



## RESEARCH ARTICLE

### IS PERIPHERAL BLOOD SMEAR EXAMINATION AN OBSOLETE ART

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#### ABSTRACT

**AIMS** - Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population. Once anemia is established, a morphological typing of anemia helps the clinician to approach the cause of anemia. The automated hematology analyzers give accurate and <1% coefficient of variation for the RBC indices and hence have replaced the manual methods. The expertise needed to see the peripheral blood smear is definitely far more than that needed to run the analyzers. The additional peripheral blood smear review performed by hematologist hardly ever provided unique information and provided incremental helpful information in only 4% of the cases. A total of 600 cases of anemia were studied over a period of two years. Anemia typing was done by using RBC indices with RDW and then with additional **peripheral blood smears** examination followed by correlation of results. Morphological typing of anemia in cases of Microcytic hypochromic anemia, Normocytic Normochromic anemia with normal RDW and Macrocytic anemia using RBC indices and RDW from XT-2000i, an automated hematology analyzer showed very high specificity and high sensitivity. However cases with raised RDW were wrongly typed on indices alone and had a low sensitivity and specificity indicating additional peripheral blood smear examination was essential in such cases.

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#### INTRODUCTION

Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in women in reproductive age group (42%) and the lowest prevalence is in men (12.7%). The population group with the greatest number of individuals affected is non-pregnant women (468.4 million) (De Benoist *et al.*, 2008).

World Health Organization (WHO) defines the lower limit of normal for hemoglobin concentration at sea level to be 12.0 g/dl in women and 13.0 g/dl in men.

One third of the world population suffers from anemia. India is one of the countries with very high prevalence rates. NFHS-3 (National Family Health Survey) reveals the prevalence of anemia to be 70-80% in children, 70% in pregnant women and 24% in adult men.

In 1930, Wintrobe conducted a study on more than 1,000 persons and tabulated his finding according to morphology into four groups; Macrocytic, Normocytic, simple Microcytic and hypochromic Microcytic. He further went on to describe the types of cases, their clinical diagnosis and gave a

morphological classification of anemia, which is still being used with little modification (Wintrobe, 1934). From the therapeutic point of view, it is not useful to know the cause of anemia but it is important to differentiate the anemia in accordance with the type of disorder in the hematopoietic system. Once anemia is established, a morphological typing of anemia helps the clinician to approach the cause of anemia. Morphological typing of anemia 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 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 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 anemia based on peripheral blood smear examination. The additional peripheral blood smear review performed by hematologist hardly ever provided unique

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information and provided incremental helpful information in only 4% of the cases (Jen P et al, 1983).

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 (Bourner et al, 2005). They are also more efficient and cost effective 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 (Chapman M et al, 2000).

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 leukocyte differential as the initial screening and detection system for hematological abnormalities in modern hospitals and clinics (Lantis KL et al, 2003).

The traditional review of all automated hematology instrument results by preparation, staining and microscopic examination of a blood film examination has disappeared in most institutions (Pierre RV, 2002). The reasons are the more accurate detection of specimens with distributional or morphological abnormalities than by the traditional eye count method (Novis DS et al, 2006).

The present study is being carried out to find the correlation of morphological typing of anemia based on RBC indices obtained from XT-2000i, an automated analyzer with peripheral blood smear examination and to find out if the rate of manual peripheral blood smear review in cases of anemia already morphologically typed on automated analyzer can be reduced.

### Aims and Objectives

To correlate morphological typing of anemia based on RBC indices (MCV, MCH and MCHC) and RDW with morphological typing of anemia based on peripheral blood smear examination.

## MATERIALS AND METHODS

This study was undertaken at the Department of Pathology, Command Hospital Air Force, Bangalore and a total of 600 cases of anemia including both in and out patients were studied over a period of two years between Oct 2010 to Sep 2012. Anemia typing was done by two methods – Firstly using RBC indices with RDW only obtained by Sysmex XT-2000i an automated hematology analyzer. Secondly with indices and peripheral blood smear examination.

### Inclusion Criteria

- All patients with anemia as per WHO reference values were included in the study.

The study is approved by Ethical Committee Command Hospital (Air Force) Bangalore.

