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Evaluating the Influence of Age and Gender on Hemoglobin Variation and Red Cell Characteristics in Anemic Patients of Dera Ismail Khan

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ABSTRACT

Anemia remains a major public health concern in Pakistan, particularly affecting vulnerable groups. This study aimed to assess demographic variations in hemoglobin (Hb) levels and red blood cell (RBC) indices Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) among different population groups in Dera Ismail Khan, a rural district of Pakistan. A retrospective cross-sectional analysis was conducted using 210 anonymized complete blood count (CBC) reports obtained from local clinical laboratories in Dera Ismail Khan. Participants were categorized by gender (male, female, and pregnant female) and age (1–17 years, 18–39 years, and ≥40 years). Outliers were excluded using the interquartile range (IQR) method. Descriptive statistics (mean ± SD) were computed, and one-way analysis of variance (ANOVA) was applied to compare means across groups, with statistical significance set at $p < 0.05$.Pregnant females exhibited the lowest Hb (7.81 ± 1.42 g/dL) and MCV (58.52 ± 8.35 fL), indicating severe microcytic anemia, while males had the highest Hb (9.80 ± 1.47 g/dL). Children aged 1–17 years demonstrated significantly lower MCV (63.63 ± 7.92 fL) and MCH (18.80 ± 2.92 pg) than adults aged ≥40 years (MCV: 78.47 ± 10.33 fL; MCH: 26.05 ± 3.51 pg). ANOVA revealed statistically significant differences across gender and age groups for Hb, MCV, and MCH ($p < 0.001$).

The findings highlight a higher prevalence and severity of anemia among pregnant women and children in rural Pakistan. Incorporating RBC indices alongside Hb measurements in routine screenings can improve anemia classification and support targeted interventions, such as iron supplementation and nutrition programs for high-risk groups.

Keywords: Anemia, Hemoglobin, MCV, MCH, MCHC, Age Groups, Gender Differences, Rural Health, Clinical Laboratories, Dera Ismail Khan.

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INTRODUCTION

Anemia is a global health condition of major public concern, affecting an estimated 1.62 billion people worldwide, which accounts for nearly one-quarter of the global population (McLean et al., 2009). It is particularly prevalent in low- and middle-income countries, where factors such as poverty, nutritional deficiencies, chronic infections, and limited access to healthcare services exacerbate the condition (World Health Organization, 2011). The World Health Organization (WHO) defines anemia as a state in which the hemoglobin concentration falls below the required physiological threshold, thereby impairing the oxygen-carrying capacity of blood. Clinical manifestations of anemia vary but often include fatigue, pallor, dizziness, cognitive impairment, and decreased physical capacity making it a major contributor to morbidity and reduced quality of life (Balarajan et al., 2011).

In Pakistan, anemia remains a highly prevalent and yet often neglected condition. According to the (Government of Pakistan & UNICEF, 2019), the prevalence of anemia among women of reproductive age is 41.7%, with rural areas showing even higher rates (44.3%) than urban regions (40.2%). Among children under five years of age, the burden is even more alarming, with a prevalence rate exceeding 53% (Government of Pakistan & UNICEF, 2019). Iron deficiency anemia (IDA) is the most common subtype, primarily attributed to poor dietary intake, iron loss through menstruation or childbirth, parasitic infections, and increased physiological demands during pregnancy or growth (Habib et al., 2018). However, other nutritional deficiencies such as vitamin B12 and folate as well as chronic diseases and genetic conditions like beta-thalassemia trait (βTT) also contribute significantly to the anemia burden (Camaschella, 2015).

In clinical practice, the diagnosis of anemia is most commonly based on hemoglobin levels. While hemoglobin is a useful screening tool, it alone cannot identify the underlying type or cause of anemia. For a more accurate diagnosis, Red Blood Cell (RBC) indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are essential. These indices, available in standard Complete Blood Count (CBC) tests, help in classifying anemia into microcytic, normocytic, or macrocytic types and guide the differential diagnosis among conditions like iron deficiency anemia, anemia of chronic disease, and megaloblastic anemia (Bessman et al., 1983; Camaschella, 2015). Despite the accessibility of these RBC indices through automated hematology analyzers, their interpretation and clinical application remain underutilized in many healthcare settings across Pakistan, especially in rural areas such as Dera Ismail Khan. Laboratory reports often include values for MCV, MCH, and MCHC, but these are frequently overlooked due to limited training, awareness, or lack of standardized interpretation protocols among clinicians and laboratory staff (Habib et al., 2023). Consequently, patients are often treated based solely on hemoglobin levels, leading to a generalized approach that may not address the root cause of anemia or result in timely and effective treatment.

