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Original Article

Establishment of Reference Ranges for CBC Parameters in Adult Pakistani Population

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ABSTRACT

Background: Complete blood count interpretation depends on reference intervals that may vary by sex, ethnicity, geography, altitude, nutrition, and analytical methods. Reliance on international reference ranges may be inappropriate for local Pakistani populations, particularly in high-altitude regions. Objective: To establish sex-specific reference intervals for selected complete blood count parameters among apparently healthy adults from Quetta and surrounding areas. Methods: This cross-sectional observational study was conducted in the Department of Pathology, Combined Military Hospital, Quetta, from December 2020 to November 2021. A total of 300 apparently healthy adults aged 20–55 years were recruited, including 150 males and 150 females. Venous blood samples were collected in EDTA tubes and analyzed using a SYSMEX XP-100 automated hematology analyzer. Hemoglobin, total leukocyte count, platelet count, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were recorded. Reference intervals were calculated using the 2.5th and 97.5th percentiles. Results: Males showed higher mean hemoglobin, red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin, while females showed higher total leukocyte count, platelet count, and mean corpuscular hemoglobin concentration. Male and female hemoglobin reference intervals were 12.6–15.9 g/dL and 10.3–13.1 g/dL, respectively. Conclusion: Sex-specific CBC reference intervals differed from commonly used international ranges, supporting locally derived interpretation in this population. Keywords: Complete blood count; Hemoglobin; Reference interval; Red blood cell count; Pakistani adults; Hematological parameters.

INTRODUCTION

Complete blood count is one of the most frequently requested laboratory investigations in clinical practice and provides essential information for screening, diagnosis, disease classification, treatment monitoring, and follow-up of a wide range of hematological and systemic disorders. Interpretation of CBC parameters depends on reference intervals that define the expected range of values in a healthy population. These intervals are not fixed biological constants; rather, they vary according to age, sex, ethnicity, genetic background, altitude, nutritional status, environmental exposure, physiological status, and analytical methods used by laboratories. Therefore, inappropriate reliance on reference intervals generated from dissimilar populations may lead to misclassification of normal individuals as abnormal or failure to detect true abnormalities in a local population (1–5).

Reference intervals are particularly important in hematology because small differences in hemoglobin concentration, red blood cell indices, leukocyte count, and platelet count may influence clinical decisions

regarding anemia, infection, inflammation, thrombocytopenia, polycythemia, and other hematological conditions. International reference limits commonly used in many laboratories have largely been derived from Western or other non-local populations. However, these intervals may not accurately reflect hematological patterns in Pakistani adults because of differences in geography, ethnicity, altitude, dietary habits, socioeconomic conditions, and background health status. In Pakistan, locally generated reference interval data remain limited, and available studies suggest that hematological parameters in healthy Pakistani adults may differ from international textbook-based or manufacturer-provided ranges (6–8).

Sex-specific variation is another essential consideration when establishing hematological reference intervals. Previous studies have shown that hemoglobin concentration, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, platelet count, and leukocyte count may differ significantly between males and females because of physiological, hormonal, nutritional, and reproductive factors. For this reason, sex-stratified reference intervals are often more clinically useful than a single combined range, particularly for parameters such as hemoglobin and red blood cell indices (6,9–11).

Geographical altitude may also influence hematological parameters, especially hemoglobin concentration and red blood cell count. Populations residing at higher altitude may demonstrate adaptive hematological changes related to reduced oxygen pressure, including relatively higher erythrocyte mass and hemoglobin levels. Quetta and surrounding areas of Balochistan are situated at approximately 1,679 meters above sea level, making it clinically relevant to evaluate whether CBC reference intervals in this population differ from commonly used international reference standards and from values reported in populations living at lower altitudes (12–15).

Although several international studies have established hematological reference intervals in healthy adults, fewer data are available for adult Pakistani populations, and even fewer address populations residing in high-altitude Pakistani regions. This creates a practical diagnostic gap for clinicians and laboratories that interpret CBC reports using generalized international ranges. Establishing locally relevant CBC reference intervals may improve diagnostic accuracy, reduce unnecessary clinical concern, and support more appropriate interpretation of routine hematological parameters in local clinical settings.

