International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2013 Vol. 3 (1) January-April, pp.292-299/Santosh et al.
Research Article

A STUDY ON RED CELL DISTRIBUTION WIDTH IN RELATION TO OTHER IRON (RED CELL) INDICES WITH SPECIAL REFERENCE TO RETICULOCYTE COUNT BEFORE AND AFTER ORAL IRON THERAPY IN IRON DEFICIENCY ANEMIA

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ABSTRACT
Iron deficiency anemia is the most common nutritional deficiency in children and is widespread in pediatric populations through out the world. It is especially prevalent in infancy. Anemia is defined as reduction of red cell volume or hemoglobin concentration below the range of values occurring in the healthy children (Welch *et al.,* 1992).
Aims and objectives behind the study were to study red cell indices in iron deficiency anemia in relation to RDW.
1. To determine sensitivity of RDW in the early diagnosis of iron deficiency.
2. To study changes and outcome of the above parameters before and after oral iron therapy.
The children who were treated in Kempegowda institute of Medical sciences and Research Centre Bangalore. Both Inpatient and outpatient recruited into the study on a purposive sampling basis. The sample consisted of 60 iron deficient children during one year period of data collection.
The mean baseline RDW was 16.48%, 17.03%, 15.88% and 17.28% in group 1, group 2, group 3 and group 4 respectively. After iron therapy the decrease in RDW is statistically significant in all the Groups.
Majority of anemic children in this study falls below 6 years, and male predominance was seen, RDW was more sensitive in detecting mild and moderate iron deficiency anemia than Red cell indices and Peripheral smear examination

Key Words: *Red Cell Distribution Width, Peripheral Smear, Anemia.*

INTRODUCTION

Iron deficiency anemia is the most common nutritional deficiency in children and is widespread in pediatric populations throughout the world. It is especially prevalent in infancy. Anemia is defined as reduction of red cell volume or hemoglobin concentration below the range of values occurring in the healthy children (Welch *et al.,* 1992). In United States, approximately 5% of children from one to five years of age suffer from iron deficiency anemia (De Gruchy *et al.*). In developing countries, how ever the prevalence of anemia reaches and in some countries exceeds 50% in one year old children (ICDS, 2000; De Gruchy *et al.,*) and 46% in 5-12 years old children. The estimated global prevalence of IDA among school age children is 37% and 43% in younger children and infants respectively (ICDS, 2000).

Over the past two decades, the morphologic diagnosis of anemia has been facilitated by reliable estimates of the mean volume of red blood cells. Mean cell volume (MCV) and reticulocyte count (De Moeyer et al., 1989) are the two traditional principle criteria for the initial classification of anemic disorders.Price Jones (Judish *et al.,* 1966) first quantified anisocytosis as the coefficient of variation of red blood cell size in a Price Jones curve. Quantification of anisocytosis using red blood cell volume distribution histogram was then developed (Price-Jones, 1933). More recently, the RDW has been derived by the use of an analog computation technique using the new automated blood cell analyzer (Bessman *et al.,* 1983). The total numbers of erythrocytes counted are classified by size by an automatic, continuously variable threshold circuit. This threshold begins at a level equivalent to 360 femtolitres and moves progressively lower until 20% of all erythrocytes present have a size greater than the threshold. The cell size at which

this occurs is recorded as 20th percentile value (A). The threshold continues downwards until 80% of all the erythrocytes have a size greater than the threshold. The cell size at which this occurs is recorded as the 80th percentile value (B), and the values are computed through the equation.

RDW= {(A-B) / (A+B)} K (constant)

The RDW thus represents the coefficient of variation of the red blood cell volume distribution and can be considered an index of heterogenecity, the equivalent of anisocytosis observed in the peripheral blood smear (De Moeyer et al., 1989)

Aims and Objectives

3. To study red cell indices in iron deficiency anemia in relation to RDW.
4. To determine sensitivity of RDW in the early diagnosis of iron deficiency.
5. To study changes and outcome of the above parameters before and after oral iron therapy.
6.

MATERIALS AND METHODS

The children who were treated in Kempegowdainsititute of Medical scinces and Research Centre Bangalore. Both Inpatient and outpatient recruited into the study on a purposive sampling basis. The sample consisted of 60 iron deficient children during one year period of data collection.

