



# **St. Paul's Hospital Millennium Medical College**

**Study of the 99th Percentile Value of High Sensitivity Cardiac Troponin Level  
in Healthy Addis Ababa Population, Addis Ababa, Ethiopia, July, 2022.**

**By**

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**A research paper submitted to Adult Cardiology Unit, department of Internal Medicine,  
Saint Paul's Hospital Millennium Medical College, in partial fulfillment for completion of  
subspecialty in Adult Cardiology, Addis Ababa, Ethiopia, July 2022.**

**July, 2022. Addis Ababa, Ethiopia.**

**St. Paul's Hospital Millennium Medical College**  
**Department of Internal Medicine, Adult Cardiology Unit.**

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## Abbreviations and Acronyms

|         |                                                     |                    |                                              |
|---------|-----------------------------------------------------|--------------------|----------------------------------------------|
| ACC     | American college of cardiology                      | cTn                | Cardiac troponin                             |
| ACCF    | American College of Cardiology Foundation           | CV                 | Cardiovascular risk                          |
| ACS     | Acute coronary syndrome                             | CV                 | Co efficient of variation                    |
| AHA     | American heart association                          | CVD                | Cardiovascular disease                       |
| ALERT   | All Africa leprosy, tuberculosis and rehabilitation | DALYs              | Disability adjusted life years               |
| ARIC    |                                                     | DBP                | Diastolic blood pressure                     |
| BMI     | Body mass index                                     | DHS                |                                              |
| BNP     | Brain natriuretic peptide                           | DM                 | Diabetes Mellitus                            |
| BP      | Blood pressure                                      | ECG                | Electrocardiography                          |
| BUN     | Blood urea nitrogen                                 | EDTA               |                                              |
| CBC     | Complete blood count                                | eGFR               | Estimated glomerular filtration rate         |
| CHF     | Congestive heart failure                            | ETB                | Ethiopian birr                               |
| CHS     |                                                     | ESC                | European society of cardiology               |
| CI      | Confidence interval                                 | FEV <sub>1</sub>   | Forced expiratory volume in 1 second         |
| CKD     | Chronic kidney disease                              | FEV <sub>1</sub> % | Forced expiratory volume in 1 second percent |
| CKD-EPI | Chronic kidney disease-Epidemiology Collaboration   | GBD                | Global burden of disease                     |
| CM      | Centimeter                                          | Hb                 | Hemoglobin                                   |
| CMR     | Cardiac magnetic resonance imaging                  | HBA1 <sub>C</sub>  | Glycated Hemoglobin                          |
| CT      | Computed tomography                                 | HDL-C              | High density lipoprotein cholesterol         |

### Abbreviations and acronymy continued.....

|           |                                              |                 |                                                  |
|-----------|----------------------------------------------|-----------------|--------------------------------------------------|
| Hs-cTn    | High sensitivity cardiac troponin            | PLT             | Platelet                                         |
| IDL-C     | Intermediate density lipoprotein cholesterol | RBC             | Red blood cell                                   |
| IHD       | Ischemic heart disease                       | RHD             | Rheumatic heart disease                          |
| Kg        | kilogram                                     | SBP             | Systolic blood pressure                          |
| LDL-C     | Low density lipoprotein cholesterol          | S <sub>cr</sub> | Serum creatinine                                 |
| LV        | Left ventricle                               | SPHMMC          | Saint Paul's hospital millennium medical college |
| LVEF      | Left ventricle ejection fraction             | SPSS            | Statistical package for social sciences          |
| LVH       | Left ventricle hypertrophy                   | SSG             |                                                  |
| MetS      | Metabolic syndrome                           | URL             | Upper reference limit                            |
| MI        | Myocardial infarction                        | USA             | United states of America                         |
| Mg/dl     | Milligram per deciliter                      | VC              | Vital capacity                                   |
| Ng/dl     | Nano gram per deciliter                      | VLDL-C          | Very low density lipoprotein cholesterol         |
| Ng/dl     | Nano gram per liter                          | WBC             | White blood cell                                 |
| Non-HDL-C | Non- High density lipoprotein cholesterol    | WHF             | World heart federation                           |
| NT-proBNP | N terminal pro-brain natriuretic peptide     | WHO             | World health organization                        |
| NVP       | Negative predictive value                    |                 |                                                  |
| OR        | Odds ratio                                   |                 |                                                  |
| PCI       | Percutaneous coronary intervention           |                 |                                                  |
| Pg/L      | Pictogram per liter                          |                 |                                                  |
| Pg/ml     | Pictogram per milliliter                     |                 |                                                  |

### **Conversion factors**

mg/dL cholesterol=mmol/L $\times$ 38.6

mg/dL triglycerides= mmol/L $\times$ 88.5

mg/dL glucose= mmol/L $\times$ 18

pg/ml troponin= 10000\* $\mu$ /dl troponin

## **Abstract**

**Background:** The use of high sensitivity cardiac troponin (hs-cTn) test has led to early diagnosis and management of ACS which lead to an invaluable armament to clinician. These values are extrapolated from the normal healthy individuals applying upper level of the 99 percentile. Diagnosis, management and prognostication of variety of cardiovascular disorders (CVD) specifically acute coronary syndromes (ACS) had been one of breakthroughs in the field of cardiology. However, their use should be based on comparison with normal range values obtained from the population, and it's considered abnormal when the value is more than the 99th percentile. To date, there are no such nomograms in Ethiopia. Hence, this research was conducted to determine hs-cTn values and associated factors in healthy adults as well prevalence of cardiovascular factors in apparently healthy adults.

**Method:** A prospective study was conducted to determine the 99<sup>th</sup> percentile level of high sensitive cardiac troponin (hs-cTn) assay in healthy Addis Ababa population. A total of 210 apparently healthy in the primary samples were included for screening out of which 120 were identified as healthy. The general population 99<sup>th</sup> percentile hs-cTn I level was found to be 45.74 pg/ml, with statistically significant difference between male and female groups ( $p=0.002$ ) as well with increasing age ( $p=0.012$ ) with age group  $\geq 65$  having higher values..

**Conclusion and Recommendation:** The 99<sup>th</sup> percentile value of hs-cTn value of Addis Ababa healthy population determined to be 45.74 pg/ml with 44.97 pg/ml & 31.93 pg/ml for male and female respectively.

**Key words:** High sensitive Cardiac Troponin assay , 99<sup>th</sup> percentile cTn, prospective study, SPHMMC, ALERT center/hospital, Addis Ababa, Ethiopia.

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# 1 Introduction

## 1.1 Background information

The study area was Addis Ababa, capital of Ethiopia with estimated population of 3,686,068 million of which 34.6% are in the age group between 15-45 years as of 2020, with 11 sub cities and 869 Kebeles (smallest administrative unit). (1)

According to Ministry of health Ethiopia; heart failure was 4<sup>th</sup> most common cause of death in all age group as of 2020 and 34% of all cause of deaths were as a result of heart problem, for which specific cause were difficult to ascertain. (2)

There are 4 federally administered hospitals in Addis Ababa under administration of Ministry of Health Ethiopia. The study was conducted at ALERT hospital, selected randomly with lottery methods. (3)

The study of level of high sensitive cardiac troponin (hs-cTn) assay to diagnose, manage and prognosticate cardiovascular diseases especially acute coronary syndromes (ACS) is one of paradigm shift in cardiovascular medicine, to use the hs-cTn test the 99<sup>th</sup> percentile reference values should be determined from healthy adult's for every settings which can be employed as a cutoff point. Any value with consistent clinical presentations greater than the 99<sup>th</sup> percentile developed in that settings is recommended to diagnosis of ACS.(4)

There have been no such studies conducted in Ethiopia. Hence, this research was conducted to determine the 99<sup>th</sup> percentile value of hs-cTn in healthy adults and associated factors to use for diagnosis and management of ACS. The prevalence of cardiovascular risk factor and associated factors was studied on apparently healthy adults too.

