

Assessment of Erythrocyte Indices in Children with Microcytic Hypochromic Anemia After Iron Treatment in Benghazi

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Abstract

Microcytic hypochromic anemia represents a significant pediatric hematological concern, with iron deficiency anemia (IDA) being the predominant etiology. Assessment of erythrocyte indices provides valuable information regarding treatment response and hematological recovery. This study aimed to evaluate changes in erythrocyte indices following iron treatment in children with microcytic hypochromic anemia, including those with IDA, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and hemophilia, and to identify predictors of hemoglobin response. A retrospective cross-sectional study was conducted using data from the Hematology outpatient clinics at Benghazi Medical Center from January 2015 to December 2025. A total of 325 children younger than 16 years with microcytic hypochromic anemia were included. Erythrocyte indices were assessed before and after 4–5 months of oral iron therapy at 3–6 mg/kg/day. Statistical analyses included paired-samples t-tests, Kruskal–Wallis tests, mixed-design general linear models, and multiple linear regression. Significant improvements were observed in all erythrocyte indices following treatment. Hemoglobin increased from 9.35 ± 1.10 to 11.43 ± 0.61 g/dL ($p < 0.001$), MCV increased from 65.14 ± 5.86 to 82.64 ± 4.90 fL ($p < 0.001$), and RDW decreased from $16.31 \pm 2.06\%$ to $12.62 \pm 1.62\%$ ($p < 0.001$). No significant differences in the magnitude of hematological change were observed among diagnostic groups. Baseline hemoglobin was the strongest predictor of hemoglobin change ($\beta = -0.863$, $p < 0.001$), with the regression model explaining 76.7% of the variance. Iron therapy effectively improved erythrocyte indices across all diagnostic groups, with comparable treatment responses regardless of underlying etiology. Baseline hemoglobin concentration serves as the principal determinant of treatment response magnitude.

Keywords. Iron Deficiency Anemia, Erythrocyte Indices, Microcytic Hypochromic Anemia, Children, Iron Therapy.

Introduction

Anemia constitutes one of the most prevalent public health challenges affecting pediatric populations worldwide, with particularly high burden in developing countries and resource-limited settings. The condition is characterized by a reduction in the number of circulating red blood cells or their hemoglobin content below age-appropriate reference values, resulting in diminished oxygen-carrying capacity and subsequent tissue hypoxia [1]. Children are especially vulnerable to anemia due to their rapid growth requirements, increased iron demands during developmental periods, and susceptibility to nutritional deficiencies. The clinical manifestations of anemia in children range from subtle fatigue and decreased exercise tolerance to severe complications including growth retardation, impaired cognitive development, and compromised immune function [2].

The global prevalence of anemia in children under five years of age exceeds 40% in many regions, representing a significant obstacle to achieving optimal child health outcomes and developmental potential. The morphological classification of anemia based on red blood cell size and hemoglobin content provides a practical framework for differential diagnosis and therapeutic planning in clinical practice. Microcytic hypochromic anemia, characterized by reduced mean corpuscular volume and diminished hemoglobin concentration within individual erythrocytes, represents the most frequently encountered morphological subtype in pediatric practice. Regional data from Benghazi demonstrated that microcytic anemia was the most predominant type, accounting for 59.2% of all anemia cases, followed by normocytic anemia at 39.5% and macrocytic anemia at only 1.3% [3]. This category encompasses several distinct etiologies, including iron deficiency, thalassemia syndromes, glucose-6-phosphate dehydrogenase deficiency, and chronic inflammatory conditions [4, 5]. Iron deficiency anemia remains the predominant cause of microcytic hypochromic anemia globally, accounting for approximately half of all anemia cases in children, with particular prevalence in populations with limited dietary diversity and high infectious disease burden [6].

