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Original Article

Discriminatory Ability of Hemoglobin and Red Cell Indices in Differentiating Iron Deficiency Anemia and Beta-Thalassemia Trait in a Rural Population of Sindh

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ABSTRACT

Anemia is a significant health issue in Pakistan, especially in the rural regions where nutritional deficiencies are prevalent. In resource-constrained environments such as rural Sindh, new diagnostic tests are very expensive. Thus, red cell indices that are readily available and easy to use can provide a viable and cost-effective method of distinguishing the types of anemia. **Objective:** To assess the most appropriate diagnostic indicators of hemoglobin and red cell distribution indices to distinguish the type of anemia in patients of the rural population of Sindh. **Methods:** A Cross-sectional descriptive study was conducted at Indus Medical College and Hospital, the University of Modern Sciences, Tando Muhammad Khan, Sindh, Pakistan. 254 patients who were suspected of having anemia were enrolled. The parameters of complete blood count, such as hemoglobin, RBC, MCV, MCH, MCHC, and RDW, were assessed using an automated hematology analyzer. **Results:** Among 254 participants, 44.90% had normal hemoglobin levels, 20.50% had iron deficiency anemia (IDA), 5.10% had beta thalassemia trait (BTT), 26.40% other etiologies of anemia, and 3.10% severe anemia. The average level of hemoglobin in males (12.26 ± 2.18 g/dL) was much greater than in females (10.58 ± 2.04 g/dL, $p<0.001$). The discriminatory index of the RBC showed a very good ability to distinguish between IDA and BTT, with MCV, MCH, and RDWI showing significant differences. **Conclusion:** The red cell distribution indices are accurate, inexpensive, and easily accessible parameters that can be used to distinguish between the various types of anemia in resource-constrained rural areas.

INTRODUCTION

Anemia remains a significant public health challenge globally, with particular prevalence in developing countries, including Pakistan [1]. According to the World Health Organization (WHO), approximately 2 billion people worldwide are anemic, with iron deficiency being the most common cause. In Pakistan, the prevalence of anemia exceeds 45% in certain populations, with higher rates in rural communities where nutritional deficiencies are endemic [2]. Iron deficiency anemia (IDA) and beta thalassemia trait (βTT) are among the most common

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causes of microcytic hypochromic anemia, both presenting with similar morphological features on peripheral blood examination [3]. This diagnostic overlap poses a clinical dilemma with substantial ramifications for clinical management and counseling, especially in populations where there is high carrier prevalence for hemoglobinopathies [4]. Limited access to advanced diagnostic facilities, high prevalence of nutritional deficiencies, high carrier rates of thalassemia mutations, poor screening and early detection programs, and poor health infrastructure are some of the major problems associated with hematological disorders in rural Sindh, which are common in developing Pakistan [5]. Traditionally, the diagnosis of IDA from BTT has been based on time-consuming and costly methods, such as hemoglobin electrophoresis, serum iron measurement, and measurement of ferritin and TIBC [6]. These modalities are not always available in rural healthcare, causing delays in diagnoses and inappropriate therapeutic options [7]. Derived red cell indices (DRCs) have proven to be valuable in the diagnosis of anemia in recent advances in laboratory practice in hematology [8]. Modern hematology analyzers automatically calculate parameters like red cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width index (RDWI) with very little extra expense [9]. These are compound indices that contain information on several hematological variables and are able to discriminate among the different forms of anaemia [10].

The rationale of the study was that the prevalence of anemia in rural Sindh is high, and diagnostic infrastructure is not available, so it is necessary to develop a practical and inexpensive diagnostic protocol with available red cell indices. The study aimed to assess the discriminatory power of haemoglobin and indices of red cells for the type of anaemia in rural Sindh.

