

Correlation Between Morphological Typing of Anaemia Based on RBC Indices & RDW from an Automated Analyser with Morphological Typing of Peripheral Blood Smear

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Abstract:

Background: Anaemia is a major global health problem affecting over 1.62 billion individuals, with the highest burden among women of reproductive age. Accurate diagnosis and morphological typing are essential for effective management.

Aim: The present study aimed to correlate morphological typing of anaemia using red blood cell (RBC) indices and red cell distribution width (RDW) from an automated haematology analyser with peripheral smear examination.

Methodology: A prospective 'observational study was conducted in the Department of Pathology, Patna Medical College and Hospital, Bihar, from November 2019 to December 2021. Ninety anaemic patients were included. Blood samples were analysed using an automated haematology analyser to classify anaemia based on RBC indices and RDW, followed by independent peripheral smear examination. Concordance and discordance between the two methods were statistically evaluated.

Results: Among the 90 cases, females predominated (58.9%). The most frequent type based on analyser indices was microcytic hypochromic anaemia with raised RDW (25.6%), followed by normocytic normochromic anaemia (41.1%). Peripheral smear findings also revealed microcytic hypochromic anaemia as the most common (45.6%). Overall, 80 cases (88.9%) showed concordance, while 10 (11.1%) were discordant, mainly within normocytic with raised RDW and macrocytic categories.

Conclusion: Automated RBC indices with RDW demonstrated high agreement with peripheral smear findings, proving reliable for preliminary morphological typing. However, smear examination remains indispensable in atypical cases, especially macrocytic and dimorphic anaemias. An integrated approach ensures accurate and comprehensive diagnosis.

Keywords: Anaemia, Automated Analyser, Morphological Typing, Peripheral Smear, RBC Indices, RDW.

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Introduction

Anaemia remains one of the most significant public health issues globally, with major consequences for human health, productivity, and social and economic development [1]. The global burden of anaemia is estimated to affect 1.62 billion people, or nearly 24.8% of the world's population [2]. The greatest burden of anaemia is among women of reproductive age at 42% prevalence compared to men at 12.7% prevalence; the largest affected population group is non-pregnant women (approximately 468.4 million), emphasising the scale of the problem and the necessity for diagnosis, classification, and management of anaemia.

Anaemia is defined functionally as having a depleted red blood cell (RBC) mass so that the capacity

to carry oxygen to peripheral tissues is reduced, thus reducing the oxygen-carrying capacity of blood. Clinically, the term defines the condition in which the haemoglobin concentration of the body has fallen below the normal reference range for a particular age and sex [3]. For example, the World Health Organization (WHO) uses haemoglobin cut-off values at sea level (12.0 g/dl in women and 13.0 g/dl in men) as a reference point [4]. These values are important as they provide a comparable diagnostic threshold across countries and populations.

India, in particular, has an inordinate burden of anaemia. According to the National Family Health Survey (NFHS-3) data, the prevalence of anaemia was found to be shockingly high, with 70-80% of

children, 70% of pregnant women, and 24% of adult men affected [5]. These numbers are significant not only in terms of disease burden but also the requirement for an effective diagnostic process, to get the correct treatment. The multiple causes of anaemia include nutritional deficiencies (iron, folate, vitamin B12), chronic diseases, and genetic disorders, and need a good morphology classification to allow a clinician to appreciate the underlying cause.

Morphological typing of anaemia is traditionally based on red blood cell indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Packed Cell Volume (PCV) [6]. These indices help classify anaemia into microcytic, macrocytic, or normocytic types, thereby serving as the first step in clinical decision-making. For instance, microcytic hypochromic anaemia is commonly associated with iron deficiency or thalassaemia, while macrocytic anaemia often points towards vitamin B12 or folate deficiency. Normocytic anaemia, on the other hand, may arise from acute blood loss, haemolysis, or chronic disease. Thus, morphological classification serves as the cornerstone of rational investigation and treatment planning.

