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CORRELATION OF AUTOMATED HEMATOLOGY ANALYZER GENERATED RBC INDICES AND HISTOGRAM WITH MANUAL PERIPHERAL SMEAR EXAMINATION IN ANEMIA

Haematology

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ABSTRACT

Background: Anemia is a common medical condition, mostly diagnosed in developing nations. Peripheral smear examination is the gold standard for diagnosing most RBC disorders, which may fail to get diagnosed by automated cell counters. Few studies exist that compare automated analyzer based histogram, complete blood counts (CBC) parameters with peripheral smear examination. Present study was carried out to correlate these parameters. **Objectives:** The current study was conducted with the aim to correlate RBC indices and histograms obtained from automated analyzer with peripheral smear examination in cases of anemia. **Materials And Methods:** In the haematology laboratory, blood samples from 775 anemic patients were obtained during a six-month period. The haematology analyzer-based metrics (RBC indices & histogram) and the peripheral smear examination were used to type each blood sample for anemia. The acquired diagnosis was examined to determine the significance of the mean difference in various parameters. **Results:** 775 cases examined on peripheral blood smear showed the highest number of cases to have Normocytic normochromic anemia (57%), followed by Microcytic hypochromic anemia (35%). Dimorphic anemia (4.3%), macrocytic anemia (3.1%) and haemolytic anemia (0.6%). Analysis by erythrocyte indices and histogram showed similar frequency of various types of anemia, however the percentages varied in individual methods. The correlation between the diagnosis made by these methods was statistically significant, $p<0.0001$. **Conclusion:** Histogram based data provide early intimation of subtle changes in numerical parameters, subjective to evaluation. RBC histogram and indices should be interpreted in the light of peripheral smear.

KEYWORDS

Anemia, RBC indices, peripheral smear, histogram

INTRODUCTION

Anemia is a common medical condition prevalent worldwide, with most cases being reported in developing nations[1] . For treatment purposes, the precise classification of the various kinds of anemia is crucial. To determine the cause, many laboratory tests are run, with the most crucial ones being the peripheral smear (PS) analysis of red blood cell (RBC) morphology, red blood cell indices, and histogram.

Automated cell counts are a crucial component of the pathology lab for the analysis of CBC. Additionally, they offer RBC histograms and RBC indices to analyze various red blood cell morphological variations. If they are appropriately studied, they have been proven to be highly helpful in detecting a variety of haematological diseases including red blood cell abnormalities[2]. Since decades, peripheral blood smear examination has played a significant role in the research of a variety of haematological illnesses. It is also a key diagnostic tool, particularly for the etiopathological workup of numerous haematological disorders[2].

For several RBC disorders that may not otherwise be detected by automated cell counts, gold standard is still peripheral smear examination. They are crucial for the automated analyzers' quality control[2]. The histogram shows a double peak for the small population of microcytic hypochromic cells because the MCV average value does not accurately represent the variability of the RBC population. A double peak histogram curve, on the other hand, merely denotes the presence of a dual cell population and must be further connected with peripheral smear results[3].

Numerous studies have been conducted over the years to evaluate the usefulness and precision of parameters provided by automated cell counters in general as well as with regard to the diagnosis of various forms of anemia. The usefulness of the histogram, CBC parameters, and peripheral smear testing have only been briefly evaluated in few series[1]. The goal of the current study was to correlate these factors.

METHODOLOGY:

In this comparative double blinded study, we have analyzed 775 samples received during the period of six months from March 2022 to August 2022. Samples were evaluated for RBCs indices along with histograms obtained through automated hematology analyzer SYSMEX XN 1000 and peripheral blood smear stained by Leishman stain.

Inclusion Criteria: All cases of anemia, irrespective of age and gender, with serum haemoglobin levels as per WHO reference range were included in the study.

