# Do We Need Pakistan-Specific Reference Ranges in Laboratory Medicine?

Muhammad Saeed[1]   Usman Waheed[2,3]   Akhlaaq Wazeer[3]   Noore Saba[2,3]

[1] Department of Pathology, Tehsil Headquarters Hospital, Murree, Pakistan
[2] Peshawar Regional Blood Centre, Provincial Ministry of Health, Khyber Pakhtunkhwa, Pakistan
[3] Department of Pathology and Transfusion Medicine, Divisional Headquarters Teaching Hospital, Mirpur, Pakistan

Address for correspondence  Noore Saba, MBBS, MPH, MPhil, Peshawar Regional Blood Centre, Provincial Ministry of Health, Khyber Pakhtunkhwa, Pakistan (e-mail: dr.nooresaba@gmail.com).

Evidence-based laboratory medicine is indispensable for health care practices. Reference values or reference ranges are necessary for the interpretation of clinical laboratory tests and subsequent patient care. Nearly 70% of medical decisions by physicians are solely based on information provided by laboratory test results.[1] As a preliminary investigation into the topic of reference ranges, Gräsbeck and Fellman published a study in 1968 titled "Normal Value and Statistics".[2] The term "reference values" was used as a result of the realization that the concept of "normal values" was insufficient and even partially inaccurate in later years.

A test result by itself is of little significance except when it is reported with adequate information for its interpretation. Classically, this information is delivered in the form of a reference range or reference interval or normal value. According to the recommendations by International Federation of Clinical Chemistry (IFCC), the term "interval" is preferred to "range."[3] The term "range" should only relate to the difference between an interval's upper and lower limits. For instance, the sodium content in serum would have a reference range of 10 mmol/L and a reference interval of 135 to 145 mmol/L.

Comparison of a patient's laboratory test result versus a reference or "normal" range is vital to medical decision-making. Physicians compare laboratory report values to specified reference ranges to make decisions about a person's health status in clinical diagnosis, treatment, and monitoring. Reference ranges are also required by professional accreditation and regulatory bodies such as International Organization for Standardization 15189 which recommends that each laboratory should reevaluate its own reference interval on a regular basis.[3] Likewise, in the European Directive 98/79 on in vitro diagnostic medical devices, diagnostic kit producers are required to give their customers the proper reference intervals to use with their assay kit reagents.[4] Unfortunately, due to the challenges involved in obtaining a sufficient number of healthy people and the high cost of sample analysis, the majority of clinical laboratories are unable to develop their own reference ranges.[5]

For reference range formulation, a preselected reference population is sampled, measurements are taken, and then reference ranges are calculated according to the direct approach, a classic way to produce reference values.[6] The use of normal laboratory data recorded in the laboratory information system to derive reference intervals in an indirect one has, however, become more and more popular. The study to compare the traditional (direct) and alternative (indirect) methodologies for the determination of reference intervals is now being worked on by the IFCC and Laboratory Medicine Committee on Reference Intervals and Decision Limits. Most of the reference intervals in use refer to the central 95% of the reference population of study subjects. By definition, 5% of all findings from "healthy" individuals will deviate from the published reference value and be marked as abnormal.[6,7] Clinical and Laboratory Standards Institute guidelines advise establishing a reference value by choosing a statistically significant group with a minimum of 120 healthy reference subjects. International organizations also endorse population-specific clinical laboratory reference intervals, as gender, age, ethnicity, race, diet, geographic location, and other factors can affect the physiological value of a biochemical parameter.[8]

Several studies conducted in Asian and African populations have also shown differences in reference intervals compared with established Western values, and significant differences in the reference values by sex among various population groups are present.[9]

Despite its enormous clinical importance, most health care facilities in many developing countries, including Pakistan, rely on reference intervals available either in textbooks or diagnostic kit inserts. Given that dietary habits, sex, geographic location, and ethnic diversity are sources of variation, it is necessary to establish a population-specific reference interval for the population of Pakistan.

Although defining and establishing reference values for a particular population is an expensive and time-consuming project, each laboratory should arrange its reference intervals according to the recommendations of the IFCC.[10,11]

Significant public health issues like obesity, cardiovascular disease, diabetes, and infectious diseases are currently plaguing developing nations like Pakistan. The clinical laboratory is critical in this area for the early diagnosis of serious illnesses as well as for providing insightful data on an individual's health by comparing it with established reference intervals. However, there is a dearth of information available on the reference values in the Pakistani population. Therefore, we conducted a pilot study in the Islamabad Capital Territory to formulate reference intervals for fasting plasma glucose (FPG) and renal function tests (RFTs) that included urea, creatinine, and uric acid. A total of 400 healthy adults were selected for the study based primarily on careful consideration of inclusion and exclusion criteria. The study participants belonged to different geographical regions of Islamabad Capital Territory. Most of them belonged to sector G-6 (14.1%) followed by sector G-10 (9.4%).

