

# Comparative Study of Automated Cell Counter Generated Data and Peripheral Smear Evaluation in Cases of Anaemia

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## Abstract

**Introduction:** In developing countries Anemia is major public health problem. Females are more commonly affected by anemia. Iron deficiency is the main cause of anemia. It was estimated that preschool children and pregnant woman are more commonly affected by anemia. **Subjects and Methods:** It is hospital based prospective observational study. Total number of cases was 400. The study duration is for a period of one and half years. The study was carried out at our tertiary care center in Department of Pathology and in patients of clinical departments of Netaji Subhash Chandra Bose Medical College & Hospital, Jabalpur (M.P.) Patients diagnosed anemia in central laboratory of age above 14 year and Indoor patients in NSCB Medical College & Hospital Jabalpur were included in this study. Ethical permission was taken from Institutional Ethics Committee. **Result:** In our study 15-25 year of age group are more affected in both females 105 (45.5%) and males 80 (47.3%). As per the above table, on the basis of interpretation of auto analyzer generated data, the most common type of anemia in females is microcytic hypochromic 102 (68.5%) and in male macrocytic anemia 74(56.5%) that is statistically significant  $p < 0.05$ . **Conclusion:** Our study concludes that the two methods of morphological typing of anemia having “moderate agreement” with each other according to kappa ( $\kappa$ ) Analysis.

**Keywords:** Anemia, Peripheral Blood Smear, Automated Cell Counter.

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## Introduction

Anemia is major public health problem over worldwide, especially among females in developing countries. ‘Anemia is defined as a reduction of total circulating red cell mass below normal limits.’<sup>[1]</sup> It is estimated that approximately 33% of the world’s population has anemia with iron deficiency considered to be the leading cause of anemia accounts for almost 9% of the world’s population living years with a disability burden. It has also been estimated that worldwide 273 million preschool-age children having anemia (43% of all children), 32 million pregnant women having anemia (38% of all pregnant women); and 496 million non-pregnant women having anemia (29% of all non-pregnant women). Anemia is most prevalent in central and west Africa and south Asia.<sup>[2]</sup>

“The basic test performed on the peripheral blood- “Complete Blood Count” (CBC) is one of the most informative single investigation expressing the health and disease status of the body in whole menu of laboratory medicine. A long journey travelled by this single investigation from the era of only hematocrit/hemoglobins diagnostic tool to the most sophisti-

cated multiparts, multiparameters automation” in hematology. “Automation, because of its accuracy has changed principles and methodologies, approaches and conclusions of various disciplines of medicine. Few branches are modified to the extent that their entire philosophy is so much reoriented that it needs to be rewritten and hematology is one of them, although the fact remains that Automation is not a replacement for the study of peripheral smear, it just a compliment to manual microscopy like ECG and X-ray chest are compliment to manual auscultation in clinical medicine”.<sup>[3]</sup> Automated Analyzers are a very important part of hematology laboratory for evaluation of complete blood count (CBC). Although Peripheral smears evaluation is still the gold standard for diagnosing some of the RBC disorders which might not be diagnosed by automated cell counters. It plays an important role in quality check of automated analyzers. Nowadays most of hospitals are replacing traditional manual methods by automated analyzers for measuring various hematological parameters as the initial screening tool.<sup>[4]</sup>

## Aims & Objectives

1. To evaluate the merits and demerits of Conventional methods of evaluation versus Modern Technology of Automation in cases of anemia.
2. To establish anemia assessment methodology with precision, cost effectiveness with minimum battery of investigations.
3. To make better use of multiple parameter already available with cell counter generated data for guiding towards type of anemia.
4. To establish the best combination of conventional and Modern technology for diagnostic approach to anemia.

## Subjects and Methods

This is hospital based prospective observational study was undertaken with 400 cases to find out characteristics of anemia on the basis of automated analyzer and peripheral blood smear.

