STUDY OF PERIPHERAL BLOOD SMEAR FINDINGS IN PATIENTS OF ANEMIA AND TO COMPARE IT WITH AUTOMATED HEMATOLOGY ANALYZER GENERATED RED CELL PARAMETERS

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ABSTRACT

Introduction

Anemia is one of the common medical conditions prevalent in our society. The correct categorization of varying types of anemia is essential for therapeutic purposes. Various laboratory tests are done to find out its underlying cause, but peripheral blood smear (PBS) study of red blood cells (RBCs) morphology is important along with study of red blood cell indices in the classification of anemia.

Objectives

This study was done to evaluate RBC morphology on peripheral blood smear examination in patients of anemia and to compare these findings with cell counter generated red blood cells indices comprising of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Hemoglobin (MCH).

Methodology

Red blood cell morphology on peripheral blood smear was studied in 350 patients of anemia irrespective of age and gender received under six months duration from January to June, 2019 and findings were compared with cell counter generated red cell parameters generated by automated hematology analyzer.

Result

Most common anemia seen on smear examination was microcytic hypochromic anemia which accounted for 210 (60%) cases followed by dimorphic anemia in 73 (20.86%) cases. Most of the patients were female, comprising of 227 (64.85%) cases. Highest numbers of patients were in the age group of 21-30 years (20.30%). Sensitivity of MCV, MCHC and MCH was 78%, 14% and 80% respectively in detection of microcytic hypochromic anemia.The sensitivity of MCV and MCH was found to be 100 % in detection of macrocytic anemia. Sensitivity of MCHC was only 10% for detection of macrocytic anemia. The sensitivity of MCV, MCHC and MCH was 78%, 100% and 67% respectively in detection of normocytic normochromic anemia.

Conclusion

The peripheral blood smear examination should always be interpreted along with the red blood cell indices generated by an automated analyzer in order to classify various types of anemia.

KEYWORDS

Anemia, peripheral blood smear, RBC morphology

INTRODUCTION

Anemia is a common medical problem faced by Nepalese population which is often ignored. Anemia leads to tissue hypoxia due to reduced oxygen-carrying capacity of the blood. In anemic patients red blood cell mass is decreased. Anemia affects 1.62 billion people worldwide which correspond to 24.8% of the world population. The preschool age group children show high prevalence of anemia (47.4%) followed by pregnant women (41.8%).[1]

The most common cause of anemia is nutritional deficiency of iron, vitamin B12 or folic acid. But many clinical conditions can also lead to anemia like decreased production of RBCs in disease causing bone marrow failure and increased destruction of RBCs in clinical conditions leading to hemolysis. Anemia also could be multifactorial in origin. So, every patient of anemia should be investigated to find out the cause and the type for proper medical treatment. However there is lack of consistency in the protocols for proper screening for anemia.[2-4]

The commonest laboratory test for diagnosis of anemia is hemoglobin estimation. In clinical practice, anemia is diagnosed by reduced hemoglobin concentration of the blood below the normal range and reduced hematocrit level (the ratio of packed red cells to total blood volume).[5]

Peripheral blood smear (PBS) examination is a cheap and quick method for categorizing different types of anemias by studying the RBCs morphology. Smear examination can also identify abnormal, atypical WBCs, any abnormality in platelets and can give an assumption of total WBCs count and platelet counts. Peripheral blood smear examination in addition with complete blood count (CBC) by the automated hematology analyzers can provide a more accurate report on categorizing different types of anemia. Smear examination also helps in cross checking the results of RBCs indices generated by the analyzer. Moreover, the patient doesn't need to give a separate blood sample for smear examination. The smear slides are immediately made from the blood sample collected for CBC. The reports are also dispatched on the same day within a few hours after collection. So, the technique is simple and cheap. Both the patient, as well as, the treating physician does not need to wait a long for the reports.

