

Research Article

CLINICAL, HEMATOLOGICAL, AND HISTOPATHOLOGICAL CORRELATES OF DRUG-RESPONSIVE ANEMIA IN CHILDREN: EVALUATION OF THERAPEUTIC RESPONSE AND PHARMACOLOGICAL FACTORS

¹Dr. Sejpal Jaykumar Jashvantrai, ²Dr. Jasmine Kholi, ³Dr. Raval Hitesh Ganabhai, ⁴Dr. Jayesh Patel Vashrambhai Patel

¹Assistant Professor, Department of Pharmacology, ICARE Institute of Medical sciences & research, and Dr. Bidhan Chandra Roy hospital, Haldia

²Assistant Professor, Department of Pathology, ICARE Institute of Medical sciences & research, and Dr. Bidhan Chandra Roy hospital, Haldia

³Assistant Professor, Department of Pediatrics, K D Medical college hospital & research, center, Mathura, U.P

⁴Assistant Professor, Department of Obstetrics and gynecology, ICARE Institute of Medical sciences & Research, and Dr. Bidhan Chandra Roy hospital, Haldia

Corresponding Author

Dr. Jayesh Patel Vashrambhai Patel
jayeshpatel1985@gmail.com

ABSTRACT

Background: Anemia is a frequent hematological disorder in childhood and may result from nutritional deficiencies, chronic disease, hemolysis, blood loss, or bone marrow pathology. A substantial proportion of pediatric anemia is amenable to specific pharmacological therapy, particularly supplementation with iron, folic acid, and vitamin B12. Correlation of clinical features with hematological parameters and, where indicated, bone marrow findings may facilitate etiological diagnosis and help predict therapeutic response.

Objectives: To evaluate the clinical, hematological, and histopathological characteristics of drug-responsive anemia in children and to assess therapeutic response and pharmacological factors associated with hematological recovery.

Materials and Methods: This prospective observational study included 150 children aged 6 months to 18 years with anemia attending a tertiary-care hospital. Detailed clinical assessment, complete blood count, peripheral

smear, reticulocyte count, iron profile, serum ferritin, vitamin B12, and folate levels were evaluated as clinically indicated. Bone marrow aspiration/biopsy was performed only in selected children in whom the diagnosis remained uncertain or marrow pathology was suspected. Treatment was based on the identified or strongly suspected deficiency and included oral or parenteral iron, folic acid, vitamin B12, or combined hematinic therapy. Response was evaluated at 2, 4, and 12 weeks. A rise in hemoglobin of ≥ 1 g/dL by 4 weeks was considered a satisfactory early response.

Results: The mean age of the study population was 7.6 ± 4.3 years, with 54.7% males. Pallor (94.7%), fatigue (62.0%), reduced appetite (46.0%), pica (34.0%), and poor weight gain (30.0%) were common manifestations. Microcytic hypochromic anemia was the predominant hematological pattern (62.0%), followed by macrocytic (18.0%), normocytic (14.0%), and dimorphic anemia (6.0%). Iron deficiency was the commonest identified etiology (60.7%), while vitamin B12/folate

deficiency accounted for 18.0%. Bone marrow examination was performed in 32 selected children and demonstrated micronormoblastic erythropoiesis with reduced iron stores in 15, megaloblastic erythropoiesis in 10, dimorphic erythropoiesis in 4, and other/reactive patterns in 3. Overall, 126 (84.0%) children demonstrated a satisfactory therapeutic response at 4 weeks. Responders showed a mean hemoglobin increase from 8.1 ± 1.3 to 10.2 ± 1.4 g/dL ($p < 0.001$). Good adherence and confirmed nutritional deficiency were positively associated with response, whereas poor adherence, gastrointestinal intolerance, chronic inflammation, and mixed deficiencies were associated with delayed or inadequate response.

Conclusion: Nutritional deficiency, particularly iron deficiency, constitutes an important pharmacologically reversible cause of childhood anemia. Clinical findings combined with red-cell indices, peripheral smear, ferritin and appropriate micronutrient assays generally permit diagnosis without invasive testing. Bone marrow examination has a complementary role in selected diagnostically difficult cases. Appropriate drug selection, adequate dosage, adherence, and correction of the underlying cause are important determinants of hematological recovery.