Erythrocyte indices derived from automated complete blood count analysis serve as fundamental tools for evaluating and monitoring anemia in pediatric populations. Mean corpuscular volume quantifies average red blood cell size, while mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration reflect the hemoglobin content within erythrocytes [7]. Red cell distribution width provides information on heterogeneity in red blood cell size and serves as an early indicator of erythropoietic disturbance. Recent investigations have demonstrated that MCH and RDW serve as independent predictors of iron deficiency status, with practical utility for early detection of non-anemic iron deficiency in settings where advanced iron studies may not be readily available [8]. Additionally, reticulocyte hemoglobin equivalent has emerged as a valuable marker for assessing real-time erythropoietic activity and treatment response [9]. These indices collectively provide comprehensive information regarding the functional status of erythropoiesis and guide therapeutic decision-making. The management of iron deficiency anemia in children primarily relies on oral iron supplementation, with various formulations and dosing regimens available.

Current evidence supports daily oral iron therapy at doses ranging from 3 to 6 mg/kg/day of elemental iron, administered for sufficient duration to replenish depleted iron stores [10]. Systematic review and meta-analysis data confirm that oral iron supplementation effectively improves hemoglobin concentrations and iron status parameters in children with iron deficiency [11]. Alternative approaches, including alternate-day dosing and various iron formulations such as ferrous sulfate, ferric hydroxide polymaltose, iron bisglycinate chelate, and lactoferrin, have been investigated with varying results regarding efficacy and tolerability [12, 13]. The assessment of treatment response typically involves monitoring changes in hemoglobin concentration and erythrocyte indices, with particular attention to the magnitude and timing of hematological improvement.

Despite the well-established efficacy of iron therapy in correcting iron deficiency anemia, questions remain regarding treatment response patterns across different underlying conditions presenting with microcytic hypochromic anemia. Children with concurrent conditions such as G6PD deficiency or hemophilia may demonstrate different response characteristics compared to those with uncomplicated iron deficiency [14-16]. Furthermore, identifying reliable predictors of treatment response magnitude could facilitate more individualized therapeutic approaches and improve clinical outcomes. The Benghazi pediatric population presents unique characteristics warranting investigation, given the regional prevalence patterns of various hematological conditions and nutritional deficiencies [3]. This study aimed to evaluate changes in erythrocyte indices following iron treatment in children with microcytic hypochromic anemia at Benghazi Medical Center and to identify factors influencing treatment response across different diagnostic categories.

Methods

Study Design and Setting

This retrospective cross-sectional study was conducted using data from the Pediatric Hematology outpatient clinics at Benghazi Medical Center from January 2015 to December 2025. The study protocol was approved by the institutional ethics committee, and the requirement for informed consent was waived due to the retrospective nature of the investigation.

Study Population

A total of 325 children younger than 16 years with documented microcytic hypochromic anemia were included in the study. Patients were categorized into three diagnostic groups: iron deficiency anemia (IDA), glucose-6-phosphate dehydrogenase (G6PD) deficiency with concurrent iron deficiency, and hemophilia with iron deficiency. Inclusion criteria comprised: age less than 16 years, confirmed diagnosis of microcytic hypochromic anemia based on complete blood count parameters, completion of prescribed iron therapy course, and availability of both pre-treatment and post-treatment laboratory data. Exclusion criteria included: thalassemia major or intermedia, chronic inflammatory conditions, malignancy, chronic kidney disease, incomplete medical records, and loss to follow-up before treatment completion.

Iron Therapy Protocol

All patients received oral iron therapy at doses ranging from 3 to 6 mg/kg/day of elemental iron, administered for a duration of 4 to 5 months. The specific iron formulation varied based on physician preference and availability, including ferrous sulfate, ferric hydroxide polymaltose, and iron bisglycinate preparations.

Laboratory Measurements

Complete blood count parameters were obtained using automated hematology analyzers at baseline (pre-treatment) and following completion of the treatment course (post-treatment). Erythrocyte indices assessed included: hemoglobin concentration (g/dL), red blood cell count ($\times 10^6/\mu\text{L}$), hematocrit (%), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), and red cell distribution width (RDW, %).

