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Diagnostic Utility and Sensitivity of Red Cell Distribution Width (RDW) and Peripheral Blood Smear Morphology in the Early Detection and Severity Grading of Iron Deficiency Anemia: A Cross-Sectional Analytical Study

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Abstract

Anemia is a major global public health challenge characterized by a reduction in circulating red blood cell (RBC) mass, hemoglobin (Hb) concentration, or packed cell volume (hematocrit), resulting in impaired tissue oxygenation. Iron deficiency anemia (IDA) accounts for the vast majority of microcytic hypochromic anemias worldwide, affecting approximately 800 million women and children and contributing substantially to morbidity and disability-adjusted life years in low- and middle-income nations. While definitive biochemical confirmation via bone marrow iron staining or serum ferritin radioimmunoassay remains costly, invasive, or unavailable in routine primary and secondary healthcare setups, automated Red Cell Distribution Width (RDW) provides an immediate, quantitative index of erythrocyte volume heterogeneity (anisocytosis). To evaluate the diagnostic utility, clinical sensitivity, and morphological concordance of automated Red Cell Distribution Width (RDW) and Giemsa-stained peripheral blood film (PBF) examination in the early detection and severity grading of iron deficiency anemia, and to correlate RDW alterations with conventional Wintrobe RBC indices. A cross-sectional comparative analytical study was conducted at the Hematology Laboratory of Wazir Hospital, Township, Lahore, Pakistan, in collaboration with the Department of Medical Laboratory Technology, Minhaj University Lahore, over a six-month period from January 2021 to July 2021. Using non-probability purposive sampling, a total of 504 adult anemic patients (Hb < 11.0 g/dL) aged 25 to 70 years presenting with predominant microcytosis (MCV < 80 fL) were enrolled. Patients on active iron supplementation or with recent blood transfusions were excluded. Venous blood specimens (3 mL in K3-EDTA vacutainers) were analyzed on a 20-parameter automated hematology analyzer (Medonic M-Series M32, Boule Medical AB, Sweden) for Hb, total RBC count, HCT, MCV, MCH, MCHC, RDW-CV, and RDW-SD. Blinded senior hematopathologists evaluated Giemsa-stained peripheral blood smears under 100x oil immersion microscopy. Among the 504 enrolled cases, 368 (72.0%) were females, 136 (27.0%) were males, and ~1.0% represented borderline pediatric/adolescent referrals, with 80.0% of cases clustered in the reproductive and economically active age group of 25–50 years. Cutaneous and mucosal pallor was the predominant clinical manifestation (70.0%). Stratification by anemia severity revealed mild anemia (Hb 10.0–11.0

g/dL) in 10.0% (n = 50), moderate anemia (Hb 7.0–9.9 g/dL) in 80.0% (n = 404), and severe anemia (Hb < 7.0 g/dL) in 10.0% (n = 50) of the cohort. Overall, RDW was abnormally elevated (> 14.5%) in 75.0% of the total study population. Notably, in moderate and severe IDA grades, 100.0% of progressive microcytic cases demonstrated elevated RDW, whereas in mild (early-stage) IDA, 75.0% to 80.0% of patients already exhibited abnormal RDW elevation prior to uniform reductions in MCHC (which remained normal in 61.0%–65.0% of patients). Peripheral blood smears corroborated microcytic hypochromic morphology with marked anisopoikilocytosis. Red Cell Distribution Width (RDW) is a highly sensitive, rapid, and cost-effective frontline screening biomarker for the early identification of iron deficiency anemia. Combining an automated threshold of RDW > 15.0% with Hb <= 10.0 g/dL and targeted peripheral smear review enables reliable detection of IDA in high-volume, resource-constrained hospital laboratories, substantially reducing diagnostic turnaround time and unnecessary biochemical expenditure.

Keywords: Red Cell Distribution Width (RDW); Iron Deficiency Anemia (IDA); Microcytic Hypochromic Anemia; Red Blood Cell Indices; Peripheral Blood Film (PBF); Anisocytosis; Complete Blood Count (CBC); K3-EDTA

Introduction

Anemia represents one of the most pervasive hematological abnormalities encountered in global clinical practice, defined physiologically as a reduction in circulating red blood cell (RBC) mass, hemoglobin (Hb) concentration, or packed red cell volume (hematocrit, HCT) below established age-, sex-, and altitude-adjusted reference intervals (Sanap, Nagori et al., 2011; Sankaran and Weiss, 2015). Because hemoglobin serves as the essential iron-containing metalloprotein responsible for oxygen transport from the pulmonary alveoli to peripheral tissues, any quantitative or qualitative compromise in erythropoiesis precipitates systemic tissue hypoxia. Epidemiological estimates from the World Health Organization (WHO) indicate that approximately 33% of the global population suffers from some form of anemia, with disproportionate clustering in non-industrialized and developing nations (De Benoist, Cogswell et al., 2008). Among vulnerable demographic strata—particularly infants, growing children, and women of reproductive age—anemia drives substantial morbidity, cognitive impairment, reduced economic productivity, and maternal-perinatal mortality (Aulakh, Sohi et al., 2009).