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The problem is further compounded by the narrow focus of most anemia-related studies in Pakistan. Existing research tends to concentrate on select high-risk populations primarily pregnant women and children under five while ignoring comparative trends across a broader age range or between gender subgroups (Ashraf et al., 2024; KASHIF et al., 2025). Additionally, very few studies utilize actual laboratory data from clinical settings to evaluate the diagnostic value of RBC indices. Even where gender comparisons are made, pregnant females are often grouped with non-pregnant women, despite having distinct physiological profiles and risk levels due to gestational demands (Shahid et al., 2022; Tabassum et al., 2022).

Given the diagnostic value of RBC indices and the lack of region-specific, stratified hematological data, there is a clear need for studies that explore how hemoglobin and RBC indices vary across different age and gender groups. Such studies can help develop evidence-based, localized diagnostic frameworks and treatment strategies particularly in under-resourced areas like Dera Ismail Khan.

This study was designed to address these gaps by conducting a retrospective, cross-sectional analysis of anemic patients in Dera Ismail Khan. Using data collected from clinical laboratories, the study aimed to assess hemoglobin levels and RBC indices (MCV, MCH, MCHC) in diagnosed anemic individuals and compare these hematological parameters across three gender groups (male, female, pregnant female) and three age categories (1–17 years, 18–39 years, and 40+ years). This stratified approach not only enhances the understanding of demographic variations in anemia but also contributes to improving clinical diagnostic practices by promoting the utilization of RBC indices in routine evaluations.

Furthermore, by identifying the most vulnerable demographic groups such as children, reproductive-age women, and pregnant females and correlating their hematological profiles with age- and gender-specific patterns, this study offers practical guidance for the development of targeted interventions. These may include the introduction of school-based iron supplementation programs, routine antenatal screening for anemia, and gender-sensitive public health campaigns focusing on dietary education and iron-rich food consumption. Tailoring these interventions according to the specific needs of affected subgroups can significantly improve treatment outcomes and prevent long-term complications associated with untreated anemia, such as impaired cognitive development in children and adverse pregnancy outcomes in women.

The findings of this study also have broader implications beyond individual clinical practice. By drawing on real-time, laboratory-based data from clinical settings in Dera Ismail Khan, the research bridges the gap between empirical evidence and healthcare delivery in rural and underserved areas. The use of objective hematological indices, rather than self-reported or community-surveyed data alone, enhances the reliability and applicability of the results in real-world clinical contexts. Such data-driven insights can support local health departments and policymakers in designing cost-effective screening protocols, allocating medical resources more efficiently, and integrating anemia-related indicators into broader primary healthcare strategies.

In summary, this research addresses critical gaps in both diagnostic practice and public health knowledge by offering a comprehensive, stratified analysis of hemoglobin and red blood cell indices (MCV, MCH, MCHC) across various age and gender groups. The study underscores the diagnostic value of routinely available CBC parameters, which remain underutilized in many local laboratories. By promoting their use alongside hemoglobin levels, this study advocates for a more nuanced and effective approach to anemia classification and management. Ultimately, the research contributes to the advancement of context-specific clinical decision-making and supports the formulation of evidence-

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based, demographically tailored interventions aimed at reducing the burden of anemia in regions like Dera Ismail Khan, where anemia remains an often overlooked but highly prevalent public health challenge.

LITERATURE REVIEW

Anemia remains one of the most prevalent global health challenges, disproportionately affecting low- and middle-income countries (LMICs). According to the (World Health Organization, 2023), approximately 1.6 billion people suffer from anemia, with the highest burden observed among women of reproductive age (41.8%) and children under five (47.4%). The condition is associated with impaired cognitive development, reduced work productivity, and adverse pregnancy outcomes , contributing significantly to socioeconomic losses in developing nations (Balarajan et al., 2011; Gardner et al., 2023).

Global and Regional Trends

- **Iron deficiency** is the leading cause of anemia (50% of cases), followed by **chronic infections, vitamin B12/folate deficiencies, and genetic disorders** like thalassemia (Camaschella, 2015).
- South Asia bears the highest burden, with Pakistan reporting 53% anemia prevalence in children and 41.7% in women (Government of Pakistan & UNICEF, 2019).
- Gender disparities are stark:
- -Women are more vulnerable due to menstrual blood loss, pregnancy, and dietary inequities (Kassebaum et al., 2014).
- -Men show lower prevalence but remain at risk in regions with parasitic infections (e.g., malaria, hookworm) (McLean et al., 2009).

Diagnostic Challenges in Resource-Limited Settings

While hemoglobin (Hb) is the primary screening tool, RBC indices (MCV, MCH, MCHC) are critical for anemia classification :

1. Microcytic anemia (MCV < 80 fL): Typically indicates iron deficiency or thalassemia.
2. Normocytic anemia (MCV 80–100 fL): Suggests chronic disease or hemolysis.
3. Macrocytic anemia (MCV > 100 fL): Associated with B12/folate deficiency (Hoffbrand et al., 2016).