## METHOD

The venous blood sample was collected by venepuncture in EDTA vacutainers following standard protocol. The sample was aspirated into Sysmex XT-2000i, automated 5 – part differential hematology analyzer which analyses the sample and provides the complete blood count, RBC indices, RDW, retic count, PDW, scattergram and distribution curves. Using the above data, morphological typing of anemia was done 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 and Macrocytic Anemia. Microcytic, Macrocytic and Normocytic was decided by the MCV values and hypochromic and normochromic was given based on the MCH values. Normal or raised RDW was mentioned to indicate the degree of anisocytosis. The reference values were taken from Wintrobe's Clinical hematology – 12<sup>th</sup> Edition

Peripheral blood smear was prepared by finger prick method using standard precautions and smear was stained immediately following drying with Leishman stain. Peripheral smear was the examined and morphological typing of anemia was done as Microcytic Hypochromic Anemia, Normocytic Normochromic Anemia, Macrocytic Anemia and Dimorphic anemia.

### Analysis

Statistical analysis was done using Chi Square test and probability was calculated. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value was determined for the two methods.

## RESULTS

### Morphological typing of anemia based on RBC indices and RDW

Out of the 600 cases on which morphological typing of anemia was done using RBC indices and RDW, majority of the as case belonged to Microcytic Hypochromic Anemia of raised RDW(43.8%) followed by Normocytic Normochromic Anemia with normal RDW(36.5%).

**Table 1** Morphological typing of anemia based on RBC indices and RDW

| Morphological type of anemia                   | Male | Female | Total |
|--|------|--------|-------|
| Microcytic Hypochromic Anemia with normal RDW  | 06   | 19     | 25    |
| Microcytic Hypochromic Anemia with raised RDW  | 31   | 232    | 263   |
| Normocytic Normochromic Anemia with normal RDW | 131  | 88     | 219   |
| Normocytic Normochromic anemia with raised RDW | 28   | 40     | 68    |
| Macrocytic Anemia                              | 23   | 02     | 25    |
| Total  | 381  | 219    | 600   |

### Morphological typing of anemia based on peripheral blood smear examination

Out of the 600 cases on which morphological typing of anemia was done using peripheral blood smear examination, majority

of the case belonged to Microcytic Hypochromic Anemia(49.8%) followed by Normocytic Normochromic Anemia(36.5%).

**Table 2** Morphological typing of anemia based on peripheral blood smear examination.

| Morphological Type Of Anemia   | Male | Female | Total |
|--------------------------------|------|--------|-------|
| Microcytic Hypochromic Anemia  | 42   | 257    | 299   |
| Normocytic Normochromic Anemia | 131  | 88     | 219   |
| Macrocytic Anemia              | 23   | 02     | 25    |
| Dimorphic Anemia               | 23   | 34     | 57    |
| Total                          | 219  | 381    | 600   |

### Correlation between morphological typing of anemia using RBC indices and RDW with morphological typing of anemia using peripheral blood smear examination

**Table 3** Correlation between morphological typing of anemia using RBC indices and RDW with morphological typing of anemia using peripheral blood smear examination.

| Morphological Typing Of Anemia using RBC indices & RDW (Number of Cases) | Morphological Typing Of Anemia using PBS examination (Number of Cases) |
|--|--|
| Microcytic Hypochromic Anemia with normal RDW (25)                       | Microcytic Hypochromic Anemia (299)                                    |
| Microcytic Hypochromic Anemia with raised RDW (263)                      |  |
| Normocytic Normochromic Anemia with raised RDW (11)                      | Dimorphic Anemia (57)  |
| Normocytic Normochromic Anemia with raised RDW (57)                      |  |
| Normocytic Normochromic Anemia with normal RDW (219)                     | Normocytic Normochromic Anemia (219)                                   |
| Macrocytic Anemia (25)   | Macrocytic Anemia (25)   |
| Total – 600  | Total – 600  |

### Cases with concordant and non-concordant results.

**Table 4** Cases with concordant and non-concordant results.

| Concordant Typing | Non-concordant Typing | Total |
|-------------------|-----------------------|-------|
| 532               | 68                    | 600   |

**Table 5** Comparison of need for peripheral blood smear review rate based on MCV and leucocyte differential count:-

|   | Present Study | Paul Froom et al (2009) | Novis et al (2006) |
|---|---------------|-------------------------|--------------------|
| Total number of cases                                 | 600           | 39,759                  | 95,141             |
| Number of cases needing peripheral blood smear review | 69(11.5%)     | 5526 (13.9%)            | 6147(6.4%)         |

**Table 6** Comparison of need for peripheral blood smear review rate based on leucocyte differential count.

|   | Present Study | Paul Froom et al (2009) | Novis et al (2006) | Lantis et al (2003) | Samuel O Ike (2010) |
|---|---------------|-------------------------|--------------------|---------------------|---------------------|
| Total number of cases                                 | 600           | 39,759                  | 95,141             | 204                 | 60                  |
| Number of cases needing peripheral blood smear review | 11(1.8%)      | 1194 (3.0%)             | 9276(9.7%)         | 15(7.4%)            | 4(6.6%)             |

Out of 600 cases, 88% (532) of the cases showed concordant typing of anemia with indices obtained by hematology analyzer and peripheral blood smear examination. Only ~ 11%(68) cases showed discordant typing, which needed peripheral blood smear examination for typing of anemia correctly. Out of the 532 cases, with concordant typing using RBC indices alone and with peripheral blood smear examination, majority of the cases

were Microcytic hypochromic anemia (49.1%) followed by Normocytic normochromic anemia(46.5%).