Therefore, the study was undertaken to study the morphological findings of the red blood cells (RBCs) in peripheral blood smear in order to categorize different types of anemia and to compare it with RBC indices measured with an automated hematology analyzer.

METHODOLOGY

This prospective study was conducted from January to June 2019 in the Department of Pathology, Birat Medical College & Teaching Hospital (BMCTH), Morang, Nepal after the approval obtained from the Institutional Review Committee. Peripheral smear examination of the blood samples received in laboratory Birat Medical College & Teaching Hospital (BMCTH), Morang, Nepal of patients who were diagnosed anemic on hemoglobin estimation were studied irrespective of age and sex. Taking all aseptic precautions blood samples were collected from the peripheral veins in an anticoagulant vial in the right proportion. Ethylene diamine tetra-acetic Acid (EDTA) was the anticoagulant used. Both Peripheral smear and CBC by the automated analyzer were analyzed within 2 to 3 hours of blood collection because delay in preparation of blood smear may lead to artifactual changes in WBC, RBC and pseudo-thrombocytopenia may occur due to formation of platelet aggregates.[6] Peripheral blood smear slides of anemia were studied for the morphological abnormalities in the red blood cells associated with different types of anemia and the findings were noted down.

By studying the hemoglobin level on the automated analyzer the criteria for defining anemia was undertaken according to guidelines as given by the World Health Organization (WHO).[7] Anemia was diagnosed in Children (6 - 59 months of age) with Hemoglobin (Hb) < 11gm/dl , Children (5 - 11 years of age) with Hb< 11.5gm/dl, Children (12-14 years of age) with Hb< 12gm/dl, Non-pregnant women (15 years of age and above) with Hb< 12gm/dl, Pregnant women with Hb<11gm /dl and Men (15 years of age and above) with Hb< 13gm/dl .

The morphological changes in the red blood cells like hypochromia, microcytosis, macrocytosis, polychromatophilia, elliptocytosis, target cell changes, tear drop changes, fragmented - red blood cell change, any red blood cell inclusion bodies or presence of any nucleated red blood cell were observed by microscopic examination. The PBS reported by one of the pathologists was also counter checked by another pathologist involved in this research work. These findings were correlated with the findings observed by the automated analyzer (Benesphera H33s). Differential values of MCV between 80fl and 100fl were categorized as normocytic normochromic. Values of MCV <80fl and >100fl were categorized as microcytic hypochromic and macrocytic normochromic respectively. Normal value for MCH was considered as 29±2 picogram, values below and above this were categorized as microcytic and macrocytic anemia respectively. Similarly normal value for MCHCis 34 ± 2 g/dl. Values below and above this were categorized as microcytic and macrocytic anemia respectively. The data were entered in Microsoft excel sheet and were analyzed in terms of sensitivity and age distribution.

RESULTS

The peripheral blood smear and RBCs indices of 350 patients of anemia were analyzed and correlated. The age group of patients ranged from 6 months to 90 years. Highest number of the patients were in the age group 21-30 years (20.30%) followed by 31-40 years (15.71%). Out of 350 patients, 227 (64.85%) were female and 123(35.16%) were male. (Table 1)

Table 1: Age distribution

Age group (Years)	Number of patients (Percentage)
1 – 10	20 (5.71)
11-21	40 (11.42)
21-30	71 (20.30)
31-40	55 (15.71)
41-50	45 (12.85)
51-60	45 (12.85)
61- 70	42 (12.0)
71-80	22 (6.30)
81-90	10 (2.86)

Most common anemia in our study on smear examination was microcytic hypochromic anemia(Figure 1) and the least common anemia observed was hemolytic anemia (Figure 2).

