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Co-Relative Study on Peripheral Blood Smears in Anemia with Automated Cell Counter Generated Red Cell Parameters

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Abstract: ***Background*** *– Being one of the most common disorder faced by our society, the correct diagnosis of anemia is important for better clinical outcome.*
Aim & Objectives – The present study was designed to compare the findings of peripheral smear with cell counter generated parameters i.e. RBC indices and histograms.
Material and methods – 500 patients with Hb<11.5g/dl were selected for the comparison of PS findings with that of cell counter parameters.
Results – There was much discrepancy between the diagnosis of some anemias like hemolytic and macrocytic anemias on peripheral smear and cell counter generated parameters.
Conclusion – The CBC should always be interpreted in the light of peripheral smear examination despite all the advances in laboratory sciences.
Keywords –*Peripheral smear examination, RBC indices, Histograms.*

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I. Introduction

Anemia is one of the most common problems faced by our society which is often ignored worldwide both in developed and developing countries. It is a condition that occurs when the red blood cells do not carry enough oxygen to the tissues of the body. Preschool children, pregnant women and adolescents constitute vulnerable group of anemia. [1] It has been associated with increased morbidity and mortality. Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in preschool-age children (47.4%), and the lowest prevalence is in men (12.7%). However, the population group with the greatest number of individuals affected is pregnant women (41.8%).[2] From being the most common nutritional deficiency disorder in the world, other causes of anemia like hemolysis are also common. [3] Therefore correct diagnosis of type of anemia is very important for the right therapy to be administered. Peripheral smear (PS) examination is one of the basic and informative tools for screening, diagnosis and monitoring of type of anemias and to look for therapeutic response. It has been a window for hematological outgoings since decades and has been a major diagnostic tool in the etiopathological workup of anemias. [4] Cell counters have entered medical laboratory services in a ubiquitous manner with increasing efficacy and decreasing cost all over the world. Over the past few years, complete blood count (CBC) by the automated hematology analyzers and microscopic examination of peripheral smear have complemented each other to provide a comprehensive report on patient's blood sample. [5,6,7]. There is still a need to depend on manual techniques for primary calibration despite the sophistication of present day instruments. This highlights the importance of maintaining the manual technical skills, despite the urge to leave it all to the machines. So despite the advent of cell counters, the importance of peripheral smear examination by a pathologist cannot be replaced. Therefore the aforesaid study was undertaken to correlate the findings of peripheral blood smear examination with cell counter generated parameters in different types of anemia.

II. Aim & Objectives

The present study was carried out with the following aims & objectives –

Aim –

To correlate the findings of different types of anemia on peripheral smear examination and cell counter generated parameters.

III. Objectives –

1. To diagnose the type of anemia by peripheral smear examination
2. To diagnose the type of anemia by cell counter generated parameters
3. To correlate the findings of peripheral smear examination with cell counter generated parameters.

IV. Materials And Methods

This prospective study for a duration of 2 years is described for its materials and methods for the set objectives -

Place of study –

The study was carried out in the Department of Pathology (Division of Hematology, Central clinical laboratory, Acharya Vinoba Bhave Rural Hospital), Jawaharlal Nehru Medical College, Sawangi (M), Wardha after approval obtained from Institutional Ethics Committee.

Sample Size –

500 patients attending outpatient department (OPD) as well as admitted in various wards of Acharya Vinoba Bhave Rural Hospital (AVBRH) clinically suspected to be anemic.

Selection criteria –

Patients attending outpatient department (OPD) as well as admitted in various wards of AVBRH clinically suspected to be anemic were advised complete blood counts (CBC) and PS examination. Of these 500 patients with hemoglobin levels below 11.5gm/dl were selected and included in this study.

Known cases of hematological malignancies were excluded from the study.

Two ml sample of the patients was collected in Ethylene diamine tetra acetic acid (EDTA) bulb, was processed for CBC in ABX Pentra XLR (Make - Horiba) and PS was prepared from the same sample.

The peripheral smears prepared were stained by standard protocol with leishman stain[viii].

After preparation, each peripheral smear was examined for the morphological abnormalities associated with different types of anemia and results were noted.

The cell counter generated parameters which included red blood cell (RBC) indices (MCV, MCHC) were analyzed. Values of MCV between 80fl and 100fl, <80fl and >100fl were categorized as normocytic normochromic, microcytic hypochromic and macrocytic normochromic respectively.

The histograms were analyzed for their position (normal, left shift, right shift) and shape (normal bell shaped, bimodal peak, skewing to left or right, U shaped curve). The normal bell shaped histogram positioned normally was considered as normocytic normochromic, left shift indicated microcytic anemia and right shift indicated macrocytic anemia. A bimodal peak indicated dimorphic anemia and a broad base with left shift was considered as hemolytic anemia.

The diagnoses obtained by both the methods i.e. PS examination and cell counter generated parameters were correlated.

V. Results

In the present study titled "Co-relative study on peripheral blood smears in anemia with automated cell counter generated red cell parameters" carried out with the above mentioned objectives in the Hematology division of Central clinical laboratory, Department of Pathology, AVBRH, the peripheral smear, RBC indices and histograms of 500 patients having hemoglobin value less than 11.5gm/dl were analyzed and correlated. The following results were obtained –

The age group of patients included in this study ranged from 05 to 70 years. Majority of patients (268, 53.6%) were in the age group of 21-50 years age group. Out of 500 patients 311(62.2%) were female and 189 (37.8%) were male.

