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ESTABLISHING NORMAL REFERENCE RANGES FOR WHITE BLOOD CELL AND PLATELET COUNTS IN ADULT MALES: A REGIONAL STUDY IN MUZAFFARABAD DISTRICT.

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ABSTRACT

BACKGROUND: Many studies have established normal reference values for hematological indices in various populations, but these values can be influenced by factors like sex, age, ethnicity, and socio-environmental conditions. It is crucial to develop specific reference values for each population. For the Pakistani population, existing hematological reference values are based on Western populations and may not be suitable. **OBJECTIVE:** To determine normal reference values for white blood cells and platelets for adult male population in district Muzaffarabad. **METHODOLOGY:** This cross-sectional study took place in District Muzaffarabad from July 2022 to February 2023, involving 384 healthy adult men, selected through stratified disproportionate random sampling. Data was collected through a structured questionnaire. The collected blood samples were analyzed using the Sysmex Hematology Analyzer KX21, Japan. Reference values for Total Leukocyte and platelets count were established and compared with existing reference values. **RESULTS:** The mean age of 29.11 years, about 71.87% n=276 were from rural areas, 44.27% n=170 belonged to the lower socioeconomic class, 66.67% n=256 had completed high school education. The mean Total Leukocyte count TLC was 7,579/µl, with a range of 4,280 to 10,380/µl, while the mean platelet count PLT# was 284,271/µl, ranging from 195,891 to 365,678/µl. The independent t-test revealed no statistically significant differences in the mean Leukocytes and Platelets across different age groups, areas of residence, education levels, and socioeconomic statuses $p>0.05$. **CONCLUSION:** Normal reference values for TLC and Platelets for adult male population in district Muzaffarabad are different from the reference values which are currently used.

KEYWORDS: Leukocytes, Platelets, Reference values, Hemoglobin.

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INTRODUCTION

Leukocytes, or White blood cells WBCs, are important components of the immune system, playing a pivotal role in both inborn and adaptive immune responses.[1,2] Originating from the Greek words leukos white and kytos cell, WBCs are responsible for defending the body against infection and disease.2 The types and total count of white blood cells WBCs provide essential information about our health. Abnormalities in WBC counts can signal various health conditions, including leukemia, AIDS, autoimmune disorders, immune deficiencies, and other blood-related diseases.[1]

The leukocytes circulate through the bloodstream, white blood cells play a key role in managing both inflammatory and cellular responses to injuries or the presence of pathogens. Their primary functions include identifying and eliminating foreign invaders like bacteria and viruses, as well as modulating the body's overall immune reactions.[2] The range of physiological parameters typically seen in healthy individuals is known as normal reference values, which healthcare professionals rely on to interpret test results.[3] These values indicate the range within which 95% of a reference population's measurements fall. Ideally, normal reference values for hematological indices are determined through random sampling of populations that are similar in age, gender, ethnicity, and factors such as environmental, nutritional, and social conditions. These ranges are usually expressed as the mean ±2 standard deviations SD and are affected by factors like sex, age, body build, ethnic background, altitude, and various environmental and nutritional conditions.[4–6] Using inappropriate reference values can lead to unnecessary tests or missed diagnoses.[5] Therefore, establishing specific normal reference values for each population is crucial for accurate clinical assessments. This study highlights the need for research within the localpopulation to establish reference values for hematological parameters, as current values are often based on Western populations and may not accurately reflect the Pakistani context.[7]

The total number of white blood cells in the blood, known as the Total Leukocyte Count TLC, typically ranges between 4,000 and 11,000/µl.2 When TLC is elevated, a condition called leukocytosis, it may result from normal immune responses or indicate an underlying health issue. Conversely, a reduced TLC, known as leukopenia, signals a potentially compromised immune system.[2,8] White blood cells WBCs play a crucial role in defending the body against infections and are categorized into five types: monocytes, lymphocytes, neutrophils, basophils, and eosinophils.1 The normal range for platelet count PLT# is between 150,000 and 400,000/µl.[9] Thrombocytopenia, a reduced platelet count, may occur due to either increased destruction or decreased production of platelets, while thrombocytosis, an elevated platelet count, can arise from increased production, certain disorders, or other conditions.[10]