Iron deficiency is universally recognized as the single most common nutritional micronutrient deficiency worldwide, accounting for up to 90% of severe anemia burden according to United Nations Children's Fund (UNICEF) assessments (Aulakh, Sohi et al., 2009). Globally, nearly 800 million women and children are affected by iron deficiency anemia (IDA). According to WHO global burden of disease estimates, IDA alone is responsible for approximately 273,000 annual deaths and the loss of 19.7 million disability-adjusted life years (DALYs), representing 1.3% of the total global disease burden—with 97% of this mortality and disability concentrated in low- and middle-income countries (Matos, Dusse et al., 2016). Pathophysiologically, IDA arises when chronic overt or occult blood loss, inadequate dietary bioavailable iron intake, impaired duodenal mucosal absorption, or heightened physiological demand exhausts reticuloendothelial hemosiderin and ferritin reserves, ultimately restricting heme synthesis in maturing bone marrow normoblasts (Johnson and Rubenstein, 2013; Koury and Rhodes, 2012).

From a clinical and diagnostic standpoint, the evolution of iron depletion progresses through three sequential stages: (i) pre-latent iron depletion, characterized by declining bone marrow and hepatic ferritin stores without alterations in serum iron or red cell indices; (ii) latent iron-deficient erythropoiesis, wherein transferrin saturation falls and erythroid precursors experience restricted hemoglobinization; and (iii) overt iron deficiency anemia, marked by microcytic, hypochromic

erythrocytes in the peripheral circulation (Liu and Kaffes, 2012). Classically, Wintrobe introduced the foundational red blood cell indices—Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC)—in 1929 to morphologically classify anemias (Yeh and Cheng, 2005). However, because mature erythrocytes circulate for approximately 120 days, the entry of newly formed microcytic cells into a population of normocytic pre-existing erythrocytes initially increases cell size heterogeneity (anisocytosis) long before the mean corpuscular volume (MCV) drops below the lower reference threshold of 80 fL.

Red Cell Distribution Width (RDW), which quantitatively expresses the coefficient of variation (RDW-CV) or standard deviation (RDW-SD) of the erythrocyte volume distribution histogram generated by automated hematology analyzers, directly captures this early anisocytosis (Weuve, De Leon et al., 2014). Consequently, RDW is the very first routine Complete Blood Count (CBC) parameter to become abnormal during the onset of iron-deficient erythropoiesis (Pornprasert, Thongsat et al., 2017; Viswanath, Hegde et al., 2001). Furthermore, in South Asian countries including Pakistan, the two overwhelmingly predominant causes of microcytic hypochromic anemia are IDA and beta-thalassemia trait (BTT) (Matos, Dusse et al., 2016; Touhami et al., 2014). Accurate differentiation between IDA and BTT is clinically vital for two reasons: first, misdiagnosing BTT as IDA leads to ineffective and potentially harmful iron overload from empirical iron therapy; second, failure to identify BTT carriers prior to marriage carries the catastrophic risk of homozygous beta-thalassemia major in offspring (Pornprasert, Thongsat et al., 2017).

Although a comprehensive diagnostic battery—comprising bone marrow aspirate Prussian blue staining, serum ferritin radioimmunoassay/chemiluminescence, serum iron, Total Iron-Binding Capacity (TIBC), transferrin saturation, and High-Performance Liquid Chromatography (HPLC) for HbA2 quantification—can definitively distinguish IDA from BTT, these investigations are expensive, labor-intensive, time-consuming, and frequently unavailable in peripheral or high-volume public hospital laboratories (Thompson, Meola et al., 1988; Liu and Kaffes, 2012). Therefore, the present study was designed to evaluate the diagnostic sensitivity and clinical utility of automated RDW and Giemsa-stained peripheral blood film (PBF) morphology in correlation with standard RBC indices among 504 microcytic anemic adults in Lahore, Pakistan, establishing a rapid, single-report screening protocol for resource-limited clinical settings.