**Study Design:** A hospital based prospective observational Study.

**Study Duration:** one and half years.

**Study Area:** The study was done at our tertiary care center in Department of Pathology and in patients of clinical departments of Netaji Subhash Chandra Bose Medical College & Hospital, Jabalpur (M.P.).

**Study Population:** 400 adult patients admitted in clinical departments in our tertiary care center of Jabalpur region.

### Inclusion criteria

1. Patients diagnosed anemia in central laboratory of age above 14 year.
2. Patients willing for and giving consent to participate in the study.
3. Indoor patients in NSCB Medical College & Hospital Jabalpur.

### Exclusion criteria

1. Patients not willing to participate in the study.
2. Patients of Leukemia.
3. Outdoor patients in NSCB Medical College & Hospital Jabalpur.
4. Patients of the age 14year and below.

**Ethical Permission:** permission was taken from Institutional Ethics Committee to conduct this study.

## Method of Data Collection

Anemic patients as per WHO reference range were enrolled for the study, patient details and history was taken as per

my proforma. An informed consent was taken in written from patients and counselled about procedure. With all aseptic precaution, 2 ml blood sample was obtained from peripheral vein by venepuncture and stored in anticoagulant (EDTA) vacutainer/vial and sent to central laboratory as soon as possible. The blood sample was first run in automated analyzer BC-3600 (mindray), 3 – part differential automated hematology analyzer which analysed the blood sample and generate the multiparameter data (RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD) obtained as print out with complete blood count. On the basis of RBC indices, morphological typing of anemia was done. Same blood sample used immediately for preparing a peripheral blood smear and stained with Leishman stain for examination. On the basis of peripheral blood smear examination, morphological typing of anemia has been done.

## Data Analysis Plan

The data was analysed with the help of SPSS 20 software for windows. Appropriate univariate and bivariate analysis is carried out by use of ANOVA test, Cohen's kappa test ( $\kappa$ ), student's t-test and chi-square ( $\chi^2$ ) test are applied to check the hypothesis according to the type of data. The critical value for the significance of the results are considered at 0.05 level i.e.  $p < 0.05$  was considered significant.

In our study 15-25 year of age group are more affected in both females 105 (45.5%) and males 80 (47.3%) and least affected age group is  $>76$  years for both male 1(0.6%) and female 1(0.4%) [Table 1]. In our study, case of anemia on the basis of auto-analyzer data are categorized into various morphological types like 51 (12.8%) cases are Normocytic normochromic, 149 (37.3%) cases are Microcytic hypochromic, 131 (32.8%) cases are Macrocytic, 64 (16%) cases are Dimorphic and 5 (1.3%) cases are of Hemolytic anemia although typing of anemia on the basis of peripheral blood smear examination categorized as 14 (3.5%) cases are normocytic normochromic, 116 (29%) cases are microcytic hypochromic, 106 (26.5%) cases are macrocytic, 134 (33.5%) cases are dimorphic and 30 (7.5%) cases are of Hemolytic anemia [Table 2].

As per the above table, on the basis of interpretation of auto analyzer generated data, the most common type of anemia in females is microcytic hypochromic 102 (68.5%) and in male macrocytic anemia 74(56.5%) that is statistically significant  $p < 0.05$  [Table 3]. As per the above table, on the basis of peripheral blood smear examination, the most common type of anemia in females is microcytic hypochromic 84 (72.4%) and in male macrocytic anemia 58(54.7%) that is statistically significant  $p < 0.05$  [Table 4]. The impression made by PBS and Automated analyzer generated data is statistically analysed, for the agreement analysis between two methods with kappa, the value of kappa was calculated as  $0.538 \pm 0.029$

