Research Article

Assessing Testosterone Epidemiology Using Distributional Approaches: Evidence From Large-Scale Longitudinal Laboratory Dataset in Pakistan

Sibtain Ahmed[1*], Minal Imran[2], Imran Siddiqui[1]

[1]*Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan*
[2]*Aga Khan University Medical College, Karachi, Pakistan*

Article Info

[*]***Corresponding Author:***
Sibtain Ahmed
Assistant Professor & Consultant Chemical Pathologist
The Aga Khan University
Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan
Telephone: +92 21 34861951
E-mail: Sibtain.ahmed@aku.edu

Keywords

Testosterone, Trends, Pakistan, Laboratory, Population

Abstract

Background: Testosterone is the main circulating androgen in adult males and serves a crucial role in reproductive function, skeletal health, metabolic regulation, and overall physiological stability. In recent decades, concern has emerged regarding possible secular changes in serum testosterone concentrations, independent of aging. However, findings across populations remain inconsistent, and most evidence originates from high-income settings.

Objective: This study aimed to evaluate long-term temporal patterns in serum testosterone levels among adult men, with particular emphasis on distributional shifts and population-wide hormonal instability, rather than solely on a mean decline.

Methods: A retrospective observational analysis was conducted using anonymized laboratory-based serum testosterone measurements from adult males aged ≥18 years between 2008 and 2025. Only the earliest measurement per individual was included to reduce repeat testing bias. Annual descriptive statistics were calculated, and temporal trends were assessed using linear regression. Age-stratified analysis, percentile drift evaluation, and reference interval shifts were used to explore changes across the testosterone distribution over time.

Results: A total of 104,559 testosterone measurements were analysed over 17 years. Linear regression demonstrated a statistically significant but clinically minimal association between calendar year and testosterone concentration ($\beta = +1.97$ ng/dL/year, $p < 0.001$; $R2 = 0.002$). Substantial overlap in testosterone distribution was observedacross age groups, with notable inter year variability. Percentile drift analyses indicated parallel movement across the distribution, suggesting population-wide hormonal fluctuation rather than a uniform secular decline.

Conclusion: Mean testosterone levels did not demonstrate a consistent age-independent decline. Instead, findings support a temporal instability and persistent low exposure subgroups, emphasising the importance of distributional approaches in understanding contemporary hormonal epidemiology.

Introduction

In adult males, testosterone serves as the dominant circulating androgen and is essential for normal reproductive physiology as well as for maintaining skeletal strength, muscle integrity, and metabolic function [1]. Beyond its reproductive effects, testosterone contributes to bone mineralization, preservation of lean body mass, regulation of erythropoiesis, and cardiometabolic homeostasis throughout adulthood [2, 3]. Reduced testosterone concentrations have been associated with adverse outcomes such as osteoporosis, metabolic dysfunction, frailty, and increased overall morbidity, prompting greater clinical and public health interest in long-term hormonal patterns among men [4].

Over recent decades, concern has emerged regarding potential age-independent changes in serum testosterone concentrations in male populations[5,6]. Multiple hypotheses have been proposed to explain these observations, including increasing rates of obesity, exposure to environmental endocrine-disrupting chemicals, reduced physical activity, psychosocial stressors, and evolving utilization patterns [7]. However, published findings have varied substantially, with no consistent evidence supporting a uniform or progressive decline across all populations. A major limitation of prior research is its reliance on population averages, which may fail to capture clinically important changes occurring at different points within the testosterone distribution, particularly among individuals with persistently low levels. In addition, most available data are derived from high-income countries, limiting the applicability of existing findings to other regions with distinct demographic and environmental characteristics [8].

To address these limitations, the present study analyses serum testosterone measurements obtained from a large laboratory-based cohort over a 17-year period. By evaluating age-stratified trends, distributional shifts, percentile drift, and temporal changes in reference intervals, this study seeks to determine whether observed hormonal patterns reflect a simple linear decline or a broader population-level instability with earlier exposure to suboptimal testosterone levels.

