

**TO EVALUATE ANAEMIA BY ERYTHROCYTE INDICES, RED CELL DISTRIBUTION WIDTH AND HAEMOGLOBIN ELECTROPHORESIS WITH SPECIAL REFERENCE TO THALASSEMIA IN PAEDIATRIC AGE GROUP**Mahendra Singh<sup>1</sup>, Anuradha Gautam<sup>2</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT:** Anemia is not a diagnosis; it is a manifestation of an underlying disorder. Thus, even mild, asymptomatic anemia should be investigated so that the primary problem can be diagnosed and treated. Laboratory evaluation begins with a CBC, including WBC and platelet counts, RBC indices and morphology (MCV, MCH, MCHC, RBC distribution width [RDW]), and examination of the peripheral smear. In many instances routine test like Hb, TLC, DLC, GBP fail to decide anemia especially in early cases and also fail to decide the type of anaemia. In such situations the RBC indices and RDW are very useful. These become abnormal even before changes in routine hemogram are appreciable. Thalassaemia minor poses problems in diagnosis because GBP reveals no features of hemolysis rather it has microcytic hypochromic picture which has similarity with iron deficiency anemia. It is difficult to differentiate between two by only GBP. Several decision making rules have been proposed for differentiation. **METHOD:** The present study was carried out in 100 cases to evaluate anaemia in different age groups based on RBC Indices and RDW and to evaluate sensitivity of RBC indices and RDW in diagnosis of anaemia. Cases showing positivity by various rules and RDW in favour of thalassaemia minor were subjected to Hb electrophoresis for confirmation of diagnosis. **RESULTS:** RBC indices are more sensitive for diagnosis of microcytic hypochromic anemia, normocytic normochromic anemia and macrocytic anemia than PBS alone. RDW-CV is superior to all in use to differentiate iron deficiency anemia and thalassaemia minor having high sensitivity 87.3% and specificity 90.5%.

**KEYWORDS:** RBC indices, RDW (red cell distribution width), Iron deficiency anemia, Thalassaemia.

**INTRODUCTION:** Anemia is defined as fall of hemoglobin concentration or the hematocrit below the lower limit of the 95% reference interval for the individual's age, sex and geographic location.

The concept of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) was first given by Wintrobe in 1929. Since then many studies have been done to classify anemia. The red blood cell indices (RBC indices) are part of a routine blood test called the complete blood count (CBC). The RBC indices measure the size, shape, and physical characteristics of the red blood cells. Along with the red blood cell count (RBC), the RBC indices are used to diagnose various forms of anemia.

A person with a low RBC and/or any abnormal RBC indices has some form of anemia. Thalassaemia is a quantitative disorder of hemoglobin. Hb electrophoresis is a reliable, rapid, reproducible and easy method to separate various Hb fractions depending on their charge and these fractions are then quantitated.

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**MATERIALS AND METHODS:** The present study was conducted in the Department of Pathology, G.S.V.M. Medical College, Kanpur, from December 2010 to September 2012. The study included 100 cases of pediatric age group (0-18 years) presenting with anemia were selected. Careful history and physical examination regarding organomegaly lymphadenopathy were elicited.

Cases showing anemia due to drugs intake, leukemia and blood loss were excluded in this study. Each case was evaluated using Hemoglobin levels, RBC indices (PCV, Red Blood Cell count and absolute values), Reticulocyte count, peripheral smear examination, bone marrow examination using Leishman stain and Hemoglobin Electrophoresis (in selected cases) was done.

**OBSERVATION:** In the present study 100 cases were studied showing anemia was most common in 13-18 years (45%) followed 6 to 12 year (30%). Along with these cases 50 healthy subject with similar age group were taken as control group.

| Age (Yrs.)   | No of Cases | Percentage   |
|--------------|-------------|--------------|
| < 1          | 09          | 09 %         |
| 1-5          | 16          | 16 %         |
| 6-12         | 30          | 30 %         |
| 13-18        | 45          | 45 %         |
| <b>Total</b> | <b>100</b>  | <b>100 %</b> |

**Table 1: AGE INCIDENCE**

The most common age group showing anemia was 13-18 yrs. (45%).

