|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
NLM (National Library of Medicine ID: 101738825)
Index Copernicus Value 2019: 79.34
Volume 5, Issue 4; April: 2021; Page No. 08-11

Original Research Article

A STUDY TO ACCESS THE CORRELATION OF MORPHOLOGICAL TYPING OF ANEMIA USING RDW WITH RBC INDICES AND PERIPHERAL BLOOD SMEAR

Lalit Aseri[1], Vanita Kumar[2]
[1] Postgraduate Resident, [2] Senior Professor & Head,
[1,2] Department of Pathology, Sardar Patel Medical College and Associated Group of Hospitals, Bikaner, Rajasthan, India.

Article Info: Received 06 February 2021; Accepted 3 April 2021
DOI: https://doi.org/10.32553/ijmbs.v5i4.1857
Corresponding author: Dr. Lalit Aseri
Conflict of interest: No conflict of interest.

Abstract

Background: Anemia as a disease entity was well known to ancient Greek and Indian physicians. Anemia affects an estimated 2.36 billion individuals globally, especially women and children. Clinically useful approach classifies anemia according to alterations in red cell morphology. 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.

Methods: This study is a two years prospective study conducted between Jan 2019 to Dec 2020 at the Department of Pathology, Sardar Patel Medical College and Associated Group of Hospitals, Bikaner. All the patients with the clinical suspicion of anemia who attended PBM and associated group of hospitals during study period, were included and investigated. The RBC indices and RDW was done using a 5 part automated hematology analyser and PBF was prepared, stained with leishmann's stain and reported for morphological typing of Anemia.

Results: We received a total of 542 cases with clinical features of anemia during the study period. Microcytic hypochromic anemia was the most common type of anemia and females were affected more than males. Overall 83.21% of the cases showed concordant typing of anemia with RBC indices alone and 16.79% cases showed discordant typing, which need to be typed correctly with peripheral blood smear examination. In 91 cases of non concordant typing of anemia, most of the cases were typed as Normocytic normochromic anemia with raised RDW with RBC indices alone.

Conclusions: In our study the findings can be summarized in one line – Peripheral blood smear examination even today cannot be totally replaced by automated hematology analysers as they provide so much additional information which cannot be summarized completely by the mere numerical calculations of an automated analyser

Keywords: Anemia, RBC Indices, RDW, Peripheral Blood Smear

Introduction

Anemia as a disease entity was well known to ancient Greek and Indian physicians. The word Anemia is derived from Greek word 'Annaime' (an - not, naime - blood) meaning lack of blood which is decrease in normal number of RBCs or less than normal quantity of hemoglobin in blood.[1,2] Its prevalence is inordinately higher among developing nations, because of low socioeconomic status and indigent access to healthcare services.[3] Anemia affects an estimated 2.36 billion individuals globally, especially women and children.[4] The prevalence of anemia is estimated to be higher in India when compared to all other developing countries.[5] Also, it is the second leading cause of maternal deaths in the country.[6]

Clinically useful approach classifies anemia according to alterations in red cell morphology. Morphologic characteristic providing etiologic clues include red cell size (normocytic, microcytic, or macrocytic) degree of hemoglobinization, reflected in the color of red cells (normochromic or hypochromic), and shape. In general, microcytic hypochromic anemias are caused by disorders of haemoglobin synthesis (most often iron deficiency), while macrocytic anemias often stem from abnormalities that impair the maturation of erythroid precursors in the bone marrow. Normochromic, normocytic anemias have diverse etiologies in some of these anemias, specific abnormalities of red cell shape provide an important clue as to the cause.[7] 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.[8]

Methods

This study is a two years prospective study conducted between Jan 2019 to Dec 2020 at the Department of Pathology, Sardar Patel Medical College and Associated Group of Hospitals, Bikaner. All the patients with the clinical suspicion of anemia who attended PBM and associated group of hospitals during study period, were included and investigated. Anemia typing was done by two methods – Firstly using RBC indices with RDW. Secondly on peripheral blood smear examination. The venous blood samples were collected by venepuncture in vaccutainer containing EDTA anticoagulant. The sample is first aspirated into automated 5 – part differential hematology analyser and a peripheral blood smear was prepared as per protocol and stained with Leishmann's stain and examined under light microscope. The reference values are taken from Wintrobe's Clinical Hematology.