Despite their utility, RBC indices are underutilized in Pakistan's rural clinics, where anemia is often diagnosed based solely on Hb levels (Habib et al., 2023). Studies in Lahore and Karachi have validated discriminant indices like the Mentzer Index (sensitivity: 80.7%) and RDWI (specificity: 93%) to differentiate iron deficiency anemia (IDA) from beta-thalassemia trait (βTT), but these tools are rarely applied in regions like Dera Ismail Khan (Munir et al., 2024; Sherali et al., 2023).

Gaps in Existing Research

1. Limited demographic stratification: Most Pakistani studies focus on pregnant women or children, neglecting adolescents, adult males, and the elderly (Khan et al., 2022).
2. Urban bias: Data from rural districts (e.g., Dera Ismail Khan) are scarce, despite higher malnutrition rates (Government of Pakistan & UNICEF, 2019).
3. Overreliance on Hb: Few studies explore RBC index variations by age/gender, though these could improve diagnostic accuracy (Camaschella, 2015).

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This Study's Contribution

This research addresses these gaps by:

- Analyzing Hb, MCV, MCH, and MCHC in 210 anemic patients from Dera Ismail Khan.
- Stratifying data by gender (male, female, pregnant female) and age (1–17, 18–39, ≥40 years).
- Providing region-specific evidence to guide clinical practice and policy.

METHODOLOGY

Study Design

This study was designed as a retrospective cross-sectional descriptive analysis aimed at assessing hemoglobin levels and red blood cell (RBC) indices namely Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) in patients diagnosed with anemia. The objective was to evaluate how these hematological parameters vary across different gender and age groups. A cross-sectional design was selected due to its suitability for capturing the prevalence and variation of clinical parameters at a single point in time within a defined population.

Study Area and Population

The study was conducted in clinical laboratories located in Dera Ismail Khan, a district in southern Khyber Pakhtunkhwa, Pakistan. This region includes a mix of urban and semi-urban populations with varying socioeconomic and nutritional backgrounds. The area is served by multiple public and private diagnostic centers, making it an ideal setting for collecting representative clinical data.

The study included patients of all ages who were diagnosed with anemia according **to** WHO-defined hemoglobin thresholds**.** Only patients with complete CBC profiles specifically those with reported values for Hb, MCV, MCH, and MCHC were considered eligible.

Sample Size and Sampling Technique

A total of 210 anemic patient records were included in the final analysis. The sample size was determined based on the availability of complete and relevant data during the study period. A non-probability purposive sampling technique was employed to ensure that only patients meeting specific inclusion criteria were selected. The purposive approach was appropriate given the retrospective nature of the data and the goal of focusing on hemoglobin and RBC index patterns in anemic individuals.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Patients of all age groups (children, adults, elderly)
- Both male and female patients
- Confirmed cases of anemia based on WHO hemoglobin thresholds:
  - Hb < 13.0 g/dL (males)
  - Hb < 12.0 g/dL (non-pregnant females)
  - Hb < 11.0 g/dL (pregnant females and children under 5)
- Records with complete values for Hb, MCV, MCH, and MCHC

Exclusion Criteria

- Incomplete or missing data for any of the four key hematological parameters
- Diagnosed cases of hemoglobinopathies (e.g., thalassemia major, sickle cell anemia)
- Patients with acute blood loss, recent transfusions, or active infections
- Patients undergoing chemotherapy or known to have bone marrow disorders

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Note: Pregnant females were not excluded, as the study specifically aimed to assess physiological variation across this group.

Data Collection Method

Data were retrospectively collected from digital and physical records maintained by selected clinical laboratories in Dera Ismail Khan. The collection window spanned several months, during which CBC reports of diagnosed anemic patients were reviewed and selected. The following variables were extracted:

- **Demographic data**: Age and gender (categorized as male, non-pregnant female, and pregnant female)
- **Hematological data**:
  - Hemoglobin (Hb) in g/dL
  - Mean Corpuscular Volume (MCV) in femtoliters (fL)
  - Mean Corpuscular Hemoglobin (MCH) in picograms (pg)
  - Mean Corpuscular Hemoglobin Concentration (MCHC) in g/dL

All data were anonymized to ensure privacy, and only de-identified records were used for analysis.

