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Correlation of RBC indices and RBC histogram obtained by automated hematology analyser with peripheral smear in the diagnosis of anemia

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ABSTRACT

Background and Objectives: The impact of early diagnosis and management of anemia on patient outcome has been tremendous. Although conventional microscopic examination of blood using peripheral smear is unequivocal, the advent of automated hematology analyzer has revolutionized the field of clinical hematology and its practice. This study highlights the efficiency and utility of specific parameters obtained by hematology analyzer and its comparison with peripheral smear in the diagnosis of anemia, also determines the sensitivity of the same.

Materials and Methods: 501 Ethylenediamine tetra acetic acid blood samples collected at a tertiary care teaching hospital in Karnataka during one and half years from February 2021 to August 2022, were run on the Sysmex 6-part automated hematology analyzer and simultaneous peripheral smears were prepared and stained using Leishman stain based on standard operating procedures. The histogram and peripheral smear findings were noted.

Results: Among the 501 cases, there were 269(53.7%) cases of Normocytic Normochromic Anemia in which 82.9% showed normal curve, 145(28.9%) cases of Microcytic Hypochromic Anemia in which 92.4% showed shift to left, 31(6.2%) cases of Dimorphic Anemia in which 61.3% showed normal curve contrary to the expected double peak, 24(4.8%) cases of Macrocytic Anemia in which 83.3% showed shift to right, 23(4.6%) cases of Pancytopenia in which 39.1% showed normal curve and 9 cases of Normocytic Hypochromic Anemia.

Conclusion: The Automated hematology Analyzer provides highly valuable information with limited sample and is an essential tool in diagnosis, classification and management of anemia.

1. Introduction

Anaemia is a global problem affecting the population in both developing as well as developed countries. About one third of the global population is anemic. The conventional microscopic examination of peripheral smear for red blood cell morphology provides crucial information about the patient's clinical condition and has always been the cornerstone of diagnostic haematology.[1] However, laboratory haematology has been labour intensive and time consuming.[2]

Over the past few years, complete blood count by the automated haematology analyser and peripheral blood smear have complemented each other to provide a comprehensive report on the patient's blood sample. The advent of automated haematology cell counter has improved accuracy and precision, reduced subjective errors and improved safety in handling blood samples.[3] The RBC histogram is commonly used with peripheral smear as an aid in monitoring and interpreting abnormal morphologic changes particularly in dimorphic red cell population.[1] The modern-day analysers use the principles of

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electrical impedance, conductivity, cytochemical staining, light scatter and flow cytometry.[2]

Peripheral smear examination is sensitive and a gold standard when it comes to the diagnosis of red cell disorders. Red cell histogram and red cell indices obtained from haematology automated analyser act as an adjunct to the visual examination of peripheral smears.[2]

2. Materials and Methods

This study was a descriptive prospective study wherein 501 blood samples in ethylene diamine tetra acetic acid were collected and run on the Sysmex 6-part machine. Peripheral smears were also prepared from the same. The samples collected were from both men and women in the age group between 18 to 75 years that fit the diagnostic criteria for anemia. Patients with haematological malignancies, infections, leukemoid reaction or platelet disorders were excluded. RBC indices and other parameters were noted along with the type of histogram. These results were compared with the peripheral smear report. The degree of correlation was ascertained. Results were analysed using the Excel Spreadsheet and SPSS software Version 22. It was represented in the form of proportions, frequencies and tables.