The categorization of type of anemia in patients (*n=500*) based on PS examination is as follows –

Table 1: Categorization of anemia on the basis of peripheral smear examination

SN	Category of anemia	No. of cases
1	Normocytic normochromic	100 (20%)
2	Microcytic hypochromic	200 (40%)
3	Macrocytic normochromic	50 (10%)
4	Dimorphic	30 (06%)
5	Hemolytic	120 (24%)

All the patients who fulfilled the inclusion and exclusion criteria were selected for the study and were examined for the morphological type of anemia on peripheral smear. Peripheral smear showed normal morphology of red cells i.e. normocytic normochromic anemia in 100 (20%) cases. Peripheral smear examination in 200 (40%) cases showed red blood cells of small size and reduced central pallor i.e. microcytic hypochromic red cells indicating the diagnosis of microcytic hypochromic anemia. 10% (50) cases showed larger than normal red cells suggesting the diagnosis of macrocytic anemia. PS of 30 cases showed dual population of red cells in the form of microcytes as well as macrocytes i.e. dimorphic blood picture leading us to

the diagnosis of dimorphic anemia. Presence of normocytic as well as microcytic red cells along with poikilocytosis showing fragmented red cells and pencil cells was seen in peripheral smear of 120 (24%) cases.

Table 2: Categorization of anemia based on RBC indices and histogram

SN	Category of anemia	No. of cases
1	Normocytic normochromic	94 (18.8%)
2	Microcytic hypochromic	222 (44.4%)
3	Macrocytic normochromic	80 (16%)
4	Dimorphic	25 (05%)
5	Hemolytic	79 (15.8%)

All the patients who fulfilled the inclusion and exclusion criteria were selected for the study, and their red blood cell indices along with histograms generated by cell counters were analyzed for the type of anemia. As shown in table 2, there were 94 cases diagnosed as normocytic normochromic anemia, 222 (44.4%) cases diagnosed as microcytic hypochromic anemia, 80 (16%) cases categorized as macrocytic normochromic anemia, 25 (05%) cases diagnosed as dimorphic anemia and 79 (15.8%) cases were categorized as hemolytic anemia on cell counter generated values and histograms.

Table 3: Comparison of findings of PS examination and cell counter generated values

SN	Category of anemia	No. of cases on peripheral smear examination	No. of cases on cell counter values
1	Normocytic normochromic	100 (20%)	94 (18.8%)
2	Microcytic hypochromic	200 (40%)	222 (44.4%)
3	Macrocytic normochromic	50 (10%)	80 (16%)
4	Dimorphic	30 (06%)	25 (05%)
5	Hemolytic	120 (24%)	79 (15.8%)

Table 3 shows the comparison of findings of peripheral smear examination and cell counter generated values of red blood cell indices and histogram. When these values were analyzed we found that there were significant correlation in normocytic normochromic, microcytic hypochromic and dimorphic anemia diagnosed on PS as well as by cell counter generated parameters. Hemolytic anemia and macrocytic anemia were the 2 categories of anemia with a discrepancy in the impression analyzed by peripheral smear examination and cell counter generated parameters i.e. red blood cell indices and histogram.

VI. Discussion

Anemia has been a major health problem worldwide, particularly among the females of reproductive age group and preschool going children. Microscopic examination and morphological assessment are an essential part of CBC reporting that provides crucial information apart from the cell counts. This study correlated the morphological findings of peripheral smear examination with the findings obtained from cell counters.

In the present study majority of anemic patients were found in 21-50 year age group which is in concordance with the published figures[ix]. On peripheral smear examination 100 out of 500 cases were of normocytic normochromic anemia while cell counter generated parameters reveled normocytic normochromic anemia in 94 cases which showed that findings of red cell indices and histograms were comparable to the findings of peripheral smear.

The presence of giant platelets or platelet clumps, fragmented red cells in hemolytic anemias where cell counters consider them as a microcyte could explain the difference observed in the cases of microcytic hypochromic anemias. So peripheral smear examination is must to rule out these errors. This finding is in accordance to the studies done by Poonamet. al[x] and Sandhya et. al.[xi]

Macrocytic anemia was observed in 50 cases on PS examination whereas red cell indices and histograms diagnosed 80 cases of macrocytic anemia out of total 500 cases. This difference could have arose due to the presence of polychromatic red cells in hemolytic anemias which raised the MCV of the sample misleading us to the diagnosis of macrocytic anemia.

The difference in the diagnosis of few cases of hemolytic anemia could be due to the presence of fragmented red cells and polychromatic red cells due to which some cases fell into the categories of microcytic and macrocytic anemias respectively. These findings point the limitation of cell counters in the diagnosis of hemolytic anemias. Some published studies also report such limitation.[10,11]

In this study not much difference was observed in the diagnosis of dimorphic anemia.

VII. Conclusion

By this study we conclude that even in the era of molecular diagnoses and automated cell counters, peripheral blood smear examination remains an important diagnostic tool. The CBC done with the help of cell counters should always be interpreted in the light of peripheral smear examination as this could help us in getting the correct diagnosis not only in the cases of hemolytic anemias but also macrocytic anemia where cell counters are incapable of arriving at the correct diagnosis at times.

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