Section: General Medicine

Original Research Article

A DESCRIPTIVE OBSERVATIONAL STUDY FOR ASSESSMENT OF AGREEMENT BETWEEN BEDSIDE PROVISIONAL DIAGNOSIS OF ANEMIA ON THE BASIS OF COMPLETE BLOOD COUNT BY AUTOMATED HEMATOLOGY ANALYZER AND FINAL DIAGNOSIS OF ANEMIA IN SMS HOSPITAL, JAIPUR

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ABSTRACT

Background: Anemia, a global health concern affecting 2.36 billion people, is often diagnosed using automated complete blood count (CBC) at bedside, but its agreement with final diagnosis based on biochemical parameters remains underexplored. This study assesses the diagnostic concordance between bedside CBC-based provisional anemia diagnosis and final diagnosis at SMS Hospital, Jaipur.

Materials and Methods: A descriptive observational study was conducted from January 2023 to December 2024, involving 100 patients (>18 years) with newly diagnosed anemia based on CBC. Patients with acute blood loss, recent transfusions, acute leukaemia, or hematinic therapy were excluded. Bedside diagnoses using CBC parameters (e.g., hemoglobin, MCV, RDW) were compared with final diagnoses using biochemical tests (e.g., ferritin, B12, folate) and additional investigations (e.g., bone marrow aspiration, endoscopy). Kappa statistics assessed agreement, Pearson's correlation evaluated Ret-He and ferritin, and sensitivity/specificity of RDW and Ret-He were calculated. Immature reticulocyte fraction (IRF) changes during treatment were monitored.

Results: Of 100 patients (62% female, mean age 42.3 ± 15.7 years), 65% were rural, and 64% were from lower socioeconomic classes. Moderate anemia (7-9.9 g/dL) was most common (62%), with microcytic hypochromic anemia (45%) predominant, especially in females. Agreement between bedside and final diagnoses was highest for iron deficiency anemia (IDA) (90%, Kappa = 0.70), followed by B12 deficiency (85.7%, Kappa = 0.68) and folate deficiency (80%, Kappa = 0.60). Lower agreement was noted for chronic disease (75%, Kappa = 0.55) and hemolytic anemia (70%, Kappa = 0.50). RDW showed 88.9% sensitivity and 79.2% specificity for IDA. Ret-He correlated strongly with ferritin ($r = 0.78$, $p < 0.001$), with a ≤25 pg cutoff yielding 91.1% sensitivity and 83% specificity. IRF increased 146.2% by week 4 of IDA treatment ($p = 0.001$). Chronic kidney disease (11.4%) and gastrointestinal bleeding (13.6%) were key contributors.

Conclusion: Bedside CBC is a sensitive screening tool, particularly for nutritional anemias, but requires peripheral blood smear and biochemical confirmation due to low specificity. RDW, Ret-He, and IRF enhance diagnostic and monitoring accuracy. Targeted interventions for IDA and socioeconomic factors are crucial in resource-limited settings.

Keywords: Anemia, Complete Blood Count, Iron Deficiency, RDW, Ret-He, Diagnostic Agreement.

INTRODUCTION

Anemia is defined as a condition where the number of red blood cells or their hemoglobin concentration is below normal levels, impairing the blood's oxygen-carrying capacity to tissues. This leads to symptoms including fatigue, weakness, dizziness, and shortness of breath. Optimal hemoglobin levels vary by factors such as age, sex, altitude, smoking, and pregnancy. Common causes encompass nutritional deficiencies (e.g., iron, folate, vitamin B12, and vitamin A), hemoglobinopathies, and infections like malaria, tuberculosis, HIV, and parasitic diseases.[1]

Globally, anemia impacts approximately 2.36 billion people, disproportionately affecting women and children, with South Asia bearing the highest burden due to gaps in nutrition interventions.[2] Root causes include increased red blood cell destruction (e.g., from malaria), blood loss (e.g., helminthiasis or hemorrhage), and reduced production (e.g., from iron or vitamin B12 deficiencies and chronic infections). Iron deficiency accounts for about 50% of cases, exacerbated by poor dietary iron bioavailability in phytate- and fiber-rich diets.[3,4] In pregnancy, anemia doubles maternal mortality risk,[5] while broader consequences include motor/mental impairments in children and reduced adult productivity, costing up to 4% of GDP.[6]