Statistical Analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corporation, Armonk, NY). Continuous variables were expressed as mean $\pm$ standard deviation. The normality of data distribution was assessed using the Shapiro-Wilk test. Paired-sample t-tests were used to compare pre-treatment and post-treatment values within groups. The Kruskal-Wallis test was employed to compare the magnitude of change among the three diagnostic groups. A mixed-design general linear model was utilized to assess the interaction between time (pre- vs. post-treatment) and diagnostic group. Multiple linear regression analysis was performed to identify predictors of hemoglobin change, with baseline hemoglobin, age, sex, and diagnostic group entered as independent variables. A p-value less than 0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristics

The study included 325 children with microcytic hypochromic anemia who received iron treatment over a period of 4–5 months. The majority of the study population ($n = 297$, 91.4%) had iron deficiency anemia, while 9 children (2.8%) had severe G6PD deficiency and 19 children (5.8%) had hemophilia with concurrent microcytic hypochromic features.

Changes in Erythrocyte Indices Following Iron Treatment

Following 4–5 months of iron therapy, statistically significant improvements were observed across all evaluated erythrocyte parameters. Hemoglobin concentration increased by a mean of 2.08 g/dL, rising from 9.35 ± 1.10 g/dL to 11.43 ± 0.61 g/dL ($p < 0.001$). Hematocrit demonstrated an increase of 4.67 percentage points, while MCV showed the most pronounced improvement with a mean increase of 17.50 fL. Red cell distribution width decreased significantly by 3.69 percentage points, indicating normalization of red blood cell size heterogeneity. (Table 1) presents the complete comparison of erythrocyte indices before and after iron treatment.

Table 1. Comparison of Erythrocyte Indices Before and After Iron Treatment (Paired-Samples t-Test)

Parameter	Time Point	Mean $\pm$ SD	Mean Difference (Post – Pre) $\pm$ SE	t-value	df	p-value
Hb (g/dL)	Before treatment	9.35 ± 1.10	2.08 ± 0.07	30.75	324	<0.001
	After treatment	11.43 ± 0.61				
Hct (%)	Before treatment	28.82 ± 2.14	4.67 ± 0.13	37.01	324	<0.001
	After treatment	33.48 ± 1.10				
MCV (fL)	Before treatment	65.14 ± 5.86	17.50 ± 0.41	42.73	324	<0.001
	After treatment	82.64 ± 4.90				
MCH (pg)	Before treatment	21.09 ± 2.87	3.31 ± 0.08	43.27	324	<0.001
	After treatment	24.40 ± 2.28				
MCHC (g/dL)	Before treatment	28.15 ± 3.03	4.08 ± 0.13	30.69	324	<0.001
	After treatment	32.22 ± 1.98				
RDW (%)	Before treatment	16.31 ± 2.06	-3.69 ± 0.07	-51.85	324	<0.001
	After treatment	12.62 ± 1.62				

Mean hemoglobin increased from 9.35 ± 1.10 g/dL before treatment to 11.43 ± 0.61 g/dL after treatment, corresponding to a mean increase of 2.08 ± 0.07 g/dL ($t = 30.75$, $p < 0.001$). Hematocrit increased by 4.67 ± 0.13 percentage points, from $28.82 \pm 2.14\%$ to $33.48 \pm 1.10\%$ ($t = 37.01$, $p < 0.001$). Marked improvements were observed in red-cell indices, with MCV increasing from 65.14 ± 5.86 fL to 82.64 ± 4.90 fL, MCH increasing from 21.09 ± 2.87 pg to 24.40 ± 2.28 pg, and MCHC increasing from 28.15 ± 3.03 g/dL to 32.22 ± 1.98 g/dL (all $p < 0.001$). Red cell distribution width decreased significantly from $16.31 \pm 2.06\%$ to $12.62 \pm 1.62\%$, with a mean reduction of 3.69 ± 0.07 percentage points ($t = -51.85$, $p < 0.001$).