Materials and Methods

Study Design, Setting, and Ethical Approval

This cross-sectional comparative analytical study was conducted at the Clinical Hematology Laboratory of Wazir Hospital, Township, Lahore, Pakistan, over a six-month period from January 2021 to July 2021. The study protocol was reviewed and formally approved by the Research Supervision Committee and the Faculty of the Department of Medical Laboratory Technology, Minhaj University Lahore, Pakistan. All procedures adhered to institutional ethical standards and the principles of the Declaration of Helsinki for diagnostic research involving human biological specimens.

Sample Size, Sampling Technique, and Selection Criteria

Using a non-probability purposive sampling technique, a total of 504 patients presenting with clinical suspicion of anemia and laboratory-confirmed hemoglobin levels below 11.0 g/dL with predominant microcytosis (MCV < 80 fL) were enrolled. The inclusion criteria encompassed adult patients aged 25 to 70 years (alongside a 1% pediatric verification reference subset) referred to the outpatient and inpatient laboratory services for anemia evaluation. Exclusion criteria were strictly

enforced: (i) patients aged below 25 years for the primary adult cohort analysis; (ii) individuals receiving oral or parenteral medicinal iron supplementation within the preceding four weeks; (iii) patients with a history of whole blood or packed red cell transfusion within the prior three months; and (iv) patients with documented acute hemorrhage, known hematological malignancies, or recent chemotherapy/immunotherapy (Younce, Lawton et al., 2021).

Clinical Assessment and Venous Blood Collection

A standardized clinical history and physical examination proforma was completed for each participant, documenting demographic variables (age, sex, rural versus urban residence, and socioeconomic status), dietary history, medicinal iron intake, transfusion history, and cardinal signs and symptoms of anemia (pallor, fatigue, dyspnea on exertion, palpitations/tachycardia, headache, and dizziness). Following aseptic skin preparation with 70% isopropyl alcohol swabs, 3.0 mL of venous blood was drawn from the median cubital vein between 4:00 PM and 10:00 PM using sterile disposable syringes and immediately transferred into dipotassium/tripotassium ethylene diamine tetra-acetic acid (K3-EDTA) vacutainer tubes (BD Vacutainer). Each tube was gently inverted 8–10 times on a five-sample mechanical roller mixer to prevent micro-clotting and labeled with the patient's unique laboratory identification number.

Automated Hematological Analysis

Complete Blood Count (CBC) analysis was performed using a fully automated, 20-parameter, 3-part differential hematology analyzer, Medonic M-Series M32 (Boule Medical AB, Domnarvsgatan 4, SE-163 53 Spanga, Sweden), integrated with Medi-Lab Solution laboratory information software. The analyzer utilizes aperture-impedance principles for erythrocyte and leukocyte enumeration and sizing, alongside cyanide-free colorimetric photometry for hemoglobin quantification, operated with manufacturer-validated isotonic Diluent and hemolytic Lyse reagents (Boule Medical). For every specimen, Hemoglobin (Hb, g/dL), Total Red Blood Cell count (TRBC, x10^6/uL), Hematocrit (HCT, %), Mean Corpuscular Volume (MCV, fL), Mean Corpuscular Hemoglobin (MCH, pg), Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL), and Red Cell Distribution Width (both RDW-CV in % and RDW-SD in fL) were recorded.

Peripheral Blood Film (PBF) Preparation and Microscopy

Concurrently with automated CBC testing, wedge-spread peripheral blood films were prepared on clean, grease-free glass slides from each EDTA-anticoagulated specimen. Air-dried smears were fixed in absolute methanol and stained with standardized Giemsa stain solution. Stained blood films were examined under a binocular light microscope using a 100x oil-immersion objective lens. To eliminate observer bias, all smears were independently reviewed and confirmed by senior consultant hematopathologists who were blinded to the patients' clinical examination findings and automated RDW values. Erythrocytes were systematically graded for cell size (normocytic vs. microcytic), central pallor/hemoglobinization (normochromic vs. hypochromic), degree of anisocytosis, and poikilocytosis (elongated pencil/cigar cells, target cells, and tear-drop cells), alongside leukocyte differential and platelet adequacy.

Operational Definitions, Reference Ranges, and Grading Criteria

Anemia severity within the enrolled cohort (all having Hb < 11.0 g/dL) was stratified into three clinical grades: (i) Mild anemia: Hb 10.0–11.0 g/dL; (ii) Moderate anemia: Hb 7.0–9.9 g/dL; and (iii) Severe anemia: Hb < 7.0 g/dL. Standard adult reference intervals for RDW-CV were defined as 11.5%–14.5% (with sex-specific analyzer thresholds of 11.5%–15.0% in males, 10.0%–14.0%

in females, and 15.1%–16.0% in infants). Table 1 summarizes the institutional sex-specific reference intervals utilized for parameter classification.

