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Prevalence of Anemia and Patterns of Red Cell Morphology Among Primary School Boys in Rural Pakistan: A Cross-Sectional Study

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Abstract—Objective*: Anemia is a major public health problem in school-aged children and may impair physical performance, attention and academic achievement. This study evaluated the prevalence and morphological pattern of anemia by red cell indices among primary school boys of rural areas in Pakistan.* ***Materials and Methods****: We conducted a school-based cross-sectional study in a school enrolled from 84 boys aged 5-11 years and a government primary school in Tariqabad Shergarh Mardan, Khyber Pakhtunkhwa region, Pakistan. We obtained venous blood in Ethylenediaminetetraacetic acid (EDTA) capped tubes and analyzed hemoglobin and red cell indices by using an automated hematology analyzer. We categorized anemia based on hemoglobin cutoffs of the World Health Organization and defined the morphological patterns by the red cell indices.* ***Results****: Among 84 study participants, 32 (38.0%) of them were found to be anemic. However, prevalence differed by age with the highest prevalence being at 6 years (91.7%) and lowest at 11 years (8.3%). Microcytic hypochromic anemia was the most common pattern affecting 24 out of 32 anemic children (75.0%) followed by normocytic hypochromic anemia in 7 (21.9%) and normocytic normochromic anemia in 1 (3.1%). Mild anemia was found in 14 children (43.8%) and moderate anemia was found in 18 (56.3%); there was no severe anemia.* ***Conclusion****: Anemia was prevalent, with significant variation by age and dominance of microcytic hypochromic morphology, indicating the need for early screening and targeted interventions for nutritional intervention.*

Keywords*— Anemia; Hemoglobin; Red blood cells; Red cell indices; Microcytic hypochromic anemia; School children.*

I. Introduction

Hemoglobin (Hb) and red blood cells (RBCs) are fundamental in aerobic metabolism because they transport oxygen from the lungs to peripheral tissues, and helps to remove carbon dioxide in order to maintain normal cell function and acid-base balance [1-3]. In a clinical practice, hemoglobin concentration is the most commonly used laboratory indicator for determination of anemia, estimation of adequacy of erythropoiesis, tracking of blood loss, and transfusion decision, if needed when required [4, 5]. During childhood rapid growth raises the nutritional demands and makes the disturbances of hemoglobin synthesis and RBC production particularly important. Consequently, anemia during the school years may impair the physical endurance, attention, cognitive development, and school performance [6, 7].

Anemia is a major global public health problem and particularly affects populations living in low and middle income countries. The World Health Organization (WHO) has defined anemia using age and sex-specific hemoglobin cut-offs and classified the severity to facilitate surveillance, comparison between populations and evaluation of prevention programs [8, 9]. Global estimates illustrate that populations in significant proportion of anemia, which may not experience the same burden and causes in different regions. Nutritional deficiencies, infections, inflammation, parasitic infestation and hemoglobin disorders are all factors that contribute to this variability [10]. In children, anemia symptoms may include fatigue, low tolerance and poor concentration, and less favorable school performance. Even iron deficiency in the absence of overt anemia, may have a negative impact on neurocognitive function and hence, supports the value of early detection in school aged populations [11, 12].

Because there are various causes of anemia and the symptoms overlap, it is essential to characterize the anemia using laboratory methods to ensure proper evaluation and follow up. Iron deficiency is the leading cause of microcytic hypochromic anemia worldwide, however anemia can also be caused by inflammation or chronic disease, hemoglobinopathies, parasitic infection and vitamin B12 or folate deficiency; the latter being more likely to cause macrocytosis [13, 14]. In settings where ferritin, transferrin saturation, vitamin B12, folate or hemoglobin electrophoresis are not routinely available, complete blood count (CBC) parameters and RBC indices are a practical first-line method for classifying the patterns of anemia and directing differential diagnosis and referral [15].