Methods

This study was a retrospective observational study based on serum testosterone measurements obtained from a large clinical laboratory database. The dataset included all adult male testosterone tests performed between 2008 and 2025. All data were anonymized prior to analysis, and no patient level identifiers were accessible. The Aga Khan University (AKU) Laboratory receives specimens through a nationwide network of 13 outreach laboratories and more than 300 collection centers across Pakistan, encompassing urban and rural regions. This extensive coverage supports the representativeness of the study population with respect to the broader adult male population of Pakistan. The study was approved by the institutional ethical review committee of Aga Khan University (2025-11907-36009).

The study population consisted of adult men aged 18 years and above who underwent serum testosterone testing during the study period. Where multiple measurements were available for the same individual, only the earliest recorded test was used for analysis in order to minimize bias related to repeat testing andclinical follow-up. The age at the time of testing and the calendar year of measurement were recorded for all included samples. As this was a laboratory-based retrospective dataset, detailed clinical variables such as thyroid status or other endocrine diagnoses were not consistently available. Therefore, no additional filtering based on hormonal comorbidities was performed, and the analysis was intended to capture population-level distributional patterns of testosterone within routine clinical testing Serum testosterone concentrations were measured as part of routine clinical care using standardized laboratory assays. Serum total testosterone concentrations were measured using electrochemiluminescence immunoassay (ECLIA) on Roche diagnostic platforms during the study period. Testing was initially performed on the Roche Elecsys e170 analyzer, subsequently on the Elecsys e411 platform, and currently on the Cobas e801 analyzer. Results were reported in nanograms per deciliter. Although assay platforms evolved over the study period, all measurements were performed in accredited laboratory settings with established internal and external quality control procedures. Descriptive statistics were calculated annually, including sample size, mean, median, and standard deviation. Temporal trends were assessed using linear regression, with testosterone concentration as the dependent variable and calendar year as the independent variable. Effect size, statistical significance, and explained variance (R^2) were reported to distinguish statistical from clinical relevance. Age-stratified analyses were performed to minimize confounding by aging. Distributional changes were assessed using percentile drift analyses, comparisons between early and late testing cohorts, and longitudinal evaluation of reference interval shifts. Analyses were conducted using Python (pandas, matplotlib). A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 104,559 adult male testosterone measurements were analysed over a 17-year period (2009–2025). Linear regression demonstrated a statistically significant but clinically minimal association between calendar year and testosterone concentration ($\beta = +1.97$ ng/dL/year, $p < 0.001$; $R^2 = 0.002$), indicating that calendar time alone explained a negligible proportion of hormonal variability.

Age-stratified analyses revealed substantial overlap in testosterone distributions between younger and older men, with repeated periods in which younger adults exhibited testosterone levels comparable to those traditionally associated with ageing. Temporal fluctuations were observed across all age groups, suggesting population-wide instability rather than age-restricted decline.

These findings indicate that loss of hormonal robustness and earlier exposure to suboptimal testosterone levels, rather than simple linear decline, may represent the dominant contemporary pattern. Percentile drift analyses revealed parallel movement across the entire testosterone distribution, suggesting population-wide hormonal changes. Early versus late cohort comparisons demonstrated shifts in distributional profiles, while reference interval analyses showed temporal variability in both lower and upper limits as shown in Table

1. Overlayedhistograms comparing testosterone distributions between early and late testing cohorts, supporting temporal shifts beyond age-related effects as shown in Figure 1. Longitudinal changes in the 10th, 25th, 50th, 75th, and 90th percentiles of serum testosterone across calendar years, demonstrating population-wide distributional drift as depicted in Figure 2. Year-wise movement of the lower (2.5th percentile) and upper (97.5th percentile) reference limits, highlighting instability of static reference intervals over time as described in Figure 3. Prevalence of Low Testosterone (<300 ng/dL) by year is defined in Table 2.

Table 1: Annual Distribution of Adult Male Testosterone Levels.

