ORIGINAL RESEARCH

Study of anemia pattern and its correlation with hematological parameters in a tertiary care center

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ABSTRACT
Background: Anemia is characterized by low hemoglobin (Hb) concentration and insufficient oxygen-carrying capacity to meet the body's physiological needs. Present study was aimed to study anemia pattern and its correlation with hematological parameters in a tertiary care center. **Material and Methods:** Present study was cross sectional, observational study, conducted in patients of age 18-60 years, either gender, with hemoglobin less than 11 gm/dl, sample processed at central clinical laboratory at our hospital. All samples underwent peripheral blood smear examination as well as through hematology analyzer. **Results:** In present study, among 2366 samples examined, 926 were eligible for study. Majority were from 51-60 years age group (35.85 %), females (55.51 %). According to severity of anemia, majority had mild (9.1-11 gm %) anemia (61.23 %), followed by moderate (7.1-9 gm %) (26.46 %) & severe anemia (<7 gm %) (12.31 %). Typing of anemia on basis of peripheral smear examination was done. Common types were microcytic hypochromic (53.24 %) & normocytic normochromic (27.54 %) while less common were macrocytic (9.61 %), dimorphic (5.94 %), hemolytic (3.13 %) & pancytopenia (0.54 %). Typing of anemia on basis of red cell indices and histogram was done. Common types were microcytic hypochromic (57.24 %) & normocytic normochromic (22.89 %) while less common were macrocytic (9.83 %), dimorphic (7.24 %), hemolytic (2.38 %) & pancytopenia (0.43 %). We compared anemia pattern by peripheral smear findings versus red cell indices findings, both groups were comparable & no statistically significant difference was observed. **Conclusion:** Red cell indices should be used as a screening method to pick up pathological samples, followed by a peripheral smear examination for confirmatory diagnosis.
Keywords: anemia pattern, peripheral smear findings, red cell indices, hematology analyser
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INTRODUCTION
Anemia is characterized by low hemoglobin (Hb) concentration and insufficient oxygen-carrying capacity to meet the body's physiological needs. Anemia is conventionally identified when the hemoglobin concentration in blood falls under an age-specific and sex-specific threshold or cutoff.[1,2] Iron deficiency anemia is the commonest nutritional disorder found all over the world, predominantly in the developing countries, mostly, affecting young children of 6-24 months of age, adolescents, women of reproductive age group and pregnant/ lactating women.[3,4]
Anemia can be classified from three points of view: pathogenesis, red cell morphology, and clinical presentation. Classification of anemia is done based on the analysis of total RBC count, Hemoglobin, packed cell volume and red cell indices such as mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red cell distribution width.[5,6]
Peripheral blood smear (PBS) examination is a cheap and quick method for categorizing different types of anaemias by studying the RBCs morphology. Peripheral blood smear examination in addition with complete blood count (CBC) by the automated hematology analysers can provide a more accurate report on categorizing different types of anemia.[7] Present study was aimed to study anemia pattern and its correlation with hematological parameters in a tertiary care center.

MATERIAL AND METHODS
Present study was cross sectional, observational study, conducted in department of pathology, at XXX medical college & hospital, XXX, India. Study duration was of 3 months (October 2022 to December 2022). Study approval was obtained from institutional ethical committee.
Inclusion criteria
- Patients of age 18-60 years, either gender, with hemoglobin less than 11 gm/dl, sample processed at central clinical laboratory at our hospital
Exclusion criteria
- Patients with leukaemia, malignancies

- Pregnant women

Only reports were reviewed and identity was kept confidential, no consent was needed. Peripheral blood smear was prepared according to standard operating procedures and stained by Leishman stain, was done for every patient. All peripheral blood films were prepared manually and were stained by a single trained person to minimize variation in smear spreading and staining due to interpersonal differences in technique.

