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Original Research Article

A Study to Evaluate the Morphological Pattern of Anemia by RBC Indices and Peripheral Smear at a Tertiary Care Center in Rajasthan

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Abstract:

Background: Anemia is a common problem worldwide. Morphological typing of anemia provides a lot of information about the underlaying cause and guides the treatment. Red cell indices which is a quick and easy to determine, can be used to determine the morphological type of anemia.

Methods: This is a prospective study for one year. All the peripheral blood smears received by the department during study period were studied and the smears with pathological diagnosis of anemia were included in the study. A complete hemogram was done for all samples to get the RBC indices and PBF examination was done and both the findings were compared and analysed.

Results: We received a total of 479 samples for PBF examination. 354 (74%) smears were reported as having anaemia and were included in the study. There was a female predominance in our study with M: F ratio of 1: 1.94. The age of patients ranged from 12 years to 59 years with the median age of 41 years. On complete blood count based on haemoglobin concentration, 162 (45.76%) cases showed mild anaemia, 144 (40.68%) showed moderate and 48 (13.56%) showed severe anaemia. According to age, the most common affected age group was 6th decade in our study with 39.55% cases followed by third decade with 22.60% cases. Mild anaemia was most prevalent in all the age groups as compared to moderate and severe anemia and the difference between the number of cases with mild, moderate and severe anaemia were not statistically significant (p-value = 0.3002). On basis of red cells indices, the anaemia was grouped in Microcytic hypochromic anemia with raised RDW with 125 cases which was the most common finding in our study. There were 59 cases of Microcytic hypochromic anemia with normal RDW, 67 cases of Normocytic normochromic anemia with normal RDW, 49 cases of Normocytic normochromic anemia with raised RDW and 54 cases of Macrocytic anemia.

Conclusions: Our present study demonstrates that red cell indices are a good initial screening for typing the anemia with good sensitivity and specificity. However, the role of peripheral blood smear by an experienced pathologist cannot be died in identifying the morphology of red cells in anemia. We feel that in cases with mismatch in CBC and clinical data of patients and in confusing cases, smear examination should be carried out.

Keywords: Anemia, RBC indices, PBF

Introduction

WHO defines anemia as reduction in red blood cell count or haemoglobin level below the normal percentage for that age.[1] Anemia is a widespread public health concern globally, affecting people of all ages and socioeconomic backgrounds. According to the World Health Organization (WHO) in 2021, it was estimated that around 1.62 billion people, or approximately 24.8% of the global population, had anemia.[2] India has been grappling with a significant burden of anemia for decades. In India, anemia is mainly caused by nutritional deficiencies, particularly iron-deficiency anemia (IDA).

The National Family Health Survey (NFHS) conducted between 2015 and 2016, published that the prevalence of anemia among Indian women aged 15-49 years was around 53%. This indicates that more than half of women in the reproductive age group were anemic. Among children aged 6-59 months, the prevalence of anemia was approximately 58%. This high prevalence is concerning as anemia during early childhood can have long-lasting developmental impacts. Anemia also affects men in India, but the prevalence is generally lower compared to women and children. [3]

According to the World Health Organization (WHO), anemia is defined as a condition in which the hemoglobin content is below normal.[1] Anemias can be categorized based on the size and hemoglobin content of red blood cells. They are divided into normocytic, microcytic, or macrocytic anemias depending on size, and normochromic or hypochromic anemias based on hemoglobinization levels. These classifications offer insights into potential causes. Microcytic hypochromic anemias, primarily caused by hemoglobin synthesis disorders like iron deficiency, are characterized by small and pale cells. In contrast, macrocytic anemias result from bone marrow issues hindering red cell maturation. Normocytic, normochromic anemias have varied causes. Clinical features of anaemia and vague and non-specific. The work-up should include physical exams and laboratory tests, such as evaluations of hematocrit, hemoglobin and red blood cell indices. The red blood cell indices should include the cell count, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW)[4]. In fact, the hemoglobin concentration is the parameter that is most commonly used as an indicator of the pathophysiological consequences of anemia. However, this variable is not very specific or sensitive. Hemoglobin levels can be altered in different pathologic conditions, such as infectious and inflammatory processes, hemorrhage, protein-caloric malnutrition, associated to medications and smoking. The MCV guides the diagnosis of anemia and helps in its classification.[5] However, the MCV value, that is, the mean size of the red cells (macrocytic, microcytic and normocytic), should be used together with the RDW, thus directing the interpretation of the variation in the size of red blood cells.

Materials and Methods

This is a prospective study carried out in the Department of Pathology, PDU Medical College, and Associated Group of Hospitals, Churu for one yearsfrom January 2022 to December 2022. All the peripheral blood smears received by the department during study period were studied and the smears with pathological diagnosis of anemia were included in the study.