RBC indices, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are used to summarize RBC size and hemoglobinization and aid in the morphological

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classification of anemia as microcytic, normocytic and macrocytic [16]. Since Wintrobe first described these indices, they have continued to be central to the interpretation of anemia because they link quantitative measurements to potential pathophysiologic mechanisms [17]. Modern hematology analyzers are used to create these indices quickly and reproducibly from routine CBC measurements. Their more practical use is to look for patterns that are indicative of iron deficiency, macrocytic processes, or anemia of chronic disease. The additional parameters such as red cell distribution width may provide even finer refinement of the interpretation as an indication of anisocytosis, sometimes before significant changes in MCV can be discerned [18]. Although the use of RBC indices cannot replace etiologic testing, these indices can be used to identify high-risk populations, to help provide low-cost triage, and to help standardize school-based anemia screening in resource-limited settings [19].

Pakistan bears a high burden of anemia though, prevalence and patterns vary according to age, geographical, dietary and infective exposure and access to healthcare. National surveys give good baseline estimates but do not necessarily reflect significant local variation, particularly in rural and peri-urban communities [20, 21]. School based data that incorporate hemoglobin measurement and analysis of RBC indices to classify anemia could therefore be used to further strengthen district level planning based on the occurrence of the common anemia patterns, for cost-effective action on screening. Several studies have been published from Khyber Pakhtunkhwa and nearby areas showing the presence of anemia and iron deficiency in school aged children, however, the findings of published studies vary as to sampling frame, diagnostic technique, and the extent of morphological characterization [22, 23].

In the present study, we studied boys in a government primary school, Tariqabad Shergarh, District Mardan, Khyber Pakhtunkhwa, Pakistan. Using an automated hematology analyzer, we estimated hemoglobin and RBC parameters to calculate the prevalence of anemia and classify the morphological patterns according to routine CBC indices. We also looked at the RBC status count in morphology groups to offer further context for the interpretation of microcytic hypochromic patterns that may either reflect iron deficiency, thalassemia trait, or be due to any other cause. Our objectives were to determine the prevalence of anemia among this school population and also to classify the anemia morphologically by using RBC indices obtained from CBC testing.

II. Materials and Methods

A. Study design

We performed a school based cross sectional study on male children aged 5-11 years enrolled in a government primary school of Tariqabad Shergarh, District Mardan, Khyber Pakhtunkhwa, Pakistan. The aim of the study was to know the prevalence of anemia and the morphological pattern using hemoglobin levels with red cell indices. We collected the blood (84 venous blood samples) over a period of 6 months. Each research subject was assigned a unique study code in order to ensure confidentiality and assure accurate linkage of data forms to lab results.

B. Data collection and sample handling

We used a structured proforma to record age and hematologic findings of this participant. We measured venous blood into ethylenediaminetetraacetic acid (EDTA) blood collection tubes by following aseptic procedures. After collection, we gently inverted all tubes to get blood and anticoagulant mixed in it. We shipped the samples in sealed biohazard bags and processed the samples within acceptable stability limits. We exclude samples where there is evidence of clotting, leaking or labeling problems.

C. Laboratory analysis

We examined all specimens at Dymind automated hematology analyzer of Saeed Medical complex, Mardan. We measured hemoglobin, RBC count, MCV, MCH and MCHC. The analyzer was a hematocounter with electrical impedance as the method of determining RBC count and cell volume, and a Colorimetric method at 525 nm for determining hemoglobin. Using these parameters, we were able to classify anemia morphologically with reference to standard reference ranges recorded in the study proforma.

D. Statistical analysis

We spread all data into a study data set, and conducted descriptive analysis. In that case, we expressed categorical variables as frequencies and percentages. We classified anemia based on hemoglobin level and described morphological patterns based on the values of MCV, MCH and MCHC. We recorded all samples with the same criteria to help to make classification the same.

III. Results

We examined 84 records of children aged 5-11 years who had a complete blood count. The sample was distributed equally among all age groups where the number of children in each group was 12 (14.28%) (Table I).

TABLE I. Shows the Total samples of School Children

S. No	Age	No of Sample
1	5	12 (14.28%)
2	6	12 (14.28%)
3	7	12 (14.28%)
4	8	12 (14.28%)
5	9	12 (14.28%)
6	10	12 (14.28%)
7	11	12 (14.28%)
	Total Samples	84 (100%)

Overall, 32 children out of 84 (38.0%) were anemic while 52 (61.9%) had normal hemoglobin levels. The prevalence of anemia varied with age (Table II). The highest prevalence was at 6 years of age with 11 of the 12 children (91.67%) found to be anemic. This was followed by 5 years and 6 children out of 12 (50.0%) were classified as anemic. Intermediate levels of prevalence's were noted at 8 years (5/12; 41.67%) and at 9 years of age (4/12; 33.3%). Lower prevalence's were observed at 10 years (3/12; 25.0%), 7 years (2/12; 16.67%) and the lowest prevalence was found at 11 years (1/12; 8.33%).

