e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2021; 13(6); 913-921

Original Research Article

Retrospective Analysis of Morphological Types of Anemia Based on Peripheral Smear Findings

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Received: 12-10-2021 / Revised: 15-11-2021 / Accepted: 20-12-2021

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Conflict of interest: Nil

Abstract:

Introduction: Anemia is a frequently encountered condition that occurs among all age groups and continues to be a significant health issue globally. The peripheral smear test is an affordable yet useful diagnostic tool for the detection of anemia along with any related changes in the red blood cells.

Objective: The present study seeks to conduct a retrospective analysis of various categories of anemia as revealed by peripheral smear testing among patients attending a tertiary care diagnostic center.

Methodology: This retrospective observational hospital-based study was carried out for one year. A total of 200 cases of patients with anemia along with their hematological and peripheral smear results were selected for inclusion in the study. Relevant data pertaining to demographic information, hemoglobin concentration, red blood cell indices, and peripheral smear results were obtained and analyzed using SPSS version 25.0.

Results: The largest number of patients fell within the age group of 21 to 30 years (26.0%), and females made up 59.0% of the total patient pool. Hemoglobin levels within the range of 8.1 to 10.0 g/dL were prevalent among the patients (37.0%). The most prevalent morphological type of anemia was microcytic hypochromic anemia, found in 48.0% of cases, followed by normocytic normochromic anemia (21.0%). Some of the peripheral smear abnormalities include anisocytosis (61.0%), microcytosis (56.0%), and hypochromia (53.0%). The average hemoglobin level is 8.24 ± 1.86 g/dL.

Conclusion: This study confirmed that peripheral smear analysis continues to be an efficient and valid technique for morphological categorization of anemia. The most common form of anemia in this study was microcytic hypochromic anemia, which suggests a high occurrence of iron-deficiency anemia. Peripheral smear analysis.

Keywords: Anemia, Peripheral Smear, Microcytic Hypochromic Anemia, Hematological Parameters, Red Blood Cell Morphology, Retrospective Study.

Introduction

Anemia is one of the commonest hemato logical disorders in the world and constitutes a serious health problem which can affect people of all ages. It is a clinical condition wherein there is reduced concentration of hemoglobin or red blood cells or the capacity of oxygenation in blood causing deficiency in the delivery of oxygen to body tissues [1]. Anemia can result from a variety of causes including nutritional problems, other medical conditions, disorders related to bone marrow, destruction of red blood cells (hemolysis), hemorrhage and genetic factors [2]. Early diagnosis and classification of anemia are crucial for proper management and avoidance of complications [3]. A peripheral blood smear test is a relatively inexpensive yet highly effective procedure to assess the morphological characteristics of the red blood cells and classify different types of anemia depending on cellular abnormalities like microcytosis, macrocytosis, hypochromia, anisocytosis and poikilocytosis [4]. Retrospective analysis of results of a peripheral blood smear test will provide information about the types of anemia prevalent in a particular community as well as their characteristic features. Thus, this study was done to conduct a retrospective analysis of anemia types based on peripheral blood smear test results among patients visiting a tertiary diagnostic center [5].

Background of the Study

Anemia remains a global health concern, especially in developing nations due to nutritional deficiency, infections, and poor access to health care services, which predisposes people to the condition [6]. The disease causes malnutrition, impairs mental and physical development, and reduces productivity at home and work environments; hence, its proper diagnosis is imperative [7]. Modern diagnostic tests exist for the diagnosis of anemia; however, peripheral smear evaluation remains one of the most useful techniques in routine hematology because of its ability to give prompt results about red blood cell morphologies and help identify the anemia type [8]. Examination of the red blood cells' morphology through peripheral smears can detect abnormalities like microcytosis, macrocytosis, hypochromia, anisocytosis, and poikilocytosis that are helpful in distinguishing anemia associated with nutritional deficiency, hemolysis, or chronic conditions [9]. Evaluation of peripheral smear findings retrospectively can help understand the distribution and pattern of anemia among hospitalized patients. This will assist in formulating better diagnostic strategies and treatment plans [10]. This study, therefore, aims to assess different types of anemia through peripheral smear findings among patients in a tertiary diagnostic center.

