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Original Article

Correlation between Morphological Typing of Anemia Based On RBC Indices and RDW Obtained from Sysmex KX-21, an Automated Hematological Analyser with Peripheral Blood Smear Examination

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ABSTRACT

INTRODUCTION - Anemia affects millions of people globally. Once anemia is diagnosed clinically, a morphological typing of anemia pathologically helps the treating physician to come up to the cause of anemia. In the new era of laboratory diagnostics, the automated hematology analyzers give accurate results for the RBC indices and hence have replaced the manual methods in the majority. The expertise needed to see the peripheral blood smear is unquestionably far more than that needed to run the analyzers. Moreover, in morphological typing of anemia based on peripheral blood smear examination, there is intra and inter observer variation. Further peripheral blood smear review performed by pathologists hardly ever provide unique information and provide incremental helpful information in few of the cases. Hence, the aim of the present study was to correlate morphological typing of anemia based on RBC indices and RDW obtained from KX-21, an 3 –part automated hematology analyzer with peripheral blood smear evaluation and to discover if the rate of manual peripheral blood smear review in cases of anemia already morphologically typed on automated analyzer can be reduced.

METHODS - A total of 1100 cases of anemia were studied over a period of five years between January 2017 to December 2021 at Central Diagnostic Laboratory, Dhiraj hospital, Department of Pathology, Smt. B. K. Shah Medical Institute & Research Centre, Piparia, Vadodara. Anemia typing was done by two methods – to start with using RBC indices & RDW only. Subsequently on peripheral blood smear examination. The results were then correlated and analyzed.

RESULTS - All the cases were typed as Microcytic Hypochromic Anemia with normal and raised RDW, Macrocytic Anemia and Normocytic Normochromic Anemia with normal RDW (91.82 %) on RBC indices with RDW alone. Then all these were confirmed as the same by peripheral blood smear examination. However all cases typed as Normocytic Normochromic Anemia with raised RDW (8.18 %) on RBC indices with RDW alone were typed as either Dimorphic Anemia or Microcytic Hypochromic Anemia with polychromasia on subsequent peripheral blood smear examination.

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 3 part automated hematology analyzer shows very high specificity and high sensitivity. In cases of Normocytic normochromic anemia, the cases with normal RDW showed high specificity and sensitivity but the cases with high RDW were wrongly typed on indices alone and had a low sensitivity and specificity signifying that additional peripheral blood smear examination as an extremely essential tool in morphological typing of anemia.

Key Words: *Red cell Distribution Width, KX-21, 3 part automated hematology analyzer; Peripheral blood smear examination, Morphological Typing of anemia, RBC Indices*

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INTRODUCTION

Anemia affects millions of people globally. Once anemia is diagnosed clinically, a morphological typing of anemia pathologically helps the treating physician to come up to the cause of anemia. Morphological typing of anemia is based on Laboratory parameters are from RBC indices – MCH, MCV and PCV. The manual methods of measuring RBC indices are tedious, slow, time consuming and have human errors. The automated hematology analyzers give more accurate validation for the RBC indices and hence have replaced the manual methods. Thus we can definitely conclude that the hematology analyzers have a potential and possible role to replace peripheral blood smear examination.

The expertise needed to examine the peripheral blood smear is definitely far more than that needed to run test into the analyzers. Moreover there is intra and inter observer variation in morphological typing of anemia based on peripheral blood smear examination. The expertise needed to see the peripheral blood smear is unquestionably far more than that needed to run the analyzers. Moreover, in morphological typing of anemia based on peripheral blood smear examination, there is intra and inter observer variation. The additional peripheral blood smear review performed by pathologist hardly ever provided unique information and provided incremental helpful information in only 4% of the cases [1].

Cell counting with these automated hematology analyzers is quick, objective, statistically significant and not subjected to the distributional bias from the manual count. Hence automated instruments increase accuracy, speed of analysis, minimizes levels of human manipulation for test entry, sampling, sample dilution and analysis [2]. They are also more resourceful and cost effective than the manual method. Most of these cell counters can process 120- 150 samples per hour [3].

The automated hematology analyzer with complete blood count results has replaced the traditional manual or individual parameter assay methods for hematological parameters as the initial screening and detection system for hematological abnormalities in modern hospitals and clinics [4]. The traditional review of all automated hematology instrument results by preparation, staining and microscopic examination of a blood film examination has moved out in most institutions[5]. The reasons are the more accurate detection of specimens with distributional or morphological abnormalities by the traditional eye count method [6].

