



ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2024; 7(2): 97-101
Received: 27-04-2024
Accepted: 31-05-2024

Noorah Sabah Kadhum
AL-Najaf Health Directorate,
Al-Najaf, Iraq

Anne Khazal Yaseen Rijub-
Agha
Consultant Pathologist,
National Center of Teaching
Laboratories Medical City,
Baghdad, Iraq

Determination of sex and age specific 99th percentile upper reference limit of high sensitivity cardiac Troponin I in a sample of apparently healthy Iraqi adults

Noorah Sabah Kadhum and Anne Khazal Yaseen Rijub-Agha

DOI: <https://doi.org/10.33545/pathol.2024.v7.i2b.574>

Abstract

Background: Cardiovascular disease is the leading global cause of death, necessitating early detection and treatment for improved patient outcomes. Despite advancements in biomarkers, the aging population increases disease prevalence, highlighting the need for novel, more specific biomarkers like troponins, which are crucial for diagnosis. Aim of study Determination of sex and age specific 99th percentile upper reference limit of high sensitivity cardiac troponin I in a sample of apparently healthy Iraqi adults.

Methods: An analytical cross-sectional study was conducted from May 28 to October 30, 2023, at Baghdad Teaching Hospital, involving 300 healthy volunteers (150 females, 150 males). Routine anthropometric measurements, blood pressure, and biochemistry tests (fasting glucose, HbA1c, lipid profile) were performed, with participants meeting reference intervals included in the study for cardiac troponin measurement.

Results: In this study, cardiac troponin levels were significantly lower in females (median 0.7 pg/ml) compared to males (median 1.9 pg/ml) with a p-value < 0.001. The overall 99th percentile URL was 4.50 pg/ml, with males at 5.02 pg/ml and females at 3.39 pg/ml. Age-specific upper limits were: 20-29 years (2.69 pg/ml), 30-39 years (3.43 pg/ml), 40-49 years (3.79 pg/ml), and 50-72 years (5.90 pg/ml).

Conclusion: The research found that high-sensitivity cardiac troponin I limits were lower than worldwide norms, helping to comprehend baseline troponin levels in healthy persons. The research found that men had higher 99th percentile URL troponin levels than females.

Keywords: Determination, sex, age specific 99th percentile, cardiac troponin I

Introduction

Cardiovascular diseases (CVDs) are the foremost cause of mortality worldwide, claiming approximately 17.9 million lives annually. These conditions, which include coronary artery disease (CAD), heart failure, and atherosclerosis, evolve gradually and often remain undetected for long periods. Atherosclerosis in particular, characterized by the thickening of arterial walls, is a significant cause of death globally, primarily due to elevated cholesterol levels greater than 150 mg/dL [1]. Among the various manifestations of CVD, congestive heart failure (CHF) stands out, marked by the heart's reduced capacity to pump blood effectively, often resulting in fluid accumulation in the lungs. This syndrome is divided into heart failure with reduced ejection fraction (HFREF) and heart failure with preserved ejection fraction (HFPEF), the latter due to increased myocardial stiffness [2]. Acute coronary syndrome (ACS) represents another severe category of CVD, encompassing conditions such as CHD and CAD, where atheromatous plaque accumulation leads to myocardial infarction (MI). Depending on the electrocardiogram (ECG) results, MIs are classified as either ST elevation myocardial infarctions (STEMI) or non-ST elevation myocardial infarctions (NSTEMI), with STEMI associated with the development of Q waves on the ECG [3]. The epidemiological data underscore the significance of CVD, remaining a leading cause of death in the US since 1975 and responsible for over 633,842 deaths in 2015. The global burden of these diseases was similarly high in 2015, with an estimated 17.7 million deaths [4]. Despite advancements in medical diagnostics and treatments that have reduced the mortality rates from MIs, the overall risk associated with heart diseases remains alarmingly high, particularly as individuals age. Notably, the risk increases significantly in men at younger ages but the gender disparity diminishes post-menopause [5].

