptors, CRP, ESR, ultrasound abdomen, urine analysis, stool analysis and all relevant inflammatory markers performed at Shifa International hospital Islamabad. All these investigations were carried out before and after the follow-up period of the study. Out of (n=41) all children (n=23) who had serum ferritin levels of over 1000ng/ml (N = 7 to 140 ng/ml) were advised iron chelation therapy by deferoxamine.

Transfusion protocols of all (n=41) children were evaluated also revisions were necessary. Twenty-three (n=23) parents managed to start the chelation therapy. These children (n=41) were followed up regularly by their platelet counts monthly (June-Dec 2021). Out of the total cohort of (n=41) eleven (n=11) children still had a platelet count of over $1000x10^9$/l after six months hence BCR ABL1, JAK2 V617F, CAL-R and MPN-1 mutations mutation analysis was done at AFIT. SPSS 21 was used for data analysis and result compilation. Categorical data was represented in the form of frequencies and percentages. As the data was normally distributed and the same parameters were measured in the subject before and after the follow-up, hence paired sample T-test was used for the comparison of the means.

Results

There were 23 male and 18 female children with Thalassemia, having mean ages of 4.05±0.76 and 4.6±0.79 years, respectively. No significant differences were observed in various variables, including hemoglobin levels (Hb), platelet and WBC count, or serum ferritin levels, when comparing the two genders. (Table I)

The interval between successive transfusions varied between 15 days and 7 weeks in different patients. In 36.59% of patients, the frequency of transfusion was 30 days. (Figure 2)

Initially, all of the 41 patients had raised levels of ferritin and platelet count with a mean value of 2833.92±1484.9ng/ml and 1260.56±215.4 $x10^9/mm^3$ respectively as in Table II. Chelation was performed among 23 subjects as the rest did not cooperate for the process along-side the transfusions were revised and implemented. After six months of careful observation and chelation, thirty subjects (73%) approached normal platelet count and a highly significant fall in the serum

Table I: Comparison of different variables on the basis of gender.				
Variables		Male (n=23)	Females (n=18)	p-value*
Age (year)		4.05±0.76	4.6±0.79	0.82
Haemoglobin (g/dl)	Initial	10.76±1.5	10.38±1.42	0.42
	After 6 months	11.79±1.48	10.87±2.83	0.20
Platelet count x 10^9/L	Initial	1258.2±211.54	1263.56±226.36	0.94
	After 6 months	479.87±268.06	515.33±278.36	0.68
Ferritin (ng/ml)	Initial	2830.35±1511.6	2838.5±1493.68	0.99
	After 6 months	1910.96±1227.11	1764.0±1078.42	0.69
WBC x 10^9/L	Initial	6.83±1.85	6.32±1.75	0.38
	After 6 months	6.46±1.56	6.07±1.54	0.43
**p-value calculated by using an independent sample T-test.*				

ferritin levels were noted along with significant raise in Hb levels.

In the remaining eleven children (27%) who did not show decrease in platelet count, BCR ABL 1, JAK2 V617F, CAL-R and MPN-1 mutations were analyzed and came out all negative for these subjects. Out of thirty (n=30) children whose platelet count reverted back to normal included (n=23) children put on iron chelation therapy. Secondary thrombocytosis parameters were negative (n=41) in children. Out of twenty-three (n=23) children put on chelation therapy with raised ferritin levels twenty (n=20) achieved normal ferritin levels at the end of the study. (Table III)

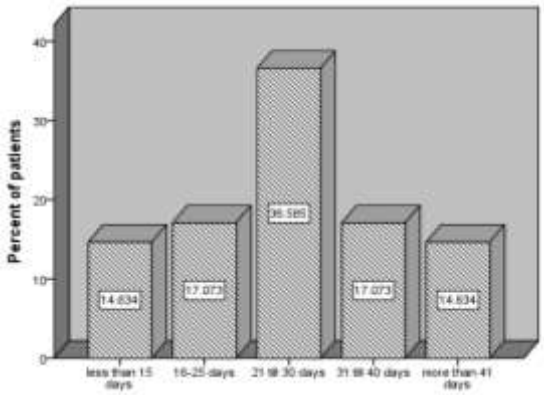

Figure 2. Frequency of transfusions in the subjects under study.

