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DIAGNOSTIC VALUE OF HAEMATOLOGICAL INDICES IN SCREENING FOR IRON DEFICIENCY ANAEMIA AMONG FEMALES IN LAHORE

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Abstract

Iron deficiency anaemia (IDA) is the most common nutritional disorder worldwide and disproportionately affects women of reproductive age. In Pakistan, the prevalence of anaemia among females exceeds 40%. However, confirmatory tests such as serum ferritin and total iron-binding capacity are often inaccessible due to financial and logistical constraints. As a result, a complete blood count (CBC) is a universally available and cost-effective alternative for the early detection of IDA. This cross-sectional study evaluated the diagnostic effectiveness of routine haematological indices, including haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW), in 50 females aged 18 to 45 years with clinically suspected IDA and haemoglobin levels below 12 g/dL. Venous blood samples were collected in EDTA tubes and analysed using an automated haematology analyser. Participants with haemoglobinopathies, anaemia of chronic disease, pregnancy, or those receiving current haematinic therapy were excluded from the study, which was conducted over 8 months at the Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan. Among the participants, 62% were anaemic, with a mean haemoglobin level of 10.01 g/dL. Elevated RDW was observed in 82% of cases, indicating anisocytosis, while MCV and MCH remained within normal reference ranges, which is consistent with early-stage normocytic IDA. The mean platelet count was $387.5\times10^3/\mu L$, approaching thrombocytosis and further supporting the diagnosis of iron deficiency. A weak correlation was noted between haemoglobin and MCV, reinforcing the predominance of a normocytic pattern. These findings indicate that elevated RDW, combined with reduced haemoglobin in the absence of microcytosis or hypochromia, is a sensitive indicator of early iron deficiency anaemia. Routine CBC parameters, particularly RDW, provide an accessible, reliable, and clinically valuable screening tool for IDA, especially in resource-limited settings where advanced iron studies are not routinely available.

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1. INTRODUCTION:

Iron deficiency anaemia (IDA) is the most common nutritional deficiency disorder worldwide, affecting approximately two billion people. Iron deficiency accounts for nearly 50% of all anaemia cases. Women of reproductive age bear a disproportionate burden of this condition, primarily due to menstrual blood loss, inadequate diets, and frequent pregnancies. In Pakistan, the situation is particularly alarming, with anaemia prevalence exceeding 50% among women of reproductive age in certain regions, where iron deficiency is the predominant underlying cause. Beyond its effects on blood health, IDA has wide-ranging negative impacts on cognitive development, physical work capacity, maternal and perinatal outcomes, and economic productivity. This makes it not just a clinical issue but a significant public health challenge. Despite its high prevalence, IDA is often underdiagnosed, especially in low-resource healthcare settings. Tests needed for confirmation—such as serum ferritin, serum iron, and total iron-binding capacity (TIBC)—are frequently unavailable or unaffordable at the primary care level throughout much of Pakistan. In contrast, the complete blood count (CBC) is universally accessible, inexpensive, and routinely performed at all levels of the healthcare system. CBC-derived red cell indices—haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW)—reflect progressive changes in red blood cells that occur during the three distinct stages of iron deficiency.

Understanding the stages of iron deficiency is crucial for recognizing the diagnostic limitations of standard CBC interpretation. In the initial stage of iron depletion, serum ferritin levels decline while haemoglobin and red cell indices remain normal, making this stage clinically silent and often undetected. In the second stage—iron-deficient erythropoiesis—TIBC rises, and serum iron decreases, leading the bone marrow to produce a mixed population of red blood cells of varying sizes. At this stage, RDW, which measures variation in red blood cell size, increases before any changes in MCV are detectable. The classic presentation of microcytic hypochromic anaemia, commonly discussed in clinical education, does not appear until the third and final stage of overt IDA, when iron deficiency has been present for a significant duration. Thus, relying solely on microcytosis and hypochromia as diagnostic criteria risks missing a considerable number of patients in the earlier, more treatable stages of the disease.

This study aims to evaluate the diagnostic utility of CBC-derived haematological indices in females with suspected IDA, with a specific focus on identifying which parameters show the earliest and most consistent abnormalities. Given that CBC is often the only commonly available haematological test in many district-level and primary healthcare facilities across Pakistan, this study seeks to provide clinically relevant, data-driven guidance for diagnosing IDA in resource-limited settings.

