

FREQUENCY AND MORPHOLOGICAL CLASSIFICATION OF ANEMIA BASED ON CBC AND PERIPHERAL SMEAR

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Abstract

Background: Anemia is one of the most prevalent hematological disorders worldwide, affecting approximately 1.62 billion people globally, with a disproportionately higher burden in low- and middle-income countries such as Pakistan. Despite its well-recognized national prevalence, region-specific data on the frequency and morphological classification of anemia in Dera Ismail Khan (a district characterized by limited healthcare access, poor nutritional status, and high rates of infectious disease) remain largely absent from the published literature.

Objectives: This study was conducted to determine the frequency and morphological classification of anemia using Complete Blood Count (CBC) parameters and peripheral blood smear examination in the population of Dera Ismail Khan, and to identify the predominant morphological type of anemia across different age groups.

Materials and Methods: A cross-sectional descriptive study was conducted at the Department of Pathology / Hematology Laboratory, DHQ Teaching Hospital MTI, Dera Ismail Khan, over a period of 4 months (March 2026 June 2026). A total of 500 confirmed anemic patients of all age groups, and both sexes were enrolled using a consecutive non-probability sampling technique. Anemia was confirmed based on WHO-defined hemoglobin cut-off values. Venous blood samples were collected in EDTA anticoagulant tubes and processed for CBC analysis using a calibrated automated hematology analyzer. Peripheral blood smears were prepared and stained with Leishman stain and examined under oil-immersion microscopy. Anemia was morphologically classified into microcytic (MCV < 80 fL), normocytic (MCV 80–100 fL), and macrocytic (MCV > 100 fL) types based on the combined interpretation of CBC indices and peripheral smear morphology. Data were analyzed using SPSS version 25.0, and results were expressed as frequencies and percentages.

Results: Of the 500 confirmed anemic patients, microcytic anemia was the most prevalent morphological type, identified in 384 patients (76.8%). Normocytic anemia was the second most frequent type, diagnosed in 104 patients (20.8%). Macrocytic anemia was the least common, observed in only 12 patients (2.4%). Age-stratified analysis revealed that the 20–39 years age group had the highest burden of anemia, comprising 290 patients (58.0%) of the total study population, followed by the 40–59 years group (134 patients; 26.8%), the 0–19 years group (50 patients; 10.0%), and the 60+ years group (26 patients; 5.2%). Microcytic anemia was the dominant morphological type across all age groups, ranging from 76.2% to 82.0%. Macrocytic anemia was absent in the 0–19 years group and was most relatively prominent in the 60+ years group (3.8%). Peripheral blood smear findings corroborated CBC-based morphological classification in all cases, with microcytic hypochromic red cells, anisocytosis, and poikilocytosis predominating in the microcytic group, normochromic normocytic morphology in the normocytic group, and macro-ovalocytes with hyper segmented neutrophils in several macrocytic cases.

Conclusion: Microcytic anemia (most likely driven predominantly by iron deficiency) is the overwhelmingly dominant morphological type of anemia in the population of Dera Ismail Khan, accounting for nearly 77% of all anemia cases. The heaviest disease burden falls on individuals in the 20–39 years age group, suggesting a major contribution from iron deficiency anemia in women of reproductive age. Normocytic anemia likely reflects the growing burden of chronic disease-related anemia, particularly in older age groups, while macrocytic anemia, though infrequent overall, warrants clinical attention in elderly patients where vitamin B12 deficiency is an

important reversible cause. The combined use of CBC and peripheral blood smear provides superior diagnostic accuracy compared to hemoglobin measurement alone and should be adopted as the standard approach for anemia diagnosis in this setting. These findings provide the first systematic, morphologically classified baseline dataset on anemia for Dera Ismail Khan and have direct implications for clinical management, laboratory practice, and public health policy in the district.

