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Original Research Article

Comparison between Morphological Typing of Anemia based on RBC Indices Obtained from an Automated Haematology Analyzer with Peripheral Blood Smear Examination

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Abstract

The automated hematology analyzer providing CBC has become the keystone of modern hematology laboratories and has replaced the traditional manual methods. Morphological typing of anemia is routinely done based on RBC indices like MCV, MCH, MCHC and RDW and also by peripheral smear examination. In this study morphological typing of different types of anemia were done by indices obtained from automated analyzer and by peripheral smear and were compared.400 cases of anemia were analyzed by sysmex XT 1800i analyzer and results compared and correlated. Out of 400 cases 351 cases showed a concordant typing and 49 cases showed a non-concordant typing and 12.25% of cases required a peripheral smear review for the correct typing of anemia. The sensitivity and specificity of the sysmex XT 1800i analyzer were calculated as 97% and 91% respectively for the detection of microcytes, 96.5% and 97.5% respectively for the detection of macrocytes and 91% and 90.5% respectively for the detection of normocytes. Most of the non-concordant cases belonged to the category of normocytic normochromic anemia with raised RDW. Morphological typing of anemia from the RBC indices and RDW showed a high sensitivity and specificity, except for some cases with a raised RDW which required an additional peripheral smear examination for the correct typing. So manual peripheral smear examination still holds important role in the modern laboratory practice as it provides us with many information for the proper diagnosis and management.

Keywords: Morphological typing of anemia, sysmex XT 1800i, five-part automated hematology analyzer, peripheral smear examination, RBC indices, red cell distribution width.

INTRODUCTION

Anemia is defined as an insufficient RBC mass to adequately deliver oxygen to peripheral tissues which is present when hemoglobin level is below the normal range for the age and sex of the individual [1]. Once anemia is diagnosed, a morphological typing of anemia helps the clinician to get a clue to the probable cause of anemia. Morphological typing of anemia now a days done based on RBC indices including MCV, MCH, MCHC and RDW obtained from automatic cell counters. Cell counting with analyzers is more rapid, objective, statistically significant (8000 or more cells are counted) and is not subjected to the distributional bias that we encounter in the manual count. Hence automated instruments has increased accuracy, can analyze more number of samples with greater speed and precision and minimizes the requirement of human manipulation for test entry, sampling, sample dilution and analysis [2].

The automated hematology analyzer with complete blood count (CBC) results has become the cornerstone of modern laboratory and has replaced the traditional manual or individual assay methods for common hematological parameters in modern hospital and clinical settings. The practice of review of all automated hematology instrument results by preparation of a stained blood film and microscopic examination is no longer done in most institutions. The reasons are the quick and accurate diagnosis of specimens with distributional or morphological abnormalities by the analyzers than by the traditional eye count method. Peripheral smear is labor intensive but it is cost effective and more sensitive method than hematology analyzer. Hematology analyzer detects normal or low level of hemoglobin but in some situations it is not able to detect the presence of various abnormal cells that are diagnostic of specific conditions. So a manual examination of the peripheral smear can give more information which are useful in

the proper morphological classification of anemia. The aim of this study is to classify and diagnose different types of anemia morphologically from peripheral smear and from RBC indices and thereby affirming the results of automated analyzer by comparing with the routine peripheral smear examination. The number of non-concordant cases would give us an idea of the number of anemic cases that are requiring a peripheral smear review for their proper morphological typing and also will help us determine the sensitivity and specificity of the analyzer in detecting the different morphological types of red blood cells.

OBJECTIVES

- To compare the morphological typing of anemia done from RBC indices generated by an automated hematology analyzer and from peripheral blood smear examination.
- To assess the sensitivity and specificity of the five-part automated hematology analyzer in measuring the MCV thereby helping in typing of anemia.

MATERIALS & METHODS

STUDY DESIGN: DIAGNOSTIC TEST VALIDATION STUDY using SYSMEX XT 1800i five part automated hematology analyzer SAMPLE SIZE: 400

INCLUSION CRITERIA

All patients with hemoglobin below the WHO reference values and above the age of 5 years whose blood samples were received at the clinical pathology lab, Government medical college, Thrissur for CBC and peripheral smear examination.