The reference value was constructed using 2.5th and 97.5th percentiles as lower and upper limits at a 95% confidence interval in accordance with the Clinical and Laboratory Standards Institute for determining reference intervals. The study indicated that the reference interval of FPG was 55 to 115 mg/dL. For urea, it was 12 to 55 (mg/dL), for creatinine it was 0.4 to 1.5 (mg/dL) in males while 0.3 to 1.3 (mg/dL) in females. For uric acid, it was 3.0 to 8.5 mg/dL in males while 2.0 to 7.3 mg/dL in females. Our results deviated from lower limits as well as from upper limits of already established reference intervals in the region.

In contrast, reference intervals established by studies reported from the neighboring countries are different, although the genetic makeup and socioeconomic status remain more or less similar. For instance, a study on 10,665 individuals from Chennai, India, established that the reference interval for FPG was 78 to 110 mg/dL, urea 11 to 31 mg/dL, creatinine 0.6 to 0.9 mg/dL, and for uric acid, it was 3.5 to 8.2 mg/dL.[12] Similarly, two independent studies from Iran[13,14] established reference intervals for FPG (78–109 mg/dL; 63.4–104.9 mg/dL) and uric acid (2.80–7.59 mg/dL; 2.7–10.1 mg/dL) both of which deviated from our findings.

The present study showed significant variations in the level of FPG and RFT of a healthy Islamabad population in comparison with the reference intervals currently used in most clinical laboratories. This was a pilot study on a limited number of parameters. Given the significance, gender-based research should be done to find out what causes the biological variation and to determine most at risk for contracting the disease. The process of generating reference intervals is intricate, and the selection of the most effective method depends on a variety of biological and technological aspects pertaining to populations and variations in laboratory equipment. Knowing the benefits and drawbacks of the reference interval paves the way for a major improvement in clinical practice's everyday accuracy. The results of the current study should act as a catalyst for decision makers to initiate a larger sample-sized study with a multipronged approach to establish reference intervals/ranges for all territorial regions of the country.

Conflict of Interest
None declared.

## References

1 Hallworth MJ. The '70% claim': what is the evidence base? Ann Clin Biochem 2011;48(Pt 6):487–488

2 Gräsbeck R, Fellman J. Normal values and statistics. Scand J Clin Lab Invest 1968;21(03):193–195

3 Dybkaer R, Solberg HE. Presentation of observed values related to reference values. J Clin Chem Clin Biochem 1987;25:657–662

4 Pereira P. ISO 15189:2012 Medical Laboratories - Requirements for Quality and Competence. International Organization for Standardization, 3rd ed. 2020. accessed 14 July 2022 at: https://www.westgard.com/iso-15189-2012-requirements-1.htm

5 Ceriotti F, Hinzmann R, Panteghini M. Reference intervals: the way forward. Ann Clin Biochem 2009;46(Pt 1):8–17

6 Directive 98/79/EC of European Parliament and the Council of 27 October 1998 on in vitro diagnostic medical devices. Official J Eur Commun 1998;L331/1–L331/37

7 Huma T, Waheed U. The need to establish reference ranges. J Pub Health Bio Sci. 2013;2(02):188–190

8 Maphephu SM, Kasvosve I. Reference intervals for serum total cholesterol, HDL cholesterol and non-HDL cholesterol in Batswana adults. S Afr Med J 2011;101(08):546–547

9 Mekonnen Z, Amuamuta A, Mulu W, et al. Clinical chemistry reference intervals of healthy adult populations in Gojjam Zones of Amhara National Regional State, Northwest Ethiopia. PLoS One 2017;12(09):e0184665

10 Ozarda Y. Establishing and using reference intervals. Turkish J Biochem 2020;45(01):1–10

11 International Federation of Clinical Chemistry. Expert panel on reference intervals and decision limits (C-RIDL). Accessed June 20, 2022, at: https://www.ifcc.org/ifcc-scientific-division/sd-committees/c-ridl/

12 Sairam S, Domalapalli S, Muthu S, et al. Hematological and biochemical parameters in apparently healthy Indian population: defining reference intervals. Indian J Clin Biochem 2014;29(03):290–297

13 Ghazizadeh H, Kathryn Bohn M, Ghaffarian Zirak R, et al. Comprehensive laboratory reference intervals for routine biochemical markers and pro-oxidant-antioxidant balance (PAB) in male adults. J Clin Lab Anal 2020;34(11):e23470

14 Ghazizadeh H, Yaghooti-Khorasani M, Khodabandeh AK, et al. Reference intervals for routine biochemical markers and body mass index: a study based on healthcare center database in northeastern Iran. IUBMB Life 2021;73(02):390–397