The blood samples were collected in EDTA tubes.The complete hemogram was done for all the samples for peripheral blood smear using Sysmex XN-1000B4 6 part fully automated hematology analyzer. The evaluated parameters included the hemoglobin concentration and red blood cell indices- Mean Cell Volume (MCV), Mean cell hemoglobin (MCH), Mean cell hemoglobin concentration (MCHC), and hematocrit (PCV). Clinical history was noted from clinical records. The smears were methanol fixed and stained with Leishman stain and observed under light microscope.

Inclusion Criteria

- All blood samples received for peripheral blood examination.
- A pathological diagnosis of anaemia was made on PBF examination.

Exclusion Criteria

- Smears with pathological diagnosis other than anemia.
- Patients without complete clinical records.
- Improper smears.

The reference values for the studies were taken as per the WHO guideline as follows:

Table 1: Hemoglobin concentrations (g/dL) for the diagnosis of anemia and assessment of severity according to the World Health Organization

Age	Mild	Moderate	Severe
6–59 months	10–10.9	7–9.9	<7
5–11 years	11–11.4	8–10.9	<8
12–14 years	11–11.9	8–10.9	<8
Female >14 years	11–11.9	8–10.9	<8
Male >14 years	11–12.9	8–10.9	<8

- The reference range of Mean Cell Volume (MCV) was taken as 80-100 fl, mean cell hemoglobin (MCH) was taken as 27-32pg, for mean cell hemoglobin concentration 32-36g/dl.
- Microcytic anemia was taken as MCV value less than 80 fl and MCH less than 27. Macrocytic was taken when MCV is greater than 100 fl.
- Normocytic Normochromic was taken when all hematological indices are within range.

Statistical Analysis

Qualitative variables were summarized using percentages and proportions. Quantitative variables were summarized using mean with standard deviation. Data was entered in MS Excel.

Results

We received a total of 479 samples for PBF examination during the study period. Of these, 354 (74%) smears were reported as having anaemia and

were included in the study. There was a female predominance in our study with 121(34%) male patients and 234(66%) females with M: F ratio of 1: 1.94. [Table no. 2]The age of patients ranged from 12 years to 59 years with the median age of 41 years.

On complete blood count based on haemoglobin concentration, 162 (45.76%) cases showed mild anaemia, 144 (40.68%) showed moderate and 48 (13.56%) showed severe anaemia.

According to age, the most common affected age group was 6th decade in our study with 39.55% cases followed by third decade with 22.60% cases. [Table no. 2] Mild anaemia was most prevalent in all the age groups as compared to moderate and severe anemia and the difference between the number of cases with mild, moderate and severe anaemia were not statistically significant (p-value = 0.3002).

In cases of mild anaemia, 47 cases had MCV less than 80fl, 9 cases had MCV greater than 100fl and in rest 106 cases the MCV was in the normal range. MCH was reduced in 42 cases and was normal in rest of the cases.

In cases of moderate anaemia, 93 cases had MCV less than 80fl, 31 cases had MCV greater than 100fl and in rest 20 cases the MCV was in the normal range. MCH was reduced in 91 cases and was normal in rest of the cases. In cases of severe anaemia, 27 cases had MCV less than 80fl, 13 cases had MCV greater than 100fl and in rest 8 cases the MCV was in the normal range. MCH was reduced in 26 cases and was normal in rest of the cases.

