ORIGINAL ARTICLE

REFERENCE VALUES FOR HAEMOGLOBIN LEVEL AND RED CELL COUNT FOR ADULT MALE POPULATION OF MUZAFFARABAD

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Background: For each population, its own normal reference values should be established. Reference values for haemoglobin concentration (Hb con) and red blood cells count (RBC#) have been worked out for different populations. This study was conducted to determine normal reference values for Hb con and RBC# for adult male population in district Muzaffarabad. **Methods:** This cross-sectional study was conducted in district Muzaffarabad from Jul 2019 to Feb 2020. A total of 384 healthy adult local males, aged 18–50 years, were enrolled through stratified random sampling. Collection of information and blood sampling were carried out in the community. Levels of Hb con and RBC# were assessed using Sysmex Haematology Analyzer. Comparison of determined reference values was made between different groups of study population and with reference values already being used. **Results:** Mean age of study population was 29.11±9.26 years with a range of 18–50 years. Mean value of Hb was 13.05 g/dL (9.73–16.37 g/dl) and RBC# 4.58 million/µL (3.4–5.76 million/µL) respectively. Determined values were compared between different groups of study population and with the reference values being used. **Conclusion:** Normal reference values for Hb con and RBC# for adult male population in district Muzaffarabad were different from values which are being used.

Keywords: Reference values, Haematological indices, Haemoglobin, Red blood cell count, Male, Muzaffarabad

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INTRODUCTION

Haemoglobin (Hb) is the iron containing metalloprotein in red blood cells (RBCs). Hb transports oxygen from the lungs to the body tissues. Aerobic metabolism provides energy and functions of the cells are powered. Hb also transports carbon dioxide (CO_2) from the tissues to the lungs. It transports about 20–25% of CO_2 as carbaminohaemoglobin. It also transports nitric oxide which binds with globin protein, released simultaneously with O_2.[1] Hb concentration is expressed as g/dL.[2] Reference range for Hb con being used for adult male Pakistani population is 13–17 g/dL.[3]

RBCs are the principal means of O_2 delivery to the tissues. O_2 is taken up in the lungs by RBCs and delivered to the tissues while passing through the capillaries. In human adults, about 2.4 million new RBCs are produced each second. Mature RBCs are elliptical biconcave disks. Most organelles and nuclei are absent in RBCs in adults and maximum space is occupied by Hb, which is responsible for the red colour of blood. In adult life RBCs undergo development in the bone marrow. They circulate in the body for 100–120 days, before they are engulfed by macrophages and their components are recycled. RBCs are most numerous among blood cells and body as well. About 70–84% of the cells in the human body are RBCs.[4]

Red Blood Cell count (RBC#) is tested as a part of complete blood count. RBC# is expressed in million/µL. Hb con is affected by number of RBC#.[2] Reference range for RBC# being used for adult male Pakistani population is 4.5–5.5 million/Cmm.[3]

Studies conducted in different populations of the world[5–12] have worked out the reference ranges for haematological indices in adults and children. Many factors affect the values of haematological indices even in healthy populations and these factors include sex, age, body build, ethnic background, altitude, nutrition, environment and social conditions. The reference values for haematological indices are different for different populations in the world. Therefore, establishment of its own reference values have been stressed for each population.[5–12]

Reference values for haematological indices used for Pakistani population are the reference values derived from populations of western countries.[3,13] Previous studies on Pakistani population groups[3,13] have determined that the reference values derived for these populations are different from the values which are being used. The objectives of this study were to determine the reference values for Hb con and RBC# for adult male population in district Muzaffarabad.

MATERIAL AND METHODS

This cross-sectional study was conducted based on the blood samples of adult males in the age range from 18–50 years, residing in urban and rural areas of district Muzaffarabad, from July 2019 to February 2020. Approval was obtained from Ethical Review Committee of Khyber Girls Medical College, Peshawar and

Executive Director, Abbas Institute of Medical Sciences, Muzaffarabad. Sample size was 384, which was calculated with population proportion sample size formula[14]. Stratified random sampling technique[15] was used. Target population was 165,460 (21% of total population of district Muzaffarabad). Percentage of target population in each of 28 union councils of district Muzaffarabad was calculated using optimum allocation (disproportionate allocation) stratified random sampling strategy[15].

Written informed consent was obtained from all participants. Healthy adult males were included in this study. Exclusion criteria were high altitude (>8,000 feet[16]) dwelling, acute or chronic diseases, use of drugs which interfere with Hb con or RBC#, blood loss during last 3 months, blood transfusion during last 12 months, blood donation during last 3 months, surgery during last 3 months, tobacco smoking, family history of haematological disorders and exposure to hazardous chemicals.