Year	n	Mean (ng/dL)	Median (ng/dL)	SD
2008	4352	369.9	348.0	204.8
2009	4739	389.2	363.4	221.3
2010	5128	377.4	349.2	218.6
2011	5031	378.2	354.5	215.1
2012	5012	382.5	356.5	210.3
2013	5045	365.1	333.2	210.6
2014	5046	405.2	377.4	218.7
2015	4915	387.0	361.8	216.8
2016	5600	386.5	355.5	223.1
2017	5797	397.4	367.8	218.7
2018	6035	390.2	364.0	216.2
2019	6522	407.3	381.5	219.1
2020	5698	402.8	379.1	208.7
2021	6786	401.1	374.3	209.8
2022	6467	390.2	366.4	203.7
2023	7310	393.2	370.0	202.5
2024	8178	406.0	380.0	207.8
2025	6898	421.0	398.0	211.2

Table 2: Prevalence of Low Testosterone (<300 ng/dL) by Year.

Year	Total tests (n)	<300 ng/dL (n)	<300 ng/dL (%)
2008	4352	1673	38.4
2009	4739	1678	35.4
2010	5128	1974	38.5
2011	5031	1857	36.9
2012	5012	1802	36.0
2013	5045	2093	41.5
2014	5046	1634	32.4
2015	4915	1757	35.7
2016	5600	2070	37.0
2017	5797	2008	34.6

Year	Total tests (n)	<300 ng/dL (n)	<300 ng/dL (%)
2018	6035	2139	35.4
2019	6522	2109	32.3
2020	5698	1773	31.1
2021	6786	2182	32.2
2022	6467	2228	34.5
2023	7310	2390	32.7
2024	8178	2495	30.5
2025	6898	1914	27.7

Figure 1: Early vs Late Cohort Testosterone Distribution.

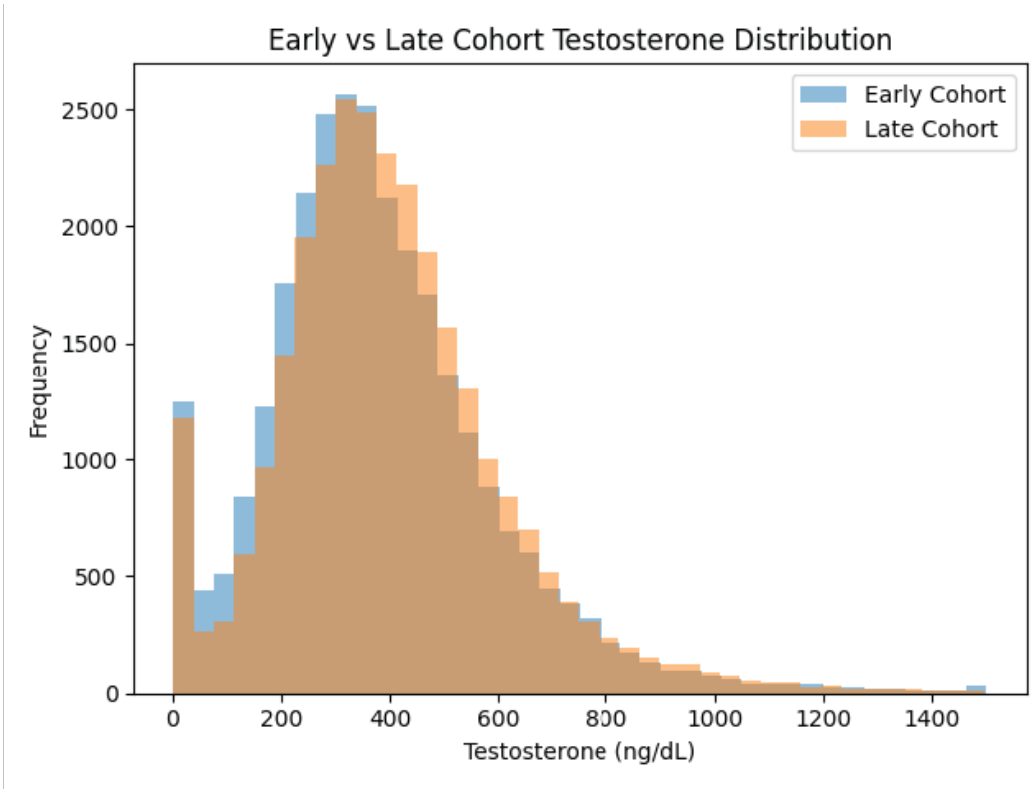


Figure 2: Percentile Drift of Serum Testosterone Over Time.