3. Results

A total of 501 cases of anaemia were evaluated with respect to peripheral smear and RBC indices along with histogram generated by the automated haematology analyser. Anaemia was diagnosed in 283 females and 218 males which was 56.5% and 43.5% of the total cases respectively (Figure 1). The maximum number of cases of anaemia was seen in the age group of 20 to 40 years with 245 cases which was 48.9% of the total. The second most affected age group was 40 to 60 years with 144 cases that was 28.7% of the total (Table 1). Among both females and males, the worst affected age group was between 20 and 40years. It was observed that males were more affected than females beyond the age of 40 years (Table 2). Among the 501 cases of anaemia which were evaluated, it was observed that the most common type was Normocytic Normochromic Anaemia with 269 cases that is 53.7%, followed by 145 cases (28.9%) of Microcytic Hypochromic Anaemia, 31 cases (6.2%) of Dimorphic Anaemia, 24 cases (4.8%) of Macrocytic Anaemia, 23 cases (4.6%) of Pancytopenia and the least being 9 cases of Normocytic Hypochromic Anaemia (Table 3). Among the cases of Normocytic Normochromic Anaemia, there were 142 females and 127 males, Microcytic Hypochromic Anaemia was seen in 101 females and 44 males, Dimorphic Anaemia was seen in 20 females and 11 males, Macrocytic anaemia was seen in 4 females and 20 males, Pancytopenia in 11 females and 12 males and Normocytic Hypochromic Anaemia was seen in 5 females and 4 males respectively. It was observed that though generally more common in females, both macrocytic anaemia and pancytopenia were slightly more common in males. Among the 501 histograms obtained, 270 (53.9%) showed a normal curve followed by 186 cases (37.1%) which showed a shift to left. These were the 2 most predominant patterns observed on histogram as was seen on peripheral smear (Table 4). Among the 269 cases of Normocytic Normochromic Anaemia, 223 showed normal curve (82.9%) and this was the most predominant pattern. The remaining 44 cases (16.45%) showed shift to left and only 2 cases showed double peak. The mean MCV, MCH and MCHC were found to be within normal limits while the mean RDW was 15.7 and the mean Hb was 9.7 (Table 5). Among the 145 cases of Microcytic Hypochromic Anemia,133 cases (92.4%) showed shift to left which was the most common pattern followed by 9(6.3%) cases showing a normal curve,1 case showed double peak and the remaining 2 cases showed the Multiple Peaks (MP) and RL flags respectively (Figure 2). The mean MCV and MCH were found to be decreased and mean MCHC was 31.0. The mean RDW was 19.2 while the mean Hb was 8.4. The lowest MCV value of 49.6 was observed in a case of Microcytic Hypochromic Anaemia. However, a case with MCV 80.9 was also reported as Microcytic Hypochromic based on red cell morphology on peripheral smear (Table 6). Among the 31 cases of Dimorphic anaemia, 19 showed a normal curve (61.3%), 9 cases (29%) showed the double peak pattern and 3 cases (9.7%) showed a shift to left (Figure 3). The mean MCV, MCH and MCHC were found to be within normal limits while the mean RDW was 20.1. The mean Hb was 7.7. The lowest Hb value that is 2.4 was observed here. Among the 24 cases of Macrocytic Anemia, 20 of them showed shift to right (83.3%) followed by 3 of the cases(12.5%) which showed a normal curve and 1 case(4.2%) showing double peak. The mean MCV was 104.3 while the mean Hb was 9.8. The mean RDW was 16.The maximum MCV observed was 116.9. Among the 23 cases of Pancytopenia, the most common pattern observed was normal curve [39.1%, 9 cases], followed by shift to right [21.7%, 5 cases], shift to left, double peak [13%, 3 cases each] and least was shift to right with RL, shift to left with RL and double peak with RL[1 case each]. The mean MCV was 93.1 while the mean RDW CV was 20.3. The mean Hb was 6.4. The maximum value of MCV that is 125.0 and also the highest RDW CV of 35.6 was seen in Pancytopenia (Table 7). Among the 9 cases of Normocytic Hypochromic Anemia of which 7(77.8%) showed a normal curve and remaining 2 cases (22.2%) showed a shift to left. The mean MCV was 80.5 while the mean RDW CV was 16.3. The mean Hb was 8.8. The sensitivity, specificity, positive predictive value and negative predictive value were calculated for the different histograms in specific anaemias. The shift to left curve for diagnosing

Microcytic Hypochromic Anaemia showed the greatest sensitivity of 91.72%. The double peak for Dimorphic Anaemia showed the least sensitivity of 29.03%. However, it showed the greatest specificity of 98.09%. The normal curve for Normocytic Normochromic Anaemia was found to be least specific with specificity of 79.74%.(Table 8) The chi sqaure statistic is 683.039 and the p value was observed to be <0.00001 which was statistically significant.