Morphological Evaluation of Anemia Using Peripheral Smear: Peripheral smear examination is one of the common methods employed for the assessment of anemia based on its morphological evaluation, where the morphology of RBCs is assessed based on their size, shape, color, and any abnormality present within [11]. Through peripheral smear evaluation, different types of morphologies associated with anemia, like microcytic hypochromic, macrocytic, normocytic normochromic, dimorphic, and hemolytic anemia, are recognized [12]. This test is useful in recognizing any abnormalities in RBCs, like anisocytosis, poikilocytosis, target cells, tear drop cells, and hypochromia, which can help diagnose the cause and severity of anemia. As peripheral smear test is easy and economical to perform, it holds great significance in diagnosing anemia [13].

Research Objectives

The research objectives of the study are:

- To retrospectively evaluate the different morphological types of anemia based on peripheral smear findings among anemic patients attending a tertiary care hospital.
- To analyze the demographic and hematological profile of anemic patients with respect to age, gender, hemoglobin levels, and red blood cell indices.
- To identify and classify the common peripheral smear abnormalities associated with various types of anemia.

Methodology: The current study was undertaken to retrospectively analyze the anemias with regard to their peripheral smear characteristics in the patients referred to a tertiary diagnostic center. The study method was planned to conduct a systematic analysis of hematology reports and peripheral blood smear reports with respect to the identification of different morphological features of anemias.

Study Design: This research is considered a hospital-based retrospective observational study. The previously documented hematology and peripheral smear findings of anemic cases were studied and analyzed to determine the type of anemia according to the morphology seen in the peripheral blood smear results.

Study Area: The experiment was conducted in the Department of Pathology, Gouri Devi Institute of Medical Sciences and Hospital, Durgapur, West Bengal, India

Study Duration: The study was conducted over a period of one year.

Study Participants

The study subjects consisted of patients whose blood samples underwent peripheral smear analysis and were found to have anemia.

Inclusion Criteria

- Patients of any age group and sex suffering from anemia.
- Patients who had their complete hematological history, along with levels of hemoglobin and blood smear findings.
- Patients whose blood smears had definite morphological features suggesting anemia.

Exclusion Criteria

- Patients with missing laboratory results.
- Samples that were hemolyzed or improperly stored.
- Patients with hematological cancers or other severe systemic diseases that might affect smear evaluation.
- Repeat records of the same patient during the study period.

Sample Size: The number of patient files examined for the study that had been diagnosed with anemia was 200. The sample size was determined to be adequate enough to evaluate the prevalence and morphology of different types of anemia in relation to their peripheral smear results.

Procedure: Following the approval from the concerned authorities of the institution, the lab reports for patients with anemia were retrieved retrospectively from the department of hematology. Information regarding demographics and

hematology of the patients, such as age, sex, hemoglobin level, red blood cell indices, and results of peripheral smear examination was documented in a data sheet.

The results of the peripheral smear examination were examined and classified as follows:

- Microcytic hypochromic anemia
- Macrocytic anemia
- Normocytic normochromic anemia
- Dimorphic anemia
- Hemolytic anemia and other abnormal smear patterns

Peripheral smear examination was conducted in accordance with the standard procedures employed in laboratory practice and stained with the help of Leishman stain/Wright-Giemsa stain. The study of the morphology of red blood cells, white blood cells, and platelets was done with the help of light microscopy. The acquired data were systematically analyzed and classified based on anemia types and demographics.

Statistical Analysis: The collected data were analyzed by importing them into Microsoft Excel. Further analysis of the collected data was done using Statistical Package for Social Sciences (SPSS) version 25.0. The analysis of the collected data was done using various descriptive statistics techniques including frequency, percentage, mean, and standard deviation. Presentation of various categories of anemia according to the results of peripheral smear was done through use of tables and figures. Relevant statistical tests including Chi-Square test were used where appropriate with a p-value < 0.05 being statistically significant.

Results

The present retrospective study was conducted in order to analyze various forms of anemia on the basis of peripheral smear analysis in 200 patients suffering from anemia. Data collected were subjected to analysis to find out demographic characteristics, hemoglobin levels, morphological types of anemia, and peripheral smear abnormalities related to anemia. The results of the analysis are given below in tabular form and their interpretations.