The objective of this study was to establish sex-specific reference intervals for selected complete blood count parameters among apparently healthy adults from Quetta and surrounding areas using automated hematology analysis. The study specifically evaluated hemoglobin, hematocrit, red blood cell count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total leukocyte count, and compared these parameters between male and female participants.

MATERIAL AND METHODS

This cross-sectional observational study was conducted in the Department of Pathology, Combined Military Hospital, Quetta, over a one-year period from December 2020 to November 2021. The study was designed to establish reference intervals for complete blood count parameters in apparently healthy adults residing in Quetta and surrounding areas. The setting was selected because Quetta is located at approximately 1,679 meters above sea level, and altitude-related hematological adaptation may influence red blood cell indices and hemoglobin concentration.

The required sample size was determined according to the Clinical and Laboratory Standards Institute guidance for establishing reference intervals, which recommends a minimum of 120 reference individuals per subgroup when non-parametric percentile-based reference intervals are estimated. On this basis, at least 240 participants were required for sex-stratified analysis, including 120 males and 120 females. A total of 300 apparently healthy adults were recruited using non-probability purposive

sampling, comprising 150 males and 150 females. Participants were aged 20 to 55 years and were selected after assessment of eligibility through clinical history and screening for conditions likely to influence hematological parameters.

Apparently healthy adult individuals were eligible for inclusion if they were within the defined age range and had no known acute or chronic illness likely to affect complete blood count values. Individuals were excluded if they had a history of drug use affecting hematological parameters, recent blood transfusion, jaundice, anemia, hepatitis B or C infection, diabetes mellitus, hypertension, ischemic heart disease, or any other significant comorbid condition. Pregnant women, lactating women, and menstruating women at the time of sampling were also excluded to reduce physiological variation in hematological values. Demographic details and relevant clinical history, including ethnicity, socioeconomic status, medication history, anemia history, and comorbid disease status, were recorded before sample collection.

Ethical approval was obtained from the institutional ethical review committee before participant recruitment, with approval reference number CMH-QTA-IRB/041. Written informed consent was obtained from all participants before enrollment. All procedures were performed in accordance with ethical principles for human participant research, and participant confidentiality was maintained during data collection, analysis, and reporting.

A 2 mL venous blood sample was collected from the antecubital fossa of each participant under aseptic conditions into an EDTA anticoagulant tube. Samples were analyzed within one hour of collection using an automated hematology analyzer, SYSMEX XP-100. The CBC parameters recorded included hemoglobin, hematocrit, red blood cell count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total leukocyte count. The analyzer was calibrated and standardized using commercially prepared calibrators according to the manufacturer's instructions. To support analytical precision and data integrity, 40 samples were reanalyzed for quality checking, and calibration procedures were performed to reduce measurement error related to the electrical and analytical systems of the instrument.

Data were entered and analyzed using Statistical Package for the Social Sciences version 25.0. Continuous variables were summarized using mean and standard deviation. Categorical variables, including sex, were summarized using frequency and percentage. Reference intervals were estimated using the 2.5th and 97.5th percentiles for each CBC parameter. Male and female participants were analyzed separately because sex-related differences in hematological parameters were expected and were clinically relevant for interpretation. Comparisons of CBC parameters between males and females were performed using the independent-samples t-test. A p-value of ≤ 0.05 was considered statistically significant. Outliers outside the acceptable analytical range were identified before reference interval estimation, and final reference limits were calculated after excluding values judged unsuitable for reference interval construction.

RESULTS

A total of 300 apparently healthy adults were included in the study, comprising 150 males and 150 females. The mean age of the study population was 35.1 ± 8.05 years, with a reported age range of 20–53 years.

Table 1. Baseline Characteristics of the Study Population

Variable	Overall
Total participants, n	300
Male, n (%)	150 (50.0)
Female, n (%)	150 (50.0)
Age, years, Mean ± SD	35.1 ± 8.05
Age range, years	20–53

The study population had equal representation of male and female participants, with each sex contributing 150 participants. The mean age was 35.1 ± 8.05 years, indicating that the analyzed sample represented young to middle-aged adults.