Keywords: Anemia, Complete Blood Count, Peripheral Blood Smear, Microcytic Anemia, Normocytic Anemia, Macrocytic Anemia, Iron Deficiency, Morphological Classification, Dera Ismail Khan, Pakistan

Background of the Study

Anemia is one of the most common hematological disorders encountered in clinical practice, defined as a reduction in hemoglobin (Hb) concentration, red blood cell (RBC) count, or hematocrit (Hct) below the normal range expected for a person's age, sex, and physiological status. Because hemoglobin is the principal carrier of oxygen in the blood, a deficiency in its concentration directly compromises the oxygen-carrying capacity of the circulatory system. This results in tissue hypoxia and triggers a wide spectrum of clinical manifestations, ranging from mild fatigue, pallor, and weakness to more severe presentations such as exertional dyspnea, palpitations, dizziness, and, in advanced or chronic cases, cardiac strain and failure.

Anemia is not a disease in itself but rather a sign of an underlying pathological process. Its causes are diverse and multifactorial, encompassing nutritional deficiencies, chronic blood loss, hemolysis, bone marrow disorders, chronic systemic disease, genetic hemoglobinopathies, and infectious or parasitic infestations. The clinical and public health importance of anemia lies not only in its high global prevalence but also in its disproportionate burden on vulnerable populations, including infants, children, women of reproductive age, pregnant women, and individuals from low socioeconomic backgrounds.

Classification and Diagnostic Approach

The diagnosis and classification of anemia rely primarily on two complementary laboratory tools: the Complete Blood Count (CBC) and the peripheral blood smear examination.

The CBC, generated through automated hematology analyzers, provides quantitative data on hemoglobin concentration, hematocrit, total RBC count, and the red cell indices Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). The MCV, in particular, serves as the cornerstone of the morphological classification of anemia, allowing it to be broadly categorized into three groups:

- Microcytic anemia (MCV below the normal reference range), most associated with iron deficiency, thalassemia trait, chronic disease, and sideroblastic anemia.
- Normocytic anemia (MCV within the normal reference range), often seen in anemia

of chronic disease, acute blood loss, hemolytic anemias, and marrow infiltrative or aplastic processes.

- Macrocytic anemia (MCV above the normal reference range), typically linked to vitamin B12 or folate deficiency, hypothyroidism, liver disease, myelodysplastic syndromes, and certain drug-induced effects.

Etiological Context in Pakistan

In the Pakistani context, anemia is driven by a complex interplay of factors. Nutritional deficiencies particularly of iron, vitamin B12, and folate remain the predominant cause, fueled by poor dietary diversity, low consumption of iron-rich foods, and limited awareness of nutritional needs, especially among women and children. Infectious diseases such as malaria, tuberculosis, and chronic urinary or gastrointestinal infections contribute to anemia of chronic disease, while parasitic infestations, particularly soil-transmitted helminths and hookworm in rural and peri-urban communities, cause chronic occult blood loss and iron depletion. Compounding these biological determinants are socioeconomic factors: poverty, food insecurity, low literacy, limited access to antenatal care, large family sizes, and inadequate healthcare infrastructure all act synergistically to sustain a high anemia burden, particularly in underdeveloped and semi-rural regions.

Rationale of the Study

Anemia continues to represent a major public health concern in Pakistan, with its burden disproportionately concentrated in underdeveloped and resource-limited areas where nutritional deficiencies, poor sanitation, and infectious or parasitic diseases remain widespread. Despite the well-recognized scale of the problem at the national level, there remains a distinct lack of updated, comprehensive, region-specific data describing the frequency and morphological classification of anemia in Dera Ismail Khan.

This study was therefore designed to:

- Generate baseline, region-specific statistical data on the frequency of anemia among the population of Dera Ismail Khan.
- Identify and describe the most frequently occurring morphological types of anemia (microcytic, normocytic, and macrocytic) within this population.
- Strengthen the understanding of the relationship between quantitative CBC parameters and qualitative peripheral smear findings, reinforcing the diagnostic value of combining both methods.

Statement of the Problem

Although the high prevalence of anemia in Pakistan is well established at the national level, there remains a significant lack of localized data describing the prevalence and morphological classification of anemia specifically within Dera Ismail Khan. This absence of region-specific evidence creates practical difficulties for clinicians and public health planners, who must often rely on extrapolated data from

other regions that may not accurately reflect the local disease burden, etiological pattern, or population-specific risk factors.