EXCLUSION CRITERIA

Patients with hemoglobin within the normal range for that particular age and sex and pediatric patients below 5 years of age with anemia.

Peripheral blood smear stained with Leishman stain examined and the sample also processed with an automated hematology analyzer. Morphological typing of anemia done using RBC indices as Microcytic Hypochromic Anemia with normal RDW, Microcytic Hypochromic Anemia with raised RDW, Normocytic Normochromic Anemia with normal RDW, Normocytic Normochromic Anemia with raised RDW, Macrocytic Anemia. Morphological typing of anemia were done with Peripheral smear as Microcytic hypochromic anemia, Normocytic normochromic anemia, Macrocytic anemia, Dimorphic anemia.

RESULTS

Total of 400 cases were included in the study 234 females and 166 males. In this study commonest morphological type of anemia in females was microcytic hypochromic anemia (53%) and the commonest one in males was normocytic normochromic anemia (48%)

Table-1: Gender wise distribution of the cases

SEX	MALE	FEMALE	TOTAL
FREQUENCY	166	234	400

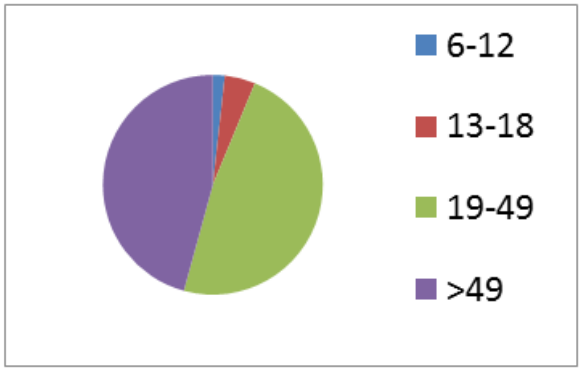

Fig-1: Age wise distribution of Anemia cases

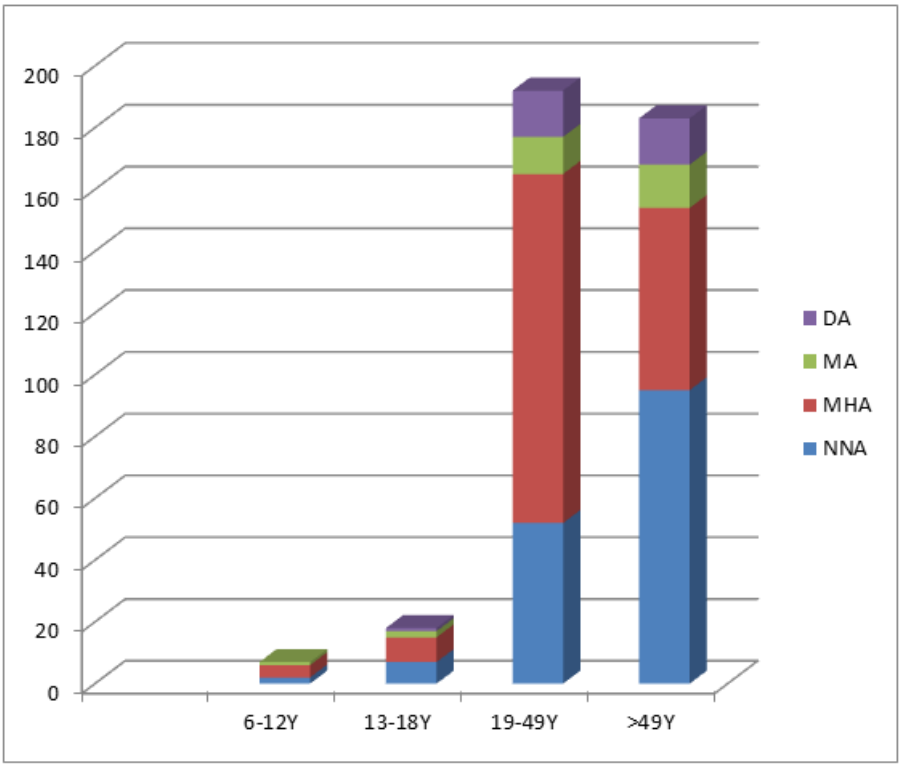

Fig-2: Distribution of different morphological types of anemia in the age categories

