Letter to the editor

Population-Specific Testosterone Reference Intervals in Pakistani Men: Application of the RefineR Algorithm

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Dear Editor,

Serum testosterone levels are essential for assessing normal physiological processes in males as well as identifying pathological conditions such as Hypogonadism [1]. Interpretation of these values relies heavily on reference intervals established for specific populations and age groups, taking cognisance of age, race, nutritional status among others due to physiological variations [2,3]. However, in Pakistan, laboratories often adopt reference intervals derived from international populations, which may not accurately represent local ethnic and physiological differences. Although reference intervals can also be established using direct methods involving healthy participants, limited adherence to guidelines from the Clinical and Laboratory Standards Institute may affect their reliability [4]. Recently, indirect statistical approaches have emerged and are supported by the International Federation of Clinical Chemistry and Laboratory Medicine as efficient methods for estimating reference intervals using existing laboratory data [5,6]. Therefore, this study utilizes patient data to derive reference intervals for serum testosterone and compares them with existing Pakistani and international values.

This study was conducted at the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, Aga Khan University (AKU). Data mining of serum testosterone results from 2008-2025 was done on a target population ranging from 19-39 (Mean: 29.82 ± 5.32) years of age after approval from institutional ethical review committee. This age group was selected because testosterone levels typically peak in early adulthood (around 20–30 years) and begin to gradually decline from approximately 30–40 years onward, reflecting the transition from physiological maturity to age-related hormonal decline. [7,8]. Serum total testosterone concentrations were measured using electrochemiluminescence immunoassay (ECLIA) on Roche platforms throughout the study period. Testing was initially performed on the Roche Elecsys e170 analyzer, followed by the Roche Elecsys e411, and currently on the Roche Cobas e801 analyzer. All analyses were conducted according to the manufacturer's protocols in an accredited laboratory with routine internal quality control and participation in external quality assessment program to ensure analytical reliability.

The corresponding clarification has been added to the Methods sectionThe program used is the RefineR package (version 1.0.0) due to its low mean error in separating non pathological values from pathological ones and further using them to derive reference intervals [9]. The AKU clinical lab handles sample from all over the country through its network on more than 300 phlebotomy centers spread across all of Pakistan, hence our sample was representative of the geographical distribution and all ethnicities of the country. Due to the retrospective nature of the dataset, pre-analytical variables such as timing of sample collection (e.g., morning vs non-morning samples) and fasting status could not be standardized. In cases where multiple test request forms existed for the same individual, only the results from the initial sample were considered for the final analysis. This study was conducted in accordance with the ethical principles for medical research involving human subjects as outlined in the Declaration of Helsinki. Ethical approval was obtained from the institutional review committee of Aga Khan University (ERC#2025-11907-36009), and all data were fully anonymized prior to analysis.

Given the right-skewed distribution of testosterone values, a Box–Cox transformation was applied to approximate a Gaussian distribution as required by the RefineR algorithm. The algorithm iteratively models the central distribution corresponding to the non-pathological population. Using the Box-Cox transformation method, the 2.5th percentile and 97.5th percentile were used as the lower and upper reference limit with a confidence interval of 95% as shown in Figure 1 was estimated for 75,547 values. No explicit exclusion of pathological values was performed; instead, the RefineR algorithm statistically models the central non-pathological distribution within the dataset, thereby minimizing the influence of outliers and pathological results. From this dataset, the estimated reference interval for serum testosterone in Pakistani males aged 19–39 years was 166–942 ng/dL (5.76–32.69 nmol/L). The distribution of values demonstrated a right-skewed pattern with a clear central peak representing the presumed healthy population, from which the algorithm derived the reference limits. The calculated reference interval was subsequently compared with previously reported international reference ranges in Table 1.

Figure 1: Estimated Reference Intervals (from Patients' Samples).