Objectives of the Study

General Objective

To determine the prevalence and morphological classification of anemia using Complete Blood Count (CBC) and peripheral blood smear examination among the population of Dera Ismail Khan.

Specific Objectives

1. To determine the frequency of anemia among the study population.
2. To categorize anemia into microcytic, normocytic, and macrocytic types based on CBC parameters, particularly the Mean Corpuscular Volume (MCV).
3. To assess red blood cell morphology including anisocytosis, poikilocytosis, hypochromia, and macrocytosis through peripheral blood smear examination.
4. To determine the correlation between CBC findings and peripheral blood smear morphological characteristics.
5. To identify the predominant morphological type of anemia among the local population of Dera Ismail Khan.

MATERIALS AND METHODS:

Study Design

The present study was conducted as a cross-sectional descriptive study. In a cross-sectional study design, data are collected from a defined population at a single point in time, or over a relatively short period, without any follow-up or longitudinal tracking of individual participants.

Study Setting

The study was conducted at the Department of Pathology / Hematology Laboratory, D.H.Q Teaching Hospital MTI, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

Study Duration

The study was carried out over a period of 4 months, from March 2026 to June 2026.

Target Population

The target population for this study comprised all patients of either sex and any age group who attended the Outpatient Department (OPD) or were admitted to the Inpatient Department (IPD) of D.H.Q Teaching Hospital, Dera Ismail Khan

Sampling Technique

A non-probability consecutive sampling technique was employed for patient recruitment. Under this approach, all patients who met the pre-defined inclusion criteria and presented to the hematology laboratory during the study period were enrolled sequentially and continuously until the required sample size was achieved.

Sample Size

The minimum required sample size was determined using the standard formula for estimating a population proportion:

$$n = Z^2 \times P \times (1 - P) / d^2$$

Where:

- n = minimum required sample size
- Z = Z-statistic corresponding to the 95% confidence level = 1.96.
- P = estimated prevalence of anemia in the study population = 0.50 (50%), taken as 50% in the absence of a prior local prevalence estimate for Dera Ismail Khan, as this value maximizes the estimated sample size and minimizes the risk of under-sampling
- d = acceptable margin of error = 0.05 (5%)

Calculation:

$$n = (1.96)^2 \times 0.50 \times (1 - 0.50) / (0.05)^2 \quad n = 3.8416 \times 0.25 / 0.0025 \quad n = 0.9604 / 0.0025$$

$$n = 384 \text{ (minimum required)}$$

A total of 500 confirmed anemic patients were ultimately enrolled in the study, exceeding the calculated minimum of 384.

Inclusion Criteria

Patients were considered eligible for enrollment in the study if they satisfied all the following inclusion criteria:

1. Patients of all age groups (including neonates, infants, children, adolescents, adults, and elderly individuals) and of both sexes (male and female).
2. Patients confirmed as anemic based on hemoglobin concentration falling below the WHO-defined sex- and age-specific cut-off values for anemia, as follows:
3. Patients referred to the hematology laboratory for CBC testing by a registered medical practitioner during the defined study period.
4. Patients or their legal guardians (in the case of minors) willing to provide informed consent for participation in the study.
5. Patients from whom a satisfactory venous blood sample of adequate volume could be collected for both CBC analysis and peripheral smear preparation.

Exclusion Criteria

The following categories of patients were excluded from the study to minimize confounding of CBC indices and peripheral smear morphology:

1. Patients who had received a blood transfusion within the preceding three months, as transfused red cells may alter CBC parameters and peripheral smear morphology, obscure the patient's true hematological status and prevent accurate morphological classification.
2. Patients who were currently receiving hematinics (including oral or parenteral iron preparations, vitamin B12 injections, or folate supplementation) at the time of blood sample collection, as active treatment may partially normalize CBC indices while the

underlying deficiency state persists, producing atypical or unclassifiable morphological patterns.