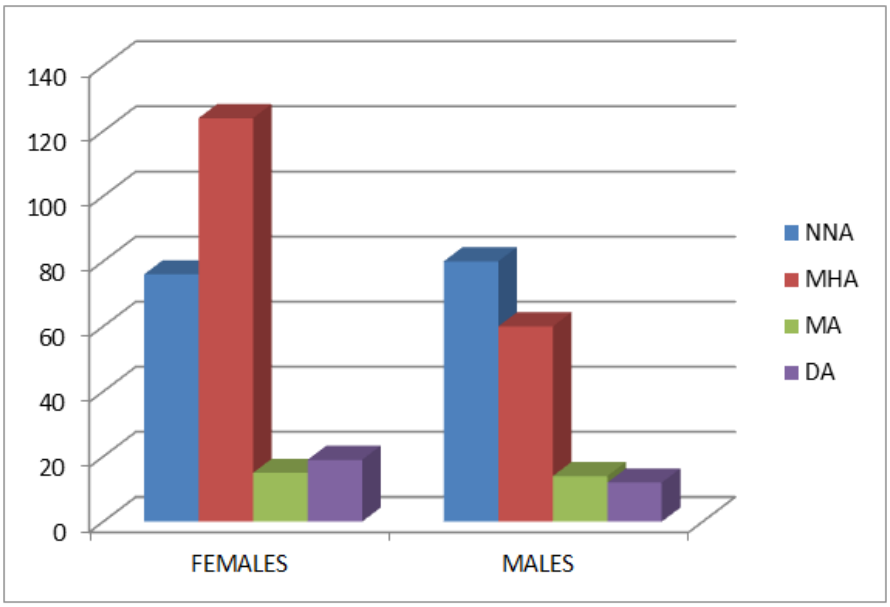

Fig-3: Gender wise Distribution of Anemia

Morphological typing of anemia based on RBC indices and RDW obtained from sysmex 1800i (five part automated hematology analyzer) was done in 400 cases and Table2 shows the morphological types and frequency of anemia.

Table-2: Morphological typing using RBC indices and RDW

Morphological type of anemia	Frequency
Normocytic normochromic anemia with normal RDW	98
Normocytic normochromic anemia with raised RDW	67
Microcytic hypochromic anemia with normal RDW	95
Microcytic hypochromic anemia with raised RDW	103
Macrocytic anemia	37

All these cases were also typed using peripheral smear examination and majority of the cases were microcytic hypochromic anemia (184 cases) which accounted for 46% followed by normocytic normochromic anemia (156 cases).

Table-3: Morphological typing using peripheral smear

Morphological type of anemia	**Frequency**
Normocyti normochromic	**156**
Microcytic hypochromic	**184**
Macrocytic	**29**
Dimorphic	**31**
Total	**400**