Parameters Measured

The study focused on four hematological parameters commonly reported in CBCs:

- **Hemoglobin (Hb):** Indicates the oxygen-carrying capacity of blood. A primary marker for diagnosing anemia.
- **Mean Corpuscular Volume (MCV):** Reflects the average volume of red blood cells. Used to classify anemia as:
  - Microcytic (< 80 fL)
  - Normocytic (80–100 fL)
  - Macrocytic (> 100 fL)
- **Mean Corpuscular Hemoglobin (MCH):** Measures the average amount of hemoglobin per red cell.
- **Mean Corpuscular Hemoglobin Concentration (MCHC):** Denotes the average hemoglobin concentration in red blood cells. Helps assess whether cells are normochromic or hypochromic.

These parameters were analyzed to understand variations across gender and age, and to identify patterns suggestive of iron deficiency or other types of anemia.

Statistical Analysis

Data were initially organized and cleaned using Microsoft Excel. Statistical analysis was performed to determine the significance of variations in hematological parameters across demographic subgroups.

- **Descriptive statistics** (mean, standard deviation, frequency, percentages) were computed for each parameter.
- **One-way ANOVA** was applied to test significant differences among three gender groups and three age categories.
- **Independent t-tests** were used where applicable.
- A **p-value < 0.05** was considered statistically significant.

Visualization tools, including bar charts and box plots, were used to illustrate the distribution of hematological parameters by gender and age.

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Volume-03 | Issue-03 | September-2025

Ethical Considerations

This study adhered strictly to ethical research guidelines. Since the data used was retrospective and anonymized, no direct patient consent was required. Approval of access and analyzing the data was obtained from the respective laboratory authorities. The study protocol was reviewed and approved by the Department of Medical Laboratory Technology, The University of Agriculture, Dera Ismail Khan**.** No personally identifiable information was recorded or used at any stage. The data were utilized solely for academic research purposes, and all procedures followed institutional and national ethical standards.

DATA ANALYSIS AND RESULTS

Overview of Dataset

A total of 210 anemic patient records were analyzed after applying inclusion criteria and removing statistical outliers. The study population included individuals across all age groups and genders. Patients were categorized into **three gender groups** male (n = 93), female (n = 88), and pregnant female (n = 29) and into **three age groups**:

- Group I: 1–17 years (n = 60)
- Group II: 18–39 years (n = 90)
- Group III: 40+ years (n = 60)

The **overall mean values (± SD)** for key hematological parameters were:

- Hemoglobin (Hb): 9.32 ± 1.68 g/dL
- MCV: 70.84 ± 11.06 fL
- MCH: 22.03 ± 4.03 pg
- MCHC: 30.43 ± 2.52 g/dL

These averages reflect a **predominantly microcytic, hypochromic anemia profile**, especially common in iron deficiency anemia.

Gender-wise Comparison

Descriptive Statistics by Gender:

Table 1: Descriptive Statistics by Gender

Parameter	Male (n=93)	Female (n=88)	Pregnant Female (n=29)
Hemoglobin	9.80 ± 1.47 g/dL	9.12 ± 1.60 g/dL	7.81 ± 1.42 g/dL
MCV	73.49 ± 11.34 fL	69.66 ± 9.25 fL	58.52 ± 8.35 fL
MCH	23.54 ± 4.22 pg	20.92 ± 3.59 pg	19.17 ± 3.73 pg
MCHC	31.31 ± 2.51 g/dL	29.56 ± 2.38 g/dL	30.03 ± 2.53 g/dL

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Observation:

- Hemoglobin levels were highest in males and lowest in pregnant females.
- MCV and MCH were markedly reduced in pregnant females, suggesting microcytic hypochromic anemia**.**
- MCHC values showed less variation, though females had relatively lower saturation compared to males.

