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Association of red cell distribution width in diagnosis and severity of iron deficiency anaemia

Prabha Panthi¹, Sushna Maharjan², Nirbija Dhakal³, Subash Koirala⁴,
Roshna Thapa⁵

¹Lecturer, ²Professor, ⁵Resident; Department of Pathology Chitwan Medical College and Teaching Hospital, Nepal

³Consultant Hospital for Advanced Medicine and Surgery (HAMS), department of Pathology, Nepal

⁴Lecturer, Tribhuvan University, Department of Community medicine, Nepal



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Abstract

Introduction: Iron deficiency anaemia (IDA) is the most prevalent micronutrient deficiency in the world. Detection of IDA largely depends upon quantification of biochemical markers like serum ferritin, transferrin saturation, which are not routinely available and affordable in developing countries. Our study aims to show relationship between red cell distribution width (RDW) and the severity of IDA.

Method: A cross-sectional study was conducted at Chitwan Medical College from 01 Jun 2022 to 31 May 2023 on patients who presented with microcytic anaemia after receiving ethical clearance from the Institutional Review Committee. Patients with a recent history of iron therapy, blood transfusion or leucocytosis were excluded from the study. Patients' information was retrieved from the Medical Information Data Analysis System software, and IBM SPSS Statistics 20 was used to evaluate the results. The receiver operating characteristic curve was used to assess the efficiency of RDW in diagnosing IDA.

Result: In total, 204 cases were studied and found a significant association between age group and IDA status ($\chi^2=9.55$, $p=0.008$). Receiver's operating characteristic curve found at a cut-off of 14.5% RDW, sensitivity was 97% and specificity 27% and at a cut-off of 21%, sensitivity was 17% and specificity 90%, indicating that with increased value of RDW, the extent of specificity for diagnosis of IDA also increases.

Conclusion: The RDW can be a useful tool to be evaluated in conjunction with other clinical and laboratory results to increase diagnostic accuracy, but it is not a reliable diagnostic tool for IDA on its own.

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Correspondence

Prabha Panthi, Department of Pathology, Chitwan Medical College and Teaching Hospital, Nepal. Email: panthi.prabha@cmc.edu.np, Telephone: +977 9849635074

Introduction

Iron deficiency anaemia (IDA) is the most prevalent micronutrient deficiency in the world. In an underdeveloped nation like Nepal, anaemia is a serious public health issue. Among the women of reproductive age, the prevalence of IDA in South and Southeast Asian countries is 52.5%.¹ The frequency of anaemia among women in the reproductive age group increased from 35% to 41% between 2011 and 2016 in Nepal, indicating that anaemia is a major health issue.^{2,3}

Anaemia is categorised morphologically based on red blood cell (RBC) size and degree of haemoglobinization. Microcytic hypochromic anaemia is caused by thalassemia, iron deficiency, and anaemia of chronic illnesses, with iron deficiency being the most frequent cause.^{4,5} Iron deficiency anaemia can be diagnosed with tests like blood smear, iron profile, and complete blood count. Though bone marrow examination is the gold standard, bone marrow examination and iron profile are often limited by cost or availability.^{6,7} The majority of automated haematolysers determine Red Cell Distribution Width (RDW), equivalent to "anisocytosis" in blood smear, that measures heterogeneity in red cell size.⁸⁻¹⁰

According to several studies, RDW is a more sensitive measure to determine the potential cause of microcytic anaemia; hence, it can be used as a low-cost initial investigation to distinguish IDA from other causes of microcytic anaemias.^{10,11} A significant number of patients with IDA visit our hospital. Therefore, the purpose of this study is to find out the relationship between RDW in the diagnosis and degree of severity of IDA.

Method

This study analysed cases of microcytic anaemia with Mean Corpuscle Volume (MCV) <80 fL and haemoglobin (Hb) <11 g/dL diagnosed at the Pathology Department of Chitwan Medical College Teaching Hospital (CMCTH) from 01 Jun 2022 to 31 May 2023. Ethical clearance was

obtained (Ref: CMC-IRC/078/079-113). All consecutive patients who underwent iron profile testing were included in the study. IDA was diagnosed based on the 2020 WHO guidelines¹², which require transferrin saturation <16% and serum ferritin <12 ng/ml. Patients with a recent history of iron therapy, blood transfusions, or abnormal white blood cell counts were excluded from the analysis.

Patients fulfilling the inclusion criteria for the study were evaluated for RDW, red cell indices like MCV, mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), Hb, serum iron, serum ferritin, serum iron binding capacity, and transferrin saturation. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes to measure Hb, red cell indices, and RDW using an automatic haematology analyser (Yumizen H2500 Horibas). Serum ferritin was analysed by Chemiluminescence immunoassay analyser (CLIA), while serum iron and transferrin iron binding capacity were measured using a Dimension Rxl assay based on photometric principles.