Data were collected using an objectively structured questionnaire. Blood samples were obtained by trained team members. Detailed medical history was taken and general physical, systemic examination was performed on each subject.

Four (4) mL venous blood was withdrawn from each subject in EDTA vials and analysed within 4 hours of sampling[8] at AIMS, Muzaffarabad.

Sysmex Hematology Analyzer XP-100 (Serial No. A–4847) and Sysmex specified reagents were used. Procedure was performed strictly in accordance with manufacturer's instructions, while observing strict quality control of the machine.[2,17]

Data were analyzed using SPSS-22. Means and standard deviations were calculated. Independent sample Student's *t*-test was used to see the mean differences, and $p<0.05$ was considered statistically significant.

RESULTS

Mean age of study population was 29.11±9.26 years with a range of 18–50 years. Majority (276, 71.87%) of the study population belonged to rural areas and 108 (28.13%) lived in the urban areas. Majority of study population (170, 44.27%) belonged to lower socioeconomic class, followed by lower middle class (148, 38.54%), upper middle class (51, 13.28%) and upper class (15, 3.91%) (Table-1).

Mean values of Hb con was 13.05 (range: 9.73–16.37) g/dL, and RBC# was 4.58 (range: 3.4–5.76) million/µL. (Table-2).

No significant difference was found on Student's *t*-test between different groups of study population except, in Hb con mean values of lower and lower middle class ($p=0.00$). (Table-3).

Hb con and RBC# in this study were different from the reference values currently being used. Determined values of Hb con and RBC# are lower and have wider range than the normal reference values in use. (Table-4).

Table-1: Demographics of study population (n=384)

Variables	Frequency	Percentage
Area of residence		
Rural	276	71.87
Urban	108	28.13
Socioeconomic status		
Lower class	170	44.27
Lower middle class	148	38.54
Upper middle class	51	13.28
Upper class	15	3.91

Table-2: Hb conc and RBC# in study population

Variable	Mean±SD	Mean±2 SDs	Range
Hb con (g/dL)	13.05±1.66	13.05±3.32	9.73–16.37
RBC# (million/µL)	4.58±0.59	4.58±1.18	3.40–5.76

Table-3: Comparison of Hb con and RBC# values in age, residence, education and socioeconomic groups

Variables	Hb con (g/dL)	RBC# (million/µL)
Age group		
Up to 34 years	12.96±1.65	4.56±0.60
>34 years	13.28±1.68	4.62±0.56
p	0.767	0.452
Residence		
Rural	12.94±1.69	4.55±0.61
Urban	13.33±1.57	4.66±0.56
p	0.306	0.15
Educational Status		
Up to HSSC	12.58±1.47	4.48±0.60
More than HSSC	13.97±1.63	4.77±0.52
p	0.146	0.33
Socioeconomic Status		
Lower class	11.75±0.82	4.55±0.61
Lower middle class	13.46±1.13	4.66±0.56
p	0.000	0.71

Table-4: Comparison between measured range values and reference values in use

Variable	Measured values	Reference values in use
Hb con (g/dL)	9.73–16.37	13–17
RBC# (million/µL)	3.4–5.76	4.5–5.5

DISCUSSION

It is necessary to establish normal reference values for haematological indices in a population for screening, diagnosis, and monitoring of associated pathological conditions. The normal reference values for haematological indices are influenced by many factors, e.g., ethnicity, environmental factors, age, gender, nutritional factors, social factors, and genetic influences necessitating the establishment of normal reference values for each population.[3,5–13]

Reference values for haematological parameters have been determined in different populations of the world. Studies conducted by Addai-Mensah O *et al*[5] in three regions of Ghana, Mulu W *et al*[6] in Gojjam zones in Amhara region, Ethiopia,

Shaheen NA *et al*[7] in Saudi Arabia, Siraj N *et al*[8] in Asmara, Omarine Nlinwe N *et al*[9] in Bamenda, North West Region of Cameroon, Rosenfeld LG *et al*[10] in Brazil, and Iftikhar R *et al*[11] in Nyala, Darfur determined the values of haematological indices in healthy adult populations. Study by Ouma JO *et al*[12] in Kombewa Sub-County, Kisumu, Western Kenya, determined the reference values of haematological parameters among infants aged 1 to 17 months. Reference values for haematological indices for Pakistani population are derived from the populations of western countries. Reference values from different Pakistani populations were determined in a very small number of studies.[3,13]

