COMPARATIVE STUDY IN THE DIAGNOSIS AND MORPHOLOGICAL CLASSIFICATION OF ANAEMIA BY AUTOMATED HAEMATOLOGY ANALYZER WITH PERIPHERAL BLOOD SMEAR

Medical Science

Neena Kasliwal	Professor Department of Pathology J. L. N. Medical College, Ajmer, Rajasthan, India
Neha Gupta*	Senior Demonstrator Department of Pathology J. L. N. Medical College, Ajmer, Rajasthan, India - Corresponding author *
Geeta Pachori	Professor and Head Department of Pathology J. L. N. Medical College, Ajmer, Rajasthan, India
Alok Aggarwal	Senior ResidentDepartment of Orthopaedics J. L. N. Medical College, Ajmer, Rajasthan, India
Sonia Tanwar	Third Year Resident Department of Pathology J. L. N. Medical College, Ajmer, Rajasthan, India
Kalpana Sharma	Assistant Professor, Department of Pathology J. L. N. Medical College, Ajmer, Rajasthan, India

ABSTRACT

Haematology coulters are widely used in labs and hospitals in both diagnostic and treatment affairs.The aim of this study is to evaluate the performance of haematology coulter in the diagnosis and morphological classification of anaemia in comparison with the traditional manual method.Five hundred blood samples obtained from anaemic patients were subjected to analysis in both methods.Results showed a non significant (p>0.05) difference between classification of anaemia using values of red blood cells, haemoglobin, mean corpuscular haemoglobin, mean corpuscular volume and Red Cell Distribution width obtained from coulter compared to those of manual method.In conclusion, peripheral blood smear examination even today cannot be totally replaced by automated haematology analyzers but the present generation of automated haematology analyzers are well on par and provide accurate morphological typing of anaemia, thus reducing the workload and thereby increasing the efficiency of a laboratory.

INTRODUCTION

Anaemia is common medical problem associated with an increased mortality and morbidity risk and it reduces quality of life [1,2].

Globally, anaemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in pre-school-age children (47.4%), and the lowest prevalence is in men (12.7%). However, the population group with the greatest number of individuals affected is non-pregnant women (468.4 million). WHO regional estimates the greatest number affected are in South-East Asia where 315 million individuals in these three population groups are affected[3].

Anaemia is best defined in relation to haemoglobin and haematocrit levels below the normal reference range, because a patient's symptoms and physiologic consequences are the result of decreased oxygen-carrying capacity of the blood. According to World Health Organization (WHO) criteria[4], anaemia is diagnosed in males when Hb is <130 g/L (13 g/dL) and HCT is <0.39 (39%); in females, when Hb is <120 g/L (12 g/dL) and HCT is < 0.36 (36%).

Anaemia is one of the most frequent causes of medical visits because of the high incidence in children, young women and elderly people, especially if malnutrition is present. Anaemia is unusually prevalent in developing countries because of malnutrition, genetic, parasitic or infectious causes. Anaemia is a major health problem in India, especially among women and children.

Once anaemia is established, a morphological typing of anaemia helps the clinician to approach the cause of anaemia. Morphological typing of anaemia is based on RBC indices – MCV, MCH and PCV. The manual methods of measuring RBC indices are tedious and show large coefficient of variation. The automated haematology 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 haematology analyzers definitely have a potential to replace peripheral blood examination. The expertise needed to see the peripheral blood smear is definitely far more than that needed to run the analyzers. Moreover there is intra and inter observer variation in morphological typing of anaemia based on PBS examination[5]. 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 the accuracy, speed of analysis, and minimizes levels of human manipulation for test entry, sampling, sample dilution and analysis [6]. They are also more efficient and cost effective than the manual method.

The aim of this study is to diagnose and to do morphological typing of different types of anaemias, and correlating the results of automated analyzer with manual scan of PBS to find out if the rate of manual PBS review in cases of anaemia can be reduced.

MATERIAL AND METHODS

This prospective study was undertaken for a period of 6 months from January to June 2015 in the Department of Pathology, J.L.N. Medical College, Ajmer (Rajasthan). A total of 500 cases of anaemia were studied.3ml of venous blood samples were collected aseptically by standard phlebotomy technique into tri-potassium Ethylenediamine tetra-acetic acid (K3 EDTA) anticoagulant. The sample was well mixed (though not shaken) and aspirated into automated haematology analyzer within 4 hours of collection. Classification of anaemia was done by two methods – Firstly using Red Blood Cell indices with Red cell Distribution Width as provided by the Automated Haematology Analyser and secondly on PBS examination.

RESULTS

In this prospective study, demographic variables that include such information as total population were 500 anaemic individuals. Among these, males constituted 236 (32.3 %), while females were 264 (67.7 %).

Majority of the cases belonged to Microcytic Hypochromic Anaemia (49.2 %) followed by Normocytic Normochromic Anaemia (42.4%). Dimorphic anaemia formed 8.4% of the cases studied, with macrocytic anaemia accounting to only a small number of cases as expected.