Keywords: Pediatric anemia; iron deficiency anemia; hematinics; bone marrow; therapeutic response; iron therapy; vitamin B12; pharmacological treatment.

INTRODUCTION

Anemia in childhood is a clinically important condition characterized by a hemoglobin concentration below the age- and sex-appropriate reference threshold. Its etiology is heterogeneous and includes nutritional deficiencies, infections, inflammatory disorders, hemoglobinopathies, blood loss, hemolytic disorders, and primary or secondary bone marrow abnormalities. The World Health Organization has updated hemoglobin thresholds for defining anemia, emphasizing age- and population-specific interpretation of

hemoglobin measurements [1]. Nutritional deficiencies remain particularly important in pediatric practice. Iron deficiency is the most frequent nutritional cause of anemia in children and may arise from inadequate dietary intake, increased requirements during rapid growth, impaired intestinal absorption, or chronic blood loss [2,3]. Vitamin B12 and folate deficiencies constitute other potentially reversible causes and may produce macrocytic or megaloblastic hematological abnormalities. The hematological phenotype often provides an initial indication of the underlying mechanism. Low mean corpuscular volume (MCV), reduced mean corpuscular hemoglobin (MCH), elevated red-cell distribution width (RDW), microcytosis, and hypochromia favor iron deficiency, whereas macrocytosis and hypersegmented neutrophils raise suspicion of megaloblastic anemia. Serum ferritin is useful for assessing iron stores, although its interpretation requires caution in the presence of inflammation [2,4]. Reticulocyte hemoglobin and soluble transferrin receptor measurements can provide additional information when conventional iron indices are inconclusive [4].

Therapeutic response itself can contribute to diagnosis. In children with iron-deficiency anemia, an appropriate rise in hemoglobin following therapeutic iron supports iron deficiency as the underlying cause [5]. Conversely, an inadequate response should prompt assessment of adherence, dosage, continuing blood loss, malabsorption, inflammation, mixed nutritional deficiencies, or an alternative diagnosis. Bone marrow examination is not routinely required for uncomplicated nutritional anemia. Nevertheless, in children with unexplained severe anemia, associated cytopenias, abnormal peripheral blood morphology, organomegaly, or failure to respond appropriately to treatment, marrow evaluation can help characterize erythropoiesis and exclude marrow failure, infiltration, or other hematological disease [6, 7].

Few clinical studies evaluate clinical presentation, hematological phenotype, selected marrow morphology, pharmacological therapy, and subsequent hematological response within a single pediatric cohort. Therefore, the present study was designed to assess these parameters collectively and identify factors influencing therapeutic response in children with drug-responsive anemia.

MATERIALS AND METHODS

Study design and setting

This prospective observational study was conducted in the Departments of Pediatrics and Pathology of a tertiary-care teaching hospital in India over a period of 18 months.

Study population

A sample of 150 children presenting to the outpatient department or admitted to pediatric wards and diagnosed with anemia according to age-appropriate hemoglobin thresholds were screened for inclusion.

Inclusion criteria

Children aged 6 months to 18 years with laboratory-confirmed anemia; children undergoing etiological evaluation for suspected nutritional or hematinic-responsive anemia; and parents/guardians providing written informed consent were included. Assent was obtained from older children wherever applicable.

Exclusion criteria

Children with previously diagnosed hematological malignancy, aplastic anemia, major congenital marrow disorders, or anemia requiring immediate definitive treatment unrelated to hematinic therapy were excluded. Children who were lost to follow-up before assessment of therapeutic response were excluded from response analysis.

Clinical evaluation

Demographic characteristics, socioeconomic and dietary history, duration of symptoms, pica, fatigue, appetite, growth pattern, recurrent infections, gastrointestinal symptoms, drug history, previous hematinic supplementation, bleeding history, and relevant family history were recorded. Clinical examination included assessment for pallor, icterus, edema, glossitis,

angular stomatitis, koilonychia, lymphadenopathy, hepatomegaly, splenomegaly, anthropometric status, and evidence of systemic disease.