Study by Shaikh MS *et al*[3] determined reference intervals for routine and special haematological parameters for adult population in Karachi. They concluded that determined values of the parameters were different from the values in practice. Reference values of haematological indices for healthy adult population determined by Mazhar N *et al*[13] from Lahore, Karachi, Quetta, Rawalpindi and Abbottabad were found different from international reference values in use. Measured reference values of Hb con and RBC# in this study are different from the reference values currently in use for adult male population in district Muzaffarabad. Results of studies in Pakistani populations[3,13] and different populations of the rest of the world[5–12] favour the results of this study.

CONCLUSION

The reference values for Hb con and RBC# for adult male population in district Muzaffarabad are different from the values already in use. Determined values of Hb con and RBC# are lower and have wider range than the normal reference values currently in use.

LIMITATIONS

This study was conducted in district Muzaffarabad only and cannot be generalized for whole adult population of Kashmir. This study reflects reference range for male population only and lacks information about females. This study is focused on people 18–50 years old. Studies with larger sample size including female population, all age groups and parameters will further clear the picture.

REFERENCES

1. Dybas J, Bokamper MJ, Marzec KM, Mak PJ. Probing the structure-function relationship of hemoglobin in living human red blood cells. Spectrochim Acta A Mol Biomol Spectrosc 2020;239:118530.
2. Means RT, (Ed). Wintrobe's clinical hematology. 15th ed. Philadelphia: Wolters Kluwer; 2023.
3. Shaikh MS, Ahmed S, Khalid A, Hayat MH, Shaikh MU, Ali N. Establishment of population specific reference intervals in healthy Pakistani adults for 21 routine and special haematology analytes. EJIFCC 2022;33(3):220–32.
4. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 2016;14(8):1002533.
5. Addai-Mensah O, Gyamfi D, Duneeh RV, Danquah KO, Annani-Akollor ME, Boateng L, *et al*. Determination of haematological reference ranges in healthy adults in three regions in Ghana. BioMed Res Int 2019;2019:7467512.
6. Mulu W, Abera B, Mekonnen Z, Adem Y, Yimer M, Zenebe Y, *et al*. Haematological and CD4+ T cells reference ranges in healthy adult populations in Gojjam zones in Amhara region, Ethiopia. PloS One 2017;12(7):e0181268.
7. Shaheen NA, Rehan H, Moghairi A, Gmati G, Damlaj M, Salama H, *et al*. Hematological indices in the adult Saudi population: Reference intervals by gender, age, and region. Front Med (Lausanne) 2022;9:901937.
8. 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):55.
9. Omarine Nlinwe N, Larissa Kumenyuy Y, Precious Funwi C. Establishment of hematological reference values among healthy adults in Bamenda, North West region of Cameroon. Anemia 2021;2021:6690926.
10. Rosenfeld LG, Malta DC, Szwarcwald CL, Bacal NS, Cuder MAM, Pereira CA, *et al*. Reference values for blood count laboratory tests in the Brazilian adult population, National Health Survey. Rev Bras Epidemiol 2019;22(Suppl 2):E190003.
11. Iftikhar R, Khan NU, Iqbal Z, Kamran SM, Anwar MI. Haematological Parameters in Different African Populations: An Experience From United Nations Level 3 Hospital. Pak Armed Forces Med J 2017;67(6):1068–72.
12. Ouma JO, Mulama DH, Otieno L, Owuoth J, Ogutu B, Oyieko J, *et al*. Clinical laboratory hematology reference values among infants aged 1month to 17 months in Kombewa Sub-County, Kisumu: A cross sectional study of rural population in Western Kenya. PloS One 2021;16(3):e0244786.
13. 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(11):3040–2.
14. Population Proportion –Sample Size. Available at: https://select-statistics.co.uk/calculators/sample-size-calculator-population-proportion.
15. Elfil M, Negida A. Sampling methods in Clinical Research; an Educational Review. Emerg (Tehran) 2017;5(1):e52.
16. Burtscher J, Swenson ER, Hackett PH, Millet GP, Burtscher M. Flying to high-altitude destinations: is the risk of acute mountain sickness greater? J Travel Med 2023;30(4):taad011.
17. Keohane EM, Otto CN, Walenga JM. Rodak's Hematology: Clinical Principles and Applications. 6th ed. St. Louis, Missouri: Elsevier; 2020.

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