**Table 1: Gender and age wise distribution of anemia**

Age group (years)	Sex		Total (%)
	F (%)	M (%)	
15 – 25	105 (45.5)	80 (47.3)	185 (46.3)
26 – 35	56 (24.2%)	47 (27.8%)	103 (25.8%)
36 – 45	37 (16.0%)	16 (9.5%)	53 (13.3%)
46 – 55	12 (5.2%)	9 (5.3%)	21 (5.3%)
56 – 65	16 (6.9%)	13 (7.7%)	29 (7.3%)
66 – 75	4 (1.7%)	3 (1.8%)	7 (1.8%)
> 76	1 (0.4%)	1 (0.6%)	2 (0.5%)
Total	231 (100%)	169 (100%)	400 (100%)

**Table 2: Morphological typing of anemia on the basis of automated parameters and PBS**

Interpretation	Automated Parameters		Manual Peripheral Smear	
	Frequency (f)	Percent (%)	Frequency (f)	Percent (%)
Normocytic mochromic	51	12.8	14	3.5
Microcytic hypochromic	149	37.3	116	29.0
Macrocytic	131	32.8	106	26.5
Dimorphic	64	16.0	134	33.5
Hemolytic	5	1.3	30	7.5
Total	400	100	400	100

**Table 3: Gender wise distribution of different anemia interpreted on the basis of auto analyzer generated data**

Sex	F	n	Interpretation of auto analyzer generated data					Total	Chi-	Result
			Normocy <sup>1</sup> nor- mochrom	Microcyti hypochro	Macrocyt	Dimorphi	Hemolyti			
			34	102	57	36	2	231	Chi-	
		%	66.7%	68.5%	43.5%	56.3%	40%	57.8%		
	M	n	17	47	74	28	3	169		
		%	33.3%	31.5%	56.5%	43.8%	60%	42.3%		
Total		n	51	149	131	64	5	400		
		%	100%	100%	100%	100%	100%	100%		

that is statistically significant ( $p = 0.001$ ), for this measure of agreement. The above analysis shows 53.8% agreement between two methods, which is statistically significant [Table 5].

## Discussion

Morphological typing of anemia is done by PBS, helps clinician and hematologist in making early diagnosis and appropriate therapeutic intervention although automated hematology

analyzer has improved accuracy, precision and reduced the subjective error and time consumption. Even in era of automation and development of sophisticated auto analyzers, peripheral smear is still used as a basic and important diagnostic tool for anemia, leukemia and other hematological disorders. [5]

In present study it is observed that females of reproductive age group between 15 - 45 years are mostly affected with anemia. The same results also found in studies of Rao BSS et al, Shruti Singla et al, Mukaya JE et al and Dr. Faiza H et al. [6-9] [Table 1].

**Table 4: Gender wise distribution of different morphological anemia diagnosed by peripheral blood smear examination**

			Manual Peripheral Smear					Total	Chi-	Result
			Normocytic normochromic	Microcytic hypochromic	Macrocyt	Dimorphi	Hemolyti			
Sex	F	N	7	84	48	77	15	231	Chi-	
		%	50%	72.4%	45.3%	57.5%	50%	57.8%		
	M	N	7	32	58	57	15	169		
		%	50%	27.6%	54.7%	42.5%	50%	42.3%		
Total		N	14	116	106	134	30	400		
		%	100%	100%	100%	100%	100%	100%		

**Table 5: Comparison between manual Peripheral Smear and auto analyzer interpretation in cases of different morphological anemia**

Type of anemia	PBS	Auto analyzer
Normocytic normochromic (%)	14 (3.5%)	51 (12.8%)
Microcytic hypochromic (%)	116 (29%)	149 (37.3%)
Macrocytic (%)	106 (26.5%)	131 (32.8%)
Dimorphic (%)	134 (33.5%)	64 (16%)
Hemolytic (%)	30 (7.5%)	05 (1.3%)
Total	400	400

In present study, it is observed that various type of anemia on the basis of auto analyzer found that 12.8% case are normocytic normochromic, 37.3% cases are microcytic hypochromic, 32.8% macrocytic, 16% cases are dimorphic and 1.3% case are hemolytic anemia although morphological typing of anemia is done on the basis of peripheral smear examination and found that 14 (3.5%) case are normocytic normochromic, 116 (29%) case are microcytic hypochromic, 106 (26.5%) case are macrocytic, 134 (33.5%) case are dimorphic and 30 (7.5%) case of Hemolytic anemia. That is comparable with following studies Chavda et al, Sinha R et al, Sandhya V et al and Jain A et al. [10-13] [Table 2].