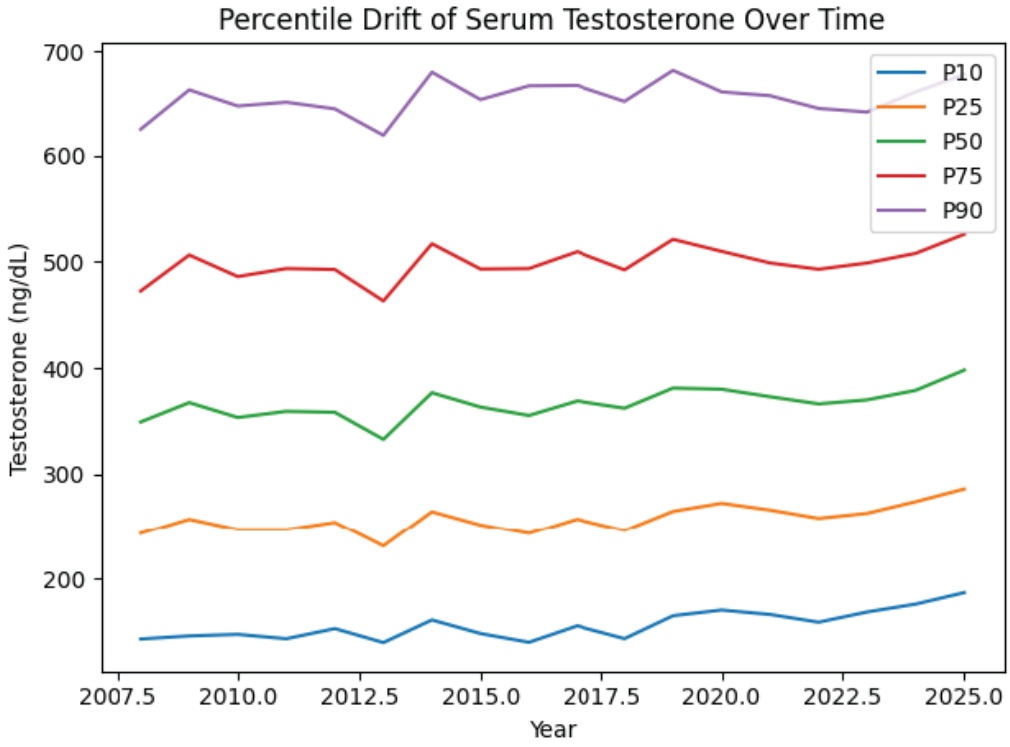
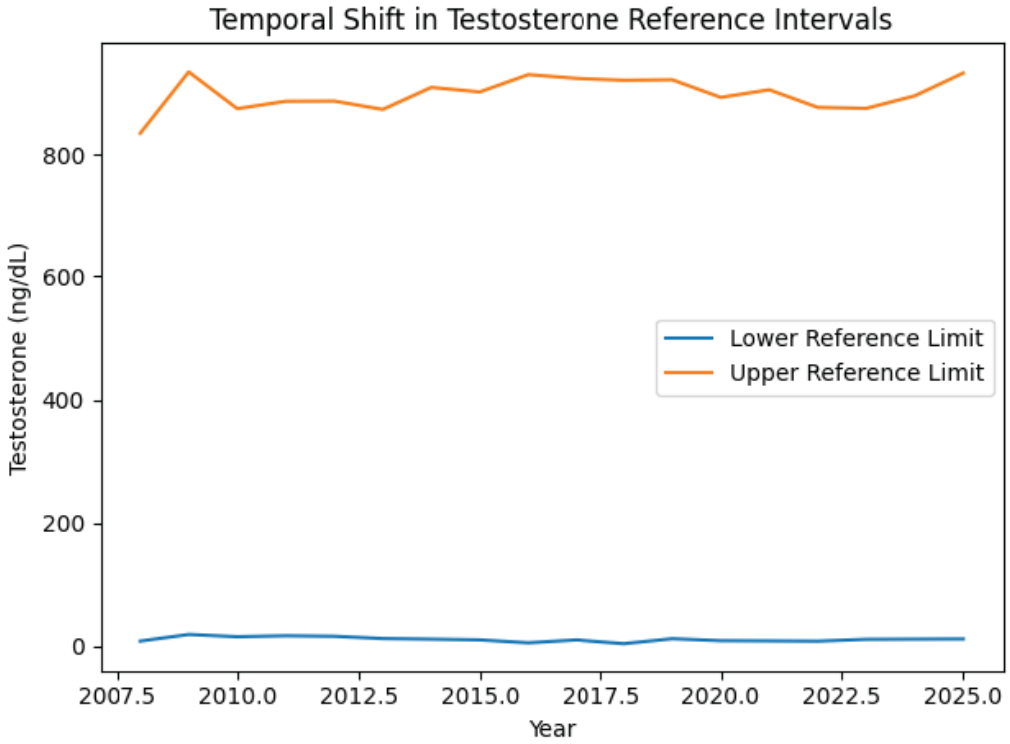