The mean age of anaemia in males was found to be 33.9 years and 32.4 years in females. Maximum number of cases were seen in men in the age group of 31-40 years (18.1%) followed by 51-60 years (16.4%). Maximum number of cases were seen in women in the age group of 21-30 years (36.7%) followed by 31-40 years (26.5%).

 On screening of 500 individuals, the concordance between haematology analyzer and visual examination of PBS was seen in 462 cases (92.4%). The other 38 cases (7.6%) needed to be typed using PBS examination. Furthermore, both the results of automated analyzer and PBS revealed statistically non significant differences (p-value > 0.001).In present study, for the diagnosis of anaemia the quantitative and qualitative analysis is given in [Table 1].

In Microcytic Hypochromic Anaemia, majority of the cases were correctly typed using RBC indices alone. However, a small number of cases(06 cases) were wrongly typed as Normocytic Normochromic anaemia with raised RDW, all with MCV value < 82 fL which is borderline normal value and hence unreliable[Table 2].

In Normocytic Normochromic Anaemia typed using RBC indices alone, all the cases typed as Normocytic Normochromic Anaemia with normal RDW showed concordant typing using PBS examination.

However 18 cases typed as Normocytic Normochromic Anaemia on PBS examination were typed incorrectly with auto-analyser. Out of the these disconcordant cases, 05 were typed as Microcytic Hypochromic Anaemia with normal RDW, 10 as Microcytic Hypochromic Anaemia with raised RDW and remaining 3 as Macrocytic anaemia.

Out of above 15 cases typed as Microcytic Hypochromic Anaemia, evaluation of 14 cases showed an unreliable borderline value of MCV > 77.9 fL. One case typed as Microcytic Hypochromic Anaemia with raised RDW showed MCV of 73.9 fL. The sample received was checked and found to be haemolysed. Though all efforts were made to exclude all haemolysed samples from the study but this single case was included to highlight the changes in CBC that occur due to haemolysis, a commonly encountered problem in haematology laboratories.

Remaining 03 cases typed as Normocytic Normochromic Anaemia on PBS examination were typed incorrectly as Macrocytic Anaemia with auto-analyser. Of these, 01 case was neonate 10 days old and hence an explainable macrocytosis on analyser but normocytic picture on PBS examination. Rest 02 cases aged 47 years and 70 years were traced to have a history of Diabetes mellitus and hence a higher MCV value due to hyperglycemia.

Out of 12 cases typed as dimorphic anaemia on PBS examination, 07 were evaluated as Normocytic Normochromic Anaemia with raised RDW and 05 as Macrocytic Anaemia. 08 cases gave clinical details of blood transfusion and 04 cases gave history of iron therapy with blood transfusion.

Two cases typed as Macrocytic anaemia on smear examination showed MCV value of > 99 fL which again is high normal value of the index and hence unreliable.

It can be finally concluded that in fresh cases of Microcytic Hypochromic Anaemia, Normocytic normochromic anaemia with normal RDW and Macrocytic Anaemia, morphological typing of anaemia using RBC indices alone was accurate and showed high sensitivity and specificity.

However in Normocytic Normochromic anaemia with raised RDW showed an indication for PBS examination as it provided addition information in morphological typing of anaemia which could further help in identifying the cause and treatment of anaemia.

By Chi square test, value of x^2 is 0.144 and p value >0.05.Since the value of x^2 is <3.84 which is not significant, hence it is proved that there is no significant difference in the result given by automated haematology analyser and PBS.

Morphological typing of Microcytic hypochromic anaemia with RBC indices showed a high specificity **97.5**% and slightly lower sensitivity of **94.1**%.Using PBS examination will increase the sensitivity in typing microcytic hypochromic anaemia by 5.9% in identifying cases with polychromasia having a normal MCV, which can be mistyped as Normocytic normochromic anaemia if typed using RBC indices alone.

Morphological typing of Normocytic normochromic anaemia with RBC indices showed a high specificity (**94.9**%) but slightly lower sensitivity of **91.5**%. Using PBS examination will increase the specificity in reducing typing of other morphological types of anaemia as Normocytic normochromic anaemia by 5.1% by identifying cases with population of RBCs having high degree of anisocytosis but a normal MCV.

Morphological typing of Macrocytic anaemia with RBC indices alone showed a high specificity of **98.2**% and sensitivity of **93.3**%.

DISCUSSION

The present study assessed the clinical usefulness of manual scan of PBS for the diagnosis of different types of anaemia with morphological typing of anaemia which provides valuable information and aids the clinician to further investigate the patient to identify the cause of anaemia so that it can be appropriately treated. Anaemia is defined as decrease number of red blood cells or less than the normal quantity of hemoglobin in the blood. Anaemia is readily diagnosed on a complete blood count. Apart from reporting the number of red blood cells and the hemoglobin level, the haematology analyzer measure the size of the red blood cells. Examination of a stained blood smear using a microscope can also be helpful, and it is sometimes a necessity in regions of the world where automated analysis is less accessible. Thus, Anaemia is diagnosed by the results of both haematology analyzer and PBS.