The present study was being carried out to find the correlation of morphological typing of anemia based on RBC indices obtained from KX-21, a 3 part 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.

This study intended to create a guide to laboratory personnel and clinicians with sufficient accuracy to presumptively diagnose morphological classes of anemia directly from the automated hematology cell counter forms and to correlate with morphological features of peripheral smear examination.

AIMS

To study whether the judicious application of automated hematology analyzer is an add-on to well-informed morphological assessment by Peripheral smear examination in anemia patients.

OBJECTIVES

1. Interpretation of histograms in normal persons and patients with different types of anemia.
2. Morphological typing of anemia using RBC indices and RDW obtained from Sysmex KX-21, an automated hematology analyzer
3. Comparison of automated histogram patterns with morphological features noticed on peripheral smear examination.

MATERIAL & METHODS

This study was undertaken at the Department of Pathology, Smt. B. K. Shah Medical Institute & Research Centre, Piparia, Vadodara. Anemia typing was done by two methods – to start with using RBC indices & RDW only. Subsequently on peripheral blood smear examination. The results were then correlated and analyzed.

- Study site: Central Diagnostic Laboratory, Dhiraj hospital, Department of Pathology, Smt.B.KShah Medical Institute & Research Centre, Piparia, Vadodara
- Duration of study: Five years, January 2017 to December 2021
- Study Design: This will be a cross sectional observational study.
- Study procedure:
- 3 ml of EDTA blood sample were collected and histograms were obtained after thorough mixing. The automated analyzer used in this hospital Sysmex KX 21 i.e. 3 part differential automated analyzer was used for the study.
- A simultaneous peripheral smears were also be prepared according to standard operating procedures and stained

by Fields stain.

- This peripheral smears were reported by Pathologist .The pathologist however ,would not be privy to histogram during the reporting of peripheral smear.
- Data analysis:
- The data were collected and statistically comparative study will be done.
- Ethical approval: The study was prior approved by Ethical Committee of the Institute HRRP & SVIEC.

Patient samples with hemoglobin levels indicating anemia (hemoglobin value below the WHO - reference values) were chosen for the study.

The sample was venous blood collected by venepuncture in vaccutainer containing EDTA anticoagulant. The sample was first aspirated into KX-21, automated 3 – part differential hematology analyzer which analyses the sample and provided a print out with complete blood count, RBC indices, RDW, histogram and distribution curves.

Morphological typing of anemia was done using RBC indices and RDW (Table:1):

- 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 Based on RBC indices
- Microcytic hypochromic when MCV and MCH are below the reference values for that particular age and sex.

Table : 1: The reference values are taken from Wintrobe's Clinical hematology – 12th Edition [7]

Parameters	Reference values (2-6y)	Reference values (6-12y)	Reference values Male (12-18y)	Reference values Female (12-18y)	Reference values Male (18-49y)	Reference values Female (18-49)	Reference values Male (>49y)	Reference values Female (>49)
Hb (g/dl)	11.5-13.5	11.5-15.5	13-16	12-16	13.5 – 17	12 – 16	13.5 – 17	12 – 16
Hct (%)	34-40	35-45	37-49	36-46	41 – 53	36 – 46	41 – 53	36 – 46
TRBC (million/cumm)	3.9-5.3	4.0 - 5.2	4.5-5.3	4.1-5.1	4.5 - 5.9	4.0 - 5.2	4.5 - 5.9	4.0 - 5.2
MCV (fl)	75-87	77-95	80-100	78-98	80 – 100	80 – 100	80 – 100	80 – 100
MCH (pg)	24 – 30	25 – 33	25-35	25-35	26 – 34	26 – 34	26 – 34	26 – 34
MCHC (%)	31-37	31-37	31-37	31-37	31-37	31-37	31-37	31-37
RDW (CV) (%)	<14.6	<14.6	<14.6	<14.6	<14.6	<14.6	<14.6	<14.6
RDW (SD) (fl)	35-45	35-45	35-45	35-45	35-45	35-45	35-45	35-45
TLC (cells/cumm)	11000-16000	4500-13500	4500-13000	4500-13000	4000-11000	4000-11000	4000-11000	4000-11000
Platelet (lakh / cumm)	1.5-4.5	1.5-4.5	1.5-4.5	1.5-4.5	1.5-4.5	1.5-4.5	1.5-4.5	1.5-4.5

Peripheral blood smears were then prepared immediately, stained with Fields stain and examined by expert.