## **1.2 Statement of the problem**

According to world health organization ( WHO) June, 2021 port, Cardiovascular diseases (CVDs) are leading cause of death globally with an estimated 17.9 million people died from CVDs in 2019, accounting for 32% of all global deaths, of this 85% were due to heart attack and stroke. Low- and middle income countries took the lion share with more than three quarters of CVD death. CVDs include; coronary heart disease; cerebrovascular diseases, peripheral arterial disease; Rheumatic heart disease; congenital heart disease and deep vein thrombosis and pulmonary embolism. According to WHO most important behavioral risk factors of heart attack and stroke are unhealthy diet, tobacco use and unhealthy use of alcohol.(5)

According to Global Burden of Disease ( GBD) study 2019, prevalent cases total CVD nearly doubled from 271 million in 1990 to 523 million in 2019; of the total 197 million were accounted as a result of ischemic heart diseases (IHD) and the number of CVD deaths increased steadily from 12.1 million in 1990 to 18.6 million in 2019. Ukraine, Tajikistan and many islands of Oceania were with extremely high rates of IHD at country level with partly explained with higher exposure to risk factors including tobacco and excessive alcohol use and restricted access to preventive health care. According to this study modifiable risk factors which predispose with development of CVD were; high systolic blood pressure ( SBP), high fasting plasma glucose, high low density lipoprotein cholesterol, high body mass index (BMI), ambient and household air pollution, tobacco use, dietary risks and low physical activity.(6)

Study on the burden of cardiovascular disease In Ethiopia from 1990-017; evidence from global Burden of disease study, published in July, 2020, using GBD 2017 study data, showed that the age standardized CVD prevalence, disability-adjusted life years (DALYs) and mortality were 5534, 3549.6 and 183.63 per 100, 000 population respectively with top three CVDs were; IHD,

Rheumatic heart diseases (RHD) and stroke in descending orders; with leading risk factors for CVD in Ethiopia being , high blood pressure ( 26%), dietary risk factors ( 28%) and air pollution inclusive of household air pollution and ambient particulate matter (9%).(7)

Now a public health problem CVDs are managed at different level of health facilities with multifaceted strategies which are known to decrease mortality and improve the wellbeing of individuals with the disease; one of the key element in decreasing the mortality and morbidity of CVDs is early diagnosis with various armamentarium including Troponin level when appropriate. One of key paradigm shift in CV medicine id use of high sensitivity cardiac troponin test to diagnosis heart attack which accounts for more than 85% of death as a result of CVDs however to use it cutoff point should be developed by measuring from apparently healthy person and identifying risk associated with increased level so that 99<sup>th</sup> percentile will be developed and used for specific setting. (8)

Studies determining the level of cardiac troponin are lacking in Ethiopia and hence this research answer the key question on the 99<sup>th</sup> percentile of cardiac troponin using high sensitivity assay in healthy individuals and associated risk factors as well the prevalence of CV risk factor in apparently healthy individuals.

### 1.3 Literature review

Cardiac troponin (cTn) consists of 3 protein's; cTnC, cTnI & cTnT depending on their functions, cTn are released with injury to myocytes starting 4-6 hours, peaking 18-24 hours and remains detectable up to 14 days then after, based on this dynamic release changes variety of CVD are diagnosed, managed and prognosis made, with current paradigm evolution in cardiology using high sensitive cTn (hsTn) assay lower limit values are detected (  $<0.001\text{ng/mL}$  or  $< 1\text{ pg/mL}$ ) and allows at least 50% ( some  $\geq 95\%$ ) apparently healthy individuals below 99<sup>th</sup> percentile to have measurable level of troponin. (8,9)

The high sensitive cardiac troponin assay shortens time interval to next measurement to 1-2 hours and still achieves NPVs  $\geq 99.5\%$ . Using cutoff well below 99<sup>th</sup> percentile, approximately 20% to 25 % patients will have low or undetectable level with corresponding  $>99\%$  NPV. However timing and nature of symptoms and the risk of population requires consideration.(10)

The causes and mechanisms of increased cardiac troponin T and I (cTnT and cTnI) concentrations are numerous and are not limited to acute myocardial infarction (AMI) (ischemic necrosis of cardiac myocytes). Any type of reversible or irreversible cardiomyocyte injury can result in elevated serum cTnT and cTnI levels, Table2-1.(11–18)

**Table 1.1 Classification of the main causes of troponins elevation not associated with myocardial infarction.**

Possible causes of increased cTn in blood serum in addition to AMI.

| Myocardial damage in cardiac pathologies                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Myocardial damage in non-cardiac and systemic pathologies                                                                                                                                                                                                                                                                                                                                                                                                                                           | False positive causes of increased troponins                                                                                                                                                                                                                                                                                               |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>• Inflammatory heart diseases ( endocarditis, myocarditis, pericarditis)</li> <li>• Cardiac dysrhythmias ( Atrial fibrillation (AF), Super ventricular tachycardia, others)</li> <li>• Cardiomyopathies (all types) and heart failure (HF).</li> <li>• Drugs with direct cardiotoxic properties (antitumor chemotherapy drugs, adenomimetics, cocaine, Methamphetamines and others)</li> <li>• Cardiac surgery 9 Coronary bypass, percutaneous coronary interventions, Catheter ablation, etc....)</li> <li>• Takotsubo syndrome</li> <li>• Peripheral vascular disease</li> </ul> | <ul style="list-style-type: none"> <li>• Heavy physical exertion (marathon) &amp; stressful situations.</li> <li>• Systemic inflammatory reactions= ( sepsis)</li> <li>• Chronic diseases (CKD, COPD, DM, oncologic diseases, etc....)</li> <li>• Pulmonary embolism</li> <li>• Diseases of CNS (Stroke, SAH, etc....), neuromuscular diseases</li> <li>• Systemic hypoxia in various critical pathologic conditions (severe anemia, respiratory disorders, etc....)</li> <li>• COVID-19</li> </ul> | <ul style="list-style-type: none"> <li>• Heterophilic antibodies</li> <li>• Rheumatoid factor</li> <li>• Alkaline phosphatase</li> <li>• Biotin</li> <li>• Hemolysis</li> <li>• Fibrin clot in serum</li> <li>• Cross sectional (non-specific) reactions of anti-cardiac troponin diagnostic antibodies with skeletal troponins</li> </ul> |

According to metaanalysis and systematic review of 9 community and institution based studies conducted on 2021, the result showed that there were heterogeneity in CVD in different regions of Ethiopia with prevalence ranging between 1-20% with no difference between male and female. (19)

Unmatched case control; study done in Deber Berehane referral hospital to determine cause of CVD IHD accounted for 44.9% and hypertension 44.1% with most frequent risk factors for CVD being older age, smoking, physical activities and abdominal obesity. (20)

Among diabetic patient the dyslipidemia, physical inactivity and hypertension were commonest CVD risk factor in institution based cross sectional study conducted in adult diabetic patient in Eastern Ethiopia. (21)

Clinical assessment of CVD associated risk factors in Jimma town, community based cross sectional study, at least 70% of respondents have one or more of even CVD risk factors, with 23.8% being hypertensive, 26.8% overweight/ obese, 13% smoker and 6.2 were diabetics.(22)

