

Study of anaemia in women of reproductive age group

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Abstract

Background: Women of reproductive age group are prone to anaemia owing to menstrual blood loss, poor dietary intake, increase in blood supply during pregnancy, infections etc. **Aim:** The present study will help in typing and diagnosis of anaemia, which in turn help to reduce the consequences. **Materials and Methods:** The present prospective study was carried out on a study population of women between 15 – 45 years of age that attended the clinical pathology laboratory from Dec 2019 to March 2021 at the Department of Pathology, GITAM Institute of Medical Sciences and Research, Andhra Pradesh, India. Venous blood was collected in EDTA anticoagulant containing container for hematological investigations. Investigations were carried out through an automated hemocytometer and also manually. As a part of complete blood picture, all the red blood cell indices along with cell counts, differential leucocyte counts were obtained by automated analyzer. Peripheral blood smears stained with Leishman's stain were studied in all the cases. The smears were then examined under 40X (high power) and 100X (oil immersion). Bone marrow aspiration studies, serum ferritin levels, serum iron levels were studied wherever possible in identified cases. **Results:** Majority of the women in the present study have normal hemoglobin levels (50.3%), 17.2 % of women show mild anaemia, 24.9% show moderate anaemia and 7.6% show severe degree of anaemia. The present study show statistically significant values (P<0.001) of MCV and RDW values in relation to hemoglobin levels in both pregnant and non-pregnant women. **Conclusion:** Red cell parameters like MCV and RDW are helpful in early diagnosis and early effective treatment of anaemia.

Keywords: Haemoglobin, Anaemia, blood smear, RBC, Pregnant women, reproductive age

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Introduction

Anaemia is a global health problem affecting both the developing and developed countries with major consequences for human health as well as social and economic development [1]. It is estimated that in India it ranges from 33% to 89% among pregnant woman and is more than 60% in adolescent girls.

National Family Health Survey – 4 (NFHS), under Ministry of Health and Family Welfare, Government of India, conducted series of surveys based on a sample households that are representative at national and state level. It conducted interviews on women in the age group 15-49 and men in the age group 15-54 years. NFHS produced population based estimates of anaemia, HIV prevalence, blood glucose, blood pressure, height and weight measurements among women age 15 -49 and men age 15-54. Among young children age 6-71months, anaemia, height and weight are measured. The survey estimates the prevalence of anaemia 55.3% among women of reproductive age group and 53.2% in non-pregnant non lactating women [2]. Nutritional anaemia is more common type affecting

Iron deficiency anaemia is the most common type met with in routine clinical practice in women of reproductive age groups and is evidenced by microcytic hypochromic blood picture. Women of reproductive age group are prone to anaemia owing to menstrual blood loss, poor dietary intake, increase in blood supply during pregnancy, infections etc [3]. The consequences of anaemia for women include increased risk of low birth weight or prematurity, perinatal and neonatal mortality, inadequate iron stores for the newborn, increased risk of maternal morbidity and mortality, and lowered physical activity, mental concentration, and productivity[4]. In India and other developing countries, the incidence of nutritional anaemia in reproductive age group ranges from 60 – 80% compared to 10 – 20% in developed countries[5]. In Asia the prevalence of nutritional anaemia is particularly high in countries such as Bangladesh (74-80%), India (34- 69%)[6]. Since several decades, it has been known to be important problem in most tropical countries[7]. Anaemia is defined as a reduction of the total circulating red cell mass below normal limits. For practical purposes, any of the three concentration measurements are used to establish the presence of anaemia-Hemoglobin level (gm/dl), hematocrit (%), RBC count ($10^{12}/L$).

In the past, these parameters were measured using manual physical and chemical techniques, but now these assays are determined by electronic cell counters and Hb analyzers. In most of the current analyses, RBC number, Hb concentration and mean corpuscular volume (MCV) are directly measured. These values are used to calculate the hematocrit, the mean corpuscular hemoglobin (MCH) and the MCH concentration (MCHC). The electronic counters also generate an index of red cell size and the red cell distribution width (RDW). The RDW is a quantitative measure of the variation in red cell size, and higher the values, the more heterogeneous the RBC

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population size. The mean normal hemoglobin and hematocrit value and the lower limits of the normal range depend on the age and the gender of the subjects, as well as their altitude of residence[8]. This study will help in typing and diagnosis of anaemia, which in turn help in reduce the consequences.

Material and methods

The present prospective study was carried out on a study population of women between 15 – 45 years of age that attended the clinical pathology laboratory from Dec 2019 to March 2021 at the Department of Pathology, GITAM Institute of Medical Sciences and Research, Andhra Pradesh, India.