Table 2. Sex-Wise Comparison of Complete Blood Count Parameters

Parameter	Male, Mean ± SD	Female, Mean ± SD	p-value
Hemoglobin, g/dL	14.2 ± 0.8	11.8 ± 0.7	0.001
Total leukocyte count, $\times10^9$/L	8.3 ± 1.0	8.7 ± 0.9	0.001
Platelet count, $\times10^9$/L	223.8 ± 26.8	254.1 ± 30.7	0.001
Red blood cell count, $\times10^{12}$/L	4.8 ± 0.5	4.5 ± 0.4	0.001
Mean corpuscular volume, fL	76.7 ± 0.5	73.3 ± 0.8	0.001
Mean corpuscular hemoglobin, pg	26.3 ± 1.7	24.9 ± 0.4	0.001
Mean corpuscular hemoglobin concentration, g/dL	32.9 ± 0.9	37.4 ± 0.9	0.001

Independent-samples t-test was used for male–female comparisons.

Sex-wise comparison showed statistically different CBC parameter distributions between male and female participants. Males had higher mean hemoglobin, red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin values than females. Mean hemoglobin was 14.2 ± 0.8 g/dL in males compared with 11.8 ± 0.7 g/dL in females, while mean red blood cell count was 4.8 ± 0.5 $\times10^{12}$/L in males and 4.5 ± 0.4 $\times10^{12}$/L in females. In contrast, females had higher mean total leukocyte count, platelet count, and mean corpuscular hemoglobin concentration. Mean platelet count was 254.1 ± 30.7 $\times10^9$/L in females compared with 223.8 ± 26.8 $\times10^9$/L in males.

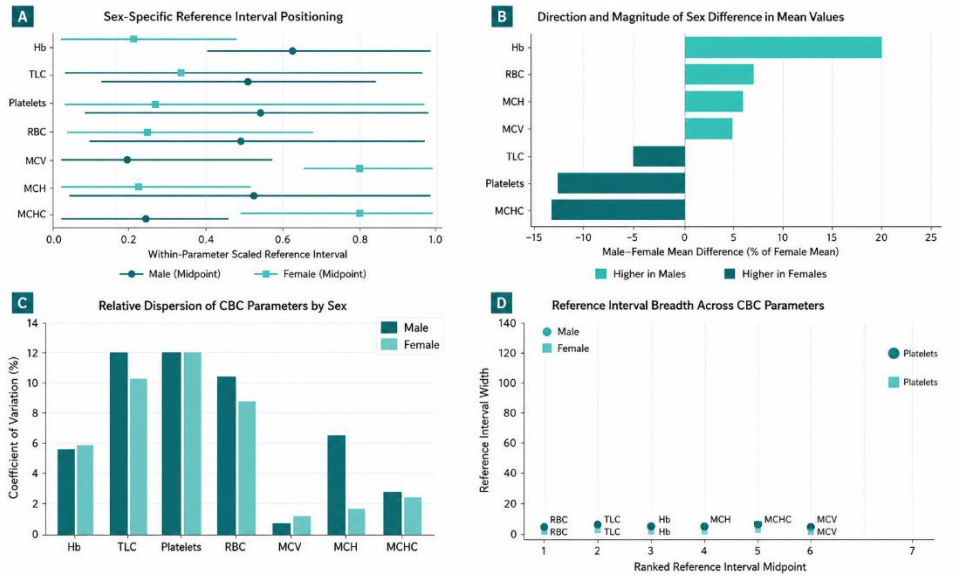

Figure 1 Sex-Specific Complete Blood Count Reference Patterns Among Apparently Healthy Adults. The panelled figure demonstrates sex-specific variation in complete blood count parameters using reported means, standard deviations, and 2.5th–97.5th percentile reference intervals. Male participants showed higher mean hemoglobin by 20.3%, red blood cell count by 6.7%, mean corpuscular hemoglobin by 5.6%, and mean corpuscular volume by 4.6% compared with females, while females showed higher total leukocyte count by 4.6%, platelet count by 13.5%, and mean corpuscular hemoglobin concentration by 12.0%. Relative dispersion was greatest for platelet count and total leukocyte count in both sexes, while mean corpuscular volume showed the narrowest variability. The reference interval breadth was widest for platelet count, measuring 120.5 $\times10^9$/L in males and 100.7 $\times10^9$/L in females, whereas red cell indices showed comparatively narrower reference limits. These patterns support sex-stratified interpretation of CBC values and highlight that hemoglobin, erythrocyte indices, platelet count, and MCHC show clinically relevant sex-linked distribution differences in this apparently healthy adult population.