In India, high anemia prevalence stems from low micronutrient levels and socioeconomic factors, necessitating a comprehensive understanding of causes for effective management.[7] WHO diagnostic criteria include hemoglobin <11 g/dL for children 6 months to 5 years and <12 g/dL for 6-14 years, though values vary by ethnicity, gender, and physiology.[8,9] Modern classification relies on blood indices (MCV, MCH, MCHC) and reticulocyte production index, with MCV and RDW enabling six categories for efficient differential diagnosis via automated analyzers.[10]

Automated complete blood count (CBC) provides valuable data for provisional bedside anemia diagnosis, yet clinicians often overlook it in favor of costly investigations. This study assesses agreement between CBC-based provisional diagnoses and final diagnoses using biochemical parameters, aiming to develop a bedside algorithm to minimize expensive tests. No similar study exists to our knowledge. Present study was conducted to assess agreement between bedside provisional diagnosis of anemia on the basis of complete blood count by automated hematology analyzer and final diagnosis of anemia.

Objectives

1. To determine the prevalence of different types of anemia in SMS Jaipur.
2. To determine the usefulness of RDW and RBC Histogram in diagnosis of anemia.
3. To correlate Ret-He equivalent with serum ferritin in diagnosis of iron deficiency anemia.
4. To see how early IRF value changes during treatment.

MATERIALS AND METHODS

Study Universe and Design: The study universe comprised all patients attending medicine IPD and OPD at S.M.S. Hospital, Jaipur. This hospital-based, descriptive observational study was conducted in the Department of Medicine, S.M.S. Medical College, Jaipur (Rajasthan), India, from January 2023 to December 2024.

Sample Size: A sample of 100 suspected anemia cases was calculated at 95% confidence, assuming 50% agreement between CBC parameters and biochemical parameters, with a 10% absolute allowable error (to maximize sample size).

Method of Data Collection: Simple random sampling was used. After informed written consent, patients meeting inclusion criteria were evaluated via:

1. Screening for clinical anemia evidence using a prestructured proforma, emphasizing history of melena, menorrhagia, hematuria, rectal bleeding, diet, addictions, steatorrhea, and bone pain.
2. General physical examination focusing on pallor, lymphadenopathy, nail changes, knuckle pigmentation, and rash.
3. Systemic examination stressing hepatosplenomegaly.
4. CBC analysis for provisional diagnosis.
5. Additional investigations (e.g., ferritin, folate, vitamin B12, urine/stool tests, LFT, RFT, tTG IgA, Coombs test, ANA, LDH, infections like malaria/brucella/leptospira, bone marrow aspiration/biopsy if needed) for final diagnosis.
6. Comparison of provisional and final diagnoses.

Inclusion Criteria

1. Newly diagnosed anemia patients based on CBC.
2. Patients >18 years, both sexes.
3. Willing participants.

Exclusion Criteria

1. Acute blood loss.
2. Blood transfusion within 3 months.
3. Acute leukaemia.
4. Patients on hematinic therapy.

Ethics Clearance: Clearance was obtained from the Institutional Research Review Board and Ethics Committee. Permissions from the Head of Department were secured. Participants received information sheets, provided written consent, and had confidentiality maintained.

Study Tools

1. General information schedule.
2. CBC by automated analyzer.
3. Chest X-ray and ECG.
4. Biochemical tests (e.g., ferritin, folate, B12, etc.) and bone marrow if required.

Outcome Variables: Proportions of individuals and mean ± SD for quantitative variables.

Statistical Analysis

Continuous data summarized as mean ± SD, counts as proportions. Kappa statistics assessed agreement between bedside and final diagnoses. Pearson's correlation evaluated continuous variables. Significance level: 95%.

