Research Article

Significance of red cell distribution width in the diagnosis of iron deficiency anemia: an observational study from India

Abstract

Objective: To study the importance of Red cell distribution width (RDW) and peripheral smear in Iron Deficiency anemia (IDA) and its correlation with various RBC indices.

Method and material: Hundred cases of microcytic (MCV <80fl) anemia (Hb<11 gm %), diagnosed as IDA were enrolled in the study, after getting parental consent. These children's were evaluated for clinical examination, red cell indices, RDW, serum iron and serum total iron binding capacity (TIBC) and transferrin saturation and peripheral blood film (PBF) examination for red cell morphology.

Results: The mean RDW in mild, moderate and severe grade of iron deficiency anemia were 16.83±1.24, 18.52%+1.54 and 21.4%+3.15 respectively. The RDW was high in 98% cases of IDA. As the severity of IDA increased the values of RDW increased, serum iron decreased (p<0.001), TIBC increased (p<0.001) and serum transferrin saturation decreased (p<0.001). There was progressive decrease in MCV with increase in severity of anemia and there was corresponding increase in RDW. In mild IDA, the sensitivity of RDW was found to be very high (91%) in comparison to PBF changes (43.47%) cases and was statistically highly significant (p<0.001). In moderate IDA and severe IDA the sensitivity of RDW & PBF changes both were found to be very high. The positive predictive value of RDW and peripheral blood film changes was (100%) in all grades of IDA. The negative predictive value of RDW in mild grade of IDA was 88.23% as compared to 53.57% in PBF. In moderate grade of IDA, NPV of RDW was 100% as compared to 83% in case of PBF. While in severe grade of IDA the NPV of RDW and PBF was recorded to be 100%.

Conclusion: The RDW was found to be highly sensitive and effective tool for early diagnosis of mild iron deficiency anaemia therefore; it is recommended that the RDW can be used as an effective tool for the diagnosis of iron deficiency anaemia in early stages, in a large number of samples at major hospitals thereby reducing manpower and time consumption.

Keywords: red cell distribution width, iron deficiency anemia, rbc indices, microcytic anemia, serum iron, serum iron binding capacity, transferrin saturation

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Abbreviations: RDW, Red cell distribution width; IDA, Iron Deficiency Anemia; MCV, Mean Corpuscular Volume; TIBC, Total Iron Binding Capacity; PBF, Peripheral Blood Film; NFHS, National Family Health Survey; UNICEF, United Nations Children's Fund; IRB, Institutional Research Board; EDTA, Ethylene Diamino-Tetra Acetate; MCHC, Mean Corpuscular Hemoglobin Concentration; TRBC, Total Red Blood Cells

Introduction

Anemia is a major health problem in developing countries and results in significant morbidity and mortality in infants and children.[1] Iron deficiency is the most commonly seen micronutrient nutritional deficiency among Indian children's and has contributed significantly for increase in morbidity and mortality in our country.[2] The most severe consequence of iron deficiency seen is iron deficiency anemia (IDA). In the recent survey conducted by National family health survey (NFHS– III) (2005– 06) showed that approximately 79.2% of Indian young children had anemia.[3,4] According to the United Nations Children's Fund (UNICEF), 90% of all types of anemia in the world are due to iron deficiency.[5] The etiology in most of the IDA is multi-factorial and usually IDA is attributed to inadequate iron intake, poor bioavailability of the iron that is taken or high nutritional requirements

during childhood and during rapid growth, chronic intestinal blood losses due to helminth infections, or severe malarial infections in tropical countries.[6,7]

Iron deficiency anemia produces spectrum of symptoms including weakness, fatigue, growth retardation, tachypnea, tachycardia, permanent reduction of cognitive functions and variety of behavioral disturbances like irritability, short attention span, and lack of interest in the surroundings. The other sign and symptoms includes edema, epithelial changes including angular stomatitis, atrophy of lingual papillae, post-cricoid esophageal webs and gastritis, nail changes like thinning, flattening and finally the development of koilonychias (concave or spoon shaped nails). Finger nails may become brittle, fragile or longitudinally ridged. The iron deficient subjects are unusually prone to infection related to defective cell mediated immunity and impaired bacterial killing by phagocytes. There has also been studies showing long term implication of IDA on the neuro developmental outcome. All of these behavioral disturbances are ameliorated with the initiation of iron therapy.[8–10]