As for the morphological pattern, microcytic hypochromic anemia was the most prevalent presentation in the anemic children, presenting in 24 of 32 cases (75.0%). Normocytic

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hypochromic anemia was present in 7 (21.88%), and the normocytic normochromic in 1 (3.12%) case, respectively (Table III).

TABLE II. Illustrate Age Wise Distribution of Anemia

S. No	Age	No of Samples	Anemic	Normal
1	5	12	6(50%)	6 (50%)
2	6	12	11 (91.67%)	1 (8.33%)
3	7	12	2 (16.67%)	10 (83.33%)
4	8	12	5 (41.67%)	7 (58.33%)
5	9	12	4 (33.3%)	8 (66.7%)
6	10	12	3 (25%)	9 (75%)
7	11	12	1 (8.33%)	11(91.67%)
	Total	84	32 (38%)	52 (61.9%)

TABLE III. Illustrate Distribution of anemia by red cell indices

S. No	Microcytic hypochromic	Normocytic hypochromic	Normocytic normochromic
1	24/32 (75%)	7/32(21.88%)	1/32 (3.12%)

When it comes to their degree, 14 (43.8%) of the 32 anemic children had a mild degree of anemia; 18 (56.3%) had a moderate degree of anemia. We did not find any severe anemia (Table IV).

TABLE IV. Illustrate Severity distribution among anemic participants

S. No	Severity	N	% of anemic
1	Mild	14	43.8%
2	Moderate	18	56.3%
3	Severe	0	0.00%

Assessment of RBC count by morphology revealed that there was further heterogeneity within the microcytic hypochromic group (Table V). Amongst these 24 children, 15 (62.50%) had normal RBC count and 9 (37.50%) had abnormal RBC count. In contrast, all children with normocytic hypochromic anemia and the 1 child with normocytic normochromic anemia had normal RBC counts. These results suggest that anemia was prevalent, age-related and mainly microcytic hypochromic in the tested population.

TABLE V. Shows Normal vs abnormal RBC count among anemic children by morphology

S. No	Morphology	N	Normal RBCs count	Abnormal RBCs count
1	Microcytic hypochromic	24	15 (62.50%)	9 (37.50%)
2	Normocytic hypochromic	7	7 (100.00%)	0 (0.00%)
3	Normocytic normochromic	1	1 (100.00%)	0 (0.00%)

IV. Discussion

In this school based cross sectional study on boys 5-11 years of age, from Tariqabad Shergarh, Mardan, the incidence of anemia was high, i.e., 32 of 84 boys (38.0%) were found to be suffering (Table II). This burden is important from a programmatic point of view because WHO deems a prevalence of 20%-39.9% to be a moderate public health problem and helps to argue for the necessity of prevention, early detection, and targeted intervention, but not merely relying on the presentation of symptoms alone[8]. Our findings therefore strengthen the case for the importance of anemia as a child health priority in this setting and demonstrate local evidence that may be used in support of school linked screening strategies.

We found significant differences in the prevalence of anemia between age groups (Table II). The highest percentage was at 6 years while the lowest percentage was at 11 years. This finding and others support the possibility of anemia risk clustering during the early primary school years. It is also possible that younger children have increased micronutrient requirements for rapid growth, more variable dietary intake, and data which may vary in their exposure to infection or inflammation. Due to our small sample size for each age group and the fact that our study was a cross-sectoral study these age specific patterns should be viewed with caution. Nevertheless, they point to an important hypothesis for larger studies in the future and the idea that early school entry is perhaps an appropriate point to screen for.

A key contribution of this study is the fact that we did not stop at hemoglobin measurement and classified anemia morphologically using RBC indices (Table III). Microcytic hypochromic anemia was three times more frequent, indicating that iron restricted erythropoiesis is likely to be a major contributor in this population. This interpretation is consistent with nutritional and environmental risks that are common in low resource communities. However, it is not possible to offer etiology solely based on morphology. Microcytosis may also indicate some hemoglobinopathies or combination of causes of anemias. Therefore, the value of RBC index-based classification in this scenario is primarily in better triaging, focusing nutritional assessment and identifying those children who may be suitable candidates for confirmatory tests, where possible.