Table 2: Gender wise distribution of cases in study group

Gender	Total Cases	Percentage
Males	121	34
Females	234	66
Total	354	100

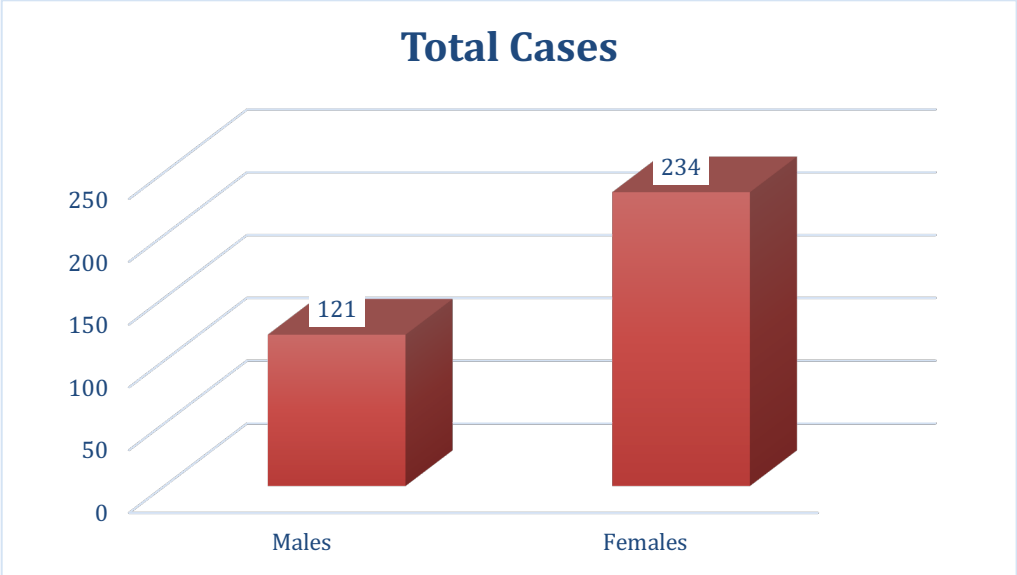

Figure 1: Gender wise distribution of cases

Table 3: Age group wise distribution of cases

Age in years	Mild	Moderate	Severe	Total	Percentage
11 – 20	11	7	3	21	5.93%
21 – 30	25	41	14	80	22.60%
31 – 40	26	20	7	53	15.00%
41 - 50	28	25	7	60	17.00%
51 - 60	72	51	17	140	39.55%
Total	162	144	48	354	100.08%

Chi-squared 9.522; DF 8; P = 0.3002

On basis of red cells indices, the anaemia was grouped in Microcytic hypochromic anemia with raised RDW with 125 cases which was the most common finding in our study. There were 59 cases of Microcytic hypochromic anemia with normal RDW, 67 cases of Normocytic normochromic anemia with normal RDW, 49 cases of Normocytic normochromic anemia with raised RDW and 54 cases of Macrocytic anemia. [Table no.]

Table 4: Morphological type of anemia according to red cell indices with RDW

Morphological type of anemia	Frequency
Normocytic normochromic anemia with normal RDW	67
Normocytic normochromic anemia with raised RDW	49
Microcytic hypochromic anemia with normal RDW	59
Microcytic hypochromic anemia with raised RDW	125
Macrocytic anemia	54
Total	354

On peripheral smear examination, most common finding was microcytic hypochromic anaemia in 169 (47.69%) cases followed bynormocytic normochromic anaemia was reported in 121 (34.27%) cases, macrocytic anemia in 45 (12.71%) cases and dimorphic anemia in 19 (5.37%) cases. The distribution of cases in each group of smears is shown in Table no.

Table 5: Morphological type of anemia according to peripheral smear examination

Peripheral smear examination	Mild	Moderate	Severe	Total
Normocytic normochromic	105	12	4	121
Microcytic hypochromic	45	96	27	169
Macrocytic	6	28	11	45
Dimorphic	5	8	6	19
	162	144	48	354

Table 6: Comparison of cases between typing by red cell indices va peripheral smear

Typing by RBC Indices And RDW	No. of Cases	Typing from Peripheral Smear	No. of Cases
Normocytic normochromic anemia with normal RDW	67	Normocytic normochromic anemia	63
		Microcytic hypochromic anemia	4
Normocytic normochromic anemia with raised RDW	49	Normocytic normochromic anemia	37
		Microcytic hypochromic anemia	2
		Macrocytic anemia	1
		Dimorphic anemia	9
Microcytic hypochromic anemia with normal RDW	59	Normocytic normochromic anemia	9
		Microcytic hypochromic anemia	50
Microcytic hypochromic anemia with raised RDW	125	Normocytic normochromic anemia	4
		Microcytic hypochromic anemia	114
		Dimorphic anemia	7
Macrocytic anemia	54	Normocytic normochromic anemia	2
		Macrocytic anemia	49
		Dimorphic anemia	3

On comparing the morphological typing of anemia from RBC indices and RDW values with that of peripheral smear findings, out of total 67 of the cases which were reported as normocytic normochromic anemia with normal RDW, 63 cases were confirmed to be the same normocytic normochromic anaemia on the peripheral smear, while 4 cases with MCV as 80 - 83 fl were typed as microcytic hypochromic on peripheral smear examination. Out of the 49 cases of normocytic normochromic anemia with raised RDW 37 cases were typed as normocytic normochromic anemia on PBF examination, 2 as microcytic hypochromic anemia (MCV was 79fL), one as macrocytic anemia (MCV was 99 fL) and 9 cases as dimorphic anemia.

Out of 59 cases of microcytic hypochromic anemia with normal RDW 50 cases were confirmed to be the same on peripheral smear while 9 cases were typed as microcytic hypochromic anemia (most with MCV in the range 75-79 fL).

In cases of microcytic hypochromic anemia with raised RDW on RBC indices, out of 125 cases, 114 were confirmed to be the same on peripheral smear, however 4 cases were reported as normocytic normochromic anemia (MCV 78/79 fL) and 7cases were reported as dimorphic anemia.

Out of the 54 cases typed as macrocytic anemia from the indices and RDW 49 cases were confirmed to be the same on peripheral smear, 2 as normocytic normochromic anemia with marked polychromasia and 3 as dimorphic anemia.