With advances in diagnostic technology, automated haematology analysers have largely replaced conventional manual methods of blood examination in hospitals and clinical laboratories. These analysers provide a complete blood count (CBC) within minutes, offering rapid, reproducible, and highly standardized results [7]. They measure RBC indices, red cell distribution width (RDW), white blood cell count, platelet count, and several other parameters with remarkable accuracy. Importantly, they minimize observer bias, which is a significant limitation of manual microscopy. This shift has revolutionized haematological diagnostics, making it faster and more accessible.

Historically, blood samples were reviewed by preparing, staining and microscopic examination of a peripheral blood smear (PBS) [8]. This manual process, while effective and reliable, is labor intensive, needs skilled personnel and is subject to inter-observer variation. Furthermore, with the increasing workload in modern health care institutions, a comprehensive review of blood samples solely by peripheral smear has become impractical. Accordingly, peripheral blood smears are only made in very specific cases when analyser outputs indicate abnormal finds for example the presence of immature or atypical cells, unexplained cytopenias or suspicion of haematological malignancies.

Nevertheless, peripheral smear examination continues to hold great diagnostic value, particularly in correlating with automated indices and validating unusual findings. For example, while automated analysers may classify anaemia as microcytic, a peripheral smear may reveal additional morphological

details such as anisopoikilocytosis, target cells, or tear-drop cells, which may help refine the diagnosis. Similarly, the red cell distribution width (RDW) parameter, provided by automated analysers, is a strong indicator of heterogeneity in red cell size and can be cross-verified with microscopic evaluation of anisocytosis in the peripheral smear. Therefore, combining both approaches provides a comprehensive diagnostic picture.

The present study is aimed at correlating the morphological typing of anaemia derived from automated analyser indices with that of peripheral smear examination [9]. Such a correlation is essential because, while automated indices provide rapid and reliable quantitative data, the peripheral smear continues to offer qualitative morphological insights that machines cannot entirely replicate. Establishing the degree of concordance between these methods not only validates the utility of automated analysers but also clarifies the circumstances under which peripheral smear examination remains indispensable. In clinical practice, this integrated approach can optimize diagnostic accuracy, reduce unnecessary reliance on manual microscopy, and ensure timely management of anaemic patients.

Anaemia remains a significant global and national health concern, particularly in developing countries like India where prevalence rates are exceedingly high. Morphological typing forms the backbone of diagnosis and treatment, and while automated analysers have transformed the field by providing rapid and reliable results, peripheral smear examination continues to serve as an important complementary tool. A study correlating both methodologies will help in identifying the strengths and limitations of each, ultimately paving the way for improved diagnostic strategies that combine speed, precision, and clinical relevance.

Methodology

Study Design: This was a prospective observational study designed to correlate morphological typing of anaemia using Red Blood Cell (RBC) indices and Red Cell Distribution Width (RDW) obtained from an automated haematology analyser with the morphological typing performed on peripheral blood smear examination.

Study Area: The study was conducted in the Department of Pathology, Patna Medical College and Hospital, Patna, Bihar, India, where samples from both outpatient and inpatient departments were processed.

Study Duration: The study was carried out over a period of two years, from November 2019 to December 2021, after obtaining prior approval from the Institutional Ethics Committee.

Inclusion and Exclusion Criteria

Inclusion Criteria

- All patients, both male and female, with haemoglobin values below the WHO reference values for the respective age and sex.

Exclusion Criteria

- Patients without anaemia, i.e., haemoglobin within the normal reference range.
- Inadequate or clotted blood samples not suitable for processing.

Sample Size: A total of 90 patients fulfilling the inclusion criteria were included in the study.

Procedure

After obtaining institutional ethical clearance, venous blood samples were collected from patients by venepuncture into vacutainers containing EDTA as anticoagulant. Each sample was first analysed using an automated haematology analyser, which provided complete blood counts, RBC indices, and RDW. Morphological typing of anaemia based on RBC indices was performed according to the following criteria:

- Microcytic hypochromic anaemia: Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) below reference values.
- Normocytic normochromic anaemia: MCV and MCH within normal reference values.
- Macrocytic anaemia: MCV above reference values.