3. Patients with a known pre-existing diagnosis of hematological malignancy, including acute or chronic leukemia, lymphoma, multiple myeloma, or myelodysplastic syndrome, as these conditions independently alter red cell morphology and CBC parameters through mechanisms unrelated to the etiologies of common nutritional or disease-related anemia.
4. Patients with end-stage renal disease on maintenance hemodialysis, as erythropoietin deficiency associated with advanced renal failure produces a characteristic normocytic anemia that would confound the morphological classification exercise.
5. Patients diagnosed with primary bone marrow failure (aplastic anemia) or infiltrative bone marrow disease, as these conditions produce distinctive pancytopenic pictures that fall outside the scope of nutritional and morphological anemia classification.
6. Patients who refused to participate in the study or from whom informed consent could not be obtained.
7. Blood samples that were hemolyzed, clotted, or otherwise unsuitable for accurate CBC analysis or peripheral smear preparation.

Data Collection Instrument

A pre-designed, structured data collection proforma was developed specifically for this study, validated by the research supervisor, and piloted on a small group of patients prior to the commencement of formal data collection. The proforma was used to record all relevant demographic, clinical, and laboratory data for each enrolled patient. The following information was captured on the proforma for every patient:

- Patient identification: name, age, sex.
- CBC parameters: Hb, Hct, RBC count, MCV, MCH, MCHC, RDW, WBC count, platelet count (as generated by the automated analyzer)
- Peripheral blood smear findings: red cell size, color, shape, and any abnormal morphological features observed on microscopy
- Morphological classification of anemia: microcytic, normocytic, or macrocytic, based on combined CBC and smear interpretation

RESULTS AND DISCUSSION

This chapter presents the results obtained from the analysis of Complete Blood Count (CBC) parameters and peripheral blood smear examination of anemic patients enrolled in this study from Dera Ismail Khan. The findings are organized systematically to address each specific objective of the study. Following the presentation of results, a detailed discussion is provided in which the findings are interpreted in the context of the existing

literature on the prevalence and morphological classification of anemia in Pakistan and globally. The chapter concludes with a synthesis of the overall study findings and their clinical and public health implications.

A total of 500 confirmed anemic patients were enrolled in this study based on WHO-defined hemoglobin cut-off values. Each patient's blood sample was analyzed for CBC parameters, and peripheral blood smear examination was performed to determine the morphological type of anemia. Patients were categorized into three morphological groups Microcytic Anemia, Normocytic Anemia, and Macrocytic Anemia and further stratified into four age brackets: 0–19 years, 20–39 years, 40–59 years, and 60+ years.

Overall Distribution of Morphological Types of Anemia

The primary finding of this study is the overall distribution of the three morphological types of anemia across the entire study population of 500 patients. As summarized in Table 4.1 below, microcytic anemia was overwhelmingly the most prevalent morphological type, followed by normocytic anemia, and macrocytic anemia was the least frequently observed type.

Table 4.1: Overall Distribution of Morphological Types of Anemia (n = 500)

Morphological Type	Number of Cases	Percentage (%)
Microcytic Anemia	384	76.8%
Normocytic Anemia	104	20.8%
Macrocytic Anemia	12	2.4%
Total	500	100%

Out of the total 500 anemic patients, 384 patients (76.8%) were classified as having microcytic anemia based on MCV values below 80 fL, confirmed by peripheral smear findings of microcytic hypochromic red cells with or without anisocytosis and poikilocytosis. 104 patients (20.8%) were classified as having normocytic anemia, with MCV values in the normal range of 80–100 fL and peripheral smear showing normocytic normochromic red cell morphology. 12 patients (2.4%) were found to have macrocytic anemia, with MCV values exceeding 100 fL and peripheral smear demonstrating enlarged red cells, with hyper segmented neutrophils noted in several cases suggestive of an underlying megaloblastic process.