A detailed clinical history was taken from the study population and the procedure was well explained prior to the execution. Venous blood was collected in EDTA anticoagulant containing container for hematological investigations. Investigations were carried out through an automated hemocytometer and also manually. As a part of complete blood picture, all the red blood cell indices along with cell counts, differential leucocyte counts were obtained by automated analyzer. Peripheral blood smears stained with Leishman's stain were studied in all the cases. The smears were then examined under 40X (high power) and 100X (oil immersion). Bone marrow aspiration studies, serum ferritin levels, serum iron levels were studied wherever possible in identified cases.

Inclusion criteria

Women of reproductive age group (15 – 45 years) including the pregnant women are included in the present study

Exclusion criteria

Patients who received blood transfusions were excluded. Patients with known hematological ailments are excluded from the study.

Results

In the present study, majority of the cases were reported in the age group of 21-25 years with 28.6% and 67% of the women in the study population were non-pregnant while 33% are pregnant. Majority of the women in the present study have normal hemoglobin levels (50.3%), 17.2 % of women show mild anaemia, 24.9% show moderate anaemia and 7.6% show severe degree of anaemia (Table 1).

In the present study, the minimum value of hemoglobin observed is 2 g/dl and the maximum value is 18.4 g/dl. The mean hemoglobin value observed was 11.17 g/dl (Table 2). Statistical analysis showed the

positive relation of MCV with the hemoglobin levels ($r = 0.556$) (Table 3 and Figure 1).

In the present study the data is statistically evaluated and the mean MCV values were calculated for various degrees of anaemia. From the mean and standard error values, 95% confidence intervals are calculated and the lower (LL) and upper limits (UL) of MCV values for various degrees of anaemia are calculated (Table 4 and Table 5). Our study show statistically significant values ($P < 0.001$) in study of MCV values in relation to hemoglobin levels in both pregnant and non-pregnant women (Table 6).

Deviations in the MCV values

In the present study, out of 503 women with normal hemoglobin levels, 47 women show decreased MCV values constituting to 9.3%. Out of 172 women with mild degree of anaemia, normal levels of MCV are observed in 42 women (24.4 %). Out of 249 women with moderate degree of anaemia, MCV values in normal range are observed in 39 women (15.6%). Out of 76 women with severe degree of anaemia, normal MCV values are observed in 15 women (19.7%). These deviations from the normal range are seen in 14.6% of non-pregnant women and 13.5 % of pregnant women. Through Pearson's correlation analysis, it was found that, RDW with Hb levels showed significant ($P < 0.001$) negative correlation ($r: -0.564$) (Figure 2). In the present study, mean RDW values for the degrees of anaemia are calculated. From the mean and standard error values, 95% confidence intervals are calculated and the lower and upper limits of RDW values for various degrees of anaemia are evaluated (Table 7 and Table 8). The present study show statistically significant values ($P < 0.001$) of RDW values in relation to hemoglobin levels in both pregnant and non-pregnant women (Table 9).

Deviations of RDW values

In the present study, out of 503 women with normal hemoglobin levels 10 women (1.9%) show increased RDW levels. Out of 241 samples that are validated as microcytic hypochromic anaemia, 2% women showed RDW values within normal limits. Out of 266 pregnant women in the present study, 41 women (15.4%) show increased RDW values with normal hemoglobin values. In the present study, normocytic normochromic blood picture (NNBP) is observed in 457 women (45.7%), normocytic normochromic anaemia (NNA) in 279 women (27.9%), microcytic hypochromic anaemia (MHA) in 241 women (24.1%), dimorphic anaemia (DA) in 22 women (2.2%) and a single case of hemolytic anaemia (HA) is observed (Table 10). Figure 3a-3f showed the RBC morphological findings of current study.

Table 1: Distribution of study cases based on Age, Pregnancy status and Severity of anaemia

Age	Frequency	Percentage (%)
15-20	191	19.1
21-25	286	28.6
26-30	177	17.7
31-35	115	11.5
36-40	122	12.2
41-45	109	10.9
Total	1000	100.0
Pregnancy Status	Frequency	Percentage
Non Pregnancy	669	66.9
Pregnancy	331	33.1
Total	1000	100.0
Degree of Anaemia	Frequency	Percentage
Severe	76	7.6
Moderate	249	24.9
Mild	172	17.2
Normal	503	50.3
Total	1000	100.0

Table 2: Table showing distribution of hemoglobin (Hb) values

Variable	N	Minimum	Maximum	Mean	SD
Hb	1000	2.0	18.4	11.17	2.08

Table 3: Table showing correlation of MCV with Hb levels.