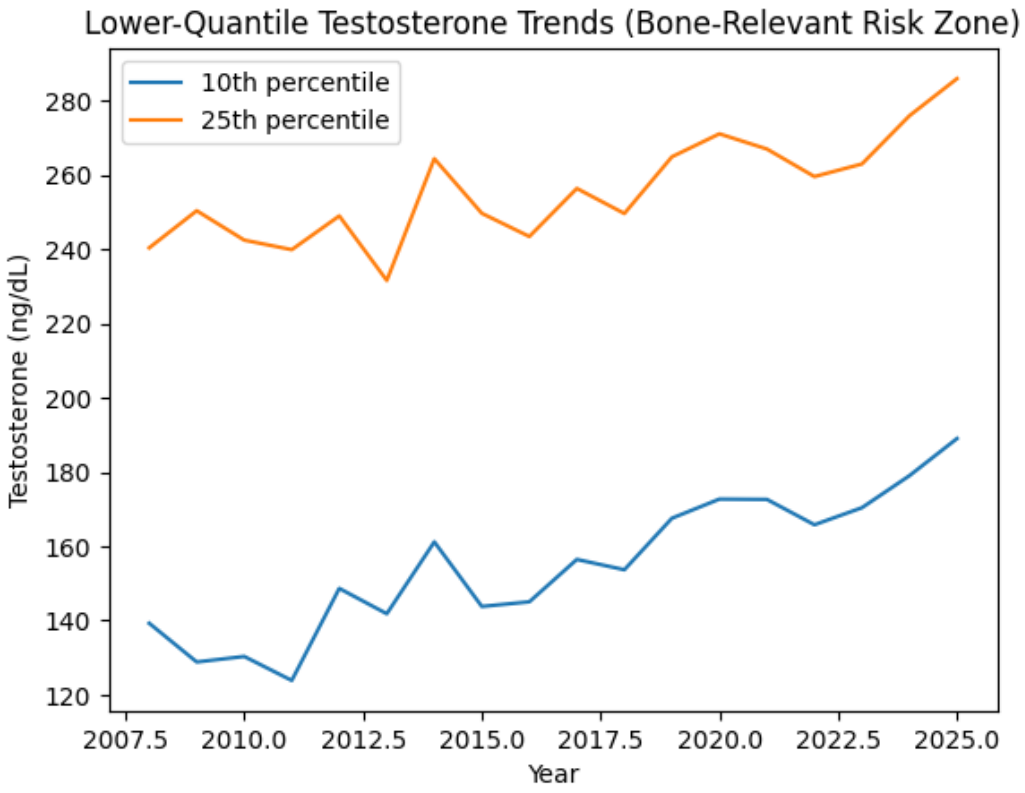
Figure 3: Temporal Shift in Testosterone Reference Intervals.



Wise movement of the lower (2.5th percentile) and upper (97.5th percentile) reference limits, highlighting instability of static reference intervals over time.

The Figure 4 demonstrates marked inter-year variability rather than a monotonic decline, with periods of hormonal suppression followed by partial recovery. Despite fluctuations in mean values,wide dispersion persists across all years, indicating substantial population heterogeneity relevant to long-term skeletal health.