Corresponding Author:
Noorah Sabah Kadhum
AL-Najaf Health Directorate,
Al-Najaf, Iraq

Risk factors for CVD are varied, ranging from lifestyle choices such as tobacco use, which is projected to cause around 10 million deaths by 2030, to dietary habits that include high intakes of saturated and trans fats. Physical inactivity, a consequence of modern mechanization, along with metabolic factors like high cholesterol and hypertension, significantly contributes to the risk of developing CVDs [6]. The pathophysiology of CVD is complex, involving genetic and environmental factors that disrupt crucial cellular processes and lead to conditions like atherosclerosis. This process starts early in life with the formation of fatty streaks, which develop into plaques that can obstruct blood flow and, if calcified, exacerbate cardiovascular complications [7]. Understanding the mechanisms and risk factors of CVD is crucial for developing effective preventive and therapeutic strategies. This dissertation focuses on establishing age and sex-specific 99th percentile upper reference limits for hs-cTnI in a sample of apparently healthy Iraqi adults, aiming to enhance diagnostic precision and improve clinical outcomes within the region [8]. Aim of study: Determination of sex and age specific 99th percentile upper reference limit of high sensitivity cardiac troponin I in a sample of apparently healthy Iraqi adults.

Methods

The methodology of this analytical cross-sectional study involved comprehensive data collection and ethical protocols, conducted from May 28 to October 30, 2023, at Baghdad Teaching Hospital. A total of 315 individuals were initially considered, with 15 excluded due to non-adherence to inclusion criteria, resulting in 300 participants equally divided by gender. Ethical clearance was secured from the University of Baghdad’s College of Medicine, Department of Clinical Biochemistry, and the Iraqi Board for Medical Specializations, ensuring adherence to ethical standards. Additionally, verbal consent was obtained from all participants, emphasizing ethical compliance and participant safety. Participant selection was rigorously controlled; inclusion criteria were strictly defined to ensure the health status of the volunteers. Individuals aged 20 to 75 without cardiovascular diseases or medication use that could alter biochemical markers were included. They underwent a detailed screening that included physical examinations and routine biochemical tests such as fasting blood glucose, glycated hemoglobin (HbA1c), and a lipid profile. Only those with results within normal reference ranges for all tests were enrolled in the study. Key exclusion criteria included pregnancy, recent blood transfusion or donation, hypertension (blood pressure ≥ 140/90 mmHg), diabetes (HbA1c ≥ 5.7% or fasting blood sugar ≥ 100 mg/dL), and dyslipidemia (triglycerides ≥ 150mg/dl or total cholesterol ≥ 200 mg/dL). Additionally, individuals classified as overweight or obese (BMI ≥ 25 kg/m²) were excluded to minimize confounding variables that could influence the study’s outcomes on cardiac health. For data collection, standardized protocols were followed for anthropometric measurements (body weight and height) and blood pressure, which were routinely performed for all attendees of the internal medicine outpatient clinic. Biochemical tests were conducted on participants who had fasted for at least 12 hours to ensure accuracy in the readings of fasting blood glucose, HbA1c, and lipid profiles. Blood sample collection was meticulously carried out, with 5 ml of blood drawn from the antecubital vein. Samples were properly handled and processed, with 2 ml placed in EDTA tubes for HbA1c

testing and the remaining 3 ml in plain tubes for fasting blood sugar, lipid profiles, and cardiac troponin analysis. Samples were left to coagulate for about 20 minutes before being centrifuged at 3500 RPM for 30 minutes to separate the serum, which was then immediately analyzed. The analytical process involved the use of state-of-the-art equipment, such as the Abbott architect i1000SR® for high-sensitivity cardiac troponin I assays and other fully automated analyzers for different biochemical markers. These instruments ensured high precision and accuracy in the measurement of various biochemical parameters critical to the study. Statistical analyses were conducted using R software, with continuous variables expressed as means and standard deviations or medians and ranges, and categorical variables as frequencies and percentages. The robust method was employed to calculate the 99th percentile reference interval for cardiac troponin, ensuring statistical reliability and robustness.