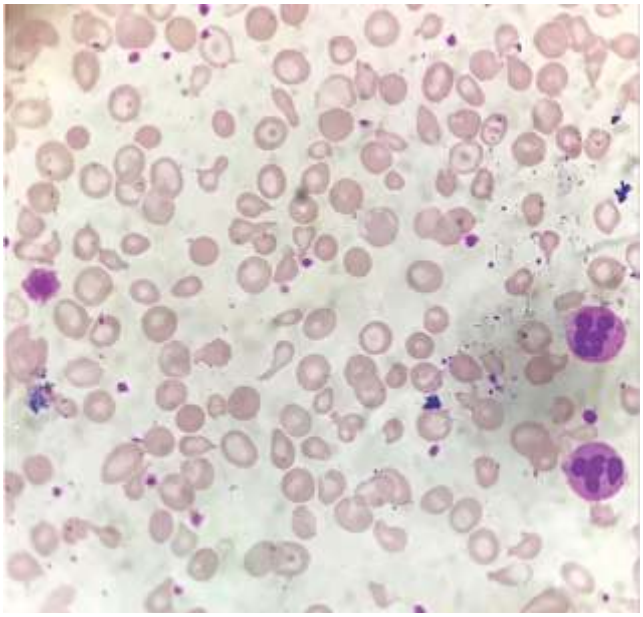

Figure 1 : Peripheral blood smear of microcytic hypochromic anemia showing microcytes, teardrop cells and elliptocytes.

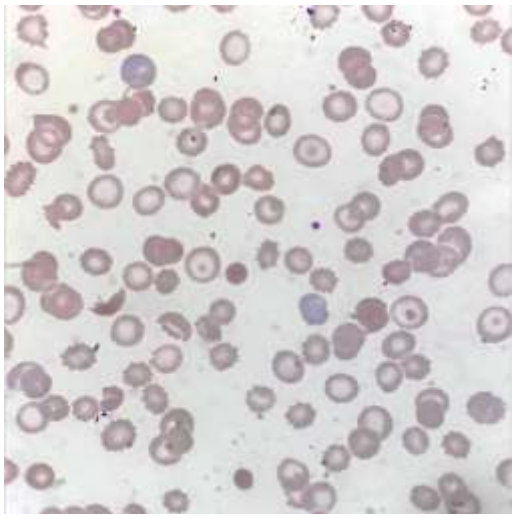

Figure 2: Peripheral blood smear of hemolytic anemia showing microcytes, target cells, polychromatophils, acanthocytes and fragmented red blood cells.

Out of 350 cases of anemia on peripheral blood smear examination, 210 (60%) cases showed microcytic hypochromic anemia, followed by dimorphic, normocytic normochromic, macrocytic and hemolytic anemia respectively. (Table 2)

Table 2: Categorization of anemia on peripheral smear examination

SN	Type of Anemia	Number of cases (Percentage)
1	Microcytic hypochromic	210(60%)
2	Dimorphic(both microcytic and macrocytic)	73(20.86%)
3	Normocytic normochromic	52(14.86%)
4	Macrocytic normochromic	10(2.85%)
5	Hemolytic	5(1.43%)

Among 210 cases of microcytic hypochromic anemia on PBS, a total of 164 cases revealed a similar picture on a cell counter with MCV value <80fl. Rest, all 46 cases on the cell counter showed normal MCV value suggesting normocytic normochromic anemia. None of the cases of microcytic hypochromic anemia on the cell counter showed high MCV value. Similarly, the number of cases of dimorphic and other anemias values correlating with cell counter are demonstrated in the Table 3:

Table 3: Comparison of findings of peripheral blood smear examination and cell counter generated parameters

Peripheral blood smear examination of red blood cell (RBC) morphology	Number of cases (n)	Cell counter generated parameters (n)		
		MCV (n)	MCHC (n)	MCH (n)
Microcytic hypochromic blood picture on smear	210	Decrease (164) Normal (46)	Decrease (30) Normal (180)	Decrease (168) Normal (42)
Dimorphic(both microcytic and macrocytic) blood picture on smear	73	Normal (32) Decrease (30) Increase (11)	Normal (73)	Normal (29) High (5) Low (39)
Normocytic normochromi blood picture on smear	52	Normal (41) Decrease (11)	Normal (52)	Normal (35) High (10) Low (7)
Macrocytic blood picture on smear	10	High (10)	High (1) Normal (9)	High (10)
Hemolytic blood picture on smear	5	Low (3) Normal (2)	Normal (5)	Low (3) Decrease (2)