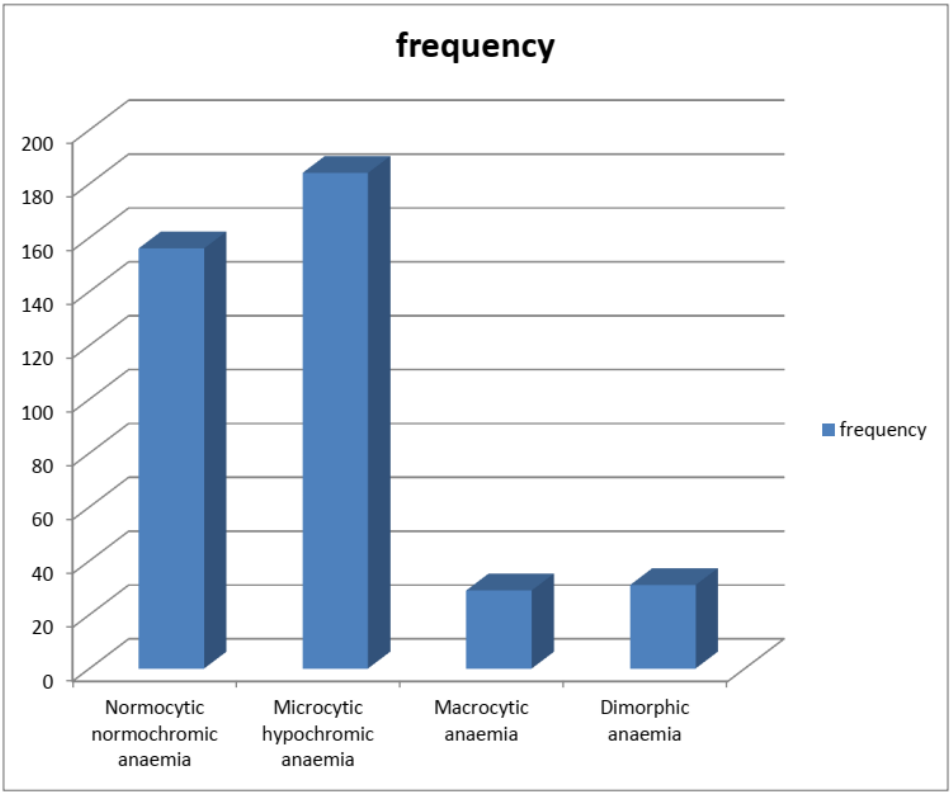

Fig-4: Frequency of different types of Anemia

Table-4: Comparison between morphological typing of anemia based on RBC indices and RDW with peripheral smear examination

TYPING BY RBC INDICES AND RDW	TYPING FROM PERIPHERAL SMEAR
Normocytic normochromic anemia with normal RDW (98)	Normocytic normochromic anemia (96)
	Microcytic hypochromic anemia (2)
Normocytic normochromic anemia with raised RDW (67)	Normocytic normochromic anemia (47)
	Microcytic hypochromic anemia (2)
	Macrocytic anemia (1)
	Dimorphic anemia (17)
Microcytic hypochromic anemia with normal RDW (95)	Normocytic normochromic anemia (10)
	Microcytic hypochromic anemia (85)
Microcytic hypochromic anemia with raised RDW 103)	Normocytic normochromic anemia (2)
	Microcytic hypochromic anemia (95)
	Dimorphic anemia (6)
Macrocytic anemia (37)	Normocytic normochromic anemia (1)
	Macrocytic anemia (28)
	Dimorphic anemia (8)

On comparing the morphological typing of anemia from RBC indices and RDW with that of peripheral smear majority of the cases typed as normocytic normochromic anemia with normal RDW (98%) were confirmed to be the same on the peripheral smear as normocytic normochromic anemia while 2 cases who had MCV as 80 fl were typed as microcytic hypochromic on peripheral smear examination. Out of the 67 cases of normocytic normochromic anemia with raised RDW 47 cases were typed as normocytic normochromic anemia, 2 as microcytic hypochromic anemia (MCV was 79fL), one as macrocytic anemia (MCV was 99 fL) and 17 cases as dimorphic anemia. 95 cases of microcytic hypochromic anemia with normal RDW 85 cases were confirmed to be the same on peripheral smear while 10 cases were typed as normocytic normochromic anemia (most with MCV in the range 75-79 fL) Majority of the cases typed as microcytic hypochromic anemia with raised RDW (92%) were confirmed to be the same on peripheral smear, 2 cases were typed as normocytic normochromic anemia (MCV 78/79 fL) and 6 typed as dimorphic anemia.Out of the 37 cases typed as macrocytic anemia from the indices and RDW 28 cases were confirmed to be the same on peripheral smear, 1 as normocytic normochromic anemia with marked polychromasia and 8 as dimorphic anemia. Out of the 400 cases studied 351 cases accounting to 87.75% showed concordance between typing using RBC indices and RDW and using peripheral smear examination. Only 12.25% of cases showed non-concordant typing and so had to be confirmed by the peripheral smear.