We further assessed the status of the RBCs in morphological categories (Table V). Among children with microcytic hypochromic anemia, it was found that most of them had normal RBC counts, but more than one third of them had abnormal counts. By contrast, the RBC counts of all the children with normocytic hypochromic anemia and 1 normocytic normochromic case were normal. This finding indicates heterogeneity of this microcytic group. In practical aspects, microcytosis with variable Patterns of RBC can be reasons for more careful follow up especially where iron deficiency and inherited microcytosis may coexist. Although we did not measure ferritin, C-reactive protein, transferrin saturation, or hemoglobin variants, the evidence of variation in RBC count helps put into perspective the cautious interpretation of microcytic hypochromic anemia and suggests the potential value of low-cost CBC-based discrimination tools in future work[24, 25].

The pattern of severity in our study is also of public health importance. All cases of anemia were mild and moderate, and no severe anemia was noted (Table IV). This finding should not diminish concern, because mild and moderate anemia can still have an impact on child health, functional performance and learning. At the same time, not having severe anemia may show the possibility of early intervention before more advanced disease occurs. In a school environment, this profile facilitates on-site practical responses, including screening, nutritional counseling, and appropriate supplementation as well as follow-up referral for selected cases.

The findings also highlight the usefulness of the routine hematology services provided at district level. In many low-resource settings an accessibility advantage of CBC testing over biochemical assessment of iron status or special testing for hemoglobin variants is present. A morphology-based approach can therefore be used to improve the quality of first-line evaluation without significantly increasing cost. By identifying children with microcytic patterns, as well as abnormal RBC count profiles, schools and local laboratories may be in a better prepared position to focus on specific interventions to increase the child's likelihood of success with dietary and supplemental nutritional interventions, and/or refer some children for further on assessment if resources are available. This approach is of particular use in communities where delayed attendance at health facilities is a problem and school-based contact provides an efficient opportunity to offer early identification and follow up care.

Our study has limitations. First, it studied only boys from one school, which limits the generalizability of the study to girls, other schools and other districts. Second, we relied on estimations derived from CBC with no biochemical or molecular confirmation of the etiology, meaning that what we found is descriptive of patterns, not causes. Third, in the cross-sectional design, no causal inference can be made. Despite these limitations, the study is useful in providing local evidence, and demonstrates how routine LDH indices may be useful in reinforcing school-based evidence of anemia in areas where diagnostic resources are limited.

V. Conclusion

This study demonstrates that anemia is a pertinent health issue among boys of primary schools in this rural region of Mardan, Pakistan. More than one third of participants had an anemia with a significant variation with regard to age and predominant microcytic hypochromic morphology. Most of the cases were mild and moderate, indicating a high, but possible, burden if caught early. These results have supported school-based screening, nutrition emphasizing prevention and structured follow-up of the microcytic cases, especially for those in low resource settings where the routine confirmatory testing is not readily available. Larger studies that involve girls and multiple schools as well as additional laboratory evaluation are required to clarify etiology and promote more precise prevention and referral strategies.

References

[1]. W. Grzegorzewski, et al., "Hemoglobin-Based Oxygen Carriers: Selected Advances and Challenges in the Design of Safe Oxygen Therapeutics (A Focused Review)," *International Journal of Molecular Sciences*, vol. 26, issue 19, pp. 9775, 2025.

[2]. E. Hopkins, T. Sanvictores, and S. Sharma, Physiology, acid base balance, 2018.

[3]. S. Anbalagan, "Hemoglobin as an oxygen gasoreceptor," *Acta Biochim Pol*, vol. 72, pp. 15546, 2025.

[4]. E. Brihi, Normal and abnormal complete blood count with differential. 2024.

[5]. J. L. Carson, et al., "Red Blood Cell Transfusion: 2023 AABB International Guidelines," *Jama*, vol. 330, issue 19, pp. 1892-1902, 2023.

[6]. J.W. De Neve, et al., "Relationship between adolescent anemia and school attendance observed during a nationally representative survey in India," Commun Med (Lond), vol. 4, issue 1, pp. 112, 2024.

