



RDW- A discriminative index in differentiation of microcytic hypochromic anemia in children below five years.

Pathology

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ABSTRACT

Background: Anemia is a major public health problem that affects populations in both developed and developing countries and is widely prevalent amongst all the ages. Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in infants and children. It has been estimated that 30% of the global population suffers from iron deficiency anemia (IDA), and most of those affected live in the developing countries. Recent NFHS-III (National Family Health Survey- III) surveys (2005-06) have shown that 70-85% (approx. 79.2%) of Indian young children have anemia. Thalassemia is one of the major autosomal recessive hereditary hemoglobinopathies prevalent in the world population, particularly in Mediterranean belt, Far-eastern and South East Asian countries. Thalassemias are a group of hemoglobinopathies caused by genetic mutations of the hemoglobin (Hb) genes, resulting in reduced production or total absence of one or more globin chains. Iron deficiency anemia is the most common microcytic hypochromic anemia worldwide. Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in young children and women of reproductive age but it can be found in people of any age-group. It has been estimated that 30% of the global population suffers from iron deficiency anemia (IDA) and most of those affected live in the developing countries like India⁷. Iron deficiency anemia in adults is caused by loss of blood, while in childhood faulty diet is to blame. Currently, the detection of IDA is largely dependent upon quantification of biochemical markers like serum ferritin (SFr), serum transferrin (STr) and zinc protoporphyrin (ZnPP) which are not routinely available and affordable in developing countries due to high costs. Results & Observation. In the present study, Mentzer Index (MI) had a sensitivity of 71.91 and 84.62 % for IDA and BTT respectively. The specificity of MI for IDA and BTT was 84.62% and 71.91% respectively. The PPV of MI for IDA and BTT was 94.12% and 46.81% respectively while the NPV of MI for IDA and BTT was 46.81 and 94.12% respectively. we can conclude that RBC count >4.69 million/mm³ and MCV < 62.9 fL favours the diagnosis of BTT while RBC count <4.69 million/mm³ and MCV >62.9 fL favours IDA.

Conclusion: A limited specificity of RDW in diagnosis of IDA among children with microcytic hypochromic anemia suggests that further studies like serum ferritin, serum iron, serum TIBC, and hemoglobin studies used in a systemic manner are still necessary to make an accurate diagnosis of the cause of microcytosis. RDW with a good sensitivity can be used as good discriminative index between IDA and BTT. It can be used to differentiate microcytic anemias into IDA and BTT, where HPLC and iron studies (S. ferritin, S.iron, TIBC) cannot be done due to factors like unavailability or high cost.

KEYWORDS:

Microcytic hypochromic anemia, red cell distribution width

INTRODUCTION

Anemia is a public health problem that affects populations in both rich and poor countries and is widely prevalent amongst all the ages. Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in infants and children. It has been estimated that 30% of the global population suffers from iron deficiency anemia (IDA), and most of those affected live in the developing countries. Recent NFHS-III (National Family Health Survey- III) surveys (2005-06) have shown that 70-85% (approx. 79.2%) of Indian young children have anemia¹.

The most commonly encountered disorders with microcytic anemia are iron deficiency anemia (IDA) and beta thalassemia trait (BTT). Other diagnoses to consider include anemia of chronic disease, lead toxicity and sideroblastic anemia. In developing countries like India, where resources are limited, thalassemia is a major health burden. BTT often shows microcytosis, a normal or an increased red blood cell (RBC) count, and an elevated level of HbA₂, which provide the basis for laboratory screening. Iron deficiency anemia is the most common hematological disease in young children and women of reproductive age but it can be found in people of any age-group. It has been estimated that 30% of the global population suffers from IDA and most

of those affected live in the developing countries like India². Iron deficiency anemia in adults is caused by loss of blood, while in childhood faulty diet is to blame.

BTT is an important differential diagnosis of IDA may closely mimic those that are present in iron deficiency anemia, the therapy is radically different. It is very important not to treat a patient with thalassemia with an iron supplement as this can lead to hemochromatosis (accumulation of iron in various organs, especially the liver). Thus reliable and efficient diagnostic ways to distinguish between thalassemia and iron restricted microcytic hypochromic anemia are desirable.

Studies have shown that iron deficiency causes delay in cognitive development and poor motor and sensory system functioning and that iron supplementation in early years may prevent these complications among children³. Conversely, there is an evidence suggesting that routine iron treatment in non-iron deficient children may have adverse consequences for morbidity and infections⁴.