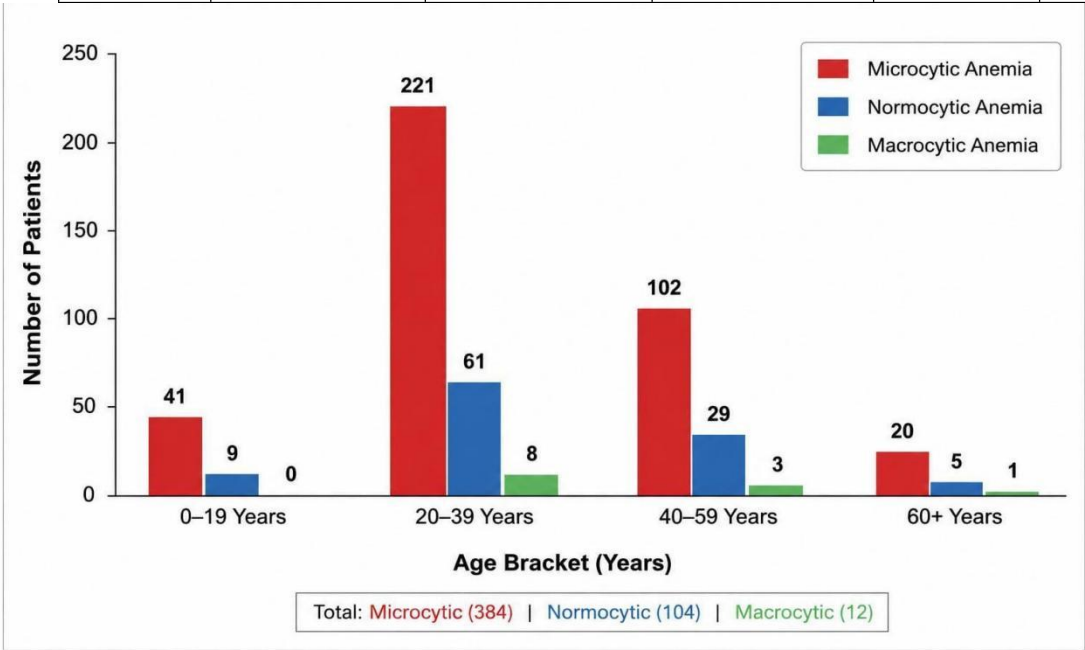
These findings unequivocally establish microcytic anemia as the dominant morphological type in the anemic population of Dera Ismail Khan, accounting for approximately three-quarters of all diagnosed anemia cases.

Distribution of Anemia Types by Age Bracket

A detailed breakdown of anemia types across different age groups was performed to identify whether the prevalence and morphological pattern of anemia varied with age. Table 4.2 presents the complete age-stratified distribution of anemia types.

Figure 4.1: Distribution of Morphological Types of Anemia by Age Bracket

Age Bracket	Microcytic Anemia	Normocytic Anemia	Macrocytic Anemia	Total per Group	% of Total
0–19 Y	41	9	0	50	10.0%
20–39 Y	221	61	8	290	58.0%
40–59 Y	102	29	3	134	26.8%
60+ Y	20	5	1	26	5.2%
Total	384	104	12	500	100%



Age Group 0–19 Years

A total of 50 patients (10.0%) belonged to the youngest age group of 0 to 19 years. Within this group, the vast majority 41 patients (82.0%) had microcytic anemia, while 9 patients (18.0%) had normocytic anemia. Notably, no cases of macrocytic anemia were

detected in this age group. The high prevalence of microcytic anemia in children and adolescents is consistent with the recognized burden of iron deficiency in this age group in Pakistan, driven by poor dietary diversity, rapid growth spurts increasing iron demand, and the high prevalence of parasitic infestations such as hookworm, which cause chronic occult intestinal blood loss and progressive iron depletion. The absence of macrocytic anemia in this group is also expected. As vitamin B12 and folate deficiencies the principal causes of macrocytic/megaloblastic anemia are relatively uncommon in the pediatric age group compared to older adults in this region.