WHO (2011) recommendation[4] of haemoglobin (Hb) levels (g/dl) to diagnose anemia is as follows:

- Children ½ – 5 years (Hb< 11)
- Children 5 – 11years (Hb< 11.5)
- Children 12 – 14years (Hb< 12)
- Pregnant females (Hb<11)
- Non-pregnant females (15 years and above) (Hb<12)
- Men (15 years and above) (Hb<13)

Exclusion Criteria: The research excluded patients with leucocytosis, leukemoid reaction, leukaemia, parasite infestations and platelet abnormalities.

RESULTS AND OBSERVATIONS

In this study peripheral blood smears from 775 patients of various forms of anemia were analyzed, along with cell counter-generated RBC indices and histograms. Pursuant to the results of the peripheral smear test, the cases were microcytic hypochromic anemia (MCHC), normocytic normochromic anemia (NCNC), macrocytic anemia, dimorphic anemia, and hemolytic anemia.

Most of the individuals in the current study were between the ages of 31 and 40 (135 cases), followed by 51-60 years (133 cases), 41-50 years (121 cases), 21-30 years (118 cases). Females (60.1%) outnumbered males (39.9%), majority study subjects in the age group of 31-40 years (Table 1).

Table 1: age and gender-wise distribution of anemia cases

AGE (in years)	SEX		TOTAL
	Female	Male	
</=1	11	29	40

2-10	18	19	37
11-20	53	10	63
21-30	97	21	118
31-40	88	47	135
41-50	76	45	121
51-60	64	69	133
61-70	42	45	87
71-80	8	21	29
81-90	9	3	12
Total	466	309	775

On peripheral blood smear findings, NCNC anemia was found to be high in number with 442 patients (57.0%), followed by MCHC anemia having 271 patients (35.0%). In both the anemias, females were high as compared to males. (Table 2)

Table 2: Types Of Anemia Based On Peripheral Blood Smear Findings

Types of Anemia based on peripheral smear	Female (%) (n=466) (60.1%)	Male (%) (n=309) (39.9%)	Total (%) (n=775) (100%)
NCNC	244 52.4%	198 64.1%	442 57.0%
MCHC	191 41.0%	80 25.9%	271 35.0%
Macrocytic	8 1.7%	16 5.2%	24 3.1%
Dimorphic	19 4.1%	14 4.5%	33 4.3%
Hemolytic	4 0.9%	1 0.3%	5 0.6%

On analyzing the data it was noted that 45.5% and 35.0% patients were of MCHC on the RBC indices and peripheral smear respectively. Similarly 49.8% were of NCNC on RBC indices and 57.0% in peripheral smear. 3.9% had features of macrocytic anemia on RBC indices and 3.1% had the same picture on peripheral smear (TABLE 3).

Table 3: Correlation Of Peripheral Smear Findings With Analyzer Generated RBC indices (n=775)

Types of Anemia	Peripheral Smear Findings, n (%)		RBC indices, n (%)		P Value
NCNC	442	57.0	386	49.8	0.404
MCHC	271	35.0	353	45.5	0.000
Macrocytic	24	3.1	30	3.9	0.406
Dimorphic	33	4.3	5	0.6	0.000
Hemolytic	5	0.6	1	0.1	0.004

In our study, of 271 cases of microcytic hypochromic anemia, 226 cases showed left shift, 22 cases showed broad-based curve and 23 showed normal curve on RBC histogram. Among 442 cases of normocytic normochromic anemia, 431 cases showed normal bell-shaped histogram, 24 cases of macrocytic anemia 19 cases showed right-shift and 5 showed broad base on RBC histogram. However, among 33 cases of dimorphic anemia, 15 showed bimodal peak while 10 cases showed broad-base and one showed normal curve on RBC histogram.(TABLE 4)

Table 4 Correlation Of Peripheral Smear Findings With Analyzer Generated Histogram (n=775)