Currently, the detection of IDA is largely dependent upon quantification of biochemical markers like serum ferritin (Sfr), serum

transferrin (STr) and zinc protoporphyrin (ZnPP) which are not routinely available and affordable in developing countries due to high costs. A definitive differential diagnosis between -TT and IDA is based on the result of HbA2 electrophoresis, serum iron levels, and a ferritin calculation⁵

Studies have shown that RDW, a measure of variations in the circulating RBCs, in addition to other hematological markers like mean corpuscular volume (MCV) and hemoglobin can be used as a differential diagnostic tool for identification of iron deficiency anemia^{6, 7,8}.

Red cell distribution width (RDW) has been proposed to be a more sensitive indicator to establish the possible origin of microcytic hypochromic anemia⁹. The RDW represents the coefficient of variation of the red blood cell volume distribution and can be considered an index of heterogeneity, the equivalent of anisocytosis observed in the peripheral blood smear¹⁰. Various previous studies have debated the role of RDW in diagnosis of IDA^{8,11,12}, with no conclusive word on the utility of RDW in diagnosing iron deficiency anemia.

Moreover, fewer studies have been conducted in children, our study shall test the efficacy of RDW for the same. Also, the standard diagnostic tests like serum ferritin, HPLC (High Performance Liquid Chromatography) have a limitation of high cost factor. We will analyze if RDW, which is a derivative of a normal CBC, can help in distinguishing the major types of microcytic anemia, in a developing country where the burden of anemia is so high and the resources are limited.

MATERIAL AND METHOD

The study was conducted in the department of Pathology, AIMSR, Bathinda on all anemic patients below 5 years presenting to Adesh Hospital, Bathinda and patients referred from urban and rural health centres linked to Adesh Hospital, Bathinda from 1st April 2015 to 31st March 2016.

Inclusion criteria comprised of age group 1-5 years, Hemoglobin<11g/dl and Mean Cell Volume (MCV)<80fl.

Patients having history of recent blood transfusions/blood loss and all patients with known chronic diseases were excluded from the study. Ethical approval from Institutional Ethical Committee of Adesh University, Bathinda was taken before start of the study.

Blood sampled in EDTA vials was used to perform the routine hematological investigations as:-

a) Complete Blood Counts including Hemoglobin, red blood cell counts, hematocrit, red cell indices including Mean Corpuscular Volume (MCV), Mean Hemoglobin Concentration (MCH) & Mean Cell Hemoglobin Concentration (MCHC) and lastly, red cell distribution width (RDW), using automated cell counter.

b) Peripheral blood smears of the same were made & stained using Leishman stain to evaluate the red cell morphology.

Iron profile in the form of serum ferritin, serum iron & serum TIBC was done. . Serum Iron and TIBC was done by Ferrozine method using kit provided by Crest Biosystems on Biochemistry fully automated analyser A-15(Biosystems). Serum Ferritin was done using immunofluorescence method on AIA-360 Tosoh autoanalyser.

Detection of hemoglobin patterns was done by High Performance Liquid Chromatography (HPLC) using BIORAD D-10 Hemoglobin Testing System.

The diagnosis of iron deficiency was established with S.iron <50µg/dl, S.TIBC>450µg/dl & S.ferritin <12 µl.

-Analysis of red cell distribution width (RDW) was done in all cases.

RESULT

The present study was conducted on a pediatric population (age group 1-5 years i.e. 12-60 months), with a hemoglobin < 11 g/dL and an MCV

of <80 fL. A total of 115 cases were analyzed. All these cases of microcytic hypochromic anemia were subgrouped as IDA and BTT on the basis of criteria already mentioned. Out of the total 115 cases, 22.6% were diagnosed as BTT and 77.4% were diagnosed as IDA. Out of the total 115 cases, the most common age group was 12-24 months with 38.3% of cases. Amongst the BTT and IDA group, the most common age group was 12-24 months (50% and 34.8% cases respectively). There was insignificant correlation between the prevalence in different age groups and the disease occurrence of BTT and IDA (p value- 0.449) Out of the total cases, 58.3% were females and 41.7% were males. Out of total BTT cases, 65.4% were females and 34.6% were males. Amongst all the IDA cases, 56.2% cases were females and 43.8% were males. However, no significant correlation between the age and the diagnosis was found. (p-value= 0.402) Almost all of the BTT cases (96.2%), presented with mild anemia with hemoglobin between 9-11g/dl (p value- 0.000, significant). While IDA patients presented with mild, moderate as well as severe degree of anemia. The mean value of hemoglobin in IDA group was statistically lower i.e. 7.68 g/dl (SD- 2.468) as compared to BTT group with a mean Hb of 10.31 g/dl (SD- 0.731) (p-value-0.000)

RDW in IDA and BTT

Table 1 : Distribution of cases according to RDW in BTT and IDA

		DIAGNOSIS				Total	Chi-square value	P-value
		BTT		IDA				
RDW-CV (%)	11.6-14	0	0.0%	2	2.2%	2	4.813	0.090
	14-18	26	100.0%	21	23.6%	47		
	More than 18	0	0.0%	66	74.2%	66		
Total	26	100.0%	89	100.0%	115			

Normal RDW-CV - 11.6 to 14% (12.8±1.2%)

In all the cases of BTT, the RDW was only slightly elevated (value 14-18%), while in 74.2% of the IDA patients had RDW-CV values more than 18%, followed by 23.6% having RDW 14-18% while only a few cases (2.2%) showed RDW in the normal range (11.6- 14%) But the p value obtained in this analysis was 0.090(>0.05), hence it was statistically insignificant. The mean value of RDW was 16.24% in BTT and 21.52% in IDA.