Age Group 20–39 Years

The 20 to 39 years age bracket was by far the largest group, comprising 290 patients (58.0%) of the total study population, making it the dominant age group in this study. Within this group:

- 221 patients (76.2%) had microcytic anemia
- 61 patients (21.0%) had normocytic anemia
- 8 patients (2.8%) had macrocytic anemia

The exceptionally high total number of anemic patients in this age group is a significant finding. This age range corresponds to the peak reproductive years in women, a period of particularly high risk for iron deficiency anemia due to monthly menstrual blood loss, frequent pregnancies, increased iron demands during gestation and lactation, and often inadequate dietary iron intake. In the socioeconomic context of Dera Ismail Khan where large family sizes, closely spaced pregnancies, limited access to antenatal care, and low awareness of nutritional requirements are prevalent this finding is not surprising and aligns with national data showing women of reproductive age as the most affected demographic group. The 8 cases of macrocytic anemia in this group likely reflect dietary deficiencies of vitamin B12 and folate, which may also co-exist with iron deficiency in women with poor overall nutritional status.

Age Group 40–59 Years

The 40 to 59 years age group comprised 134 patients (26.8%) of the study population:

- 102 patients (76.1%) had microcytic anemia
- 29 patients (21.6%) had normocytic anemia
- 3 patients (2.2%) had macrocytic anemia

The proportion of normocytic anemia in this age group was slightly higher than in the younger groups, which is consistent with the increasing prevalence of chronic systemic diseases such as diabetes mellitus, chronic kidney disease, rheumatoid arthritis, and chronic infections in middle-aged patients. Anemia of chronic disease, which characteristically produces a normocytic normochromic pattern on peripheral smear, is

closely linked to the presence of these underlying conditions. Microcytic anemia remains the dominant type even in this group, however, which may reflect the persistence of long-standing iron deficiency in women who have had repeated pregnancies throughout the 20–39-year period, carrying the deficit into their middle years.

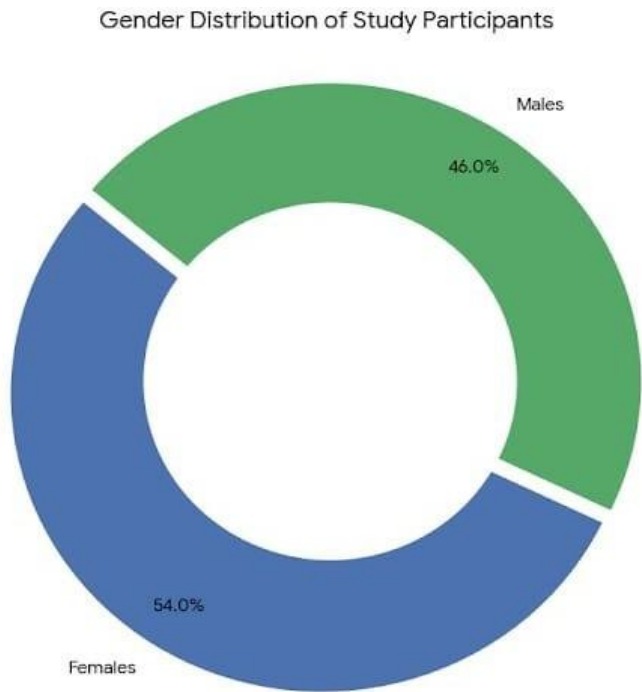
Age Group 60+ Years

The oldest age group of 60 years and above was the smallest, comprising only 26 patients (5.2%) of the total sample:

- 20 patients (76.9%) had microcytic anemia
- 5 patients (19.2%) had normocytic anemia
- 1 patient (3.8%) had macrocytic anemia

Although small in absolute number, the presence of macrocytic anemia in this age group with a relative proportion of 3.8% was the highest across all age groups. This is in keeping with the well-established increase in the prevalence of vitamin B12 deficiency in the elderly, driven by atrophic gastritis, reduced intrinsic factor secretion, and decreased absorption of dietary cobalamin. The normocytic cases in this group likely represent anemia of chronic disease and possibly early-stage renal anemia, both of which are more prevalent in the elderly population.

Figure 4.2: Gender Distribution of Study Participants



Discussion

Predominance of Microcytic Anemia

The finding that microcytic anemia accounts for 76.8% of all anemia cases in this study is both clinically significant and epidemiologically consistent with the broader published literature from Pakistan and comparable low- and middle-income settings. The peripheral smear findings of microcytic hypochromic red cells the hallmark of iron deficiency anemia and thalassemia trait corroborated the CBC classification in the overwhelming majority of these cases.

