https://doi.org/10.29309/TPMJ/2024.31.12.8185

The Professional Medical Journal
www.theprofesional.com
2024, Volume, 31 Issue, 12

ORIGINAL ARTICLE

Determination of 99th percentile upper reference limit of cardiac troponin I in healthy population of Rahim Yar Khan.

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Article Citation: Rahim A, Haider SS, Farwa U, Younes T, Khalid S, Zainab ul Ghazali. Determination of 99th percentile upper reference limit of cardiac troponin I in healthy population of Rahim Yar Khan. Professional Med J 2024; 31(12):1742-1750. https://doi.org/10.29309/TPMJ/2024.31.12.8185

ABSTRACT… Objective: To determine the 99th percentile upper reference limit (URL) of cardiac troponin I (cTnI) in healthy population of Rahim Yar Khan. **Study Design:** Cross-sectional study. **Setting:** Department of Chemical Pathology, Sheikh Zayed Hospital, Rahim Yar Khan. **Period:** February 2023 to November 2023. **Methods:** A total of 262 healthy individuals aged between 20-76 years without any known cardiac diseases were included and selection was performed on the basis of a designed questionnaire. Serum hs-cTnI was measured using a chemiluminescent technique on ACCESS II, Beckman Coulter. A non-parametric statistical technique was used to establish the 99th percentile upper reference limit (URL) of hs-cTnI in accordance with CLSI Document C28-A3c. Mann Whitney U test was applied between two subgroups taking p value <0.05 as significant. **Results:** In this study, 136 (51.9%) were males and 126 (48.1%) were females. Of the total 262 study subjects, 180(68.7%) had values greater than Limit of detection (LoD) 2.3 ng/L while 82(31.3%) had values less than LOD. Overall, Male and Female 99th percentile URL of hs-cTnI in population of Rahim Yar Khan was found to be 10.8, 13.1 and 8.3 ng/ml respectively before removing any outliers. After removing outliers by Dixon/Reed method, the 99th percentile URL found to be 8.4, 9.0 and 6.3 ng/L for overall, males and females respectively. The distribution troponin I with respect to age and gender was statistically significant with p value <0.05. **Conclusion:** On the basis of our study, it was concluded that the study population's overall high-sensitive cardiac troponin I 99th percentile URL was found to be significantly lower than both manufacturer-quoted values and results published in prior publications. High-sensitive troponin assay allows precise quantification of very low cardiac troponin concentration that helps in rapid exclusion of myocardial infarction.

Key words: High-sensitivity Cardiac Troponins (hs-cTn), 99th Percentile Upper Reference Limits (99th percentile URLs).

INTRODUCTION

Cardiovascular disease (CVD) has emerged as the leading cause of death globally.[1] Hospital admission for observation and serial cardiac troponin I testing is necessary to identify those with or without myocardial infarction, even if the majority of patients show no signs of the condition.[2] It is quite challenging to evaluate patients with chest pain presenting in Emergency Department (ED) and it leads into overcrowding, and also delays the diagnosis.[3] A well timed diagnosis and necessary intervention in such patients is very critical in improving the outcomes and are made possible by high-sensitivity cardiac troponins (hs-cTn) as advocated by the European Society of Cardiology (ESC) and the American Heart Association (AHA).[4,5]

The troponin complex in a cardiac or skeletal muscle is a heterotrimer composed of troponin C (TnC), the Ca^{2+}-binding subunit, troponin I (TnI), the inhibitory subunit, and troponin T (TnT), the Tm-binding subunit.[6,7] The concentration of free cardiac troponin I (cTn I) increases rapidly in the blood circulation whenever the integrity of the myocardial cell membrane is compromised in the setting of myocardial injury resulting from myocardial ischemia making it a useful marker in the diagnosis of Myocardial injury and infarction.[8,9,10]

According to the Fourth Universal definition of

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Article received on: 15/03/2024
Accepted for publication: 16/05/2024

Myocardial Infarction recent publication, the diagnostic criteria for acute myocardial infarction comprises of a dynamic pattern of cardiac troponin concentration by a high sensitive assay, on serial measurements with at least one value above 99th percentile of upper reference limit of normal reference population along with presence of clinical features suggesting acute myocardial ischemia.[11,12,13] This criteria along with acknowledgment of sex specific 99th percentile URL especially in females and other patient subsets is recommended by the 2021 American Heart Association, AHA/American College of Cardiology, ACC / American College of Chest Physicians guidelines and also endorsed by International Federation of Clinical Chemistry and Laboratory Medicine Committee on Clinical Applications of Cardiac Bio-Markers (IFCC C-CB).[14]