Laboratory investigations

Venous blood was collected under aseptic precautions. Investigations included complete blood count with red-cell indices, peripheral blood smear, reticulocyte count, serum ferritin, serum iron, total iron-binding capacity and transferrin saturation, serum vitamin B12 and folate, inflammatory markers, and additional etiological tests where clinically indicated. Iron deficiency was diagnosed using compatible hematological morphology and biochemical evidence of depleted iron stores, with ferritin interpreted in the context of inflammation.

Peripheral smear classification

Anemia was morphologically categorized as microcytic hypochromic, macrocytic, normocytic normochromic, or dimorphic.

Bone marrow examination

Bone marrow aspiration, with trephine biopsy when clinically required, was not performed routinely. It was restricted to selected patients with severe or unexplained anemia, associated cytopenias, atypical peripheral smear findings, suspected marrow pathology, or inadequate response to appropriate initial therapy. Marrow cellularity, myeloid-to-erythroid ratio, erythroid morphology, maturation pattern, megaloblastic changes, dysplastic changes, abnormal cells, and iron stores using Perls' Prussian blue staining were evaluated where applicable.

Pharmacological treatment

Treatment was individualized according to the suspected or confirmed cause. Children with iron-deficiency anemia received oral elemental iron, generally 3–6 mg/kg/day, according to severity and clinical circumstances. Children unable to tolerate oral therapy, those with documented malabsorption, or selected patients with inadequate response despite confirmed adherence were considered for parenteral iron. Folic acid and vitamin B12 were administered for documented or strongly suspected deficiencies, and combined hematinic therapy

was used when more than one deficiency was demonstrated. Dietary counselling and treatment of contributing conditions were undertaken when indicated.

Assessment of therapeutic response

Patients were followed at approximately 2, 4, and 12 weeks. Clinical improvement, adherence, adverse drug reactions, reticulocyte response, hemoglobin concentration, MCV, RDW, and other relevant indices were assessed. A rise in hemoglobin of ≥ 1 g/dL by approximately 4 weeks was considered a satisfactory early hematological response.

Outcome measures

The primary outcome was satisfactory hematological response at 4 weeks. Secondary outcomes included absolute hemoglobin increase, normalization or improvement of red-cell indices, clinical symptom improvement, adverse drug effects, adherence, and factors associated with inadequate response.

Statistical analysis

Continuous variables were expressed as mean $\pm$ standard deviation or median with interquartile range, as appropriate. Categorical variables were expressed as frequencies and percentages. Paired continuous measurements were compared using the paired t-test or Wilcoxon signed-rank test. Categorical variables were analyzed using the chi-square test or Fisher's exact test. Variables associated with treatment response on univariate analysis were considered for binary logistic regression. A two-sided p value <0.05 was considered statistically significant.

RESULTS

A total of 150 children with anemia were evaluated. The mean age was 7.6 ± 4.3 years; 82 (54.7%) were boys and 68 (45.3%) were girls.

Table 1. Baseline demographic and clinical characteristics

Characteristic	n	%
Age <5 years	52	34.7
Age 5–10 years	55	36.7
Age >10 years	43	28.7
Male	82	54.7
Female	68	45.3
Pallor	142	94.7
Fatigue/easy fatigability	93	62.0
Reduced appetite	69	46.0
Pica	51	34.0
Poor weight gain	45	30.0
Glossitis/angular stomatitis	29	19.3
Hepatomegaly	21	14.0
Splenomegaly	13	8.7

Pallor was the predominant clinical finding, followed by fatigue and reduced appetite. Pica was observed in approximately one-third of children and occurred predominantly among those with iron deficiency.

