e-ISSN: 0976-822X, p-ISSN:2861-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2023; 15(6); 392-398

Original Research Article

Hematological Parameters versus Peripheral Smear in Anemic Patients: A Comparative Study

Lucky Sriwastwa[1], C.P. Jaiswal[2], Sunil Kumar[3]

[1]Tutor, Department of Pathology, Nalanda Medical College and Hospital, Patna, Bihar, India

[2]Associate Professor, Department of Pathology, Nalanda Medical College and Hospital, Patna, Bihar, India

[3]Assistant Professor, Department of Pathology, Nalanda Medical College and Hospital, Patna, Bihar, India

Received: 15-03-2023 Revised: 20-04-2023 / Accepted: 21-05-2023

Corresponding author: Dr. Lucky Sriwastwa

Conflict of interest: Nil

Abstract

Aim: The present study was designed to find out the association between cell counter generated hematological parameters and peripheral smear findings in the diagnosis of anemia

Methods: This study was carried out in Department of Pathology, Nalanda Medical College and Hospital, Patna, Bihar, India for 12 months. 100 patients with low Hb level with reference to age and sex were selected for the comparison of PS findings with that of cell counter parameters.

Results: Anemia was more common in our study was age group more than 60 years (32 out of 100 cases, 32%) followed by between 41 to 50 years (26 out of 100, 26%). In males, normocytic normochromic anemia was more common (38 out of 52 cases,) and in females, microcytic hypochromic anemia was more common (34 out of 40 cases). Out of 40 cases of microcytic hypochromic anemia 33 cases showed low MCV but 6 cases showed normal MCV. When compared, the anemia diagnosed based on MCV values and by manual examination there was a significant difference. Out of 52 cases of normocytic normochromic anemia, 46 cases showed normal MCV level. 37 cases of Microcytic hypochromic anemia had high RDW which was normally expected but 26 cases of Normocytic normochromic anemia showed raised RDW.

Conclusion: Despite all the advances in laboratory sciences, peripheral smear examination remains an important diagnostic tool in diagnosis of anemia.

Keywords: Anemia, peripheral smear examination, RBC indices.

Introduction

Anaemia is characterised by a reduction in the overall number of red blood cells, haemoglobin levels, or the blood's capacity to transport oxygen. One of the most prevalent medical conditions, particularly in our nation, is anaemia. Significant morbidity and death have been linked to it. 1.62 billion individuals, or 24.8% of the world's population, are affected with anaemia. Anaemia is more common in children under the age of six (47.3%) than in pregnant women (41.8%). [1] Iron, vitamin B12, or folic acid dietary deficiencies are the most frequent causes of anaemia. However, numerous clinical illnesses can also cause anaemia, such as

diseases that cause bone marrow failure that result in reduced RBC synthesis and clinical conditions that cause hemolysis that result in increased RBC lysis. [2] Therefore, determining the kind of anaemia accurately is crucial to administering the proper medicine. It is a symptom of underlying disease rather than a diagnosis in and of itself. Red blood cell morphological variations have a significant diagnostic role in the identification of various anaemias. Using PBS, anaemia is diagnosed and information on altered RBC shape and size or the existence of any inclusion bodies is obtained by microscopically examining blood smears. RBC morphology is a crucial tool that haematologists use to choose the best tests for definitive diagnosis and to suggest appropriate clinical and laboratory follow-up. [3] Based on RBC morphology and clinical characteristics, anaemia can be assessed. On the basis of both qualitative and quantitative data, anaemia is divided into different categories. A skilled lab worker can do morphological analysis using blood smears by thinly spreading a drop of blood onto a glass slide, staining it with dyes like Giemsa, Leishman, and Wright-Giemsa, and then examining it under a microscope. [4] Most helpful is probably an initial morphologic categorization of anaemia that integrates red blood cell indices and morphologic parameters. As a result, it might be microcytic, normocytic, or macrocytic depending on the size of the cells. The most common micronutrient deficiency condition in the world, impacting 2 billion people, is microcytic anaemia linked with iron deficiency. [5] White blood cells (WBCs), red blood cells (RBCs), and platelets are among the several cell types present in the blood smear. In recent years, peripheral smear microscopic analysis and complete blood count (CBC) by automated haematology analyzers have combined to produce a thorough report on patient's blood sample. Automated haematology cell counts have increased precision and accuracy, decreased human error, and increased safety when handling blood specimens. [6] The advantage of a manual scan is that it can find morphological abnormalities that are clinically important but cannot be measured by an instrument, such as pencil cells, sickle cells, tear drop cells, and schistocytes. As a result, the haematology analyzer findings should be verified by a manual scan of a peripheral blood smear, and both results must be consistent. [7] Hence, the present study was designed to find out the association between cell counter generated hematological parameters and peripheral smear findings in the diagnosis of anemia

Materials & Methods

This study was carried out in Department of Pathology, Nalanda Medical College and Hospital, Patna, Bihar, India for 12 months. 100 patients with low Hb level with reference to age and sex were selected for the comparison of PS findings with that of cell counter parameters.