Age distribution was studied in order to determine the occurrence of anemia in different age groups. Age is an important factor that helps in deciding the nutritional status of the body and its vulnerability to different types of anemia. Age groups of the selected samples of patients were determined to systematically study the problem. Frequency and percentage distribution of samples are presented in the following table 1 and figure 1.

Table 1: Distribution of Patients According to Age Group

Age Group (Years)	Frequency (n=200)	Percentage (%)
Below 20	28	14.0%
21–30	52	26.0%
31–40	46	23.0%
41–50	38	19.0%
Above 50	36	18.0%
Total	**200**	**100.0**

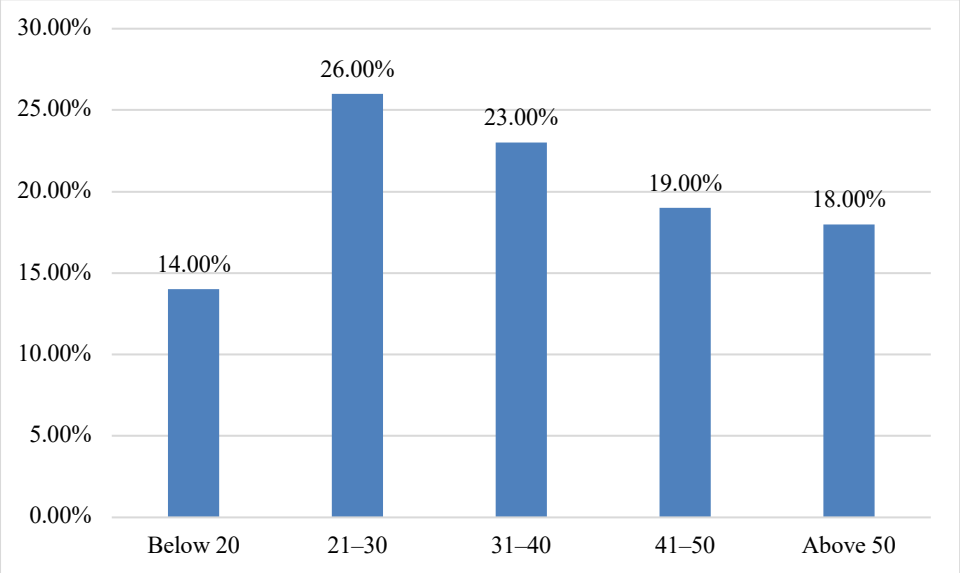

Figure 1: Graphical Representation of Percentage in Distribution of Patients According to Age Group

As per table 1 and figure 1, it can be seen that the maximum number of subjects included in the study, i.e., 52 (26.0%) patients belong to the age group 21-30 years followed by 46 (23.0%) patients belonging to the age group 31-40 years. Age group 41-50 years patients constituted 38 (19.0%) whereas those above 50 years constituted 36 (18.0%). Minimum number of patients, i.e., 28 (14.0%), belonged to age group less than 20 years. The results revealed that anemia is most common in young adults.

In order to assess the occurrence of anemia among male and female patients, the distribution of participants as per gender was determined because gender difference plays an important role in developing nutritional deficiencies, menstrual bleeding, and anemia. The distribution of participants per gender is provided below.

Table 2: Gender-wise Distribution of Study Participants

Gender	Frequency (n=200)	Percentage (%)
Male	82	41.0
Female	118	59.0
Total	**200**	**100.0**