In 2006 published study to determine the prevalence of and determinant of cTnT elevation in general population which involved 3557 subjects, sample weight adjusted prevalence of cTnT elevation ( $\geq 0.01\mu\text{g/L}$ ) was 0.07%. with univariate analysis elevation of cTnT was associated with old age, black race, male sex, coronary artery calcium by electron beam CT, a composite marker of CHF, LVH, CKD and DM (  $P<0.001$  for each ).(23)

In a multi-center study to establish the 99<sup>th</sup> percentile value of high sensitivity troponin I in normally healthy Chinese population, the overall 99<sup>th</sup> percentile was 29.42 pg/ml with males and females having value of 29.40 pg/ml and 19.46 pg/ml respectively ( $P<0.001$ ). (24)

In a study to assess minimally elevated cTnT and NT-proBNP levels in elderly apparently healthy individuals in US, cTnT was detectable in 4.1% of populations with range of 0.001 to 0.01 ng/ml, compared to those with undetectable those with detectable cardiac troponin were older, male, likely to be hypertensive, diabetic, have lower HDL-C level and poorer renal function.(25)

According to research evaluating evidence behind sex specific cutoffs values in using high sensitive cardiac troponin, showed that 21-50% health populations will have detectable troponin values with variation occurring with different age group strata and sex group.(26)

In scientific paper published on October 2018, in cardio-Healthy individuals, large proportion of individuals <60years had detectable hs-cTnI compared to hs-cTnT ( $p<0.001$ ), the 99<sup>th</sup> percentile URL of hs-cTnT (Roche) & hc-cTnI (Abbott) were 15.2 & 21.1 ng/L respectively with males had higher values than females. (27)

What was most important in conducting this kind of research is to have a criteria that fulfills what constitute normal healthy population and adequacy of sample size taking in to account various literatures so that the inferences made could be extrapolated to general population under which sample was taken in the study. To define normal healthy populations various studies used only health questionnaire's and baseline clinical characteristics, whereas several performed thorough evaluation including blood tests, imaging modalities before enrolling individuals in the study equating them as normal healthy individuals, stringent criteria was decisive factor in evaluation of an assay, hence this paper used stringent inclusive and exclusive criteria with the use of healthy questionnaires, physical examination, blood tests ( CBC, creatinine, HbA1C, lipid profile) and echocardiography, to maximum extent possible define normal healthy individual's, table 2.2.(27–31)

**Table 1.2 Common Clinical characteristics obtained via questionnaires, surrogate biomarkers (blood), and other tests used in the process of defining healthy reference sample groups.**

| <b>Reported variables included in questionnaires</b>                                                                                                  | <b>Surrogate Biomarkers</b>                        | <b>Other tests</b>                                                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Cardiac disease</b>                                                                                                                                | <b>Electrolyte</b>                                 | <b>Echocardiography</b>                                                                                                                                                    |
| <ul style="list-style-type: none"> <li>• Coronary artery disease</li> <li>• Congestive heart disease</li> <li>• Previous revascularization</li> </ul> | Urea<br>Creatinine<br>Creatinine clearance<br>eGFR | <ul style="list-style-type: none"> <li>• LVEF</li> <li>• LV mass</li> <li>• Valvular regurgitation</li> <li>• Valvular stenosis</li> <li>• Diastolic parameters</li> </ul> |
| <b>Hypertension</b>                                                                                                                                   |                                                    |                                                                                                                                                                            |
| <b>Dyslipidemia</b>                                                                                                                                   | <b>Calcium</b>                                     |                                                                                                                                                                            |
| <b>Renal disease</b>                                                                                                                                  | <b>Cholesterol</b>                                 | <b>Stress Echocardiography</b>                                                                                                                                             |
| <b>Pregnancy</b>                                                                                                                                      | <b>Triglycerides</b>                               | <b>Electrocardiogram</b>                                                                                                                                                   |
| <b>Current cold or infection</b>                                                                                                                      | <b>HDL, LDL</b>                                    | <b>Heart rate</b>                                                                                                                                                          |
| <b>Diabetes</b>                                                                                                                                       | <b>Transferrin</b>                                 | <b>Blood pressure</b>                                                                                                                                                      |
| <b>Tobacco use</b>                                                                                                                                    | <b>Urine total protein</b>                         | <b>Physical exam</b>                                                                                                                                                       |
| <b>Cardioactive drugs</b>                                                                                                                             | <b>HbA1c</b>                                       | <b>Weight</b>                                                                                                                                                              |
| <b>Immediate family history of cardiovascular disease</b>                                                                                             | <b>Urine albumin</b>                               | <b>Height</b>                                                                                                                                                              |
| <b>Alcohol intake</b>                                                                                                                                 | <b>NT-proBNP</b>                                   | <b>Spirometry</b>                                                                                                                                                          |
| <b>Cancer</b>                                                                                                                                         | <b>Phosphates</b>                                  | <ul style="list-style-type: none"> <li>• FEV<sub>1</sub></li> <li>• VC</li> <li>• FEV<sub>1</sub>%</li> </ul>                                                              |
| <b>Thyroid disease</b>                                                                                                                                | <b>Liver enzymes</b>                               |                                                                                                                                                                            |
| <b>Hospitalization in the past 3 months</b>                                                                                                           | <b>Creatinine kinase-MB</b>                        |                                                                                                                                                                            |
| <b>Abnormal BMI ( &gt;35 or &lt;18)</b>                                                                                                               | <b>Hemoglobin</b>                                  |                                                                                                                                                                            |
| <b>Systemic or acute chronic disease</b>                                                                                                              | <b>WBC count</b>                                   |                                                                                                                                                                            |
| <b>Previous stroke</b>                                                                                                                                | <b>Glucose</b>                                     |                                                                                                                                                                            |
|                                                                                                                                                       | <b>Fasting blood glucose</b>                       |                                                                                                                                                                            |
|                                                                                                                                                       | <b>Platelets</b>                                   |                                                                                                                                                                            |
|                                                                                                                                                       | <b>Iron</b>                                        |                                                                                                                                                                            |
|                                                                                                                                                       | <b>Ferritin</b>                                    |                                                                                                                                                                            |

Adequacy of sample size to determine the 99<sup>th</sup> percentile of biological marker is still matter of debt, where as a minimum of 100 sample size is agreed in most literatures, in one of study to use the recommendation of cardiac troponin assay adequacy was defined as having a sample size of 300 individuals for determination of the 99<sup>th</sup> percentile in general and 600 ( 300 male and 300 female ) individuals were recommended as adequate to compare the difference in the 99<sup>th</sup> percentile and conclude between male and female, with regards to this total of 210 individuals were screened in this study with 124 considered as normal health fulfilling all-inclusive criteria, as there was limitations of funding and timing constraint added with extra burden imposed by COVID pandemic to screen and include more normal healthy individuals who fulfill the inclusive criteria.(28)

Before the advent of cTn protein assay ACS was diagnosed according to the definition of the World Health Organization and also based on clinical symptoms, ECG and comprehensive myocardial enzymes assay, however currently using hs-cTn protein's microscopic ischemia can be detected especially when combined with imaging modalities. Staring from 200 and 2007 ESC, ACCF and AHA release 1<sup>st</sup> , 2<sup>nd</sup> & 3<sup>rd</sup> definition of myocardial infarction with replacement of cardiac enzymes with CTn protein with updated definition by ESC/ACC/AHA/WHF EXPERT CONSENSUS DOCUMENT; Fourth Universal Definition of Myocardial Infarction 2018, using the 99<sup>th</sup> percentile and rise and fall of cTn value for diagnosis and management of MI.(32–34)

## 2 Significance of the study

Cardiovascular diseases especially ischemic heart disease is major public health problem worldwide. This study will determine the 99<sup>th</sup> percentile value of hs-cTn in healthy individual to be used as reference for diagnosis and management of ACS as well determine the prevalence of CV risk factors in apparently healthy individuals.

