ORIGINAL ARTICLE

Determination of Upper Reference Limit for High-Sensitivity Troponin T in the Healthy Population of Balochistan

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ABSTRACT

Objective: To determine the 99th percentile upper reference limit of high-sensitivity cardiac troponin T in the healthy population of North and South Balochistan, Pakistan, with age-and gender- stratification.

Study Design: Cross-sectional, Observational.

Place and Duration of the Study: Combined Military Hospital, Quetta, between July 1, 2024, to June 30, 2025.

Materials and Methods: Following the acquisition of informed consent, 217 healthy participants (110 men and 107 women) underwent cardiovascular evaluation included vital signs, chest auscultation, ECG, eGFR, and NT-proBNP to exclude subclinical disease. High-sensitivity cardiac troponin T (hs-cTnT) was measured using the Roche Diagnostics Cobas e-411 platform, with electrochemiluminescence technology. The 99th percentile upper reference limit (URL) for hs-cTnT was determined using non-parametric statistical methods, with age and gender-stratified comparisons.

Results: This study included 110 male (50.7%) and 107 female (49.3 %) participants. The median age, accompanied by interquartile ranges was 41.50 (32.00 – 53.00) years for males and 43.00 (32.00 – 57.00) years for females. The 99th percentile URL of hs-cTnT was 20.94 ng/L overall, with gender-specific values of 34.00 ng/L in men and 16.00 ng/L in women ($p < 0.001$). Median hs-cTnT concentrations were significantly higher in males [12.00 (8.00 - 29.25) ng/L] compared to females [4.00 (3.00 - 10.00) ng/L].

Conclusion: This study established the 99th percentile upper reference limit of hs-cTnT in a healthy population from Balochistan at 20.94 ng/L. Significant differences were observed by gender and age, with men and older individuals showing higher values, underscoring the need for gender- and age-specific diagnostic thresholds. No statistically significant differences were observed between Pathan and Baloch participants in this sample.

Key Words*: Acute Coronary Syndrome*, *High-Sensitivity Cardiac Troponin T, 99th Percentile, Myocardial Infarction, Upper Reference Limit.*

Introduction

Within this global health challenge, high-sensitivity cardiac troponin T (hs-cTnT) assays have become crucial for the early diagnosis and risk stratification of acute myocardial infarction, a major contributor to CVD morbidity and mortality.[1]

The gold standard biomarker for identifying myocardial damage is high-sensitivity cardiac troponin T assay. It is highly sensitive and specific, being released into the bloodstream following myocardial injury, thus reflecting even minimal damage to cardiac myocytes.[2] According to current international recommendations, the 99th percentile upper reference limit (URL) is crucial to the clinical interpretation of hs-cTnT findings and serves as a threshold for detecting acute myocardial infarction (AMI).[3] The introduction of hs-cTnT has significantly enhanced diagnostic capabilities, allowing for the detection of lower troponin concentrations in individuals with non-ST elevation myocardial infarction or those with mild symptoms.[4,5] Hs-cTnT levels rise within hours of myocardial injury; early detection enables timely intervention, often improving patient outcomes. Furthermore, high levels of hs-cTnT are associated with a higher risk of unfavorable outcomes like cardiac failure, arrhythmias, and death, making it a potent prognostic marker.[6] Another feature of high-sensitivity troponin tests is gender-specific upper reference limits. Because troponin levels are partly attributed to differences in cardiac mass and baseline troponin distribution, with higher values

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Received: November 1, 2025 ; Revised: March 11, 2026
Accepted: March 16, 2026

https://doi.org/10.57234/jiimc.june26.2825

commonly observed in males.[7]

The 99th percentile upper reference limit (URL) for men is significantly higher than that for women, according to studies on reference intervals utilizing both high sensitivity troponin I and T (hs-Trop I, hs-Trop T) evaluation.[8,9] Recent studies emphasize the necessity of establishing gender and age-specific thresholds to improve the diagnostic accuracy of the hs-cTnT assay.[10] However, the generally applied universal cutoff value of 14 ng/L may not adequately reflect physiological variation across different populations.[11] Increasing evidence highlights significant biological variation in hs-cTnT concentrations based on demographic and geographic factors, including age, gender, and ethnicity.[11]