RESULTS

The study included 100 patients (62 females, 38 males) with a mean age of 42.3 ± 15.7 years. Anemia was more prevalent in females, especially pre-menopausal (18-40 years: 25 females vs. 10 males), and distributed evenly in males across ages. Rural residence dominated (65%), with mean BMI 22.8 ± 1.5. Comorbidities were low: smokers (6%), alcoholics (8%), COPD (3%), diabetes (6%), hypertension (15%). Most patients (64%) were from lower socioeconomic classes (Class III: 26%, Class IV: 38%).

Fatigue (93.2%) and pallor (88.6%) were the most common symptoms, followed by dyspnea (51.1%), dizziness (34.1%), menorrhagia (32.5% of females), and melena (17%). Physical findings included pallor (91%), nail changes (25%), hepatosplenomegaly (18%), knuckle pigmentation (12%), and lymphadenopathy (11%). CBC parameters showed mean hemoglobin 8.5 ± 1.9 g/dL, MCV 75.2 ± 12.3 fL, MCH 24.8 ± 5.1 pg, MCHC 30.1 ± 3.2 g/dL, RDW-CV 18.5 ± 3.4%, RDW-SD 20.8 ± 4.32, RBC count 4.2 ± 0.8 × 10^6/µL, TLC 7.6 ± 2.1, platelets 1.9 ± 0.6 lakh, and reticulocyte count 0.3 ± 0.16%.

Biochemical parameters included mean ferritin 45.3 ± 20.1 ng/mL, B12 320.5 ± 112.2 pg/mL, folate 8.2 ± 3.5 ng/mL, LDH 251.7 ± 80.4 U/L, and bilirubin 1.2 ± 0.4 mg/dL.

Additional investigations revealed: stool occult blood positive in 24.4% (12/49), upper GI endoscopy in 36.3% (8/22), colonoscopy in 31.3% (5/16), bone marrow aspiration in 63.7% (7/11), Coombs test in 20.7% (6/29), and ANA in 16.6% (4/24).

Underlying conditions included chronic kidney disease (11.4%), gastrointestinal bleeding (13.6%), chronic liver disease (9.1%), autoimmune disorders (6.8%), and infections (5.7%).

Microcytic hypochromic anemia was most common (45%, 34 females vs. 11 males), followed by macrocytic (22%, 8 females vs. 14 males), dimorphic (14%), normocytic normochromic (11%), and hemolytic (8%). Severe anemia (<7 g/dL) correlated with low ferritin (<100 ng/mL in 9/11 cases) and folate deficiency (<5 ng/mL in 6/8 deficient cases). B12 deficiency was more in moderate anemia (15 cases).

Table 1: Distribution of Cases according to Severity of Anemia

Grades of Anemia	Female Patients	Male Patients	Total
Mild (>10 g/dL)	4	21	25
Moderate (7-9.9 g/dL)	46	16	62
Severe (<7 g/dL)	12	1	13
Total	62	38	100

Table 2: Morphological Spectrum of Anemia in this Study

PBF Typing	Female	Male	Total
Dimorphic Anemia	6	8	14
Hemolytic Anemia	5	3	8
Macrocytic Anemia	8	14	22
Microcytic Hypochromic Anemia	34	11	45
Normocytic Normochromic Anemia	9	2	11
Total	62	38	100

Table 3: Diagnostic Utility of RDW in Anemia Diagnosis

Anemia Type	Mean RDW-CV (%)	SD	Normal RDW-CV (n)	Elevated RDW-CV (n)	p-value
Iron Deficiency Anemia	18.5	2.1	5	40	0.001
Vitamin B12 Deficiency	17.8	1.9	3	15	0.001
Folate Deficiency	17.2	1.8	2	6	0.001
Anemia of Chronic Disease	14.5	1.2	12	3	0.01
Hemolytic Anemia	16.8	1.7	3	5	0.1