Correlation with Hb			
Variable	N	r-value	P-value
MCV	1000	0.556	<0.001

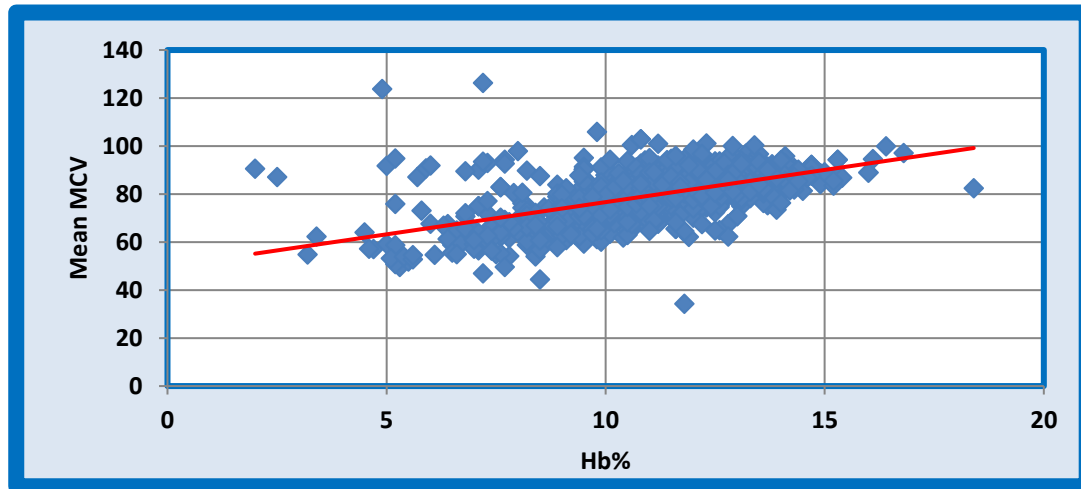


Figure 1: Scatter plot showing relation of MCV with Hb levels.

Table 4: Distribution of MCV values according to the degree of anaemia

Degree of Anaemia	MCV				
	N	Minimum	Maximum	Mean	SD
Severe	76	46.9	126.3	68.86	16.16
Moderate	249	44.3	105.8	72.94	8.59
Mild	172	64.6	102.8	78.93	7.19
Normal	503	34.2	101.0	84.40	6.61

F=153.2; P<0.001

Table 5: Statistical analysis of MCV values in relation to degree of anaemia

Degree of Anaemia	MCV		95% CI	
	Mean	SE	LL	UL
Severe	68.86	1.85	65.23	72.49
Moderate	72.94	0.54	71.88	74.01
Mild	78.93	0.55	75.86	79.01
Normal	84.40	0.29	80.83	84.98

Table 6: statistical analysis of MCV in relation to Hb values in both pregnant and non-pregnant women.

Pregnancy Status	Degree of Anaemia	MCV						95% CI	
		N	Minimum	Maximum	Mean	SD	SE	LL	UL
NON PREG	Severe	67	46.9	126.3	69.21	16.28	1.99	65.31	73.11
	Moderate	202	44.3	105.8	73.30	8.21	0.58	72.17	74.43
	Mild	122	64.6	100.9	78.06	6.28	0.57	78.95	79.17
	Normal	278	62.3	101.0	84.88	6.23	0.37	80.15	85.61
F=108.25; P<0.001									
PREG	Severe	9	49.5	94.8	66.23	15.83	5.28	55.89	76.58
	Moderate	47	58.8	97.8	71.43	10.01	1.46	68.56	74.29
	Mild	50	69.1	102.8	80.06	8.75	1.24	78.63	80.49
	Normal	225	34.2	100.3	81.81	7.03	0.47	81.90	84.73
F=41.68; P<0.001									