Materials and Methods

2.1 Study Design

This study was conducted as a cross-sectional descriptive investigation to evaluate the diagnostic utility of routine haematological indices in females with clinically suspected iron deficiency anaemia (IDA). A cross-sectional design was chosen because it effectively addresses prevalence and patterns, focusing on characterizing hematological profiles at a specific moment instead of monitoring changes over time or assessing therapeutic outcomes. There was no intervention, follow-up, or control group, as the study aimed not to compare anaemic and non-anaemic females but

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to identify the haematological signature of IDA in a symptomatic female population. Individual parameter values were compared to normative reference ranges established by the instrument manufacturer and the World Health Organization (WHO). Descriptive statistics were used to summarize the distribution of all hematological parameters, which is methodologically appropriate given the study's sample size and descriptive nature.

2.2 Study Setting and Duration

The study was conducted at the Research Laboratory of the Institute of Microbiology and Molecular Genetics (MMG) at the University of the Punjab in collboartion with [3]TML Labs & Diagnostics Health Care Network (Trustmed Lab), Lahore, Pakistan, over a period of eight months. A clinical laboratory setting was intentionally chosen to replicate the real-world conditions where iron deficiency anaemia (IDA) screening is most commonly performed, ensuring that the findings are directly applicable to routine diagnostic practices. All venous blood samples were collected from the median cubital vein using 5 mL syringes and were transferred into EDTA-anticoagulated vacutainer tubes. The samples were processed using the Sysmex XP-300 automated hematology analyzer (Sysmex Corporation, Kobe, Japan), which is a validated instrument routinely employed in clinical laboratory diagnostics. To ensure internal consistency and eliminate inter-instrument variability, a recognised source of discrepancies in haematological studies, particularly for mean corpuscular volume (MCV) and red cell distribution width (RDW) measurements, all 50 samples were analysed on a single instrument under uniform calibration conditions throughout the study period.

2.3 Study Population and Sample Size

The study enrolled 50 female patients aged 18 to 45 years who exhibited clinical symptoms of anemia, such as fatigue, pallor, and generalized weakness, with a hemoglobin (Hb) level below 12 g/dL. This age range was specifically chosen to align with the reproductive years, during which women are at a higher physiological risk for iron deficiency anemia (IDA) due to monthly menstrual blood loss. In cases of menorrhagia, blood loss can reach up to 80 mL per cycle, often exceeding dietary iron intake and leading to progressive iron depletion. In the context of Pakistan, these physiological risks are further intensified by poor dietary diversity, food insecurity, limited consumption of iron-rich foods, and suboptimal healthcare-seeking behaviors. Nationally, it is reported that approximately 41% of women of reproductive age in Pakistan have low hemoglobin levels, with IDA identified as the primary underlying cause. This highlights the clinical and epidemiological significance of focusing on this target population. Participants were recruited using consecutive purposive sampling. This method involved enrolling all eligible females who presented to the laboratory during the study period, continuing until the predetermined sample size of 50 was reached. This approach minimized selection bias by ensuring that no eligible participant was preferentially excluded. A sample size of 50 is considered adequate for descriptive studies aimed at characterizing the distribution of hematological parameters within a defined clinical population. In such studies, the primary goal is to estimate means and standard deviations rather than to detect small effect sizes or test inferential hypotheses.

2.4 Inclusion and Exclusion Criteria

Participants were included in the study if they were female, aged between 18 and 45 years, exhibited symptoms consistent with anaemia, and had a haemoglobin level below 12 g/dL. Those were excluded if they had a confirmed or suspected hemoglobinopathy, anaemia of chronic disease, were actively pregnant, or were currently receiving haematinic

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supplementation. These conditions could independently affect red cell indices and confound the interpretation of CBC-derived parameters.

2.5 Parameters Assessed

The following haematological indices were measured and analyzed for each participant: haemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width coefficient of variation (RDW-CV) and standard deviation (RDW-SD), platelet count (PLT), and associated platelet indices. Data were recorded, tabulated, and analyzed using Microsoft Excel, with results presented as means, standard deviations, and percentage frequencies.

Results

3.1 Study Population and Gender Distribution

A total of 50 participants were enrolled in this cross-sectional study. The haematological parameters derived from complete blood counts (CBCs) were analysed for each participant and compared with established WHO reference ranges and instrument-specific normative values. Results are presented sequentially based on demographic characteristics, followed by individual haematological parameters. Among the 50 participants, 34 (68%) were female and 16 (32%) were male, resulting in an approximately 2:1 female-to-male ratio. This disproportionate distribution likely reflects the higher prevalence of iron deficiency anaemia (IDA) among women of reproductive age, along with a greater tendency for females to seek healthcare in the clinical setting from which the samples were obtained. It is important to consider this gender imbalance when interpreting sex-sensitive parameters, such as haemoglobin concentration and red cell indices, since reference ranges differ between males and females.