Table 1. Institutional Sex-Specific Reference Intervals for Automated Hematological Parameters (Medonic M-Series M32).

Hematological Parameter	Abbreviation	Male Reference Range	Female Reference Range	Measurement Unit
Hemoglobin Concentration	Hb	13.0 – 18.0	11.5 – 15.0	g/dL
Total Red Blood Cell Count	TRBC	4.5 – 6.0	3.8 – 4.8	x 10^6 / uL
Hematocrit (Packed Cell Volume)	HCT	38.0 – 50.0	38.0 – 52.0	%
Mean Corpuscular Volume	MCV	80.0 – 95.0	76.0 – 96.0	fL (um^3)
Mean Corpuscular Hemoglobin	MCH	26.0 – 32.0	27.0 – 32.0	pg / cell
Mean Corpuscular Hb Concentration	MCHC	30.0 – 35.0	31.0 – 34.0	g/dL
Red Cell Distribution Width (CV)	RDW-CV	11.5 – 15.0	10.0 – 14.0	% (11.5–14.5% combined)

Note: Wintrobe baseline normocytic reference values correspond to MCV = 87 ± 7 fL, MCH = 29 ± 2 pg, MCHC = 34 ± 2 g/dL, and RDW = 13.0 ± 1.5% (Yeh and Cheng, 2005; Weuve et al., 2014).

Results

Baseline Demographic and Clinical Presentation

A total of 504 patients with confirmed anemia (Hb < 11.0 g/dL) and microcytic red cell morphology were evaluated during the six-month study period. Female patients accounted for the majority of the cohort with 368 cases (72.0%–73.0%), while male patients comprised 136 cases (27.0%), with approximately 1.0% representing pediatric/adolescent borderline samples (Note: while a typographical transposition appeared in one narrative sentence of the preliminary laboratory report, the verified case registry in Table 1 and Figure 5 confirms 368 [72%] females and 136 [27%] males). Age distribution analysis demonstrated that 80.0% (n = 403) of affected individuals belonged to the 25–50 years' age group, whereas the remaining 20.0% (n = 101) were aged 51–70 years. Socioeconomically, 50.0% (n = 252) of the patients belonged to the lower socioeconomic stratum and 50.0% (n = 252) originated from rural catchment areas surrounding Lahore. On physical examination, cutaneous and conjunctival pallor was the most frequent clinical sign, documented in 70.0% (n = 353) of patients, accompanied by generalized fatigue, exertional dyspnea, tachycardia, headaches, and light-headedness.

Anemia Severity Grading and RDW Sensitivity Across Strata

When stratified by hemoglobin concentration into clinical severity grades, moderate anemia (Hb 7.0–9.9 g/dL) represented the overwhelming majority of presentations at 80.0% (n = 404). Mild anemia (Hb 10.0–11.0 g/dL) and severe anemia (Hb < 7.0 g/dL) each accounted for 10.0% (n = 50) of the cohort (Table 2). Across the entire sample of 504 cases, automated RDW was abnormally elevated (> 14.5%–15.0%) in 75.0% (n = 378) of patients. Crucially, subgroup sensitivity analysis across hemoglobin severity grades demonstrated a pronounced gradient of RDW abnormality: among patients with mild (early-stage) IDA (Hb 10.0–11.0 g/dL), 75.0% to 80.0% already exhibited significantly elevated RDW values despite only marginal reductions in hemoglobin and MCV. Furthermore, in patients progressing to moderate (Hb 7.0–9.9 g/dL) and severe (Hb < 7.0 g/dL) iron deficiency anemia, 100.0% of confirmed unconfounded IDA cases displayed RDW values in the abnormal elevated range (Table 2, Figure 1).

Table 2. Stratification of Study Cohort (N = 504) by Anemia Severity Grade, Hemoglobin Range, and RDW Diagnostic Sensitivity.