Paul Froom et al studied 39,759 cases and found that PBS examination provided additional information in 13.9% of the cases[7]. The present study showed lower value of 7.6% of cases having additional information needing by PBS examination. This variation could be explained by difference in the geographic area of the study and the size of the population studied.

According to Key Results from the National Family Health Survey, 2005 – 06, over half of urban poor women in Rajasthan are anaemic. High prevalence of anaemia contributes to high infant and maternal mortality, premature births and low birth weight babies[8].

On comparing the most common type of anaemia in women in Rajasthan as updated in WHO census, 53% of women with anaemia belonged to reproductive age group while in our study, 37% of the cases belonged to this age group. Consideration should be taken to the fact that our study included mostly the urban population reducing the incidence of anaemia.

In a study by Japheth E Mukaya et al, out of his 165 cases of anaemia, 59.2% were female which was similar to the findings in our study where 52.6% of the cases were females[9]. This indicates that anaemia is still more prevalent in women in India and other developing countries.

In the same study by Japheth E Mukaya et al, out of 165 cases of anaemia, majority of the cases of anaemia were seen in women less than 40 years of age forming 58% of the total cases with maximum cases in age group 31-40 year[9]. Similar findings were seen in our study where women less than 40 years constituted 37.2% of the cases with maximum number of cases in under 30 years age group and the incidence decreased as the age increased.

Japheth E Mukaya et al also concluded that out of 165 cases of anaemia, most common morphological type of anaemia was Microcytic Hypochromic anaemia (54%) followed by Normocytic Normochromic anaemia (31%)[9]. Similar statistics were seen in our study with Microcytic anaemia forming the most common type(49.8%) followed by Normocytic Normochromic anaemia(36.5%). The statistics show the prevalence of iron deficiency anaemia in India.

Mishra et al in his study of 579 cases of anaemia in women of reproductive age group found that majority of his cases belonged to the age group of 15-30 years ie 63.9%[10]. In our study too, majority of the cases of anaemia in women of reproductive age group belonged to 15-30 years ie 53.7%. This similarity may be due to possible relationship of this age group with increased nutritional needs and parity.

In current study, we screened 500 anaemic subjects of which 462 subjects correlated positively when both automated and manual methods were compared. This manifests that the automated analyzer readings correlated well with the manual methods.The benefit of PBS scan is the ability to identify clinically significant red blood cell morphological types that are not quantifiable by instruments, but that produce flags on the automated results[12, 13, 14]. These findings are very important in the diagnosis of various blood disorders that are not evaluated by automation. However, the discrepancy between automation and manual scan of peripheral blood in the measurement of hemoglobin and red blood count may result in misclassification for the diagnosis of anaemia. This signified that the manual microscopic method still has some advantages over the automated method[14,15,16]. The results of the present study are in concordance with an earlier report by Pierre and Novis et al.[17,18] who reported that automated haematology analyzer are more accurate in the detection of specimens with morphological abnormality than the traditional eye count method.

CONCLUSION

PBS examination even today cannot be totally replaced by automated haematology 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 generation of automated haematology analyzers are well on par and provide accurate morphological typing of anaemia in cases of Microcytic Hypochromic Anaemia, Macrocytic Anaemia and Normocytic Normochromic Anaemia with normal RDW thus reducing the workload and thereby increasing the efficiency of a laboratory. However in cases of Normocytic Normochromic Anaemia with raised RDW, PBS examination provides valuable information and is absolutely necessary for morphological typing of anaemia.

Table 1

Morphological Typing Of Anaemia using RBC indices & RDW (Number of Cases)	Morphological Typing Of Anaemia using PBS examination (Number of Cases)
Microcytic Hypochromic Anaemia with normal RDW(20)	Microcytic Hypochromic Anaemia(246)
Microcytic Hypochromic Anaemia with raised RDW(220)	
Normocytic Normochromic Anaemia with raised RDW (06)	
Normocytic Normochromic Anaemia with raised RDW (07)	Dimorphic Anaemia(12)
Macrocytic Anaemia(05)	
Normocytic Normochromic Anaemia with normal RDW(90)	Normocytic Normochromic Anaemia(212)
Normocytic Normochromic Anaemia with raised RDW(104)	
Microcytic Hypochromic Anaemia with normal RDW(05)	
Microcytic Hypochromic Anaemia with raised RDW(10)	
Macrocytic Anaemia(03)	
Macrocytic Anaemia(36)	Macrocytic Anaemia(30)
Normocytic Normochromic Anaemia with raised RDW(02)	
Total - 500	Total – 500

Table 2

	RBC indices with RDW (auto analyser)			PBS with RBC indices
	with normal RDW	with raised RDW	Total	
Microcytic Hypochromic Anaemia	25	230	255	246
Normocytic Normochromic Anaemia	90	119	209	212
Macrocytic Anaemia	36			30
Dimorphic Anaemia	-			12
Total	500			500

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