A study conducted in Multan, Pakistan, in 2022, reported that the 99th percentile hs-cTnT value was 18.20 ng/L in all cases, with men having higher levels (30.46 ng/L) than women (16.77 ng/L).[10] Similarly, research from Shenyang, China, employing improved selection criteria, established 99th percentile upper reference values of 18 ng/L for males and 13 ng/L for females.[6] The data underscore the significant influence of demographic factors, particularly gender, age and ethnicity on troponin concentrations. Data from the United States also demonstrate higher sex- and age-stratified URLs for fifth-generation hs-cTnT assays, generally ranging from approximately 18–20 ng/L.[12] Implementing tailored diagnostic thresholds could improve the specificity of myocardial infarction diagnoses, minimize overdiagnosis, and promote effective patient management. Continued research is crucial to validate these demographic-based cutoffs and refine clinical guidelines for diverse populations. Significant variation in the 99th percentile URL has been observed in population-based investigations, casting doubt on the applicability of a set diagnostic cutoff in a variety of groups. In resource-constrained and demographically unique regions such as Balochistan, Pakistan, the lack of population-specific reference data may compromise diagnostic accuracy and clinical decision-making.

To overcome this gap, our study aimed to establish age, gender and ethnicity specific 99th percentile upper reference limit for hs-cTnT using a cross-sectional design that reflects the demographic variety of North and South Balochistan. This study intends to improve the diagnostic accuracy of myocardial infarction by developing localized reference values, thereby supporting evidence-based cardiovascular decision-making in this underserved region.

Materials and Methods

This cross-sectional study was carried out at the CMH Quetta, Pakistan, from July 1, 2024, to June 30, 2025. The Institutional Ethical Review Board (IERB) approved the study protocol (Ethical Approval Number: CMH QTA-IERB/27/2024). The Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines recommend at least 120 individuals in each partition to perform non-parametric estimation of reference limits. In the current research, 217 presumably healthy subjects were recruited comprising 110 males and 107 females. Even though the sample size in the subgroups was slightly less than the suggested sample size to have valid estimation of extreme percentiles, strict inclusion and exclusion conditions were used to have the right sample of healthy reference population. The participants were recruited using non-probability convenience sampling method. Demographic details were recorded on a proforma. Participants were instructed to maintain their usual lifestyle and diet and avoid strenuous exercise. They fasted for 8-14 hours after dinner and consumed no more than 200 mL of water. Each participant provided two blood samples for analysis. Three milliliters of whole blood were collected in each ethylenediaminetetraacetic acid (EDTA) tube and used to estimate, HbA1c. Another 3 mL of blood was drawn into a clot activator tube for the analysis of lipid profile, serum creatinine, CK/CK-MB, Hs-cTnT and NT-Pro BNP levels. Each sample was processed on a Roche Diagnostics Cobas c-501 analyzer by photometry and e-411 by electro-chemiluminescence. Serum hs-cTnT concentrations were measured using the Roche Elecsys fifth generation high sensitivity cardiac troponin T assay on the Cobas e-411 analyzer employing electrochemiluminescence immunoassay (ECLIA) technology. Blood samples were centrifuged within 30 minutes of collection at 4000 rpm for 5 minutes, and serum was analyzed immediately. Limit of blank (LoB) of the assay is 2.5 ng/L, limit of detection (LoD)

is 3 ng/L and its measuring range is 3-10,000 ng/L. Internal quality control procedures were performed daily according to manufacturer recommendations.

All participants underwent a comprehensive cardiovascular assessment, including measurement of pulse rate, blood pressure, chest auscultation, and electrocardiography (ECG) performed to rule out diseases such as ventricular or atrial hypertrophy, myocardial infarction, and atrial fibrillation. After obtaining informed consent, this study included apparently healthy participants of both genders, aged 18 to 65 years, residing in North and South Balochistan, without any clinical cardiovascular conditions, such as atrial fibrillation, chronic heart failure, or coronary artery disease. Patients with history of hypertension, diabetes mellitus, heart failure (NT-pro BNP >125 pg/mL) was applied in accordance with European Society of Cardiology (ESC) guidelines, to exclude participants with possible underlying heart failure,[13] or chronic renal disease at stage III or greater (estimated glomerular filtration rate <60 ml/min/1.73m²) were excluded from the study to ensure the integrity of the healthy participants, because these conditions can result in hs-cTnT release independent of myocardial infarction.[11]

To statistically analyze the collected data, Statistical Package for Social Sciences (SPSS) software version 22 was used. Quantitative variable (Age, hs-cTnT) was presented as median interquartile range (IQR) after checking normality of data by Shapiro-Wilk test which showed that it was not distributed normally. Qualitative variables (Gender, Age groups, Ethnicity) were presented as frequency and percentages. The Mann Whitney U test used for the comparison of hs-cTnT with gender and Ethnicity and Kruskal Wallis test was used for the comparison of hs-cTnT with age groups.