METHODS

This was a cross-sectional descriptive study conducted between 1st Feb 2025 - 30th November 2025 among patients attending Indus Medical College and Hospital, The University of Modern Sciences, Tando Muhammad Khan, Sindh, Pakistan. The study was approved by the Institutional Ethical Review Committee, Indus Medical College, TMK, via letter no. TUMS/ERC/2025/027, dated 28th January 2025. Informed written consent was obtained from all the participants or their guardians (for pediatric patients). Participant confidentiality and data privacy were maintained throughout the study. The study population was drawn from diverse geographic locations across rural Sindh to ensure adequate representation of the target population. A total of 254 eligible patients were recruited consecutively using a non-probability sampling approach. Patients of either gender aged 1 to 95 years, presenting with clinical suspicion of anemia, having Hemoglobin levels ranging from 2.6 to 18.2 g/dL, and those patients who are willing to provide informed consent. Patients who received blood transfusion for 3 months, having active infection or inflammatory conditions, Malignancy, or chronic systemic disease affecting hematological parameters, Incomplete laboratory data, and Refusal to provide informed consent. Venous blood samples (3-5 mL) were collected in EDTA tubes under aseptic conditions by a trained phlebotomist. Complete blood count (CBC) and red cell indices were determined using an automated hematology analyzer (Sysmex KX-21) at the central pathology laboratory. Laboratory personnel were not specifically blinded to the clinical information or study classification during laboratory processing; therefore, the possibility of measurement or observer-related bias cannot be completely excluded. The parameters analyzed included: Hemoglobin (Hb) in g/dL, red blood cell count (RBC) in 10^12/L, Hematocrit/Packed Cell Volume (PCV) in %, Mean Corpuscular Volume (MCV) in fL, Mean Corpuscular Hemoglobin (MCH) in pg, Mean Corpuscular Hemoglobin Concentration (MCHC) in g/dL, and Red Cell Distribution Width (RDW) in %. Red Cell Distribution Width Index (RDWI) was calculated using the formula: RDWI=MCV×RDW/RBC. Patients were classified into anemia categories based on hemoglobin levels and red cell indices. IDA and BTT were considered presumptive classifications based on CBC-derived parameters, as confirmatory testing with serum ferritin and hemoglobin electrophoresis was not performed. Accordingly, the evaluated red cell indices were assessed as screening/discriminatory parameters rather than against a laboratory-confirmed gold-standard diagnosis. Because the classification criteria incorporated CBC-derived parameters, subsequent comparisons of these same indices between the presumptive IDA and BTT groups were considered exploratory and should not be interpreted as independent diagnostic validation. Anemia was defined according to World Health Organization (WHO) age- and sex-specific criteria: Children (5–11 years): Hb <11.5 g/dL, Adolescents (12–14 years): Hb <12.0 g/dL, adult females: Hb <12.0 g/dL, adult males: Hb <13.0 g/dL, and Elderly: Hb <12.0 g/Dl. Hemoglobin ≥10 g/dL with MCV <80 fL and RBC count ≥5.0 $\times 10^{12}$/L, suggestive of thalassemic erythropoiesis. Hemoglobin below the WHO cut-off for age and gender with MCV <80 fL and RBC count <4.5 $\times 10^{12}$/L, suggestive of iron deficiency.

Data were analyzed using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA). Quantitative variables were expressed as mean ± standard deviation (SD) with range, while qualitative variables were presented as frequencies

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and percentages. Descriptive statistics were calculated for all demographic and hematological parameters. Group comparisons were performed using an independent samples t-test for continuous variables between two groups, One-way ANOVA for comparison across multiple groups, and Chi-square test for categorical variables. Statistical significance was defined as p-value <0.05. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of individual indices were calculated using 2×2 contingency tables.

RESULTS

The majority of the participants were male compared to the females. The age distribution identified that the highest proportion was that of the adult (20-59 years), followed by adolescents, the pediatric, and the elderly. The mean age of the participants was 35.06 ± 9.75 years, which reveals that there was a considerable age variation, as various subjects had varied clinical presentations (Table 1).

Table 1: Sociodemographic Characteristics of the Participants

Demographic Variable	Frequency (%) (n= 254)
Gender Distribution	
Male	149 (58.70%)
Female	105 (41.30%)
Age Distribution	
Pediatric (1-12 years)	28 (11.0%)
Adolescent (13-19 years)	45 (17.70%)
Adult (20-59 years)	156 (61.40%)
Elderly (60+ years)	25 (9.80%)
Mean Age (Years)	35.06 ± 19.75

In 254 individuals, 44.9% had normal hemoglobin, and the rest, 55.1%, were anemic to some extent. Mild anemia was noted in 24.4%, having a mean Hb of 10.87 ± 0.59/dl, and moderate anemia was detected in 27.6%, having a mean of 8.93 ± 0.92/dl. Severe anemia with the least mean Hb value of 5.63 ± 1.14g/dl was seen in 3.1% of participants. The mean hemoglobin of the study population was 11.50-2.27g/dl, which is quite high and shows that anemia is a significant problem in this rural cohort. This prevalence indicates the great percentage of moderate-to-mild anemia, which may be due to nutritional deficiencies, chronic diseases, or socioeconomic difficulties in the study area (Table 2).

