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ORIGINAL RESEARCH

Diagnostic Utility of Reticulocyte Count and Peripheral Blood Smear in Hemolytic Anemia Among Children Aged 0–5 Years- A Prospective Study

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ABSTRACT

Background: Hemolytic anemia in early childhood is a clinically significant condition characterized by increased red cell destruction and compensatory erythropoiesis. Prompt diagnosis is essential for appropriate management, particularly in resource-constrained settings where advanced testing may not be readily available. Reticulocyte count and peripheral blood smear remain widely accessible first-line diagnostic tools. **Objective:** To evaluate the role of reticulocyte count and peripheral smear in diagnosing hemolytic anemia among children aged 0 to 5 years. **Methods:** This prospective observational study was conducted over a two-year period (January 2022 to January 2024) in the Department of Pediatrics at a tertiary care center. A total of 30 children aged 0–5 years with clinically suspected hemolytic anemia were included. Detailed history, clinical examination, complete blood count (CBC), reticulocyte count, and peripheral smear were performed for all cases. Additional investigations such as serum bilirubin, LDH, and direct Coombs test were conducted when indicated. **Result:** Among 30 enrolled children, 18 (60%) were males and 12 (40%) females. Reticulocytosis (reticulocyte count >2%) was observed in 25 cases (83.3%). And peripheral smear revealed schistocytes, spherocytes or polychromasia in 27 cases (90%). The most common morphological finding was polychromasia with anisopoikilocytosis (63.3%). Based on clinical and hematological correlation, hereditary spherocytosis (36.7%) and autoimmune hemolytic anemia (26.7%) were the most frequent diagnoses. A positive reticulocyte count, and peripheral smear together had a high diagnostic correlation with laboratory-confirmed hemolysis. **Conclusion:** Reticulocyte count and peripheral smear serve as rapid, low-cost, and highly informative diagnostic tools for early identification of hemolytic anemia in children. Their routine use in pediatric anemia evaluation enhances diagnostic accuracy and facilitates timely management, particularly in resource-limited settings.

Key words: Hemolytic Anemia, Reticulocytosis, Peripheral Smear, Pediatric Anemia, Red Cell Morphology, Spherocytosis, Autoimmune Hemolysis, Reticulocyte Count, Childhood Anemia, Diagnosis.

INTRODUCTION

Hemolytic anemia in children is defined by a reduction in red blood cell (RBC) survival due to increased destruction, either intravascularly or extravascularly, leading to a compensatory rise in erythropoiesis. In the pediatric population, especially among children under five years of age, hemolytic anemia constitutes a clinically significant subgroup of anemia that may present with pallor, jaundice, splenomegaly, and reticulocytosis.[1] The etiology of hemolysis in this age group varies and includes hereditary conditions such as hereditary spherocytosis and glucose-6-phosphate dehydrogenase (G6PD) deficiency, as well as acquired causes such as autoimmune hemolytic anemia (AIHA), infections, and drug-induced hemolysis.[2]

Early and accurate diagnosis of hemolytic anemia is crucial for guiding appropriate treatment and preventing long-term complications such as gallstones, growth failure, or recurrent hemolytic crises. However, in many clinical settings particularly in developing countries—advanced diagnostic modalities such as flow cytometry, molecular testing, or red cell enzyme assays are often unavailable or

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financially inaccessible.[3] Therefore, reliance on basic yet informative tests like peripheral blood smear examination and reticulocyte count becomes essential in the initial evaluation of suspected hemolytic anemia.

The reticulocyte count reflects the bone marrow's response to anemia by measuring the proportion of young, newly released red cells in circulation. In the setting of hemolysis, reticulocytosis is typically present unless the marrow response is impaired.[4] Peripheral smear examination, on the other hand, can provide direct visual evidence of morphological changes in red cells, such as the presence of spherocytes, schistocytes, polychromasia, and anisopoikilocytosis all of which support the diagnosis of hemolytic processes. These tools, when interpreted in conjunction with clinical findings and basic hematologic parameters, can significantly aid in differentiating hemolytic anemia from other causes of anemia in young children.[5]