Figure 4: Temporal Trend of Mean Serum Testosterone Levels in Adult Men (2008–2025).



Year-wise trends in the 10th and 25th percentiles of serum testosterone concentrations in adult men. These lower quantiles represent individuals at highest biological risk for impaired bone metabolism and fragility fractures as shown in Figure 5.

Persistently low values across the study period highlight a substantial subgroup of men chronically exposed to bone-adverse testosterone levels, even when population mean values appear stable.

Figure 5: Lower-Quantile (10th and 25th Percentile) Testosterone Trends Over Time.

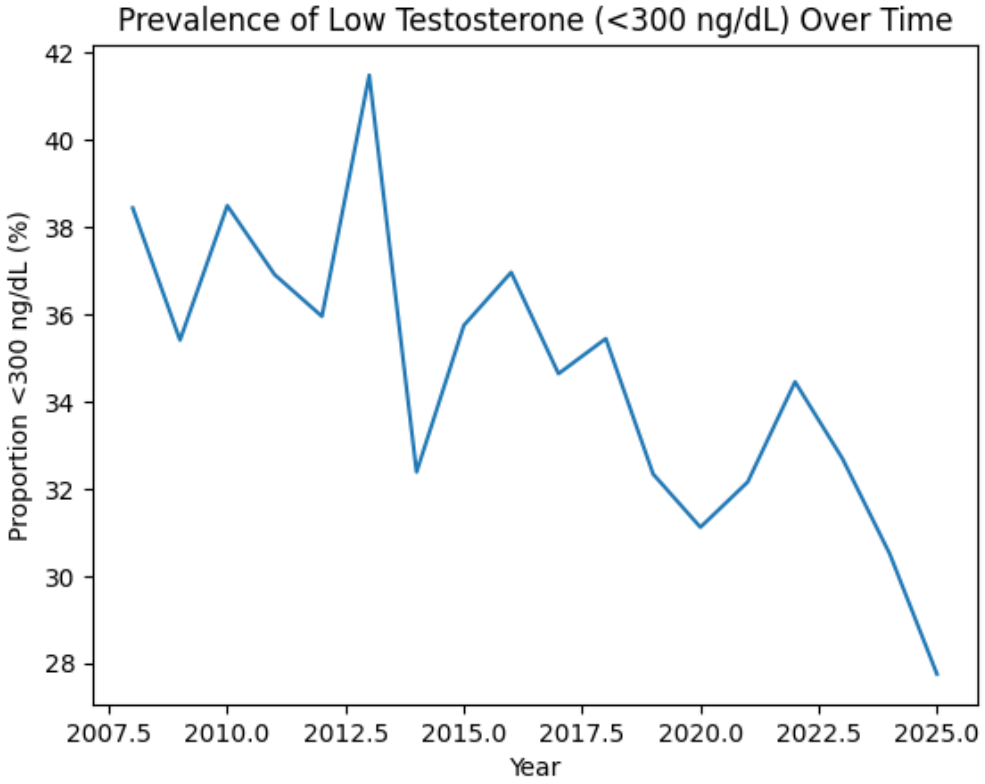
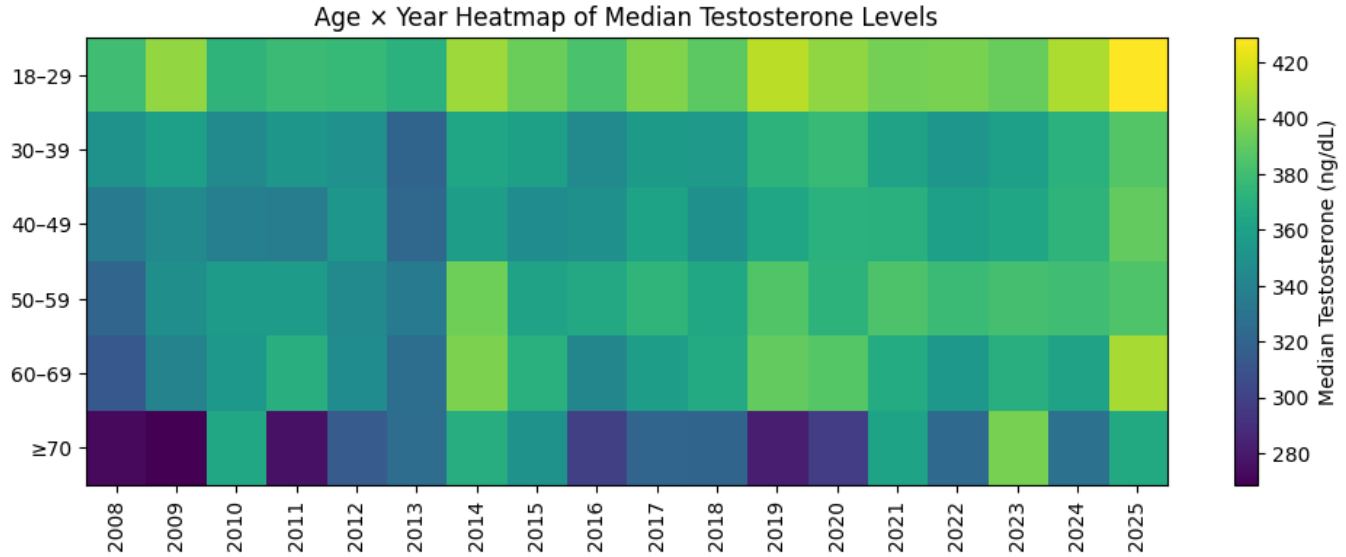