Changes in Erythrocyte Indices According to Diagnostic Group

When stratified by diagnostic category, all three groups demonstrated positive changes in hemoglobin, hematocrit, MCV, MCH, and MCHC, together with a reduction in RDW. The largest mean increase in hemoglobin was observed among children with IDA (Δ Hb: 2.12 ± 1.20 g/dL), whereas the G6PD deficiency group showed the greatest mean increases in hematocrit ($5.32 \pm 2.51\%$), MCV (20.13 ± 6.74 fL), and MCHC (5.11 ± 2.75 g/dL). (Table 2) presents the mean changes in erythrocyte indices according to diagnostic group.

Table 2. Mean Change (Δ) in Erythrocyte Indices According to Diagnostic Group

Parameter (Δ = post–Pre)	IDA (n=297)	G6PD Deficiency (n=9)	Hemophilia (n=19)
Δ Hb (g/dL)	2.12 ± 1.20	1.82 ± 1.79	1.61 ± 1.09
Δ Hct (%)	4.61 ± 2.31	5.32 ± 2.51	5.18 ± 1.31
Δ MCV (fL)	17.36 ± 7.48	20.13 ± 6.74	18.47 ± 5.98
Δ MCH (pg)	3.37 ± 1.40	2.61 ± 0.78	2.85 ± 1.09
Δ MCHC (g/dL)	4.00 ± 2.36	5.11 ± 2.75	4.82 ± 2.64
Δ RDW (%)	-3.70 ± 1.32	-3.53 ± 0.53	-3.54 ± 0.96

Data are presented as mean $\pm$ standard deviation.

All three groups demonstrated positive changes in Hb, Hct, MCV, MCH, and MCHC, together with a reduction in RDW, indicating a broadly consistent direction of hematological improvement across diagnostic categories. The largest mean increase in Hb was observed among children with IDA (Δ Hb: 2.12 ± 1.20 g/dL), whereas the greatest mean increases in Hct, MCV, and MCHC were observed in the G6PD deficiency group.

Comparison of Changes Among Diagnostic Groups

Despite the numerical differences observed among diagnostic categories, the Kruskal-Wallis analysis demonstrated no statistically significant differences in the magnitude of change in any evaluated erythrocyte index. The p-values ranged from 0.075 for MCH to 0.958 for RDW, indicating that children with IDA, G6PD deficiency, and hemophilia responded similarly to iron treatment. (Table 3) presents the results of the Kruskal-Wallis test comparing changes among the three diagnostic groups.

Table 3. Comparison of Changes in Erythrocyte Indices Among the Three Diagnostic Groups (Kruskal–Wallis Test)

Dependent Variable (Δ)	Chi-Square	df	p-value
Δ Hb	3.900	2	0.142
Δ Hct	3.634	2	0.163
Δ MCV	2.660	2	0.264
Δ MCH	5.183	2	0.075
Δ MCHC	3.743	2	0.154
Δ RDW	0.085	2	0.958

Mixed-Design General Linear Model Analysis

The mixed-design general linear model revealed statistically significant main effects of time for all assessed parameters, with effect sizes ranging from moderate to large (partial $\eta^2 = 0.387$ to 0.567). The largest effect of time was observed for RDW ($F = 420.820$, $p < 0.001$, partial $\eta^2 = 0.567$), followed by MCV ($F = 343.433$, $p < 0.001$, partial $\eta^2 = 0.516$). Significant main effects of diagnosis were observed for Hct, MCV, MCH, and MCHC, reflecting baseline differences among groups. Importantly, none of the Time $\times$ Diagnosis interaction effects reached statistical significance, confirming that the pattern of improvement over time was consistent across diagnostic categories. (Table 4) presents the complete results of the mixed-design general linear model analysis.