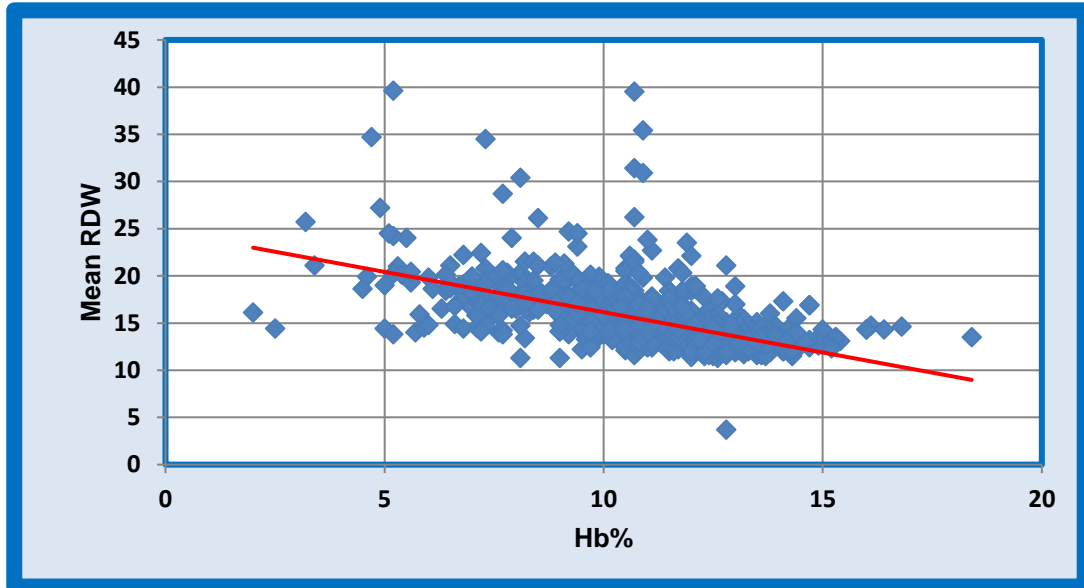


Figure 2: Scatter plot showing the relation of RDW values with the Hemoglobin levels.

Table 7: Distribution of RDW values according to the degree of anaemia

Degree of Anaemia	RDW				
	N	Minimum	Maximum	Mean	SD
Severe	76	13.8	39.6	19.02	4.73
Moderate	249	11.3	35.4	16.94	2.99
Mild	172	12.0	39.5	15.23	3.17
Normal	503	3.7	22.1	13.66	1.55

F=148.58; P<0.001

Table 8: Statistical analysis of RDW values in relation to degree of anaemia

Degree of Anaemia	RDW		95% CI	
	Mean	SE	LL	UL
Severe	19.02	0.54	17.95	20.08
Moderate	16.94	0.19	16.57	17.31
Mild	15.23	0.24	14.76	15.71
Normal	13.66	0.07	13.52	13.80

Table 9: Statistical analysis of RDW in relation to Hb values in both pregnant and non-pregnant women.

Pregnancy Status`	Degree of Anaemia	RDW						95% CI	
		N	Minimum	Maximum	Mean	SD	SE	LL	UL
Non Pregnancy	Severe	67	13.8	39.6	19.12	4.93	0.60	17.94	20.30
	Moderate	202	11.3	35.4	16.91	3.07	0.22	16.48	17.33
	Mild	122	12.0	23.8	14.84	2.17	0.20	14.46	15.23
	Normal	278	11.3	21.1	13.45	1.41	0.08	13.29	13.62
F=118.71; P<0.001									
Pregnancy	Severe	9	13.8	21.1	18.29	2.95	0.98	16.36	20.22
	Moderate	47	12.2	24.5	17.09	2.68	0.39	16.32	17.86
	Mild	50	12.1	39.5	16.19	4.69	0.66	14.89	17.49
	Normal	225	3.7	22.1	13.92	1.68	0.11	13.70	14.14
F=32.37; P<0.001									

Table 10: Table showing distribution of peripheral smear findings

Peripheral Smear	Frequency	Percent
DA+HA	1	.1
DA	22	2.2
MHA	241	24.1
NNA	279	27.9
NNBP	457	45.7
Total	1000	100.0

Bone marrow studies

Out of 1000 study population, bone marrow studies is recommended for four cases with severe anaemia which were opined as erythroid hyperplasia with nutritional anaemia. As three of these women have prior blood transfusions, they were excluded from our present study. The case included in our study showed erythroid hyperplasia with megaloblastic maturation on bone marrow aspiration studies.

Serum Ferritin:

In few cases with microcytic hypochromic blood picture, evaluation of serum ferritin levels was done to identify the iron deficiency.

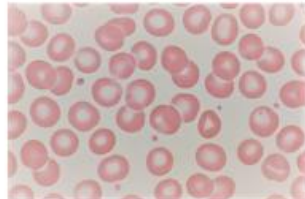


Fig.3a: peripheral smear (40X) showing Normocytic Normochromic erythrocytes.