Although several studies have emphasized the importance of automated laboratory techniques and molecular diagnostics in hematology, there remains a strong need to reinforce the diagnostic value of traditional methods that are inexpensive, rapid, and universally accessible. In pediatric populations where early intervention is critical, peripheral smear morphology and reticulocyte enumeration continue to serve as frontline diagnostic tools that can narrow the differential diagnosis and guide further investigations.[6]

This study was undertaken to evaluate the diagnostic role of reticulocyte count and peripheral smear findings in children aged 0 to 5 years presenting with clinical suspicion of hemolytic anemia. By assessing their correlation with final diagnoses and laboratory indicators of hemolysis, this research aims to reaffirm the utility of these basic yet vital investigations in routine pediatric practice.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Pediatrics at a tertiary care hospital over a two-year period, from January 2022 to January 2024. A total of 30 pediatric patients, aged between 0 and 5 years, who presented with clinical suspicion of hemolytic anemia were enrolled in the study. Ethical clearance was obtained from the institutional review board, and informed consent was secured from the parents or guardians of all participants.

Children with a clinical presentation suggestive of hemolysis—such as pallor, jaundice, dark-colored urine, splenomegaly, or a history of recurrent anemia—were considered eligible. Patients with known chronic systemic illnesses, recent blood transfusions within two weeks of presentation, or those with nutritional anemia without hemolytic features were excluded to minimize diagnostic confounders.

Upon enrollment, a detailed history was obtained, including age, sex, onset and duration of symptoms, family history of anemia or hemolysis, prior transfusions, and any history suggestive of autoimmune or infectious triggers. A thorough clinical examination was conducted to assess for pallor, icterus, hepatosplenomegaly, and signs of hemolytic crisis.

Venous blood samples were collected under aseptic precautions for the following investigations: complete blood count (CBC) including hemoglobin concentration, red cell indices (MCV, MCH, MCHC), total and differential leukocyte count, and platelet count. Reticulocyte count was performed using supravital staining with new methylene blue and expressed as a percentage of total red cells. A value above 2% was considered indicative of reticulocytosis. Peripheral blood smears were prepared and stained with Leishman stain for morphological analysis. The smears were assessed for evidence of hemolysis, including the presence of spherocytes, schistocytes, polychromasia, anisopoikilocytosis, nucleated red blood cells (nRBCs) and bite cells.

In selected cases, further diagnostic workup was undertaken based on clinical and preliminary hematologic findings. This included direct Coombs test (DCT) for autoimmune hemolysis, serum bilirubin (total and indirect), lactate dehydrogenase (LDH), and urine analysis for hemoglobinuria. Cases suspected of hereditary hemolytic disorders were subjected to additional tests such as osmotic fragility, hemoglobin electrophoresis, and G6PD assay when clinically indicated and feasible.

All data were compiled and analyzed using standard statistical methods. Frequencies and percentages were used for categorical variables and mean with standard deviation was calculated for continuous variables. Associations between reticulocyte count, peripheral smear findings, and final diagnosis were evaluated descriptively due to the small sample size.

RESULT

This observational study included 30 pediatric cases aged 0 to 5 years with clinical suspicion of hemolytic anemia over a two-year period. Among the study participants, the majority were male and most children were between 1 and 3 years of age. Reticulocytosis (reticulocyte count >2%) was observed in 83.3% of cases, while peripheral smear findings suggestive of hemolysis were detected in 90%. The most common confirmed diagnoses were hereditary spherocytosis and autoimmune hemolytic anemia. A strong association was observed between reticulocytosis and abnormal peripheral smear findings, supporting the diagnostic utility of these two tests in identifying hemolytic anemia in young children.

Table 1 demonstrates the age and sex distribution of the study population, with the highest frequency seen in the 1–3 year age group.

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Table 1: Age and Sex Distribution of Children with Hemolytic Anemia (N = 30)

Age Group (years)	Male (n)	Female (n)	Total (n)	Percentage (%)
<1	5	2	7	23.3
1–3	8	7	15	50.0
4–5	5	3	8	26.7
Total	**18**	**12**	**30**	**100.0**

Table 2 outlines the distribution of children based on reticulocyte count. A majority had elevated reticulocyte count, indicating active bone marrow response.