Morphological typing of anemia was done with PBS :

- Microcytic HypochromicAnemia
- Normocytic NormochromicAnemia
- Macrocytic Anemia
- Dimorphic anemia

Morphological typing of anemia is done using RBC indices with RDW from KX-21, and automated hematology analyzer 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.

Inclusion Criteria

- All anemic patients with hemoglobin percentage less than 11.5 gm% were included in the study.
- Patient of all age groups and both the sexes were included in the study. The patients were referred from in-patient and out-patient departments of Dhiraj Hospital were includes.

Exclusion Criteria

- Inadequate or inappropriate samples (clotted, hemolysed, poorly preserved)
- Patients without anemia i.e. hemoglobin within the normal range for that particular age and sex.

RESULT

A total of 1100 cases of anemia were studied over a period of five years. The data was obtained retrospectively and prospectively after ethical approval from January 2017 to December 2021 at Central Diagnostic Laboratory, Dhiraj hospital, Department of Pathology, Smt. B. K. Shah Medical Institute & Research Centre, Piparia, Vadodara.

The data was extracted as below:

a) Morphological typing of anemia based on RBC indices and RDW
b) Morphological typing of anemia based on peripheral blood smear examination
c) Correlation between morphological typing of anemia using RBC indices and RDW with morphological typing of anemia using peripheral blood smear examination
d) Cases with concordant and non-concordant results

a. Morphological typing of anemia based on RBC indices and RDW (Table:2)

Out of the 1100 cases on which morphological typing of anemia was done using RBC indices and RDW, majority of the case belonged to Microcytic Hypochromic Anemia of raised RDW (43.63%) followed by Normocytic Normochromic Anemia with normal RDW (34.54%).

Table 2: Morphological typing of anemia based on RBC indices and RDW

Morphological type of anemia	Total
Microcytic Hypochromic Anemia with normal RDW	55
Microcytic Hypochromic Anemia with raised RDW	480
Normocytic Normochromic Anemia with normal RDW	380
Normocytic Normochromic anemia with raised RDW	133
Macrocytic Anemia	52
Total	1100

b. Morphological typing of anemia based on peripheral blood smear examination (Table:3)

Out of the 1100 cases on which morphological typing of anemia was done using peripheral blood smear examination, majority of the case belonged to Microcytic Hypochromic Anemia (47.27%) followed by Normocytic Normochromic Anemia(37.63%). Dimorphic anemia were 10.36% of the cases whereas macrocytic anemia accounting to only a small number of cases as expected (4.72%).

Table 3: Morphological typing of anemia based on peripheral blood smear examination.

Morphological Type Of Anemia	Total
Microcytic Hypochromic Anemia	520
Normocytic Normochromic Anemia	414
Macrocytic Anemia	52
Dimorphic Anemia	114
Total	1100

c. 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 (55)	Microcytic Hypochromic Anemia (520)
Microcytic Hypochromic Anemia with raised RDW (480)	
Normocytic Normochromic Anemia with raised RDW (23)	
Normocytic Normochromic Anemia with raised RDW (90)	Dimorphic Anemia (114)
Normocytic Normochromic Anemia with normal RDW(380)	Normocytic Normochromic Anemia

	(414)
Macrocytic Anemia (52)	Macrocytic Anemia (52)
Total - 1100	Total – 1100

Correlation – On verifying the morphological typing of anemia by peripheral blood smear examination of cases already typed by RBC indices and RDW obtained by KX-21, an automated 3 parthematology analyzer. Findings were

- All cases typed as Microcytic Hypochromic Anemia with normal RDW on RBC indices with RDW alone were confirmed by peripheral blood smear examination as Microcytic Hypochromic Anemia.
- All cases typed as Microcytic Hypochromic Anemia with raised RDW on RBC indices with RDW alone were confirmed by peripheral blood smear examination as Microcytic Hypochromic Anemia.
- All cases typed as Macrocytic Anemia on RBC indices with RDW alone were confirmed by peripheral blood smear examination as MacrocyticAnemia.
- All cases typed as Normocytic Normochromic Anemia with normal RDW on RBC indices with RDW alone were confirmed by peripheral blood smear examination as Normocytic NormochromicAnemia.
- However all cases typed as Normocytic Normochromic Anemia with raised RDW (68) on RBC indices with RDW alone were typed differently on peripheral blood smear examination. Out of the 133 cases, 23 cases were typed as Microcytic Hypochromic Anemia with Polychromasia on PBS examination and the rest 114 cases were typed as Dimorphic Anemia on PBS examination.