A complete blood count CBC is an essential diagnostic test that measures various blood components, including WBCs, red blood cells, and platelets. It provides critical insights into various disease processes by evaluating these

parameters. For a more detailed analysis, a peripheral blood smear might be performed, which involves spreading a thin layer of blood on a microscope slide for examination. This test is particularly useful in diagnosing conditions such as leukopenia or malignancies like leukemia or lymphoma, allowing for a closer inspection of blood cell morphology and the detection of abnormal cells or counts.[11] Current values for the Pakistani population are largely based on data from Western populations, there is a significant need for studies like this to develop appropriate national reference values for hematological indices in Pakistan.[7,12] This study aims to establish reference values of White Blood Cells and Platelets for adult males in District Muzaffarabad and compare them with existing reference ranges. The primary objective was to determine the reference values for white blood cells and platelets in male adult population.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted from July 2022 to February 2023 in Muzaffarabad District, enrolling 384 healthy adult males aged 18-50 years through stratified random sampling. The sample size was determined using the WHO sample size calculator, considering a 95% confidence interval, a 5% margin of error, and a population of adult male about 165,460, which resulted in a required sample size of 384. Stratified random sampling was employed to ensure representation, with the sample distributed across the 28 union councils within the two tehsils of District Muzaffarabad: Muzaffarabad and Naseerabad. Disproportionate allocation ensured representative sampling from each union council. Within each council, mohallas and wards were randomly selected, and subjects within these areas were randomly chosen.

Data collection for the study was carried out using a structured questionnaire and blood sample collection, conducted in the community by trained doctors and paramedical staff. Blood samples 4 ml were collected between 9 a.m. and 11 a.m. using disposable syringes and were immediately transferred to glass vials containing EDTA as an anticoagulant. The samples were analyzed at AIMS by experienced pathologists using the Sysmex Hematology Analyzer XP-100. Before the blood sampling, participants underwent detailed medical examinations including physical and systemic examinations. Enrollment was based on specific inclusion and exclusion criteria.

The inclusion criteria required participants to be healthy adult men aged 18-50 years, residing in District Muzaffarabad, and free from any acute or chronic illnesses that could influence hematological indices. Only participants who provided informed written consent were included in the study. Exclusion criteria were set to ensure the accuracy of hematological measurements. Individuals living at altitudes above 6,500 feet 1,982 meters were excluded due to the potential impact of high elevation on hematological parameters. Additionally, those with acute or chronic diseases, recent blood loss, blood transfusions, blood donations, or surgeries within the past three months were not eligible for participation. The study also excluded individuals who smoked tobacco, abused drugs, or had a family history of hematological disorders. Participants with exposure to hazardous chemicals or those taking medications that could interfere with hematological indices were also excluded, along with any other conditions or factors that might compromise the accuracy of the measurements.

Ethical considerations were rigorously observed, with approval obtained from the Ethical Committee of Khyber Girls Medical College Peshawar and the Executive Director of Abbas Institute of Medical Sciences AIMS, Muzaffarabad.

WBCs and Platelets level was assessed with means and standard deviations, and reference ranges were calculated as mean ± 2 standard deviations. Data were entered

into a structured data collection proforma and analyzed using SPSS version 22. Independent sample t-tests were conducted to evaluate mean variations, with a $p < 0.05$ deemed significant.

RESULTS

The study stratified participants by socioeconomic status based on monthly family income in Pakistani rupees: Lower Class up to PKR 30,000, Lower Middle Class PKR 31,000 – 150,000, Upper Middle Class PKR 151,000 – 250,000, and Upper Class above PKR 250,000. The majority 44.3%, n=170 belonged to the lower socioeconomic class, followed by the lower-middle class n=148; 38.54%.

In terms of employment, students constituted a significant portion of the study population 32.55%, while the remaining participants were either self-employed or employed in government/private sectors 67.45%. Specifically, 48 participants 12.50% were in government service, 38 9.90% were teachers in public or private schools, and 34 8.85% were employed as domestic helpers or in other private jobs. The rest were self-employed in various fields.

The majority of the study population n=256; 66.67% had completed their education up to the Higher Secondary School Certificate HSSC level, while 128 participants 33.33% had pursued graduation and/or higher studies. A detailed breakdown of the educational status of the participants showed that HSSC 26.3% level was the education level of participants, followed by Matriculation 23.96%, graduation 20.83%, primary school 10.16%, Masters 9.38%, Middle school 6.25%, and M.Phil. 2.08%. Additionally, two participants 0.52% each had completed PhD and Islamic education. The detailed socio-demographic characteristics of the study participants are presented in Table 1

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Table 1. Demographics characteristics of the participants

Variables	Categories	Frequency	Percent
Age	≤ 34 yrs	279	72.3%
	>34 yrs	105	27.7%
Residence	Rural Area	276	71.9%
	Urban Area	108	28.1%
Economic status	Lower class	170	44.3%
	Lower-middle Class	148	38.5%
	Upper-middle Class	51	13.3%
	Upper Class	15	3.9%
Education	Up to HSSC	256	66.67%
	More than HSSC	128	33.33%
Professional Status	Student	125	32.55%
	Employed	259	67.45%

The hematological indices of the study population reveal significant insights into the normal ranges of RBC, WBC, and platelets PLT. The total leukocyte count TLC had an average of 7579.28±2249.57 per µl. The calculated range for WBC was between 3080.14 and 12078.42 per µl. The platelet count PLT averaged 284,271 per µl with a standard deviation of 60,668 per µl, giving a range of 162,935 to 405,607 per µl. The remaining hematological indices, including, MCV, MCH, and MCHC, are presented in Table 2.

