Research Article

Can peripheral blood smear examination be totally replaced by automated hematology analyser - with special reference to anemia?

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ABSTRACT

Background: The aims and objectives of present study was to correlate typing of anemia based on RBC indices obtained from an automated analyzer with peripheral blood smear (PBS) examination and also to find out whether the number of PBS examination can be reduced with the help of automated hematology analyzer.
Methods: A total of 2500 blood samples showing anemia as per WHO reference range were collected in central pathology lab of SVBP Hospital attached to L.L.R.M. Medical College, Meerut, Uttar Pradesh, India over a period of one year. Samples were reported by auto-analyzer and PBS examination simultaneously.
Results: Out of total 2500 cases, there were 1623 females (64.9%) and 877 males (35.1%) with male: female ratio 0.54:1. By auto-analyzer and PBS examination, MCHC anemia (49.8%) was the commonest anemia followed by NCNC anemia (36.5%) and Macrocytic anemia (4.2%). Discordant typing of anemia between two methods was found in 284 (11.4%) cases only. These cases were diagnosed as normocytic normochromic (NCNC) anemia with raised RDW by autoanalyzer while as Dimorphic Anemia (DA) on PBS examination. Also morphological changes such as RBC inclusions, spherocytes, RBC fragments, schistocytes, nucleated RBCs were seen only on PBS examination.
Conclusions: The Study concluded that even today PBS examination is very important and cannot be totally replaced by automated analyzer and both methods are complementary to each other.

Keywords: Automated hematology analyzer, Peripheral blood smear, Examination, Anemia

INTRODUCTION

Peripheral blood smear (PBS) examination is a part of the routine work of every laboratory. The manual examination of these images is tedious, time consuming and suffers from interobserver variation. This has motivated researchers to develop different algorithms and methods to automate PBS image analysis. The automated hematology analyzers give more accurate and <1% coefficient of variation for the RBC indices and hence have replaced the manual methods. Thus we can definitely conclude that the hematology analyzers definitely have a potential to replace peripheral blood smear examination.[1]

The expertise needed to see the PBS is definitely far more than that needed to run the analyzers. Moreover there is intra and inter observer variation in morphological typing of anemia based on PBS examination. The additional PBS review performed by hematologist hardly ever provided unique information and provided incremental helpful information in only 4% of the cases.[2]

Cell counting with these instruments is rapid, objective, statistically significant (8000 or more cells are counted) and not subject to the distributional bias of the manual count. Hence automated instruments increase accuracy, speed of analysis, minimizes levels of human manipulation for test entry, sampling, sample dilution and

analysis.[3] Analyzers are also more efficient than the manual method. Most of these cell counters can process 120-150 samples per hour. In addition, the precision of the automated differential makes the absolute leukocyte counts reliable and reproducible.[4]

The automated hematology analyzer with complete blood count (CBC) results has replaced the traditional manual or individual assay methods for hematological parameters and the eye count leucocyte differential as the initial screening and detection system for hematological abnormalities in modern hospitals and clinics.[5]

The present study was being carried out to find the correlation of typing of anemia based on RBC indices obtained from an automated analyzer with PBS examination and to find out if the rate of manual PBS review in cases of anemia already morphologically typed on automated analyzer can be reduced.

METHODS

The present study was a randomized cohort based prospective study in which 2500 blood samples showing anemia as per WHO reference range were collected in the central pathology lab of SVBP Hospital attached to L.L.R.M Medical College, Meerut over a period of 1st June 2014 to 1st August 2015.

In the present study, children below 5 years were excluded and the samples showing anemia as per WHO reference range in the age range 6-70 years were included.

WHO (2011) recommendations of hemoglobin (Hb) levels to diagnose anemia at sea level (g/l)-

- Children 5-11 years- Hb <115
- Children 12-14 years- Hb <120
- Non pregnant females (15 years and above)- Hb <120
- Pregnant females- Hb <110

- Men (15 years and above)- Hb < 130

Anemia typing was done by two methods – Firstly using RBC indices with RDW by autoanalyzer and secondly on PBS examination.