Figure 6: Age × Year Heatmap of Median Serum Testosterone Levels.



Heatmap illustrating median serum testosterone concentrations (ng/dL) across age bands and calendar years.

Discussion

In this large laboratory-based analysis spanning 17 years and over 104,000 adult male testosterone measurements, we found no evidence of a consistent age-independent linear decline in mean serum testosterone. Although linear regression revealed a statistically significant association between calendar year and testosterone, the effect size was small and explained a tiny portion of the overall variability. Instead, variability occurredacross all age groups, with substantial temporal fluctuation and overlapping testosterone distributions, suggesting population-wide instability rather than a uniform decline. Our findings are consistent with some recent literature that emphasizes heterogeneity in testosterone levels. For instance, meta-analysis of testosterone and health outcomes has shown considerable variability in testosterone levels across populations and limited associations with age or outcome endpoints,highlighting the complexity of population-level

hormone dynamics [8]. Such variability supports the notion that mean-level trends may not capture shifts in hormonal distributions [9].

However, some systematic reviews have reported evidence of declining trends in testosterone concentrations over several decades [5,6]. A recent comprehensive literature synthesis identified a negative linear association between testosterone and calendar year across large international cohorts, independent of age and body mass index, suggesting a possible secular decline [10]. These differences may arise from variation in study populations, measurement methods, and analytic frameworks, reinforcing the need for context-specific interpretations.

The present study builds on prior research by incorporating distributional analyses, including percentile drift and reference interval shifts. This approach revealed broad movement across the entire testosterone distribution, meaning changes were not confined to the central tendency alone.[8] Similar distributional concerns have been raised in population hormone studies, where reliance on mean values might obscure important subgroups at risk, such as those with chronically low testosterone [10,11]. Clinically, the presence of a persistent subgroup with low testosterone has important implications [12]. Low testosterone has been linked to adverse outcomes, including impaired bone health, metabolic dysfunction, and frailty [13,14]. Our findings of instability and early exposure to lower serum concentration suggest that timing and distributional context, rather than mean levels alone, may better capture biologically relevant population patterns.

This study has several limitations. First, as a laboratory-based analysis, the sample may reflect healthcare-seeking populations rather than community-based cohorts, which can introduce selection bias. Second, assay changes over time, and a lack of standardized mass spectrometry measurements across the entire period may contribute to analytical heterogeneity [15,16]. Finally, while distributional methods provide nuanced insights, they may be less comparable to traditional epidemiological metrics in other published studies. Moreover, detailed clinical variables such as comorbidities, thyroid status, body mass index, medication use, and lifestyle factors were not consistently available in the laboratory information system and therefore could not be controlled for the analysis.