Inclusion criteria

- All cases of anemia as per the World Health Organization criteria where both peripheral smear and cell counter reports available; and who have given informed consent. [8]

Exclusion criteria

- Patients <6 months of age
- Uncooperative patients and unwilling to give consent.
- Patients with hematological malignancies
- Hemolyzed samples
- Clotted samples
- Samples insufficient to run in hematology analyzer.
- Patient with history of recent blood transfusion.

After getting informed consent, detailed clinical history and demographic information from each patient were recorded. Taking all aseptic precautions

blood samples were collected from the peripheral veins in an anticoagulant vial in the right proportion. Two ml sample of the patients collected in Ethylene diamine tetra acetic acid (EDTA) vial, was processed for CBC in Beckman Coulter and Peripheral Smear was prepared from the same sample. The cell counter generated parameters which included red blood cell (RBC) indices (MCV, MCHC, MCH), RDW (Red cell distribution width) and platelet counts were analyzed. The peripheral smears prepared were stained by standard protocol with Leishman stain. After preparation, each peripheral smear was examined for the morphological abnormalities associated with different types of anemia and results were noted. The diagnosis obtained by both the methods i.e. PS examination and cell counter generated parameters were correlated.

Anemia typing was first done using automated cell counter generated RBC parameters such as RBC indices with RDW. Then, PBS was examined by the pathologist under microscope and morphological typing of anemia done. The position and the shape of RBC histograms were recorded. Pathologists were unaware of the histogram and RBC indices while reporting the PBS.

Categorization of anemia is based on RBC indices and RBC histograms by automated hematology analyzer:

- Microcytic hypochromic anemia
- Normocytic normochromic anemia
- Macrocytic anemia
- Dimorphic anemia
- Hemolytic anemia.

Position (normal, left shift, and right shift) and shape (normal bell-shaped or Gaussian, broad-shaped, bimodal peak, and showing to the left or right) of RBC histograms were noted.

Final morphological typing of anemia was done based on peripheral smear examination findings and are recorded as:

- Microcytic hypochromic anemia
- Normocytic normochromic anemia
- Macrocytic anemia
- Dimorphic anemia
- Hemolytic anemia.

Statistical Analysis

The data were entered in Microsoft excel sheet and were analyzed in terms of sensitivity and age distribution. A qualitative analysis of the data was done using Pearsons Chi square test and Fisher exact test wherever appropriate. The P = 0.05 or less was considered statistically significant

Results

Table 1: Age wise distribution and grading of anemia

Grades	**<13**	**13 - 18**	**19 - 30**	**31 - 40**	**41 - 50**	**51 - 60**	**>60**	**Total**
Mild	1	1	3	0	2	3	4	14
Moderate	5	4	3	2	10	2	12	38
Severe	1	3	4	3	14	7	16	48
Total	7	8	10	5	26	12	32	100

Anemia was more common in our study was age group more than 60 years (32 out of 100 cases, 32%) followed by between 41 to 50 years (26 out of 100, 26%).

Table 2: Gender wise distribution of type of anemia

Type	Male	Female	Total
Microcytic hypochromic	6	34	40
Normocytic normochromic	38	14	52
Macrocytic	1	1	2
Dimorphic	3	2	5
Hemolytic anemia	0	1	1
Total	48	52	100

In males, normocytic normochromic anemia was more common (38 out of 52 cases) and in females, microcytic hypochromic anemia was more common (34 out of 40 cases).

Table 3: Mean Corpuscular Volume (MCV) vs distribution of type of anemia

Type	Normal	Low	High	Total
Microcytic hypochromic	6	33	1	40
Normocytic normochromic	46	3	3	52
Macrocytic	0	0	2	2
Dimorphic	3	2	0	5
Hemolytic anemia	1	0	0	1
Total	56	38	6	100

Out of 40 cases of microcytic hypochromic anemia 33 cases showed low MCV but 6 cases showed normal MCV. When compared, the anemia diagnosed based on MCV values and by manual examination there was a significant difference. Out of 52 cases of normocytic normochromic anemia, 46 cases showed normal MCV level.

Table 4: Red cell Distribution Width (RDW) vs distribution of type of anemia

Type	Normal	Low	High	Total
Microcytic hypochromic	2	1	37	40
Normocytic normochromic	16	10	26	52
Macrocytic	0	0	2	2
Dimorphic	0	0	5	5
Hemolytic anemia	0	0	1	1
Total	18	11	71	100

37 cases of Microcytic hypochromic anemia had high RDW which was normally expected but 26 cases of Normocytic normochromic anemia showed raised RDW.