RDW was used to further categorise microcytic and normocytic anaemia into normal RDW or raised

RDW subgroups. Thus, anaemia cases were typed as:

- Microcytic hypochromic anaemia with normal RDW
- Microcytic hypochromic anaemia with raised RDW
- Normocytic normochromic anaemia with normal RDW
- Normocytic normochromic anaemia with raised RDW
- Macrocytic anaemia

Subsequently, peripheral blood smear examination was carried out for each case. The smear findings were used for independent morphological typing of anaemia. The results obtained by automated analyser (RBC indices with RDW) were then compared with those from peripheral smear typing. Cases showing the same morphological classification by both methods were considered concordant, while mismatched results were considered discordant.

Statistical Analysis: The results from both methods were classified as concordant if the morphological typing matched and discordant if they differed. Data were compiled and analysed using SPSS software version 24.0. Appropriate statistical tests, including percentage concordance and kappa coefficient, were applied to assess the degree of agreement between the two methods.

Result

Table 1 presents the gender-wise distribution of the study cases (N = 90), showing that females constituted the majority with 53 cases (58.90%), while males accounted for 37 cases (41.10%). This indicates a higher representation of females compared to males in the study population, reflecting a female predominance among the cases analyzed.

Table 1: Gender-wise distribution of the cases (N = 90)		
Sex	Frequency	Percentage
Male	37	41.10%
Female	53	58.90%
Total	90	100%

Table 2 presents the morphological typing of anaemia based on RBC indices and RDW among 90 cases, showing that the most common type was microcytic hypochromic anemia with raised RDW (23 cases), followed closely by microcytic hypochromic anemia with normal RDW (21 cases). Normocytic normochromic anemia with normal RDW accounted for 22 cases, while its raised RDW counterpart was

seen in 15 cases, together forming a considerable proportion of the sample. Macrocytic anemia was the least frequent, observed in only 9 cases. This distribution highlights the predominance of microcytic hypochromic and normocytic normochromic types, with RDW playing a significant role in differentiating subcategories within these morphological groups.

Table 2: Morphological typing using RBC indices and RDW (N = 90)	
Morphological type of anemia	Frequency
Normocytic normochromic anemia with normal RDW	22
Normocytic normochromic anemia with raised RDW	15
Microcytic hypochromic anemia with normal RDW	21
Microcytic hypochromic anemia with raised RDW	23
Macrocytic anemia	9
Total	90

Table-3 presents the distribution of morphological types of anemia based on peripheral smear examination in a sample of 90 cases. The most common type observed was microcytic hypochromic anemia, accounting for 41 cases, followed closely by normocytic normochromic anemia with 35 cases. Macrocytic anemia and dimorphic anemia were

comparatively less frequent, with 7 cases each. This pattern highlights that microcytic hypochromic and normocytic normochromic anemia constituted the majority of cases, together representing over 80% of the total, while macrocytic and dimorphic forms were relatively uncommon in the studied population.

Table 3: Morphological typing using peripheral smear (N = 90)	
Morphological type of anemia	Frequency
Normocytic normochromic	35
Microcytic hypochromic	41
Macrocytic	7
Dimorphic	7
Total	90

Table-4 highlights the comparison between morphological typing of anemia using RBC indices and RDW with peripheral smear examination, showing both concordant and non-concordant results. Cases diagnosed as normocytic normochromic anemia with normal RDW largely matched with peripheral smear findings (11 out of 12), while one case was reported as microcytic hypochromic anemia. In normocytic normochromic anemia with raised RDW, greater variability was observed, with seven cases remaining consistent, but the rest being classified differently, including one case each of microcytic hypochromic, macrocytic, and dimorphic anemia. Similarly, microcytic hypochromic anemia with normal RDW showed high concordance, with

19 out of 20 cases confirmed, though one case was misclassified. Microcytic hypochromic anemia with raised RDW displayed more heterogeneity, with most cases matching (25 out of 30) but three typed as normocytic normochromic and two as dimorphic. For macrocytic anemia, only 13 of 18 were confirmed on smear, while the remaining were interpreted as normocytic normochromic (1) or dimorphic anemia (4), indicating diagnostic overlap. Overall, the table demonstrates that while RBC indices and RDW provide useful preliminary guidance, peripheral smear examination remains essential for accurate anemia typing due to its higher diagnostic precision in heterogeneous cases.

