

## COMPARISON OF RED CELL INDICES IN IRON DEFICIENCY ANEMIA AND BETA THALASSEMIA MINOR IN CHILDREN AGE 6 MONTHS TO 2 YEARS

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### ABSTRACT

**Background:** Iron deficiency anemia (IDA) and  $\beta$ -thalassemia trait ( $\beta$ -TT) are the most common microcytic hypochromic anemias in children. Distinguishing between these conditions is essential for accurate management and genetic counseling, especially in resource-limited settings where advanced diagnostic testing may not be readily available. Red cell indices provide a simple and cost-effective method for differentiating between red blood cells. **Objective:** To determine and compare mean red cell indices in children aged 6 months to 2 years with iron deficiency anemia and  $\beta$ -thalassemia minor. **Study Design:** Cross-sectional study. **Setting:** Department of Pediatrics, Unit-C of Khyber Teaching Hospital, Peshawar, Pakistan. **Duration of Study:** 21-October-2024 to 21-April-2025. **Methods:** A total of 60 children aged 6 months to 2 years were enrolled, including 30 with IDA and 30 with  $\beta$ -TT. Children were classified as IDA if hemoglobin was  $<13$  g/dL with serum ferritin  $<10$  ng/mL, and as  $\beta$ -TT if hemoglobin was  $<13$  g/dL with HbA2  $>7\%$ . Venous blood samples were analyzed for hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and red blood cell (RBC) count. Statistical analysis was performed using SPSS (version [insert]), with  $p < 0.05$  considered significant. **Results:** The mean MCV was significantly higher in IDA ( $75.04 \pm 2.06$  fL) compared to  $\beta$ -TT ( $72.89 \pm 1.77$  fL;  $p < 0.0001$ ). MCHC was lower in IDA ( $31.59 \pm 1.49$  g/dL) than in  $\beta$ -TT ( $34.44 \pm 1.13$  g/dL;  $p < 0.0001$ ). RDW was markedly elevated in  $\beta$ -TT ( $19.39 \pm 1.71\%$ ) compared to IDA ( $14.32 \pm 1.64\%$ ;  $p < 0.0001$ ). RBC counts were higher in  $\beta$ -TT ( $4.90 \pm 0.98 \times 10^6/\mu\text{L}$ ) compared to IDA ( $4.06 \pm 0.62 \times 10^6/\mu\text{L}$ ;  $p < 0.0001$ ). **Conclusion:** Red cell indices, including MCV, MCHC, RDW, and RBC count, demonstrated significant differences between IDA and  $\beta$ -TT. These findings support their role as simple, reliable, and cost-effective discriminators in differentiating between the two conditions in pediatric populations.

**Keywords:** Iron Deficiency Anemia, Beta-Thalassemia Minor, Red Cell Indices, Pediatric Hematology, Microcytic Anemia

### INTRODUCTION

Anemia is characterized by a decrease in hemoglobin concentration, leading to reduced oxygen transport and distribution to bodily tissues (1, 2). The average rate of anemia among children worldwide is 43% (3, 4). Anemia has been identified as an important health problem in developing nations, with estimates suggesting that approximately 2 billion individuals are affected by anemia worldwide. Anemia has numerous underlying causes that are preventable. Microcytic hypochromic anaemia represents a prevalent haematological abnormality commonly encountered in clinical practice (5, 6). The two main reasons for microcytic hypochromic anemia are actually iron deficiency anemia (IDA) and thalassemia trait. Clinically, both of these diseases can present challenges in distinctiveness. IDA in young children is identified as a significant health issue and is the most common type of micronutrient deficiency worldwide (7).