Recommendation will be disseminated to practicing clinician's, Internists, Cardiologists through continuous medical educations ( CMEs), through seminars, trainings by various stakeholders who are thriving in improvement and optimization of patient care specifically; Ministry of health non communicable diseases directorate, Ethiopian Public health institute, Ethiopian cardiac professionals associations, Ethiopian Internal medicine associations, Ethiopian Public health association, Ethiopian medical association, Ethiopian laboratory technologists and technician associations.

### 3 Conceptual framework

#### Sociodemographic characteristic's

Age

Sex

Educational status

Occupational status

Marital status

Religion

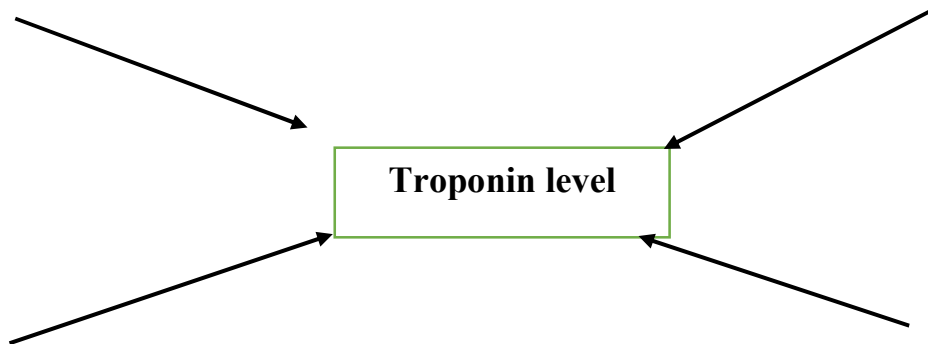
#### Clinical characteristics

Weight

Height

Waist circumference

Blood pressure level



#### Associated Clinical risk factors

HbA1c level

Creatinine level

Total cholesterol level

LDL level

HDL level

Triglyceride level

#### Associated Behavioral risk factors

Salt intake habit

Alcohol intake habit

Exercise habit

Cigarette smoking habit

*Figure 3-1 Conceptual framework depicting factors associated with level of cardiac troponin using hs-cTn.*

## **4 Objectives**

### **4.1 General Objective**

To determine 99<sup>th</sup> percentile value of high sensitivity cardiac troponin assay and associated cardiovascular risk factors in healthy adults and prevalence of cardiovascular risk factors in apparently healthy adults living in Addis Ababa, Ethiopia, July 2022.

### **4.2 Specific objectives**

- To determine the prevalence cardiovascular risk in apparently healthy adults living in Addis Ababa, Ethiopia, July 2022.
- To determine the 99<sup>th</sup> percentile of cardiac troponin by high sensitive assay in healthy adults living in Addis Ababa, Ethiopia, July 2022.
- To identify associated factors with Level of cardiac troponin by high sensitive assay in healthy adults living in Addis Ababa, Ethiopia, July 2022.

## **5 Subjects and Methods**

### **5.1 Study Area and period and Study design**

The study was conducted in Addis Ababa town, capital city of Ethiopia. It is a special administrative region with 11 sub cities the study was conducted from November 2021 to June, 2022.(35) A prospective cross-sectional study was conducted.

### **5.2 Source and study Population**

All individuals living in Addis Ababa town were the source of population.

The study population were adults living in Addis Ababa town who full fill the inclusion criteria and exclusion criteria.

### **5.3 Inclusion and exclusion criteria**

Inclusion criteria for cardiovascular risk factors in apparently healthy individuals

- Adults aged  $\geq 15$  years
- Adults who are apparently healthy.
- No history of heart disease and heart-related diseases;
- No high blood pressure, no diabetes mellitus and no history of high cholesterol

Inclusion criteria for the 99<sup>th</sup> percentile level of hs-cTn level

- Adults aged  $\geq 15$  years. (16,27,36)
- Adults who are not pregnant.
- Adults who are apparently healthy.
- No history of heart disease and heart-related diseases;
- No high blood pressure, no diabetes mellitus and no history of high cholesterol;

- No abnormal renal function test results
- No abnormal blood count test results (RBC, WBC, Hb, PLT);
- Glycated hemoglobin (HbA1c) <6.5%
- Normal echocardiographic examination

Exclusion criteria for cardiovascular risk factors in apparently healthy individuals

- Adult who are not willing to participate
- Adults who are admitted to and under follow up at ALERT hospital.
- Adults with acute medical, surgical or gynecological illness.
- Adults with suspected of having coronary syndrome; acute myocarditis

Exclusion criteria for the 99<sup>th</sup> percentile level of hs-cTn level

- Adult who are not willing to participate
- Adults with acute medical, surgical or gynecological illness.
- Adults with suspected of having coronary syndrome; acute myocarditis
- Adults with history of unstable angina; myocardial infarction; cerebrovascular disease;
- Underlying diseases such as DM, acute and chronic kidney disease, high blood pressure.
- Adults aged ≤40 years with eGFR <90 mL/min, >40 years with eGFR <60 mL/min. (37)

$$eGFR = 142 * \min(S_{cr}/K, 1)^{\alpha} * \max(S_{cr}/K, 1)^{-1.200} * 0.9938^{Age} * 1.012 \text{ [if female]}$$

***Equation 5-1eGFR CKD-EPI Creatinine Equation (2021) of National Kidney Foundation.***

#### **5.4 Sample size and sampling technique**

The sample size to determine the 99<sup>th</sup> percentile as suggested by Clerico A et. Al. review paper published in 2017 entitled “The 99th percentile of reference population for cTnI and cTnT assay:

methodology, pathophysiology and clinical implications” was minimum of 100 participants with increasing the power of study if the sample size can reach 300 participants. (28)

The study was primarily planned to be conducted at house hold level because of the COVID pandemic and time constraint courtained by principal investigator schedule and funding organization, samples which represented apparently healthy individuals in Addis Ababa were selected from apparently healthy care takes attending ALERT hospital, during period of study. The study was terminated when the total amount of selected individual reached 210 for apparently healthy individuals from this 120 individual met the inclusion and exclusion criteria for determination of the 99<sup>th</sup> percentile value of hs-cTn as per recommendation by Clerico A et. Al. the research met the minimum requirment of 100 sample size. Figure 6-1

The adequacy of sample size to determine prevalence of cardiovascular risk factors in apparently healthy adults were done using formula for estimation of single population proportion taking prevalence of cardiovascular risks among adult individuals 20% (19,27,38), margin of error 5%, and using 95% confidence level, calculated using the formula;

$$n = (Z\alpha/2)^2 P (1-P) / d^2 = 246$$

***Equation 5-2. Formula to estimate sample size with single proportion population.***

ALERT comprehensive specialized hospital was selected out of 9 tertiary hospitals in Addis Ababa town, randomly by lottery method. Selection were made using randomly generated computer number with reference to patient medical record number, and their adult care taker were included in the study. Apparently health care taker adults who came during data collection period and who fulfill the inclusion and exclusion criteria were included in the study, when there were more than 1 caretakers a lottery method was employed to select one.