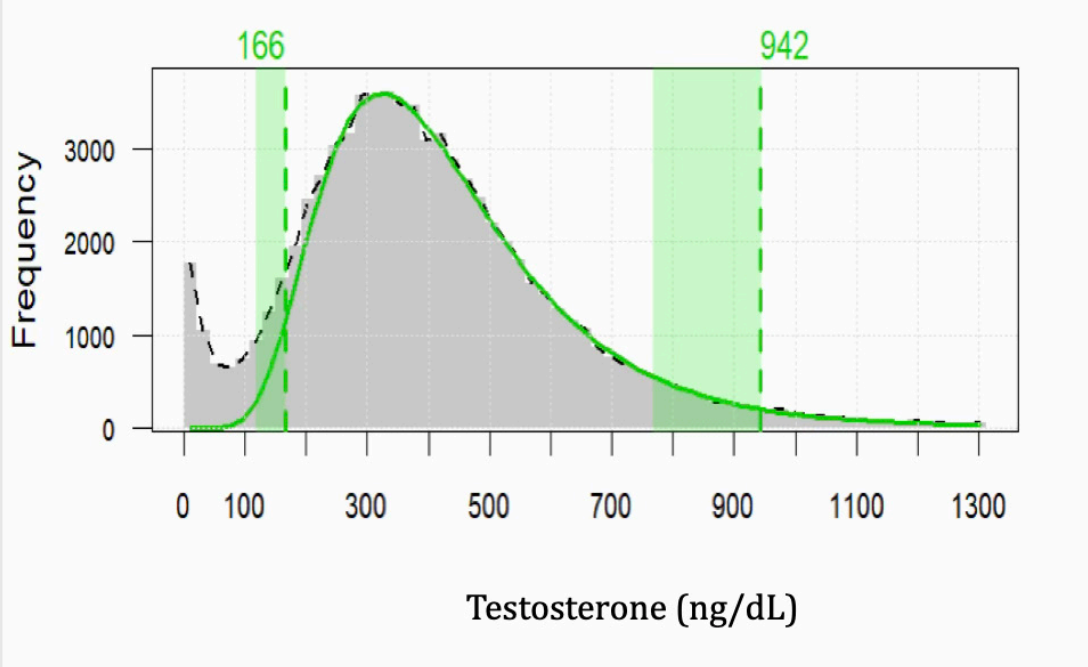


Table 1: Testosterone reference intervals reported in published studies.

Author	Study Type	Population	Age (years)	Calculation method	Testosterone reference Intervals (ng/dL)
Joeri Walravens et al, 2025[10]	Cohort	Belgium	18–39	Direct	184–749
Xue Qin et al, 2012[11]	Cohort	China	20–69	Direct	363–1066
Myrna BuenaluzSedurante et al, 2020[12]	Cohort	Philippines	21–40	Direct	211.4–1528.2
Ravi Jasuja et al, 2022[13]	Cohort	US	19–39	Direct	152.5–925
Thomas G Travison et al, 2017[14]	Cohort	US and Europe	19–39	Direct	264–916
Pruktanakul T et al, 2021[15]	Cohort	Thailand	18–65	Direct	211–970 ng/dL

In conclusion, this study establishes testosterone RIs for Pakistani males using an indirect statistical approach. The estimated reference interval in our study shows both overlap and notable differences when compared with previously published international ranges. For example, studies from European and US cohorts report lower limits ranging from approximately 152 to 264 ng/dL and upper limits between 749 and 925 ng/dL, while data from Asian populations demonstrate wider variability. In comparison, our findings demonstrate a relatively broader upper limit and comparable lower limit, which may reflect population-specific biological variation, differences in assay methods, and the use of an indirect statistical approach.

These observations highlight that while international RIs provide a useful framework, locally derived, data-driven estimates may better reflect the underlying population distribution. However, given the indirect nature of our methodology, these intervals should be interpreted cautiously and require validation using well-characterized healthy cohorts before routine clinical implementation.

Declaration of Conflict of interest
None.

Ethical Approval
Ethical approval was obtained from the institutional review committee, and all data were fully anonymized prior to analysis.

Author's Disclosures
None.

Author Statements
Sibtain Ahmed contributed to the conceptualization of the study, manuscript drafting, and overall supervision. Aiza Zeeshan contributed to data analysis and manuscript writing. Imran Siddiqui contributed to the study concept, critical revision of the manuscript, and intellectual input. All authors reviewed and approved the final version of the manuscript.

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Data Availability Statements
Available on reasonable request from corresponding author.

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