Thalassemias represent the most common etiology of hypochromic microcytic anemia, which is caused by diminished production of the globin chain of hemoglobin. Thalassemia symbolizes a quantitative deficiency in the synthesis of hemoglobin. This differs from hemoglobinopathies, including sickle cell disease, which are defined by both structural and qualitative defects in hemoglobin. Beta-thalassemia has been characterized by an inherited mutation in the beta-globin gene, resulting in a decreased production of the beta-globin chain of hemoglobin. More than 200 distinct mutations that result in thalassemia have been identified throughout the beta-globin gene, leading to significant variability in both genotype and phenotype associated with the disease (8-10). The various types of beta-thalassemia are categorized depending on specific laboratory and

clinical results. Beta-thalassemia minor, also known as a carrier state or trait, refers to a heterozygous condition typically associated with an asymptomatic presentation and mild anemia. The overall incidence of beta-thalassemia demonstrates regional variation, with the highest frequencies noted in the Mediterranean, the Middle East, and Southeast Asia. Approximately 68,000 children are diagnosed with beta-thalassemia. The estimated prevalence ranges from 80 to 90 million carriers, which makes up approximately 1.5% of the worldwide population (11). The carrier prevalence reported in Greek and Turkish individuals of Cyprus is as high as 15% (12). In IDA, MCV was  $76.48 \pm 4.85$  fL, and MCV was  $71.95 \pm 6.76$  fL in thalassemia minor (11).

The rationale of this study is to determine the mean red cell indices in iron deficiency anemia and Beta thalassemia minor and to compare them in children aged 6 months to 2 years, so that both prevalent diseases can be differentiated with a simple test that is easily available in all hospitals. An accurate Diagnosis will help in the prompt management of patients.

### METHODOLOGY

The study employed a cross-sectional design, conducted at the Department of Pediatrics, Unit C, of Khyber Teaching Hospital, Peshawar, after obtaining ethical approval from our hospital. The study was conducted from October 21, 2024, to April 21, 2025. We utilized non-probability consecutive sampling to enroll 60 patients with 30 cases each of iron deficiency anemia and beta-thalassemia minor. The sample was selected based on the mean corpuscular volume (MCV) from a previous study,  $71.95 \pm 6.76$  fL (13), with a

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power of 80% and a 95% confidence interval. Children aged between 6 months and 2 years presenting with hemoglobin levels < 13 g/dL were selected, while those with recent blood transfusions or intravenous iron therapy within four weeks were excluded from participation.

Diagnostic confirmation followed strict operational definitions: iron deficiency anemia cases were defined by serum ferritin levels of 10 ng/mL or less. In contrast, beta-thalassemia minor cases were characterized by hemoglobin electrophoresis results with HbA2 exceeding 7%. The researcher collected 2 mL of venous blood in EDTA tubes from each participant under aseptic conditions. Complete blood counts and red cell indices were analyzed on the same day using a Coulter Automated Cell Counter (LH500). The study examined five key parameters: hemoglobin concentration (g/dL), mean corpuscular volume (μL), mean corpuscular hemoglobin concentration (g/dL), red cell distribution width (%), and red blood cell count ( $\times 10^6/\mu\text{L}$ ).

SPSS 24 was used for analysis. Mean and SD were calculated for age, duration of anemia, and red cell indices. Gender, socioeconomic status, education of parents, father's professional status, residence, and family history of thalassemia were noted using frequency and percentages. Red cell indices were compared between individuals with IDF and those with beta-thalassemia using an independent t-test. Age, gender, and duration of anemia were stratified with red cell indices using the test above. P-value was significant if  $\leq 0.05$ .

## RESULTS

Our study included 60 patients, divided into two groups: Group A (iron deficiency anemia) and Group B (thalassemia minor), with 30 patients in each group. The mean age in Group A was  $14.67 \pm 6.12$  months, and in Group B, it averaged  $14.50 \pm 6.19$  months. The

duration of anemia in Group A was  $13.67 \pm 6.12$  months, and in Group B, it was  $13.50 \pm 6.19$  months.

Table 1 presents the demographic characteristics of the patients. Laboratory analysis demonstrated that hemoglobin levels were  $8.88 \pm 1.06$  g/dL in Group A versus  $8.69 \pm 0.66$  g/dL in Group B ( $p=0.39$ ). Mean corpuscular volume (MCV) showed statistically significant differences with Group A at  $75.04 \pm 2.06$  fL and Group B at  $72.89 \pm 1.76$  fL ( $p<0.0001$ ). Mean corpuscular hemoglobin concentration (MCHC) values were  $31.59 \pm 1.49$  g/dL for Group A and  $34.44 \pm 1.13$  g/dL for Group B ( $p<0.0001$ ). Red cell distribution width (RDW) was substantially different between groups, measuring  $14.32 \pm 1.64\%$  in Group A and  $19.39 \pm 1.71\%$  in Group B ( $p<0.0001$ ). Red blood cell (RBC) counts were  $4.06 \pm 0.62 \times 10^6/\mu\text{L}$  in Group A and  $4.90 \pm 0.98 \times 10^6/\mu\text{L}$  in Group B ( $p<0.0001$ ) (Table 2). Tables 3, 4, and 5 present stratifications.