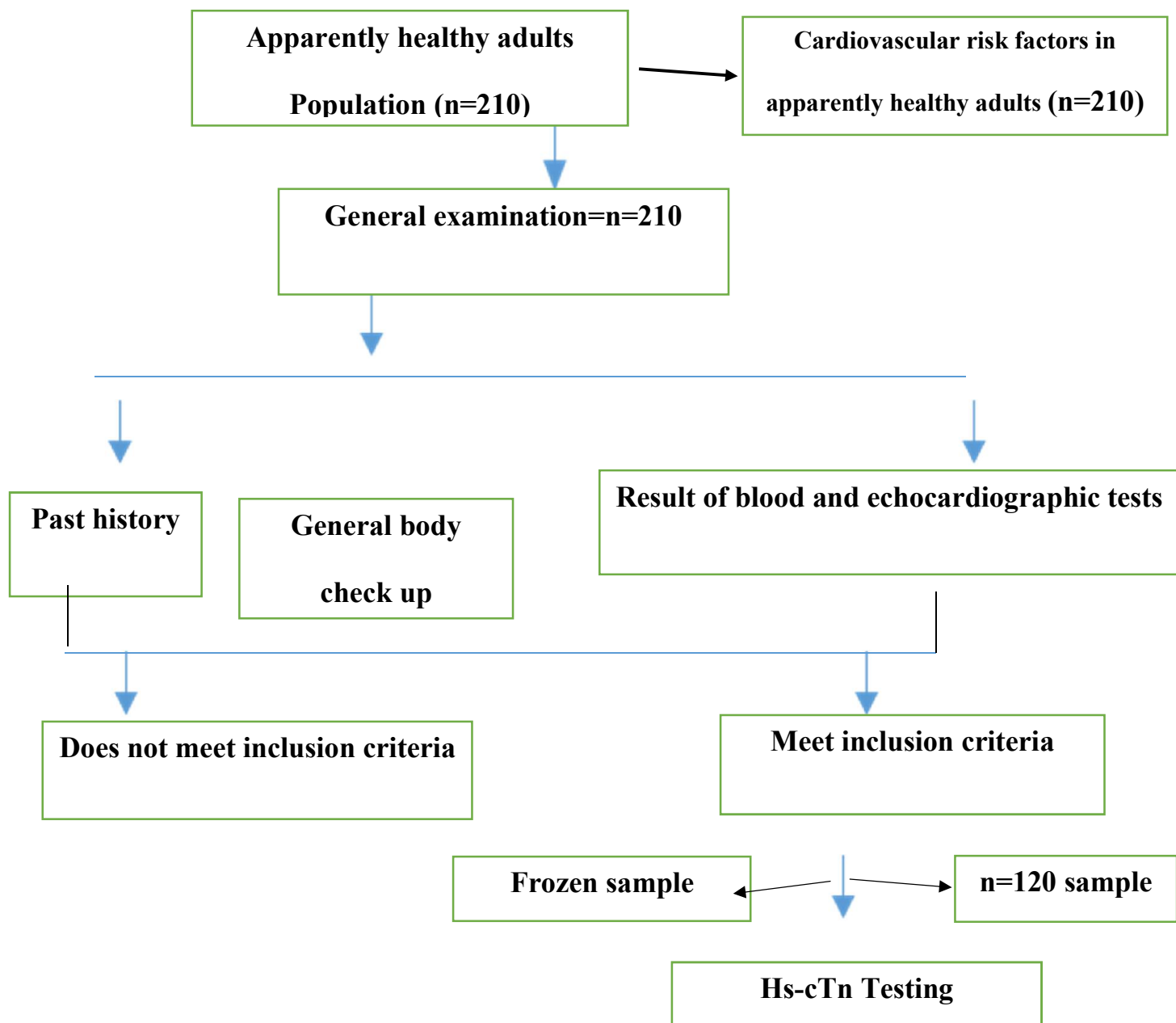


*Figure 5-1*Flowchart depicting selection of individuals in determining the 99th percentile of hs-cTn in normal healthy adults and cardiovascular risk factors in apparently healthy adults Addis Ababa, Ethiopia, July 2022.

## 5.5 Measurements

Dependent Variables for the 99<sup>th</sup> percentile value of hs-cTn.

- Cardiac troponin level using high sensitive assay

Independent variables for the 99<sup>th</sup> percentile value of hs-cTn.

### I. Sociodemographic characteristics

- Age, sex, Religion, Occupation, Level of education, marital status, yearly income ( ETB),

### II. Clinical characteristics

- Weight, height, waist circumference,
- Blood pressure( Both arms, one leg, and standing after 3 minutes)

### III. Co morbidities

- HbA<sub>1c</sub>
- Lipid profile ( total cholesterol, Triglycerides, HDL, LDL, Non-HDL)
- RFT ( Creatinine, BUN)
- Echocardiography result

### IV. Risk factors for cardiovascular diseases

- Cigarettes smoking
- Staple diet ( 12 month)
- Exercise habit
- Alcohol use
- Illicit drug use

### **Dependent Variables for the prevalence of cardiovascular risk factors**

- Sociodemographic variables; age, sex
- Behavioral factors; Cigarette smoking, exercise habit, family history of CVD
- Clinical factors; waist circumference, BMI, level of blood pressure
- Biomarkers; Lipid profiles, HbA<sub>1c</sub>, eGFR
- Cardiac Imaging; Echocardiography

### **Independent variables for the prevalence of cardiovascular risk factors**

- Sociodemographic variables; religion, occupation, marital status
- Behavioral factors; alcohol use habit
- Clinical factors; body surface area
- Biomarkers; cardiac troponin value

## **5.6 Operational definition of variables**

Apparently healthy adults= Individual's without any medical complaint subjectively as self-reported at a time of selection during data collection period.

**Healthy adults**- individual's with normal clinical screening using standard history and physical examination with eGFR ( $>90$  ml/min/1.73 m<sup>2</sup>), normal blood count results, HbA<sub>1c</sub> level  $<6.5\%$ , normal total cholesterol ( $<200$  mg/dl), normal LDL ( $<160$ mg/dl with no CV risk factors,  $<130$  mg/dl with  $\leq 1$  CV risk factors), normal HDL ( $\geq 40$  mg/dl for men &  $\geq 50$  mg/dl for women) level and normal echocardiographic screening results.

### **Educational status-**

- Illiterate= those who cannot read and write

- Primary= those who attended grade 1-6.
- Secondary- those who attended grade 7-12
- Tertiary- anyone who attended beyond 12 grade.

### **Occupational status**

- Unemployed = individuals who were not working, who were retired and housemaid during data collection period.
- Employed = individuals who were working during data collection period and who were not fulfilling criteria for unemployed.

**Exercise** = WHO, AHA and ACSM recommendation for health related benefit. Table 6-1

- Regular= activities who fulfill the criteria by WHO, AHA and ACSM.(39,40)
- Occasional= those who participate in activities that did not meet the criteria for regular exercise as per WHO, AHA and ACSM.