Table 3. Sex-Specific Reference Intervals for Complete Blood Count Parameters

Parameter	Male, 2.5th–97.5th Percentile	Female, 2.5th–97.5th Percentile
Hemoglobin, g/dL	12.6–15.9	10.3–13.1
Total leukocyte count, $\times10^9$/L	6.1–10.0	6.9–10.6
Platelet count, $\times10^9$/L	186.5–307.0	201.7–302.4
Red blood cell count, $\times10^{12}$/L	4.1–5.9	3.9–5.3
Mean corpuscular volume, fL	76.0–77.9	72.1–75.5
Mean corpuscular hemoglobin, pg	24.1–27.8	24.0–26.0

Parameter	Male, 2.5th–97.5th Percentile	Female, 2.5th–97.5th Percentile
Mean corpuscular hemoglobin concentration, g/dL	30.8–34.6	34.9–38.9

Sex-specific reference intervals were established using the 2.5th and 97.5th percentiles. The male hemoglobin reference interval was 12.6–15.9 g/dL, while the female interval was 10.3–13.1 g/dL. The male red blood cell reference interval was 4.1–5.9 $\times 10^{12}$/L, compared with 3.9–5.3 $\times 10^{12}$/L in females. Platelet count intervals were higher in females, with a reference interval of 201.7–302.4 $\times 10^{9}$/L compared with 186.5–307.0 $\times 10^{9}$/L in males. Total leukocyte count intervals were 6.1–10.0 $\times 10^{9}$/L in males and 6.9–10.6 $\times 10^{9}$/L in females.

Overall, the findings demonstrate clear sex-specific variation in hematological parameters among apparently healthy adults. The observed differences support the use of sex-stratified CBC reference intervals rather than a single combined reference range. The results particularly show higher hemoglobin, red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin values among males, while females showed higher platelet count, total leukocyte count, and mean corpuscular hemoglobin concentration.

DISCUSSION

This study established sex-specific reference intervals for selected complete blood count parameters among apparently healthy adults from Quetta and surrounding areas. The findings showed measurable differences between male and female participants across all reported CBC parameters, supporting the use of sex-stratified interpretation rather than a single combined reference range. Male participants had higher mean hemoglobin, red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin, whereas female participants had higher platelet count, total leukocyte count, and mean corpuscular hemoglobin concentration. These differences are consistent with the biological expectation that erythrocyte indices and hemoglobin concentration differ by sex because of hormonal, physiological, and nutritional influences, while platelet and leukocyte parameters may also vary across populations and demographic subgroups (6,12,13).

The male hemoglobin reference interval in this study was 12.6–15.9 g/dL, whereas the female interval was 10.3–13.1 g/dL. These values differ from commonly used international reference limits and reinforce the importance of locally derived intervals for clinical interpretation. Previous Pakistani and regional studies have also reported differences between locally observed hematological parameters and reference values derived from Western populations, highlighting the risk of applying generalized international ranges without local validation (12,21). The lower female hemoglobin interval observed in this study may reflect a combination of physiological sex differences, nutritional patterns, reproductive factors, and background iron status; however, iron indices and nutritional biomarkers were not reported, so this explanation should be interpreted cautiously.

The red blood cell count and related indices also showed sex-based variation. Males had higher red blood cell count, MCV, and MCH than females, which is broadly consistent with prior hematological reference interval studies showing higher erythrocyte parameters in males (12,13,15). The study setting is clinically relevant because Quetta and surrounding areas are located at approximately 1,679 meters above sea level. Residence at higher altitude may influence erythropoiesis through adaptive responses to lower oxygen pressure, potentially contributing to differences in hemoglobin and red blood cell parameters. However, this altitude-related interpretation should remain cautious unless participant residence, duration of altitude exposure, and city-wise recruitment are reported clearly. If participants were recruited from multiple cities, the analysis should be stratified by recruitment site or altitude category before attributing differences to high-altitude adaptation.