Table 4: RBC Histogram Patterns by Anemia Type

Anemia Type	Left Shift (n)	Right Shift (n)	Normal Curve (n)	Bimodal Curve (n)	p-value
Iron Deficiency Anemia	38	2	3	2	0.001
Vitamin B12 Deficiency	2	14	1	1	0.001
Folate Deficiency	1	6	1	0	0.001
Anemia of Chronic Disease	5	3	7	0	0.2
Hemolytic Anemia	4	2	1	1	0.26

Table 5: Sensitivity and Specificity of RDW for Iron Deficiency Anemia

Parameter	Value
True Positives (TP)	40
True Negatives (TN)	38
False Positives (FP)	10
False Negatives (FN)	5

Sensitivity	88.9%
Specificity	79.2%
Positive Predictive Value	80.0%
Negative Predictive Value	88.4%

Table 6: Correlation between Ret-He and Serum Ferritin in Iron Deficiency Anemia

Parameter	Mean ± SD	Pearson Correlation (r)	p-value
Ret-He (pg)	22.5 ± 3.2	0.78	<0.001
Serum Ferritin (ng/mL)	12.8 ± 5.1		

Table 7: Ret-He Threshold for Iron Deficiency Anemia

Ret-He Cutoff (pg)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≤25	91.1	83.0	85.4	89.5
≤22	80.0	90.6	90.0	81.0

Table 8: IRF Changes During Iron Deficiency Anemia Treatment

Time Point	Mean IRF (%)	SD	Change from Baseline (%)	p-value
Baseline	5.2	1.8	-	-
Week 1	7.8	2.1	+50.0%	0.01
Week 2	10.5	2.4	+101.9%	0.001
Week 4	12.8	2.7	+146.2%	0.001

Table 9: The Agreement between Bedside Diagnosis of Anemia and Final Diagnosis

Anemia Type	Bedside Diagnosis (n)	Final Diagnosis (n)	Agreement (%)	Kappa
Iron Deficiency Anemia	50	45	90.0%	0.70
Vitamin B12 Deficiency	21	18	85.7%	0.68
Folate Deficiency	10	8	80.0%	0.60
Anemia of Chronic Disease	12	15	75.0%	0.55
Others (e.g., Hemolytic)	9	12	70.0%	0.50

DISCUSSION

The present study evaluated the agreement between bedside provisional diagnosis of anemia based on complete blood count (CBC) parameters obtained from automated hematology analyzers and the final diagnosis established through biochemical and ancillary investigations. The study population demonstrated a female predominance (62%), particularly among premenopausal women, which is consistent with previous studies attributing the higher burden of anemia in women to menstrual blood loss, pregnancy-related iron depletion, and nutritional deficiencies.[11,12]

A majority of patients belonged to rural areas (65%) and lower socioeconomic strata (64%). This finding highlights the strong influence of socioeconomic determinants on anemia prevalence, including poor nutritional intake, inadequate healthcare access, recurrent infections, and delayed diagnosis. Previous epidemiological studies have consistently demonstrated a significant association between low socioeconomic status and increased risk of anemia, particularly in developing countries.[13]

Moderate anemia was the most common presentation in the present study, accounting for 62% of cases. Morphologically, microcytic hypochromic anemia was the predominant subtype (45%), reflecting the high burden of iron deficiency anemia (IDA). Similar observations have been reported in hospital-based studies where iron deficiency remains the leading cause of anemia, especially among women of reproductive age.[14,15]

Macrocytic anemia constituted 22% of cases and was more frequent among male patients. Vitamin B12 deficiency was identified more commonly than folate deficiency, a pattern that has been observed in several contemporary studies from developing countries where dietary deficiencies and malabsorption syndromes remain prevalent.[16] In addition, chronic disease-related etiologies including chronic kidney disease, chronic liver disease, autoimmune disorders, and gastrointestinal blood loss contributed significantly to anemia burden, emphasizing the multifactorial nature of anemia in tertiary care settings.[15]

The agreement between bedside CBC-based diagnosis and final diagnosis was highest for iron deficiency anemia (90%, Kappa = 0.70), followed by vitamin B12 deficiency anemia (85.7%, Kappa = 0.68) and folate deficiency anemia (80%, Kappa = 0.60). Lower agreement was observed for anemia of chronic disease and hemolytic anemia. These findings suggest that CBC parameters are particularly useful in identifying nutritional anemias but are less reliable in complex etiologies where biochemical confirmation is required. Similar observations have been reported by studies comparing automated analyzer-based diagnosis with definitive laboratory investigations.[14,17]