The 99th percentile upper reference limit (URL) was calculated using a non-parametric method in accordance with CLSI EP28 A3c guidelines. After arranging hs-cTnT concentrations in ascending order, the percentile rank position was determined using the formula $k = (n + 1) \times 0.99$. When the calculated rank position was non-integer, interpolation between adjacent observations was applied. Confidence intervals for the 99th percentile were estimated using bootstrap resampling with 5,000 iterations. Outlier detection was performed using Tukey's method based on the interquartile range (IQR). Observations exceeding 1.5 × IQR were examined carefully. No values were excluded after statistical and clinical review.

Results

Out of 217 participants, 110 (50.7%) were men and 107 (49.3%) women. Median age was 42.00 (32.00 – 52.00) years. Median was 41.50 (32.00 – 53.00) years for males and 43.00 (32.00 – 57.00) years for females. The 99th percentile upper reference limit (URL) for hs-cTnT in the overall population was 20.94 ng/L (90% CI: 18.5–23.8 ng/L). Gender specific analysis showed higher values in males (34.0 ng/L; 90% CI: 33.91–34.0 ng/L) compared with females (16.0 ng/L; 90% CI: 12.95–16.0 ng/L), ($p < 0.001$). Median of hs-cTnT was high in males [12.00 (8.00 - 29.25) ng/L] as compared to females [4.00 (3.00 - 10.00) ng/L] $p < 0.001$ as shown in Table I. Cardiac troponin concentrations demonstrated a significant age-related increase, with median values rising from 6.00 (3.00 – 11.00) ng/L in individuals aged 18–35 years to 11.50 (3.25 - 31.00) ng/L in those aged older than 50 years. Correspondingly, the 99th percentile increased from 15.3 to 34.0 ng/L, accompanied by broader 90% confidence intervals in older age groups, indicating greater variability. These findings were statistically significant ($p < 0.001$), highlighting age as a critical determinant of troponin levels shown in Table II. The median (IQR) hs-cTnT level in the Pathan group was 10.00 (3.00–13.75), while in the Baloch group it was 9.00 (3.00–12.00). The difference between the two groups was not statistically significant ($p = 0.439$), indicating that hs-cTnT levels were comparable across ethnicities. Furthermore, the 99th percentile upper reference limit was 33.93 in the Pathan group and 34.00 in the Baloch group, demonstrating nearly identical upper reference limits between the two ethnic groups as Shown in Table III. Confidence intervals for the 99th percentile were calculated for the overall population, gender groups, age groups, and ethnicity groups using bootstrap resampling.

Discussion

The 99th percentile URL of hs-cTnT was 20.94 ng/L in the overall population, with gender-specific values of 34.0 ng/L and 16.00 ng/L for men and women, respectively. These findings from our study revealed

https://doi.org/10.57234/jiimc.june26.2825

Table I: Distribution of High-Sensitivity Cardiac Troponin T Concentrations According to Gender (n=217)

Hs-cTnT (ng/L)	Male (n=110)	Female (n=107)	Total (n=217)	p-value
Median (IQR)	12.00 (8.00 - 29.25)	4.00 (3.00 - 10.00)	9.00 (3.00 – 13.00)	< 0.001$
99th percentile	34.00	16.00	20.94	-
90% CI	33.9–34.0	12.9–16.0	18.5–23.8	-

$= Mann Whitney U test, CI= Confidence Interval

Table II: Comparison of High-Sensitivity Cardiac Troponin T according to Age Distribution (n=217)

Hs-cTnT (ng/L)	Age in Years			*P* value
	18 - 35 (n=71)	36 – 50 (n=70)	> 50 (n=76)	
Median (IQR)	6.00 (3.00 - 11.00)	10.00 (5.50 - 13.00)	11.50 (3.25 -31.00)	< 0.001^
99th percentile	15.3	22.1	34.0	-
90% CI	13.0 -16.5	20.0 – 24.0	31.0 – 34.0	-

^ = Kruskal Wallis test, CI= Confidence Interval

Table III: Comparison of High-Sensitivity Cardiac Troponin T by Ethnicity (n=217)

Hs-cTnT (ng/L)	Pathan (n=108)	Baloch (n=109)	p
Median (IQR)	10.0 (3.00 - 13.75)	9.00 (3.00 - 12.00)	0.439$
99th percentile	33.93	34.00	-
90% CI	32.0 - 34.0	32.5 – 34.0	-