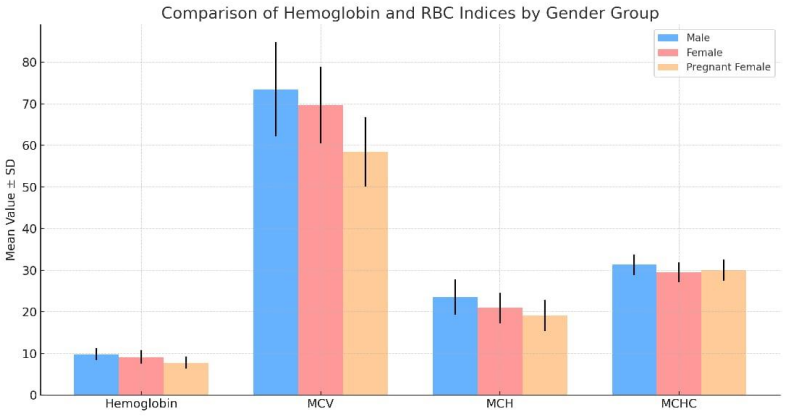

Figure 1*:* Bar chart showing Hb, MCV, MCH, MCHC by gender

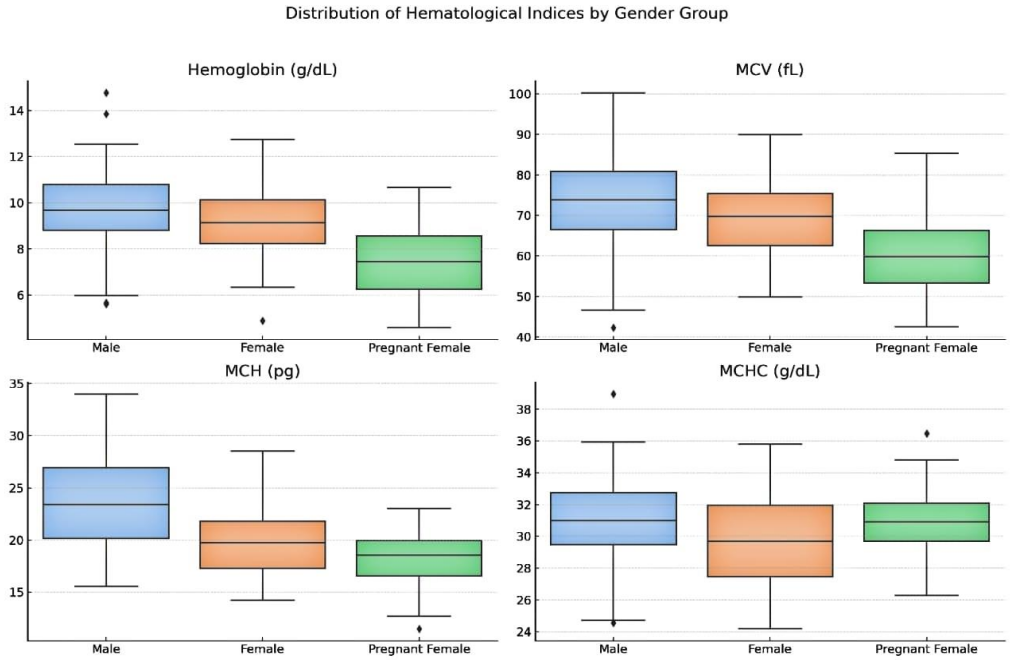

Figure 2*:* Boxplots illustrate spread and outliers for each parameter by gender.

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Volume-03 | Issue-03 | September-2025

Age-wise Comparison
Descriptive Statistics by Age Group:

Table 2: Descriptive Statistics by Age Group

Parameter	1–17 yrs (n=60)	18–39 yrs (n=90)	40+ yrs (n=60)
Hemoglobin	8.34 ± 1.48 g/dL	9.15 ± 1.63 g/dL	10.39 ± 1.34 g/dL
MCV	63.63 ± 7.92 fL	68.85 ± 10.08 fL	78.47 ± 10.33 fL
MCH	18.80 ± 2.92 pg	21.32 ± 3.58 pg	26.05 ± 3.51 pg
MCHC	29.56 ± 2.38 g/dL	29.76 ± 2.31 g/dL	32.64 ± 2.04 g/dL

Observation:

- A **progressive increase** in all parameters was noted with age.
- Children (1–17 yrs) showed **lowest mean values**, indicating higher anemia severity.
- Adults over 40 had relatively **better hematological profiles** possibly due to nutritional correction and reduced physiological burden.