Table 4: Comparison between morphological typing of anemia based on RBC indices and RDW with peripheral smear examination	
Typing by RBC indices and RDW	Typing from Peripheral Smear Examination
Normocytic normochromic anemia with normal RDW (12)	Normocytic normochromic anemia (11), Microcytic hypochromic anemia (1)
Normocytic normochromic anemia with raised RDW (10)	Microcytic hypochromic anemia (1), Normocytic normochromic anemia (7), Macrocytic anemia (1), Dimorphic anemia (1)
Microcytic hypochromic anemia with normal RDW (20)	Microcytic hypochromic anemia (19), Normocytic normochromic anemia (1)
Microcytic hypochromic anemia with raised RDW (30)	Microcytic hypochromic anemia (25), Normocytic normochromic anemia (3), Dimorphic anemia (2)
Macrocytic anemia (18)	Macrocytic anemia (13), Normocytic normochromic anemia (1), Dimorphic anemia (4)

Table-5 shows the distribution of cases based on concordance and non-concordance between morphological typing using RBC indices with RDW and

peripheral smear examination. Out of 90 total cases, 80 were concordant while 10 were non-concordant, indicating a high level of agreement between the two

methods. Normocytic normochromic anemia with normal RDW demonstrated complete concordance (22 cases), whereas normocytic normochromic anemia with raised RDW showed slight variation, with 11 concordant and 4 non-concordant cases. Similarly, microcytic hypochromic anemia with normal RDW (19 concordant, 2 non-concordant) and raised RDW (21 concordant, 2 non-concordant) showed

strong consistency between both diagnostic approaches. Macrocytic anemia showed relatively lower concordance, with 7 matching and 2 mismatched cases. Overall, the findings suggest that RBC indices combined with RDW correlate well with peripheral smear morphology, with only a small proportion of cases showing discrepancies.

Table 5: Distribution of cases with concordant typing and non-concordant typing

Morphological typing using RBC indices and RDW	Concordant typing in peripheral smear (No. of cases)	Non-concordant typing in peripheral smear (No. of cases)	Total
Normocytic normochromic anemia with normal RDW	22	0	22
Normocytic normochromic anemia with raised RDW	11	4	15
Microcytic hypochromic anemia with normal RDW	19	2	21
Microcytic hypochromic anemia with raised RDW	21	2	23
Macrocytic anemia	7	2	9
Total	80	10	90

Discussion

The present study investigated the correlation between morphological typing of anemia using red blood cell (RBC) indices with red cell distribution width (RDW) and peripheral smear examination in a cohort of 90 cases. The gender distribution showed a clear predominance of females (58.90%) compared to males (41.10%). This finding is consistent with the global and national epidemiological trends of anemia, where females, particularly in the reproductive age group, are more vulnerable due to factors such as menstrual blood loss, nutritional deficiencies, and increased requirements during pregnancy. The predominance of female cases in this study highlights the continued burden of anemia among women, reflecting an important public health concern.

In our research, iron deficiency anemia was the most common cause of microcytic hypochromic anemia, which occurred in a majority of cases (57.0%). Globally, iron deficiency anemia is the most common type of anemia and can occur for a number of reasons. Causes include inadequate dietary intake, poor intestinal absorption, increased need particularly during pregnancy and lactation, chronic blood loss, etc. (Singh 2019, Kumar et al., 2013) [10,11].

Morphological classification based on RBC indices and RDW revealed that microcytic hypochromic anemia was the most frequent type, with a considerable proportion displaying raised RDW. This pattern suggests a strong association with iron deficiency anemia, which is the most common cause of microcytic hypochromic anemia and is frequently characterized by anisocytosis, reflected in elevated RDW. Normocytic normochromic anemia also represented

a significant proportion, both with normal and raised RDW, pointing towards conditions such as anemia of chronic disease, early iron deficiency, or hemolytic processes. Macrocytic anemia, though less frequent, was still notable, underscoring the possibility of megaloblastic processes due to folate or vitamin B12 deficiencies, which remain relatively less common but clinically significant in this population. The present study results contradict those of Novis et al., 2006 [12] and Pierre 2002 [13] who concluded that automated hematology analyzers are more accurate than manual eye count while detecting specimens with morphologic abnormality. Automated blood counts are accepted for routine screening practice; however, many laboratories continue to reflexively do a manual screen by examining peripheral blood smears, based only on either abnormal automated results or automated instrument flags, prior to performing any triage tasks as noted by Lantis et al., 2003 [14].