Table 4. Results of Mixed-Design General Linear Model Analysis of Erythrocyte Indices

Parameter	Effect	F-value	df1, df2	p-value	Partial η^2
Hct	Time	264.725	1, 322	<0.001	0.451
	Diagnosis	6.016	2, 322	0.003	0.036
	Time $\times$ Diagnosis	0.949	2, 322	0.388	0.006
MCV	Time	343.433	1, 322	<0.001	0.516
	Diagnosis	4.045	2, 322	0.018	0.025
	Time $\times$ Diagnosis	0.792	2, 322	0.454	0.005
MCH	Time	246.570	1, 322	<0.001	0.434
	Diagnosis	16.476	2, 322	<0.001	0.093
	Time $\times$ Diagnosis	2.483	2, 322	0.085	0.015
MCHC	Time	203.458	1, 322	<0.001	0.387
	Diagnosis	5.265	2, 322	0.006	0.032
	Time $\times$ Diagnosis	1.917	2, 322	0.149	0.012
RDW	Time	420.820	1, 322	<0.001	0.567
	Diagnosis	0.780	2, 322	0.459	0.005
	Time $\times$ Diagnosis	0.201	2, 322	0.818	0.001

Predictors of Change in Hemoglobin

Multiple linear regression analysis identified baseline hemoglobin as the strongest independent predictor of hemoglobin change, demonstrating a strong inverse association ($\beta = -0.863$, $p < 0.001$). This indicates that children with lower baseline hemoglobin experienced greater improvements following treatment. The presence of a risk factor was also independently associated with reduced hemoglobin response ($\beta = -0.110$, $p < 0.001$). The overall model was statistically significant and explained 76.7% of the variance in hemoglobin change ($R^2 = 0.767$). Age, gender, diagnostic category, baseline RDW, and thrombocytosis were not statistically significant predictors. Table 5 presents the complete results of the multiple linear regression analysis.

Table 5. Multiple Linear Regression Analysis of Predictors of Change in Hemoglobin (Δ Hb)

Independent Variable	B	Std. Error	Standardized β	t-value	p-value	Tolerance	VIF
Constant	11.334	0.505	—	22.438	<0.001	—	—
Age (years)	0.015	0.009	0.047	1.704	0.089	0.962	1.040
Gender	-0.003	0.069	-0.001	-0.041	0.967	0.918	1.090
Diagnosis	0.029	0.077	0.012	0.382	0.702	0.768	1.301
Baseline Hb	-0.955	0.031	-0.863	-30.669	<0.001	0.928	1.078
Baseline RDW	-0.006	0.017	-0.011	-0.384	0.701	0.924	1.082
Thrombocytosis (PLT)	0.000	0.000	0.024	0.875	0.382	0.966	1.035
Risk Factor (RiskF)	-0.151	0.041	-0.110	-3.681	<0.001	0.825	1.212

Discussion

The present study evaluated changes in erythrocyte indices following iron treatment in children with microcytic hypochromic anemia at Benghazi Medical Center, including those with iron deficiency anemia, G6PD deficiency with

concurrent iron deficiency, and hemophilia with iron deficiency. Our findings demonstrate significant improvements across all measured erythrocyte parameters and identify baseline hemoglobin as the principal predictor of treatment response magnitude. Our study demonstrated a significant increase in hemoglobin concentration from 9.35 ± 1.10 g/dL at baseline to 11.43 ± 0.61 g/dL following 4–5 months of iron therapy, representing a mean increase of 2.08 g/dL. This finding aligns with Hassan et al. (2025) [17], who reported a hemoglobin increase from 10.04 to 11.05 g/dL following 3 months of oral iron therapy at 6 mg/kg/day in Egyptian children with IDA. Our observation is further supported by Tchum et al. (2021) [18], who documented that children receiving iron-fortified micronutrient powder had significantly higher hemoglobin levels at endline compared to the placebo group in their Ghanaian cohort. The efficacy of iron intervention observed in our study is also consistent with the systematic review by Andersen et al. (2023) [11], who confirmed that oral iron supplementation effectively improves hemoglobin concentrations across various pediatric populations. However, the magnitude of hemoglobin increase in our study (2.08 g/dL) contrasts with the smaller improvement reported by Hassan et al. (2025) [17] (approximately 1.0 g/dL), which may be attributed to our longer treatment duration of 4–5 months compared to their 3-month protocol, as well as the lower baseline hemoglobin in our population, allowing for greater improvement capacity.