Highly sensitive assays are available to allow detection of small changes in cardiac troponin which helps in early diagnosis.[15] It is associated with shorter stay at hospital, decreased admissions, and decreased testing to rule in/out acute MI.[16] The highly sensitive assay provides high negative predictive value (NPV) for ruling out an AMI diagnosis, allowing emergency physicians to identify patients at low risk of adverse cardiac events in the next 30 days (peacock). The use of high-sensitive assay Assays for troponin measurement are not standardized. Ideally, each laboratory should establish the 99th percentile relevant for its method and population.[17]

An assay must meet the criteria set forth by the International Federation of Clinical Chemistry (IFCC) in order to be considered high-sensitive. Specifically, the percentage coefficient of variation (%CV) at the 99th percentile URL of the cardiac troponin assay must not exceed 10%, and the cardiac troponin concentration measured in a healthy population with a nearly equal distribution of male and female members must be at or above the assay's 50% limit of detection (LOD).[18] Recent studies have displayed a high performance of Beckman Access II cTn assay in terms of analytical sensitivity and precision at 99th percentile values fulfilling the IFCC criteria.[19,20]

The Laboratory Standards Institute (CLSI) states that each laboratory should establish its own reference limits for the laboratory. Reference ranges for chemistry should be obtained from a healthy population that is typical of the group under study. Guidelines for creating a laboratory reference limit can be found in the CLSI (Clinical & Laboratory Standards Institute) publication "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory," EP28-A3c.[21,22] CLSI guidelines recommends the establishment of chemistry reference ranges with at least 120 reference healthy individuals for each partition, using a non-parametric ranking method.[23]

The purpose of the study is to determine the 99th percentile upper reference limit of cardiac troponin I in healthy population of Rahim Yar Khan and to assess the other factors influencing the hsTnI concentrations. As the estimated 99th percentile upper reference limit for troponin varies according to population, it is foremost prerequisite to calculate the 99th percentile URLs in healthy reference cohort representing the targeted population. And to our best knowledge no such study has been conducted in this area. It will provide the diagnostic cut off for troponin to rule out/rule in the cases of acute myocardial infarction in Rahim Yar Khan.

METHODS

The cross-sectional study was conducted in department of Chemical Pathology, Sheikh Zayed Hospital, Rahim Yar Khan from February 2023 to November 2023. After taking ethical approval from institutional review board ((417-/IRB/SZMC/SH)17-2-22}, data was collected. Self-proclaimed Healthy blood donors or Health care Professionals of both genders aged between 20 and 76 years, with no known history of acute or chronic disorders (cardiovascular, renal, liver, thyroid, respiratory, autoimmune, diabetes, hypertension and hyperlipidaemia), drugs or medications use were included. Following individuals were excluded, pregnant females, history of recent blood transfusion, sub clinical heart disease i.e., N-terminal-pro-B-type natriuretic peptide (NT-pro BNP) >125 ng/L for those <75 years of age and >450ng/L for those above 75 years of age

and recent acute hospitalization within the last 3 months. Prior to enrolment, they were screened for their health status through a predesigned questionnaire.

Non fasting fresh venous blood samples were collected from antecubital vein in serum vaccinator tubes with gel. Visibly haemolyzed or turbid samples were omitted. All blood specimens were stored for 30 minutes at room temperature, centrifugation was done at 3,000 rpm for 10 min after clotting, and analysis was performed within 2 hr.

Serum troponin I and pro BNP were estimated on ACCESS II Beckman Coulter using technique of chemiluminescence and three levels of controls were used for ensuring Quality control. Sampling technique was non probability consecutive sampling. The Limit of detection (LOD) and limit of quantification (LOQ) were 2.3 ng/L and 5.6 ng/L, respectively with a bias and imprecision of ≤10%. Values below the LOD were assigned a concentration of 2 ng/L for data analysis.