Type of Anemia	RBC Histogram pattern				
	Normal	Left shift	Right shift	Bimodal	Broad
NCNC (n=442)	431	2	0	0	9
MCHC (n=271)	23	226	0	0	22
Macrocytic (n=24)	0	0	19	0	5
Dimorphic (n=33)	1	4	3	15	10
Hemolytic (n=5)	0	0	0	2	3
Total, n (%)	455 (58.7%)	232 (29.9%)	22 (2.8%)	17 (2.2%)	49 (6.3%)

DISCUSSION

Anemia is a significant global public health issue, that affects around one-third of people worldwide. This being more common among women in underdeveloped nations who are of reproductive age[5]. There is controversy over the appropriate haemoglobin level thresholds to apply to classify anemia in the general population because it is a widespread issue that affects people in both industrialized and developing nations[1]. For an extended period of time, peripheral smears have been a crucial diagnostic tool for analyzing anemia.

Automated haematology cell counters have made accuracy and precision gains while lowering subjectivity mistakes[1]. The Coulter principle and Coulter counter were significant discoveries in the field of haematology, and the revolutionary laboratory techniques developed by the prolific Coulter are crucial in the etiopathological work up for various haematological conditions. According to the Coulter Principle, cells may be sized and counted by identifying and measuring changes in electrical resistance as they move through a tiny hole. The RBC histogram is a vertical bar graph that displays any cell size that falls within the size range. The cell counter counts a cell as an RBC if its volume is between 25 fL and 250 fL RBC histogram is a symmetrical bell-shaped curve[2]. The region of the peak is used to compute several RBC metrics including mean corpuscular volume (MCV) and red cell distribution width (RDW)[2]. The goal of the current study was to compare patterns in red blood cell histograms and SYSMEX XN1000 red cell indices with peripheral smear results.

In our study, we included a total of 775 anemic cases as per inclusion criteria. Of these cases, the majority of incidents affect those who were between 31–40 years (17.4%) and females outnumbered males similar to other studies[1,6]. However, this finding is contradictory to few other studies, where they have found maximum number of cases in the 21–30 years age group[7,8,9]. In line with Singhal et al.'s study, which found a higher prevalence of anemia in females (64.9%)[10], we found this to be the case in our analysis as well. Males were seen to be more impacted than females after the fifth decade, indicating that additional factors may have had a role in the development of anemia in this age group[1].

In our investigation, the most prevalent kind of anemia was normocytic normochromic anemia. Rarely red cell indices in normocytic normochromic anemia show a little increase, and they continue to fall in normal ranges. The cell population's size might vary [1]. The diagnosis made by cell counter based on RBC parameters and RBC histograms is quite comparable to the diagnosis made on peripheral smear examination, as evidenced by the observation that there were 442 cases (57.0%) on peripheral smear examination and 386 cases (49.8%) on RBC indices, with a p value of 0.404. In our study, patients of normocytic normochromic anemia were divided into 431 cases (97.5%) with normal bell-shaped curves and 9 cases (2.0%) with broad curves. The majority of normal-sized cells may have microcytic cells, which results in a bigger deviation and, thus, a higher RDW. However, discordant values were noted in study done by Rao et al[11].

Iron deficiency anemia is the most frequent cause of microcytic hypochromic anemia. Although MCHC may be normal in this case, the reduced MCV and MCH induce a left shift in the RBC histogram. Due to the high RDW, a broad-based curve indicates anisocytosis[1]. Various factors, including the presence of enormous platelets, the formation of platelet clumps, and the presence of fragmented RBCs in hemolytic anemias, which are classified as microcytic RBC by automated cell counter, may account for the discrepancy of results in classifying microcytic anemia in RBC indices with histogram and peripheral smear. So peripheral smears rule out these errors[1]. 226 cases (29.1%) of the 271 cases (35%) with microcytic hypochromic anemia on smear exhibited left shift, and 22 cases (2.8%) had a broad-based curve on the histogram. When we compare all of these results to data obtained from the analyzer, 353 cases (45.5%) showed microcytic hypochromic anemia, showing a significant difference in interpretable results (p = 0.00). These results agree with research by Sandhya et al (46%) [3] and Singhal et al (49.8%)[10].