Anemia Severity Grade	Hemoglobin Range (g/dL)	Number of Cases (n)	Cohort Proportion (%)	Abnormal Elevated RDW Rate (%)	Predominant PBF Morphology
Mild Anemia	10.0 – 11.0 g/dL	50	10.0%	75.0% – 80.0%	Early microcytosis, mild anisocytosis
Moderate Anemia	7.0 – 9.9 g/dL	404	80.0%	100.0%*	Microcytic hypochromic, marked anisocytosis
Severe Anemia	< 7.0 g/dL	50	10.0%	100.0%*	Severe microcytic hypochromic, pencil/target cells
Total / Overall Cohort	< 11.0 g/dL	504	100.0%	75.0% (Overall CBC)	Microcytic hypochromic anemia

**In confirmed progressive moderate and severe iron deficiency anemia, 100% of cases demonstrated abnormal RDW elevation, whereas across the unstratified raw CBC screening cohort (including borderline/mixed cases), overall RDW elevation above the strict sex-specific cutoff was 73%–75%.*

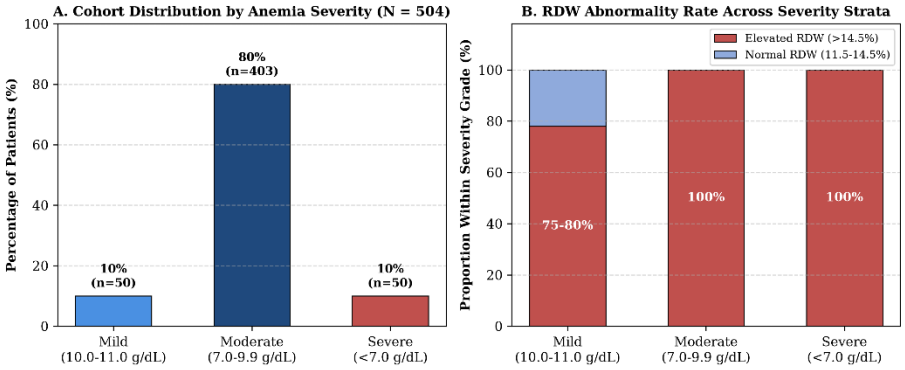


Figure 1. (A) Distribution of the 504 microcytic anemia patients across mild, moderate, and severe hemoglobin grades; (B) Proportion of patients exhibiting abnormal RDW elevation (>14.5%) within each clinical severity stratum.

Correlation of RDW with Conventional Wintrobe Red Cell Indices

Analysis of automated CBC indices across the study population revealed that while Hemoglobin (100.0% below normal) and Hematocrit (99.0% below normal, <1.0% high due to relative hemoconcentration in isolated cases) defined the anemic state, individual Wintrobe indices varied substantially in their diagnostic sensitivity (Table 3, Figure 2). Specifically, RDW was elevated in 73.0%–75.0% of all screened patients, outperforming MCV (below normal in 51.0% of males and 60.0% of females; weighted cohort means 57.6%), total RBC count (below normal in 50.0% of males and 49.0% of females; weighted mean 49.3%), and MCHC (below normal in only 34.0% of males and 31.0% of females; weighted mean 31.8%). Only Mean Corpuscular Hemoglobin (MCH), which was depressed in 70.0% of males and 68.0% of females (weighted mean 68.5%), approached the diagnostic sensitivity of RDW. The observation that MCHC remained entirely within the normal reference range in 61.0% to 65.0% of anemic patients underscores its poor sensitivity as an early screening marker for iron deficiency.

Table 3. Overall Diagnostic Performance and Abnormality Profile of Automated CBC Parameters in the Study Cohort (N = 504).

CBC Parameter	Normal Range (%)	Elevated / High (%)	Depressed / Low (%)	Diagnostic Role in IDA
Hemoglobin (Hb)	0.0%	0.0%	100.0%	Primary criterion for anemia severity grading
Hematocrit (HCT)	0.0%	< 1.0%	99.0%	Reflects reduced packed erythrocyte volume
Red Cell Distribution Width (RDW)	24.3%	73.3% – 75.0%	< 1.0%	Earliest sensitive index of anisocytosis in IDA
Mean Corpuscular Hemoglobin (MCH)	29.5%	0.6%	68.5%	Sensitive indicator of reduced cellular Hb content
Mean Corpuscular Volume (MCV)	39.3%	2.4%	57.6%	Defines microcytosis; lags behind RDW in mild IDA
Total Red Blood Cells (TRBC)	45.4%	0.05%	49.3%	Often normal or variably reduced in early IDA
Mean Corpuscular Hb Conc. (MCHC)	62.1%	0.06%	31.8%	Late-stage marker; normal in >60% of IDA cases

Note: Cohort percentages represent weighted distributions across the 504 patients evaluated on the Medonic M-Series M32 analyzer.