3.2 Mean Haematological Parameters

The mean values of all measured haematological parameters (Table 1: Figure 2). Due to the substantial differences in units and absolute magnitudes across parameters, values are presented relative to one another for clarity. Notable findings include a mean platelet count of 387.5 × 10^3/µL, the highest absolute value recorded, and a mean red cell distribution width (RDW-SD) of 48.6 fL. Other parameters, including red blood cell (RBC) count (mean 4.72 × 10^6/µL), mean platelet volume (MPV) (mean 5.77 fL), and platelet distribution width (PDW) (mean 6.96 fL), showed comparatively lower absolute values. The mean white blood cell (WBC) count of 9.47 × 10^3/µL fell within the upper limit of the normal reference range (4–11 × 10^3/µL). Importantly, the mean haemoglobin level of 10.01 g/dL was below the WHO-defined threshold of 12 g/dL for adult females, indicating a population-wide tendency toward anaemia.

3.3 WBC and RBC Distribution

The frequency distributions of WBC and RBC counts for all 50 participants are illustrated in Figure 3. **WBC Distribution**: WBC counts ranged from 4 to 14 × 10^3/µL, with the highest frequency observed in the 9–10 × 10^3/µL category (approximately 9 participants). A secondary cluster was noted in the 5–6 × 10^3/µL range. A right-sided tail extending to 14 × 10^3/µL indicated leucocytosis in a subset of participants, suggesting possible concurrent infection or an underlying inflammatory condition. The distribution displayed slight bimodality, reflecting clinical heterogeneity within the study cohort. **RBC Distribution**: RBC counts ranged from 4.0 to 5.0 × 10^6/µL, with the highest frequency found in the 4.5–5.0 × 10^6/µL category (n = 7). A particularly low value of 2.9 × 10^6/µL was recorded for one participant, marking a significant outlier consistent with pronounced erythrocytopenia. Overall, the distribution was left-skewed,

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aligning with the anaemic trend seen in the mean haemoglobin values.

3.4 Haemoglobin and Haematocrit Distribution

The frequency distributions of haemoglobin (Hb) and haematocrit (HCT) — two closely related measures of red cell mass and oxygen-carrying capacity — are presented in Figure 4.

Haemoglobin Distribution: Haemoglobin values ranged from 5 to 14 g/dL, with the highest frequency occurring in the 9–10 g/dL category (n = 7). The distribution was right-skewed, with most values below the normal female threshold of 12 g/dL. In total, 62% of participants (n = 31) had haemoglobin levels below 12 g/dL, confirming a high prevalence of anaemia within the study population. Only one participant recorded a value in the 13–14 g/dL range, indicating near-normal haemoglobin status.

Haematocrit Distribution: Haematocrit values were primarily distributed between 28% and 35%, peaking in the 30–32% range (approximately 9 participants). These values fall significantly below the established reference ranges for adult females (36–48%) and males (41–53%), which is consistent with the anaemic haemoglobin findings. A minor right tail extending towards 45–47% was observed, likely attributable to male participants or non-anaemic individuals within the cohort.

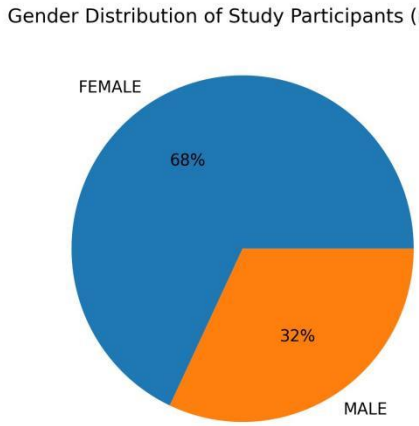


Figure 1. Gender distribution of study participants (n=50) is shown in a pie chart. There were 34 females (68%) and 16 males (32%).

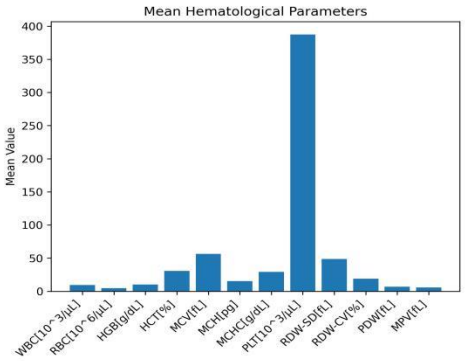


Figure 2 displays a bar chart showing the mean values of 12 hematological parameters from a total of 50 samples. The difference scale indicates that PLT and RDW-SD have the highest absolute mean values.