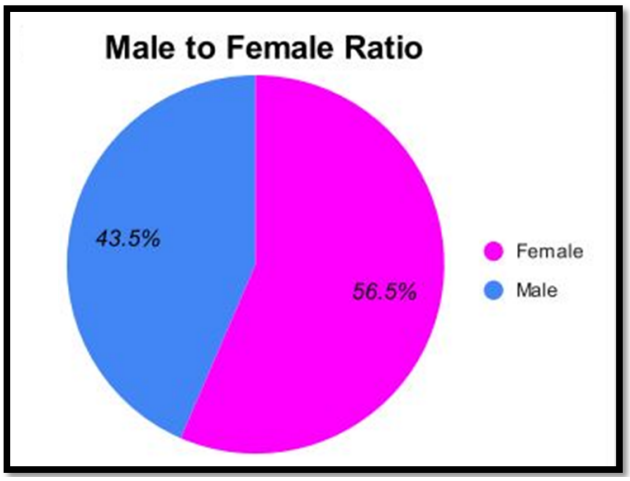

Figure 1: Percentage distribution of Anaemia in males and females

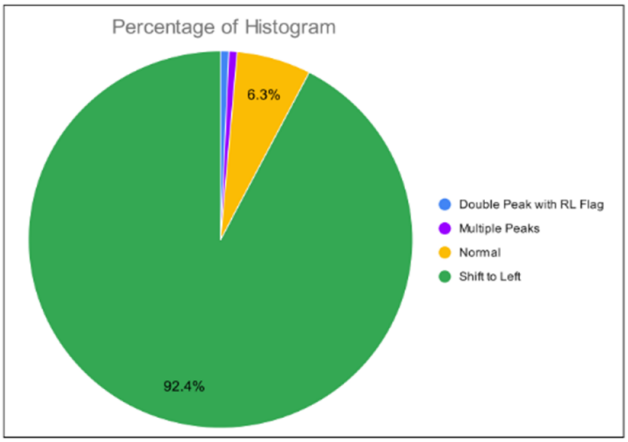

Figure 2: Percentage distribution of different types of histogram curves obtained in microcytic hypochromic anaemia

Table 1: Age distribution among study population

Age	Count	Percentage
<20 years	30	6.0%
20-40 years	245	48.9%
40-60 years	144	28.7%
>60 years	82	16.4%
Total	501	100.0%

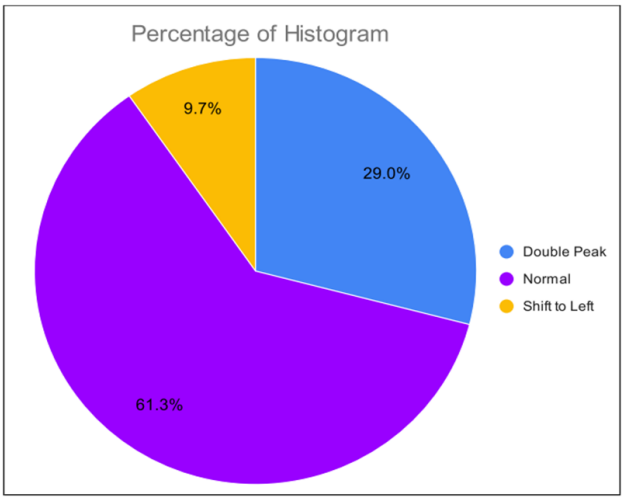

Figure 3: Percentage distribution of different types of histogram curves obtained in dimorphic anemia