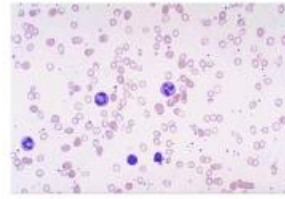


Fig.3b: peripheral smear (20X) showing microcytic hypochromic picture with anisopoikilocytosis.

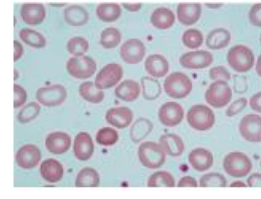


Fig.3c: peripheral smear (40X) showing dimorphic picture with normocytes and microcytes

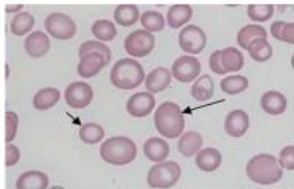


Fig.3d: peripheral smear (40 X) showing dimorphic picture with normocytes and macrocytes

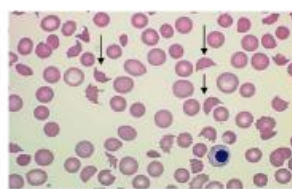


Fig.3e: peripheral smear (40X) showing hemolytic picture with schistocytes, microspherocytes and a nucleated erythrocyte.

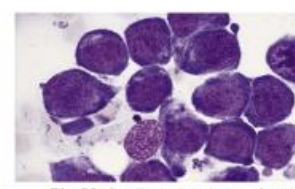


Fig.3f: bone marrow aspirate smear (100X) showing erythroid hyperplasia with megaloblastic maturation.

Figure 3a-3f: RBC Morphological findings

Discussion

Anaemia is a global health problem seen mainly in the developing countries. It is mainly seen especially in women of reproductive age group. Various hematological parameters along with the peripheral smear findings can help in diagnosing and typing of anaemia in majority of the cases without any assistance of special investigations. The present study concentrated in the study of anaemia in women of reproductive age group with correlation of hemoglobin values with red cell indices like mean corpuscular volume (MCV) and red cell distribution width (RDW).

The present study included 1000 women in reproductive age group (15 – 45 years). In the study population, majority of women are in the age group of 21 to 25 years with 28.6% followed by 26 to 30 years with 17.7%. The mean age of the females in the present study was 28.47 years. These findings correlated with the study done by Rairiker et al[9]. and Verma et al[10]. High prevalence of anaemia in this age group owes to the menstrual losses, nutritional insufficiency, pregnancy etc. Out of 1000 women in the study population, 669 women were non pregnant and 331 women were pregnant. We categorized the study population on the basis of hemoglobin values proposed by World Health Organization, into normal, mild, moderate and severe degrees of anaemia. Majority of women have normal hemoglobin values. 24.9% of women have moderate anaemia followed by 17.2% with mild and 7.6% with severe anaemia. Among the anaemic women, 15.3% has severe, 50.1% has moderate and 34.6% has mild degree of anaemia. The values obtained are correlating with those from the study done by Rairiker et al[9].

In the present study, out of 669 non pregnant women, 10% of women has severe anaemia, 30% of women has mild anaemia and 18.2% of women has moderate anaemia. Out of 333 pregnant women, 2.7% of women show severe anaemia, 14.1 % of moderate anaemia and 15% of women has mild anaemia. Red cell indices are valuable in the morphologic classification of anaemias. Since different etiologic factors result in characteristically different red cell morphology, the clinician can properly plan the management of a patient with an anaemia if he can interpret the blood counts and peripheral blood

smear well. MCV values were lowest in severe anaemia in the present study. This is confirmed by the study done by Ryan (2006).^[11] In the present study which shows statistically significant ($p = <0.001$) results, mean values of MCV has been proposed for various degrees of anaemia. The mean MCV values for various degrees of anaemia in the present study show mild deviation with the findings of Anil kumar et al.[12].