Inclution Criteria

1. Children between the age group of 6 months to 12 years with Iron deficiency anaemia (Hb%Less than 11gm%).

2. Children who do not have any serious systemic disease.

Exclution Criteria

1. Children with haemolyticanaemia.

2. Children who have severe anaemia (Hb% less than 7 gm%) and those requiring blood transfusion.

3. Children with chronic systemic illeness, malignancies.

4. Children onhaematinics.

Methodology

All the children diagnosed to have anaemia based on above mentioned criteria were chosen for the study. The children were divided into 2 age group: 6months to 6 years and 6yrs to 12 years, later these children were subdivided into mild and moderate anaemic groups based on their haemoglobin levels (Mild anaemic- Hb% of 9-11 gms% and Moderate between 7-9gms %) Thus there were 4 subgroups-

Group1 – 6 months to 6 years with mild anemia,

Group2 – 6 months to 6 years with moderate anemia.

Group3 – 6 years to 12 years with mild anemia and

Group3 – 6 years to 12 years with moderate anemia.

A detailed history was taken regarding the Presenting illness, Repeated illness if any, Family, Birth, Immunization, Nutrition and Socioeconomic status. History of Anorexia, Genralisedweakness, Fatigue, Decreased activity, Dysnoea Pica, were asked for. The children were examined system wise and signs of iron deficiency like Pallor, kilonychia, Platynychia, Glossitis, papillary atrophy, stomatitis etc. were noted. With the sub division the parameters-Haemoglobin%,RBC count, MCV,MCH, MCHC,RDW,Reticulocyte count and Peripheral smear were noted and the baseline values were compared after treatment With the sub division the parameters- Haemoglobin%, RBC count, MCV, MCH, MCHC, RDW, Reticulocyte count and Peripheral smear were noted and the baseline values were compared after treatment with 6mg/kg/day in 3 divided doses of iron syrup at 3 weeks and 6 weeks.routine deworming done on all children prior to treatment

RESULTS

It is observed in this study that of the 60 children studied, 24(40%) were in the age group of 6-13 years, and 36(60%) were between 6 months to 6 years in them 20(33.3%) were in the age group of 3-6 years, 15(25%) were 1-3 years and only 1 case was in the age group of 0.5-1 year.

Table 1: Age wise distribution of anemic children

AGE (years)	NUMBER OF CHILDREN	PERCENTAGE
0.5-1	1	1.7
1-3	15	25.0
3-6	20	33.3
6-12	24	40.0
TOTAL	60	100.0

Table 2: Mean haemiglobin changes in anemic children before and after oral iron therapy

GROUPS	No. of CASES	MEAN Hb% INITIAL	MEAN Hb% AT 3RD WEEK	MEAN Hb% AT 6TH WEEK	P-VALUE
GROUP 1	20	9.87	10.85	11.99	0.0008
GROUP 2	16	8.4	9.68	11.08	0.0004
GROUP 3	19	10.14	11.16	12.42	0.0004
GROUP 4	5	8.96	10.24	11.46	0.003

In this study, the mean baseline Hemoglobin is 9.87gms%, 8.40gms%, 10.14gms% and 8.96gms% in group1, group2, group3 and group4 respectively. It is also observed that the mean baseline values in all the Groups improved after iron therapy and are statistically significant.

Table 3: Mean rbc count changes in anemic children before and after oral iron therapy

GROUPS	No. of CASES	MEAN RBC INITIAL	MEAN RBC AT 3RD WEEK	MEAN RBC AT 6TH WEEK	P-VALUE
GROUP 1	20	4.31	4.42	4.57	0.093
GROUP 2	16	4.17	4.43	4.58	0.075
GROUP 3	19	4.08	4.25	4.49	0.0005
GROUP 4	5	4.08	4.36	4.54	0.223

In this study, the mean baseline RBC counts are 4.31mil/cumm, 4.17mil/cumm, 4.08mil/cummand 4.08mil/cumm in group 1, group 2, group 3 and group 4 respectively. The RBC counts increased in all the age groups after iron therapy but were not statistically significant except in Group 3(0.0005).