Using EDTA vacutainer tubes blood were collected and analysed in the laboratory for haematological parameters using Mindray and Horiba 5-part hematology analyzer. Total Red cell count, hemoglobin concentration and red blood cell indices- Packed cell volume, mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular haemoglobin concentration and red cell distribution width were evaluated. Based on Mean corpuscular volume morphological classification of anemia is done and confirmed by peripheral smear examination. Anemia was categorised as mild (9.1-11 gm %), moderate (7-9 gm %) & severe mild (< 7 %). Anaemia was categorised based on RBC indices as:

a) Normocytic normochromic (MCV was between 80-100fl)
b) Microcytic hypochromic (MCV was <80fl)
c) Macrocytic normochromic (MCV was >100)
d) A bimodal peak indicated dimorphic anaemia

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi- square test or Fisher exact test as applicable. P value less than 0.05 was considered as statistically significant.

RESULTS

In present study, among 2366 samples examined, 926 were eligible for study. Majority were from 51-60 years age group (35.85 %), females (55.51 %). According to severity of anemia, majority had mild (9.1-11 gm %) anemia (61.23 %), followed by moderate (7.1-9 gm %) (26.46 %) & severe anemia (<7 gm %) (12.31 %)

Table 1: General characteristics

Characteristics	Mild (9.1-11 gm %)	Moderate (7.1-9 gm %)	Severe (<7 gm %)	Total
Age group (years)				
18-30	69 (7.45 %)	36 (3.89 %)	11 (1.19 %)	116 (12.53 %)
31-40	134 (14.47 %)	54 (5.83 %)	23 (2.48 %)	211 (22.79 %)
41-50	173 (18.68 %)	68 (7.34 %)	26 (2.81 %)	267 (28.83 %)
51-60	191 (20.63 %)	87 (9.4 %)	54 (5.83 %)	332 (35.85 %)
Gender				
Male	265 (28.62 %)	99 (10.69 %)	48 (5.18 %)	412 (44.49 %)
Female	302 (32.61 %)	146 (15.77 %)	66 (7.13 %)	514 (55.51 %)
Total	567 (61.23 %)	245 (26.46 %)	114 (12.31 %)	926

Typing of anemia on basis of peripheral smear examination was done. Common types were microcytic hypochromic (53.24 %) & normocytic normochromic (27.54 %) while less common were macrocytic (9.61 %), dimorphic (5.94 %), hemolytic (3.13 %) & pancytopenia (0.54 %).

Table 2: Distribution of anemia cases based on peripheral smear

Type of anemia	Cases	Frequency (Percentage)
Microcytic hypochromic	493	53.24
Normocytic normochromic	255	27.54
Macrocytic	89	9.61
Dimorphic	55	5.94
Homolytic	29	3.13
Pancytopenia	5	0.54

Typing of anemia on basis of red cell indices and histogram was done. Common types were microcytic hypochromic (57.24 %) & normocytic normochromic (22.89 %) while less common were macrocytic (9.83 %), dimorphic (7.24 %), hemolytic (2.38 %) & pancytopenia (0.43 %).

Table 3: Distribution of anemia cases on red cell indices and histogram

Type of anemia	cases	Frequency (Percentage)
Microcytic hypochromic	530	57.24
Normocytic normochromic	212	22.89
Macrocytic	91	9.83
Dimorphic	67	7.24
haemolytic	22	2.38
Pancytopenia	4	0.43

We compared anemia pattern by peripheral smear findings versus red cell indices findings, both groups were comparable & no statistically significant difference was observed.

Table 4: Comparison of peripheral smear findings with red cell indices

Type of anemia	on peripheral smear	on red cell indices and histogram	p value
Microcytic hypochromic	493 (53.24 %)	530 (57.24 %)	0.063
Normocytic normochromic	255 (27.54 %)	212 (22.89 %)	0.078
Macrocytic	89 (9.61 %)	91 (9.83 %)	0.083
Dimorphic	55 (5.94 %)	67 (7.24 %)	0.066
haemolytic	29 (3.13 %)	22 (2.38 %)	0.075
Pancytopenia	5 (0.54 %)	4 (0.43 %)	0.057

DISCUSSION

Nutritional anemias may result from various vitamin and mineral, as well as some macronutrient deficiencies, but the most common are megaloblastic anemia, resulting from folic acid or vitamin B12 deficiency, and microcytic, hypochromic anemia, resulting from iron deficiency.[8]

In practice, classification based on basic parameters of red cell morphology such as mean corpuscular volume (MCV), allows for a quicker diagnostic approach. Mean corpuscular volume (MCV) measures the average size and volume of a red blood cell expressed in femtoliters (fl), while mean corpuscular hemoglobin (MCH) measures the mean hemoglobin per erythrocyte expressed in pictograms (pg).[9]

Smear examination can also identify abnormal, atypical WBCs, any abnormality in platelets and can give an assumption of total WBCs count and platelet counts.