Table 2. Analysis of Hematological Parameters of the Participants

Hematological Indices	Mean	SD	Mean ± 2SD	Range Values
HCT %	39.77	5.15	39.77 ± 10.30	29.47 - 50.07
MCV fL	86.81	8.04	86.81 ± 16.08	70.73 - 102.89
MCH pg/cell	28.28	3.09	28.28 ± 6.18	22.10 - 34.46
MCHC g/dl	32.34	1.91	32.34 ± 3.82	28.52 - 36.16
TLC per µl	7579.28	2249.57	7579.28 ± 4499.14	3080.14 - 12078.42
Platelets count per µl	284271	60668	284271 ± 121336	162935 - 405607

Comparison of TLC and Platelets count between various demographic groups

To assess differences based on age, area of residence, level of education, and socioeconomic status we utilized the independent sample t-test. The comparison of hematological indices, specifically total leukocyte count TLC and platelet count PLT#, between various groups of the study population reveals some different patterns, as shown in Table 3. For age-based comparison, those aged 34 years or younger had a mean TLC of 9338.85±2034.61 per µl, compared to 8645±2392.63 per µl in those older than 34 years. However, the difference was not significant p = 0.67. The platelet count was slightly higher in the younger group 28113.67±85588.18 per µl than in the older group 277168.87±96791.56 per µl, but not significant p = 0.86. In terms of area of residence, rural residents had a mean TLC of 9053.85±4094.47 per µl compared to 9411±2238.30 per µl in urban residents, with a p-value of 0.38, indicating no significant difference. The platelet count was also similar between rural 277558.18±88251.33 per µl and urban residents 287129.63±89910.25 per µl with a p-value of 0.92.

Those who completed up to the Higher Secondary School Certificate HSSC had a mean TLC of 9324.88±4118.44 per µl, while those with more than HSSC education had a slightly lower mean TLC of 8792.19±2506.14 per µl, but this variation was not significant p = 0.96. The platelet count was higher in those with up to HSSC education 288221.09±91461.04 per µl than in those with more than HSSC 263632.03±80808.69 per µl, but again, this dissimilarity was not significant p = 0.11. When comparing socioeconomic status, there was a significant difference in TLC between the lower class 7593.59±2284.52 per µl and the upper class 7807.07±1701.80 per µl with a p-value of 0.036. However, the contrast in platelet count between these two groups was not significant p = 0.843. No significant dispute was observed in TLC and PLT# between the lower and upper middle class or between the lower middle class and upper class.

This analysis highlights the variations in hematological indices across different demographics, though many differences were not statistically significant.

Table 3. Comparative Analysis of White Blood Cells and Platelets level

Hematological Indices					
Variables	**Categories**	**TLC per µl**	**p-values**	**Platelets count per µl**	**p-values**
Age	**≤ 34 years**	9338.85±2034.61	0.67	28113.67±85588.18	0.86
	> 34 years	8645±2392.63		277168.87±96791.56	
Residency	**Rural area**	9053.85±4094.47	0.38	277558.18±88251.33	0.92
	Urban area	9411±2238.30		287129.63±89910.25	
Education	**Upto HSSC**	9324.88±4118.44	0.96	288221.09±91461.04	0.11
	More than HSSC	8792.19±2506.14		263632.03±80808.69	
Economic Status	**Lower class**	7593.59±2284.52	0.03	284734.89±59035.06	0.84
	Upper class	7807.07±1701.80		268394.60±61306.93	
	Lower middle class	7653.06±2292.37	0.42	281491.92±63749	0.11
	Upper middle class	7250.47±2172.63		296780.14±55732.14	

Comparison of haematological indices in study population and normal reference values being used

The study population's haematological indices, specifically RBC, WBC, and platelet counts, show some variations when compared to normal reference values, as presented in Table 4. the normal reference values were determined from a healthy population within the district. The total leukocyte count TLC exhibited a range of 3080.14 - 12078.42 per µl, which extends beyond the usual reference range of 4500 - 11000 per µl. Platelet counts PLT# ranged from 162935 to 405607 per µl, aligning well with the established reference values of 150,000 - 400,000 per µl. These findings suggest the need for localized reference ranges to better reflect the hematological health of the population in this district.