Table 4: Mean corpuscular volume changes in anemic children before and after oral iron therapy

GROUPS	No. of CASES	MEAN MCV INITIAL	MEAN MCV AT 3RD WEEK	MEAN MCV AT 6TH WEEK	P-VALUE
GROUP 1	20	71.35	75.75	79.8	0.0003
GROUP 2	16	65.12	69.25	75.68	0.0009
GROUP 3	19	76.47	79.94	83.42	0.0001
GROUP 4	5	68.6	73	78.4	0.0757

It is observed that the mean baseline MCV was 71.35fl, 65.43fl, 76.47fl and 68.6fl in group 1, group 2, group 3 and group 4 respectively. The values showed significant changes in all the age groups after oral iron therapy at 3rd and 6th week.

Table 5: Mean corpuscular haemoglobin changes in anemic children before and after oral iron therapy

GROUPS	No. of CASES	MEAN MCH INITIAL	MEAN MCH AT 3RD WEEK	MEAN MCH AT 6TH WEEK	P-VALUE
GROUP 1	20	22.85	24.76	26.40	0.0002
GROUP 2	16	20.46	22.06	24.39	0.0026
GROUP 3	19	24.93	26.35	27.76	0.0005
GROUP 4	5	22.1	23.6	25.58	0.1603

In these children the mean baseline MCH was 22.85pg, 20.46pg, 24.93 and 22.1pg in group 1, group 2, group 3 and group 4 respectively. After oral iron therapy the values showed significant changes in all the age groups except in Group 4.

Table 6: Red cell distribution width in anemic children

RDW (%)	NUMBER OF CHILDREN	No. OF CHILDREN < 6 Years	No. OF CHILDREN >6 Years
<14.5	Nil	0	0
>14.5	60	36	24

At presentation all the children had RDW >14.5%.

Table 7: Redcell distribution width changes in anemic children before and after oral iron therapy

GROUPS	No. of CASES	MEAN RDW INITIAL	MEAN RDW AT 3RD WEEK	MEAN RDW AT 6TH WEEK	P-VALUE
GROUP 1	20	16.48	16.59	15.39	0.028
GROUP 2	16	17.03	16.87	15.38	0.000
GROUP 3	19	15.88	15.73	14.17	0.000
GROUP 4	5	17.28	16.56	15.32	0.058

The mean baseline RDW was 16.48%, 17.03%, 15.88% and 17.28% in group 1, group 2, group 3 and group 4 respectively. After iron therapy the decrease in RDW is statistically significant in all the Groups.

Table 8: Reticulocyte counts in anemic children

RETIC COUNT (%)	NO. OF CASES	No. OF CHILDREN < 6 Years	No. OF CHILDREN >6 Years
0.5-1.5	46	26	20
>1.5	14	10	04

In these anemic children, 46(77%) children had counts in normal range and 14(23%) had elevated counts.

Table 9: Mean retic counts in anemic children before and after oral iron therapy

GROUPS	No. of CASES	MEAN Retic Counts INITIAL	MEAN Retic Counts AT 3^{RD} WEEK	MEAN Retic Counts AT 6^{TH} WEEK	P-VALUE
GROUP 1	20	1.25	1.3	1.15	0.567
GROUP 2	16	1.27	1.58	1.17	0.113
GROUP 3	19	1.09	1.14	1.0	0.660
GROUP 4	5	1.16	0.96	0.84	0.352

It is seen that the mean baseline reticulocyte counts are 1.25%, 1.27%, 1.09% and q.16 in group1, group 2, group 3 and group 4 respectively.

Table 10: Peripheral smear changes in anemic children before and after oral iron therapy

Peripheral Smear	INITIAL				3^{RD} WEEK				6^{TH} WEEK			
	MC HC	NC NC	NC HC	MC NC	MC HC	NC NC	NC HC	MC NC	MC HC	NC NC	NC HC	MC NC
GROUP 1	4	11	4	1	0	18	2	0	0	19	1	0
GROUP 2	6	3	3	4	5	9	1	1	3	11	1	1
GROUP 3	5	11	0	3	1	15	0	3	1	16	0	2
GROUP 4	3	1	0	2	1	1	1	2	1	4	0	0

DISCUSSION

In this present study 60 children with Mild and Moderate anemia between the age 6 months to 12 years were divided into following 4 subgroups-

Group 1 – 6 months to 6 years with Mild anemia,
Group 2 – 6 months to 6 years with Moderate anemia,
Group 3 – 6 years to 12 years with Mild anemia and
Group 4 – 6 years to 12 years with Moderate anemia.