The high incidence of iron deficiency anemia in children emphasizes the need for early detection and prompt treatment of this easily treatable condition. An optimal non-invasive method for diagnosing iron deficiency anemia has eluded practitioners for many

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years. There are various methods to diagnose iron deficiency anemia (CBC with RBC indices including RDW, ferritin, Total Iron binding capacity (TIBC), and peripheral smear examination) but in developing countries these investigations are not always feasible to be done either due to high cost or because of lack of facilities. Some of these parameters are effected by inflammation which is commonly seen in children's of developing countries, hence making situation even more difficult.[11] The other reason being that the red cell indices are single, mean values and cannot express the variation within the population of cells, hence they are not able to reveal the presence of a small or dual population of abnormal cells. The conventional examination of peripheral blood smear is time consuming and there are both inter-observer and intra-observer variability.[12,13]

Most electronic counters calculate the co-efficient of variation in red cell volume and report it as "Red cell distribution width" (RDW). RDW is a measure of heterogeneity of distribution of red cell size. It is equivalent to "anisocytosis" in the analysis of peripheral blood smear. The generation of RDW curve by the analyst may be useful adjunct in the diagnosis of iron deficiency anemia.[14,15]

In India IDA is very common problem and there are very few Indian studies, which has been done to study the importance of RDW and peripheral smear in IDA and its correlation with various RBC indices in the pediatric age group.[16-18] So, our study was planned to evaluate the significance of red cell distribution width in the diagnosis of iron deficiency anemia.

Material & methods

This present study was conducted in the Department of Pediatric, S.N. Medical College, Jodhpur, India for 12 months. During the study period 500 cases of microcytic (MCV<80fl) anemia (Hb < 11gm%) in the age group of 6 months to 5 years were screened with serum iron and total iron binding capacity to confirm the diagnosis of IDA. The diagnosis of iron deficiency anemia was made on the basis of TS (Transferrin Saturation) < 16% and serum iron and total iron binding capacity (TIBC). Out of these 500 children's, using computer generated random numbers hundred children's were enrolled in the study. Those children who had recent blood transfusion or iron therapy were excluded. The study was approved by institutional research board (IRB) of the institution. Written consent from the parents was taken before the enrollment of the children in the study. There is no need of regulatory review.

The primary objective of the research was to study the importance of Red cell distribution width (RDW) and peripheral smear in Iron Deficiency anemia (IDA) and its correlation with various RBC indices. These hundred cases of IDA were evaluated for red cell indices, RDW, serum iron and serum iron binding capacity and transferrin saturation, peripheral blood film examination for red cell morphology. Detailed clinical history including history of medicinal iron intake & recent blood transfusion was taken in every case and detailed physical examination was done to see for any complication of anemia.

The blood samples were drawn from peripheral vein with the subject in supine position from 9 am – 2 pm as diurnal variation in serum iron levels with highest concentration in the morning and lowest in the evening. Samples were drawn irrespective of time of feeding as there is no effect of feeding on serum iron levels. In the study, sample were collected in Ethylene diamino-tetra acetate (EDTA) tube for estimation of red cell indices including RDW & smear preparation and in a plain vial for estimation of serum iron and TIBC. Hematological parameters including Hb, Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin

concentration (MCHC) , Total red blood cells (TRBC) and RDW were obtained by using automated electronic cell counter 'SYSMAX 3000' (Trans Asia Ltd.). Both RDW – CV and RDW – SD were calculated by the automated electronic cell counter 'SYSMAX 3000'.

Peripheral blood films were prepared by standard method & were stained with Leishman stain. Smears were examined and further confirmed by the senior and expert pathologists who were blind to the findings of clinical examination and reports of serum iron studies.

Peripheral smears were looked for RBC morphology including abnormal shape and size, spherocytosis punctuate basophilia, Cabot's rings, Howell jolly bodies and any granular matter in red cells.

Statistical analysis

All the data was entered in Microsoft excel sheet and SPSS version 16 for Windows was used for analysis. Chi square test was used for the comparison of RDW and peripheral blood films in diagnosis of IDA and Student't' test for the correlation of serum iron, total iron binding capacity (TIBC) and transferrin saturation with RDW.

Results

In our study 66% of the cases were males and 34% were females and maximum cases (42%) were found in the age group of 6-12 months followed by 36% in 1-3 years. 35% cases belonged to lower socioeconomic status and 79% came from rural areas. The most common complaints were pallor (84%) followed by apathy & listlessness (59%), refusal to feed (58%) and diarrhea (57%) and most common clinical signs were pallor (93%) followed by tachypnea (39%) & tachycardia (36%), hepatomegaly (28%) & edema (14%).