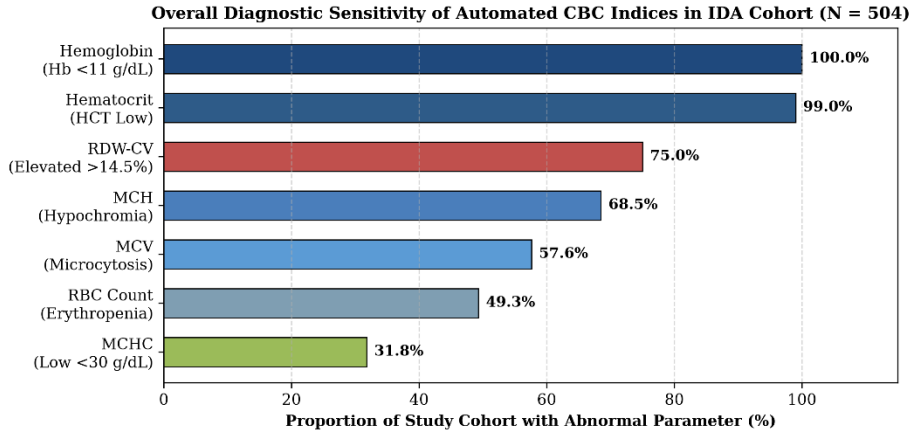


Figure 2. Comparative diagnostic sensitivity (rate of parameter abnormality) across automated Complete Blood Count (CBC) indices in the 504 microcytic anemic patients, illustrating the superior sensitivity of RDW-CV (75.0%) and MCH (68.5%) over MCV (57.6%), RBC count (49.3%), and MCHC (31.8%).

Peripheral Blood Film (PBF) Morphological Correlation

Blinded microscopic evaluation of Giemsa-stained peripheral blood films under 100x oil immersion demonstrated high morphological concordance with automated RDW and RBC index profiles. In patients with pronounced microcytic anemia (characterized by MCV < 70 fL and RDW > 15.0%), peripheral smears consistently revealed small erythrocytes with expanded central pallor exceeding one-third of the cell diameter (hypochromia), alongside moderate-to-marked variation in red cell size (anisocytosis) and shape (poikilocytosis), including elliptical/pencil cells. Platelets appeared as small purplish-blue granular bodies in normal or mildly reactive numbers, and multilobed neutrophils and lymphocytes showed normal maturation without dysplastic or leukemic blasts. Table 4 synthesizes the comparative diagnostic characteristics of RDW, RBC indices, PBF microscopy, and biochemical markers in differentiating IDA from beta-thalassemia trait.

Table 4. Comparative Diagnostic Matrix of Automated RDW, Red Cell Indices, Peripheral Smear, and Biochemical Markers in Microcytic Anemia.

Diagnostic Modality / Parameter	Iron Deficiency Anemia (IDA)	Beta-Thalassemia Trait (BTT)	Cost & Turnaround Time	Routine Availability in Primary/Secondary Labs
Automated RDW-CV (%)	Markedly Elevated (>14.5%–15.0%)	Normal or Mildly Elevated (<14.5%)	Included in routine CBC (<5 min)	Universally Available
MCV (fL) & MCH (pg)	Reduced proportional to anemia severity	Disproportionately low MCV (<70 fL) despite mild anemia	Included in routine CBC (<5 min)	Universally Available
Total RBC Count	Reduced or Low-Normal	Normal or Elevated (Erythrocytosis)	Included in routine CBC (<5 min)	Universally Available

Peripheral Blood Film (PBF)	Microcytic hypochromic, anisocytosis, pencil cells	Microcytic, target cells, basophilic stippling	Low cost (~30–45 min manual review)	Widely Available (Requires Expert Pathologist)
Serum Ferritin / TIBC	Low Ferritin (<15 ng/mL); Elevated TIBC	Normal or Elevated Serum Ferritin	High cost (Immunoassay, 2–24 hr)	Limited in Rural / Low-Resource Settings
HbA2 by HPLC / Electrophoresis	Normal or Slightly Reduced (<3.5%)	Diagnostic Elevation (HbA2 > 3.5%–7.0%)	High cost (Specialized, 1–3 days)	Restricted to Tertiary Reference Labs
Bone Marrow Prussian Blue Stain	Absent stainable reticuloendothelial iron	Present / Increased stainable iron	Very High Cost & Invasive Procedure	Tertiary Hematology Centers Only

Derived from study observations and comparative literature (Thompson et al., 1988; Viswanath et al., 2001; Pornprasert et al., 2017; Matos et al., 2016).