SPSS version 23.0 was used for statistical analysis. The distribution of data was checked by using Kolmogorov-Smirnov test. The upper reference limit (URL) 99th percentile of Hs Trop I was calculated in accordance with CLSI EP28A-3c guidelines[21] using non parametric analysis before and after removing outliers by D/R ratio. Quantitative variables (Age, Troponin I) were represented as median and interquartile range (IQR) while qualitative variables (Gender, Residence, Regular exercise, H/O smoking, Family history of cardiovascular disease) is presented in terms of frequency and percentages. All effect modifiers (Age, Gender, H/O smoking, regular exercise, family history of cardiovascular disease) were controlled through stratification. Hs-TnI values were compared by using Mann-Whitney U test. P-value < 0.05 was taken as significant. Outliers were excluded by using D/R ratio (D, absolute difference between an extreme observation; R, range of all observations).

RESULTS

A total of 262 healthy adults aged between 20 to 76 years (median 37 years, IQR 17) were enrolled in the study. Among all study subjects, 136 were males (median 40 years, IQR 14). and 126 were females (median 33 years, IQR 14). Of the total 262 participants, 204 (77.9%) were less than 50 years and 58(22.1%) were equal and greater than 50 years. 12(4.6%) were smokers, 48(18.7%) participants reported regular exercise and 61(23.3%) had a positive family history of heart disease. The median age calculated was 37 years with age range 20 to 76 years. Many study subjects (77.9%) were below 50 years old. Baseline and demographic data of study participant with median hs-TnI concentration is shown in Table-I.

Of the total 262 study subjects, 82(31.3%) demonstrated a level of hs-cTnI less than LOD i.e., 2.3 ng/L and 180(68.7%) had values of hs-cTnI >2.3ng/L. The distribution of high-sensitive cardiac troponin I levels in the study subjects was not normal and is severely skewed to the left. The 99th percentile upper reference limit of troponin I for overall population was 10.8 ng/L (95% CI: 3.05-3.41ng/L). The 99th percentile URL in our study was found higher in males (13.1ng/L, 95% CI :3.11-3.69 ng/L) and lower in females (7.8 ng/L, 95% CI: 2.84-3.23 ng/L) (Table-II). Median hs-TnI concentration was higher in males as well as in older individuals with statistically significant difference with p value <0.05. Median Troponin Concentration was not found statistically significant with respect to smoking, regular exercise and family history of CVD with p value >0.05 (Table-I).

By applying D/R ratio for exclusion of outliers, two observations were removed from total population. After their exclusion, the 99th percentile URL of cardiac hs-TnI for general, male and female population has become notably lower than estimated before excluding outliers (Table-III).

Variables	Subgroups	N %	Median ng/L (95% CI)	IQR	P-Value
Gender	Male	136 (51.9%)	3.0(3.08-3.65)	0.6	0.003
	Female	126 (48.1%)	2.9(2.70-3.07)	1.3	
Age	<50 years	204 (77.9 %)	2.9(2.81-3.18)	1.2	0.000
	≥50 years	58 (22.1 %)	3.2(3.17-4.09)	1.1	
Family H/O of CVD	Present	61(23.3%)	3.0(2.93-3.84)	0.5	0.144
	Absent	201(76.7%)	2.9(2.88-3.24)	1.5	
H/O Smoking	Smoker	12(4.6%)	2.0(2.01-2.93)	1.4	0.061
	Non-smoker	250(95.4%)	2.9(2.98-3.35)	1.4	
Regular Exercise	Present	48(18.7)%	3.0(2.77-3.46)	1.5	0.781
	Absent	214(81.3%)	2.9(2.94-3.34)	1.4	

Table-I. The distribution of hs-cTnI with respect to gender, age, family history of CVD, smoking, and regular exercise (n=262)

	99th Percentile URL (95% CI)	Median ng/L (IQR)
Overall	10.8(2.96-3.31)	2.9(1.4)
Male	13.1(3.08-3.65)	3(0.6)
Female	8.3(2.67-3.04)	2.9(1.2)

Table-II. Median hs-cTnI concentrations and 99th percentiles (ng/L) in both genders (n=262)

High-Sensitive Cardiac Troponin I URL (ng/L)						
	Overall		Male		Female	
	99th Percentile	N	99th Percentile	N	99th Percentile	N
Before excluding outliers	10.8	262	13.1	136	8.3	126
After excluding outliers	9.0	260	8.4	134	6.3	125

Table-III. 99th percentile URL of high-sensitive cTnI before and after exclusion of outliers (n=262)

DISCUSSION

For the first time, this study reported the 99th percentile URL of troponin I in the population of Rahim yar khan.