It is observed that typing of anemia by auto analyzer generated data, the most common type of anemia in females is microcytic hypochromic 102 (68.5%) and in male macrocytic anemia 74 (56.5%) that is statistically significant  $p < 0.05$ . This is comparable with Swaroop Raj BV et al. [14] [Table 3].

It is observed that typing of anemia by peripheral blood smear examination, the most common type of anemia in females is microcytic hypochromic 84 (72.4%) and in male macrocytic anemia 58 (54.7%) that is statistically significant  $p < 0.05$ . This is comparable with Swaroop Raj BV et al. [14] [Table 4].

Morphological typing of anemia by PBS examination which is already typed by automated analyzer generated data, Total 51 cases are typed by auto analyzer as normocytic normochromic anemia, while on peripheral blood smear examination it is confirmed that 21 cases are dimorphic, 12 cases are microcytic hypochromic, 9 cases are macrocytic and only 9 cases of nor-

mocytic normochromic anemia. Auto analyzer typed 12.8% cases as normocytic normochromic anemia while only 3.5% cases were diagnosed as normocytic normochromic anemia by PBS examination. Mostly in normocytic normochromic anemia, the red blood cell indices remain in normal range with few cases showing mild variation in indices. The red cell population may be variable in size that may be due to treatment therapy or blood transfusion. There may be some microcytic or macrocytic cells are present with predominance of normocytic cells that results high RDW but MCV in normal range. So interpretation made by red cell indices were comparable with diagnosis made by peripheral blood smear examination. Microcytic hypochromic anemia 37.3% Cases were typed by auto analyzer, while 29% cases were diagnosed by PBS examination as microcytic hypochromic anemia. The difference in result of analysis between auto analyzer and peripheral smear examination is due to the presence of giant platelets, platelet clumps and fragmented red cells in hemolytic disorders, therefore auto analyzer counts them as microcyte. While it is rule out by peripheral smear examination.

Auto analyzer diagnosed macrocytic anemia in 32.8% cases, while 26.5% cases were diagnosed as macrocytic anemia by PBS examination. The difference in result of PBS examination and red cell indices interpretation is due to counting of polychromatophils as macrocytes in hemolytic anemias with raised MCV. In present study Auto analyzer diagnosed 16% cases of dimorphic anemia, while on peripheral blood smear examination 33.5% cases were diagnosed as dimorphic

anemia. Because sometimes auto analyzer may interpret few cases of dimorphic anemia as macrocytic, microcytic and normocytic anemia as per the predominance of RBC population. Dimorphic anemias are associated with various RBC population, for the better approach to diagnosis of anemia, morphological finding of PBS used along with automated analyzer generated numerical data and graphical distribution of red cells. Only 1.3% cases are typed by auto analyzer as hemolytic anemia, while peripheral blood smear examination diagnosed 7.5% cases as hemolytic anemia. This is one of the limitation of auto analyzers that only limited number of cases of hemolytic anemias were interpreted by auto analyzer as compared to PBS examination. Our study is comparable with studies done by Garg M et al, Chavda J et al, Sinha R et al, Sandhya V et al and Jain A et al. [5,10-13] [Table 5].

## Conclusion

Our study concludes that the two methods of morphological typing of anemia having “moderate agreement” with each other according to kappa ( $\kappa$ ) Analysis. Even in the age of molecular analysis and automation in hematology, peripheral smear evaluation still remains a gold standard diagnostic tool. Complete blood count done by automated analyzers should always be interpreted in light of peripheral smear examination.

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