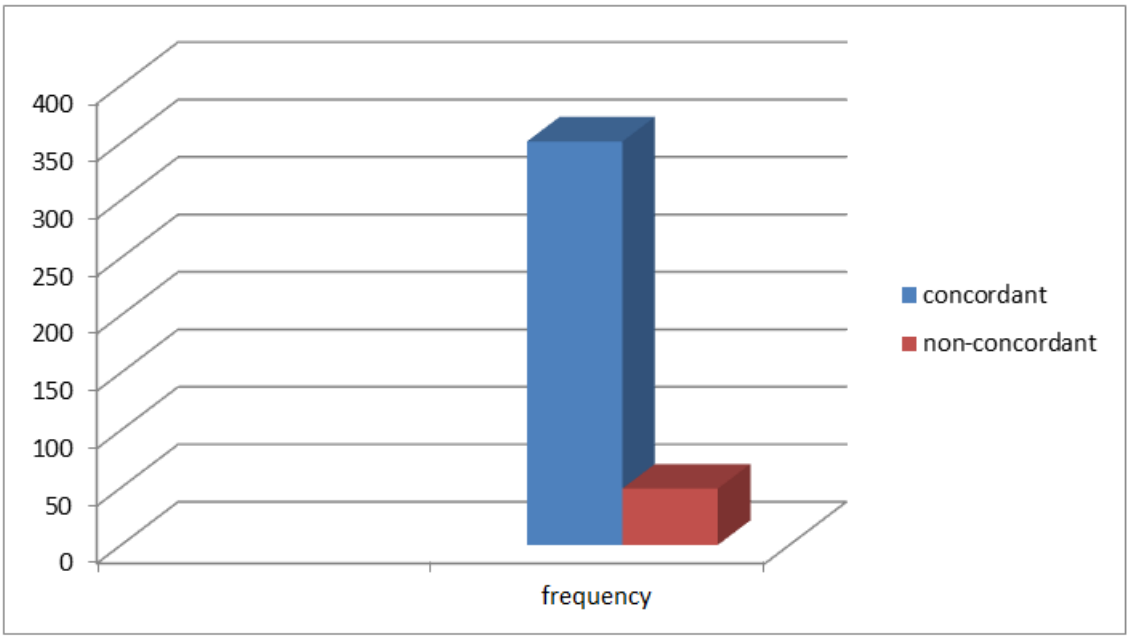

Fig-5: Cases with concordant and non-concordant results

Table-5: Distribution of cases with concordant typing and non-concordant typing

Morphological typing using RBC indices and RDW	Concordant typing in peripheral smear (no. of cases)	Non-concordant typing in peripheral smear (no. of cases)	Total
Normocytic normochromic anemia with normal RDW (1)	96	2	98
Normocytic normochromic anemia with raised RDW (2)	47	20	67
Microcytic hypochromic anemia with normal RDW (3)	85	10	95
Microcytic hypochromic anemia with raised RDW (4)	95	8	103
Macrocytic anemia (5)	28	9	37
Total	351	49	400

Discordance mostly seen in cases typed as normocytic normochromic anemia with raised RDW 29.85% of them were typed differently on peripheral smear (25.37% of cases were typed as dimorphic anemia).

Among the cases typed as macrocytic anemia from RBC indices and RDW, 21.62% of cases turned out as dimorphic anemia and 2.7% of cases turned out as normocytic normochromic anemia with polychromasia.

Table-6: Sensitivity and specificity of the five part analyzer in identifying microcytes

True Positives	False Positives	True Negative	False Negative	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
179	19	197	5	97.28%	91.20%	90.4%	97.5%

Table-7: Sensitivity and specificity of the five part analyzer in identifying macrocytes

True Positives	False Positives	True Negative	False Negative	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
28	9	362	1	96.55%	97.5%	75.68%	97.5%

Table-8: Sensitivity and specificity of the five part analyzer in identifying normocytes

True Positives	False Positives	True Negative	False Negative	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
142	23	221	14	91.03%	90.57%	86.06%	94.04%

DISCUSSION

In the current study of 400 cases 351 cases of anemia typed by the RBC indices and RDW were confirmed to be the same in the peripheral smear examination while 49 cases (12.25%) were typed differently. In other words 12.25% of cases required a peripheral smear review showing that manual review of blood smears was helpful at least in some cases as it provided additional information for the proper morphological classification of anemia which in turn provided clues to the probable cause of anemia and thus aiding in the adequate treatment required. The benefit of manual screening is in its ability to identify clinically important specific cell types (pencil cells, tear drop cells, burr cells, schistocytes, target cells, sickle cells, blast cells etc.) that are not quantifiable by analyzers, but that produce flags on the automated results as stated by Cornbleet in 1997 [3], Dutcher in 1985 and Gulati in 2001 from the observations of their studies [4, 5].