1. Key parameter Summary with clinical observations:

Parameter	Mean Value	Normal Range	Observation

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WBC [10^3/µL]	9.47	4.0–11.0	High-normal; several cases exceed 11.0
RBC [10^3/µL]	4.72	3.8–5.2 (F) / 4.5–5.9 (M)	Within an acceptable range
HGB [g/dL]	10.01	12.0–16.0 (F) / 13.5–17.5 (M)	Low — indicative of anaemia
HCT [%]	30.90	36–48 (F) / 41–53 (M)	Below normal — confirms anaemia
MCV [fL]	56.36	80–100 Mucolytic — iron deficiency is suggested by MCV [fL]	
MCH [pg]	15.17	27–33	Low — hypochromic pattern
MCHC [g/dL]	29.17	32–36	Decreased — the same as microcytic anaemia
PLT [10^3/µL]	387.50	150–450	Elevated but within range
RDW-SD [fL]	48.60	37–54	Within normal limits
Parameter	**Mean Value**	**Normal Range**	**Observation**
RDW-CV [%]	18.94	11.5–14.5	Elevated — increased anisocytosis
PDW [fL]	6.96	9–17	Low — uniform platelet size
MPV [fL]	5.77	7.5–12.5	Low — smaller platelet volume

Mean haematological parameter values, reference intervals, and observation of the patient.

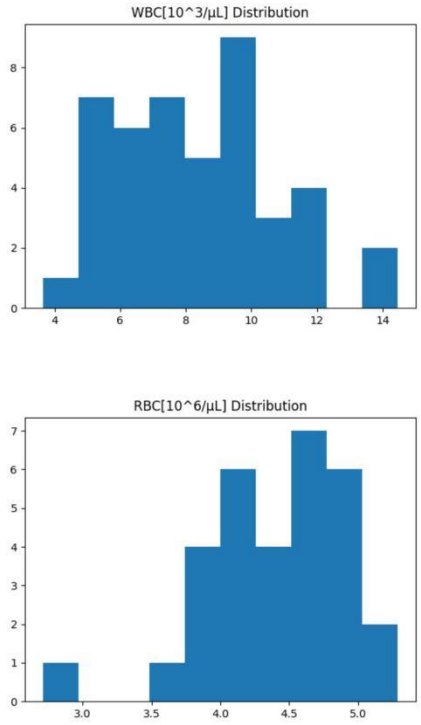


Figure 3 illustrates the frequency distribution histograms for WBC (upper) and RBC (lower). The X-axis represents the measured values, while the Y-axis indicates the number of patients.

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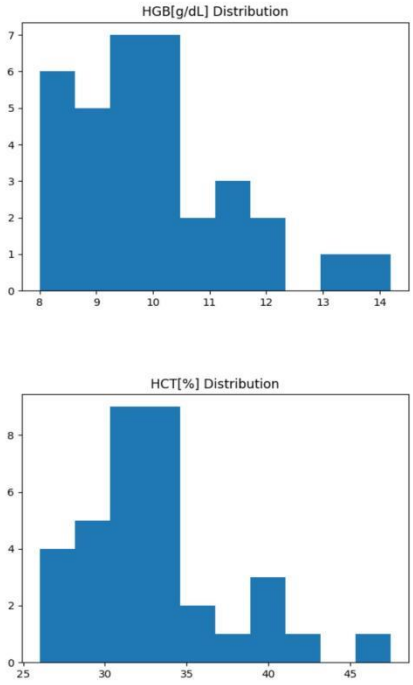


Figure 4. Upper: Frequency histogram of HGB distribution; Lower: frequency histogram of HCT distribution. Most values fall below the clinical normal ranges, indicating a general state of anaemia.