4. Discussion

Wintrobe first proposed the terms mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in 1929 to describe the dimensions and haemoglobin content of red blood cells. These numbers, known as red cell indices, are helpful in determining the cause of anaemias. If the values for haemoglobin, haematocrit (packed cell volume), and red blood cell count are known, red cell indices can be determined. Since electronic cell counters are now widely available, red cell indices are now automatically determined in all blood count calculations.[4]

A third of the world's population suffers from anaemia, which increases illness and mortality, lowers productivity at work, and impairs neurological growth. Anaemia is a disorder when the body's levels of haemoglobin (Hb) and/or red blood cells (RBCs) are abnormally low and insufficient to meet physiological demands of the individual.[5,6] Anaemia is linked to higher rates of morbidity and mortality in both women and children, poorer birth outcomes, less productivity in the workplace, and delayed cognitive and behavioural growth in kids. Women of reproductive age (WRA) and pre-schoolers (PSC) are severely impacted.[7,8]

The diameters of various blood cells are plotted on the X-axis of an automated haematology analyzer along with their relative numbers on the Y-axis to produce blood cell histograms. The distribution curves for Red Blood Cells (RBC), White Blood Cells (WBC) and Platelets must be carefully analysed in order to interpret histograms. A good interpretation of this histogram provides a wealth of information on many haematological conditions than mere cell counts, helping to narrow down the differential diagnosis at a very early stage even before higher level investigations are ordered.[9,10]

Table 2: Age and sex distribution among study population

Age	Sex				Total	
	Female		Male			
	Count	Percentage	Count	Percentage	Count	Percentage
<20	24	8.5%	6	2.8%	30	6.0%
20-40	164	58.0%	81	37.2%	245	48.9%
40-60	65	23.0%	79	36.2%	144	28.7%
>60	30	10.6%	52	23.9%	82	16.4%
Total	283	100.0%	218	100.0%	501	100.0%

Table 3: Different types of anaemia reported on peripheral smear

Type of anaemia on peripheral smear	Number of cases
Normocytic normochromic anaemia	269
Microcytic hypochromic anaemia	145
Dimorphic anaemia	31
Macrocytic anaemia	24
Pancytopenia	23
Normocytic hypochromic	9

Table 4: Distribution of various histograms in study population

Histogram Position among study population		
Histogram	**Count**	**Percentage**
Double Peak	18	3.6%
Multiple Peaks	1	.2%
Normal	270	53.9%
Shift to Left	186	37.1%
Shift to Right	26	5.2%
Total	501	100.0%

Table 5: RBC indices obtained in normocytic normochromic anaemia

	Peripheral Smear Report RBC Normocytic Normochromic Anaemia				
	Minimum	Maximum	Mean	Median	Standard Deviation
HB	4.2	12.9	9.7	10.0	1.8
RBC Count	1.49	9.24	3.49	3.62	.77
PCV	10.2	41.0	28.4	29.3	5.4
MCV	70.3	98.3	82.6	81.9	5.6
MCH	21.5	38.8	28.2	28.0	2.5
MCHC	24.1	38.5	34.1	34.3	1.7
RDWCV	11.3	31.0	15.7	15.0	3.0

Table 6: RBC indices obtained in microcytic hypochromic anaemia

Mean Blood indices in Microcytic Hypochromic Anaemia Peripheral Smear Report RBC Microcytic Hypochromic Anaemia					
	Minimum	Maximum	Mean	Median	Standard Deviation
HB	3.0	12.6	8.4	8.7	2.1
RBC Count	1.87	6.55	4.02	4.03	.90
PCV	9.8	38.9	27.0	27.4	6.0
MCV	49.6	80.9	67.4	68.7	6.5
MCH	12.9	29.7	21.0	21.2	3.2
MCHC	23.8	37.6	31.0	31.6	2.6
RDWCV	12.4	32.8	19.2	18.5	3.9