Morphological typing of anemia was done using RBC indices with RDW from automated haematology analyser alone and then verified with the typing done by peripheral blood smear examination. The results were considered concordant if typing done by both methods indicated the same morphological type of anemia, otherwise results were considered discordant. Statistical analysis was done using Chi Square test and probability was calculated. Sensitivity is defined as percentage of cases in which a particular morphological type of anemia was done correctly by the method. Specificity is defined as percentage of cases in which the other morphological types were excluded correctly by the method. The values of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) were calculated and analysed.

Results

We received a total of 542 cases with clinical features of anemia that were referred to our department during the study period and were investigated and data analysed. As shown in Image 1, out of the 542 cases, microcytic hypochromic anemia was the most common type of anemia with 350 cases (64.58%) followed by normocytic normochromic anemia (27.31%). We found that anemia is more common in females with 343 cases and 199 cases were males with mean age of anemia predominance in males was found to be 29.48 years and female mean age was found to be 27.33 years. In females, Microcytic hypochromic anemia was the most common morphological type of anemia as expected accounting for 80.17% of the cases. In males, Normocytic hypochromic anemia was the most common morphological type of anemia accounting for 50.25% of the cases. The age and gender distribution of anemia in our study is shown in Image no. 2 and gender distribution of morphological types of anemia in Image No. 3.

Morphological typing of anemia, was done using RBC indices and RDW, and using peripheral blood smear examination, and the results are summarized in Table No. 1. All cases typed as Microcytic Hypochromic Anemia (288), Macrocytic Anemia (15), Normocytic Normochromic Anemia with normal RDW (148), on RBC indices with RDW alone were confirmed by peripheral blood smear examination as having similar diagnosis. However all cases typed as Normocytic Normochromic Anemia with raised RDW (91) on RBC indices with RDW alone were typed differently on peripheral blood smear examination. Out of the 91 cases, 22 cases were typed as Dimorphic Anemia on PBS examination and the rest 69 cases were typed as Microcytic Hypochromic Anemia with Polychromasia on PBS examination. Overall 83.21% (451) of the cases showed concordant typing of anemia with RBC indices alone and 16.79% (91) cases showed discordant typing, which need to be typed correctly with peripheral blood smear examination.

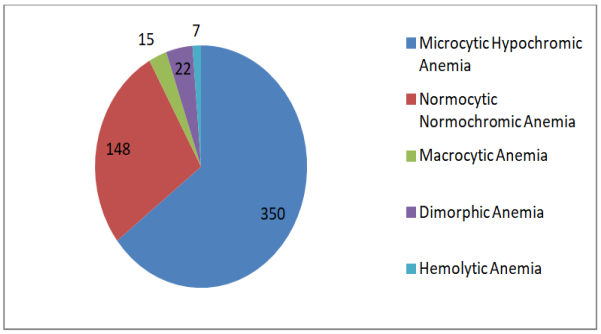

Image 1: Morphological type distribution of the cases of anemia

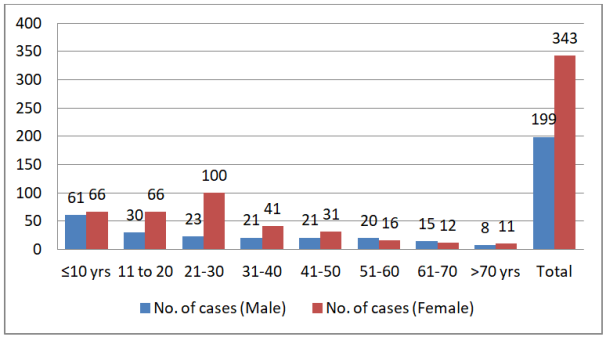

Image 2: Age and gender wise distribution of cases of anemia in the present study.