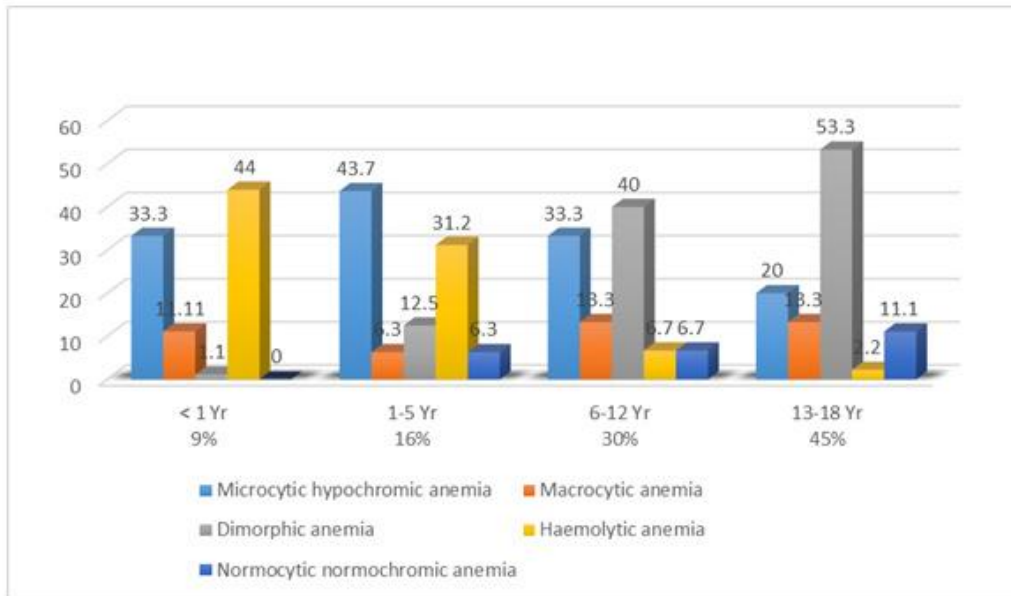
| Control Group | No of cases | Hb mean±2 SD (gm %) | MCV mean±2S SD (fl) | MCH mean±2S SD (pg.) | MCHC mean±2S SD(gm/dl) | RDW CV (%) Mean±2S SD |
|---------------|-------------|---------------------|---------------------|----------------------|------------------------|-----------------------|
| < 1Year       | 10          | 12.6±1.5            | 94.0±8.0            | 32.0±3.0             | 33.0±4.0               | 14.3±0.6              |
| 1-5 Year      | 10          | 11.8±1.5            | 88.8±5.8            | 29.8±1.6             | 33.8±1.2               | 14.3±0.6              |
| 6-12 Year     | 15          | 13.5±2.0            | 86.0±9.0            | 29±4.0               | 34±3.0                 | 14.5±1                |
| 13-18 Year    | 15          | 13.8±1.5            | 92.6±7.2            | 31.2±2.0             | 34.0±1.6               | 14.5±1                |

**Table 2: Hemoglobin, erythrocyte indices and RDW in 50 healthy subjects (control group)**

Hemoglobin & RBC Indices were found to be within the normal range for control group amongst different age groups.

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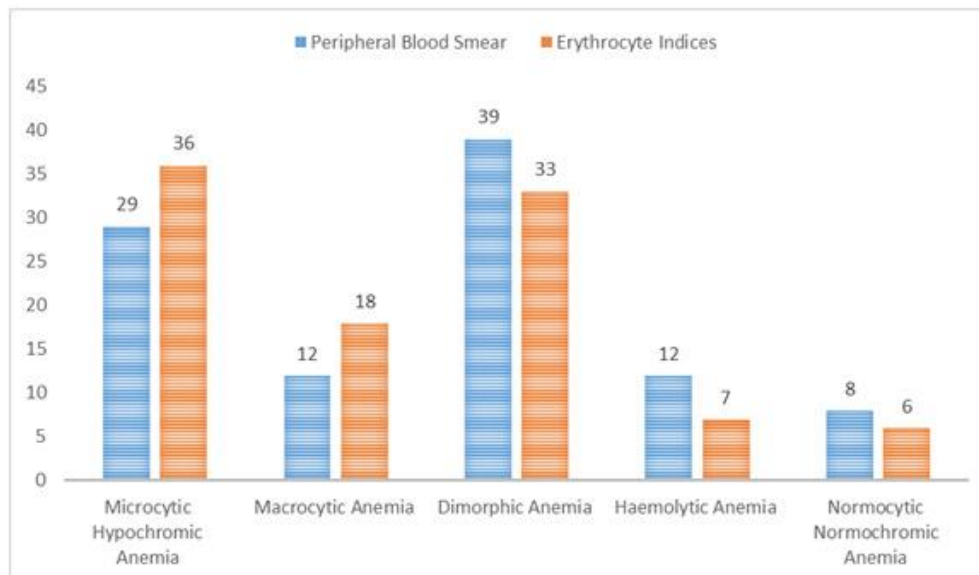
### DIFFERENT ANAEMIAS IDENTIFIED BY PERIPHERAL BLOOD SMEAR EXAMINATION IN DIFFERENT AGE GROUPS:



**Graph 1**

Dimorphic anemia was more common among the children more than 6 years of age, Microcytic hypochromic anemia was more prevalent between 1-6 years of age and Hemolytic anemia was most commonly seen in infants (<1 year).

### DIFFERENT TYPES OF ANEMIA IDENTIFIED BY PERIPHERAL BLOOD SMEAR EXAMINATIONS AND ERYTHROCYTE INDICES:



**Graph 2**

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| Type of Anemia             | MCV<br>mean±2SD<br>(fl) | MCH<br>mean±2SD<br>(pg) | MCHC<br>mean ±2SD<br>(gm %) | RDW %<br>mean ±2SD |
|----------------------------|-------------------------|-------------------------|-----------------------------|--------------------|
| Microcytic                 | 68.2±10.4               | 19.9±6.2                | 30.2±1.8                    | 19.3±3.5           |
| Macrocytic                 | 113.7±12.2              | 33.1±3.2                | 31.3±2.2                    | 23.2±2.4           |
| Dimorphic                  | 80.2±10.6               | 24.2±4.4                | 29.7±1.6                    | 20.8±2.2           |
| Haemolytic                 | 74.4±15.6               | 20.8±6.1                | 30.3±5.4                    | 14.8±2.5           |
| Normocytic<br>normochromic | 85.2±6.1                | 29.8±3.2                | 32.8±2.2                    | 18.2±1.6           |

**Table 3: RBC INDICES AND RDW IN DIFFERENT TYPES OF ANEMIA**

The differences in values of MCV, MCH and MCHC in hemolytic and microcytic anemia are insignificant ( $p>0.05$ ) while the difference in RDW values is significant ( $p<0.001$ ).

| Types of          | MCV mean ± 2SD fl | MCH mean ± 2SD pg | MCHC mean ± 2SD |
|-------------------|-------------------|-------------------|-----------------|
| Megaloblastic     | 118.5±13.4        | 33.8±3.1          | 32.1±2.4        |
| Non-Megaloblastic | 112.4±12.6        | 32.9±2.8          | 31.9±2.1        |

**TABLE 4: RED CELL INDICES IN DIFFERENT TYPES OF MACROCYTIC ANEMIA**

Values of erythrocytic indices were showing no significant difference ( $p>0.05$ ) in Megaloblastic as well as Non- Megaloblastic anemia.

| Type of Anemia         | No.of Cases | Percentage of total<br>Thalassemia Cases |
|------------------------|-------------|--|
| Thalassemia Major      | 3           | 25.0 %                                   |
| Thalassemia Intermedia | 2           | 16.67 %                                  |
| Thalassemia Minor      | 7           | 58.33 %                                  |
| <b>Total</b>           | 12          | 100 %                                    |

**TABLE 5: Hemolytic anaemias identified by peripheral blood smear examination, clinical picture and hemoglobin electrophoresis**

Cases showing anemia due to drugs, snake bites etc. were excluded from the study, Thalassemia was the only hemolytic anemia found during study period. These observations show that out of total 12 cases Thalassemia minor were the commonest.

| Type of Anemia            | MCV mean ±2SD<br>(fl) | MCH<br>mean ± 2SD<br>(pGg) | MCHC mean ± 2SD<br>(gm/dl) | RDW<br>mean±2SD<br>(%) |
|---------------------------|-----------------------|----------------------------|----------------------------|------------------------|
| Thalassemia               | 64.6±4.2              | 20.1±2.4                   | 31.5±1.2                   | 14.8±                  |
| Iron Deficiency<br>Anemia | 69.7±9.8              | 18.9±2.88                  | 29.2±1.8                   | 20.8±3.8               |