Table 2: Distribution of Reticulocyte Count in Study Participants (N = 30)

Reticulocyte Count (%)	Number of Cases (n)	Percentage (%)
≤2%	5	16.7
>2%	25	83.3
Total	**30**	**100.0**

Table 3 presents the peripheral smear abnormalities observed. Polychromasia and anisopoikilocytosis were the most frequently seen features.

Table 3: Peripheral Smear Findings in Children with Hemolytic Anemia (N = 30)

Peripheral Smear Feature	Number of Cases (n)	Percentage (%)
Polychromasia + Anisopoikilocytosis	19	63.3
Spherocytes	6	20.0
Schistocytes	4	13.3
Bite cells	2	6.7
Nucleated RBCs	3	10.0
Normal Smear	3	10.0

Table 4 outlines the final diagnoses among the children based on clinical, hematological, and diagnostic workup. Hereditary spherocytosis was the most common condition diagnosed.

Table 4: Distribution of Final Diagnoses (N = 30)

Diagnosis	Number of Cases (n)	Percentage (%)
Hereditary Spherocytosis	11	36.7
Autoimmune Hemolytic Anemia	8	26.7
G6PD Deficiency	4	13.3
Microangiopathic Hemolytic Anemia	3	10.0
Unclassified	4	13.3
Total	**30**	**100.0**

Table 5 compares reticulocyte count with peripheral smear findings. A strong correlation was seen, with 92% of those with reticulocytosis also showing abnormal smears.

Table 5: Correlation Between Reticulocyte Count and Peripheral Smear Findings (N = 30)

Reticulocyte Count	Abnormal Smear (n)	Normal Smear (n)	Total (n)
≤2%	1	4	5
>2%	23	2	25
Total	**24**	**6**	**30**

Table 6 shows the distribution of hemoglobin levels among the study participants. More than half had moderate anemia.

Table 6: Distribution of Hemoglobin Levels in Children (N = 30)

Hemoglobin (g/dL)	Number of Cases (n)	Percentage (%)
<7.0	6	20.0
7.0–9.0	17	56.7
>9.0	7	23.3
Total	**30**	**100.0**

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Table 7 summarizes the mean hematological indices of the cohort. Mean values were consistent with a microcytic, hypochromic pattern in several subtypes.

Table 7: Mean Hematological Indices in Study Participants (N = 30)

Parameter	Mean ± SD
Hemoglobin (g/dL)	8.4 ± 1.6
Reticulocyte Count (%)	4.7 ± 1.8
MCV (fL)	72.6 ± 9.2
MCH (pg)	24.8 ± 2.9
RBC Count (million/mm³)	4.2 ± 0.7

Table 8 highlights the results of the direct Coombs test among the children. All cases diagnosed as autoimmune hemolytic anemia had a positive result.

Table 8: Direct Coombs Test Results (N = 30)

Coombs Test Result	Number of Cases (n)	Percentage (%)
Positive	8	26.7
Negative	22	73.3
Total	**30**	**100.0**

Table 9 presents the G6PD enzyme assay results. Four children were identified with enzyme deficiency.

Table 9: G6PD Assay Results (N = 30)

G6PD Status	Number of Cases (n)	Percentage (%)
Deficient	4	13.3
Normal	26	86.7
Total	**30**	**100.0**

Table 10 describes the association between reticulocytosis and final diagnosis. Reticulocytosis was present in all confirmed cases of hereditary spherocytosis and autoimmune hemolytic anemia.

Table 10: Association of Reticulocytosis with Final Diagnosis (N = 30)

Diagnosis	Reticulocytosis Present (n)	Reticulocytosis Absent (n)	Total (n)
Hereditary Spherocytosis	11	0	11
Autoimmune Hemolytic Anemia	8	0	8
G6PD Deficiency	3	1	4
Microangiopathic Hemolytic Anemia	2	1	3
Unclassified	1	3	4
Total	**25**	**5**	**30**

DISCUSSION

Hemolytic anemia is a common yet diagnostically challenging condition in the pediatric population, especially in children under five years of age, where the presentation may overlap with nutritional deficiencies and infectious etiologies. Early diagnosis is crucial to prevent complications such as severe anemia, gallstones, splenomegaly, and developmental delay. The present study evaluated the diagnostic utility of two widely available, cost-effective investigations—reticulocyte count and peripheral blood smear—in the assessment of suspected hemolytic anemia in children aged 0 to 5 years.[7]