Table 2: Distribution and Categories of Hemoglobin

Hemoglobin Category	Frequency (%)	Mean ± SD (g/dL)
Normal (≥12.0)	114 (44.90%)	13.92 ± 1.94
Mild Anemia (10.0-11.9)	62 (24.40%)	10.87 ± 0.59
Moderate Anemia (7.0-9.9)	70 (27.60%)	8.931 ± 0.92
Severe Anemia (<7.0)	8 (3.10%)	5.632 ± 1.14
Total	254 (100.0%)	11.50 ± 2.27

Males had significantly higher hemoglobin, RBC count, PCV, MCH, and MCHC than females (all $p < 0.05$). MCV was numerically higher in males but was not statistically significant ($p = 0.078$). Thus, most red cell parameters differed by sex, except MCV (Table 3).

Table 3: Gender-based Comparison of Hematological Parameters

Parameter	Male (Mean ± SD) (n=149)	Female (Mean ± SD) (n=105)	p-value <0.05
Hemoglobin (g/dL)	12.26 ± 2.18	10.58 ± 2.04	<0.001*
RBC (10^12/L)	4.71 ± 0.89	4.25 ± 0.79	<0.001*
PCV (%)	36.76 ± 6.10	33.58 ± 6.29	<0.001*
MCV (fL)	80.04 ± 9.84	77.97 ± 8.82	0.078
MCH (pg)	26.35 ± 3.64	25.09 ± 3.52	0.007*
MCHC (g/dL)	32.86 ± 1.02	32.09 ± 1.39	<0.001*

(MCV, MCH, RDW, and RBC count) between individuals with iron deficiency anemia (IDA) and those with beta-thalassemia trait (BTT). The image distinctly illustrates hematological patterns that differentiate the two diseases. Patients with iron deficiency anemia demonstrated markedly reduced MCV and MCH values, indicating microcytosis and hypochromia generally resulting from inadequate iron supply for hemoglobin production. Conversely, those with BTT exhibited significantly elevated RBC counts despite reduced MCV levels, a distinguishing trait of thalassemic erythropoiesis. RDW levels were significantly increased in IDA, showing substantial anisocytosis resulting from a heterogeneous population of small and normal-sized RBCs. In distinction, RDW exhibited relative stability in BTT, corroborating the characteristic uniform microcytosis of the condition. These visual distinctions demonstrate differences in red cell indices between the presumptively classified IDA and BTT groups. particularly in low-resource environments where hemoglobin electrophoresis and sophisticated iron tests might not be easily accessible (Figure 1).

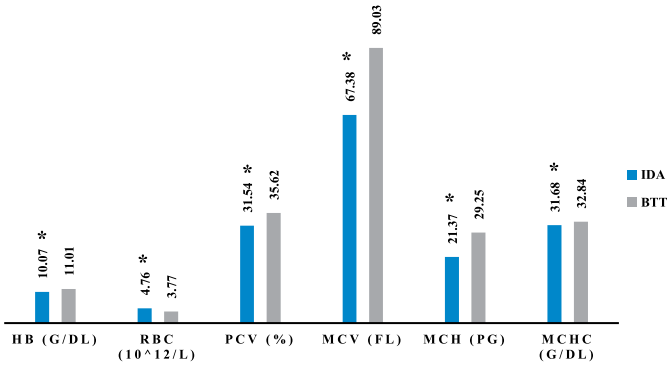


Figure 1: Comparison of Red Cell Indices in IDA vs BTT *<0.05 HB=Hemoglobin, RBC=Red blood cells, PCV=Packed cell volume, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Hemoglobin, MCHC=Mean Corpuscular Hemoglobin Concentration

Compare the discriminatory ability of different red cell indices in the differentiation of iron deficiency anemia (IDA)

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versus beta-thalassemia trait (BTT), the two diseases that often intersect hematologically. The MCV threshold of < 75 fL had a high sensitivity (92.3%), and specificity was 100%; hence, it was a favorable marker in the differentiation of BTT and IDA. Similarly, an RDWI value of less than 15 demonstrated a high observed discriminatory performance in this study, with 100% specificity and 88.5% sensitivity (Table 4).