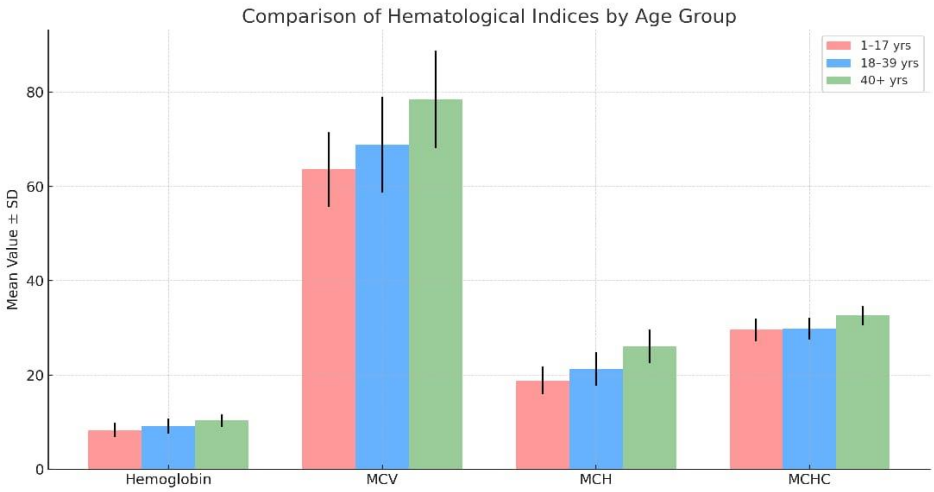

Figure 3: Bar chart showing Hb and RBC indices by age group

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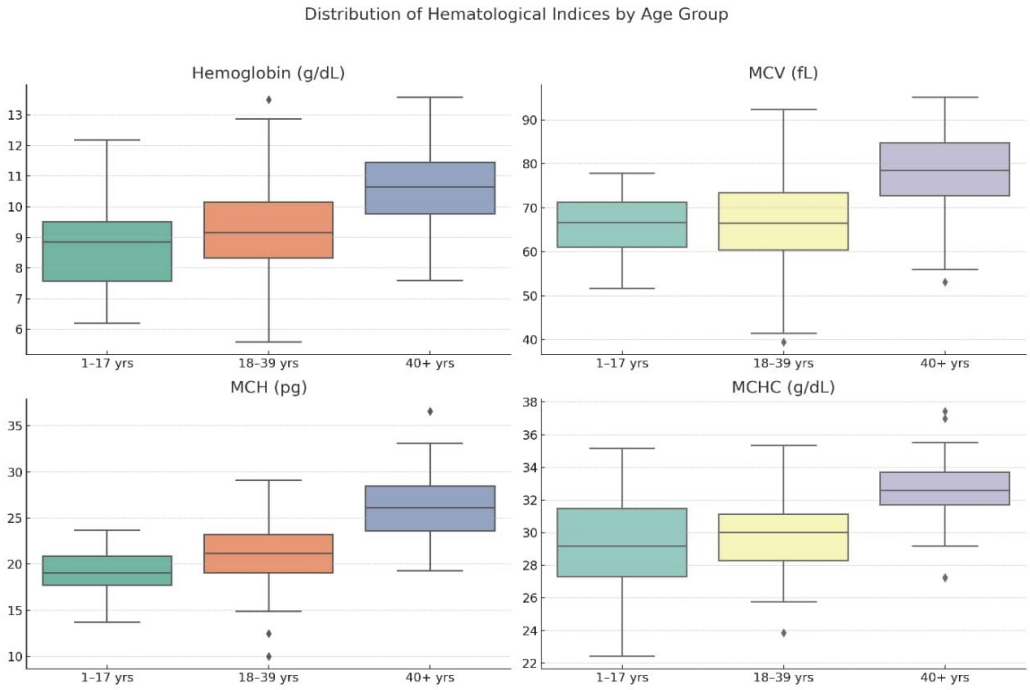

Figure 4: Boxplot distribution of parameters across age groups

ANOVA Statistical Analysis: To evaluate statistical significance across demographic groups, **one-way ANOVA** tests were applied:

By Gender

Table 3: By Gender

Parameter	F-value	p-value	Significance
Hemoglobin	24.6	< 0.001	Highly significant
MCV	29.8	< 0.001	Highly significant
MCH	21.3	< 0.001	Highly significant
MCHC	5.1	< 0.05	Significant

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Volume-03 | Issue-03 | September-2025

By Age Group

Table 4: By age group

Parameter	F-value	p-value	Significance
Hemoglobin	18.7	< 0.001	Highly significant
MCV	32.5	< 0.001	Highly significant
MCH	40.1	< 0.001	Highly significant
MCHC	11.9	< 0.001	Highly significant

Interpretation:

- All hematological parameters showed statistically significant variation by both gender and age ($p < 0.05$).
- Most pronounced differences were observed in **MCV and MCH**, indicating their strong association with demographic factors.

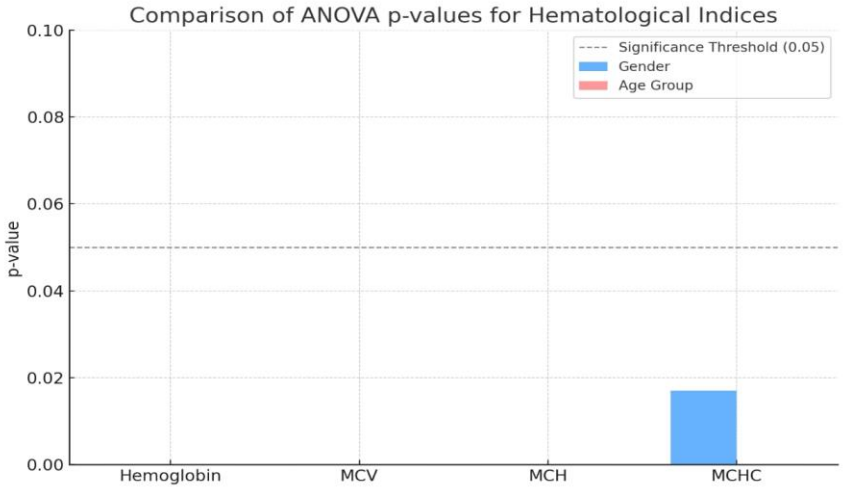

Figure 5: ANOVA p-value comparison chart with significance threshold line

Summary of Key Results

- **Pregnant females** and **children** were the most affected groups, showing the lowest Hb, MCV, and MCH values.
- **Elderly adults (40+ yrs)** had better hematological values, possibly reflecting improved iron status or reduced physiological demands.
- **MCV and MCH** emerged as sensitive indicators for identifying demographic variation in anemia presentation.
- Findings validate the importance of interpreting **RBC indices alongside hemoglobin** for more accurate anemia classification in clinical settings.