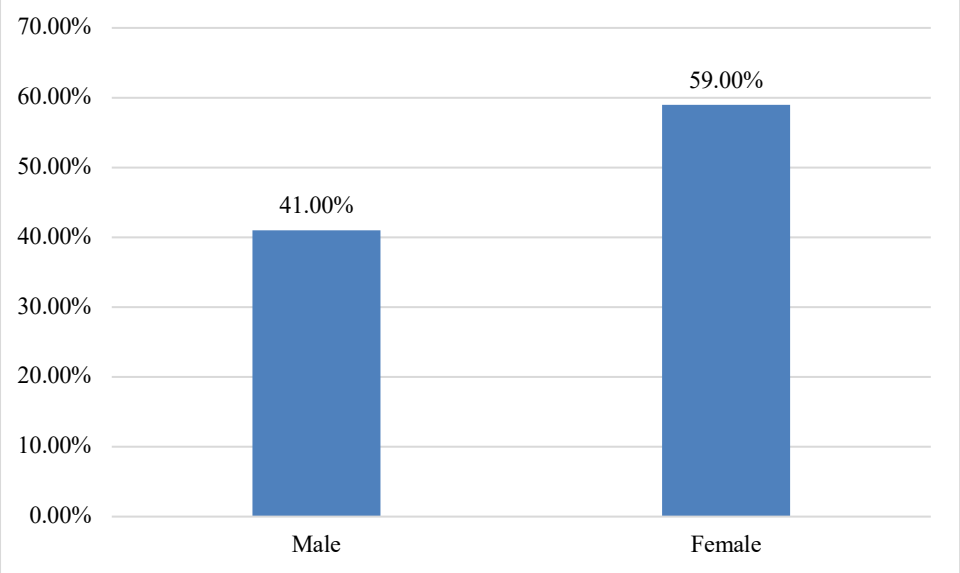

Figure 2: Graphical Representation of Percentage in Gender-wise Distribution of Study Participants

It was found out that women patients formed the majority of the sample with 118 (59.0%) subjects while men patients formed only 82 (41.0%) subjects. The higher incidence of anemia in women could possibly be attributed to deficiencies in nutrition, blood loss during menstruation, pregnancy, and higher iron needs.

Estimation of hemoglobin was used to assess the degree of anemia in the subjects included in the study. Hemoglobin concentrations served as basis in classifying subjects into various groups of anemia depending on its severity level. The classification of patients depending on their hemoglobin concentration is presented in table 3 below.

Table 3: Distribution of Patients According to Hemoglobin Levels

Hemoglobin Level (g/dL)	Frequency	Percentage (%)
<6.0	24	12.0
6.1–8.0	68	34.0
8.1–10.0	74	37.0
>10.0	34	17.0
Total	**200**	**100.0**

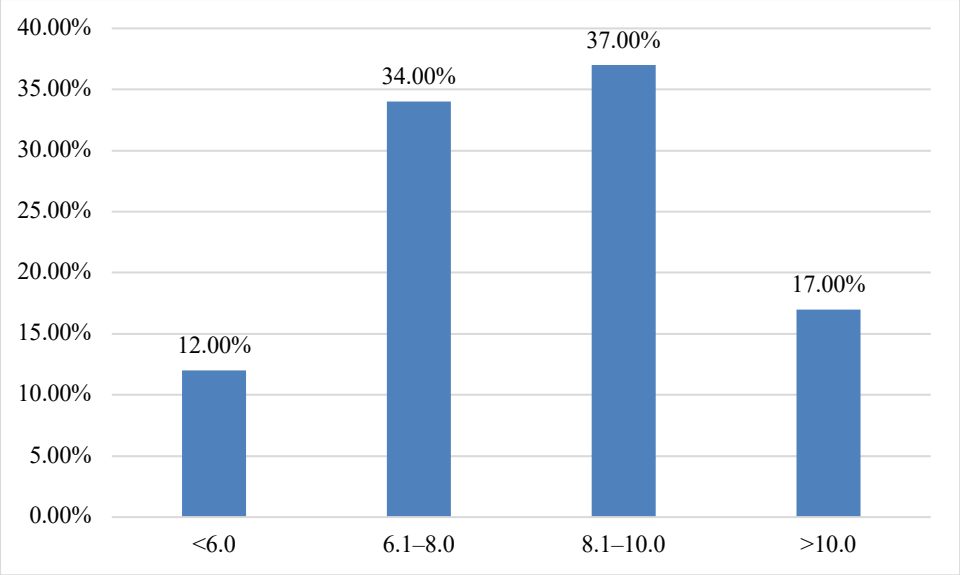

Figure 3: Graphical Representation of Percentage in Distribution of Patients According to Hemoglobin Levels

Table 3 and Figure 3 revealed that most of the patients were suffering from moderate anemia; i.e., hemoglobin was found to be 8.1-10.0 g/dL in 74 (37.0%) patients and 68 (34.0%) patients had hemoglobin ranging from 6.1-8.0 g/dL. Severe anemia was found in 24 (12.0%) patients whose hemoglobin concentration was less than 6.0 g/dL, whereas hemoglobin concentration more than 10.0 g/dL was seen in only 34 (17.0%) patients.