Table 4: Discriminatory abilities of Red Cell Indices in Differentiating IDA from BTT

Red Cell Index	Sensitivity (95% CI)	Specificity (95% CI)	PPV (%)	NPV (%)
MCV <75 fL	92.30% (81.8–97.9)	100.0% (75.3–100)	100.0 %	96.30%
MCH <23 pg	88.50% (76.6–95.6)	92.30% (64.0–99.8)	95.80%	82.10%
RDW >14%	76.90% (63.2–87.5)	69.20% (38.6–90.9)	76.20%	69.80%
RDWI <15	88.50% (76.6–95.6)	100.0% (75.3–100)	100.0%	92.40%
MCV <75 AND RBC >4.0	84.60% (71.9–93.1)	100.0% (75.3–100)	100.0%	90.10%

An MCH value of less than 23pg provided strong diagnostic power with a sensitivity of 88.50% and specificity of 92.30%. MCH value of greater than 14% showed moderate power in diagnosis (sensitivity 76.90% and specificity 69.20%). The composite criterion of MCV 75fL and RBC 4.0 had a good accuracy of 84.60% sensitivity and 100% specificity, which strengthens its use in screening as part of routine. In short, the red cell indices tested were very clinically relevant, especially those of MCV, RDWI, and the composite parameters, which allowed one to differentiate IDA and BTT, without reliance on advanced laboratory tests. Among the evaluated indices, MCV <75 fL and RDWI <15 demonstrated the highest observed discriminatory performance, with a specificity of 100% in this study.The composite criterion (MCV <75 fL combined with RBC >4.0 $\times 10^{12}$/L) also showed high specificity, reinforcing the value of combined indices in screening settings. Red cell indices varied across different age groups, with more pronounced abnormalities observed in pediatric and young adult populations. Comparison of hemoglobin, MCV, RBC count, and RDW in four age groups. The mean hemoglobin was highest in the adult age group (20–59 years), with the pediatric age group recording the lowest value. The values of MCV were gradually increasing with age, with 75.32 fL in the pediatric group and about 79–80 fL in the elderly age groups, showing maturation and stability of the erythrocyte morphology. The counts of RBC were also more or less constant in all the age groups, with slight changes but no significant differences. RDW was slightly high in the pediatric and the elderly populations, probably as a result of the variability of red cell size that is attributable to nutritional deficiencies or chronic diseases prevalent in these age groups (Table 5).

Table 5: Hematological Parameters Across Age Groups

Age Group	(n)	Mean Hb (g/dL) (Mean ± SD)	Mean MCV (fL) (Mean ± SD)	Mean RBC (10^12/L) (Mean ± SD)	Mean RDW (%) (Mean ± SD)
1-12 years (Pediatric)	28	10.21 ± 1.84	75.32 ± 8.15	4.481 ± 0.92	13.45 ± 1.92
13-19 years (Adolescent)	45	11.03 ± 2.15	77.94 ± 9.28	4.521 ± 0.86	13.62 ± 1.78
20-59 years (Adult)	156	11.84 ± 2.19	79.85 ± 9.31	4.542 ± 0.84	13.51 ± 1.85
60+ years (Elderly)	25	11.28 ± 2.41	79.76 ± 10.4	4.380 ± 0.79	13.89 ± 2.14
Total	254	11.50 ± 2.27	79.08 ± 9.40	4.50 ± 0.88	13.56 ± 1.89