Table 2. Hematological profile at presentation

Parameter	Mean $\pm$ SD
Hemoglobin (g/dL)	8.2 ± 1.5
RBC count ($\times 10^6/\mu\text{L}$)	4.10 ± 0.72
MCV (fL)	72.8 ± 11.6
MCH (pg)	23.1 ± 5.2
MCHC (g/dL)	30.8 ± 2.9

Dr. Sejpal Jaykumar Jashvantrai et al / CLINICAL, HEMATOLOGICAL, AND HISTOPATHOLOGICAL CORRELATES OF DRUG-RESPONSIVE ANEMIA IN CHILDREN: EVALUATION OF THERAPEUTIC RESPONSE AND PHARMACOLOGICAL FACTORS

RDW (%)	17.9 ± 3.2
Reticulocyte count (%)	1.3 ± 0.7

Reduced MCV and MCH with increased RDW were prominent among children subsequently categorized as having iron-deficiency anemia.

Table 3. Morphological pattern of anemia

Morphological pattern	n	%
Microcytic hypochromic	93	62.0
Macrocytic	27	18.0
Normocytic normochromic	21	14.0
Dimorphic	9	6.0
Total	150	100

Microcytic hypochromic anemia was the most frequent morphological pattern.

Table 4. Probable etiological classification

Etiology	n	%
Iron deficiency	91	60.7
Vitamin B12/folate deficiency	27	18.0
Mixed nutritional deficiency	12	8.0
Anemia associated with inflammation/chronic disease	11	7.3
Other/undetermined responsive anemia	9	6.0

Nutritional deficiencies collectively accounted for the majority of cases.

Table 5. Bone marrow findings among selected children undergoing marrow examination (n=32)

Bone marrow pattern	n	%
Micronormoblastic erythropoiesis/reduced iron stores	15	46.9
Megaloblastic erythropoiesis	10	31.3
Dimorphic erythropoiesis	4	12.5
Reactive/other pattern	3	9.4
Total	32	100

Marrow findings showed good concordance with the hematological and biochemical diagnosis in most investigated cases.

Table 6. Hematological response to treatment

Parameter	Baseline	4 weeks	12 weeks	p value*
Hemoglobin (g/dL)	8.2 ± 1.5	10.0 ± 1.6	11.7 ± 1.3	<0.001
MCV (fL)	72.8 ± 11.6	77.4 ± 10.1	82.1 ± 8.3	<0.001
MCH (pg)	23.1 ± 5.2	25.8 ± 4.6	28.0 ± 3.7	<0.001
RDW (%)	17.9 ± 3.2	17.1 ± 2.8	15.2 ± 2.1	<0.001

*Comparison between baseline and 12-week measurements. Significant improvement in hemoglobin and red-cell indices was observed during follow-up. At four weeks, 126/150 (84.0%) children achieved a satisfactory early hematological response, whereas 24 (16.0%) had an inadequate or delayed response.

Table 7. Factors associated with satisfactory therapeutic response at 4 weeks

Factor	Responders' n/N (%)	Non-responder's n/N (%)	p value
Good medication adherence	119/126 (94.4)	13/24 (54.2)	<0.001
Confirmed nutritional deficiency	114/126 (90.5)	16/24 (66.7)	0.002
Appropriate weight-based dose	121/126 (96.0)	19/24 (79.2)	0.009

Dr. Sejpal Jaykumar Jashvantraï et al / CLINICAL, HEMATOLOGICAL, AND HISTOPATHOLOGICAL CORRELATES OF DRUG-RESPONSIVE ANEMIA IN CHILDREN: EVALUATION OF THERAPEUTIC RESPONSE AND PHARMACOLOGICAL FACTORS

Gastrointestinal adverse effects	18/126 (14.3)	10/24 (41.7)	0.003
Evidence of chronic inflammation	6/126 (4.8)	7/24 (29.2)	<0.001
Mixed nutritional deficiency	7/126 (5.6)	5/24 (20.8)	0.026

Medication adherence emerged as an important modifiable factor associated with treatment response. Gastrointestinal intolerance and underlying inflammatory conditions were disproportionately represented among children with an inadequate early response.

DISCUSSION

Iron deficiency is recognized as the most frequent micronutrient deficiency contributing to childhood anemia. Aksu and Ünal described inadequate dietary iron intake, increased physiological requirements, bleeding, and impaired intestinal absorption as major causes of pediatric iron deficiency [2]. Similarly, Pasricha SR et al. emphasized the importance of iron deficiency during infancy and childhood and highlighted its potential developmental consequences [9].