**Table 6: Erythrocyte indices and RDW in thalassemia minor and iron deficiency anemia**

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Difference in MCHC and RDW were significant ( $p < 0.001$ ) while that of MCV and MCH were insignificant ( $p > 0.05$ )

| Various Rules | Sensitivity (%) | Specificity (%) | False Positive (%) | False Negative (%) |
|---------------|-----------------|-----------------|--------------------|--------------------|
| JM England    | 57.1            | 100             | 0                  | 42.9               |
| WC Mentzer    | 71.4            | 57.1            | 42.9               | 28.5               |
| I Shine       | 85.7            | 2.6             | 71.4               | 14.3               |
| RDW           | 87.3            | 90.5            | 9.5                | 12.7               |

**Table 7: Sensitivity, specificity, false positive and false negative values of various rules and RDW for diagnosing thalassemia minor in microcytic hypochromic anemia having no feature of hemolytic anemia in peripheral blood smear.**

RDW had highest sensitivity. Specificity was 100% with rule of JM England but sensitivity was less. Therefore it may be concluded that RDW is the best among all other laws.

| Hb. Disorders          | Normal Hb           |                                 |                     | Variants           |                    |
|------------------------|---------------------|---------------------------------|---------------------|--------------------|--------------------|
|                        | HbA<br>mean/ range  | HbA <sub>2</sub><br>mean/ range | HbF<br>mean/ range  | HbE<br>mean/ range | HbS<br>mean/ range |
| Thalassemia major      | 16.1<br>(3.1-53)    | 4.5<br>(2.5-8.6)                | 80.6<br>(42.6-92.8) | -                  | -                  |
| Thalassemia Intermedia | 77.9<br>(57.5-88.4) | 3.3<br>(2.6-10.4)               | 20.0<br>(10-30)     | -                  | -                  |
| Thalassemia minor      | 93.6<br>(91.4-95.1) | 5.3<br>(3.8-8.6)                | 1.6<br>(1.2-2.8)    | -                  | -                  |

**Table 8: Mean and range of various haemoglobins in different hemoglobin disorders**

HbA and HbA<sub>2</sub> were the maximum in Thalassemia minor, HbF was the maximum in Thalassemia major

**DISCUSSION:** The cases having anemia were classified by peripheral blood smear examination into five major groups- Microcytic hypochromic anemia, Macrocytic anemia, Dimorphic anemia, Hemolytic and Normocytic normochromic anemia.

In the present study it was observed that the most common age group involved in anemia was 13-18 yrs (45%). Out of total 100 cases studied dimorphic anemia was more common among the children >6 years of age, microcytic hypochromic anemia was more prevalent between 1-6 years of age and hemolytic anemia was most commonly seen in infants (< 1 year).

The most common etiology for anemia in present study was nutritional deficiency. Banerjee et al (1968), Sabah et al (2010) also concluded same cause.

In the present study 50 healthy individuals of different pediatric age groups were taken as control group. The mean values of various indices were found similar to that were reported by Dacie and Lewis (1994).

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By PBS examination 29 cases were of microcytic hypochromic anemia and 12 cases were of macrocytic anemia while RBC indices showed 36 cases as microcytic hypochromic and 18 cases as macrocytic anemia. Thus 7 cases of microcytic hypochromic anemia and 6 cases of macrocytic anemia were missed by PBS examination alone. Additional 7 cases, which were diagnosed as microcytic hypochromic by RBC indices, were having MCV ranging from 76 to 79 fl and MCHC 31 to 33.8 gm%. Iron studies in these cases showed that out of 7 cases, 3 cases were of early iron deficiency anemia. This suggests that assessment of MCV give important clue in early diagnosis of iron deficiency anemia. Similar findings were noted by Fairbank (1971) and England JM (1976).

- a) Microcytic Hypochromic anemia:** Microcytic hypochromic anemia cases showed the mean values of MCV, MCH and MCHC  $68.2 \pm 10.4$  fl,  $19.9 \pm 6.2$  pg and  $30.2 \pm 1.8$  gm/dl respectively. These values were significantly lower than control group ( $p < 0.001$ ). RDW was increased; mean value was  $19.3 \pm 3.5$ .

Among various causes of microcytic hypochromic picture, the common causes are - iron deficiency anemia and Thalassemia minor. In present study the MCV was decreased in both cases, mean MCV and MCHC values in iron deficiency anemia were  $69.7 \pm 9.8$  fl and  $29.2 \pm 1.8$  gm/dl, while for Thalassemia minor these were  $64.6 \pm 4.2$  fl and  $31.5 \pm 1.2$  gm/dl. The difference in MCV was not significant ( $p > 0.05$ ) while for MCHC it was significant ( $p < 0.001$ ). It means that MCV cannot differentiate between these two conditions but MCHC may serve an important role for screening the population for Thalassemia minor. The similar results were reported by Johnson et al (1983) and Marti HR et al (1987).