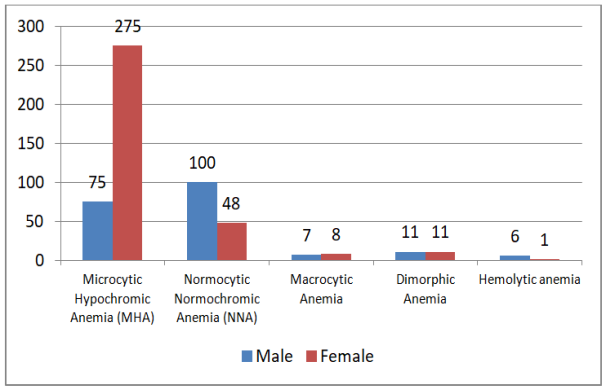

Image 3: Gender distribution of different types of anemia

Table 1: 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 (N = 288)	Microcytic Hypochromic Anemia (N = 350)
Normocytic Normochromic Anemia with raised RDW (N =91)	Dimorphic Anemia (N = 22)
Normocytic Normochromic Anemia with normal RDW (N =148)	Normocytic Normochromic Anemia (N = 148)
Macrocytic Anemia (N = 15)	Macrocytic Anemia (N = 15)
	Hemolytic Anemia (N = 7)
Total – 542	Total – 542

Distribution of cases with concordant and discordant typing

Out of the 451 cases with concordant typing, majority of the cases were Microcytic hypochromic anemia (63.85 %) followed by Normocytic normochromic anemia (32.82 %). The result is shown in table no. 2. In 91 cases of non-concordant typing of anemia, 69 cases were typed as Normocytic normochromic anemia with raised RDW with 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. The polychromatic cells (reticulocytes) being larger are responsible for increasing the MCV to fall within normal levels in such cases. 22 cases were typed as Normocytic Normochromic Anemia with raised RDW on RBC indices alone which on peripheral blood smear examination showed presence of mixed red cell population with Microcytic hypochromic cells and Macrocytic cells and hence having a normal MCV and raised RDW and hence typed correctly on peripheral blood smear examination as Dimorphic Anemia. The results are shown in Table No. 3.

Table 2: Distribution of cases with concordant typing

Morphological type of Anemia	No. of cases (Male)	No. of cases (Female)	Total
Microcytic hypochromic Anemia	60 (35.93%)	228 (80.28%)	288(63.85%)
Normocytic normochromic anemia	100 (59.88%)	48 (16.90%)	148(32.82%)
Macrocytic anemia	7 (4.19%)	8 (2.82%)	15 (3.33%)
Total	167 (100%)	284 (100%)	451 (100%)

Table 3: Cases with discordant typing

S. No.	No. of Cases		Typing with RBC indices & RDW	Typing with PBS
	Males	Females		
1	21	48	Normocytic normochromic anemia with raised RDW	Microcytic hypochromic anemia with polychromasia
3	11	11	Normocytic normochromic anemia with raised RDW	Dimorphic anemia

The statistical analysis was done with the data and summarised for Microcytic hypochromic, normocytic normochromic and macrocytic anemia, in table no. 4. The data in our study show that there is low specificity and positive predictive values for normocytic normochromic anemia. Using peripheral blood smear examination will increase the specificity in reducing typing of other morphological types of anemia as Normocytic normochromic anemia by 38.58% by identifying cases with population of RBCs having high degree of anisocytosis but a normal MCV.

Table 4: Statistical analysis of data.