Results

In this study, the sample comprised 300 individuals with a mean age of 39.6±12.3 years. Gender distribution was balanced, with 50.0% male and 50.0% female participants, presented. Anthropometric measurement and biochemical tests were performed and the mean for every test was calculated as shown in Tables.

Table 1: Age, BMI, fasting blood glucose and lipid profile in both males and females

Characteristic	Male, N=150 ¹	Female, N=150 ¹	P-Value ²
Age (years)	49.8±19.8	43.7±13.4	0.2
BMI (kg/m ²)	21.7±2.5	21.9±1.8	0.5
Fasting blood glucose (mg/dL)	96.4±11.9	90.3±10.7	< 0.001
HbA1c	5.0±0.6	5.1±0.7	0.5
Lipid profile			
Cholesterol (mg/dL)	166.7±23.0	165.4±21.3	0.6
HDL (mg/dL)	58.4±8.5	62.9±6.7	< 0.001
Triglyceride (mg/dL)	135.4±8.4	132.5±16.0	0.047
VLDL (mg/dL)	22.8±4.3	22.4±4.2	0.5

¹Mean±SD, ²Welch Two Sample t-test

In this study the cardiac troponin levels were significantly lower in females (median 0.7 pg/ml, range 0.1-6.4) compared to males (median 1.9 pg/ml, range 0.1-5.8) with a p-value of less than 0.001, as determined by the Welch Two Sample t-test. The overall reference interval for the specified characteristic was found to be 4.16 to 4.79 pg/ml, with an upper limit of 4.50 pg/ml. When stratified by gender, the upper limit for males (N=150) was 5.02 pg/ml (90% CI: 4.63, 5.42), while for females (N=150), it was 3.39 pg/ml (90% CI: 2.82, 3.91). The reference intervals were determined with a robust method according to the CLSI guidelines C28-A3c, and the confidence intervals for the values were calculated at 90% (36).

Table 2: Reference interval for cardiac troponin according to the robust statistical method

Characteristic	Age range	99th Percentile URL ¹ (pg/ml)	90% CI ² (pg/ml)
In all sample (N=300)	20-72	4.50	4.16, 4.79
In males (N=150)	20-67	5.02	4.63, 5.42
In females (N=150)	20-72	3.39	2.82, 3.91

¹99th percentile upper reference limit calculated according to the robust method, ²90% confidence interval

In Table 3.5, the reference intervals for cardiac troponin are delineated based on age categories. The 99th percentile upper limit (pg/ml) and corresponding 90% confidence intervals

(CI) for various age groups are presented as follows: 20-29 years (2.69, 2.22-3.10), 30-39 years (3.43, 2.96-3.71), 40-49 years (3.79, 3.42-4.21), and 50-72 years (5.90, 5.31-6.46).

Table 3: Reference interval for cardiac troponin according to age categories

Characteristic	Number%	99 th percentile URL ¹ (pg/ml)	90% CI ² (pg/ml)
Age categories			
20-29	45/25	2.69	2.22, 3.10
30-39	43/32	3.43	2.96, 3.71
40-49	40/37	3.79	3.42, 4.21
50-72	44/34	5.90	5.31, 6.46
¹ 99 th percentile upper reference limit calculated according to the robust method, ² 90% confidence interval			

So, there is an increment in cardiac troponin concentration by increase in age.