Our estimated 99th percentile trop I value for the overall, Male and Female population was found lower than manufacturer's reported values and previous published literature. Clerico A. et al and Min LS. et al determined the 99th percentile URLs of hs-cTnI and the values obtained were found higher than determined in our study population.[24,25] Manufacturer determined the overall, Male and Female 99th percentile URL cTnI for Access Tn assay to be 17.5, 19.8, and 11.6 ng/L respectively for the population of United States (US) as per kit insert.[26] The discrepancy in 99th percentile URL of cardiac troponin has been found in previous studies with respect to manufacturer recommendations and possible reason might be selection criteria, age range, sample type, pre analytical factors, biological parameters, statistical methods for analysis, exclusion of outliers and cTn assays between studies.

On the basis of our study, a notable difference for cTnI values has been demonstrated with respect to age and gender in a healthy population of Rahim Yar Khan. These findings are consistent with other previous studies.[27-30] This difference may be attributed to the gender-based difference in cardiac structure, prevalence and distribution of coronary artery disease.[31] Previous studies demonstrate the importance of utilizing sex-specific URLs for improved prediction and risk stratification however, clinical utility of gender specific reference interval needs to be established.[32,33] The sensitivity to diagnose myocardial infarction is much increased for females by using gender specific reference interval.[34] A randomized clinical trial is currently underway to assess the effects of a lower cutoff of cTn on the diagnosis of MI in female patients presenting with chest pain.[35]

The majority of participants in our study were below 50 years of age (n=202). However, an increase in cTnI concentrations has been observed in both genders after the age of 50 which is consistent with many previous studies.[15,25] These changes might be due to number of possible causes such as comorbidities in advancing age. In a recent study, it has been demonstrated that cardiac troponin concentrations increase with increasing age for four different types of high-sensitive assays with $p<0.001$.[36] Due to the small number of participants in the older group, we are unable to accurately calculate the 99th percentile specific for age groups. However, it has been established in previous studies that recruiting a larger number of healthy individuals over 70 years old is quite challenging.[24,29]

It was demonstrated in previous studies that 99th percentile cTnI URL is lower in Asians than Caucasians[15,18,22,24] which is consistent with our study findings. However, one study from Europe showed a lower 99th percentile URL of cTnI, 5.5 ng/L for females and 13.9 ng/L for males using Access Tn assay.[22] On the other hand, a study reported that no difference exists for US and Asian countries only after adjustment of baseline characteristics.[37]

A study in Korea using ARCHITECT STAT hs trop I assay has shown hs tropI 99th percentile URL for all participants was 18ng/L (90% CI,14–35), 20ng/L (90% CI, 15–69) for males and 19ng/L (90% CI, 11–41) for females with the median hsTnI levels higher in males than females and in individuals older than 50 years.[38] A study in Malaysian healthy blood donors(n=250) using ABOTT hs trop I assay estimates the 99th percentile URL of hs-troponin I (overall) to be 23.7 ng/L, 29.9ng/L for males and 18.6 ng/L females, respectively.[25] Katruka et al demonstrated in a study that the differences in cutoffs when using different assays may be attributed to the selection of antibodies (capture and detection ones) in designing the detection assays by the manufacturers and their ability to detect multiple forms of cardiac troponin I in circulation released from injured myocardium have created varying results of 99th percentile URL in a same population.[39] The possible reason to our lower estimates of 99th percentile URL in contrast to previous published studies could be due to pre-selection of already healthy individuals from blood donors and paramedical staff and that our study population consists a major proportion of young individuals which might not be true representative of population presenting at emergency department.