***Table 5.1WHO; AHA ACSM recommended physical activity level based on age category.***

| <b>WHO; AHA ACSM recommended physical activity level based on age category.</b>                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                       |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Adults aged 18–64</b>                                                                                                                                                                                                                                                                                              | <b>Adults aged 65 years and above</b>                                                                                                                                                                                                                                                                                 |
| <ul style="list-style-type: none"> <li>• at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous-intensity activity.</li> </ul> | <ul style="list-style-type: none"> <li>• at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous intensity activity.</li> </ul> |
| <ul style="list-style-type: none"> <li>• Aerobic activity should be performed in bouts of at least 10 minutes duration.</li> </ul>                                                                                                                                                                                    | <ul style="list-style-type: none"> <li>• Aerobic activity should be performed in bouts of at least 10 minutes duration</li> </ul>                                                                                                                                                                                     |

**Weight** = measured in kg with weight scale, with individual on clothing but shoe's removed.(41)

**Height**- measured with non-elastic tape meter with occiput, buttock and heel in same vertical level from standing on levelled floor, with individual on clothing but shoe's removed.(41)

Body mass index (BMI)- was calculated from measured height in meter squared and weight in kilogram, then categorized as underweight, normal, overweight, Class I obese, Class II Obese and class III Obese when they have calculated value in  $\text{kg/m}^2$  of <18.5, 18.5-24.9, 25-29.9, 30-34.9, 35-39.9 and  $\geq 40$  respectively.(42)

$$\text{BMI (Kg/m}^2\text{)} = \text{weight in kilograms/ height in meter squared}$$

**Equation 5-3 Formula to calculate BMI, in  $\text{kg/m}^2$ .**

Body surface area (BSA)-was calculated using simplified Mosteller, R.D. (1987) Simplified Calculation of Body-Surface Area.(43)

$$\text{BSA (m}^2\text{)} = \sqrt{[\text{height (cm)} * \text{weight (kg)}] / 3600}$$

**Equation 5-4 Mosteller simplified formula for body surface area calculation.**

**Waist circumference**- measured with non-elastic tape meter mid-way from anterior iliac spine and lowest lateral ribs standing in flat floor, with individual on clothing removed.(44) The measured waist circumference was categorized as normal if <94cm and <80cm for male and female sex respectively.(45–47)

**Blood pressure**-Blood pressure was measure according to the 2020 international society of Hypertension practice guide line, quiet room with comfortable temperature; no smoking, coffee, exercise for 30 minutes; empty bladder; relaxed for 3-5 minutes; 3 times in 1 minutes; average of last 2 were taken as final result; in both arms; after 3 minutes of standing from sitting position; using Arm-Type Fully Automatic Digital Blood Pressure Monitor (Model: DBP- 1359).(48)

**Metabolic syndrome** – defined according to IDF, Table 6.2.(46)

**Table 5.2***The new International Diabetes Federation (IDF) definition.*

| Central obesity(defined as waist circumference* with ethnicity specific values) |                                                                                                                                                                                                 |
|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Plus any two of the following four factors:                                     |                                                                                                                                                                                                 |
| Raised triglycerides                                                            | ≥150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality                                                                                                                        |
| Reduced HDL cholesterol                                                         | < 40 mg/dL (1.03 mmol/L) in males; < 50 mg/dL (1.29 mmol/L) in females<br>or specific treatment for this lipid abnormality                                                                      |
| Raised blood pressure                                                           | SBP ≥130 or DBP ≥85 mm Hg or treatment of previously diagnosed hypertension                                                                                                                     |
| Raised fasting plasma glucose (FPG)                                             | ≥100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes<br><br>If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome. |

\* If BMI is >30kg/m<sup>2</sup>, central obesity can be assumed and waist circumference does not need to be measured.

**Smoking=**

- Current smoker= those who are actively smoking & were smokers in the past 12 months.
- Past smoker- those who quit smoking for more than 12 months.
- Passive smokers- those who are exposed to cigarettes smoker for more than 4 hours per day, living with active cigarette smokers.
- Never= those who never smoked and smoked less than 100 cigarette in their life time.(49)

**Alcohol use-** any use of alcoholic beverages irrespective of duration, amount and type.

**Illicit drug use** = any use of illicit drug, Khat, weed, cocaine.

**Khat-** an evergreen shrub cultivated as a bush or small tree native to Ethiopia, East Africa, and the southern Arabian Peninsula.(50)

**Lipid profile=** included in the study were non-fasting; total cholesterol, triglyceride, HDL & LDL, all were done with direct immunoassay methods, no use of Friedewald calculation.(51)

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL-C} + \text{Triglyceride}/5)$$

**Equation 5-5. Friedewald calculation for LDL-C using TC, HDL-C & TG.**

Triglyceride classification –. (52) Table 6.3.

**Table 5.3** *Criteria proposed for clinical diagnosis of elevated triglyceride levels under fasting conditions.*

| NCEP ATP III                  |               | The Endocrine society 2010 <sup>a</sup> |                 |
|-------------------------------|---------------|-----------------------------------------|-----------------|
| Normal                        | <150 mg/dl    | Normal                                  | <150 mg/dl      |
| Borderline-high Triglycerides | 150-199 mg/dl | Mild hypertriglyceridemia               | 150-199 mg/dl   |
| High Triglycerides            | 200-499 mg/dl | Moderate hypertriglyceridemia           | 200-999 mg/dl   |
| Very high Triglycerides       | ≥500 mg/dl    | Severe hypertriglyceridemia             | 1000-1999 mg/dl |
|                               |               | Very Severe hypertriglyceridemia        | ≥2000 mg/dl     |

<sup>a</sup> The criteria developed for the present guidelines focus on the ability to assess risk for premature CVD vs. risk for pancreatitis.

## 5.7 Data collection instrument process and analysis

After training for 2 days on research objectives and tools were provided , data's were collected using structured questionnaire by 12 trained nurses for sociodemographic, CV risk factors and clinical variables and appropriate laboratory investigation for hs-cTn, lipid profile, renal function HbA1c were done after collecting 10ml venous blood at spot by the nurse, then transferred to EDTA and SSG blood collection tube 5 mm in each tube and analyzed by team members ( 37) of at ALERT specialized comprehensive hospital and Black lion specialized hospital main laboratory unit using ROCH chemistry machine and immunoassay machine.

Echocardiography was done by 1 cardiologist and 1 cardiology fellow.

The principal investigator was supervising the data collection and providing supportive supervision.

Each day, the principal investigator checked the completeness and consistency of data collected by each data collector and the data were compiled.

Then, the collected data was organized, coded, entered in computer and analyzed using SPSS version 20.0 and descriptive and analytic statistics were done based on appropriate tests and presented with tables and graphs. A p-value of less than 0.05 considered as significant. Odds ratio (OR) and 95% CI employed as measure of the strength of association.

### **5.8 Data quality assurance**

Pretest on 5 % of sample was done to assess the appropriateness of questioners to answer for the research objectives and applicability in setting of data collection place and individuals, these were excluded from the sample.

Standard operating procedures were followed during collection of venous blood samples and laboratory analysis, bare- minimum standard recommendations according to American echocardiographers association were used in performing transthoracic echocardiography.

### **5.9 Ethical consideration**

Ethical clearance was obtained from the Saint Paul's hospital millennium medical college.

Written and informed consent was obtained from all participants.

All information obtained from the individual's interview was kept anonymous.

Coincidental findings during the study was revealed to the patient if helpful for treatment outcome by principal investigator.

### **5.10 Dissemination of research outcomes**

The research outcome will be submitted to SPHMMC, Internal medicine department, cardiology unit for completion of the subspecialty course and shall also be disseminated to appropriate bodies and presented in scientific conferences when needed

## 6 Results

A total of 210 apparently healthy individuals were included in the study; which accounted 85.4% success rate for determining cardiovascular risk prevalence; after thorough screening with complete history & physical examinations; blood pressure measurement's; eGFR; HBA<sub>1C</sub>; lipid profiles and echocardiography 120 were selected for determination of the 99<sup>th</sup> percentile value of hs-cTn level and associated factors.