Discussion

Primary thrombocytosis is very rare amongst children with Thalassemia major.[5] Underlying iron deficiency in Thalassemia major is thought to be the key main reason for a raised platelet count.[6] The pathogenesis of thrombocytosis in Thalassemia major secondary to iron deficiency is not also fully understood. Various grey areas still remain unanswered.[7] EPO may stimulate TPO receptors (c-MPL) in Thalassemic children having iron deficiency resulting in thrombocytosis. But unfortunately, the underlying mechanism is still unproven and not clear.[8] Several studies have reported a relationship between ferritin status in Thalassemia major and platelet parameters.[9,10] But unlike the present study, Kadikoylu et al. found no correlation between platelet counts and serum ferritin in a stepwise logistic regression test.[11] Kuku et al also found no significant relationship between platelet counts and serum ferritin levels. Monitoring soluble serum transferrin receptors or Zinc protoporphyrin is useful in diagnosing true iron deficiency and maybe use instead of serum ferritin levels to determine iron deficiency in such patients.[12]

All our patients (n=41) with thrombocytosis were anaemic. However, Holbro et al. in their landmark study found that the platelet counts did not show a significant association to the cause of anaemia. His correlation analysis revealed

Table II: Comparison of different variables over a period of six months.			
Variables	Initial recording	6 months later	P value
Haemoglobin (g/dl)	10.59±1.46	11.37±2.20	0.001
Platelet count x 10^9/L	1260.56±215.4	465.98±254.42	<0.001
Ferritin (ng/dl)	2833.92±1484.9	1846.44±1152.35	<0.001
WBC count x 10^9/L	6.61±1.8	6.29±1.55	<0.001

Table III: Effects of chelation on the subjects.	
Total children. (n 41)	Children with recovered counts. (n 30)
Children receiving iron chelation	23
Recovery rate with iron chelation	80%

that platelet counts have a weak correlation to the degree of anaemia irrespective of the aetiology.[13] Bhoopalan et al. found this is likely due to the increased thrombopoietic activity of erythropoietin which is secreted in response to the degree of anemia.[14]

In Pediatric thrombocytosis due to Thalassemia major no extensive diagnostic workup is needed if secondary cause or reactive thrombocytosis is apparent. If, however primary thrombocytosis is suspected, the described diagnostic algorithm may be followed according to the recommendations published in a review article.[15] If a platelet count >450,000/μL persists longer than 6 weeks, then obtain a detailed family history and blood counts of each family member is suggested; genetic testing for BCR-ABL1, JAK2V617F, CALR, and MPLW515L is carried out where ever necessary. Furthermore, bone marrow biopsy may be performed. If the bone marrow shows hyperproliferation of megakaryocytes and any of the above genetic testing is positive, the patient most likely has a myeloproliferative disorder, including primary thrombocytosis. If, however the genetic testing is negative but the bone marrow shows megakaryocyte hyperproliferation or family history is positive additional genetic testing for an alternate TPO or MPL mutation is sugessted.[15] Persistent thrombocytosis is basis of hypercoagulable state which leads to high incidence of thrombosis in children.[16] Low serum ferritin levels by chelation resulted in reduced platelet count.[17] Many studies have addressed this phenomenon but have failed to find any significant correlation of serum ferritin levels with high platelet count.[18] Furthermore, all our patients with thrombocytosis had low hemoglobin but numerous studies have failed to pin the cause of secondary increase of platelet count to anemia in children.[19] We failed to determine underlying reason of persistently high platelet count in cohort of eleven children. Underlying pathology for thrombocytosis must be found as this leads to a severe hypercoagulable state with numerous complications.[20,21] This obviously can only be managed adequately if we know the cause.[22] We did not have access to particular mutations of children included in the study due to institutional policies.

Conclusion

In a significant percentage (27%) of children we could not find the underlying cause for thrombocytosis.

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