Out of 100 cases 23%, 60% & 17% were found in mild (10-11 gm%), moderate (7-9.9 gm%) and severe (<7gm%) grades of anemia respectively.

In iron deficiency anemia serum iron values ranged from 25-65μg/dl with the mean value of 43.2μg/dl and TIBC ranged from 256-750μg/dl with the mean value of 384μg/dl. Mean transferrin saturation in mild, moderate and severe anemia was 13.45%, 12.05% & 8.95% respectively.

Normal value for RDW in adults and children is 11.5%- 14.5% and for infants is 15.1-16%. Out of total 100 cases RDW was abnormal in 98% cases. In moderate and severe iron deficiency anemia all cases were having RDW in abnormal range while in mild cases of iron deficiency anemia 91.3% cases had abnormal RDW. All the cases of severe anemia were having microcytic picture in PBF while 95% cases of moderate anemia and 43.47% cases of mild anemia had changes in PBF. Overall, 84% cases were having microcytic picture (Table 1). There was progressive increase in RDW with severity of anemia and there was also progressive decrease in MCV with increase in the severity of anemia (Table 2).

The sensitivity of RDW in mild IDA was 91.3% compared to 43.47% in PBF. The specificity was recorded to be 100% by using either the RDW values or using the PBF changes. In moderate and severe IDA, the sensitivity and specificity was 100% with both RDW and PBF changes. The negative predictive value was 88.23% when RDW was used whereas it dropped down to 53.57% while using PBF microcytic changes. The positive predictive value was 100% in mild grade of anaemia by using either RDW or PBF. In moderate grade of iron deficiency anaemia the PPV and NPV of RDW was 100%. Similarly, the positive predictive value was 100% but the negative predictive value was 83% in PBF. In severe iron deficiency anaemia

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the positive predictive value and negative predictive value was 100% for RDW and PBF (Table 3). As the severity of IDA increased the values of RDW increased, serum iron decreased (p<0.001), TIBC increased (p<0.001) and serum transferrin saturation decreased (p<0.001).

Table 1 Comparison of Red cell distribution width (RDW) & peripheral blood film (PBF) findings in Iron deficiency anemia (IDA)

| Grade of Anemia | RDW | | PBF | | X2 | P |
	Normal	High (%)	Microcytic	Non Microcytic		
Mild (n=23)	2 (15±0.70)	21 (17.13±1.28)	10 (43.47%)	13 (56.53%)	11.97	<0.001
Moderate(n=60)	-	60 (18.52±1.54)	57 (95.0%)	3 (5%)	3.07	>0.1
Severe (n=17)	-	17 (21.4±3.15)	17 (100%)	0	0	0
Total (n=100)	2	98	84 (84.0%)	16 (16%)	-	0.3

Table 2 Table showing correlation between RDW, MCV and hemoglobin level in the study population

Grade of Anemia	RDW	MCV
Mild (n=23)	16.83±1.24	73.42 ± 1.8
Moderate (n=60)	18.52±1.54	69.48 ± 3.6
Severe (n=17)	21.4±3.15	64.32 ± 2.4

Table 3 Positive and Negative Predictive values of RDW and Peripheral Smear in Diagnosis of IDA

| Grade of Anaemia | RDW (Values in %) | |
	PPV	NPV
Mild	100%	88.23%
Moderate	100%	100%

Discussion

The study revealed that maximum cases were found in the age group of 6months to 3 years which is in concordance with observation made by various workers.[16-19] Considering the peculiar circumstances of diet, period of rapid and variable growth, lack of immunity and increased susceptibility to infections and effects of other extraneous influences on the haemopoietic system, anemia is quite common in infancy and early childhood.

In our study male children had predominance, with ratio 1.9:1, finding of our study is similar to other study.[18] This may be a sheer co-incidence or parents' preferential concern for the male child for better medical care due to social customs and traditions and negligence of female child. Iron deficiency anemia was seen more commonly in rural population and patients from lower socio-economic status. This can be explained because of their poor nutritional intake and also associated parasitic and helminthic infections in this group of population.