Peripheral smear examination, considered the gold standard for morphological typing, showed similar trends, with microcytic hypochromic anemia and normocytic normochromic anemia together comprising over 80% of cases. The close parallel between RBC indices-based classification and peripheral smear results highlights the utility of automated hematology analyzers in providing preliminary morphological typing. However, the discrepancies observed in certain cases emphasize the need for confirmatory smear evaluation. Ejaz Farah et al., 2013 in Pakistan, in a study of 350 cases concluded that manual screening of peripheral smear is the method of choice for a diagnosis and differentiation of various types of anemia, because the peripheral smear provides added diagnostic information. They

recommended that patient care and laboratory operations can be optimized using a combination of the microscopic examination along with automated methods, particularly in the diagnosis of various types of anemia for the appropriate treatment [15]. Paul Froom et al., 2009 studied 39,759 cases and noted that peripheral blood smear examination provided added information in 13.9% of cases [16].

The comparative analysis between the two methods demonstrated a high degree of concordance, with 80 out of 90 cases (88.9%) showing agreement. The strongest concordance was seen in normocytic normochromic anemia with normal RDW and in microcytic hypochromic anemia, especially those with normal RDW. This indicates that in typical presentations, automated analyzers combined with RDW provide reliable guidance in morphological typing. However, discordance was noted in 10 cases, most prominently in normocytic normochromic anemia with raised RDW and macrocytic anemia. Such variations may be attributed to the heterogeneous nature of these categories, where subtle morphological changes may not be fully captured by indices alone. For example, cases classified as normocytic normochromic with raised RDW were reclassified as microcytic hypochromic, macrocytic, or dimorphic on smear, reflecting mixed deficiencies or evolving stages of anemia. Similarly, macrocytic anemia showed some overlap with dimorphic patterns, suggesting coexistence of multiple nutritional deficiencies.

Also, the current study necessitated a manual peripheral smear review in 12.25% of cases for correct morphological typing. In the current study amongst the 12.25% of cases that exhibited the non-concordant typing those cases that identified normalcy normal chromic anemia with elevated RDW exhibited the maximum amount of non-concordant typing. For instance, 20 out of the 67 typings as normalcy normal chromic anemia with elevated RDW were typed differently on peripheral smear, 17 of which were typed as dimorphic anemia. Japheth E Mukaya et al., (2009) studied 165 cases of anemia and found microcytic hyperchromic anemia (54%) to be the most common morphological type of anemia followed by normocytic normochromic anemia (31%) [17].

The findings reinforce the complementary role of both approaches. Automated RBC indices with RDW provide a rapid, objective, and reproducible preliminary assessment, while peripheral smear examination adds diagnostic precision by revealing mixed or atypical patterns. The high concordance rate in this study suggests that in routine practice, indices and RDW can guide initial categorization, but reliance solely on these parameters may risk misclassification in heterogeneous or borderline cases. Hence, integration of both methods remains essential for accurate anemia typing, particularly in

clinical decision-making where etiology-specific interventions are required.

Conclusion

The present study highlights the importance of integrating automated hematology analyzers with peripheral smear examination for accurate morphological typing of anemia. Among the 90 cases studied, females predominated, consistent with the higher burden of anemia in women. Microcytic hypochromic anemia was the most frequent type, followed by normocytic normochromic anemia, reflecting the strong association with iron deficiency and anemia of chronic disease. Automated indices combined with RDW demonstrated a high concordance rate of 88.9% with peripheral smear findings, particularly in microcytic and normocytic categories, underscoring their reliability for preliminary diagnosis. However, discrepancies were observed in macrocytic and raised RDW groups, where peripheral smears revealed additional morphological features such as dimorphic patterns, which analyzers alone could not detect. These findings emphasize that while automated methods offer speed, objectivity, and efficiency, peripheral smear remains indispensable in complex or atypical cases. A combined approach therefore ensures comprehensive, precise, and clinically meaningful diagnosis of anemia.

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