However on examination of peripheral blood smear examination it was noted that the smear showed RBCs showing Microcytic Hypochromic Anemia with Polychromasia. The polychromatic cells (reticulocytes) being larger are responsible for increasing the MCV to fall within normal levels in such cases. 11 cases were wrongly typed as Normocytic Normochromic Anemia with raised RDW on RBC indices alone. However on examination of peripheral blood smear examination it was noted that the smear showed RBCs showing Microcytic Hypochromic Anemia with Polychromasia which on peripheral blood smear examination showed presence of mixed red cell population and typed as Dimorphic Anemia.

By Chi square test, value of p was calculated as < 0.001. Use of peripheral smear examination was positively associated and shows high significance with p = < 0.001.

## DISCUSSION

The first step in investigating anemia is morphological typing of anemia which provides valuable information and aids the clinician/hematologist to further investigate the patient to identify the cause of anemia so that it can be appropriately treated.

Mishra et al(2012) in his study of 579 cases of anemia in women of reproductive age group found that majority of his cases belonged to the age group of 15-30 years ie 63.9%. Japheth E Mukaya et al(2009), out of his 165 cases of anemia had majority of the cases of anemia seen in women less than 40 years of age forming 58% of the total cases. In our study too majority of the cases of anemia 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. Japheth E Mukaya et al,(2009) in his study of 165 cases of anemia had Microcytic Hypochromic anemia (54%) as the most common morphological type of anemia followed by Normocytic normochromic anemia(31%). Similar statistics were seen in our study with Microcytic anemia forming the most common type(49.8%) followed by Normocytic Normochromic anemia(36.5%). The statistics show the prevalence of iron deficiency anemia in India. Paul Froom et al(2009) studied 39,759 cases and found that peripheral blood smear examination provided additional information in 13.9% of the cases.

Novis et al(2006) studied 95,141 cases and concluded that in 6.4% of cases, peripheral blood smear examination provided additional information. The present study also showed similar results with 11.5% of cases having additional information provided by peripheral blood smear examination.

Paul Froom *et al*(2009) studied 39,759 cases and found that peripheral blood smear examination provided additional information in leucocyte differential count correction in 3% of cases. However our study showed only 1.8% cases showing erroneous leucocyte differential count.

The lower rate can be attributed to the selection bias of only anemia cases. The cause for the erroneous leucocyte differential count was found to be similar in the present study and in the study by Paul Froom *et al*(2009) where majority of erroneous readings were due to incomplete differential count by the analyzer (differential count not adding to 100%). It was primarily due to inability of the analyzer to type certain types of abnormal cells. Similar findings were seen in studies by Novis *et al* (2006), Lantis *et al*(2003) and Samuel O Ike *et al*(2010).

Reasons for erroneous low platelet counts was due to abnormal distribution of platelet, forming micro-clots which are not visible on macroscopic examination. Common erroneous high platelet count was seen in case of hemolysis which causes RBC fragments to be mistaken for platelets by the automated hematology analyzer.

## CONCLUSION

Morphological typing of anemia in cases of Microcytic hypochromic anemia with normal and raised RDW and Macrocytic anemia using RBC indices and RDW from XT-2000i, an automated hematology analyzer shows very high specificity(100%) and high sensitivity(>96%). In cases of Normocytic normochromic anemia, the cases with normal RDW showed high specificity and sensitivity(100%) but the cases with raised RDW were wrongly typed on indices alone and had a low sensitivity and specificity indicating additional peripheral blood smear examination as an absolutely necessary tool in morphological typing of anemia.

Leucocyte differential count of XT 2000i, automated hematology analyzer was accurate in most cases(95.6%) with complete differential counts without flags but however in cases where the differential count is incomplete due to cells not identifiable by the analyzer or in cases where the differential count showed flags indicating inaccuracy of differential count, a manual differential count is a must as it not only provides an accurate differential count but also provides critical information regarding the diagnosis of the patient, thus helping in treatment.

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