**Table 1: Demographics**

Demographics		n	%
Gender	Male	33	55.0%
	Female	27	45.0%
Residence	Urban	35	58.3%
	Rural	25	41.7%
Father's professional status	Employed	27	45.0%
	Unemployed	33	55.0%
Father's educational status	Educated	22	36.7%
	Uneducated	38	63.3%
Father's socioeconomic status	Low	24	40.0%
	Poor	30	50.0%
	High	6	10.0%
Family history of thalassemia	Yes	13	21.7%
	No	47	78.3%

**Table 2: Red cell indices parameters**

Parameters	Groups	N	Mean	Std. Deviation	P value
Hemoglobin (g/dl)	Group A (Iron deficiency anemia)	30	8.8863	1.06344	0.39
	Group B (Thalassemia minor)	30	8.6900	.66791	
MCV (fL)	Group A (Iron deficiency anemia)	30	75.0477	2.06383	0.0001
	Group B (Thalassemia minor)	30	72.8983	1.76649	
MCHC (g/dl)	Group A (Iron deficiency anemia)	30	31.5957	1.49308	0.0001
	Group B (Thalassemia minor)	30	34.4473	1.13306	
RDW (%)	Group A (Iron deficiency anemia)	30	14.3257	1.64952	0.0001
	Group B (Thalassemia minor)	30	19.3954	1.71256	
RBC count $\times 10^6/\mu\text{L}$	Group A (Iron deficiency anemia)	30	4.0637	.62101	0.0001
	Group B (Thalassemia minor)	30	4.9079	.98019	

**Table 3: Stratification of red cell indices in both groups with duration of anemia**

Duration of anemia (Months)	Groups	N	Mean	Std. Deviation	Std. Error Mean
5 to 15	Hemoglobin (g/dl)	Group A	8.7394	1.08170	0.75
		Group B	8.6412	.62409	
	MCV (fL)	Group A	74.4631	2.23779	0.07
		Group B	73.2565	1.50422	
	MCHC (g/dl)	Group A	31.5675	1.57924	0.0001
		Group B	34.3300	.97329	
	RDW (%)	Group A	13.7156	1.71825	0.0001
		Group B	19.2905	1.66537	
	RBC count $\times 10^6/\mu\text{L}$	Group A	4.0550	.63806	0.007
		Group B	4.9002	.98909	
	Hemoglobin (g/dl)	Group A	9.0543	1.05630	0.40
		Group B	8.6412	.62409	

	MCV (fL)	Group B	13	8.7538	.74228	0.0001
		Group A	14	75.7157	1.68000	
	MCHC (g/dl)	Group B	13	72.4300	2.02606	0.0001
		Group A	14	31.6279	1.44681	
	RDW (%)	Group B	13	34.6008	1.33960	0.0001
		Group A	14	15.0229	1.29677	
	RBC count x10 <sup>6</sup> μL	Group B	13	19.5327	1.83132	0.01
		Group A	14	4.0737	.62479	
		Group B	13	4.9180	1.00859	

**Table 4: Stratification of red cell indices in both groups by gender**

Gender		Groups	N	Mean	Std. Deviation	P value
Male	Hemoglobin (g/dl)	Group A	16	8.6769	1.15980	0.86
		Group B	17	8.6200	.65151	
	MCV (fL)	Group A	16	75.8163	1.94108	0.0001
		Group B	17	72.8718	1.96926	
	MCHC (g/dl)	Group A	16	31.1263	1.49229	0.0001
		Group B	17	34.5729	.98375	
	RDW (%)	Group A	16	14.6556	1.62646	0.0001
		Group B	17	19.5647	1.68762	
Female	RBC count x10 <sup>6</sup> μL	Group A	16	4.1291	.65314	0.03
		Group B	17	4.7900	1.01880	
	Hemoglobin (g/dl)	Group A	14	9.1257	.92468	0.29
		Group B	13	8.7815	.70435	
	MCV (fL)	Group A	14	74.1693	1.89639	0.07
		Group B	13	72.9331	1.53889	
	MCHC (g/dl)	Group A	14	32.1321	1.34995	0.0001
		Group B	13	34.2831	1.32690	
	RDW (%)	Group A	14	13.9486	1.65235	0.0001
		Group B	13	19.1741	1.78791	
	RBC count x10 <sup>6</sup> μL	Group A	14	3.9890	.59733	0.002
		Group B	13	5.0622	.94467	