Age Incidence

Out of 60 these anemic children in this study, 36 (60%) children were between 6 months to 6 years and 24 (40%) children were between 6 years to 12 years. According to the subgroups, 20(33.3%), 16(26.7%), 19(31.7%) and 5(8.3%) children were there in Group 1, 2, 3 and 4 respectively.

Simmons *et al.,* 1982; Karnad *et al.,* 1985 and Giebel *et al.,* 1998; Simmons *et al.,* 1982 in their studies showed that iron deficiency anemia is mostly encountered between 6 months to 12 years of age (Jain *et al.,* 2000) in his study revealed a high prevalence of anemia in children of 1 to 2 years of age (59.9%). ICMR study in 1985 also reported 63% between 1 to 3 years of age and 44% between 3 to 5 years of age group.

Sex Incidence

In this study 36 (60%) were male children and 24 (40%) were female children. Out of 36 male children 22 (61%) were below 6 years and 14 (39%) were between 6 to 12 years and out of 24 female children 14 (58%) were below 6 years and 10 (42%) were between 6 to 12 years. There was male predominance with ratio 1.5: 1.

There is higher rate of anemia in male children between 6 months to 6 years, but rate is higher in female children between 6 to 12 years of age group. ICMR study in 1985 has shown no sex difference below 6 years of age, but the rate is higher in female children above 6 years.

1. Lab Results

1.1 Hemoglobin

In this study, the mean baseline Hemoglobin was 9.87gms%, 8.40gms%, 10.14gms% and 8.96gms% in group 1, 2, 3 and 4 respectively.

At 1st and 2nd follow up after iron therapy at 3rd and 6th week there was significant rise .In a study done by Stanley *et al.,* 2000 showed the initial mean baseline Hemoglobin was 8.7gm/dl, and following 2 months of oral iron therapy 3 times per day mean hemoglobin was 10gm/dl. The magnitude of response is related to the degree of anemia. Hemoglobin rises at an average of 0.25gm to 0.4gm/dl/day following oral iron therapy (Stanley Zlotkin *et al.,* 2001).

1.2. RBC Counts

In this study, the mean baseline RBC counts were 4.31mil/cumm, 4.17mil/cumm, 4.08mil/cumm and 4.08mil/cumm in group1, group 2, group 3and group 4 respectively. The RBC counts increased in all the age groups but were not statistically significant except in Group 3(0.0005).

1.3. Red Cell Indices

MCV, MCH and MCHC are the red cell indices, which are generally low in Iron deficiency anemia. The MCV provides the average cell size. MCH provides the average haemoglobin per cell. MCHC provides a more accurate value of the haemoglobin level of each cell.

Out of 36 children less than 6 years, 23 had MCV less than 70 fl and 13 had MCV between 70-95 fl and out of 24 children between 6-12 years, 8 had MCV less than 75 fl and 16 had MCV between 75-95 fl.

The MCV at any given time will reflect an aggregate of the MCV of all the cells produced within last 4 months and hence do not reflect rapidly developing iron deficiency[13]. The MCV may not be useful because the long survival of red cells allows the indices to remain in the normal range for 6 to 12 weeks following the development of iron deficiency, as shown by classic phlebotomy study of Conrad and Crosby (Johnson *et al.,* 1983).

In this study, the mean baseline MCV was 71.35fl, 65.43fl, 76.47fl and 68.6fl in group1, group 2, group 3 and group 4 respectively. The values showed significant changes in all the age groups after oral iron therapy at 3rd and 6th week.

In these children the mean baseline MCH was 22.85pg, 20.46pg, 24.93 and 22.1pg in group1, group 2, group 3 and group 4 respectively. After oral iron therapy the values showed significant changes in all the age groups except in Group 4, this may be due to less number of subjects.

In these children the mean baseline MCHC was 32.28g/dl, 31.21g/dl, 32.55g/dl and 32.16g/dl in group1, group 2, group 3 and group 4 respectively. After iron therapy the values improved but not statistically significant.