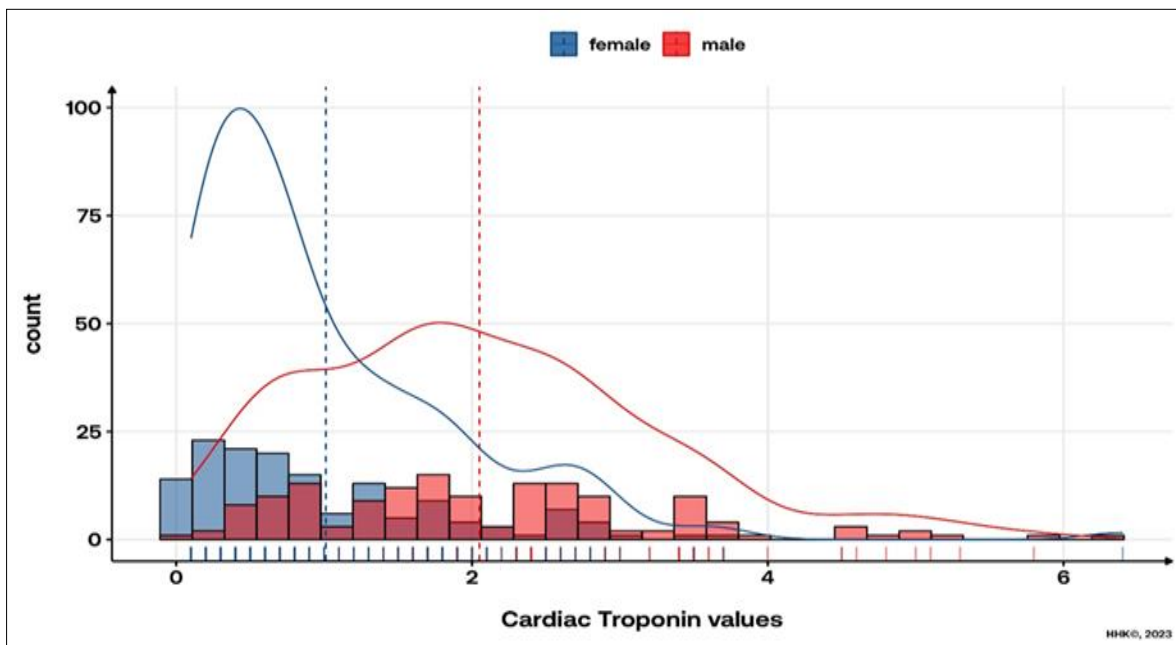


Fig 1: Histogram showing the distribution of cardiac troponin values stratified by sex