	Microcytic Hypochromic Anemia	Normocytic Normochromic Anemia	Macrocytic Anemia
True positive	288	148	15
True negative	192	303	527
False positive	0	91	0
False negative	62	0	0
Sensitivity	82.29%	100%	100%
Specificity	100%	61.42%	100%
Positive predictive value	100%	61.92%	100%
Negative predictive value	75.59%	100%	100%

Discussion

Anemia is a global problem affecting the population in both developing as well as developed countries, and there is a debate on which haemoglobin level limits should be used to define anemia in general population. About one-third of the global population is anemia.[9]

Since decades, peripheral smears have been used as a major diagnostic tool for workup of anemia. The advent of automated haematology cell counter has improved accuracy and precision and has reduced subjective errors. Microscopic examination still is required for primary calibration, for the presumptive diagnosis of anemia, leukaemia and other related disorders.[10] The present study was done to correlate peripheral smear findings with red cell indices obtained from automated hematology analyzer.

In this study it was found that there were 63.28% female and 36.72% male among all studied subjects. The overall mean age was found to be 28.12 years while for the male population it was 29.48 years and for female population it was 27.33 years. Maximum proportion of the male and female population was from <10 years and 21-30 years respectively. Overall maximum population was from <10 years (23.43%) and from 21-30 years (22.69%) age group. We see that majority of the female patients were from reproductive age group mainly because of the inadequate iron intake, chronic blood loss, malabsorption, infections and other factors like changes in lifestyle, dietary pattern, behavioural and social changes.[10,11] In our study, we observed an increased prevalence of anemia in females (63.28%). These findings are in agreement with the findings of Swaroop (2013),[12] Singhla et al. (2016)[10] and Garg et al. (2019).[13] This indicates that anemia is more prevalent in females rather than male in India.

The most common morphological type was microcytic hypochromic anemia (MHA) followed by normocytic normochromic anemia (NNA). The most common cause of microcytic hypochromic anemia is decreased iron reserve of the body, which may be due to multiple reasons like decreased iron in the diet, poor absorption of iron from gut, acute and chronic blood loss, increased demand of iron in certain situations like pregnancy or recovery from major trauma or surgery.[13] These findings regarding distribution of anemia cases are in concordance with other studies.[10,13] In

the male population NNA was more prevalent while in the female population MHA was more prevalent. These findings were evident from the reports of Automatic hematology analyzer and Peripheral blood smear examination both.

Regarding correlation between morphological typing of anemia using RBC indices and RDW with morphological typing of anemia using peripheral blood smear examination, it was found that out of 542 cases, 83.21% of the cases showed concordant typing of anemia with RBC indices alone and using peripheral blood smear examination. Only 16.79% cases (N = 91) showed discordant typing, which need to be typed correctly with peripheral blood smear examination. These findings are in agreement with the findings with the findings of Swaroop (2013).[12] Out of these 91 discordant cases, 69 cases were typed as Normocytic normochromic anemia with raised RDW with 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. The polychromatic cells (reticulocytes) being larger are responsible for increasing the MCV to fall within normal levels in such cases. 22 cases were typed as Normocytic Normochromic Anemia with raised RDW on RBC indices alone which on peripheral blood smear examination showed presence of mixed red cell population with Microcytic hypochromic cells and Normocytic normochromic cells and hence having a normal MCV and raised RDW and hence typed correctly on peripheral blood smear examination as Dimorphic Anemia. In the chi-square test use of PBS was found to be positively associated and showed high significance with $p = < 0.001$.

Conclusion

In our study the findings can be summarized in one line – Peripheral blood smear 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 analyser. The present generation of automated hematology analyzers are well on par and provide accurate morphological typing of anemia in cases of Microcytic Hypochromic Anemia, Macrocytic Anemia and Normocytic Normochromic Anemia with normal RDW thus reducing the workload and thereby increasing the efficiency of a laboratory. However in cases on Normocytic Normochromic Anemia with raised RDW, peripheral blood smear examination provides valuable information and is absolutely necessary for morphological typing of anemia.

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