### 6.1 Prevalence of cardiovascular risk factors in apparently healthy adults

The mean age participants were  $37.8 \pm 13.1$  years with female accounting for 108 (51.4). Table 8.1 depicts the sociodemographic characteristics; 152 (72.4%) were married, with only fifteen (7.1%) were unable to read. One hundred sixty-four (78.1%) were Christian by religion whereas 130 (61.9%) of participants were employed during data collection period. (Table 1)

With respect to behavioral factors (able 8-2), only twenty two (10.5%) were reported to have habit of regular exercises, 194 (92.4%) never smoked cigarette, 161 (76.7%) reported no intake of alcohol and with only 11 (5.2%) currently taking illicit drugs all taking Khat on daily bases. One hundred sixty-seven (79.5%) of participants adds table salt in their diet daily. (Table 2)

The mean height, weight and waist circumference were  $164.4 \pm 9.6$  cm,  $63.8 \pm 12.2$  kg, and  $84.3 \pm 12.4$  cm respectively, the SBP and DBP mean were  $123.7 \pm 17.8$  mmHg and  $76.5 \pm 11.5$  mmHg respectively, based on their BP measurement 81% were in optimal, normal and high normal category's. Twenty individual exhibited orthostatic hypotension.

The prevalence of obesity based on BMI, central adiposity based on waist circumference and metabolic syndrome were 8.6%, 38.6% and 26.7% respectively.

**Table 6.1***Socio-Demographic Characteristics of apparently healthy adults, Addis Ababa, Ethiopia, June 2022. (n=210).*

| Sociodemographic characteristic's |                | Frequency | percentage |
|-----------------------------------|----------------|-----------|------------|
| Age categorized in decades        | 15-24          | 33        | 15.7       |
|                                   | 25-34          | 57        | 27.1       |
|                                   | 35-44          | 61        | 29.0       |
|                                   | 45-54          | 35        | 16.7       |
|                                   | 55-64          | 16        | 7.6        |
|                                   | ≥65            | 8         | 3.8        |
| Sex                               | Male           | 102       | 48.6       |
|                                   | Female         | 108       | 51.4       |
| Marital status                    | Single         | 50        | 23.8       |
|                                   | Married        | 152       | 72.4       |
|                                   | Divorced       | 7         | 3.3        |
|                                   | Widow          | 1         | .5         |
| Educational status                | Illiterate     | 15        | 7.1        |
|                                   | Read and write | 19        | 9.0        |
|                                   | Primary        | 53        | 25.2       |
|                                   | Secondary      | 62        | 29.5       |
|                                   | Tertiary       | 61        | 29.0       |
| Occupational status               | Unemployed     | 80        | 38.1       |
|                                   | Employed       | 130       | 61.9       |
| Religion                          | Christian      | 164       | 78.1       |
|                                   | Muslim         | 46        | 21.9       |

**Table 6.2. Behavioral Characteristics of apparently healthy adults Addis Ababa, Ethiopia, July 2022.**

| Behavioral Characteristic's |                | Frequency | Percentage |
|-----------------------------|----------------|-----------|------------|
| Exercise habit              | Regular        | 22        | 10.5       |
|                             | Occasional     | 32        | 15.2       |
|                             | NO             | 156       | 74.3       |
| Smoking habit               | Current smoker | 4         | 1.9        |
|                             | Past smoker    | 8         | 3.8        |
|                             | Passive smoker | 4         | 1.9        |
|                             | Never smoked   | 194       | 92.4       |
| Alcohol Habit               | YES            | 49        | 23.3       |
|                             | NO             | 161       | 76.7       |
| Salt intake                 | YES            | 167       | 79.5       |
|                             | Occasionally   | 35        | 16.7       |
|                             | NO             | 8         | 3.8        |
| Illicit drug use            | YES            | 11        | 5.2        |
|                             | NO             | 199       | 94.8       |

**Table 6.3 Clinical Characteristics of apparently healthy adults Addis Ababa, Ethiopia, July 2022.**

| Clinical characteristic's   |                                                | Frequency | Relative frequency % |
|-----------------------------|------------------------------------------------|-----------|----------------------|
| BP categories               | Optimal (<120 & <80mmHg)                       | 96        | 45.7                 |
|                             | Normal (120-129 &/or 80-84mmHg)                | 36        | 17.1                 |
|                             | High normal (130-139 &/or 85-89mmHg)           | 38        | 18.1                 |
|                             | Grade I HTN ( 140-159 &/or 90-99mmHg)          | 14        | 6.7                  |
|                             | Grade II HTN( 160-179 &/or 100-109mmHg)        | 5         | 2.4                  |
|                             | Grade III HTN(≥180 &/or ≥110mmHg)              | 3         | 1.4                  |
|                             | Isolated Systolic HTN (≥140 & <90mmHg)         | 18        | 8.6                  |
| Orthostatic hypotension     | Present                                        | 20        | 9.5                  |
|                             | Absent                                         | 190       | 90.5                 |
| BMI                         | Underweight (<18.5 kg/m <sup>2</sup> )         | 13        | 6.2                  |
|                             | Normal ( 18.5-24.9 kg/m <sup>2</sup> )         | 133       | 63.3                 |
|                             | Overweight ( 25-29.9 kg/m <sup>2</sup> )       | 46        | 21.9                 |
|                             | Class I obesity ( 30-34.9 kg/m <sup>2</sup> )  | 17        | 8.1                  |
|                             | Class II obesity ( 35-39.9 kg/m <sup>2</sup> ) | 1         | .5                   |
| Waist circumference (n=195) | Normal ( Male <94cm & female <80cm)            | 114       | 54.3 %               |
|                             | Abnormal                                       | 81        | 38.6%                |
| Metabolic syndrome (n=197)  | Yes                                            | 56        | 28.4                 |
|                             | No                                             | 141       | 71.6                 |

The study examined lipid profile, HBA1<sub>C</sub> and renal function to assess CV risk factors based on result 7.6% and 21.4% were in diabetic and prediabetes range, 191 individuals eGFR were  $\geq 90$  ml/min/1.73 m<sup>2</sup>. The HDL-C level were poor (<40 mg/dl for male and <50 mg/dl for women) for 38.1% of individuals, whereas 108 were having TG level <150 mg/dl.

The prevalence of dyslipidemia was assessed and the result showed that elevated TC, high LDL-C, elevated TG and low HDL-C level were 21.4%, 139%, 42.9% and 38.1% respectively. With overall prevalence of dyslipidemia was 56.2%. The non-HDL-C level was abnormal in 73 individuals.