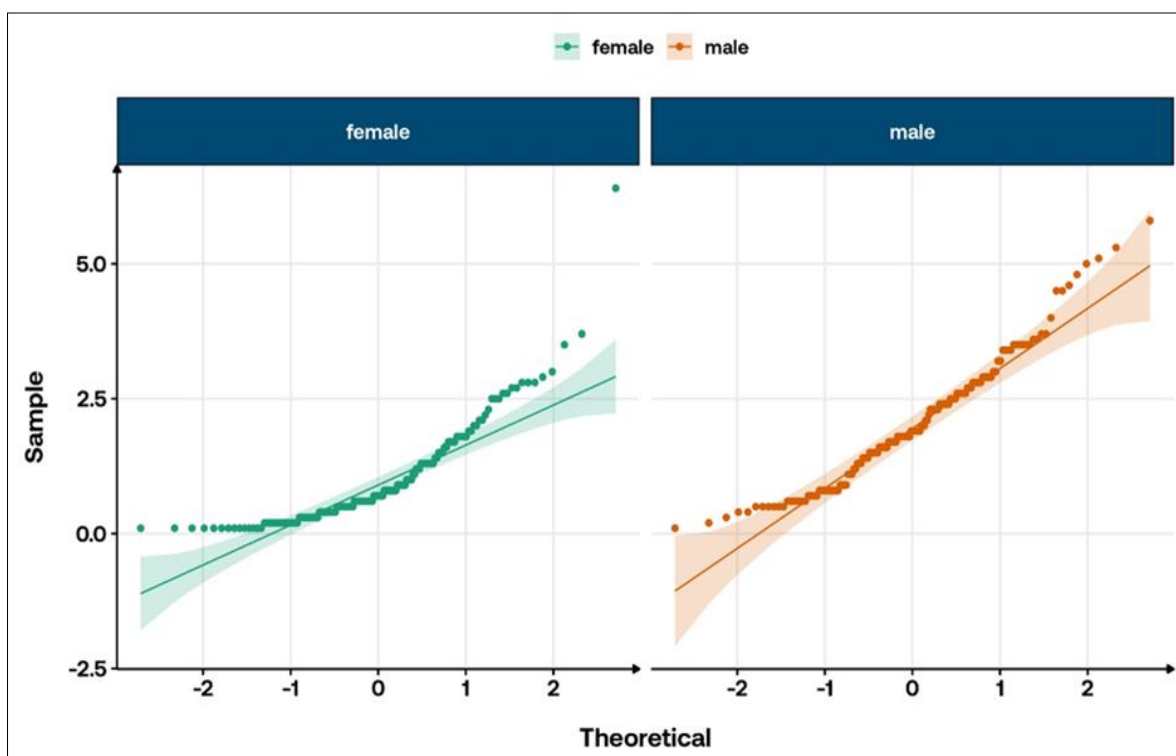


Fig 2: QQPLOT showing the deviation from normality of cardiac troponin values in both males and females.

Discussion

Globally, chest pain is a primary reason for hospitalization, often necessitating admission for observation and serial cardiac troponin testing to rule in or out myocardial infarction (MI), a crucial step in effective patient management and resource allocation in emergency departments^[9]. Cardiac troponins, particularly high-sensitivity cardiac troponin I (hs-cTnI), are the preferred biomarkers for diagnosing MI due to their sensitivity in detecting myocardial necrosis. The International Federation of Clinical Chemistry (IFCC) defines a "high-sensitive" cardiac troponin I assay based on specific criteria, including a low percentage coefficient of variation at the 99th percentile upper reference limit (URL) and detectable troponin levels in a significant percentage of healthy individuals^[10]. This study aimed to establish the assay-specific 99th percentile URL for HS-CTNI in a healthy adult population, with the overall 99th percentile URL found to be 4.50 pg/ml (90% CI 4.16-4.79). This value is considerably lower than similar studies in other regions, such as Pakistan, Malaysia, China, and Korea, which reported higher URLs ranging from 11.1 ng/L to 33.9 ng/L, illustrating significant geographical and demographic variability^[12-14]. Age and gender-specific analysis revealed that troponin levels vary with age, with older groups typically showing higher URLs. For instance, in China, individuals aged ≥ 55 years had higher URLs compared to the younger group. This study found that men generally have higher baseline troponin levels than women, with the 99th percentile URLs being 5.02 pg/ml for males and 3.39 pg/ml for females, highlighting the necessity for sex-specific reference values^[15]. The variation in troponin levels across different studies underscores the importance of regional and demographic-specific reference ranges for HS-CTNI to improve the accuracy of MI diagnosis and patient care. Such variations are influenced by factors including demographic differences, regional characteristics, and variations in assay methodologies. The need for standardized and validated reference limits is crucial for enhancing the clinical utility of hs-troponin assays globally^[16]. Additionally, this study supports the IFCC's recommendation for sex-specific 99th percentiles in cardiac biomarker evaluation to avoid underestimating MI risks in clinical settings. Employing sex-specific thresholds can improve diagnostic accuracy but also introduces potential challenges in balancing clinical sensitivity and specificity across genders^[10]. Population-specific 99th percentile URLs for HS-CTNI is vital for the precise diagnosis and management of myocardial infarction, reflecting the importance of tailoring cardiac care to demographic and regional characteristics. This approach not only improves patient outcomes but also optimizes the use of healthcare resources by ensuring that MI diagnoses are accurate and timely.