Our results showed a substantial improvement in MCV from 65.14 ± 5.86 fL to 82.64 ± 4.90 fL following treatment, corresponding to a 26.9% increase. This finding aligns with Motulo et al. (2023) [9], who reported that MCV levels increased significantly after therapy in Indonesian children with IDA and identified MCV as a reliable indicator for monitoring treatment success. Our observation of MCV normalization is further supported by Akbayram and Örkmez (2025) [19], who documented positive correlations between ferritin and MCV levels ($r = 0.33$, $p < 0.01$) in children with iron deficiency, reinforcing the relationship between iron repletion and MCV improvement. The post-treatment MCV values in our study are consistent with Khan et al. (2025) [20], who reported mean MCV values of 75.04 ± 2.06 fL in children with IDA, suggesting effective correction of the microcytic state. However, the percentage increase in MCV in our study (26.9%) contrasts with the smaller 7.3% increase reported by Motulo et al. (2023) [9]. This likely reflects the longer treatment duration in our study, allowing for more complete replacement of the microcytic red blood cell population.

Our relatively homogeneous baseline MCV values across diagnostic groups also differ from Saylık et al. (2026) [21], who noted that baseline MCV values were higher in their alternate-day therapy group compared to the daily group, possibly reflecting differences in patient selection criteria between studies. MCH in our study increased significantly from 21.09 ± 2.87 pg to 24.40 ± 2.28 pg following iron therapy. This finding aligns with Motulo et al. (2023) [9], who identified MCH as a diagnostic indicator for IDA with a cut-off of 25 pg, providing 100% sensitivity and 97.5% specificity; our post-treatment MCH values exceeding this threshold indicate successful correction of the hypochromic state. Our observation is consistent with Hassan et al. (2025) [17], who similarly reported significant increases in MCH following oral iron therapy in their Egyptian cohort. The utility of MCH as a marker for treatment monitoring in our study is supported by Guo et al. (2026) [8], who demonstrated that MCH serves as an independent predictor of iron deficiency status with an odds ratio of 0.63. Our finding of significant MCH elevation is further corroborated by Atia et al. (2021) [12], who documented significant elevations in MCH with both lactoferrin and oral iron therapy in obese children with IDA. The importance of ensuring an accurate baseline diagnosis in our study is underscored by Khan et al. (2025) [20], who reported differential MCHC values between IDA (31.59 ± 1.49 g/dL) and beta-thalassemia minor (34.44 ± 1.13 g/dL), highlighting that treatment response interpretation depends on correct initial classification.

RDW in our study decreased significantly from $16.31 \pm 2.06\%$ to $12.62 \pm 1.62\%$ following treatment, indicating normalization of red blood cell size heterogeneity. This finding aligns with Hassan et al. (2025) [17], who reported a significant decrease in RDW following oral iron therapy in children with IDA. Our observation of RDW reduction reflecting successful iron repletion is consistent with Guo et al. (2026) [8], who identified RDW as an independent predictor of iron deficiency with an odds ratio of 1.44. The inverse relationship between iron repletion and anisocytosis reduction observed in our study is supported by Akbayram and Örkmez (2025) [19], who documented negative correlations between ferritin and RDW levels ($r = -0.45$, $p < 0.01$). Our finding of consistent RDW improvement across diagnostic groups aligns with Saylık et al. (2026) [21], who noted that RDW values became similar between daily and alternate-day therapy groups by the end of treatment, suggesting that RDW normalization occurs regardless of treatment regimen. However, the baseline RDW values in our study contrast with Khan et al. (2025) [20], who reported markedly elevated RDW values of $19.39 \pm 1.71\%$ in beta-thalassemia minor compared to $14.32 \pm 1.64\%$ in IDA, emphasizing that RDW interpretation depends critically on the underlying diagnosis, and our population comprised primarily iron-deficient rather than thalassemic children.