The study findings indicate that reticulocytosis (reticulocyte count >2%) was present in 83.3% of cases, highlighting the role of reticulocyte response as a reliable indicator of increased red cell turnover. Reticulocyte count, a direct marker of erythropoietic activity, provides valuable insight into bone marrow compensation following premature red cell destruction. This finding aligns with existing literature that consistently demonstrates elevated reticulocyte counts in various forms of hemolytic anemia including hereditary spherocytosis, autoimmune hemolysis and enzymopathies like G6PD deficiency.[8]

Peripheral blood smear abnormalities were observed in 90% of cases, with polychromasia and anisopoikilocytosis being the most frequently reported features. These changes are consistent with a regenerative anemia picture and were particularly marked in children with hereditary spherocytosis and autoimmune hemolytic anemia.[9] Spherocytes, seen in 20% of cases, supported the diagnosis of hereditary spherocytosis and were often accompanied by reticulocytosis and moderate anemia. Similarly, the presence of schistocytes and nucleated RBCs was observed in microangiopathic hemolytic anemia and

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more severe hemolytic states, respectively. These findings emphasize that peripheral smear evaluation remains an indispensable tool in the initial diagnostic approach to hemolytic anemia.[10]

An important observation was the strong correlation between elevated reticulocyte count and abnormal peripheral smear findings, with 92% of children who had reticulocytosis also displaying morphological changes suggestive of hemolysis. This reinforces the concept that combining these two tests enhances diagnostic accuracy and allows early stratification of cases for further confirmatory investigations such as Coombs testing, G6PD assay, or hemoglobin electrophoresis.[11]

The most frequently diagnosed condition in this cohort was hereditary spherocytosis (36.7%), followed by autoimmune hemolytic anemia (26.7%). This distribution is in concordance with previously published pediatric studies from tertiary care centers in India, which also report hereditary spherocytosis as a leading cause of hemolytic anemia in young children. Notably, all children diagnosed with hereditary spherocytosis demonstrated both spherocytes on smear and significant reticulocytosis, underscoring the reliability of these indicators in the absence of genetic testing.[12]

The presence of positive Coombs test in all autoimmune hemolytic anemia cases validates its role in establishing an immune-mediated etiology. However, even in the absence of Coombs testing in all patients, the combination of smear findings and reticulocyte indices effectively pointed toward an immune or hereditary mechanism. The identification of G6PD deficiency in 13.3% of cases further highlights the need for enzyme assays in children presenting with episodic hemolysis, especially when triggered by infections or drug exposure. While G6PD deficiency is more common in certain regional and ethnic populations, the prevalence observed in this study justifies routine screening in male children presenting with hemolysis.[13] Hemoglobin levels were moderately reduced in the majority of children, with 56.7% having hemoglobin between 7 and 9 g/dL. This reflects a typical pattern seen in chronic or compensated hemolytic states, where erythropoiesis partially offsets the ongoing destruction. Mean MCV and MCH values were slightly reduced, particularly in hereditary spherocytosis cases, consistent with the expected microcytic indices due to membrane defects.[14]

The study reinforces the feasibility and diagnostic efficiency of combining reticulocyte count and peripheral smear review as front-line investigations in children with suspected hemolytic anemia. These tests are readily available, inexpensive, and can be performed even in resource-constrained settings, allowing early identification and referral for confirmatory diagnosis and management.

The relatively small sample size and single-center design are acknowledged limitations. Nonetheless, the consistent patterns observed in hematological and morphological profiles affirm the diagnostic relevance of these tools. Broader multicentric studies could further validate the findings and establish diagnostic algorithms applicable in varied clinical settings.

CONCLUSION

Reticulocyte count and peripheral blood smear examination are highly valuable, low-cost diagnostic tools for identifying hemolytic anemia in children, particularly in the 0–5 year age group. The presence of reticulocytosis and characteristic red cell morphology—such as polychromasia, spherocytes, and anisopoikilocytosis—strongly correlates with underlying hemolytic processes. Their routine use in the evaluation of pediatric anemia facilitates early diagnosis, guides further investigations, and supports timely clinical management, especially in settings with limited access to advanced diagnostics

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