Iron deficiency anemia is well-established as the single most common cause of microcytic hypochromic anemia in developing countries (WHO, 2021). In Pakistan, multiple studies have consistently confirmed the dominance of microcytic hypochromic anemia in clinical and community-based samples. Ahmed et al. (2018), in a study conducted in Karachi, reported microcytic hypochromic anemia as the most prevalent morphological type among anemic patients, with iron deficiency identified as the principal underlying cause. Similarly, Haque et al. (2019) found microcytic anemia to be the most common type in a hospital-based study in Peshawar, further supporting the geographic consistency of this pattern across different regions of Pakistan.

The present findings from Dera Ismail Khan add to this body of evidence and confirm that microcytic anemia (almost certainly driven predominantly by iron deficiency) represent the primary form of anemia in this underserved district. The high proportion of cases in women of reproductive age (20–39 years, the largest group) strongly implicates menstrual blood loss, repeated pregnancies, and inadequate dietary iron intake as the principal contributing factors in this local population. In addition, the high prevalence of microcytic anemia in the 0–19-year age group reflects the well-documented vulnerability of children and adolescents in resource-limited settings, where dietary diversity is often limited and parasitic infestations are prevalent.

Conclusions:

Anemia is Highly Prevalent in Dera Ismail Khan

The results of this study confirm that anemia constitutes a significant hematological burden in the population of Dera Ismail Khan. A total of 500 confirmed anemic patients were enrolled over the study period from a single tertiary care institution, reflecting a high baseline frequency of the condition in the local population. Given that this study was hospital-based, the true community prevalence is likely even higher, as a considerable proportion of anemic individuals particularly in rural and peri-urban areas of the district may not seek formal medical care due to limited healthcare access, financial

constraints, or cultural factors. These finding positions anemia as a priority public health concern in Dera Ismail Khan that requires urgent and sustained attention from both clinical and public health perspectives.

Microcytic Anemia is the Dominant Morphological Type

The most definitive conclusion of this study is that microcytic anemia is by far the most prevalent morphological type of anemia in the study population, accounting for 384 cases out of a total of 500 (76.8%). This finding was consistent across all age groups studied, with microcytic anemia representing the dominant category in the 0–19 years group (82.0%), the 20–39 years group (76.2%), the 40–59 years group (76.1%), and the 60+ years group (76.9%). The uniformity of this pattern across age groups strongly indicates that the underlying etiological factor most likely iron deficiency operates broadly across the population regardless of age, rather than being confined to a single demographic subgroup.

Recommendations for Future Research

1. **Conduct a Community-Based Prevalence Study** The present hospital-based study, while providing important baseline data, is inherently limited to the population attending the study institution. A large-scale, community-based cross-sectional prevalence study encompassing both urban and rural areas of Dera Ismail Khan district including individuals who do not seek hospital-based care would provide a more accurate and representative estimate of the true population prevalence of anemia and its morphological types.
2. **Include Confirmatory Biochemical Tests** Future studies in this region should supplement CBC and peripheral smear findings with confirmatory biochemical tests including serum ferritin, serum iron, total iron-binding capacity (TIBC), serum vitamin B12, serum folate, and red blood cell folate assays to definitively establish the etiological diagnosis in each morphological category. This would allow a more precise characterization of the causes of microcytic, normocytic, and macrocytic anemia in the local population, beyond the morphological classification achievable with CBC and smear alone.
3. **Investigate the Contribution of Thalassemia Trait** Given the known high prevalence of beta-thalassemia carrier state in Pakistan's population, future studies in Dera Ismail Khan should incorporate hemoglobin electrophoresis or high-performance liquid chromatography (HPLC) into the anemia workup protocol. This would allow the differentiation of thalassemia trait from iron deficiency anemia two conditions that present with an identical microcytic hypochromic peripheral smear and would provide valuable data on the burden of hemoglobinopathy-related anemia in the district.

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