The data were collected from the patients' requisition form for the history (blood transfusion or recent iron therapy), and other values like RBC indices, RDW, White Blood Cells and iron profile test were retrieved from the computer software Medical Information Data Analysis System (MIDAS). Collected information was further processed for descriptive analysis. Frequency, percentage, cross tabulation, measures of central tendency and dispersion were done by using software IBM SPSS Statistics 20. The Shapiro-Wilk test at 5% level of significance was used to assess the normality of the quantitative blood parameters. Descriptive statistics mean±SD/median(IQR) were used for normally distributed/skewed data, respectively. Pearson's chi-square test was used to measure the association between IDA status (presence or absence) and sociodemographic variables (age and sex). A $p \leq 0.05$ was considered statistically significant. Additionally, receiver operating characteristic (ROC) curve analysis of RDW was applied to observe the diagnostic efficiency of RDW for IDA.

Result

In total, 204 samples with Hb <11 g/dl and MCV <80 fl were analysed, of which 47.54% were classified as IDA based on serum ferritin <12 ng/ml and transferrin saturation <16%.

There was a significant association between age group and IDA status ($\chi^2=9.55$, $p=0.008$). Individuals in the 15-49 age group had a high proportion of IDA compared to those in the 0-14 and >50 age groups. The IDA was more common in females compared to males, 50.9% vs 35.6%, but the difference was statistically not significant ($p=0.068$), Table 1.

Since the distribution of data was skewed and had outliers, the median and IQR were used to represent central tendency more accurately.

The median age of individuals with non-IDA (45 years) was higher than those with IDA (38 years). Haemoglobin, MCV, and MCH were lower in IDA, while RDW was slightly higher, indicating greater variation in red blood cell size. Serum iron (18 vs. 28 $\mu\text{g/dL}$), ferritin (5.5 vs. 66 ng/mL), and transferrin saturation (4.29% vs. 11.85%) were significantly lower in IDA. The wide IQRs for serum iron and ferritin in non-IDA suggest a wide range of variability in iron levels, Table 2.

The RDW remained stable across age groups, with slightly higher values and variability in the 15–49 age group. Serum iron levels were consistent, with a slight increase in older adults. Serum ferritin varied mostly in those 50 years and older, while transferrin saturation had the highest median and widest IQR in this group

Between sexes, females had a slightly higher RDW, while serum iron levels were similar. Males exhibited significantly higher and more variable serum ferritin and transferrin saturation. Overall, RDW and serum iron remained steady across groups, while ferritin and transferrin saturation varied more, particularly with age and sex, suggesting differences in iron metabolism and storage, Table 3.

Severe anaemia (<7 g/dl) was the most prevalent, affecting 50.51% of participants. The RDW increased with severity, measuring 17.25 in mild, 17.9 in moderate, and 18.4 in severe cases, indicating greater red blood cell size variation. The high prevalence of moderate and severe anaemia, along with rising RDW, emphasises the importance of proper evaluation and confirms RDW as a reliable marker of anaemia severity, Table 4.

The ROC analysis of RDW for detecting IDA showed an area under the curve (AUC) of 67.7%, indicating a moderate level of diagnostic efficiency, which is statistically significant. With a 95% confidence level, the true AUC lies between 0.603 and 0.751, showing RDW is a reasonably good indicator of IDA. The optimal cut-off value for RDW to predict IDA was found to be 16.85%. Values exceeding 16.85 demonstrated enhanced sensitivity and specificity, with rates of 71% and 58%, respectively. At a cut-off of 14.5% RDW, sensitivity was 97% and specificity was 27%; and at a cut-off of 21% RDW, sensitivity was 17% and specificity was 90%, which indicates that with increasing value of RDW, the specificity for diagnosis of IDA also increases.

Table 1. Socio-demographic variables and iron deficiency anaemia (IDA) status, n=204

Variables	n	IDA n(%)	Non-IDA n(%)	p-value
Age group (years)				
0-14	10	5(50.0)	5(50.0)	
15-49	126	70(55.6)	56(44.4)	0.008
>50	68	22(32.4)	46(67.6)	
Gender				
Male	45	16(35.6)	29(64.4)	0.068
Female	159	81(50.9)	78(49.1)	

Table 2. Clinico-demographics parameters of patients and IDA status, n=204

Parameters	IDA, 97(47.5%) Median (Q1-Q3)	Non-IDA, 107(52.4%) Median (Q1-Q3)
Age (years)	38.0(25.0-47.0)	45.0(30.0-61.0)
Haemoglobin (g/dl)	6.9(5.8-8.0)	7.7(6.6-8.9)
MCV (fl)	63.1(56.7-68.7)	69.9(64.3-76.0)
MCH (pg)	19.8(17.3-21.6)	22.8(20.5-25.1)
RDW (%)	18.0(16.6-19.9)	16.5(14.6-18.6)
Serum iron (ug/dl)	18.0(13.5-24.0)	28.0(16.0-53.0)
Serum ferritin (ng/ml)	5.5(3.4-7.9)	66.0(23.0-259.8)
Transferrin saturation (%)	4.3(3.0-5.6)	11.85(5.74-20.8)