Table 7: RBC indices obtained in pancytopenia

	Mean Blood indices in Pancytopenia Peripheral Smear Report RBC Pancytopenia				
	Minimum	**Maximum**	**Mean**	**Median**	**Standard Deviation**
HB	3.1	12.3	6.4	5.3	2.7
RBC Count	.72	4.36	2.20	2.17	1.01
PCV	9.0	33.8	19.7	19.6	7.6
MCV	63.5	125.0	93.1	90.2	16.3
MCH	16.8	43.1	31.7	31.2	7.2
MCHC	26.4	37.6	33.7	34.4	3.1
RDWCV	13.6	35.6	20.3	18.9	6.1

Table 8: Diagnostic accuracy of different histograms in specific anaemias

Histogram in the diagnosis of specific anaemia	**True Positive**	**False Positive**	**False Negative**	**True Negative**	**Sensitivity**	**Specificity**	**NPV**	**PPV**
Normal curve for diagnosing Normocytic Normochromic anaemia	223	47	46	185	82.90%	79.74%	80.09%	82.59%
Shift to Left for diagnosing Microcytic Hypochromic anaemia	133	53	12	303	91.72%	85.11%	96.19%	71.51%
Double peak for diagnosing Dimorphic anaemia	9	9	22	461	29.03%	98.09%	95.45%	50.00%
Shift to Right for diagnosing Macrocytic anaemia	20	6	4	471	83.33%	95.74%	99.16%	76.92%

(Note: TP: True Positive, FP: False Positive, FN: False Negative, TN: True Negative, Se: Sensitivity, Sp: Specificity, NPV: Negative Predictive Value, PPV: Positive Predictive Value)

The present study was a prospective study conducted over a period of one and a half years between February 2021 and August 2022 from the Central Laboratory under the Department of Pathology at KIMS, Hubli. Data about demographics were obtained for the study population. A total of 501 cases of anaemia were evaluated with respect to peripheral smear and RBC indices along with histogram generated by the automated haematology analyser. In the present study, anaemia was diagnosed in 283 females and 218 males which was 56.5% and 43.5% of the total cases respectively which is similar to many studies conducted previously in India showing a higher prevalence in women as compared to men but as the age increased there was an increasing trend of anaemia seen in the male population. Majority of the patients with anaemia in the study were between the age groups of 20-40 years. There are significant public health repercussions from the high frequency of iron deficiency anaemia among women of reproductive age as observed in our study as well. Anaemia is thought to be responsible for 12.8% of maternal deaths in Asia. This is consistent with the study by Ratre et al. which showed a maximum prevalence of anaemia amongst the 21-30 years age group.[11] The present study is also consistent with the WHO statistics as well, wherein estimated prevalence of anaemia in developing countries is 39% which was observed in 42% of women aged between 15–59 years and in 30% of men aged between 15–59 years.[12]

The most common morphological type of anaemia in our study is normocytic normochromic anaemia followed by microcytic hypochromic anaemia A minority of the normochromic, normocytic anaemias are primary blood abnormality, while the majority are complications associated with other illnesses. Few of the causes will be by acute blood loss, polymyalgia rheumatica, renal failure, endocrine failure (hypothyroidism, hypopituitarism), marrow failure (pure red-cell aplasia, aplastic anaemia, infiltration) or anaemia of chronic disease (inflammation, neoplasia).[13] The body's diminished iron reserve, which can occur for a variety of reasons, is the most common cause of microcytic hypochromic anaemia. This may be caused by a diet low in iron, inadequate gut absorption of iron, acute and chronic blood loss, increased demand for iron during pregnancy or after major surgery or trauma recovery. The most frequent cause of microcytic hypochromic anaemia was iron deficiency.