On peripheral smear, dimorphic anemia was identified in 33 cases (4.3%) but RBC parameters were only detected in 5 cases (0.6%). In this study, there is variation in the diagnosis in cell counter generated parameters and in peripheral smear examination which is statistically significant (p=0.00). Few cases of dimorphic anemia may be mistakenly classified as microcytic, normocytic, or even macrocytic depending on the existence of the major red cell population since automated cell counters interpret anemia based on the presence of predominant RBC size [12]. Of the 33 cases of dimorphic anemia, RBC histogram showed typical bimodal peak only in 15 cases while 10 cases showed broad-based histogram. The broad-based curve can be explained by the existence of many populations of cells of various sizes, including normocytes, microcytes, and macrocytes. The

bimodal population may be a sign of haematological disorders such as early iron deficiency developing microcytic population and early folate/vitamin B12 deficiency developing macrocytic population, post-iron treatment of iron deficiency anemia, and post-iron treatment of iron deficiency combined with megaloblastic anemia. It is well recognised that the bimodal population is related to therapeutic transfusions and/or hematinic agent reactions to microcytic and macrocytic anemia. The diagnosis is supported by the additional characteristics and the bimodal curve [1]. Low levels of microcytes and macrocytes have little effect on MCV, which solely shows the mean of the distribution curve. Without MCV, MCH and MCHC provide very little information[1]. However, because dimorphic anemia is linked to an aberrant red cell population, peripheral smear analysis is extremely valuable for a more accurate interpretation of data.

On the basis of peripheral smear examination, macrocytic anemia was identified in 24 cases (3.1%), whereas RBC indices identified 30 cases (3.9%). 19 cases (2.5%) of the histogram's data indicated right shift, whereas 5 cases (0.6%) exhibited broad base. The reason for the disparity between the peripheral smear findings and the RBC indices may be because haemolytic anemia was mistaken for macrocytic anemia because the presence of polychromatophilic erythrocytes led to a high MCV value, which gave a high value for haemolytic anemia. Cold agglutinins and hyperglycemia may deceive MCV levels. Rao et al. (2.2%) [11] and Chavda et al. (3%) [13] made similar observations.

Only a small percentage of cases (0.1%) were found to have haemolytic anemia on histogram and RBC indices, compared to peripheral smear cases (0.6%) that might have fragmentation as an explanation. Cell counters classified polychromatophils as macrocytes and RBCs as microcytes[1]. Other authors also observed a similar restriction of the RBC histogram and RBC indices in the diagnosis of hemolytic anemia [14,15]. Nevertheless the broad-based histogram, which is right-skewing, has a larger RDW, a lower Hct, and a relatively high RBC count, suggests that hemolysis is a factor, in line with the finding made by Garg et al[1]. Examining the peripheral smear in these situations is crucial for the confirmation of the diagnosis and other haematological diseases. Blood smear testing is still a vital diagnostic tool even in the era of molecular analysis, claims Bain[16] in her 2005 study on the role of peripheral smear examination in the age of automation.

We observed that histograms are an effective tool for normocytic normochromic anemia. However, histogram patterns for microcytic hypochromic, dimorphic anemia, haemolytic anemia are diverse, ranging from simple to complicated curves. Therefore, peripheral smear screening is essential in all patients with reduced haemoglobin in order to detect red cell alterations. Modern, sophisticated studies of haematological problems should be comprehended in the context of both the clinical setting and peripheral blood smear results.

CONCLUSION:

RBC parameters and histograms are helpful in the identification of various kinds of anemia, according to the findings of this study. It should be utilized with manual peripheral smear examination in a complimentary manner to improve the accuracy of the diagnosis. However, the gold standard for confirming a diagnosis remains a peripheral smear examination.

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