DISCUSSION

This study looked at how well hemoglobin and red cell distribution measures could tell the difference between different types of anemia in 254 patients from rural Sindh. Several important clinical and epidemiological facts are shown by the results. In a previous study, 55.10% of the people had some kind of anemia. The most common types were iron deficiency anemia (20.50%) and other anemias (26.40%) [11]. The beta thalassemia trait was found in (5.10%) of the community, which is in line with estimates from Pakistan that the carrier rate is between 5 and 7% [12]. The study found big differences between men and women in hemoglobin levels and red cell factors. For instance, men had higher mean hemoglobin levels (12.26 ± 2.18 g/dL vs. 10.58 ± 2.04 g/dL, p<0.001)[13]. This result fits with the idea that men and women have physiologically different hemoglobin reference ranges and with WHO guidelines that say men and women should have different anemia thresholds [14]. Mean corpuscular volume proved to be the strongest individual discriminatory index between IDA and BTT. This observation is supported by the existing literature proving that MCV below 75 fL is a very good discriminating variable [15]. The RDWI was proven to have greater diagnostic strength than single RDW measurements with a statistically significant variation between the IDA and BTT groups [16]. The combined formula of MCV, RDW, and RBC count gives a combined measure of the heterogeneity of red cell population and indices of microcytosis at the same time. Although the

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reference research was conducted in a population of children (1.5-7 years), the current research involved a larger age group (1-95 years), which gives a more comprehensive idea of the epidemiology of anemia across the lifespan in rural communities. The reference study was done in an urban military hospital, but the current study targeted the rural populations in Sindh in particular, and it is in view of a huge gap in epidemiological information in the underserved rural areas. In line with the reference study, our results showed highly increased levels of RBCs in BTT patients as opposed to IDA patients. This discriminatory parameter is strong, as the reference study has indicated its similar results (RBC in BTT: 5.71+-1.2 vs IDA: 3.5+-0.5 x 1012/L)[17, 18]. The current research reported mean MCV fL of 89.03 and 67.38 fL for BTT and IDA, respectively, as compared to the values of 52.2 and 72.5 fL for BTT and IDA, respectively, in the reference study. The slightly reduced BTT of the reference study might be due to the variability of the hematology of the pediatric population [19]. The two studies have found that RDWI is a better discriminatory measure than RDW alone, and it should be implemented in the diagnostic algorithm of anemia classification [20]. In former research, RBC parameters in the male population demonstrated a high statistical level of significance among different age groups on all the investigated parameters except MCHC and RDW. The ANOVA test and multivariate analysis in the female group revealed that all hematological parameters did not have significant differences in all age groups. On sex differences, the male participants were found to have a much higher red blood count, hemoglobin content, and hematocrit. In a different study, the number of RBCs, Hb, and HCT needed a number of age divisions, especially Hb and HCT during childhood and adolescence. There were sex variations in the number of RBCs, Hb, and HCT between puberty and adulthood. MCV, MCH, and RDW, but not MCHC, were among the RBC parameters that needed an age and sex partition [21]. The cutoffs MCV <75 fL and RDWI <15 were pre-specified from previously published literature and then evaluated in the present dataset. Because these thresholds were assessed in the same cohort used for analysis, they are exploratory and may be overfitted. No independent external validation cohort was available.

This research has several limitations. First, the single-center design and consecutive non-probability sampling may limit the representativeness and generalizability of the findings to the broader rural population of Sindh. Second, IDA and BTT were classified using CBC-derived indices without confirmatory testing with serum ferritin or hemoglobin electrophoresis. Therefore, the findings should be interpreted as an assessment of the discriminatory potential of red cell indices rather than diagnostic accuracy against a gold-standard reference test. Third, blinding of laboratory personnel was not implemented or documented, which may have introduced potential measurement or observer bias. Fourth, sensitivity and specificity estimates might have been affected by the low number of BTT cases. Thus, the results are based on discriminating capacity and not final diagnosis. Also, sensitivity and specificity estimates might have been affected due to the low incidence of BTT cases. Further studies using gold-standard diagnostic methods and independent cohorts are warranted to validate the observed discriminatory performance of these indices.

CONCLUSIONS

The findings suggest that MCV, MCH, and RDW may serve as readily available screening and supportive discriminatory parameters for distinguishing presumptively classified iron deficiency anemia and beta-thalassemia trait in resource-constrained settings. However, these indices should not be considered substitutes for confirmatory investigations, and definitive diagnosis should be supported by appropriate laboratory testing where available. Further studies using gold-standard diagnostic methods and independent cohorts are warranted to validate the observed discriminatory performance of these indices.

Authors' Contribution

Conceptualization: AK
Methodology: MS, KM
Formal analysis: MM, FA, AK
Writing and Drafting: MK, JD
Review and Editing: MS, KM, MM, FA, AK, MK, JD

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflict of Interest

All the authors declare no conflict of interest.

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