4. Discussion

The findings of this study offer important insights into the haematological profile of iron deficiency anaemia (IDA) in females of reproductive age, supporting the diagnostic use of complete blood count (CBC)-derived indices in resource-limited settings. The overall prevalence of anaemia found was 62%, aligning with existing literature from Pakistan. This rate is comparable to studies (references 8 and 9) that report anaemia prevalence exceeding 40% among women of reproductive age in Pakistan. The higher prevalence observed in this study can be attributed to the selection criteria, as participants were recruited based on symptomatic presentation rather than through a general population survey. The mean haemoglobin level was 10.01 g/dL, indicating moderate anaemia, a severity at which clinical symptoms are usually present and iron supplementation is recommended. These findings highlight the urgent need for systematic anaemia screening programs targeting women of reproductive age in Pakistan, where IDA remains a significant yet preventable contributor to maternal morbidity. A particularly notable finding was the complete absence of microcytosis or hypochromia, despite universally reduced haemoglobin levels. Classical teachings define IDA as microcytic hypochromic anaemia; however, this morphological pattern represents a late manifestation of prolonged iron depletion rather than an early diagnostic feature. This aligns with the findings of reference 10, which demonstrated that haemoglobin levels decline before mean corpuscular volume (MCV) in iron deficiency, and with reference 11, which

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highlighted the limitations of relying solely on MCV as an early indicator of IDA. Therefore, normal MCV and mean corpuscular haemoglobin (MCH) values do not exclude iron deficiency, and an over-reliance on these indices risks overlooking early-stage IDA—precisely the stage at which intervention is most effective.

The elevation of RDW-CV in 82% of participants, with a mean of 18.94%, was the most significant finding in this study. This aligns with previous research that identified RDW as one of the earliest and most sensitive markers of iron deficiency, appearing before changes in mean corpuscular volume (MCV). As iron stores deplete, the bone marrow produces an increasingly diverse population of red blood cells, which includes both near-normal cells formed before depletion and smaller, hypochromic cells generated as iron stores become critically low. RDW quantifies this variation in cell size and rises before MCV drops below the reference threshold. This supports the recommendation for combined hemoglobin and RDW screening as a practical method for detecting iron deficiency anemia (IDA). However, it is important to note that an elevated RDW is not specific to IDA, as it may also occur in cases of mixed nutritional deficiencies, myelodysplastic syndromes, hemolytic anaemias, and post-transfusion states. If coexisting causes are suspected, confirmatory testing with serum ferritin and vitamin B12 levels is advised. Additionally, the mean platelet count of 387.5 × 10^3/µL, which approaches thrombocytosis, along with instances of overt thrombocytosis in some participants, provides further diagnostic support. Reactive thrombocytosis has been reported in 50–60% of patients with IDA and typically resolves with iron supplementation, distinguishing it from primary thrombocythemia. The combination of high RDW, low haemoglobin, and reactive thrombocytosis forms a recognisable haematological pattern that strongly suggests IDA, which can be clinically useful in the absence of confirmatory biochemical iron studies. These findings have important implications for clinical practice and public health policy. They strengthen the argument for incorporating RDW as a standard interpretive component of Complete Blood Count (CBC) reporting, alongside haemoglobin, MCV, and mean corpuscular haemoglobin (MCH). Furthermore, they underscore the persistently high burden of IDA among women of reproductive age in Pakistan, reinforcing the need for targeted nutritional interventions,

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prophylactic iron supplementation, and dietary education. Importantly, these results demonstrate that CBC—available universally across all levels of the Pakistani healthcare system—serves as a sufficient and effective first-line screening tool for IDA, without the necessity for costly biochemical iron studies.

These findings are contextualized within a well-documented regional epidemiological framework. According to the Pakistan National Nutrition Survey (NNS) of 2018, the prevalence of anaemia among women aged 15–49 is 42.7%, with IDA identified as the predominant form. Dietary deficiencies, particularly insufficient consumption of fruits and vegetables, are major contributing factors. Facility-based studies report even higher prevalence rates; for example, a study at Jinnah Postgraduate Medical Centre found an anaemia prevalence of 71.5%, with microcytic anaemia being particularly common in the 14–18 age group. Similarly, a study in rural Thatta reported that 61.3% of non-pregnant married women were anemic. This discrepancy from national survey data reflects the well-recognized clinical enrichment bias. Encouragingly, national data indicate a modest improvement, with anemia prevalence decreasing from 50.5% in 2011 to 42.7% in 2018, and mean hemoglobin levels rising from 11.7 ± 1.8 to 12.1 ± 1.6 g/dL ($p < 0.01$). Regionally, pooled analyses across South and Southeast Asia reported an anemia prevalence of 50.17%, with rates ranging from 13.3% in the Philippines to 70.3% in Nepal. A multi-country Demographic and Health Surveys (DHS) analysis indicated an overall prevalence of 52.5%, with India contributing the highest burden at 57%. This regional evidence positions the current findings within a continued public health crisis and emphasizes the urgent need for accessible CBC-based diagnostic strategies, especially where advanced iron studies are unavailable to the majority of the population.

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