$= Mann Whitney U test

that males have considerably greater hs-cTnT levels than females, consistent with results from a U.S. study where the 99th percentile URL varied from 18.4 to 20.2 ng/L in all cases.[12] These results corroborate research by Anwar et al. and Apple et al. that indicates males have greater troponin levels, indicating the necessity of gender-specific reference ranges.[14,15] These results are also in line with a Singaporean study that found a 99th percentile cut-off value of 25.6 ng/L overall and 32.7 ng/L and 17.9 ng/L for males and females, respectively.[16] Another study by Romiti et al., in 2019, in Italy, compared various studies that looked at gender-specific 99th percentile cut-offs for hs-cTnT and discovered that men had higher values.[17] Similar to our results, another study by Khan et al. in Multan, Pakistan in 2022 found that the values of high sensitivity troponin T (hs-cTnT) were 18.20 ng/L in all patients, 30.46 ng/L for men, and 16.77 ng/L for women. However, they concluded that there were substantial differences in hs-cTnT levels across genders but not between age groups.[10]

We observed a considerable difference between the age groups in our study. Troponin levels increased with age, as evidenced by the rise in median values from 6 ng/L in younger individuals to 11.5 ng/L in older age groups. This trend was statistically significant ($p < .0001$) and corroborated findings from other studies that highlight age as a critical determinant of troponin levels. Our study found that the range of hs-cTnT values in the general population gradually increased with age.[18] These findings are also consistent with a study that showed hs-cTnT in three large cohorts and found this cut-off to be higher in males and increased with age. These findings are also consistent with a study that analyzed hs-cTnT in three large cohorts and discovered that the cut-off was greater in males and increased with age.[11] Another study in 2013, found a strong relationship between age, gender and 99th percentile URL values of hs-cTnI among healthy reference individuals.[19] Even in the absence of acute coronary syndrome or other diseases like diabetes and hypertension, older people have elevated troponin levels.[20]

Another study by Isiksacan et al. in 2018 reported that the values of hs-cTnT were significantly higher in men over 40 years of age (24 ng/L) compared to those under 40 years of age (10 ng/L).[21] These findings are consistent with our results that the broader confidence intervals in older age groups suggest greater variability, which may necessitate age-specific reference limits for accurate clinical assessment. With advancing age, subclinical myocardial changes such as fibrosis, increased left ventricular wall stress, and cardiomyocyte apoptosis contribute to higher baseline troponin release even in the absence of overt disease.[11,19] Declining renal clearance in older adults also prolongs troponin circulation.[20] Furthermore, cumulative exposure to cardiovascular risk factors across the lifespan leads to subtle myocardial injury, reflected in elevated hs-cTnT.[18] These findings are consistent with large cohort studies showing progressive increases in troponin concentrations with age, underscoring the need for age-adjusted diagnostic thresholds to avoid misclassification of myocardial infarction in older

individuals.

Our study found no significant difference for hs-cTnT levels between Pathan and Baloch participants, indicating that ethnicity does not require adjustment of diagnostic thresholds in this population ($p = 0.439$). This aligns with the research conducted by Burgio et al., in 2024, which suggested limited evidence for ethnicity-specific reference limits.[22]

Limitations

This study was conducted at a single tertiary care center using non-probability convenience sampling. Convenience sampling may limit representativeness of the general Balochistan population and may introduce selection bias including a potential healthy volunteer effect. The sample size was also smaller than the sample size recommended size in CLSI guidelines to provide stable estimation of extreme percentiles. According to CLSI EP28-A3c guidelines, at least 120 individuals per partition are recommended for non-parametric estimation of reference limits. In the present study, the gender-specific groups (110 males and 107 females) were slightly below this recommended number; therefore, the estimated reference limits should be interpreted cautiously.

Conclusion

Our study found significant gender-specific differences in high-sensitivity cardiac troponin T levels, with males exhibiting higher levels than females. Additionally, age significantly influences troponin levels, with median values increasing in older age groups, indicating the necessity for age-adjusted diagnostic thresholds. However, no statistically significant differences were observed between Pathan and Baloch participants in this sample.

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CONFLICT OF INTEREST
Authors declared no conflicts of Interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
Authors have declared no specific grant for this research from any funding agency in public, commercial or nonprofit sector.

DATA SHARING STATEMENT
The data that support the findings of this study are available from the corresponding author upon request.

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