The platelet count reference intervals were 186.5–307.0 $\times 10^{9}$/L in males and 201.7–302.4 $\times 10^{9}$/L in females. Mean platelet count was also higher among females. This finding differs from some textbook or manufacturer-provided ranges and is broadly comparable with studies from non-Western populations

that reported locally variable platelet reference limits (17–19). Such variation may reflect ethnicity, environment, nutritional status, sampling approach, analytical platform, and population health characteristics. Because platelet count is frequently used to screen for thrombocytopenia, inflammatory disorders, hematological disease, and treatment-related complications, locally relevant reference intervals may reduce unnecessary clinical concern when values fall outside imported reference standards but remain within locally expected limits.

The total leukocyte count interval was 6.1–10.0 $\times 10^9$/L in males and 6.9–10.6 $\times 10^9$/L in females. Although the difference between sexes was statistically significant in the manuscript, clinical interpretation should consider the narrow magnitude of difference, the purposive sampling method, and the lack of differential leukocyte count reporting. Leukocyte parameters are influenced by infection, inflammation, stress, smoking, medication use, and subclinical disease. The exclusion criteria removed several major comorbid conditions, but the manuscript does not report inflammatory markers, peripheral smear confirmation for all participants, or detailed screening for subclinical illness. Therefore, the leukocyte reference intervals should be interpreted as population-specific estimates from apparently healthy adults rather than definitive national reference standards.

A major strength of this study is its focus on locally relevant CBC reference interval development in an adult Pakistani population, using a sample size consistent with CLSI guidance for sex-stratified reference interval estimation. The equal inclusion of males and females also strengthens sex-specific comparison. The use of an automated hematology analyzer, calibration procedures, and reanalysis of selected samples further supports analytical reliability. However, several methodological and reporting limitations require attention. The study used non-probability purposive sampling, which limits representativeness. The manuscript also contains an unresolved denominator inconsistency, with 300 participants reported in the main results but 246 participants reported in one table. The authors should clarify whether 54 observations were excluded as outliers, whether exclusions were parameter-specific or participant-level, and how final denominators were handled for each reference interval.

Additional limitations include the absence of confidence intervals around the 2.5th and 97.5th percentile reference limits, lack of detailed nutritional and iron-status assessment, absence of age-stratified reference intervals, and incomplete reporting of residence duration or altitude exposure. The manuscript also requires clarification of whether all participants were recruited from Quetta and surrounding areas or whether samples were collected from other cities. Without this clarification, broad national claims should be avoided. Future multicenter studies should include larger probability-based samples from different provinces, altitude zones, ethnic groups, and age categories, with standardized screening for nutritional deficiency, infection, inflammation, and chronic disease. Such studies would allow more robust national or region-specific hematological reference intervals for Pakistani adults.

CONCLUSION

This study provides sex-specific reference intervals for selected complete blood count parameters among apparently healthy adults from Quetta and surrounding areas. The findings demonstrate clear differences between male and female participants, with higher hemoglobin, red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin among males, and higher platelet count, total leukocyte count, and mean corpuscular hemoglobin concentration among females. These results support the clinical value of locally derived and sex-stratified CBC reference intervals rather than uncritical reliance on international reference standards. However, the findings should be interpreted within the limits of the study design, sampling method, and unresolved denominator reporting; larger multicenter studies are needed before national adult Pakistani reference intervals can be definitively established.