In 22 cases of dimorphic anaemia and a single case of hemolytic anaemia we validated in the present study, the MCV values are higher than the normal range. MCV values as high as 126.3 fl are recorded in these samples. The high values indicate the increased erythrocyte volume resulting in macrocytosis. Defects in nuclear maturation as seen in megaloblastic anaemias due to vitamin B12 or folate deficiency result in large oval erythrocytes (macro ovalocytes). Red cell distribution width (RDW) is the red cell measurement that quantitates the cellular volume, heterogeneity, reflecting the range of red cell sizes within the blood sample. Normal values for RDW-CV are 12.8+/- 1.2%. In the present study, 47% of women show RDW values within normal range and 53% women has higher RDW values. The values are correlating with those from the study done by Anil Kumar et al.[12]. The mean values for various degree of anaemia are calculated for both pregnant and non-pregnant women. The mean RDW values for mild, moderate and severe anaemia are 15.23, 16.94 and 19.02 respectively. The values are in correlation with the studies done by Anil Kumar et al.[12], Vishwanath et al.[13] and Carmen et al.[14]. The mean values of RDW for various degrees of anaemia in pregnant women are 16.19, 17.9 and 18.29 for mild, moderate and severe anaemia. The values correlated with those of the study done by Geethanjali et al.[15].

In the present study, out of 503 women with normal hemoglobin levels 1.9% women show increased RDW levels. Since RDW is a measure of variation in the red cell volume, it is expected to increase in the early stages of anaemia even with normal other indices. In the pre latent and latent phases of iron deficiency anaemia, the MCV values are normal and the RDW shows mild increase due to the appearance of micro erythrocytes in the peripheral blood [16].

According to Park et al.[17] nutritional deficiency whether iron, folate or vitamin B12 always causes increased RDW. Out of 266 pregnant women in the present study, 41 women (15.4%) show increased RDW values with normal hemoglobin values. RDW increased significantly during pregnancy, mainly in III trimester or at onset of labor. The unexpected rise in RDW during pregnancy suggest increased bone marrow activity. Studies reported it as a useful indicator of impeding parturition[18]. Comparison of RDW between iron deficient & non-iron deficient pregnant women found that RDW appears to be a reliable and useful parameter for detection of iron deficiency during pregnancy[19,20]. The hemolytic anaemia is diagnosed in a 38 year old woman with 4.9 gm% hemoglobin, 123.7 fl MCV and 27.2 % of RDW. Peripheral smear showed fragmented red cells, microspherocytosis among others. Other special investigations are pursued in view of hemolytic anaemia. Hemolytic picture is associated with low mean corpuscular volume of erythrocytes. As the proportion of fragmented red cells are low, the changes in MCV values are not that conspicuous.

A single case of bone marrow aspiration study is included in the present study. The 31 year old woman presented with chronic weakness. Her complete blood counts showed Hb of 2.5 gm%, MCV - 87 fl and RDW -14.2%. Peripheral smear showed normocytic normochromic anaemia. Serum Ferritin levels are within normal limits. Bone marrow aspiration revealed erythroid hyperplasia with megaloblastic maturation. In the present study, 149 samples showed microcytic homogenous picture with low MCV values and normal RDW values. These cases probably are due to a chronic disease. 298 samples showed microcytic heterogenous picture with low MCV values and increased RDW values and these are most probably due to iron deficiency. 475 samples showed normocytic homogenous picture with MCV and RDW values in our present study. These include women with normal hemoglobin levels. Low hemoglobin levels in this category could be explained by acute blood loss or chronic diseases or early deficiencies. 69 samples showed normocytic heterogenous picture with normal MCV values and increased RDW. These cases are mostly due to mixed anaemia or early folate deficiency or early iron deficiency anaemia. 5 samples showed macrocytic homogenous picture with increased MCV and normal RDW values. These women probably are in early folate or vitamin B12 deficiency. 4 samples showed macrocytic heterogenous picture with increased MCV and increased RDW values and these women most probably have vitamin B12 or folate deficiency with megaloblastic anaemia

Conclusion

- 1000 women in the age group of 15 to 45 years are taken into the study and the hemoglobin levels along with mean corpuscular volume and red cell distribution width levels are observed.
- These samples are also validated through peripheral blood smear examination.
- We evaluated how RDW complements the MCV to improve the classification of anaemia from the blood count alone and to suggest the physiologic basis of anaemia based on the mean and heterogeneity of red cell size.
- Morphological changes of blood cells, inclusions, parasites are visualized in peripheral smear examination only.
- Thus application of red cell indices along with visualization through peripheral smear examination can help in establishing type of anaemia even without use of special investigations.
- Early or mixed nutritional anaemias show high RDW with normal MCV values
- In hypo proliferative anaemia like anaemia of chronic diseases, RDW is usually normal, independent of MCV values.
- In macrocytic anaemia, both the RDW and MCV values are increased.

- The six categories described according to David Bessman's "improved classification of anaemia by MCV and RDW" yield an accurate differential diagnosis of anaemia.
- The red cell parameters like MCV and RDW are helpful in early diagnosis and early effective treatment of anaemia.

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