**Table 5: Stratification of red cell indices in both groups with duration of age**

Age groups (Months)		Groups	N	Mean	Std. Deviation	P value
6 to 12	Hemoglobin (g/dl)	Group A	13	8.6269	1.08942	0.97
		Group B	13	8.6392	.57196	
	MCV (fL)	Group A	13	74.4600	2.33278	0.08
		Group B	13	73.1046	1.46953	
	MCHC (g/dl)	Group A	13	31.5477	1.75056	0.0001
		Group B	13	34.1892	1.05132	
	RDW (%)	Group A	13	13.6000	1.68690	0.0001
		Group B	13	19.1773	1.81208	
13 to 18	RBC count x10 <sup>6</sup> μL	Group A	13	3.9330	.61876	0.009
		Group B	13	4.9111	1.08363	
	Hemoglobin (g/dl)	Group A	6	9.2550	.71113	0.20
		Group B	8	8.6900	.83469	
	MCV (fL)	Group A	6	75.0467	1.97291	0.08
		Group B	8	73.1063	1.82772	
	MCHC (g/dl)	Group A	6	32.2633	.91938	0.0001
		Group B	8	34.3075	.66461	
19 to 24	RDW (%)	Group A	6	14.3700	1.42039	0.0001
		Group B	8	20.0419	1.42999	
	RBC count x10 <sup>6</sup> μL	Group A	6	4.2470	.63044	0.06
		Group B	8	4.9847	.69654	
	Hemoglobin (g/dl)	Group A	11	8.9918	1.19219	0.62
		Group B	9	8.7633	.71172	
	MCV (fL)	Group A	11	75.7427	1.70158	0.001
		Group B	9	72.4156	2.18514	
	MCHC (g/dl)	Group A	11	31.2882	1.41086	0.0001
		Group B	9	34.9444	1.48448	
	RDW (%)	Group A	11	15.1591	1.42033	0.0001
		Group B	9	15.1591	1.42033	

RBC count $\times 10^6/\mu\text{L}$	Group B	9	19.1359	1.82918	0.09
	Group A	11	4.1182	.64391	
	Group B	9	4.8352	1.13358	

## DISCUSSION

The findings of this study provide valuable insights into the hematological differences between iron deficiency anemia (IDA) and beta-thalassemia minor ( $\beta\text{TT}$ ) in pediatric populations.

Our study found that hemoglobin levels were slightly higher in IDA ( $8.88 \pm 1.06$  g/dL) compared to  $\beta\text{TT}$  ( $8.69 \pm 0.66$  g/dL), although this difference was not statistically significant ( $p = 0.39$ ). This aligns with the findings of Indrasari et al., who reported hemoglobin levels of  $10.96 \pm 2.13$  g/dL in IDA and  $8.53 \pm 1.62$  g/dL in  $\beta\text{TT}$ , suggesting that  $\beta\text{TT}$  tends to present with slightly lower hemoglobin concentrations (13). Similarly, Arshad et al observed comparable hemoglobin values ( $7.2 \pm 0.7$  g/dL in IDA vs.  $7.3 \pm 0.8$  g/dL in  $\beta\text{TT}$ ), reinforcing the notion that hemoglobin alone is not a reliable discriminator between these two conditions (14).