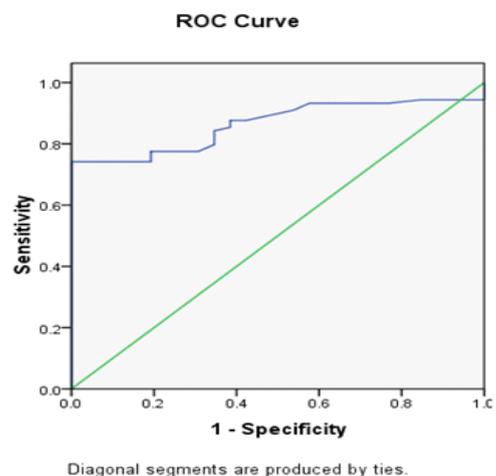


Figure 1: ROC of RDW for the differentiation of BTT from IDA

Receiver operator curves (ROC) were constructed using RDW values of the children among two groups. The sensitivity, specificity, PPV and NPV were calculated using different cut-off of 15, 16 and 17% and the following results were obtained.

Table 2: Sensitivity, specificity, PPV, NPV for IDA and BTT according to different cut-off value of RDW-CV

Cut-off value of RDW-CV (%)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
>15	IDA	94.38	7.69	77.78	28.57
	BTT	7.69	94.38	28.57	77.78
>16	IDA	87.64	57.69	87.64	57.69
	BTT	57.69	87.64	57.69	87.64
>17	IDA	77.53	80.57	93.24	51.22
	BTT	80.77	77.53	51.22	93.24

RDW cut-off value of 16 % was the value with a best combination of sensitivity and specificity for IDA and BTT. At this RDW cut-off value of >16%, the sensitivity and specificity of RDW in differentiating IDA from BTT was found to be 87.64% and 57.69% respectively with a positive and negative predictive value of 87.64% and 57.69 % respectively.

RDW v/s degree of severity of anemia in IDA

Table 3: Mean value of RDW in mild, moderate and severe anemia in IDA

Anemia vs RDW	No. of patients	Mean value of RDW	SD	Minimum	Maximum	P-VALUE
0-7 (severe)	32	25.03	5.704	16	38	0.000
7-9.0 (moderate)	24	19.80	3.901	13	31	
9-11.0 (mild)	33	19.37	4.074	14	31	

Amongst the IDA patients, the mean value of RDW was 19.37% in patients having mild anemia, 19.80% in patients with moderate anemia and 25.03% in severe anemia. Hence, the RDW increases with the severity of anemia in the IDA patients. The p value was 0.000. Hence it is highly significant statistically.

RDW v/s degree of severity of anemia in BTT

Table 4: Mean value of RDW in mild, moderate and severe anemia in BTT

Anemia vs RDW	No. of patients	Mean value of RDW	SD	Minimum	Maximum	P-VALUE
7-9.0	1	18.00	.	18	18	0.089
9-11.0	25	16.17	1.003	15	18	

Most of the patients in BTT group presented with mild anemia, showed a mean RDW value of 16.17% while only 1 patient had moderate anemia in BTT group had RDW value of 18.00%.

Though this increase in the value of RDW according to the severity of anemia was statistically insignificant in BTT group. (p value 0.089)

DISCUSSION:

In our study, almost all of the BTT cases (96.2%), presented with mild anemia with hemoglobin between 9-11g/dL. (p value- 0.000, significant). While IDA patients presented with mild, moderate as well as severe degree of anemia. The mean value of hemoglobin in IDA group was statistically lower i.e. 7.68 g/dL (SD- 2.468) as compared to BTT group with a mean Hb of 10.31 g/dL (SD- 0.731) (p- value- 0.000). Similar results were found in almost all of the studies analyzed, viz Vehapoglu et al2, Ehsani et al13, Soliman et al14, Matos et al15.

Amongst the IDA patients, the mean value of RDW was 19.37% in patients having mild anemia, 19.80% in patients with moderate anemia and 25.03% in severe anemia. Hence, the RDW increases with the severity of anemia in the IDA patients. The p value was 0.000. Hence, it is highly significant statistically. Most of the patients in BTT group presented with mild anemia, showed a mean RDW value of 16.17% while only 1 patient had moderate anemia in BTT group had RDW value of 18.00%. Though this increase in the value of RDW according to the severity of anemia was statistically insignificant in BTT group

(p-value 0.089).