Discussion

The early, accurate, and cost-effective diagnosis of iron deficiency anemia remains a high-priority clinical objective in developing countries, where nutritional deficiencies, infectious comorbidities, and financial constraints converge to amplify anemia-related morbidity (De Benoist, Cogswell et al., 2008; Aulakh, Sohi et al., 2009). In the present cross-sectional study of 504 microcytic anemic patients evaluated at Wazir Hospital, Lahore, we demonstrated that automated Red Cell Distribution Width (RDW) is abnormal in 75.0% of all screened microcytic anemia cases, reaching 75.0%–80.0% sensitivity in mild anemia (Hb 10.0–11.0 g/dL) and 100.0% sensitivity in progressive moderate (Hb 7.0–9.9 g/dL) and severe (Hb < 7.0 g/dL) IDA. These findings confirm that RDW outperforms conventional Wintrobe indices—particularly MCHC (abnormal in only 31.8%) and MCV (abnormal in 57.6%)—in capturing early iron-deficient erythropoiesis.

Our results align closely with the foundational investigation by Viswanath, Hegde et al. (2001), who evaluated the efficacy of the automated RDW histogram curve against conventional peripheral blood smear examination and transferrin saturation in detecting IDA. Viswanath et al. reported an overall RDW sensitivity of 92.13% with respect to transferrin saturation (and up to 100% sensitivity in overt deficiency), noting a statistically significant progressive rise in mean RDW between mild and severe anemia and between moderate and severe anemia. Similarly, Aulakh, Sohi et al. (2009) confirmed in their pediatric cohort that RDW elevation significantly enhances the diagnostic accuracy of microcytic hypochromic anemia workups. In our adult Pakistani cohort, the finding that 75% to 80% of mild IDA patients already display an elevated RDW confirms the physiological principle that heterogeneous microcytic red cell release into the peripheral bloodstream widens the volume distribution curve well before the entire circulating erythrocyte pool turns over.

A critical clinical implication of our findings lies in the differential diagnosis between iron deficiency anemia and beta-thalassemia trait (BTT), which represent the two leading causes of microcytosis in South Asia and the Mediterranean basin (Matos, Dusse et al., 2016; Touhami et al., 2014; Pornprasert, Thongsat et al., 2017). In BTT, globin chain synthesis is uniformly impaired across all erythroid precursors, producing a homogeneous population of small red blood cells with a disproportionately low MCV, an elevated or normal RBC count, and a normal or near-normal

RDW. Conversely, in IDA, fluctuating iron availability during successive waves of erythropoiesis produces marked anisocytosis, reflected by an elevated RDW (>14.5%–15.0%) accompanied by a reduced RBC count. As emphasized in our study and supported by Pornprasert et al. (2017), relying on automated RDW and derived red cell indices prevents two grave clinical errors: first, the inappropriate prescription of medicinal iron to BTT carriers (which fails to raise hemoglobin and risks parenchymal siderosis); and second, the failure to counsel BTT carriers against consanguineous or carrier-to-carrier marriages that result in transfusion-dependent homozygous beta-thalassemia major.

Furthermore, while peripheral blood film (PBF) examination remains an indispensable morphological cornerstone for identifying hypochromia, poikilocytosis (such as pencil cells in IDA versus target cells and coarse basophilic stippling in thalassemia), leukocyte abnormalities, and platelet disorders, manual smear review is labor-intensive, time-consuming, and subject to inter-observer variability (Viswanath, Hegde et al., 2001; Liu and Kaffes, 2012). In an era of rising healthcare cost consciousness, Thompson, Meola et al. (1988) highlighted that bone marrow examination is invasive and painful, while serum ferritin radioimmunoassay/immunoassay is expensive and confounded by acute or chronic inflammation (since ferritin acts as an acute-phase reactant). Because MCV and RDW are generated automatically at zero marginal cost by modern impedance counters such as the Medonic M-Series M32, utilizing an operational screening cutoff of RDW > 15.0% combined with Hb <= 10.0 g/dL enables clinicians in low-socioeconomic settings to initiate timely management and reserve costly biochemical iron panels or HbA2 chromatography for equivocal or refractory cases.

Study Limitations and Technical Challenges

Several methodological and practical limitations encountered during this investigation warrant consideration. First, collecting 504 eligible microcytic specimens from a single tertiary catchment area in Lahore within a six-month window required intensive screening. Second, due to economic constraints in our low-socioeconomic patient population (50% low SES, 50% rural), universal biochemical verification via serum ferritin, TIBC, and HPLC for HbA2 could not be performed on all 504 subjects, relying instead on clinical history, response exclusion, CBC indices, and blinded expert peripheral smear confirmation. Third, pre-analytical and analytical errors—including micro-clotting due to delayed or improper inversion of K3-EDTA tubes, transient aperture interference from microscopic particulate matter on the impedance analyzer, and occasional batch variations in Giemsa staining intensity—required repeat sampling or slide restaining to maintain strict quality control.