- b) Macrocytic anemia:** In 18% cases MCV was  $> 100$  fl. Cases showing macrocytosis grouped into 2 broad groups i.e. megaloblastic and non- megaloblastic anemia after examining the peripheral blood smear and bone marrow aspiration smear. In present study values of MCV, MCV and MCHC in cases with megaloblastic anemia were  $118.5 \pm 13.4$  fl,  $33.8 \pm 3.1$  pg and  $32.1 \pm 2.4$  gm/dl. Similar values were reported by Hoffbrand V (1997), Unnikrishnan V (2008).

MCV, MCH and MCHC in non-megaloblastic anemia were  $112.4 \pm 12.6$  fl,  $32.9 \pm 2.8$  pg and  $31.9 \pm 2.1$  gm/dl respectively. These values showed no significant difference ( $p > 0.05$ ). So these two types can't be differentiated by erythrocyte indices alone.

- c) Dimorphic Anemia:** Values of MCV, MCH, MCHC and RDW in dimorphic anemia cases were  $80.2 \pm 10.6$  fl,  $24.2 \pm 4.4$  pg,  $29.7 \pm 1.6$  gm/dl and  $20.8 \pm 2.2$ . The diagnosis of dimorphic anemia rests on PBS examination. Erythrocyte indices were not helpful. Due to presence of dual population of cells MCV remains within normal limit or shifts slightly towards higher side if macrocytes predominate or towards lower side, if microcytes predominate. Same result was concluded by Kakkar et al (2009).
- d) Normocytic Normochromic Anemia:** Present study showed the values of MCV, MCH, MCHC and RDW in normocytic normochromic anemia  $85.2 \pm 6.1$  fl,  $29.8 \pm 3.2$  pg,  $32.8 \pm 2.2$  gm/dl and  $18.2 \pm 1.6$  respectively. Normocytic normochromic picture is seen in disorders of diverse etiology e.g. aplastic anemia, intracorpuscular and extracorpuscular defects of RBC, collagen disorders etc. But it is not possible to trace the specific cause by erythrocyte indices.
- e) Hemolytic Anemia:** In hemolytic anemia values of MCV, MCH, MCHC and RDW were  $74.4 \pm 15.6$

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fl,  $20.8 \pm 6.1$  pg,  $30.3 \pm 5.4$  gm/dl and  $14.8 \pm 2.5$  respectively. These data favour microcytic picture but classical picture reported with hemolytic anemia is normocytic or macrocytic. The cause of this discrepancy may be that in present study thalassemia was the major cause of hemolytic anemia.

The mean value of MCV in Thalassemia major was  $65.48 \pm 6.3$  fl which corresponds to the values reported by Baty (1932) and Smith (1948).

Out of total 100 cases, 12 cases (12%) were suspected to have various hemoglobin disorders. Suspicion was raised by hematological findings and erythrocyte indices. As the cases showing anemia due to drugs, snake bites etc. were excluded from the study, the only hemoglobin disorder diagnosed in present study was Thalassemia.

Sunna et al (1996) and Lau et al (1997) estimated prevalence of beta - Thalassemia and found it 4.45% and 3.4% respectively. Madan N (2010) found frequency of beta thalassemia trait in India between 1-17% (average 4.5%). Indian Council of Medical Research, New Delhi (1993) found that  $\beta$ -Thalassemia is the commonest haemoglobin disorder in India and estimated prevalence between 2.7 to 14.9% with an average of 4.5% in different regions of India. In present study prevalence of Thalassemia was 12% with maximum number of cases (7 out of 12) were Thalassemia minor constituting 58.33% among cases of  $\beta$ -Thalassemia. No case of  $\alpha$ -Thalassemia was found during study.