Peripheral smear analysis helped in identifying different types of anemia from the morphological perspective. Morphological identification of red blood cells offered crucial diagnostic information about the actual type of anemia. Types of anemia based on the results of peripheral smears have been described below.

Table 4: Morphological Classification of Anemia Based on Peripheral Smear Findings

Type of Anemia	Frequency	Percentage (%)
Microcytic Hypochromic Anemia	96	48.0
Normocytic Normochromic Anemia	42	21.0
Macrocytic Anemia	26	13.0
Dimorphic Anemia	28	14.0
Hemolytic Anemia	8	4.0
Total	**200**	**100.0**

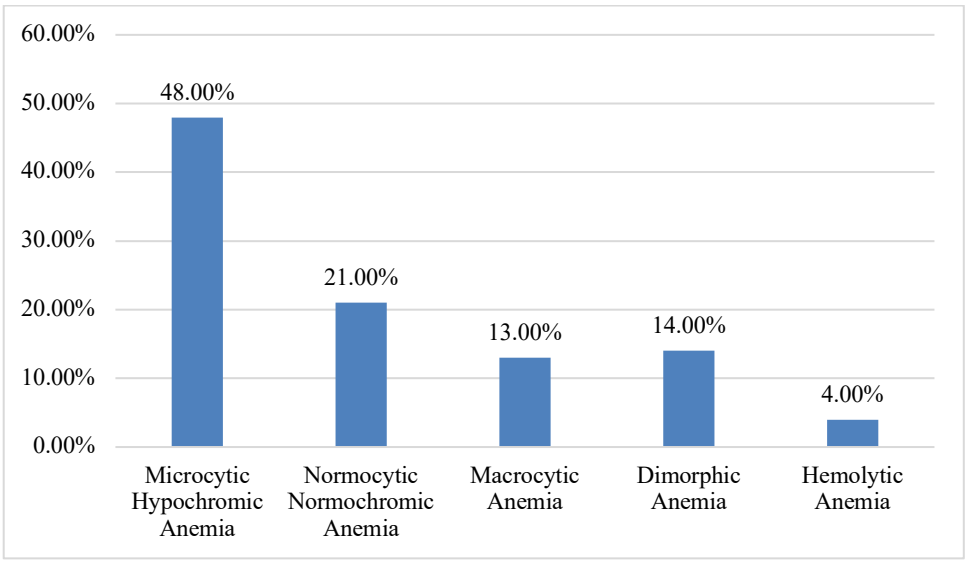

Figure 4: Graphical Representation of Percentage in Morphological Classification of Anemia Based on Peripheral Smear Findings

Based on the analysis, it was revealed that the microcytic hypochromic anemia was the prevalent form of anemia, with 96 patients (48.0%) being affected by it. Normocytic normochromic anemia comprised 42 patients (21.0%), whereas dimorphic anemia and macrocytic anemia affected 28 patients (14.0%) and 26 patients (13.0%), respectively. Hemolytic anemia was the least prevalent type of anemia, affecting only 8 patients (4.0%). This prevalence rate of microcytic hypochromic anemia suggests a relatively high prevalence rate of iron deficiency anemia in the study population.

The peripheral smear analysis provided the results that helped detect morphological abnormalities in red blood cells. Morphological abnormalities serve as diagnostic indicators for various hematological disorders and nutritional deficiencies. The results of peripheral smears are provided below.