[7]. W.H. Organization, Daily iron supplementation in children 6–23 months of age, 2022.

[8]. W.H. Organization, Guideline on haemoglobin cutoffs to define anaemia in individuals and populations, 2024: World Health Organization.

[9]. W.M. Gardner, et al., "Prevalence, years lived with disability, and trends in anaemia burden by severity and cause, 1990–2021: Findings from the Global Burden of Disease Study 2021," *The Lancet Haematology*, vol. 10, issue 9, pp. e713-e734, 2023.

[10]. G.A. Stevens, et al., "National, regional, and global estimates of anaemia by severity in women and children for 2000–19: A pooled analysis of population-representative data," *The Lancet Global Health*, vol. 10, issue 5, pp. e627-e639, 2022.

[11]. D. Fiani, et al., "Iron Deficiency Without Anemia and Reduced Basal Ganglia Iron Content in Youths. JAMA Network Open, vol. 8, issue 6, pp. e2516687-e2516687, 2025.

[12]. Y. Cheung, et al., "Impact of iron deficiency on attention among school-aged adolescents in Hong Kong," *Hong Kong Medical Journal*, vol. 31, issue 2, pp. 139-147, 2025.

[13]. H.S. Chaudhry and M.R. Kasarla, Microcytic hypochromic anemia. 2017.

[14]. S.S. Shafaat, et al., "A Cross-Sectional Study for the Spectrum of Clinical Diagnosis in Patients Presenting with Macrocytosis," Cureus, vol. 16, issue 2, 2024.

[15]. A. Alkamali, et al., "Evaluating the Mentzer index for screening of iron deficiency anemia and beta thalassemia among infants visiting primary health centers in Dubai, United Arab Emirates: A retrospective study," *Cureus*, vol. 16, issue 8, 2024.

[16]. H. Choi, et al., "Mean corpuscular volume as a prognostic factor for 30-day mortality in major trauma patients: a retrospective cohort study," *Scientific reports*, vol. 14, issue 1, pp. 3951. 2024.

[17]. R.C. Shean, M.C. Williams, and A.V. Rets, "Advances in Hematology Analyzers Technology," *Clinics in Laboratory Medicine*, vol. 44, issue 3, pp. 377-386, 2024.

[18]. V.S. Dhurde, et al., "Diagnostic performance of red cell indices in detecting iron deficiency and iron deficiency anemia among rural adolescent girls aged 14–19 years in Nagpur District," *PLOS Global Public Health*, vol. 5, issue 9, pp. e0005108, 2025.

[19]. V. Roccaforte, et al., "Use of iron deficiency flag of the hematology analyzer Sysmex XN-3000 for early iron-deficient erythropoiesis diagnosis," *Journal of Laboratory and Precision Medicine*, 2024. 9.

[20]. N. Wing, Ministry of National Health Services, Regulations and Coordination, Government of Pakistan. National nutrition survey, 2018.

[21]. A. Habib, et al., "Prevalence and risk factors for iron deficiency anemia among children under five and women of reproductive age in Pakistan: Findings from the National Nutrition Survey 2018," *Nutrients*, vol. 15, issue 15, pp. 3361. 2023.

[22]. M.S. Sayab Khan, et al., "Prevalence of iron deficiency anemia in school going children at district Bannu Khyber Pakhtunkhwa, Pakistan," *Pakistan Journal of Medical & Health Sciences*, vol. 16, issue 11, pp. 872-872. 2022.

[23]. M. Sohaib, et al., "New Risk Factors Defined for Anemia Among School-Going Children in Peshawar, Pakistan," *Pakistan Journal of Medicine and Dentistry*, vol. 12, issue 2, pp. 36-42, 2023.

[24]. A. Sherali, et al., "Screening of Iron deficiency Anemia in children using Mentzer index in Pakistan: A cross sectional study," *Global Pediatric Health*, vol. 10, pp. 2333794X221130986, 2023.

[25]. S. Naseem, et al., "Diagnostic Accuracy of Mentzer Index in Diagnosis of Iron Deficiency Anemia Taking Serum Feritin as The Gold Standard," *Indus Journal of Bioscience Research*, vol. 3, issue 5, pp. 939-943, 2025.

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