REFERENCES

1. Harris KM, McDade TW. The biosocial approach to human development, behavior, and health across the life course. RSF Russell Sage Found J Soc Sci. 2018;4(4):2-6.
2. Jones GR, Haeckel R, Loh TP, Sikaris K, Streichert T, Katayev A, et al. Indirect methods for reference interval determination: review and recommendations. Clin Chem Lab Med. 2019;57(1):20-9.
3. Brott DA, Goodman MJ, Hermann RP, Merz M, Calvo R, Poorkhalkali N, et al. Are laboratory parameter values similar to the healthy volunteer reference range in all patient populations? Drug Des Devel Ther. 2018;12:2757-64.
4. Koochakzadeh L, Mahbod M, Pakzad R, Jafari D, Khoshhal F, Yekta A, et al. Establishing normal ranges of hematological parameters from an Iranian healthy population: a population-based cross-sectional study of hospital data. Acta Med Iran. 2018;56(12):571-6.
5. Wiegele M, Kimberger O, Schaden E, Marhofer P, Baierl A, Willschke H, et al. Establishing reference ranges of cord blood: point-of-care hemostatic function assessment in preterm and term neonates. Pediatr Res. 2021;90(2):452-8.
6. Lewandowski KC, Tadros-Zins M, Horzelski W, Grzesiak M, Lewinski A. Establishing reference ranges for aldosterone, renin and aldosterone-to-renin ratio for women in the third trimester of pregnancy. Exp Clin Endocrinol Diabetes. 2022.
7. Zheng W, Zhang L, Tian Z, Zhang L, Liang X, Li G. Establishing reference ranges of serum lipid level during pregnancy and evaluating its association with perinatal outcomes: a cohort study. Int J Gynaecol Obstet. 2021.
8. Placzkowska S, Terpinska M, Piwowar A. The importance of establishing reference intervals: is it still a current problem for laboratory and doctors? Clin Lab. 2020;66(8).
9. Jiang W, Kang L, Lu HZ, Pan X, Lin Q, Pan Q, et al. Normal values for CD4 and CD8 lymphocyte subsets in healthy Chinese adults from Shanghai. Clin Diagn Lab Immunol. 2004;11(4):811-3.
10. Bukhari KT, Zafar H. Blood complete picture examination: a surrogate test for screening hepatitis C viral infection. Asian J Agric Biol. 2013;1(2):76-8.
11. Farkas JD. The complete blood count to diagnose septic shock. J Thorac Dis. 2020;12 Suppl 1:S16.
12. Usman K, Syed ZA, Rao AA. Reference range values of haematological parameters in healthy Pakistani adults. Pak J Physiol. 2007;3(1):19-22.
13. Siraj N, Issac J, Anwar M, Mehari Y, Russom S, Kahsay S, et al. Establishment of hematological reference intervals for healthy adults in Asmara. BMC Res Notes. 2018;11(1):1-6.
14. Ramezani A, Shams M, Zarinfar N, Banifazl M, Aghakhani A, Eslamifar A, et al. Hematological reference values for healthy males in the central part of Iran. Iran J Pathol. 2014;9(1):50-5.
15. Awad KM, Bashir AA, Osman AA, Malek MA, Alborai AA, Ali IA, et al. Reference values for hemoglobin and red blood cells indices in Sudanese in Khartoum State. Int J Health Sci Res. 2019;9(1):210-4.
16. Yassein RB, Alseedig NO, Abdallah SK, Mohammed AA, Alballah NA, Syid MA. Hematological parameters among Sudanese patients with chronic renal failure. Int J Res Granthaalayah. 2016;4(1):50-4.
17. Bain BJ, Bates I, Laffan M, Lewis S. Reference ranges and normal values. In: Dacie and Lewis Practical Haematology. 11th ed. London: Churchill Livingstone; 2011. p. 14-17.

18. Nweke JN, Jeremiah ZA. Immunohaematological reference values of apparently healthy adult Nigerians in Benin City, Edo State. Int J Med Eval Phys Rep. 2021;5(1):50-63.

19. Faya A, Charles M, Sembajwe LF, Dika HI. Haematological profile of healthy adult blood donors in Mwanza, Tanzania. Tanzan J Health Res. 2018;20(3).

20. Hoffbrand AV, Pettit JE, Moss PAH. Essential Haematology. Oxford: Blackwell Science; 2001.

21. Mazhar N, Rafi S, Farhan S, Yaseen S, Ahmed N. Normal reference values of complete blood count in healthy adult population of Pakistan: a multicentre study. Pak J Med Health Sci. 2021;15:3040-2.