Table 5: Peripheral Smear Findings Among Study Participants

Peripheral Smear Findings	Frequency	Percentage (%)
Anisocytosis	122	61.0
Poikilocytosis	98	49.0
Hypochromia	106	53.0
Macrocytosis	30	15.0
Microcytosis	112	56.0
Target Cells	18	9.0
Tear Drop Cells	10	5.0

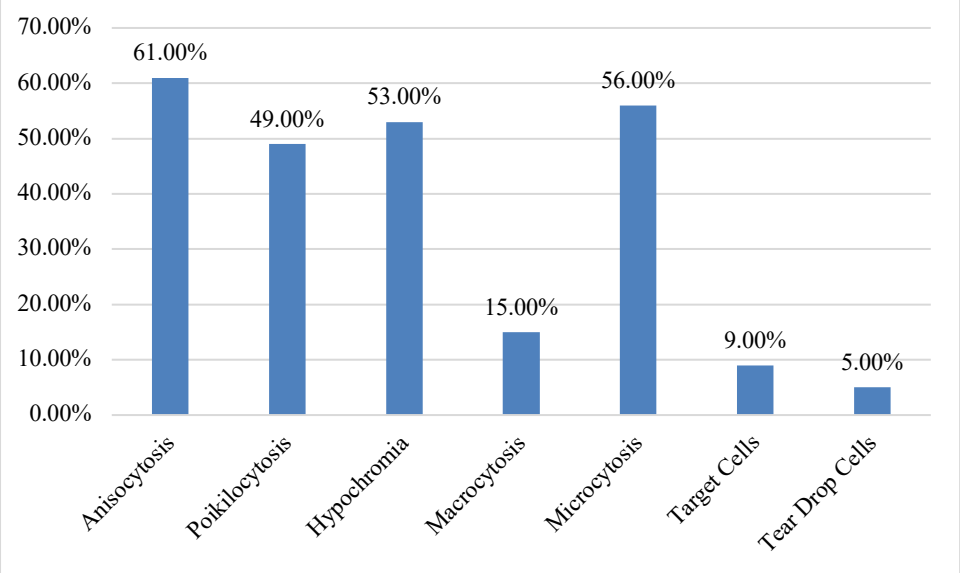

Figure 5: Graphical Representation of Percentage in Peripheral Smear Findings Among Study Participants

In terms of the peripheral smear anomalies, the most frequent was anisocytosis in 122 (61.0%) subjects, followed by microcytosis in 112 (56.0%) subjects and hypochromia in 106 (53.0%) subjects. Poikilocytosis was noted in 98 (49.0%) subjects. Macrocytosis, target cells, and tear drop cells were detected in 30 (15.0%), 18 (9.0%), and 10 (5.0%) subjects respectively. This indicated notable morphological diversity among anemic patients.

To determine the relationship between the gender and the type of anemia, we compared the morphological patterns of anemia in both genders. The results are presented in the table below.

Table 6: Association Between Gender and Type of Anemia

Type of Anemia	Male	Female	Total
Microcytic Hypochromic	34	62	96
Normocytic Normochromic	22	20	42
Macrocytic	10	16	26
Dimorphic	12	16	28
Hemolytic	4	4	8
Total	**82**	**118**	**200**

From the results shown in Table 6, microcytic hypochromic anemia was common among women, occurring in 62 women while only 34 men had the disease. Normocytic normochromic anemia was observed among 22 men and 20 women. Dimorphic anemia and macrocytic anemia were also found to be common among females. The incidence of hemolytic anemia was almost similar for both males and females, being present among four patients for each group.

Hematological properties of participants from the study were assessed with regard to the average red blood cell indices. This was done using mean and standard deviation values in order to show variations in hematological properties among different patients.

Table 7: Mean and Standard Deviation of Hematological Parameters

Hematological Parameter	Mean ± Standard Deviation
Hemoglobin (g/dL)	8.24 ± 1.86
MCV (fL)	74.62 ± 10.24
MCH (pg)	24.18 ± 4.12
MCHC (g/dL)	30.54 ± 2.36
RBC Count (million/mm³)	3.68 ± 0.82

The average hemoglobin content in the studied subjects was 8.24 ± 1.86 g/dL, depicting mild anemia for most subjects. The average MCV reading was 74.62 ± 10.24 fL, signifying that microcytic anemia is more prevalent. Low average MCH and MCHC readings of 24.18 ± 4.12 pg and 30.54 ± 2.36 g/dL, respectively, depicted hypochromic cells. The average RBC reading was 3.68 ± 0.82 million/mm³, representing low erythrocyte count among anemic subjects