Conclusion

An apparently healthy Iraqi sample was used to define the 99th percentile URL for high-sensitivity cardiac troponin I. These restrictions help explain baseline troponin levels in healthy persons in this environment, which were lower than worldwide norms. Iraqi men exhibited higher 99th percentile URL troponin I levels than females, highlighting the relevance of country-specific reference values in gender-specific situations. Gender affects troponin levels, therefore clinical interpretation must account for it. The IFCC TF-CB recommends sex-specific 99th percentiles to prevent underestimating myocardial infarction risk, which the

research supports.

Conflict of Interest

Not available

Financial Support

Not available

References

1. World health organization, Cardiovascular diseases. Accessed 11 Nov 2023. Available at: [https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-\(cvds\)](https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds))
2. Rifai N. Tietz textbook of laboratory medicine. Elsevier Health Sciences. Seventh Edition; c2023, p. 178, 550, 554-6, 558.
3. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease, professional edition e-book. Elsevier health sciences; c2021. p. 547.
4. Gao Z, Chen Z, Sun A, Deng X. Gender differences in cardiovascular disease. *Med Novel Technol Devices*. 2019 Dec 1;4:100025.
5. Nason E. An overview of cardiovascular disease and research. United States on 05 Dec 2023. CID: 20.500.12592/q1gdcs. Retrieved from: <https://policycommons.net/artifacts/4832973/an-overview-of-cardiovascular-disease-and-research/5669805/>
6. O'gara PT, Kushner FG, Ascheim DD, Casey DE, Chung MK, De Lemos JA, et al. ACCF/AHA guideline for the management of ST-elevation myocardial infarction: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2013 Jan 29;61(4).
7. Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Ganiats TG, Holmes DR, et al. AHA/ACC guideline for the management of patients with non-ST-elevation acute coronary syndromes: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014 Dec 23;64(24).
8. Lopez EO, Brian D. Ballard; Arif Jan. Cardiovascular Disease. *Life-Sciences Literature*; c2018. Accessed 10 Nov 2023. Available at: <https://europepmc.org/article/MED/30571040/NBK430685#free-full-text>
9. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2016 Jan 14;37(3):26.
10. Lim SM, Thambiah SC, Zahari SY, Sham MDM, Omar RMDM, Hambali ZMD, et al. Determination of the 99th percentile upper reference limit for high-sensitivity cardiac troponin I in Malaysian population. *Malays J Pathol*. 2017 Aug 1;39(2):135.
11. He B, Wang K, Xu P, Zhou Q, Xu J. Determination of age-and sex-specific 99th percentile upper reference limits for high-sensitivity cardiac troponin I in healthy Chinese adults. *Cardiology*. 2022 Feb 25;147(3):261-70.
12. Ji M, Moon HW, Hur M, Yun YM. Determination of

- high-sensitivity cardiac troponin I 99th percentile upper reference limits in a healthy Korean population. *Clin Biochem.* 2016 Jul 1;49(10-11):756-61.
13. Mueller T, Egger M, Leitner I, Gabriel C, Holtmayer M, Dieplinger B. Reference values of galectin 3 and troponins derived from a single cohort of healthy blood donors. *Clin Chim Acta.* 2016;456:19–23.
 14. Wildi K, Gimenez MR, Terenbold R, Reichlin T, Jaeger C, Heinzlmann A, et al. Misdiagnosis of myocardial infarction related to limitations of the current regulatory approach to define clinical decision values for cardiac troponin. *Circulation.* 2015;131:2032-40.
 15. Omland T, Lemos DJA, Holmen OL, Dalen H, Benth JS, Nygård S, et al. Impact of sex on the prognostic value of high-sensitivity cardiac troponin I in the general population: The HUNT study. *Clin Chem.* 2015 Apr 1;61(4):646-56.

How to Cite This Article

Kadhun NS, Rijub-Agha AKY. Determination of sex and age specific 99th percentile upper reference limit of high sensitivity cardiac Troponin I in a sample of apparently healthy Iraqi adults. *International Journal of Clinical and Diagnostic Pathology.* 2024;7(2):97-101.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.