Similar to Gupta et al16, we also observed an inverse relationship of RDW with the hemoglobin value (p=0.000) in iron deficiency anemia, while no such correlation was observed in non-iron deficiency (p=0.089) anemia.

In all the cases of BTT, the RDW was only slightly elevated (value 14-18 %), while in 74.2% of the IDA patients had RDW-CV values more than 18%, followed by 23.6.% having RDW 14-18% while only a few cases (2.2%) showed RDW in the normal range (11.6- 14 %) RDW cut-off value of 16 % was the value with a best combination of sensitivity and specificity for IDA and BTT. At this RDW cut-off value of >16%, the sensitivity and specificity of RDW in differentiating IDA from BTT was found to be 87.64% and 57.69% respectively with a positive and negative predictive value of 87.64% and 57.69 % respectively The sensitivity & specificity of RDW to differentiate IDA from BTT according to different studies is as depicted in the table 5.

Table 5: Comparison of sensitivity, specificity of RDW for differentiation of IDA from BTT of present study with studies conducted by other authors

	Cut value of RDW-CV(%)	Sensitivity	Specificity
Present Study(2015-16)	>16	87.64	57.69
Flynn et al ¹⁷	>13.4	94	51
Zeben et al ¹¹	>14.5	94	59
Thompson et al ¹²	>15	71	54
Aulakh et al ¹⁸	>17.4	81	53
Kim et al ¹⁹	>15	83.3	57.7
Okan et al ²⁰	>14	98	6
Lima et al ²¹	>21	90.0	77
Sazawal et al ²²	>15 with Hb<10g/dL	99	90
Vishwanath et al ⁸	>14.5(in children) >16(in infants)	92.1	90.1
Gupta et al16	>17.1	61.3	92.5

which showed a limited specificity of

RDW though the sensitivity was good. Studies by Vishwanath et al8, and Sazawal et al22, observed a higher sensitivity and specificity.

The findings and recommendations of western studies on RDW in iron deficiency anemia and beta-thalassemia trait may not be entirely applicable in a country like ours with a much higher percentage of IDA. Children with BTT, who have associated IDA, are likely to have a consequent high RDW and stand to be labeled as iron deficient initially. Careful follow up after adequate iron therapy following which the RDW may come back to normal but the low indices persist, may be required to then further investigate them for the appropriate heterozygous hemoglobinopathy.

It is well known that in certain conditions of concomitant diseases (for example, iron deficiency anemia and chronic disease anemia), even the results of gold standard tests may suffer the interference of the intercurrent disease, making diagnosis more difficult. Therefore, the results of our study indicate a limited usefulness of RDW as an auxiliary parameter for differentiation of these types of anemia, because a better discrimination between both nosological entities by RDW would depend on the absence of other disorders favoring microcytosis. Hence, in the absence of intercurrent disorders, it may be an important tool for differential diagnosis between iron deficiency anemia and thalassemia minor (alpha and beta), and it must be valued as an auxiliary laboratory parameter for this purpose.

A limited specificity of RDW in diagnosis of IDA and limited sensitivity in diagnosis of BTT among children with microcytic hypochromic children suggests that further studies like serum ferritin, serum iron, serum TIBC, bone marrow biopsy and hemoglobin studies used in a systemic manner are still necessary to make an accurate

diagnosis of the cause of microcytosis.

CONCLUSION:

Cut-off value of 16 % offered the best combination of sensitivity and specificity for IDA and BTT. At this RDW cut-off value of >16%, the sensitivity and specificity of RDW in differentiating IDA from BTT was found to be 87.64% and 57.69% respectively with a positive and negative predictive value of 87.64% and 57.69 % respectively. A limited specificity of RDW in diagnosis of IDA among children with microcytic hypochromic anemia suggests that further studies like serum ferritin, serum iron, serum TIBC, and hemoglobin studies used in a systemic manner are still necessary to make an accurate diagnosis of the cause of microcytosis. RDW with a good sensitivity can be used as good discriminative index between IDA and BTT. It can be used to differentiate microcytic anemias into IDA and BTT, where HPLC and iron studies (S. ferritin, S.iron, TIBC) cannot be done due to factors like unavailability or high cost. Though hemoglobin studies and iron profile still remain the gold standard. However, preliminary screening of microcytic hypochromic anemia cases on routine complete blood count report with the help of RDW can avoid unnecessary iron therapy in children with suspected BTT as it may cause iron overload and systemic damage.

CONFLICT OF INTEREST

The authors had no conflict of interest to declare in relation to this article.

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