Table 3. Clinico-demographic parameter, RDW and serum iron profile in IDA patients, n=204

Variables	RDW (%)	Serum iron (ug/dl)	Serum ferritin (ng/ml)	Transferrin Saturation (%)
Median (Q1-Q3)				
Age, years				
0-14	16.6(15.0-18.7)	17.5(12.5-34.3)	27.5(7.2-83.8)	5.4(2.9-8.8)
15-49	17.8(15.9-19.9)	21.0(15.0-32.3)	10.4(4.2-56.2)	5.2(3.7-11.0)
≥50	16.8(15.0-18.8)	22.5(14.0-38.0)	25.7(7.8-139.05)	7.7(4.5-20.5)
Sex				
Male	16.5(14.7-18.3)	20.0(14.5-40.0)	28.1(7.0-277.6)	7.9(4.4-17.6)
Female	17.2(16.0-19.5)	21.0(14.0-34.0)	11.4(5.2-61.0)	5.3(3.9-11.9)

Table 4. Severity of anaemia and RDW value in IDA patients, n=97

Severity of anaemia (Hb g/dl)	n(%)	RDW Median (Q3-Q1)
Mild (10-11)	6(6.2)	17.3(17.2-15.0)
Moderate (7-9.9)	42(43.3)	17.9(19.1-15.4)
Severe (<7)	49(50.5)	18.4(19.6-16.0)

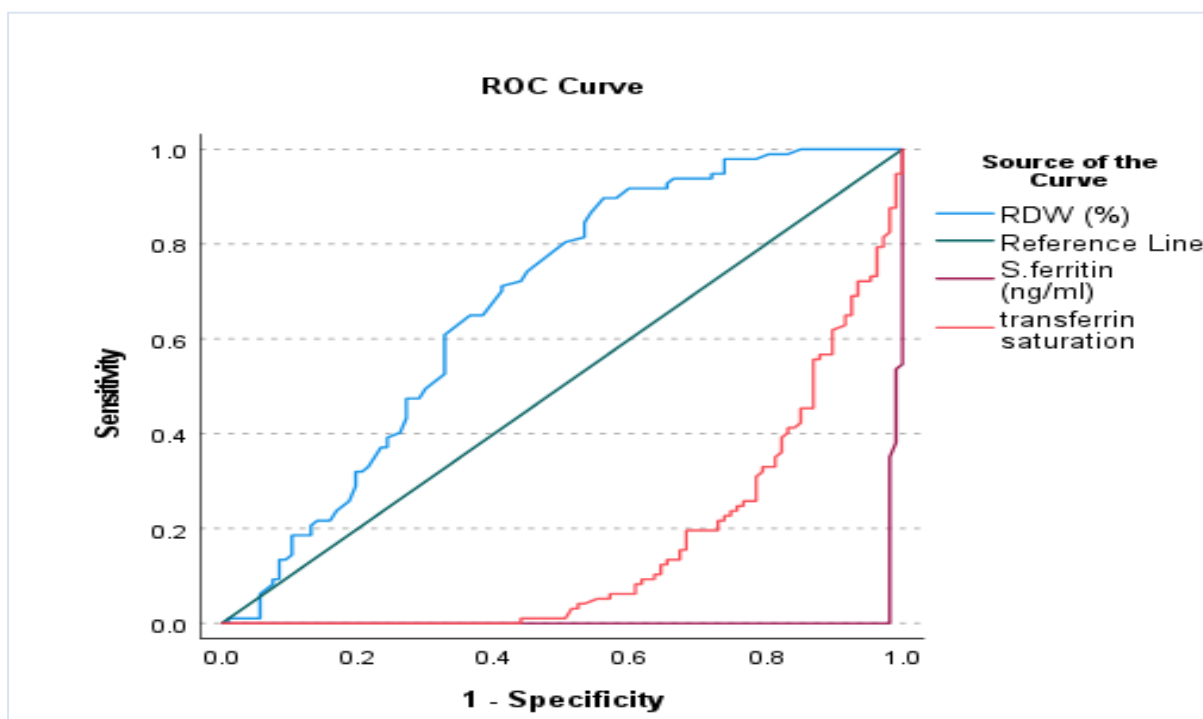


Figure.1 The ROC curve to measure the efficiency of RDW in the diagnosis of IDA, n=204