In conclusion, while simple linear regression suggests only minimal temporal change in testosterone levels, our population-level distributional analyses highlight instability and persistent low exposures across age groups. These findings challenge the interpretation of secular hormonal decline based solely on mean values and emphasize the need for broader distributional perspectives in hormonal epidemiology.

Data Availability

Data is available on reasonable requests from the corresponding author.

Conflicts of interest

None.

Funding

None.

Ethical Approval

The study was approved by the institutional ethical review committee of Aga Khan University (2025-11907-36009).

References

1. Finkelstein JS, Lee H, Burnett-Bowie S-AM, et al. Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med 2013;369:1011–1022. https://doi.org/10.1056/NEJMoa1206168.
2. Basaria S. Male hypogonadism. Lancet 2014;383:1250–1263. https://doi.org/10.1016/S0140-6736(13)61126-5.
3. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103:1715–1744. https://doi.org/10.1210/jc.2018-00229.
4. Cruickshank M, Hudson J, Hernández R, et al. The effects and safety of testosterone replacement therapy for men with hypogonadism: the TestES evidence synthesis and economic evaluation. Health Technol Assess 2024;28:1–210. https://doi.org/10.3310/JRYT3981.
5. Fantus RJ, Greenberg DR, Chang C, et al. Novel reference range values for serum testosterone: a cross-sectional study of 200,000 males. J Endocrinol Invest 2024;47:2261–2268. https://doi.org/10.1007/s40618-024-02319-0.
6. Feldman HA, Longcope C, Derby CA, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J Clin Endocrinol Metab 2002;87:589–598. https://doi.org/10.1210/jcem.87.2.8201.
7. Kim SH, Park JJ, Kim KH, et al. Efficacy of testosterone replacement therapy for treating metabolic disturbances in late-onset hypogonadism: a systematic review and meta-analysis. Int Urol Nephrol 2021;53:1733–1746. https://doi.org/10.1007/s11255-021-02876-w.
8. Mazur A, Westerman R, Mueller U. Is rising obesity causing a secular (age-independent) decline in testosterone among American men? PLoS One 2013;8:e76178. https://doi.org/10.1371/journal.pone.0076178.
9. Krishnan S, Aldana-Bitar J, Golub I, et al. Testosterone therapy and the risk of cardiovascular disease in older, hypogonadal men. Prog Cardiovasc Dis 2024;84:14–18. https://doi.org/10.1016/j.pcad.2024.02.015.
10. Midzak AS, Chen H, Papadopoulos V, et al. Leydig cell aging and the mechanisms of reduced testosterone synthesis. Mol Cell Endocrinol 2009;299:23–31. https://doi.org/10.1016/j.mce.2008.07.016.
11. Pantalone KM, George J, Ji X, et al. Testosterone replacement therapy and the risk of adverse cardiovascular outcomes and mortality. Basic Clin Androl 2019;29:5. https://doi.org/10.1186/s12610-019-0085-7.
12. Sood A, Hosseinpour A, Sood A, et al. Cardiovascular Outcomes of Hypogonadal Men Receiving Testosterone Replacement Therapy: A Meta-analysis of Randomized Controlled Trials. Endocr Pract 2024;30:2–10. https://doi.org/10.1016/j.eprac.2023.09.012.

13. Travison TG, Araujo AB, O'Donnell AB, et al. A population-level decline in serum testosterone levels in American men. J Clin Endocrinol Metab 2007;92:196–202. https://doi.org/10.1210/jc.2006-1375
14. Travison TG, Vesper HW, Orwoll E, et al. Harmonized Reference Ranges for Circulating Testosterone Levels in Men of Four Cohort Studies in the United States and Europe. J Clin Endocrinol Metab 2017;102:1161–1173.
15. Wu FCW, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. N Engl J Med 2010;363:123–135. https://doi.org/10.1056/NEJMoa0911101.
16. Yeap BB, Marriott RJ, Dwivedi G, et al. Associations of Testosterone and Related Hormones With All-Cause and Cardiovascular Mortality and Incident Cardiovascular Disease in Men : Individual Participant Data Meta-analyses. Ann Intern Med 2024;177:768–781. https://doi.org/10.7326/M23-2781.