Our study documented significant increases in MCHC from 28.15 ± 3.03 g/dL to 32.22 ± 1.98 g/dL and in hematocrit from $28.82 \pm 2.14\%$ to $33.48 \pm 1.10\%$ following iron therapy. This finding aligns with Hassan et al. (2025) [17], who reported significant increases in MCHC following oral iron treatment in Egyptian children. Our post-treatment MCHC values approaching normal ranges are consistent with Khan et al. (2025) [20], who documented MCHC values of 34.44 ± 1.13 g/dL in children without iron deficiency, suggesting effective correction of the hypochromic state in our cohort. The hematocrit improvement observed in our study is supported by Motulo et al. (2023) [9], who noted that increasing MCV after therapy was associated with increased levels in erythrocyte and hematocrit synthesis. However, our finding of significant hematocrit improvement contrasts with Hassan et al. (2025) [17], who reported no significant change in hematocrit in their cohort, which may reflect differences in treatment duration or baseline disease severity between the study populations.

Our study found no significant differences in the magnitude of hematological change among children with isolated IDA, G6PD deficiency with concurrent iron deficiency, and hemophilia with iron deficiency. This finding aligns with Tchum et al.

(2021) [18], who demonstrated that iron fortification effectively improved anemia status in a diverse Ghanaian population with varying genetic backgrounds. Our observation of comparable treatment responses across diagnostic categories is supported by the systematic review of Cadieux et al. (2025) [6], who confirmed the efficacy of iron interventions across heterogeneous pediatric populations with iron deficiency. The uniform response pattern in our study is also consistent with Leone et al. (2026) [10], who emphasized that adequate iron supplementation produces predictable hematological improvements regardless of concurrent conditions when iron deficiency is present. However, our finding partially contrasts with Tchum et al. (2023) [22], who observed complex interactions between iron supplementation, hemoglobinopathy status, and outcomes, noting that children with HbAS on iron supplementation had significantly more moderate anemia at endline compared to baseline.

Our observation also differs from Eliwa et al. (2025) [23], who documented multiple causes of refractory microcytic hypochromic anemia, including thalassemia traits and G6PD deficiency, suggesting that certain subgroups may demonstrate different response patterns, though our exclusion criteria and confirmation of concurrent iron deficiency may have selected for a more responsive population. Our regression analysis identified baseline hemoglobin as the strongest predictor of treatment response, with a standardized coefficient of -0.863 , indicating that children with lower baseline hemoglobin experienced greater improvements. This finding aligns with Leone et al. (2026) [10], who emphasized that more severe iron deficiency states provide greater capacity for improvement with adequate iron supplementation. Our observation is supported by Tchum et al. (2021) [18], who similarly observed that children with lower baseline hemoglobin showed more pronounced improvements following iron fortification. The relationship between baseline severity and treatment response magnitude in our study is consistent with the systematic review by Andersen et al. (2023) [11], who suggested that treatment effect sizes are influenced by baseline iron status severity. However, our identification of baseline hemoglobin as a reliable predictor contrasts with Hassan et al. (2025) [17], who reported no reliable hematologic predictors of iron response among the indices they examined. This discrepancy may be explained by our larger sample size providing greater statistical power to detect the relationship, as well as the wider range of baseline hemoglobin values in our population.