Discussion

This retrospective study was done to analyze different morphological types of anemia based on peripheral smear analysis of 200 patients suffering from anemia. The results of the study revealed that the microcytic hypochromic anemia was the most prevalent type in 48.0% of cases, while 21.0% of cases suffered from normocytic normochromic anemia and 14.0% of cases had dimorphic anemia. Similar results were obtained in a study carried out by Tefferi A. (2003) [14]. In his study, he noted that the peripheral smear analysis is one of the vital diagnostic methods used to identify morphological patterns of anemia and differentiate iron deficiency anemia from other diseases. Similarly, in a study carried out by Subramanian D. N. et al., (2009) [15] it was found that the prevalence of microcytosis and iron deficiency anemia was very high in their study group. These results have helped in achieving the aims of the study.

The analysis of demographic and hematological profiles obtained during the study pointed out that females made up for 59.0% of the total population, suggesting that anemia is more prevalent among women than men. Most patients were in the 21-30 years age group, and their condition was characterized by moderate anemia, which manifested itself through low levels of hemoglobin ranging from 8.1–10.0 g/dL. Mean value of hemoglobin in the patient group was estimated to be 8.24 ± 1.86 g/dL; moreover, low MCV, MCH, and MCHC suggested prevalence of hypochromic microcytic anemia. Similar observations have been made in the study conducted by Tettamanti M. et al., (2010) [16] according to which there existed great variations in terms of prevalence of anemia depending on the age and gender groups, with females being more susceptible to nutritional anemia. Moreover, in another study Malcovati L. et al. (2011) [17] emphasized the significance of hemoglobin and erythrocyte parameters in the diagnosis of anemia. The current results were similar to those obtained by Carlson A. P. et al. (2006) [18] in the analysis of retrograde evaluation of hematological parameters and their impact on anemia severity and prognosis.

The peripheral smear abnormalities identified in the current research work included anisocytosis (61.0%), microcytosis (56.0%), hypochromia (53.0%), and poikilocytosis (49.0%), suggesting notable variability in the morphology of red blood cells in anemic patients. Such abnormalities were helpful in distinguishing anemia types and determining the presence of nutritional deficiencies. Jones M. L. and Allison R. W. (2007) [19] noted similar results, highlighting the need for peripheral smear analysis in diagnosing the morphological changes in red blood cells like anisocytosis, poikilocytosis, and hypochromia. In addition to this, Oh R. C. et al. (2008) [20] pointed out that assessing the morphological changes in the peripheral smear was helpful in detecting the existence of macrocytosis and other abnormal changes in red blood cells even before conducting further tests. Thus, the results obtained in the current research work proved to be highly relevant in confirming the diagnostic value of peripheral smear assessment in classifying anemia types.

Conclusion

The present retrospective study successfully determined different morphological types of anemia using peripheral smear analysis in 200

patients presenting anemia at a tertiary care diagnostic center. The results of the study showed that microcytic hypochromic anemia was the commonest type of anemia in the study sample, suggesting that iron-deficiency anemia was prevalent among the patients. More females and younger adults were affected, with most of the participants presenting moderate anemia characterized by decreased hemoglobin concentration and abnormal red blood cell indices. Several morphological changes, including anisocytosis, microcytosis, hypochromia, and poikilocytosis, were observed in peripheral smears and played a crucial role in determining the morphological types of anemia. In addition, peripheral smear analysis was a reliable method for diagnosing anemia types due to its simplicity and cost-effectiveness in detecting hematological disorders. The purpose of the study was successfully achieved and confirmed the clinical significance of peripheral smear analysis in the diagnosis and morphological identification of anemia.