Correlating with peripheral blood smear examination, the sensitivity of MCV was 78%, MCHC was 14% and of MCH was 80% in detection of microcytic hypochromic anemia.The sensitivity of MCV and MCH was found to be 100 % in detection of macrocytic anemia. Sensitivity of MCHC was only 10% for detection of macrocytic anemia. The sensitivity of MCV, MCHC and MCH was 78%, 100% and 67 % respectively in detection of normocytic normochromic anemia. The sensitivity of MCV, MCH was 60% each and MCHC was found to be 0% in detection of hemolytic anemia respectively. (Table 4)

Table 4: Comparison of findings of Peripheral Smear examination and sensitivity on cell counter generated parameters

Peripheral blood smear examination of red blood cell (RBC) morphology	Sensitivity of Cell counter generated parameters (%)		
	MCV	MCHC	MCH
Microcytic hypochromic blood picture on smear	78	14	80
Normocytic normochromic blood picture on smear	78	100	67
Macrocytic blood picture on smear	100	10	100
Hemolytic blood picture on smear	60	0	60

DISCUSSION

In this study we have enrolled all age groups diagnosed as anemia on hemoglobin estimation. It was seen that, out of 350 cases, the majority of cases 171 (48.85%) fall in the adult age group of 21 to 50 years. Among them 227 (64.86%) were female patients and 123 were male (35.14%). These results were in concordance with the studies conducted by Kumar et al and Japheth et al.[8,9] Among female patients the majority fall in the reproductive age group. Blood loss during menstrual cycle and high nutrient demands during pregnancy and lactation can lead to nutritional anemia among women in reproductive age group.

The most common morphological type of anemia on smear examination was microcytic hypochromic anemia comprising 210 (60%) of all cases. This was almost similar to study done by Verma et al, Kumari et al, Mishra et al and Jain A et al.[10-13] They also found microcytic hypochromic anemia as the commonest anemia comprising of 55.4%, 53.33% , 47% and 40% respectively.

As it is known that MCV denotes the volume of the "average" red blood cell (RBC) in a sample and MCH is the amount of hemoglobin per red blood cell. MCH value is affected by both microcytosis and hypochromia. A decrease in value of MCH is associated with microcytic anemia and an increase in value of MCH is associated with macrocytic anemia.[14] Out of 210 cases which showed microcytic hypochromic anemia on smear examination, value of MCV and MCH were 78% and 80% sensitive respectively. However MCH and MCV were seen normal in many other cases. The reason for this could be that the microcytosis is seen on the smear much earlier than decrease in red blood indices value. But none of our cases revealed a falsely elevated MCV or MCH value in cases of microcytic anemia.

Kafle S et al[15] found that out of 100 peripheral smears of patients with microcytic, hypochromic anemia 49% were of iron deficiency anemia. Similarly another study conducted by Al alimi et al showed that the overall prevalence of iron deficiency anemia was 30.4%.[16] In our study also the majority of anemia cases were of microcytic type. Iron deficiency is the main cause of anemia; so an iron profile should be done in patients of microcytic anemia to confirm iron deficiency anemia. Red blood cell indices values are not helpful in diagnosis of dimorphic anemia. It may remain normal. We found normal MCV value in 43% cases of dimorphic anemia, normal MCH value in all cases and normal MCHC value in 39.72% cases. This anemia shows a mixed deficiency of both iron and vitamin B12. RBCs morphology in smear reveals both microcytic and macrocytic pictures. Therefore in such cases peripheral blood smear examination becomes very important. Study done by Deepthi A et al showed that red blood cell parameters are not reliable for combined deficiencies of iron and folic acid or vitamin B12. Because here one type of deficiency may be dominant and it may mask the parameters of other deficiency.[17]

Constantino et al have suggested that we can diagnose dimorphic anemia on a cell counter by correlating red cell parameters with red blood cell distribution width and histogram.[18] However this correlation was not the aim of our study and was not done. In dimorphic anemia serum assays of vitamin B12 or folate or iron is more reliable to confirm the cause and establish the diagnosis.