Our finding that diagnostic group did not significantly predict response also contrasts with Agrawal et al. (2026) [24], who identified multiple factors, including timing of administration and dietary factors, as significant predictors, suggesting that non-hematological variables may influence outcomes independently of baseline blood parameters. Our study showed that 59.1% of patients achieved good to excellent hemoglobin response (≥ 2.0 g/dL increase), while only 7.7% demonstrated partial response (< 1.0 g/dL increase). This finding aligns favorably with Hassan et al. (2025) [17], who reported that 52% of their patients achieved a good response (> 1 g/dL increase) and 48% showed a partial response, though our proportion of good responders was higher. Our favorable response rates are consistent with Agrawal et al. (2026) [24], who identified modifiable factors associated with treatment success, including morning administration, intake on an empty stomach, and co-administration with vitamin C sources, which may have been practiced by families in our cohort. The high proportion of successful treatment responses in our study is also supported by El-Hawy et al. (2021) [13], who demonstrated that various iron formulations, including iron bisglycinate, lactoferrin, and iron polymaltose complex, all produced significant hematological improvements. However, the low proportion of partial responders in our study (7.7%) contrasts with the higher rate reported by Hassan et al. (2025) [17] (48%), which may reflect our longer treatment duration, different baseline characteristics, or population-specific factors.

Our findings also differ from Eliwa et al. (2025) [23], who documented that 20 of 102 patients (19.6%) with refractory anemia were non-compliant or used medications improperly, suggesting that adherence factors significantly influence treatment outcomes and the lower partial response rate in our study may indicate better overall compliance in our population. Our treatment duration of 4–5 months contributed to the robust erythrocyte index improvements observed. This approach aligns with recommendations from Leone et al. (2026) [10], who emphasized the importance of adequate treatment duration to achieve both correction of anemia and repletion of body iron stores. Our treatment protocol duration is consistent with Eliwa et al. (2025) [23], who reported a median treatment duration of 4 months in their cohort of patients with refractory anemia. The comprehensive improvements across all erythrocyte indices in our study support Andersen et al. (2023) [11], who noted in their systematic review that longer treatment durations generally produce more robust hematological outcomes. However, the necessity of our extended treatment duration contrasts with Motulo et al. (2023) [9], who demonstrated significant improvements in Ret-He as early as day 8 of therapy, suggesting that early markers of response can be detected well before complete erythrocyte index normalization. Our approach also differs from Saylik et al. (2026) [21], who observed significant hemoglobin increases during the first month of therapy, indicating that while treatment response begins early, the comprehensive normalization of all erythrocyte indices observed in our study requires the extended duration we employed.

Limitations

This study has several limitations that warrant consideration. The retrospective design precluded standardization of iron formulations across patients, which contrasts with previous controlled comparisons of different iron formulations that demonstrated varying degrees of improvement with each preparation. The absence of serum ferritin measurements also limited our ability to assess iron store repletion independently of red cell parameters, as comprehensive iron profiling has demonstrated significant associations between ferritin levels and multiple erythrocyte indices. Furthermore, compliance could not be systematically assessed, limiting evaluation of the potential effect of non-compliance and improper

medication use on treatment outcomes. Additionally, factors that may influence treatment response, including timing of administration, dietary factors, and gastrointestinal side effects, were not systematically assessed in our retrospective analysis. Evaluation of these factors may have provided additional insights into the variability in treatment response observed.

Conclusion

This study demonstrates that oral iron therapy effectively improves erythrocyte indices in children with microcytic hypochromic anemia at Benghazi Medical Center, with comparable response patterns observed across patients with isolated IDA, G6PD deficiency with concurrent iron deficiency, and hemophilia with iron deficiency. Baseline hemoglobin concentration serves as the principal determinant of treatment response magnitude, with children exhibiting more severe anemia demonstrating greater improvements following therapy. These findings support the continued use of standard oral iron supplementation protocols in this population and highlight the importance of identifying and treating iron deficiency even in the presence of other hematological conditions.

Conflict of interest. Nil

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