Discussion

Peripheral blood smears have been used as a window to track haematological developments for many years. Regularly analysing peripheral blood smears has been a key diagnostic tool and helped interpretation of various haematological illnesses. [9] Cell counters are now widely used in medical laboratory services, and their effectiveness and price are both rising. In recent years, microscopic analysis of peripheral smears and automated haematology analyzers' counts (CBCs) have combined to produce a thorough report on patient blood samples. [10-12] For the diagnosis of anaemia, red blood cell (RBC) parameters and histograms are essential, but peripheral blood smear (PBS) testing is also highly significant since it offers vital details regarding patients' clinical circumstances. [13,14] Since a few years ago, there has been a significant improvement in accuracy and precision due to the use of automated haematology analyzers, which

has decreased subjective mistakes in the diagnosis of anaemia. [15]

In contrast to Khan et al. [16], who found that 52.63% of men and 47.36% of women had normocytic normochromic anaemia, normocytic normochromic anaemia was more prevalent in men (38 out of 52 cases) and microcytic hypochromic anaemia in women (34 out of 40 cases), which was similar to Singla et al. [15] In our study, anaemia was more prevalent in the age group over 60 (32 out of 100 cases, or 32%) and between 41 and 50 (26 out of 100 cases, or 26%). Microcytic hypochromic anaemia was more prevalent in women (34 out of 40 cases), but normocytic normochromic anaemia was more prevalent in men (38 out of 52 cases). In our study it was found that milder forms of anemia was seen in females and more severe forms seen in males which was in contrast to the study done by Khan et al [10] in which mild anemia was common in males and severe anemia in females.

Out of 40 cases of microcytic hypochromic anemia 33 cases showed low MCV but 6 cases showed normal MCV. When compared, the anemia diagnosed based on MCV values and by manual examination there was a significant difference. Out of 52 cases of normocytic normochromic anemia, 46 cases showed normal MCV level. 37 cases of Microcytic hypochromic anemia had high RDW which was normally expected but 26 cases of Normocytic normochromic anemia showed raised RDW. The result of the present study was similar to the study by Kaur et al. [17] Singla et al [15] in their study found 49. 2%) cases showed low MCV, 44.4% cases showed normal MCV and 6.4% showed high MCV values. 37 cases of Microcytic hypochromic anemia had high RDW which was normally expected but 26 cases of Normocytic normochromic anemia showed raised RDW which was statistically significant with p value <0.01. But 6 cases of microcytic hypochromic anemia showed normal MCV and 3 cases of normocytic normochromic anemia showed high MCV values and those cases had mild variation from their normal reference ranges. Bhasin A et al [18] found normocytic normochromic anemia as predominant finding comprising 66%.

Even if automated haematology analyzers are becoming more capable, manual slide examination is still important to spot some morphologic anomalies that could go unnoticed by automated approaches. [19] It is acknowledged that optical microscopy is preferable for differentiating cells, but automated technologies are superior for counting red blood cells, HB, MCV, MCH, and RDW. This will guarantee suitable and proper care for patients. [20] Our study found that the microscopic examination of peripheral blood smear is gold standard in the diagnosis of various types of anemia. Similar to their findings, Farah et al. [21] and Lantis et al. [22] concluded that automated haematology analyzers were suitable for screening applications since they shorten turnaround times and lower labour costs. The optimum procedure, however, was indicated to be a manual scan by peripheral smear to identify and distinguish between various kinds of anaemia. Thrombopoietin, erythropoietin, IL6, and IL11 levels in blood were measured by Akan et al. [23], but none of these cytokines had any impact on reactive thrombocytosis in iron deficient anaemia. According to research by Bilac et al. [24], thrombocytosis may be explained by the amino acid sequence homology between thrombopoietin and erythropoietin. Contrarily, Recke et al [25] proposed that thrombopoietin and erythropoietin did not participate.

Conclusion

Peripheral smear analysis continues to be a crucial diagnostic technique in the diagnosis of anaemia despite all the developments in laboratory sciences.

However, questions about the reliability of histocytograms were raised by the correlation between histogram patterns and peripheral smear diagnosis in dimorphic anaemia. Thus, it was determined that peripheral smear examination, together with clinical history, is a significant diagnostic tool when treating patients with haematological problems in the era of molecular analysis and automation. The visual evaluation of peripheral smears is aided by red cell histograms as well as numerical measures like MCV, MCH, MCHC, and RDW.