Peripheral blood smear examination remains an indispensable adjunct to automated hematology analyzers. Although modern analyzers provide rapid and reproducible data, morphological examination continues to play a critical role in identifying dimorphic populations, hemolytic features, abnormal

red cell morphology, and other diagnostic clues that may not be fully captured by automated systems.[18]

Red cell distribution width (RDW) demonstrated excellent utility in nutritional anemia. Elevated RDW was significantly associated with iron deficiency, vitamin B12 deficiency, and folate deficiency. In the present study, RDW exhibited a sensitivity of 88.9% and specificity of 79.2% for diagnosing iron deficiency anemia. These findings are consistent with previous reports suggesting that anisocytosis represented by increased RDW is an early marker of nutritional deficiency states.[19,20]

The RBC histogram also showed characteristic patterns across different anemia subtypes. A left-shifted histogram predominated in iron deficiency anemia, whereas right-shifted curves were commonly observed in macrocytic anemias due to vitamin B12 and folate deficiency. These histogram patterns may provide useful bedside clues for preliminary categorization of anemia and support the diagnostic interpretation of CBC results.[18,20]

Serum ferritin remained the most reliable biochemical marker for confirming iron deficiency anemia. Reticulocyte hemoglobin equivalent (Ret-He) demonstrated a strong positive correlation with serum ferritin levels ($r = 0.78$, $p < 0.001$), supporting its utility as a rapid and non-invasive marker of functional iron availability. The high sensitivity and specificity observed at a cutoff value of ≤25 pg further support the incorporation of Ret-He into routine anemia evaluation algorithms.[15]

Another important observation was the progressive increase in immature reticulocyte fraction (IRF) during treatment of iron deficiency anemia. IRF increased significantly from baseline by week 1 and continued to rise through week 4, indicating early marrow response to therapy. Previous studies have similarly demonstrated that IRF serves as an early indicator of erythropoietic recovery and may be useful for monitoring treatment response before changes in hemoglobin become evident.[21,22]

The findings of the present study have important clinical implications. In resource-limited settings, bedside CBC interpretation supported by RDW, RBC histogram analysis, Ret-He, and IRF can substantially improve diagnostic accuracy while reducing unnecessary investigations. Nevertheless, biochemical confirmation remains essential for definitive diagnosis, particularly in anemia of chronic disease, hemolytic anemia, and mixed deficiency states. Public health measures focusing on iron supplementation, nutritional education, food fortification, and early screening among high-risk populations are necessary to reduce the burden of anemia.[12,13]

The study has certain limitations. Being a single-center hospital-based study, the findings may not be generalizable to the broader population. Automated hematology analyzers may occasionally overestimate diagnostic accuracy, particularly in mixed anemia states.[23] Furthermore, long-term follow-up and cost-effectiveness analyses were beyond the scope of the present study and warrant future investigation.[24,25]

CONCLUSION

In 100 patients (62% female, mean age 42.3 years), moderate anemia (62%) and IDA (45%) predominated, with rural/low-SES bias. CBC showed high sensitivity/low specificity, best agreement for nutritional anemias (80-90%). RDW, histograms, Ret-He, and IRF enhanced diagnostics. Confirmatory tests are vital.

Bedside CBC is useful for screening but requires integration with PBS and biomarkers for accuracy, especially in resource-limited settings. Targeted interventions for IDA and socioeconomic drivers are recommended to curb anemia.

Recommendations

- Enhance diagnostics with CBC screening, PBS confirmation, RDW/Ret-He for IDA, and micronutrient tests.
- Strengthen assessments via history/exams and targeted investigations.
- Prioritize treatments for IDA/chronic causes, monitoring with IRF.
- Implement public health measures like fortification and awareness.
- Train on tools and research cost/long-term impacts.

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