Overall, in our study the sensitivity of red cell indices in predicting type of anemia was 95.6% and specificity of 93.4%.

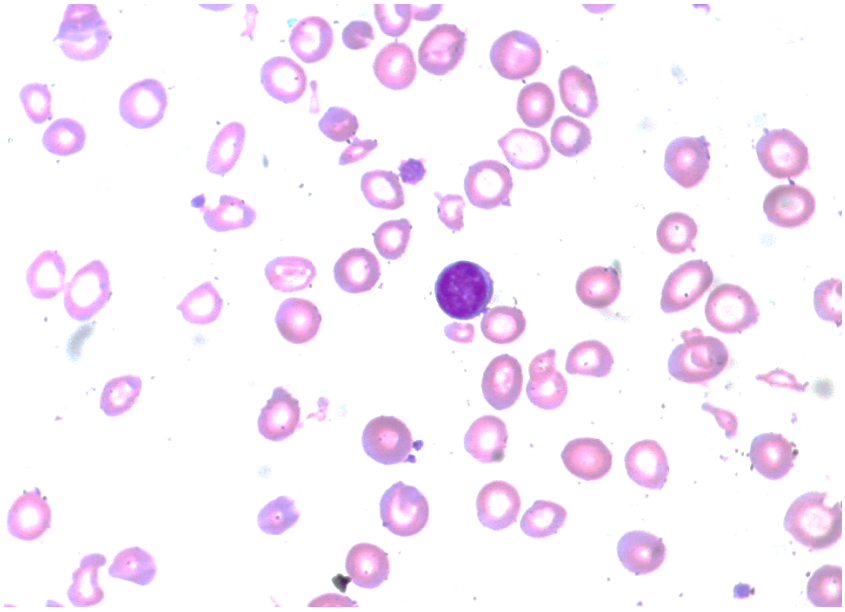

Figure 2: Showing microcytic and hypochromic anemia

Figure 3: Showing RBCs of different size, few macrocytes and few microcytes with profound hypochromia, suggestive of dimorphic anaemia

Discussion

Anemia in India is a significant public health concern that has far-reaching consequences for the well-being of the population, particularly among women and children. Nutritional anemias are the most common cause of anemia. [6] According to global data, approximately 2.15 billion individuals are affected by iron deficiency anemia, a condition where the body lacks a sufficient amount of iron. Typically, the human body absorbs 5-10% of dietary iron, but in cases of iron deficiency, this absorption rate can increase three to fivefold. Dietary factors play a crucial role in the development of iron deficiency anaemia, with reduced consumption of vegetables, red meat, and fruits being associated with a higher risk. As people age, blood production declines owing to alterations in bone marrow composition. This includes an upsurge in adipose (fat) tissue and a reduction in hematopoitic cells. In the elderly population, the bone marrow tends to respond less effectively to erythropoietin stimulation, exacerbating the occurrence of anaemia.In our study, we focused on anaemia in all age groups. In our study maximum number of cases were in 6th decade of life, which is in concordance with the study done by Nasrin A Quereshi et al [7] where they reported maximum patinets of anaemia in age >40 yrs. Nidhi Gupta et al [8] also reported similar findings, where the maximum number of cases were clustered in 5th and 6th decade of life. However, Swami et al [9] in their study reported maximum cases in 4thdecade of life. The variation may be due to the population sample selected, geographical and socioeconomic variations in this and their study.

We found female predominance in our study with M: F ratio of 1: 1.94. Most other studies done in India also reported higher female involvement in anaemia [7,8,9]

In the present study in all the age groups, mild anemia was more predominant as compared to severe anaemia. The ratio of cases with severe anemia increases with the age however the difference was not statistically significant in our study. Nasrin A Quereshi et al [7] also reported similar findings and found that mild anemia is more common in all the age groups. Nidhi Gupta et al [8] and Swami et al [9] also observed similar results. Among the various types of anemia, microcytic hypochromic anemia was the most prevalent, followed by normocytic normochromic anemia. Pasricha et al.'s [10] study in rural areas revealed that microcytic hypochromic blood patterns are predominant in children, often attributed to iron deficiency.Our findings were in agreement with the study done by Swami et al [9] and Revathi Shree R et al. [10] who also reported similar results.

In our study, overall, the sensitivity of red cell indices in predicting type of anemia was 95.6% and specificity of 93.4%. Similar findings were reported by Meenu Venukumar & Suma M.T [11] in their study.

Conclusion

Our present study demonstrates that red cell indices are a good initial screening for typing the anemia with good sensitivity and specificity. However the role of peripheral blood smear by anexperienced pathologist cannot be died in identifying the

morphology of red cells in anemia. We feel that in cases with mismatch in CBC and clinical data of patients and in confusing cases, smear examination should be carried out.

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