Table 4. Comparison of Current Reference values with Our Study's Participants

Hematological indices	Reference values of study's population	Current reference values according to NCBI-NIH**13**
TLC per µl	3080.14 - 12078.42	4500 -11000
PLT# per µl	162935 - 405607	150,000 - 400,000

DISCUSSION

The study aimed to establish normal reference ranges for TLC and PLT# in the adult male population of District Muzaffarabad, where specific data had previously been lacking. The findings revealed significant deviations from

international reference values, similar to the results reported by Usman K et al. in their study of the adult male population in Multan, as well as by Pasha W. et al. 7,11 Additionally, the total leukocyte count TLC varied from 3080.14 to 12078.42/µl, extending beyond the standard range of 4000 to 11000/µl. The platelet count PLT# closely aligned with the standard reference range but showed a broader distribution compared to Usman K et al.'s findings.11 These deviations from standard reference values are consistent with the findings of Yassin et al., who reported significant differences between the determined hematological indices reference values for young adults and the standard normal reference values. This study highlights that the reference ranges commonly used may not always accurately reflect the hematological health of specific populations.[14]

The total leukocyte count TLC ranged from 3080.14 to 12078.42/µl, the reference values reported by Hee et al. indicate slightly narrower ranges. For males, their reported WBC range is 4000 to 10300/µl, and for females, it is 3500 to 9600/µl.[15] Our study's upper limit for TLC extends beyond the upper range reported by Hee et al. for both males and females, reflecting a broader range in our population. The lower end of our range falls within the reference range for males but is slightly below the lower end for females. Hee et al. reported platelet counts ranging from 216,000 to 469,000/µl for both males and females. In contrast, our study found a broader range for platelet counts, spanning from 162,935 to 405,607/µl. While our lower limit is lower than Eun Hee et al.'s reported range, and our upper limit is also slightly lower. This indicates that the platelet counts in our study's population generally fall within the range reported by Eun Hee et al., but with some variation, especially at the lower end of the spectrum.[15]

Based on Rosenfeld's findings, the mean red blood cell RBC count for men was reported as 5.0 million/µl with a range of 4.3 to 5.8 million/µl. In comparison, our study found a mean RBC count of 4.58 million/µl, which is slightly lower but falls within the broad range of 3.40 to 5.76 million/µl observed in our population. For hemoglobin levels, Rosenfeld reported a mean of 14.9g/dL for men, ranging from 13.0 to 16.9g/dL. Our study, however, found a mean hemoglobin concentration of 13.05g/dL, ranging from 9.73 to 16.37g/dL, indicating that the mean is lower, the range covers a similar span. Rosenfeld reported a mean of 6142/µl for men with a range of 2843 to 9440/µl. Our study, in contrast, found a mean total leukocyte count TLC of 7579.28/µl, with a wider range of 3080.14 to 12078.42/µl. Although the mean WBC count in our study is higher, the broader range reflects greater variability in our population compared to Rosenfeld's narrower range, it might be due to geographic and environmental factors, nutritional status, study design and methodology.[16]

Age, residency, education, and socioeconomic status were analyzed for their impact on hematological indices. The mean TLC was higher in younger individuals ≤ 34 years compared to older ones > 34 years, but these variations were not significant statistically $p = 0.67$. Similarly, there was no significant difference in platelets count between these age groups $p = 0.86$. Analysis by residency revealed no significant differences in TLC and platelet counts between rural and urban areas $p = 0.38$ and $p = 0.92$, respectively. Educational attainment and socioeconomic status also did not show significant variations in platelet counts, although TLC was higher in the upper socioeconomic class as compared to the lower class $p = 0.036$ which is in line with study by Tamuno-Opubo et al., reporting no significant difference for PLT# while significant difference for TLC in different socioeconomic classes [17]. The differences in hematological indices observed in our study suggest that localized reference ranges may better reflect the specific

health profile of the Muzaffarabad population. The findings underscore the importance of developing region-specific reference values to improve the accuracy of clinical assessments and ensure more precise diagnostic and treatment strategies. As our study was limited to District Muzaffarabad, the findings cannot be generalized to the entire adult population of AJK. The study provides reference ranges specifically for males aged 18–50 years, without offering insights into females or other age groups. Additionally, not all hematological indices were examined, and participant health status was determined through history and clinical examination rather than laboratory confirmation. Future research should include a larger, more diverse sample encompassing females, all age groups, and incorporating laboratory tests to exclude underlying conditions, ensuring a more comprehensive understanding of hematological indices in the region.

CONCLUSION

The study identified localized reference values for TLC, PLT#, and other hematological parameters specific to the adult male population of District Muzaffarabad. The results indicated significant deviations from international reference ranges. The total leukocyte count TLC and platelet count PLT# were observed to have a narrower range compared to standard reference values. The total leukocytes count was significantly different among different socioeconomic classes. These findings highlight the need for region-specific reference ranges to more accurately reflect the hematological profiles of local populations.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

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