In study by Fatima SH et al.,[10] prevalence of anemia was 26.2% (872/3326), high prevalence of anemia was observed in females (44.04%) compared to males (5.72%) (p<0.0001). The prevalence was more in reproductive age group (58.6%). Moderate anemia (58.6%) was more prevalent followed by mild anemia (36.6%). Microcytic hypochromic blood picture predominates with the incidence of 53.6% followed by normocytic blood picture 42.2%.

Revathi S.R et al.,[11] studied 4350 samples, 45.5% patients were diagnosed as anemic. Microcytic hypochromic blood picture accounts for 48% which is the predominant type followed by other types such as normocytic anemia, dimorphic smear and macrocytic anemia. Males were commonly affected when compared to females. The frequency of anemia increases as age advances. Anemia is associated with various conditions such as nutritional deficiency, blood loss, infections and chronic diseases the predominant cause being iron deficiency.

In study by Reena K et al.,[12] overall prevalence of anemia was 71.58%. Moderate degree of Anemia was the most common followed by mild degree. Anemia was present in 39.92% of men and in 60.08% of women. Microcytic hypochromic anemia is the most common type of anemia in adult, more in reproductive age group indicating iron deficiency as the main cause. It was followed by normocytic normochromic anemia both in men and women.

In study by Koteswari M et al.,[13] microcytic hypochromic anemia was the most common type and females are more commonly affected than males. Majority of the patients (46.8%) had moderate degree of anemia with hemoglobin levels 6-9 gm/ dl was seen in 691 patients. Mild degree of anemia (42.7%) with hemoglobin > 9 gm/dl, was seen in 631 patients. Severe degree of anemia (10.5%) with hemoglobin <6 was seen in 155 patients.

In study by Nasrin A et al.,[14] overall prevalence of anemia was 43.48%. Mild degree of Anemia was present in 46.34%, which was the most common followed by moderate degree which was present in 43.44% and severe anemia was present in 10.22%, which was the least common of degree of anemia. Anemia was present in 50.25% of men and in 49.74% of women. Microcytic hypochromic anemia is the most common type of anemia in overall age groups -46.50%. Normocytic normochromic anemia without anisocytosis was the most common type of anemia -42.25% in adult patient. Mild degree of anemia is more common in males -52.21% and moderate and severe anemia was more in females-50.98% and 51.67% respectively.

A dimorphic blood film can be seen when iron deficiency anemia responds to iron therapy, after the transfusion of normal blood to a patient with a hypochromic anemia, sideroblastic anemia, macrocytic anemia post transfusion, unmasking of iron deficiency following treatment of megaloblastic anemia, delayed transfusion reactions and dual deficiency of iron and either vitamin B12 or folic acid.[15]

Elevated ferritin level, serum transferrin, transferrin receptor (TfR), TIBC, erythrocyte sedimentation rate, and C-reactive protein concentrations, and reduced serum iron concentrations and transferrin saturation are usually associated with anemia of chronic disease.[16]

The automated hematology analyzer has replaced the traditional manual methods for hematological parameters as the initial screening and detection system for hematological abnormalities in modern clinical setups. Even in the age of molecular analysis, the blood smear remains an important diagnostic tool and sophisticated modern investigations of hematologic disorders should be interpreted in the light of peripheral-blood features as well as the clinical context.[17]

We could analyze the peripheral blood smears objectively, it well be instrumental in not only reducing interobserver variation, and in making the diagnosis but may also serve as a quality control for automated counters using the regression models we got for different parameters.

CONCLUSION

Though anemia pattern were comparable on peripheral smear findings versus red cell indices findings, Red cell indices should be used as a screening method to pick up pathological samples, followed by a peripheral smear examination for confirmatory diagnosis. The peripheral blood smear has been the main diagnostic aid in establishing the etiology of anemia.

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