In present study cases with Thalassemia major showed mean values of HbA, HbF and HbA<sub>2</sub> 16.1% (3.1-53%), 80.6% (42.6-92.8%) and 4.5% (2.5-8.6%) respectively. Silvestroni et al (1968) concluded that homozygous  $\beta^0$ -Thalassemia is associated with predominance of HbF with total absence of HbA and variable amount of HbA<sub>2</sub>. In present study no case showed absence of HbA so none of the cases were of homozygous  $\beta^0$ -Thalassemia. Kattamis et al (1979) concluded that in individuals with homozygous  $\beta^0$ -Thalassemia of Mediterranean variety and those with doubly heterozygous  $\beta^0/\beta^+$  Thalassemia, amount of HbA are variable, HbF is increased and HbA<sub>2</sub> is normal, decreased or elevated. The mean levels of HbA<sub>2</sub> were 4.1 % (2.4-8.7%) and 1.8% (0.6-3.4%) in  $\beta^+/ \beta^+$  and  $\beta^0/ \beta^+$  thalassemia respectively. So our study has level of HbA<sub>2</sub> very close to  $\beta^+/ \beta^+$  thalassemia. But as such no definite criteria's are laid down till now for diagnosis or differentiation of  $\beta^+/ \beta^+$  and  $\beta^0/ \beta^+$  Thalassemia by levels of HbA<sub>2</sub>.

Thalassemia intermedia cases showed mean values of HbA, HbF and HbA<sub>2</sub> 77.9% (57.5-88.3%), 20% (10-30%) and 3.3% (2.6-4%) respectively. Involvement of  $\delta\beta$ -gene usually results in fall of or total absence of HbA<sub>2</sub>. No case in our study corresponded to this level of HbA<sub>2</sub>, so most likely genotype in our study should be  $\beta^+/ \beta^+$  black or  $\beta^0/ \beta^+$ .

In Thalassemia minor the mean values of HbA, HbF and HbA<sub>2</sub> were 93.6% (91.4-95.1 %), 1.6% (1.22-8%) and 5.3% (3.8-8.6%) respectively. Kunkel et al (1957) reported that in thalassemia minor hemoglobin electrophoresis demonstrates the predominance of HbA, increased level of HbA<sub>2</sub> (3.5-8.0%) and normal or minimally increased level of HbF. Fortova et al (1995) demonstrated range of HbA<sub>2</sub> for thalassemia minor between  $6.04 \pm 0.96$  %. In our study the levels of HbA<sub>2</sub> correspond well with all these reported values.

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**ROLE OF VARIOUS RULES AND RDW IN DIFFERENTIATION OF THALASSEMIA MINOR FROM IRON DEFICIENCY ANEMIA:** Thalassemia minor poses problem in diagnosis because GBP reveals no feature of hemolysis rather it has microcytic hypochromic picture which has similarity with iron deficiency anemia. Basophilic stippling and target cells tend to be more prominent in thalassemia minor than in iron deficiency. But it is very difficult to differentiate between two by only GBP, so several decision making rules have been proposed for differentiation will be discussed here.

In present study on applying different rules on suspected cases of Thalassemia minor- JM England proved to be superior among these by having 100% specificity but sensitivity was less. Shine's rule had sensitivity of 85.7% but specificity was only 28.6%. So application of Shine's rule detected 71.4% false positive cases. Mentzer's law was having sensitivity and specificity 71.4% and 57.1 % respectively and among all other rule it was more accurate having good sensitivity and specificity both. ICSH (1978) reported that accuracy of various rules was not more than 70% to 90%.

The major cause of the inconsistency performance of these rules can be the combined presence of iron deficiency and Thalassemia minor. In iron deficiency TRBC has lesser value than thalassemia minor so rule taking MCV and TRBC both or TRBC alone in calculation has higher specificity. But in Shine's law which takes only MCV and MCH in calculation came out to be most sensitive but least specific because higher values of TRBC do not influence this rule.

RDW served the best role in differentiation between Thalassemia minor and iron deficiency anemia having high sensitivity (87.3%) and specificity (90.5%). Normal value of RDW - CV in our study was  $14.3 \pm 0.6$ . The value of RDW in Thalassemia minor and iron deficiency anemia was  $14.8\% \pm 2.5$  and  $20.8\% \pm 3.8$  respectively, showing significant difference ( $p < 0.001$ ). RDW remained normal in Thalassemia minor while it was increased in iron deficiency anemia. Same findings were reported by Johnson et al (1983), Marti and Fisher (1987).

**CONCLUSION:** Dimorphic anemia is the commonest anemia in the study. Erythrocyte indices are more sensitive for diagnosis of Microcytic hypochromic, Normocytic normochromic and Macrocytic anemia than peripheral blood smear examination alone but can't diagnose Dimorphic and Hemolytic anemia and can't differentiate between megaloblastic and non-megaloblastic anemia. Prevalence of Thalassemia minor is the maximum among all Thalassemia. None of the indices is 100% sensitive and 100% specific. But among all indices RDW is the most reliable in differentiation of beta Thalassemia minor and Iron deficiency anemia.

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