Viswanath et al. showed that in the diagnosis of mild and moderate iron deficiency anemia, RDW had a higher sensitivity than PS and RDW was suggestive of iron deficiency in 100%, 82.05% and 100% of patient with mild, moderate and severe anemia respectively.[17] Similarly Sazawal et al. in recently published well conducted study conducted in 2091 children's showed that RDW >15% with hemoglobin ≤ 10.0 g/dL identifies iron deficient anemic children without need for iron status markers, hence highlighting the role of RDW.[18] On the other hand, there is a report of a limited specificity of RDW for diagnosis of IDA among children with microcytic hypochromic anemia from our country.[16]

RDW is generally done in a standard CBC in the developed countries for early detection of anemia, but this is not the case in developing countries like India. In the developing countries like India, IDA is diagnosed with low hemoglobin, peripheral smear examination, decreased transferrin saturation, MCH and MCV (hypochromia and microcytosis) and decreased serum ferritin. These investigations are either expensive or require sophisticated laboratories, hence are not feasible to be done routinely.[20] As our study and other studies has shown that RDW determination have high sensitivity and specificity for early diagnosis of IDA, the use of RDW can be promoted for early detection and starting of treatment of IDA. Presently this test is not available everywhere in the developing countries and usually only available in medical college level or tertiary care health care facility. The widespread implementation initially will be costly because of equipment and required training of personals but in long run the charges will be less and we can screen large number of patient in less time, hence it will be a cost effective intervention. It is also an easily doable as these instruments doesn't require much trained personals. There are numerous programs in India designed to address the major morbidity and mortality of iron deficiency anemia in the region including National Nutritional Anemia Control Programme, National Nutritional Anemia Prophylaxis Programme, Adolescent Girls Anemia Control Programme, WIFS.[Weekly Iron Folic Acid Supplementation], Iron Plus Initiative, to tackle this problem[11,21-23] but they are meeting with variable success.

There are various reasons for this which includes weak intersectoral coordination, poor knowledge about anemia, late detection of anemia, inadequate supplies of iron pills, inadequate coverage, poor antenatal coverage, poor compliance and lack of motivation of women, lack of monitoring and supervision, lack of orientation of health workers and weak implementation of parasitic infection control programs.[24] The diagnosis of IDA on the basis of RDW will help in earlier detection of IDA and start iron supplementation when compared to late detection of IDA using other methods. The other thing that it will save time and money of Government as many children's and mothers can be screened in short span of time and RDW can also be used as a response to treatment given, hence indirectly helping in confirming the efficacy of treatment.In developing countries there are clearly other barriers in the patient population for getting appropriate diagnosis/treatment but using this technique of RDW estimation, health care personal can identify IDA in early stages and start treatment and at least prevent mortality and morbidity because of IDA and this helping in implementation of health programmes of Government.

Limitation of the study includes small sample size, not able to analyse RDW of all 500 children's as we included only 100 children's in the study as this would have increased the power of the study and better correlation would been generated, using a fixed value of hemoglobin for anemia irrespective of age, using RDW in the population that already was diagnosed with IDA, lack of analysis of all the patients with low hemoglobin levels that were referred to our center in the time frame to see if RDW was more helpful than peripheral smear in the definition of iron deficiency. A study needs to be done with large pediatric population that includes analysis of all the patients with low hemoglobin levels to see if RDW is more

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helpful than peripheral smear in the definition of iron deficiency and also to correlate RDW with Hb levels and MCV and suggest how to screen anemic patient using RDW, Hb and MCV and use them to make the diagnosis of IDA without the need of serum iron and serum iron binding capacity and transferrin saturation.[25]

Finally we conclude that RDW is more sensitive than peripheral blood smear in the diagnosis of iron deficiency anemia, it is recommended that the RDW can be used with other investigations like PBF and MCV as an effective tool for the diagnosis of iron deficiency anemia in early stages, in a large number of samples at major hospitals thereby reducing manpower and time consumption.

Authorship contribution ship

Dhananjay Singh Shekhawat, Mukesh Choudhary and Deepak Sharma: Substantial contributions to the conception or design of the work and the acquisition, analysis, or interpretation of data for the work; AND

Dhananjay Singh Shekhawat and Dhanraj: Drafting the work or revising it critically for important intellectual content; AND

Dhananjay Singh Shekhawat, Mukesh Choudhary, Deepak Sharma, and Dhanraj-Final approval of the version to be published; AND

Dhananjay Singh Shekhawat, Mukesh Choudhary, Deepak Sharma, and Dhanraj-Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of interests

The authors disclose no conflicts of interest.

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