The predominance of microcytic hypochromic anemia in the present study is consistent with the high proportion of iron-deficiency cases. Low MCV and MCH together with elevated RDW were characteristic hematological findings. These parameters are useful as inexpensive initial indicators, particularly in resource-limited settings. However, morphology alone cannot establish the etiology, as thalassemia and chronic inflammatory disorders may also produce microcytosis [8].

Serum ferritin remains a useful indicator of iron stores but should be interpreted carefully in children with infection or inflammation because ferritin is an acute-phase reactant. Contemporary recommendations therefore favor assessment of ferritin in conjunction with inflammatory markers and, when necessary, additional parameters such as transferrin saturation, reticulocyte hemoglobin, or soluble transferrin receptor [5, 10].

Macrocytic anemia constituted 18% of the present cohort and was mainly associated with vitamin B12 or folate deficiency. The corresponding marrow specimens showed megaloblastic erythropoiesis in most investigated children, demonstrating the value

of morphological correlation in selected diagnostically challenging cases. Dimorphic anemia, although less common, was clinically relevant because coexistence of iron and vitamin deficiencies may obscure the classical red-cell indices of either deficiency when present alone.

Bone marrow examination was deliberately restricted to selected children. This is important because routine bone marrow examination is neither necessary nor justified in uncomplicated iron-deficiency anemia. Current pediatric literature indicates that diagnosis can generally be established using clinical features, complete blood count, peripheral smear, ferritin and other iron parameters, together with the response to treatment [6, 11]. Marrow examination becomes valuable when anemia is unexplained, severe, associated with additional cytopenias or atypical cells, or fails to respond to appropriately administered therapy.

Therapeutic response was favorable in the majority of children, with 84% demonstrating a hemoglobin increase of at least 1 g/dL at four weeks. A rise in hemoglobin following therapeutic iron is itself supportive of iron-deficiency anemia. Aksu and Ünal reported that an approximately 1 g/dL increase in hemoglobin after one month of therapeutic iron supports the diagnosis [2].

A progressive rise in hemoglobin was accompanied by improvement in MCV and MCH. RDW declined more gradually, which is biologically plausible because circulating erythrocytes produced before and after initiation of therapy coexist during the recovery period.

Medication adherence was strongly associated with therapeutic success. Children with good adherence were substantially more likely to

achieve the expected hematological response. This finding highlights an important distinction between pharmacological efficacy and effectiveness in clinical practice. Iron preparations can produce gastrointestinal adverse effects, including abdominal discomfort, nausea, constipation, and altered stool characteristics, potentially reducing adherence. Selection of a tolerable formulation, appropriate counselling, and follow-up therefore contribute materially to therapeutic success [12].

Gastrointestinal intolerance was more frequent among non-responders. Children who do not tolerate or respond adequately to oral iron require reassessment rather than automatic escalation of dosage. Incorrect diagnosis, inadequate dosing, poor adherence, persistent bleeding, celiac disease, inflammatory bowel disease, or another cause of impaired absorption should be considered. Intravenous iron has an established role in selected pediatric patients with intolerance, malabsorption, chronic inflammatory gastrointestinal conditions, or persistent iron deficiency despite appropriate oral therapy [13].

Mixed nutritional deficiency was another factor associated with delayed response. Administration of iron alone in a child with concurrent vitamin B12 or folate deficiency may produce only partial hematological correction. A detailed dietary history, peripheral smear examination, and selective micronutrient testing are therefore valuable when the clinical or hematological response is less than expected [14].

The association between chronic inflammation and inadequate early response reflects the complex regulation of iron metabolism during inflammatory states. Inflammation-mediated changes in hepcidin can restrict intestinal iron absorption and iron availability to erythroid precursors despite apparently adequate body iron stores. Consequently, treatment should address the underlying inflammatory disorder in addition to correcting nutritional deficiencies [15].