A striking difference was observed in our study in mean corpuscular volume (MCV), where IDA patients showed notably higher values ( $75.04 \pm 2.06$  fL) compared to  $\beta\text{TT}$  ( $72.89 \pm 1.76$  fL;  $p < 0.0001$ ). This finding is consistent with Ferrara et al, who reported MCV values of  $63.9 \pm 2.5$  fL in IDA versus  $72.1 \pm 2.0$  fL in  $\beta\text{TT}$  (15). Odhwani et al showed that MCV was  $70.0 \pm 5.52$  fL in IDA and  $66.0 \pm 4.4$  fL in  $\beta\text{TT}$ .<sup>16</sup> The consistently lower MCV in  $\beta\text{TT}$  across studies can be attributed to the inherent defect in hemoglobin synthesis, resulting in smaller red cells. In contrast, in IDA, microcytosis develops more gradually as iron stores are depleted (16).

The mean corpuscular hemoglobin concentration (MCHC) demonstrated a significant distinction between groups in our study, with  $\beta\text{TT}$  showing higher values ( $34.44 \pm 1.13$  g/dL) compared to IDA ( $31.59 \pm 1.49$  g/dL;  $p < 0.0001$ ). This finding contrasts with Budania et al., who reported MCHC values of  $31.2 \pm 0.4$  g/dL in  $\beta\text{TT}$  and  $31.1 \pm 2.7$  g/dL in IDA, showing less pronounced differences (17). However, our findings can be compared to those of Arshad et al., as they showed that MCHC was  $32.3 \pm 2.1$  g/dL in IDA and  $36.1 \pm 3.4$  g/dL in  $\beta\text{TT}$  (14). Red cell distribution width (RDW) with  $\beta\text{TT}$  showed markedly higher values ( $19.39 \pm 1.71\%$ ) compared to IDA ( $14.32 \pm 1.64\%$ ;  $p < 0.0001$ ). This finding contradicts several previous studies, including Ferrara et al., who found no substantial difference in RDW between groups ( $17.2 \pm 2.1\%$  in  $\beta\text{TT}$  vs.  $18.2 \pm 2.1\%$  in IDA), and Odhwani et al., who reported RDW at  $17.7 \pm 1.7\%$  in IDA versus  $16.9 \pm 1.27\%$  in  $\beta\text{TT}$ .<sup>16</sup> However, our results are affirmed by Indrasari et al, who observed RDW  $14.6 \pm 3.28\%$  in IDA and  $20.15 \pm 4.77\%$  in  $\beta\text{TT}$  (13). Arshad et al.'s findings regarding RDW also support our results. They reported RDW levels of  $15.4 \pm 3.31$  in the  $\beta\text{TT}$  group and  $13.8 \pm 2.7$  in the IDA group (14).

The red blood cell (RBC) count in our study was higher in  $\beta\text{TT}$  ( $4.90 \pm 0.98 \times 10^6/\mu\text{L}$ ) compared to IDA ( $4.06 \pm 0.62 \times 10^6/\mu\text{L}$ ,  $p < 0.0001$ ). This finding is strongly supported by multiple studies, including Arshad et al., who reported RBC counts of  $3.5 \pm 0.5 \times 10^6/\mu\text{L}$  in IDA versus  $5.80 \pm 0.7 \times 10^6/\mu\text{L}$  in  $\beta\text{TT}$  (14). Odhwani et al with  $4.5 \pm 0.35 \times 10^6/\mu\text{L}$  in IDA versus  $5.3 \pm 0.33 \times 10^6/\mu\text{L}$  in  $\beta\text{TT}$  (16). The elevated RBC count in  $\beta\text{TT}$  represents a compensatory mechanism for chronic anemia, whereas in IDA, the bone marrow's ability to produce red cells is impaired by iron deficiency.

## CONCLUSION

We determined mean red indices for IDF and  $\beta\text{TT}$  pediatric patients. We conclude that upon comparison, MCV, MCHC, RDW, and RBC count showed notable differences between the two conditions, which validates their use as simple and reliable discriminators between IDF

and  $\beta\text{TT}$ .

## DECLARATIONS

### Data Availability Statement

All data generated or analysed during the study are included in the manuscript.

### Ethics approval and consent to participate

Approved by the department Concerned. (IRB)

### Consent for publication

Approved

### Funding

Not applicable

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTION

### GUL KHAN (Postgraduate Resident)

Conception of Study, Data Collection, Manuscript drafting, Review of manuscript, and final approval of manuscript.

### ABDUL KHALIQ (Associate Professor)

Supervision, Critical input, and final approval of manuscript

### MUHAMMAD KASHIF (Assistant Professor)

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