Among the 501 histograms obtained, 270 (53.9%) showed a normal curve consistent with normocytic normochromic anaemia wherein out of 269 cases of

Normocytic Normochromic Anaemia, 223 showed normal curve (82.9%) which was the most predominant pattern. The RBC indices evaluated in the present study showed the mean MCV, MCH and MCHC within normal limits with the MCV ranging between 70.3 to 98.3, 21.5 to 38.8 and 24.1 to 38.5 respectively while the mean RDW was 15.7 and the mean Hb was 9.7 respectively in case of normocytic normochromic anaemia. The results were consistent with some of the previous studies conducted on similar lines where in patients although were not in majority but the histogram showed a normal curve with a broad base and the RBC indices were within normal limits.[14–16]

Out of 501 cases, 186 cases showed a shift to left in histogram which can be closely associated with microcytic hypochromic anaemia in which out of 145 cases of Microcytic Hypochromic Anemia, 133 cases (92.4%) i.e., majority showed shift to left as the most common pattern seen. In case of microcytic hypochromic anaemia, the RBC indices were also evaluated and the MCV values were observed in the range of 49.6 to 80.9 with the mean MCV and MCH were found to be decreased and mean MCHC was 31.0. The mean RDW was 19.2 while the mean Hb was 8.4. In microcytic hypochromic anaemia, MCHC may be normal, but MCV and MCH are decreased. The above observations were consistent with previous studies as mentioned in the table below with patients with microcytic hypochromic anaemia having a left shift with broad base in all the studies with reduction in MCV as well as MCH lower than normal.[17]

Among the 31 cases of Dimorphic anaemia, 19 showed a normal curve (61.3%) contrary to the expected double peak which is classical for this type of anaemia, 9 cases (29%) showed the double peak pattern and 3 cases (9.7%) showed a shift to left. Most of the studies conducted on similar lines showed a similar histogram with RBC indices as observed in our study.[17]

Among the 24 cases of Macrocytic Anaemia, 20 of them showed shift to right (83.3%) which was the most common pattern observed here followed by 3 of the cases (12.5%) which showed a normal curve and 1 case (4.2%) showing double peak. These were the 2 most predominant patterns observed on histogram as was seen on peripheral smear.

The present study evaluated pancytopenia and out of 23 cases, 9 of them (39.1%) showed a normal curve which was the most common pattern observed. 5 of the cases (21.7%) showed a shift to right, 3(13%) showed shift to left and 3 cases (13%) showed double peak with a mean MCV of 93.1 while the mean RDW CV was 20.3. The mean Hb is 6.4. Fewer studies have evaluated pancytopenia and majority of the studies have broad base in contrast to normal curve seen in this study.[17] However RBC indices showed similar results.

In this study there were 9 cases of Normocytic Hypochromic Anaemia of which 7(77.8%) showed a normal curve and remaining 2 cases (22.2%) showed a shift to left. The mean MCV was 80.5 while the mean RDW CV was 16.3. The mean Hb was 8.8.

This type of anaemia has not been evaluated in any of the previous studies. And the histogram showed normal and left shift with normal RBC indices.

Diagnostic accuracy was evaluated in this study and it was observed that histogram showed high sensitivity and high specificity for Normocytic Normochromic Anaemia with normal curves, left shift for microcytic hypochromic anaemia and bimodal distribution curve with high specificity for dimorphic anaemia and right shift curve for macrocytic anaemia.

5. Conclusion

Along with peripheral smear examination, diagnosis of RBC disorders is supplemented by histogram as they provide further information with regard to regarding RBC morphology, blood indices and haemoglobin values. The various IP flags also provide clues to aid our diagnosis. The speed and reliability of analysers allow more time for the hematopathologist to evaluate abnormal blood smears and correlate with histograms with more confidence and efficiency.

By doing so the likelihood of arriving at the most accurate diagnosis is increased significantly. This would result in more quality information that would help the clinician to manage the patient in the most effective way possible. Ultimately this would help to reduce the burden caused by anaemia on many levels. It would also prevent wastage of resources and ensure that appropriate treatment is administered judiciously to each patient dependent on the underlying cause of anaemia.

The use of automated haematology analysers should be encouraged and appropriate training should also be made available for its efficient utilisation. Interpretation of histograms must be inculcated in routine haematology practice.

6. Source of Funding

None.

7. Conflict of Interest

None.

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