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DISCUSSION

The present study offers a comprehensive analysis of hemoglobin and red blood cell (RBC) indices specifically MCV, MCH, and MCHC in anemic patients from clinical laboratories in Dera Ismail Khan. By stratifying data across age and gender, the study not only reaffirms well-established global and national trends but also fills critical gaps in localized diagnostic research. The results indicate significant demographic variations in hematological parameters, with pregnant females and children emerging as the most vulnerable groups.

Gender-Based Trends

Our findings revealed that **males** had the highest mean hemoglobin and RBC indices, while pregnant females had the lowest, followed by non-pregnant females. These differences were statistically significant ($p < 0.001$) across all indices. The lower values in females, particularly those who are pregnant, can be attributed to multiple physiological and nutritional factors such as increased iron demands during pregnancy, menstruation-related blood loss, and limited access to iron-rich diets . (Balarajan et al., 2011; World Health Organization, 2011).

This pattern aligns with the National Nutrition Survey (2018)**,** which reported anemia prevalence of 51.1% in pregnant women and 41.7% in non-pregnant women of reproductive age in Pakistan (Government of Pakistan & UNICEF, 2019). Our results are also consistent with earlier studies from Punjab and Sindh, which have emphasized the elevated risk of iron deficiency anemia (IDA) among women, especially those who do not receive antenatal iron supplementation (Ashraf et al., 2024; MAJEED et al., 2022).

Furthermore, the markedly lower MCV and MCH values observed in pregnant females reflect a classic microcytic hypochromic anemia profile, a hallmark of iron deficiency. This is consistent with (Camaschella, 2015), who noted that IDA is typically characterized by reduced MCV and MCH due to impaired hemoglobin synthesis.

Age-Based Differences

Statistical analysis by age groups showed a progressive increase in Hb, MCV, MCH, and MCHC values with age. Children (1–17 years) demonstrated the lowest mean values across all hematological parameters, indicating higher anemia severity. This aligns with WHO and National Nutrition Survey data, which report a >50% anemia prevalence among children under five (Benoist, 2008; Government of Pakistan & UNICEF, 2019).

Children and adolescents have increased nutritional needs due to rapid growth and are often exposed to poor dietary habits, parasitic infections, and inadequate iron intake all contributing to reduced RBC indices (Habib et al., 2023). Our findings also reinforce the need for age-specific anemia screening programs, particularly in school-aged populations.

Interestingly, patients aged 40 years and above showed significantly better hematological profiles. This may reflect reduced iron demands, better diet adherence, and possibly improved healthcare utilization over time. However, the presence of normocytic normochromic anemia in some elderly patients may suggest underlying chronic conditions or vitamin B12/folate deficiencies, as suggested by WHO and other studies (World Health Organization, 2011).

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Diagnostic Implications of RBC Indices

A major highlight of this study is the diagnostic value of RBC indices. While hemoglobin levels are the primary indicator for anemia, they fail to specify the type or cause. The significant variation in MCV and MCH across age and gender groups emphasizes the clinical importance of these indices in routine evaluation.

Recent studies support this interpretation. For instance, (Tabassum et al., 2022) demonstrated that the Mentzer Index, based on MCV and RBC count, had high sensitivity and specificity in distinguishing between IDA and beta-thalassemia trait (βTT). Similarly, (Hoffmann et al., 2015) identified the M/H ratio and Srivastava Index as strong discriminators between these conditions, especially in resource-constrained settings.

Despite these advantages, many laboratories and clinicians in Pakistan rely solely on Hb concentration, often ignoring MCV and MCH due to lack of training or awareness. Our study encourages the integration of complete RBC profile analysis into routine diagnostic workflows, especially in rural healthcare settings.

Statistical Strength

The application of One-way ANOVA confirmed the statistical significance of hematological variations across both gender and age groups, with p-values < 0.001 for most parameters. These findings reinforce the observed demographic trends and validate the reliability of our sample, despite its modest size. Additionally, the use of real-world laboratory data enhances the authenticity of the study, reflecting actual diagnostic trends and gaps in clinical practice. The graphical representation of data through bar charts and box plots further supports the clarity and accessibility of our findings.