d. Cases with concordant and non-concordant results (Table:4)

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

Concordant Typing	Non-concordant Typing	Total
1091	90	1100

Out of 1100 cases, only 8.18% cases showed discordant typing, which need to be typed correctly with peripheral blood smear examination.

Distribution of cases with concordant typing

Out of the 1091 cases, with concordant typing using RBC indices alone and with peripheral blood smear examination, majority of the cases were Microcytic hypochromic anemia followed by Normocytic normochromicanemia.

Cases with discordant typing

90 cases showed non concordant typing of anemia. Out of these cases 23 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.

57 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.

Table 5: Cases with discordant typing

No. Of Cases	Typing with RBC indices & RDW	Typing with PBS
23	Normocytic normochromic anemia with raised RDW	Microcytic hypochromic anemia with polychromasia
67	Normocytic normochromic anemia with raised RDW	Dimorphic anemia

Out of the 1100 cases, correct morphological typing of anemia was done with RBC indices alone in 91.82% which was verified on peripheral blood smear examination.

Only 90 cases (8.18%) were wrongly typed on RBC indices and needed to be typed using peripheral blood smear examination.

Table 6: Distribution of cases correctly typed by RBC indices and peripheral blood smear examination.

Method	Correct Typing	Wrong Typing	Total
RBC indices with RDW	1010	90	1100
PBS with RBC indices	1100	00	1100

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

DISCUSSION

A total of 1100 cases of anemia were studied over a period of five years between January 2017 to December 2021 at Central Diagnostic Laboratory, Dhiraj hospital, Department of Pathology, Smt.B.KShah Medical Institute & Research Centre, Piparia, Vadodara.

In a study by Japheth E Mukaya et al, out of his 165 cases of anemia, most common morphological type of anemia was Microcytic Hypochromic anemia (54%) followed by Normocytic normochromic anemia(31%)[8]. 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.

Mishra et al 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%[9]. 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 maybe due to possible relationship of this age group with increased nutritional needs and parity.

Novis et al studied 95,141 cases and concluded that in 6.4% of cases, peripheral blood smear examination provided additional information [6]. The present study showed similar findings with 9.6% of cases needing peripheral blood smear examination.

CONCLUSION

The current study was undertaken to correlate the morphological typing of anemia using RBC indices and RDW obtained from KX-21, 3 part automated hematology analyzer with peripheral blood smear examination to evaluate if peripheral blood smear review performed by pathologist provides unique and additional information in morphological typing of anemia.

In Microcytic Hypochromic Anemia, majority of the cases were appropriately typed using RBC indices alone. However in a small percentage of cases where treatment was started with iron therapy showed presence of Microcytic hypochromic anemia with polychromasia which led to wrong typing as Normocytic normochromic anemia with raised RDW.

In Macrocytic anemia the typing done using RBC indices matched with typing by peripheral blood smear evaluation in all the cases and thus showing high sensitivity and specificity of typing Macrocytic anemia using RBC indices alone.

In Normocytic Normochromic Anemia typed using RBC indices alone, the cases typed as Normocytic Normochromic Anemia with normal RDW showed concordant typing using peripheral blood smear examination.

It can be finally concluded that in fresh cases of Microcytic Hypochromic Anemia and Macrocytic Anemia, morphological typing of anemia using RBC indices alone was accurate and showed 100% sensitivity and > 96% specificity. In Normocytic normochromic anemia with normal RDW also showed high sensitivity and specificity. However in Normocytic normochromic anemia with raised RDW showed a 100% indication for peripheral blood smear examination as it provided addition information in morphological typing of anemia which could further help in identifying the cause and treatment of anemia.

SUMMARY

In summary, peripheral blood smear examination cannot be totally replaced by automated hematology analyzers as they provide so much additional information which cannot be summarized entirely by the simple numerical calculations of an automated analyzer but the present generation of automated hematology analyzers are well on equivalence 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 and competency of a clinical 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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