Current study outcome is in contrast with the early reports by Novis *et al*., [6] and Pierre [7] whose conclusion was that automated hematology analyzers are more accurate in the detection of specimens with morphological abnormality than the traditional eye count method. Automated blood counts are widely accepted for routine screening practice but many laboratories still reflexively perform manual screening of peripheral blood smears, based solely on abnormal automated results or instrument flags before undertaking any triage steps as stated by Lantis *et al*., in 2003 [8].

Ejaz Farah *et al*., in Pakistan, from his study of 350 cases concluded that for diagnosing and differentiating different types of anemia manual screening of peripheral smear is the method of choice because peripheral smear provides additional diagnostic information. They suggested that patient care and lab operations should be optimized by using microscopic examination in conjunction with automated methods, especially in the diagnosis of different types of anemia for their appropriate treatment [9]. Paul Froom et al studied 39,759 cases and found that peripheral blood smear examination provided additional information in 13.9% of the cases [10]. Likewise the current study required a manual peripheral smear review in 12.25% of cases for proper morphological typing .In the current study amongst the 12.25% of cases that showed the non-concordant typing those cases typed as normocytic normochromic anemia with raised RDW showed the maximum non-concordance. 20 out of 67 cases typed as normocytic normochromic anemia with raised RDW were typed differently on peripheral smear; 17 of which were typed as dimorphic anemia. Japheth E Mukaya *et al*., conducted a study of 165 cases of anemia and found that microcytic hypochromic anemia (54%) is the most common morphological type of anemia followed by normocytic normochromic anemia (31%) [11].

Microcytic anemia associated with iron deficiency turned out to be the most prevalent micronutrient deficiency disease in the world affecting 2 billion people in the studies conducted by McClung JP *et al*., in 2006 and DeMaeyer E. M. in 1985 [12, 13]. In the current study also most of the cases were showing microcytic hypochromic anemia (184 cases out of 400) about 46% followed by normocytic normochromic anemia (156 cases out of 400) ie about 39%. In the current study microcytic hypochromic anemia was found to be the prevalent morphological type of anemia overall and in the younger age groups while in the older age group majority of the cases belonged to the morphological category of normocytic normochromic anemia. In the current study of 400 cases 234 were females and 166 were males. Amongst the females patients majority had a microcytic hypochromic pattern of anemia accounting to about 53% (124 cases out of 234) while in the male category 36 % of patients had a microcytic hypochromic pattern. The predominant pattern of anemia amongst the male patients was normocytic normochromic accounting to about 48% (80 cases out of 166) while in the female category 32.4% of patients had normocytic normochromic pattern. Thus among the females the commonest pattern was microcytic hypochromic and amongst the males the commonest pattern was normocytic normochromic.

CONCLUSION

The RBC indices and RDW obtained from the five-part analyzer Sysmex XT 1800i is valuable and accurate that it enabled in the correct morphological classification of anemia in the majority of cases. But peripheral blood smear examination, commonest and oldest method used to morphologically type anemia still holds an important role in the modern laboratory practice and remains the gold standard. It cannot be totally replaced by readings from an automated hematology analyzer as it can provide several relevant information that help in the proper diagnosis of a disease. More importantly in cases with a raised RDW a peripheral smear review is mandatory for reaching the correct diagnosis. The sensitivity and specificity of the five part analyzer sysmex XT1800i is calculated as 97% and 91% respectively for the detection of microcytes, 96.5% and 97.5% respectively for the detection of macrocytes and 91% and 90.5% respectively for the detection of normocytes. The sensitivity and specificity of the analyzer can be improved by proper calibration of the instrument which in turn increases the accuracy and also by running a good quality control program which in turn can increase the precision.

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