Discussion

The current study demonstrated a moderate level of statistically significant association between RDW and IDA diagnosis and its status. Individuals with IDA had higher RDW values compared to those without IDA. A similar result was found in a study performed in children between ages 6 months and 5 years, and other studies, with the conclusion that RDW is a relatively highly sensitive and effective tool for early diagnosis of IDA.^{13,14}

Our study found that with an increase in the value of RDW, the specificity for the diagnosis of IDA also increases. As per the ROC curve, with values exceeding 16.85%, there is enhanced sensitivity and specificity with rates of 71% and 58% respectively, and at a cut-off of 21% RDW, sensitivity was 17% and specificity 90%. Similar findings were seen in 1026 Indian patients, which showed that RDW at a cut-off of 16.4%, the sensitivity was 94.2% and at a cut-off of 21%, the specificity was 95%.¹⁵ Also, a study performed in 100 anaemic children, at an RDW cut-off of 18.31%, sensitivity was 92.1% and a specificity of 90.9%.¹¹ However, one of the studies concluded limited sensitivity and specificity for detecting IDA.¹⁶ Overall, our study and studies from different authors concluded that with an increase in RDW value, specificity for IDA diagnosis also increases.^{11,13-15}

We observed a slight difference in RDW values between males and females, with the female population having higher RDW as compared to males, suggesting potential gender-specific variations in iron metabolism. Similarly, other studies also showed females with a significant increase in RDW value than in males in a population of patients with different comorbidities like end-stage renal disease, diabetes mellitus and in people with low cardiorespiratory fitness.^{17,18} This gender disparity could be due to higher rates of iron deficiency in women, hormonal fluctuations, and differences in nutritional status may also contribute to this disparity. However, the studies performed only on children found a higher prevalence of IDA in the male population than in females.^{14,15,19,20} This gender variation in the child

population could be due to rapid growth in male infants and longer duration of breastfeeding as the cause of IDA. Further extensive research is needed to fully understand the underlying mechanisms behind these gender-specific and age-specific variations, and implications for the diagnosis and management of IDA.

In our study, the comparative analysis between RDW and other haematological parameters like Hb, MCV, MCH in IDA and non-IDA groups was also done, which revealed individuals with IDA had a higher level of RDW and a lower level of Hb, MCV and MCH as compared to the non-IDA group. These findings were similar to a study performed by several other authors.^{13,15,21} These studies, along with our findings, support that elevated RDW in conjunction with other haematological parameters can play a significant role in the diagnostic accuracy of early IDA, especially in resource-constrained settings in remote areas of the country like ours with no/ or limited access to special investigations.

While RDW has shown promise as a marker for IDA, our study also reveals limitations. The ROC analysis shows RDW as a moderately diagnostic significance in IDA. It underscores the need for further evaluation of RDW's performance in larger and more diverse populations. Additionally, the lack of significant association between RDW and the severity of anaemia suggests that RDW may not be a sole reliable indicator to assess the degree of anaemia in IDA. Several studies have found that RDW value could be influenced by factors other than iron deficiency, such as acute and chronic inflammation, liver disease, nutritional deficiencies and cachexia. They suggested that RDW should be interpreted with caution in patients with these conditions.²²⁻²⁴ Also, some studies found that microcytic anaemia is not only specific for IDA but could be found in other conditions, such as thalassemia, anaemia of chronic disease and lead poisoning. Additionally, they discovered that those with the thalassemia trait also had higher RDW.²⁵⁻²⁷ Thus, all these studies including ours, conclude that RDW adds useful but limited information in classifying microcytic anaemia hence, should be used in combination with other diagnostic tests like

bone marrow iron, serum ferritin, serum iron, serum total iron binding capacity, serum transferrin saturation and haemoglobin electrophoresis studies to make appropriate diagnosis of IDA with precision as a cause of microcytic anaemia.

Our study was limited by the single-centre design and the absence of follow-up data, which could impact the generalizability of findings. Overall, this study highlights the limitations of using RDW as a sole diagnostic marker for IDA. While RDW can be a valuable tool, it should be interpreted in conjunction with other clinical and laboratory findings to improve diagnostic accuracy.

Conclusion

In conclusion, red cell distribution width can be a valuable tool for iron deficiency anaemia, especially in resource-limited settings. However, further research is warranted to establish its definitive diagnostic utility and to explore its limitations. Future studies should consider larger sample sizes, diverse populations, and longitudinal follow-up to enhance our understanding of red cell distribution width in iron deficiency anaemia management.

Author contribution

Concept design: PP, SM; Literature search: PP, RT; Data collection: PP, RT; Data analysis: PP, SK; Draft manuscript: PP, SM, ND; Final manuscript and accountability: All

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Conflict of interest

None

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Supplementary material

The data and supplementary material that support the findings of this study are available from the corresponding author upon reasonable request.

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