Normocytic anemia is anemia with low hemoglobin and hematocrit range but MCV, MCH and MCHC are in the normal range. We found the sensitivity of MCV, MCHC and MCH as 78%, 100% and 67 % respectively in detection of normocytic normochromic anemia. Normocytic normochromic anemias are seen commonly in patients who suffer from chronic infections or certain systemic disorders. The prevalence of normocytic normochromic anemia varies in different studies done in different parts of the world. Alwar et al[19] found normocytic normochromic anemia as predominant finding comprising 66% and Rao et al[20] found 62%cases as normocytic anemia. Study done in Nepal by Mishra et al found prevalence of normocytic normochromic anemia as 22%.[21] We found a little less cases of normocytic anemia comprising of 14.85% . Study done by American family Physician found that if we diagnose earlier even classic cause of any type of anemia either macrocytic or microcytic or hemolytic can show features of normocytic anemia.[22] The reason for the above mentioned studies showing higher percentage of normocytic normochromic anemia could be that in these studies anemia cases might have been diagnosed earlier.

In megaloblastic anemia there is defective nuclear maturation which leads to formation of macroovalocytes. So, in such cases MCH and MCV are increased, but MCHC may remain normal. In this study we have noted that in 91.2% cases of anemiaMCHC value was normal. As we know that the mean corpuscular hemoglobin concentration (MCHC) measures the average amount of hemoglobin in the RBCs. It is measured by the hemogram analyzer. MCHC can show normal value even if hemoglobin is low due to a calculation artifact, so MCHC values are not sensitive for diagnostic purposes. It is difficult to detect types of anemia by correlating with MCHC value. In a person suffering from a combination of two or more types of anemia the MCHC value may remain normal. Afterblood transfusion the MCHC value will reflect a person's own MCHC value along with the MCHC value of the transfused RBCs. Studies have shown that MCHC values are mainly used for diagnostic purposes in congenital disorders like Hereditary Spherocytosis.[23-24] In this study we did not receive any case of Hereditary Spherocytosis

and most of the cases showed normal value. We had only five cases which showed features of hemolysis on smear. The reason for such low numbers could be that we did not study specific tests like reticulocyte count, enzyme assay and hemoglobin electrophoresis for confirmation and categorization of hemolysis.

We therefore found that study of anemia is never complete without the careful examination of a well-prepared peripheral blood smear which can reveal features of hypochromia in RBCs even before it is observed on cell counter values.

CONCLUSION

The most common anemia on peripheral blood smear was microcytic hypochromic anemia which correlated well with automated cell counter generated parameters. In cases of combined deficiency anemia, peripheral blood smear examination is mandatory as cell counter parameters only showed features of the most severe types of anemia among them, the other deficiency remains hidden. This study thus showed that peripheral blood smear examination should be considered along with automated cell counter as a reliable method to categorize anemia.

RECOMMENDATIONS

If red cell distribution width is also studied along with red blood cell indices, it would be more helpful especially in cases of dimorphic anemia.

LIMITATION OF THE STUDY

We have not correlated the findings of anemia with other biochemical parameters like serum iron studies in iron deficiency anemia, vitamin B-12 studies in megaloblastic anemia and reticulocyte count and hemoglobin electrophoresis in hemolytic anemia.

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CONFLICT OF INTEREST

None

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