Dallman *et al.,* 1980; Conrad *et al.,* 1962 and England *et al.,* 1976; Dallman *et al.,* 1980in their study showed that, when derived from electronic cell counting the MCV and the MCH are far more sensitive than the MCHC for detection of iron deficiency.

1.4. Red cell distribution width (RDW)

RDW is the standard deviation of the erythrocyte cell size divided by the average erythrocyte cell size (MCV), which provides the percentage of the erythrocytes out side of the reference range.

In this study, all the children had RDW more than 14.5%. The mean baseline RDW was 16.48%, 17.03%, 15.88% and 17.28% in group1, group 2, group 3 and group 4 respectively. After iron therapy the decrease in RDW is statistically significant in all the Groups. There was slight elevation in RDW at 3rd week in Group 1 that may be due to accelerated erythropoisis with brisk reticulocytosis. In this study RDW was found 100% sensitive in detecting both mild and moderate anemia.

Vishwanath *et al.,* 2001; England *et al.,* 1976 showed RDW was elevated and was more sensitive in detecting iron deficiency anemia than peripheral smear. In their study they concluded that RDW is 100% sensitive in mild degrees of anemia, thus aiding early diagnosis of iron deficiency anemia.

McCluer *et al.,*1985 concluded in their study that RDW was 100% sensitive and was useful in early detection of iron deficiency anemia. Bessman *et al.,* 1983 found the sensitivity of RDW to be very high, 96 to 100% in detecting iron deficiency. Patton *et al.,* 1991; McClure *et al.,* 1985 showed that an elevated RDW appears to be the earliest haematological manifestation in iron deficiency anemia.

1.5. Reticulocyte Count

In this study 77% of children were within normal range (0.5 to 105%) and 23% of children had moderately elevated reticulocyte counts. The mean baseline reticulocyte counts were 1.25%, 1.27%, 1.09% and 1.16 in group1, group 2, group 3 and group 4 respectively. There was slight increase in counts after iron therapy at 3rd week and were normal at 6th week.

In iron deficiency reticulocyte count may be normal or moderately elevated normal rnage varies from 0.5 to 1.5%. The peak reticulocyte response is reached on the 5th to 10th day after starting oral iron therapy. The reticulocyte increase is inversely proportional to the severity of the anemia.

1.6. Peripheral Smear

Unless morphological changes are pronounced the diagnosis of IDA from examination of blood films is difficult and unreliable.Initially in this study 26(43.4%) children had Normocytic Normochromic blood picture and 17(28.3%) children had Microcytic Hypochromic blood picture and remaining 17(28.3%) had Normocytic Hypochromic (7) and Microcytic Normochromic (10) blood picture. After oral iron therapy at 3 weeks, 7 children still showed Microcytic Hypochromic blood picture and there were 43 children with Normocytic Normochromic blood picture and at the end of 6 weeks 51 were Normocytic Normochromic and 4 remained Microcytic Hypochromic. Hence out of 17 children with Microcytic Hypochromic blood picture, 13 were converted to Normocytic Normochromic. In this overall study, the sensitivity of MCV to detect mild iron deficiency was 46.15% and moderate iron deficiency was 80.95% whereas, RDW was 100% sensitive in detecting both mild and moderate iron deficiency. The peripheral smear was 23% sensitive in detecting mild iron deficiency and 38% sensitive in detecting moderate iron deficiency. It was also observed that there is poor correlation between reticulocyte counts and RDW.

Summary

In the present study, 60 anaemic children were included on purposive sampling basis. Among them, 36(60%) were male and 24(40%) were female children. The maximum numbers of children were in the age group 6 months to 6 years (60%).. RDW was high in all the children (100%). Reticulocyte counts were in the normal range in 77% of children.After oral iron therapy there was statistically significant increase in Hemoglobin percentage.. RDW showed statistically significant changes in all groups. The RDW was 100% sensitive in detecting both mild and moderate anemia as compared to MCV (46.15% and 80.95%) and Peripheral smear (23% and 38%).

Conclusion

Majority of anemic children in this study falls below 6 years, and male predominance was seen, RDW was more sensitive in detecting mild and moderate iron deficiency anemia than Red cell indices and Peripheral smear examination.

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