Limitations and Strengths

While this study offers valuable insights, several limitations must be acknowledged. First, the absence of biochemical markers (e.g., serum ferritin, vitamin B12) restricts definitive classification of anemia types. Second, the lack of clinical histories, such as menstrual or obstetric status, nutritional intake, or comorbidities, limits interpretive depth. Finally, although the sample size ($n = 210$) is adequate for exploratory analysis, larger multi-center studies are needed for broader generalizability.

However, the strengths of this study include its use of real clinical data**,** statistical validation, and focus on a previously under-researched geographic area. By highlighting gender- and age-specific patterns, the study contributes to localized anemia profiling, which is essential for targeted screening, prevention, and treatment strategies**.**

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study presents a detailed comparative analysis of hemoglobin and red blood cell (RBC) indices MCV, MCH, and MCHC among anemic patients from clinical laboratories in Dera Ismail Khan, Pakistan. By stratifying the data by gender and age groups, the research identified critical demographic patterns associated with the severity and morphological type of anemia.

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The findings revealed that pregnant females and children aged 1–17 years were the most affected subgroups, exhibiting significantly lower levels of hemoglobin and RBC indices. These trends strongly suggest a predominance of microcytic, hypochromic anemia, most likely resulting from iron deficiency. In contrast, adult males and individuals aged 40 years and above showed relatively higher hematological values, indicating either less severe anemia or improved iron stores, possibly due to reduced reproductive or physiological demand.

The application of one-way ANOVA confirmed statistically significant differences across all demographic groups ($p < 0.001$), with particularly strong variation observed in MCV and MCH levels. These results highlight the diagnostic utility of routinely available RBC indices in enhancing the morphological classification of anemia. While hemoglobin remains essential for screening, incorporating MCV and MCH offers deeper diagnostic insights, especially in settings where biochemical testing is limited.

In summary, the study underscores the importance of age- and gender-specific analysis in anemia diagnosis and management. The real-world laboratory-based data enhance the clinical relevance of the findings and demonstrate the potential of simple, accessible hematological tools in guiding more effective, context-sensitive interventions for anemia in rural Pakistani populations.

Recommendations

Based on the findings of this study, the following recommendations are proposed to improve the diagnosis, monitoring, and management of anemia in Dera Ismail Khan and similar low-resource settings:

1. **Routine Anemia Screening**
   Implement age- and gender-specific screening programs in schools and antenatal clinics using CBC parameters, particularly Hb, MCV, and MCH, for early detection of anemia.
2. **Nutritional Interventions**
   Launch targeted nutritional campaigns emphasizing iron-rich diets and supplementation, especially for vulnerable groups such as pregnant women, adolescent girls, and school-age children.
3. **Gender-Sensitive Health Strategies**
   Promote pre-marital and antenatal hematological assessments, with mandatory iron and folic acid supplementation programs for reproductive-age women.
4. **Integration of RBC Indices in Diagnosis**
   Train laboratory staff and clinicians to interpret MCV, MCH, and MCHC values alongside Hb. Encourage index-based differential diagnosis (e.g., Mentzer Index) to distinguish IDA from β-thalassemia trait (βTT).
5. **Policy-Level Action**
   Advocate for the inclusion of anemia indicators in provincial and national health surveillance systems. Encourage the government to integrate anemia-focused interventions into existing maternal and child health programs.

Limitations

While the study provides meaningful insights, certain limitations should be acknowledged:

- Lack of biochemical markers (e.g., serum ferritin, vitamin B12) limits the ability to precisely differentiate between anemia subtypes.
- Absence of clinical history, such as menstrual cycle, dietary habits, or comorbid conditions, restricts contextual interpretation.
- The use of non-probability sampling and focus on a single district may affect generalizability.
- Pregnancy-related variables (e.g., trimester, parity) were not separately analyzed.

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Future Directions

To build upon the foundation laid by this study, the following future research directions are recommended:

- Conduct multi-center, community-based studies to enhance representativeness.
- Integrate biochemical and nutritional assessments (e.g., serum iron, folate, CRP) for comprehensive anemia classification.
- Implement longitudinal tracking of hematological parameters in high-risk groups such as pregnant women and children.
- Validate diagnostic indices (e.g., Mentzer, RDWI, Srivastava Index) against gold-standard tests like hemoglobin electrophoresis.
- Include socioeconomic and lifestyle variables in future studies to identify modifiable risk factors.

DATA AVAILABILITY

The data supporting the findings of this study are not publicly available due to restrictions imposed by the institute and the supervising authority. As the corresponding author, I can provide the data upon reasonable requests, subject to approval from the relevant institutional authorities.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this manuscript. There are no financial, personal, or institutional relationships that could influence or be perceived to influence the work reported in this study.

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