Our study in Rahim yar khan shows the lower 99th percentile URL of cTnI than those reported in other cities of Pakistan also. Bahadur et al from Peshawar had reported 99th percentile URL of hsTnI of 33.9, 38.4 and 15.73 ng/L for overall, males and females, respectively, using ABOTT hs TnI assay in 299 cardio healthy individuals. They obtained a higher median TnI concentration for males than females and for individuals older than 40 years but no statistically significant difference noted for trop I and age.[2] In another study conducted in Multan using hsTnT assay, the 99th percentile URL in males was found higher than females while no age-related difference was found in study.[40]

On the basis of our study, no statistically significant difference was found in troponin concentration with respect to family history of CVD. However, in a recent study, significant association has been established for cardiac Troponin levels and Family history of heart disease in apparently healthy individuals and these levels could be used for risk stratification in healthy individuals above 65 years.[41]

It has been established that smoking is considered as strong and well-known risk factor for cardiovascular disease.[42,43] A positive association exists between smoking and cardiovascular risks factors on the basis of previous studies and cTnI concentrations measured by high- sensitive assays are unexpectedly lower in smokers than non-smoker.[42,44] To support our finding that no significant difference occurs in smoker and cardiac troponin I concentrations another recent study demonstrates that association only exists for cTnT and not with cTnI in smokers with stable coronary artery disease. The clear mechanism behind this finding remains unknown.[43]

We have not found significant difference for troponins concentrations in healthy individuals who were doing regular exercise i.e., more than 2hrs of physical activity in a week than those who were doing not. Previously, it has been established that cardiac troponins are elevated than recommended URL in most healthy athletes[45] and has also been endorsed in fourth universal definition of MI[11] to recognize prolonged exercise as a cause for cardiac troponin elevation along others. but still it is not associated with worse cardiovascular outcomes in a 5 year follow up of a large-scale study.[46] A few recent studies have challenged this finding[47,48] but more evidence is needed to establish it as a risk factor for cardiovascular disease. As it has been recognized that cardiac troponin I increases after exercise[49] and including the participants with increased physical activity may increase the overall 99th percentile URL, an observation made by odeaster et al.[17] However, we have seen no significant difference for troponin concentrations in healthy individuals who were doing regular exercise i.e., more than 2hrs of physical activity in a week. As we had a small number of such individuals included in our study, the effect of exercise on troponin concentration may be less pronounced.

As troponin concentration in a population depends on multiple factors like age, sex, race, ethnicity, screening criteria for the study population, detection of outliers, statistical analysis, and type of assay used, it is therefore recommended to determine the 99th percentile URL cTnI in each population for each assay.

Our estimated cutoffs are lower than implemented 99th percentile URL in clinical setting. Following the lower cutoffs will increase the sensitivity for diagnosing an acute event of infarction and greater number of screened individuals will be more. However, application of a lower 99th percentile value needs further research in terms of patient evaluation, management or discharge presenting to emergency department. As it can misdiagnose a patient with acute or chronic MI, imposing a risk of unnecessary admissions, investigations, and psychological and financial stress on both the patient and the hospital management as well. There is definitely a need to adopt the standard and evidence-based approach for the determination of 99th percentile URL following the recent guidelines[27] considering its grave clinical significance.

This is the first time ever a 99th percentile URL is estimated specifically for the population of Rahim Yar Khan in Pakistan and the population in our study is well defined and is easily reproducible by other laboratories. We analysed the fresh serum samples within 2 hours in the morning to minimize the effect of biological variations on troponin levels.

CONCLUSION

On the basis of our study, it was concluded that high-Sensitive cardiac troponin I 99th percentile URL in overall study population was found to be significantly lower as compared to the results published in previous literature as well as manufacturers quoted result. High-sensitive troponin assay allows precise quantification of very low cardiac troponin concentration that helps in rapid exclusion of myocardial infarction. The main targeted population is from Rahim yar khan in our study so the cut offs cannot be generalised to the whole population of Pakistan.

LIMITATIONS

Our study had certain limitations. It was a single-centred study with a limited sample size. The majority of the participants were younger and were not a true representative of the actual population presenting in a clinical setting. We were unable to perform diagnostic imaging studies such as echocardiography, electrocardiography, or other imaging modalities on all of the individuals to identify subclinical heart disease due to financial limitations. Moreover, we relied on manufacturers data given in kit insert for claimed LOD and did not separately verify that.

RECOMMENDATIONS

Further large sample sized studies should be undertaken to evaluate the impact and clinical usefulness of using a lower threshold for cardiac troponin suggested by our study, in screening,

diagnosis and risk prevention of cardiovascular diseases in general population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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