The sample was venous blood collected by venepuncture in vacutainer containing EDTA anticoagulant. The samples were aspirated into hematology analyzer M series M16/ M20 and simultaneously blood smears were prepared and stained with May- Grunwald Geimsa stain.

Typing of anemia using RBC indices by autoanalyzer-

- Microcytic hypochromic (MCHC) anemia with normal RDW
- MCHC anemia with raised RDW
- Normocytic normochromic (NCNC) anemia with normal RDW.
- NCNC Anemia with raised RDW
- Macrocytic anemia (MA)

Morphological typing of anemia with PBS-

- MCHC anemia
- NCNC anemia
- Macrocytic anemia
- Dimorphic anemia

RESULTS

Out of the total 2500 cases, there were1623 females (64.9%) and 877 males (35.1%) with male: female ratio 0.54:1.

It was observed that by using autoanalyzer, most common anemia was MCHC anemia with raised RDW, 1095 cases (43.8%), followed by NCNC anemia with normal RDW, 913 cases (36.5%), NCNC anemia with raised RDW, 284 cases (11.4%), MCHC anemia with normal RDW, 104 cases (4.2%) and Macrocytic anemia, 104 cases (4.2%).

Table 1: Typing of total 2500 cases of anemia based on autoanalyzer.

Typing of anemia	No. of males (%)	No. of females (%)	Total no. of cases (%)
MCHC anemia with raised RDW	129 (14.7%)	966 (59.5%)	1095 (43.8%)
NCNC anemia with normal RDW	546 (62.3%)	367 (22.6%)	913 (36.5%)
NCNC anemia with raised RDW	117 (13.3%)	167 (10.3%)	284 (11.4%)
Macrocytic anemia	60 (6.8%)	44 (2.7%)	104 (4.2%)
MCHC anemia with normal RDW	25 (2.9%)	79 (4.9%)	104 (4.2%)
Total	**877 (35.1%)**	**1623 (64.9%)**	**2500 (100%)**

Among the males, most common anemia was NCNC anemia with normal RDW, 546 cases (62.3%), followed

by MCHC anemia with raised RDW, 129 cases (14.7%), NCNC anemia with raised RDW, 117 cases (13.3%),

Macrocytic anemia, 60 cases (6.8%) and MCHC anemia with normal RDW, 25 cases (2.9%). Among the females, most common anemia was MCHC anemia with raised RDW, 966 cases (59.5%), followed by NCNC anemia with normal RDW, 367 cases (22.6%), NCNC anemia with raised RDW, 167 cases (10.3%), MCHC anemia with normal RDW, 79 cases (4.9%) and Macrocytic anemia, 44 cases (2.7%) (Table 1). By using PBS examination, it was observed that MCHC anemia was the most common, 1245 cases (49.8%), followed by NCNC anemia, 913 cases (36.5%), Dimorphic anemia, 238 cases (9.5%) and Macrocytic anemia, 104 cases (4.2%).

Table 2: Morphological typing of total 2500 cases of anemia based on PBS examination.

Morphological type of anemia	No. of males (%)	No. of females (%)	Total no. of cases (%)
MCHC Anemia	180 (20.5%)	1068 (65.8%)	1245 (49.8%)
NCNC Anemia	527 (60.1%)	383 (23.6%)	913 (36.5%)
Dimorphic Anemia	90 (10.3%)	148 (9.2%)	238 (9.5%)
Macrocytic Anemia	80 (9.1%)	24 (1.5%)	104 (4.2%)
Total	**877 (35.1%)**	**1623 (64.9%)**	**2500 (100%)**

Among the males, most common anemia was NCNC anemia, 527 cases (60.1%), followed by MCHC anemia, 180 cases (20.5%), Dimorphic anemia, 90 cases (10.3%) and Macrocytic anemia, 80 cases (9.1%).Among the females, most common anemia was MCHC anemia, 1068 cases (65.8%), followed by NCNC anemia, 383 cases (23.6%), Dimorphic anemia, 148 cases (9.2%) and Macrocytic anemia, 24 cases (1.5%) (Table 2).