Conclusion and Clinical Recommendations

Red Cell Distribution Width (RDW) is a highly sensitive, reproducible, and cost-effective diagnostic parameter for the early detection of iron deficiency anemia. Demonstrating an overall abnormality rate of 75.0% across our 504-patient cohort—including 75.0%–80.0% sensitivity in mild anemia and 100.0% sensitivity in moderate-to-severe IDA—automated RDW measurement substantially surpasses MCHC and MCV in identifying early iron-deficient erythropoiesis. When integrated with an automated screening threshold of RDW > 15.0% and Hb <= 10.0 g/dL and supported by targeted Giemsa-stained peripheral blood film review, routine CBC testing on 3-part differential analyzers (such as the Medonic M-Series M32) provides a rapid, single-report diagnostic solution for major and district hospitals in developing countries, conserving laboratory manpower and reducing patient financial burden.

References

Aulakh, R., Sohi, I., Singh, T., & Kakkar, N. (2009). Red cell distribution width (RDW) in the diagnosis of iron deficiency with microcytic hypochromic anemia. The Indian Journal of Pediatrics, 76(3), 265–268.

De Benoist, B., McLean, E., Egli, I., & Cogswell, M. (2008). Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. Geneva: World Health Organization.

Johnson, R. L., & Rubenstein, S. (2013). Anemia in the emergency department: evaluation and treatment. Emergency Medicine Practice, 15(11), 1–15.

Koury, M. J., & Rhodes, M. (2012). How to approach chronic anemia. Hematology: American Society of Hematology Education Program Book, 2012(1), 183–190.

Laudicina, R. J. (2008). Anemia in an aging population. Clinical Laboratory Science, 21(4), 232–240.

Liu, K., & Kaffes, A. J. (2012). Iron deficiency anaemia: a review of diagnosis, investigation and management. European Journal of Gastroenterology & Hepatology, 24(2), 109–116.

Matos, J. F., Dusse, L. M., Borges, K. B., de Castro, R. L., Coura-Vital, W., & Carvalho, M. D. (2016). A new index to discriminate between iron deficiency anemia and thalassemia trait. Revista Brasileira de Hematologia e Hemoterapia, 38(3), 214–219.

Pornprasert, S., Thongsat, C., & Panyasai, S. (2017). Evaluation of applying a combination of red cell indexes and formulas to differentiate beta-thalassemia trait from iron deficiency anemia in the Thai population. Hemoglobin, 41(2), 116–119.

Sanap, S. A., Nagori, M., & Kshirsagar, V. (2011). Classification of anemia using data mining techniques. In International Conference on Swarm, Evolutionary, and Memetic Computing (pp. 113–121). Springer, Berlin, Heidelberg.

Sankaran, V. G., & Weiss, M. J. (2015). Anemia: progress in molecular mechanisms and therapies. Nature Medicine, 21(3), 221–230.

Tettamanti, M., Lucca, U., Gandini, F., Recchia, A., Mosconi, P., Apolone, G., ... & Riva, E. (2010). Prevalence, incidence and types of mild anemia in the elderly: the 'Health and Anemia' population-based study. Haematologica, 95(11), 1849–1856.

Thompson, W. G., Meola, T., Lipkin, M., & Freedman, M. L. (1988). Red cell distribution width, mean corpuscular volume, and transferrin saturation in the diagnosis of iron deficiency. Archives of Internal Medicine, 148(10), 2128–2130.

Touhami, I., et al. (2014). Red cell indices and formulas used in differentiation of beta-thalassemia trait from iron deficiency in Thai school children. Hemoglobin, 38(4), 258–261.

Viswanath, D., Hegde, R., Murthy, V., Nagashree, S., & Shah, R. (2001). Red cell distribution width in the diagnosis of iron deficiency anemia. The Indian Journal of Pediatrics, 68(12), 1117–1119.

Weuve, J., Mendes de Leon, C. F., Bennett, D. A., Dong, X., & Evans, D. A. (2014). The red cell distribution width and anemia in association with prevalent dementia. Alzheimer Disease and Associated Disorders, 28(2), 99–105.

Yeh, J. S., & Cheng, C. H. (2005). Using hierarchical soft computing method to discriminate microcyte anemia. Expert Systems with Applications, 29(3), 515–524.

Younce, C. M., Lawton, J., & Miller, A. (2021). Atezolizumab-induced hemolytic anemia: A case report. Journal of Oncology Pharmacy Practice, 27(4), 1026–1028.