References

1. World Health Organization. Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia./Edited by Bruno de Benoist, Erin McLean, Ines Egli and Mary Cogswell. In Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia./Edited by Bruno de Benoist, Erin McLean, Ines Egli and Mary Cogswell 2008.
2. Dhabangi A, Idro R, John CC, Dzik WH, Opoka R, Ssenyonga R, van Hensbroek MB. Risk factors for recurrent severe anemia among previously transfused children in Uganda: an age-matched case-control study. BMC pediatrics. 2019 Dec; 19(1):1-7.
3. Kumar A, Kushwaha R, Gupta C, Singh US. An analytical study on peripheral blood smears in anemia and correlation with cell counter generated red cell parameters. Journal of applied hematology. 2013 Oct 1;4(4):137.
4. Houwen B. Blood film preparation and staining procedures. Clinics in laboratory medicine. 2002 Mar 1;22(1): 1-4.
5. DeMaeyer EA, Adiels-Tegman M. The prevalence of anaemia in the world. World health statistics quarterly 1985; 38 (3): 302-316; 1985.
6. Sullivan E. Hematology analyzer: From workhorse to thoroughbred. Laboratory Medicine. 2006 May 1;37 (5):273-8.
7. Hattersley PG, Ragusa D. Don't Forget MorphologyThe Importance of Evaluation of Blood Smears. California medicine. 1965; 103(3):175.
8. Geneva S, World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Document Reference WHO. NMH/NHD / MNM/11.1.
9. Glader B. Anemia: General considerations. In: Greer JP, Rodgers GM, Paraskevas F, Glader B, eds. Wintrobe's Clinical Haematology. 11th ed. Philadelphia: Wolters Kluwer Company, 2004: 947-978.
10. Sullivan E. Hematology analyzer: From workhorse to thoroughbred. Lab Med. 2006; 37:273–278.
11. Van Hove L, Schisano T, et al. Anemia diagnosis, classification, and monitoring using cell-dyn technology reviewed for the new millennium. Laboratory hematology 2000 ;(6):93-108.
12. Gulati GL, Hyun BH. The automated CBC. A current perspective. Hematol Oncol Clin North Am1994; 8:593-603.
13. Kumar A, Kushwaha R, Gupta C, Singh US. An analytical study on peripheral blood smears in anemia and correlation with cell counter generated red cell parameters. Journal of applied hematology. 2013 Oct 1;4(4):137.
14. Sullivan E. Hematology analyzer: From workhorse to thoroughbred. Laboratory Medicine. 2006 May 1; 37 (5):273-8.
15. Singla S, Bedi S, Joshi K. Comparative study of anemia cases based on peripheral blood smears and cell counter generated red cell indices. Int Med J. 2017;4(1):44-8.
16. Khan N, Mallik MK, Mallik A. Evaluation of the hematological parameters in correlation with peripheral smear examination to

analyse the prevalence, type and severity of anemia in different age and sex in Shahjahanpur, Uttar Pradesh. IJHSR. 2017; 7(9):16.
17. Kaur H, Piplani S, Madam M. Prevalence of anemia and micronutrient deficiency in elderly. International Journal of Medical and dental science. 2014; 3(1):296- 302.
18. Bhasin A, Rao MY. Characteristics of anemia in elderly: a hospital-based study in South India. Indian journal of hematology and blood transfusion. 2011 Mar; 27:26-32.
19. Wei C, Wei W, Xin W et al. Development of the personalized criteria for microscopic review following four different series of hematology analyzer in a Chinese large-scale hospital. Chinese Medical Journal. 2010; 123: 3231-7.
20. Comar S, Malvezzi M, Pasquini R. Are the review criteria for automated complete blood counts of the International Society of Laboratory Hematology suitable for all hematology laboratories? Rev. Bras. Hematol. Hemoter. 2014; 36:219-25.
21. Farah E, Mehwish A, Nafisa HA. Comparative Study in the Diagnosis of Anemia by Sysmex Kx-21n Hematology Analyzer with Peripheral Blood Smear. International journal of endorsing health science research. 2013; 1(2):89-92.
22. Lantis KL, Harris RJ, Davis G, Renner N, Finn WG. Elimination of Instrument-Driven Reflex Manual Differential Leukocyte Counts Optimization of Manual Blood Smear Review Criteria in a High-Volume Automated Hematology Laboratory. American journal of clinical pathology. 2003; 119(5):656-662.
23. Akan H, Güven N, Ayogdu İ, Arat M, Beksaç M, Dalva K. Thrombopoietic cytokines in patients with iron deficiency anemia with or without thrombocytosis. Acta haematologica. 2000; 103(3):152-6.
24. Bilic E, Bilic E. Amino acid sequence homology of thrombopoietin and erythropoietin may explain thrombocytosis in children with iron deficiency anemia. Journal of pediatric hematology/oncology. 2003; 25(8):67 5-6.
25. Racke FK. EPO and TPO sequences do not explain thrombocytosis in iron deficiency anemia. Journal of pediatric hematology/oncology. 2003;25(11): 91 9.