Strengths and Limitations

The strength of the study is the integrated assessment of clinical presentation, hematological morphology, selected histopathological findings, pharmacological treatment, and longitudinal therapeutic response. Several limitations should be acknowledged. This was a single-center study, and the findings may not represent the wider pediatric population. Bone marrow examination was performed only when clinically indicated; therefore, marrow findings cannot be generalized to the entire cohort. Treatment regimens were etiologically individualized rather than standardized as in a randomized trial. Dietary intake and medication adherence were partly based on caregiver reporting, introducing the possibility of recall bias. Longer follow-up would also be necessary to evaluate complete restoration of iron stores and recurrence of anemia.

CONCLUSION

Drug-responsive anemia in children is predominantly associated with correctable nutritional deficiencies, particularly iron deficiency. Pallor, fatigue, pica, microcytosis, hypochromia, elevated RDW, and reduced iron stores constitute useful clinical and laboratory indicators of iron-deficiency anemia. Macrocytic and dimorphic patterns should prompt evaluation for vitamin B12, folate, and combined nutritional deficiencies. Most children demonstrate substantial hematological improvement following appropriately selected pharmacological therapy. Adequate dosage, good medication adherence, gastrointestinal tolerability, correction of associated deficiencies, and treatment of underlying disease are important determinants of response. Failure to achieve the expected rise in hemoglobin should prompt reassessment for non-adherence, incorrect diagnosis, malabsorption, ongoing blood loss, inflammation, or mixed deficiency. Bone

marrow examination is valuable in selected diagnostically difficult cases but should not be routinely performed in uncomplicated nutritional anemia. A structured clinicopathological approach combined with targeted hematinic therapy can therefore provide effective and rational management of pediatric anemia.

REFERENCES

1. World Health Organization. Guideline on haemoglobin cutoffs to define anaemia in individuals and populations. Geneva: World Health Organization; 2024.
2. Aksu T, Ünal Ş. Iron deficiency anemia in infancy, childhood, and adolescence. *Turk Arch Pediatr*. 2023;58(4):358-362. doi:10.5152/TurkArchPediatr.2023.23049.
3. Moscheo C, Licciardello M, Samperi P, La Spina M, Di Cataldo A, Russo G. New insights into iron deficiency anemia in children: a practical review. *Metabolites*. 2022;12(4):289. doi:10.3390/metabo12040289.
4. Cappellini MD, Musallam KM, Taher AT. Iron deficiency anaemia revisited. *J Intern Med*. 2020;287(2):153-170. doi:10.1111/joim.13004.
5. Powers JM, Buchanan GR. Diagnosis and management of iron deficiency anemia. *Hematol Oncol Clin North Am*. 2014;28(4):729-745.
6. Gallagher PG. Anemia in the pediatric patient. *Blood*. 2022;140(6):571-593.
7. Powers JM, Shamoun M, McCavit TL, Adix L, Buchanan GR. Intravenous iron therapy in pediatrics: who should get it and when is the right time? *Hematology Am Soc Hematol Educ Program*. 2023;2023(1):630-637.
8. World Health Organization. Accelerating anaemia reduction: a comprehensive framework for action. Summary report. Geneva: World Health Organization; 2024.
9. Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. *Lancet*. 2021;397(10270):233-248.
10. Mantadakis E, Chatzimichael E, Zikidou P. Iron deficiency anemia in children residing in high and low-income countries: risk factors, prevention, diagnosis and therapy. *Mediterr J Hematol Infect Dis*. 2020;12(1):e2020041.
11. World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva: World Health Organization; 2020.
12. Nead KG, Halterman JS, Kaczorowski JM, Auinger P, Weitzman M. Overweight children and adolescents: a risk group for iron deficiency. *Pediatrics*. 2004;114(1):104-108.
13. Özdemir N. Iron deficiency anemia from diagnosis to treatment in children. *Turk Pediatr Ars*. 2015;50(1):11-19.
14. Bailey RL, West KP Jr, Black RE. The epidemiology of global micronutrient deficiencies. *Ann Nutr Metab*. 2015;66(Suppl 2):22-33.
15. Camaschella C. Iron-deficiency anemia. *N Engl J Med*. 2015;372(19):1832-1843.