Table 3: Correlation between typing of anemia by autoanalyzer and PBS examination.

Typing of anemia by autoanalyzer	Morphological typing of anemia by PBS examination	No. of cases (%)
MCHC anemia with raised RDW	MCHC anemia	1095 (43.8%)
NCNC anemia with normal RDW	NCNC anemia	913 (36.5%)
NCNC anemia with raised RDW	MCHC anemia	46 (1.8%)
	Dimorphic anemia	238 (9.5%)
Macrocytic anemia	Macrocytic anemia	104 (4.2%)
MCHC anemia with normal RDW	MCHC anemia	104 (4.2%)
Total		**2500 (100%)**

In the present study, discordance in typing of anemia between two methods was found only in 284 cases (11.4%) which were diagnosed as NCNC anemia by autoanalyzer while on PBS, 238 cases (9.5%) were found as Dimorphic anemia and 46 cases (1.8%) as MCHC anemia. Rest of the 2216 cases (88.6%) correlate well both by autoanalyzer and PBS examination (Table 3).

DISCUSSION

The present study was conducted to find the correlation of typing of anemia based on RBC indices obtained from an automated analyzer with PBS examination and to find out if the rate of manual PBS review in cases of anemia already morphologically typed on automated analyzer can be reduced.

A total of 2500 cases were studied, out of which 1623 females (64.9%) were affected with anemia. This was in concordance with the study done by Swaroop Raj et al as 63.5%, Mukaya JE et al as 59.2% while National family health survey 2005-06 concluded that in Uttar-Pradesh 49.9% females were suffering from anemia.[6-8] In the present study, MCHC anemia (49.8%) was the commonest followed by NCNC anemia (36.5%). Almost similar results were obtained by Japheth E.

Mukaya et al as MCHC anemia (54%) and NCNC anemia (31%).[7] Verma M et al also reported MCHC anemia as 55.4% and 37.5% NCNC cases, Kumari et al reported 53.3% cases as MCHC anemia and NCNC cases 35.8% while Prakash Joshi et al concluded that MCHC anemia 24.74% cases and NCNC anemia 12.64% cases and Sandhya I et al concluded 61% as MCHC anemia and 17% as NCNC anemia.[9-11]

The present studies showed that PBS examination provide additional information only in 11.4% cases of

anemia. Almost similar result was obtained by Paul Froom et al as 13.9% while Novis et al reported that PBS examination provide additional information in 6.4% cases of anemia, and Djulbegovic B et al in 4-6% of cases.[12-14]

CONCLUSION

Study concluded that by both autoanalyzer and PBS examination MCHC anemia (49.8%) was the commonest anemia followed by NCNC anemia (36.5%) and Macrocytic anemia (4.2%). PBS need to be reviewed only in 11.4% cases of anemia, and provide additional information like recognition of altered RBC structure, nucleated RBCs etc. RBC injury may be identified by finding RBC fragments, portions of disrupted cells (schistocytes), or evidence of significant membrane alterations i.e. ovalocytes or spherocytic cells.

Therefore, PBS examination even today cannot be totally replaced by automated hematology analyzers as they provide so much additional information which cannot be summarized completely by the mere numerical calculations of an automated analyzer. But the present generations of automated hematology analyzers are well on par and provide accurate morphological typing of anemia in cases of MCHC Anemia, Macrocytic anemia and NCNC Anemia with normal RDW thus reducing the workload and thereby increasing the efficiency of a laboratory.

However in cases on NCNC Anemia with raised RDW, PBS examination provides valuable information and is absolutely necessary for morphological typing of anemia. Hence both methods are complementary to each other and PBS examination cannot be totally replaced by automated analyzer.

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