References

1. Aygen, B. İ. L. G. E. H. A. N., Doğanay, M., Sümerkan, B., Yildiz, O. R. H. A. N., & Kayabaş, Ü. (2002). Clinical manifestations, complications and treatment of brucellosis: a retrospective evaluation of 480 patients. Medecine et maladies infectieuses, 32(9), 485-493.
2. Buzgan, T., Karahocagil, M. K., Irmak, H., Baran, A. I., Karsen, H., Evirgen, O., & Akdeniz, H. (2010). Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. International journal of infectious diseases, 14(6), e469-e478.
3. Dhaliwal, G., Cornett, P. A., & Tierney Jr, L. M. (2004). Hemolytic anemia. American family physician, 69(11), 2599-2607.
4. Ertek, M., Yazgi, H., Kadanali, A., Özden, K., & TAŞYARAN, M. A. (2006). Complications of Brucella infection among adults: an 18-year retrospective evaluation. Turkish Journal of Medical Sciences, 36(6), 377-381.
5. Gehrs, B. C., & Friedberg, R. C. (2002). Autoimmune hemolytic anemia. American journal of hematology, 69(4), 258-271.
6. Kardos, G., Baumann, I., Passmore, S. J., Locatelli, F., Hasle, H., Schultz, K. R., ... & Niemeyer, C. M. (2003). Refractory anemia in childhood: a retrospective analysis of 67 patients with particular reference to monosomy 7. Blood, 102(6), 1997-2003.
7. Kimura, S. I., Oshima, K., Sato, K., Sato, M., Terasako, K., Nakasone, H., ... & Kanda, Y. (2010). Retrospective evaluation of the area over the neutrophil curve index to predict early infection in hematopoietic stem cell transplantation recipients. Biology of Blood and Marrow Transplantation, 16(10), 1355-1361.
8. Salisbury, A. C., Reid, K. J., Alexander, K. P., Masoudi, F. A., Lai, S. M., Chan, P. S., ... & Kosiborod, M. (2011). Diagnostic blood loss from phlebotomy and hospital-acquired anemia during acute myocardial infarction. Archives of internal medicine, 171(18), 1646-1653.
9. Scheinberg, P., Cooper, J. N., Sloand, E. M., Wu, C. O., Calado, R. T., & Young, N. S. (2010). Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. Jama, 304(12), 1358-1364.
10. Slaghekke, F., Kist, W. J., Oepkes, D., Pasman, S. A., Middeldorp, J. M., Klumper, F. J., ... & Lopriore, E. (2010). Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal diagnosis and therapy, 27(4), 181-190.
11. Spahn, D. R. (2010). Anemia and patient blood management in hip and knee surgery: a systematic review of the literature. Anesthesiology, 113(2), 482-495.
12. Thavendiranathan, P., Bagai, A., Ebidia, A., Detsky, A. S., & Choudhry, N. K. (2005). Do blood tests cause anemia in hospitalized patients? The effect of diagnostic phlebotomy on hemoglobin and hematocrit levels. Journal of general internal medicine, 20(6), 520-524.
13. Weiss, D. J., & Moritz, A. (2003). Equine immune-mediated hemolytic anemia associated with Clostridium perfringens infection. Veterinary clinical pathology, 32(1), 22-26.
14. Tefferi, A. (2003, October). Anemia in adults: a contemporary approach to diagnosis. In Mayo Clinic Proceedings (Vol. 78, No. 10, pp. 1274-1280). Elsevier.
15. Subramanian, D. N., Kitson, S., & Bhaniani, A. (2009). Microcytosis and possible early iron deficiency in paediatric inpatients: a retrospective audit. BMC pediatrics, 9(1), 36.
16. Tettamanti, M., Lucca, U., Gandini, F., Recchia, A., Mosconi, P., Apolone, G., ... & Riva, E. (2010). Prevalence, incidence and types of mild anemia in the elderly: the "Health and Anemia" population-based study. haematologica, 95(11), 1849.
17. Malcovati, L., Della Porta, M. G., Strupp, C., Ambaglio, I., Kuendgen, A., Nachtkamp, K., ... & Cazzola, M. (2011). Impact of the degree of anemia on the outcome of patients with myelodysplastic syndrome and its integration into the WHO classification-based Prognostic

Scoring System (WPSS). Haematologica, 96(10), 1433.
18. Carlson, A. P., Schermer, C. R., & Lu, S. W. (2006). Retrospective evaluation of anemia and transfusion in traumatic brain injury. Journal of Trauma and Acute Care Surgery, 61(3), 567-571.
19. Jones, M. L., & Allison, R. W. (2007). Evaluation of the ruminant complete blood cell count. Veterinary Clinics of North America: Food Animal Practice, 23(3), 377-402.
20. Oh, R. C., Holt, S. N., & Hitchcock, K. (2008). How do you evaluate macrocytosis without anemia? Journal of Family Practice, 57(8), 548-551.