**Table 6.4 Laboratory profile of apparently healthy adults, Addis Ababa, Ethiopia, July 2022.**

| Lipid profile characteristic's         |                                                               | Frequency | Relative frequency % |
|----------------------------------------|---------------------------------------------------------------|-----------|----------------------|
| Total cholesterol (n=204)              | Normal( <20 years <170md/dl, ≥ 20yrs 125-199mg/dl)            | 161       | 78.9                 |
|                                        | Borderline high(<20 years 170-199 mg/dl, ≥20yrs 200-239mg/dl) | 35        | 17.2                 |
|                                        | High( <20 years ≥200md/dl, ≥20yrs ≥240mg/dl)                  | 8         | 3.9                  |
| Level of HDL-C categorized (n=189)     | Poor ( male <40mg/dl, female <50mg/dl)                        | 72        | 38.1                 |
|                                        | Good ( male 40-59mg/dl, female 50-59mg/dl)                    | 82        | 43.4                 |
|                                        | Optimal (≥60mg/dl)                                            | 35        | 18.5                 |
| Categorized LDL-C level (n=201)        | Optimal (<100md/dl)                                           | 119       | 59.2                 |
|                                        | Near Optimal ( 100-129mg/dl)                                  | 54        | 26.9                 |
|                                        | Borderline high ( 130-159mg/dl)                               | 22        | 10.9                 |
|                                        | High (160-189 mg/dl)                                          | 5         | 2.5                  |
|                                        | Very high (≥190mg/dl)                                         | 1         | .5                   |
| Categorized triglyceride level (n=189) | NORMAL ( <150MG/DL)                                           | 108       | 57.1                 |
|                                        | BORDERLINE HIGH (150-199MG/DL)                                | 30        | 15.9                 |
|                                        | HIGH (200-499 MG/DL)                                          | 46        | 24.3                 |
|                                        | VERY HIGH (≥500MG/DL)                                         | 5         | 2.6                  |
| Categorized Non-HDL-C (n=189)          | Normal (≤19years <120mg/dl; ≥20years <130)                    | 115       | 60.8                 |
|                                        | Borderline (≤19years 120-144mg/dl)                            | 1         | .5                   |
|                                        | High ( ≤19year≥145mg/l;≥20years>130mg/dl)                     | 73        | 38.6                 |
| HbA1c based categories                 | Normal (HbA1c<5.7 %)                                          | 149       | 71.0                 |
|                                        | Prediabetes (HbA1c 5.7-6.4%)                                  | 45        | 21.4                 |
|                                        | Diabetes mellitus (HbA1c≥ 6.5%)                               | 16        | 7.6                  |
| eGFR ( n=203)                          | G1 Normal or high ( ≥90ml/min/1.73m2)                         | 191       | 94.1                 |
|                                        | G2 Mildly decreased (60-89ml/min/1.73m2)                      | 9         | 4.4                  |
|                                        | G3a Mildly to Moderately decreased (45-59ml/min/1.73m2)       | 1         | .5                   |
|                                        | G5 Kidney failure (<15ml/min/1.73m2))                         | 2         | 1.0                  |

## 6.2 The 99<sup>th</sup> percentile value of hs-cTn level

Out of 210 apparently healthy adults screened for assessment of hs-cTn level only 120 fulfill the criteria for healthy adults and these were used in the determination of the 99<sup>th</sup> percentile hs-cTn value.

The mean age was  $37.3 \pm 12.5$  years, with 60 (50%) of them were females.

**Table 6.5** *Cross tabulation of sex and age in healthy adult, Addis Ababa, Ethiopia, July 2022.*

| Demographic characteristics' |       | Sex  |        | Total |
|------------------------------|-------|------|--------|-------|
|                              |       | Male | Female |       |
| Age categories' in years     | 15-24 | 9    | 9      | 18    |
|                              | 25-34 | 14   | 21     | 35    |
|                              | 35-44 | 16   | 17     | 33    |
|                              | 45-54 | 12   | 8      | 20    |
|                              | 55-64 | 7    | 4      | 11    |
|                              | ≥65   | 2    | 1      | 3     |
| Total                        |       | 60   | 60     | 120   |

The hs-cTn mean and median values were found to be  $12.72 \pm 11.55$  pg/ml, 13.5 pg/ml respectively with the 99<sup>th</sup> percentile hs-cTn value of 45.74 pg/ml in healthy adults living in Addis Ababa, Ethiopia. The 99<sup>th</sup> percentile value of male and female participants were **44.97** pg/ml and **31.93** pg/ml respectively.

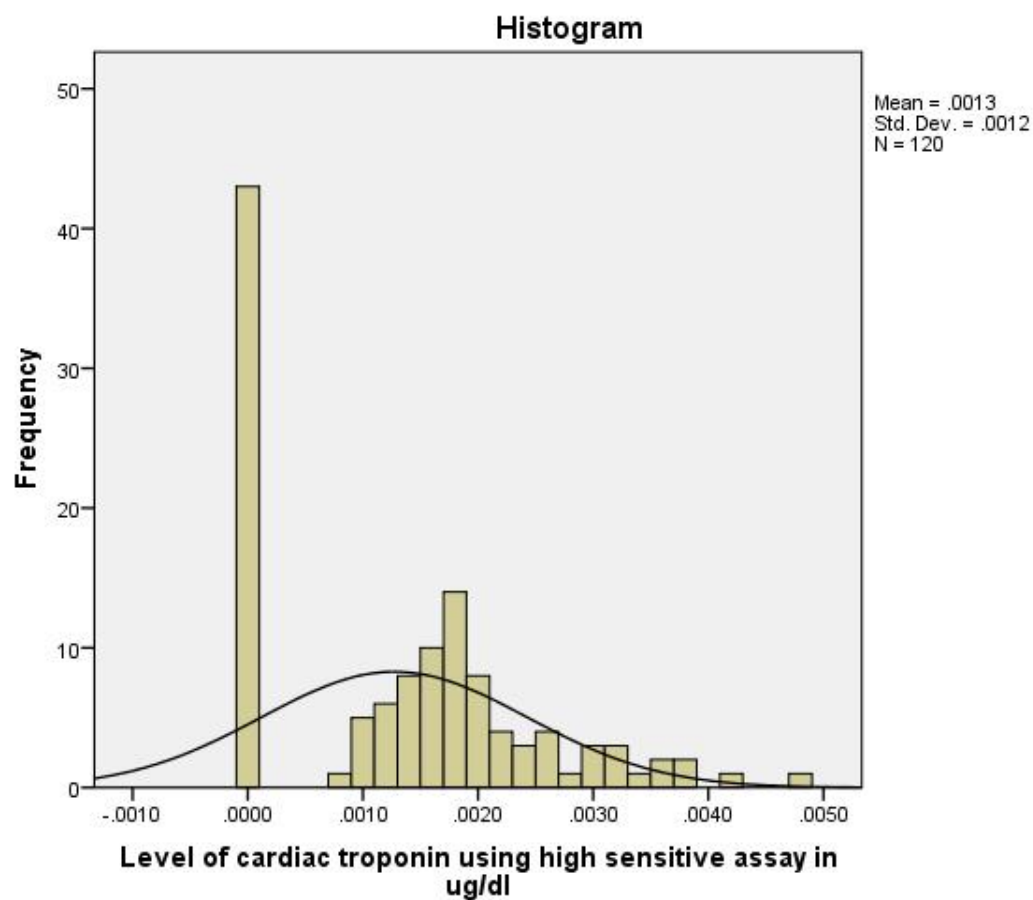


**Figure 6-1. Histogram showing the level of hs-cTn level, in healthy adult Addis Ababa, Ethiopia, July 2022.**

There was association between the mean hs-cTn value and sex with greater value in males (P=0.002) and as age increases the mean hs-cTn value increases (p=0.012). **Table 6-6**

**Table 6.6 Association of sex and age with the mean hs-cTn value in healthy adults, Addis Ababa, Ethiopia, July 2022. (n=121)**

|           |             | Frequency | Mean( pg/ml) | Median(pg/ml) | 99 <sup>th</sup> percentile | P-<br>Vale |
|-----------|-------------|-----------|--------------|---------------|-----------------------------|------------|
| Sex       | Male        | 60        | 15.90±12.495 | 17            | 44.97 pg/ml                 | .002       |
|           | Female      | 60        | 9.55±9.621   | 11            | 31.93 pg/ml                 |            |
| Age group | 15-24 years | 18        | 9.56         |               |                             | .012       |
|           | 25-34 years | 35        | 12.49        |               |                             |            |
|           | 35-44 years | 33        | 13.61        |               |                             |            |
|           | 45-54 years | 20        | 8.55         |               |                             |            |
|           | 55-64 years | 11        | 18.82        |               |                             |            |
|           | =>65 years  | 3         | 30.33        |               |                             |            |

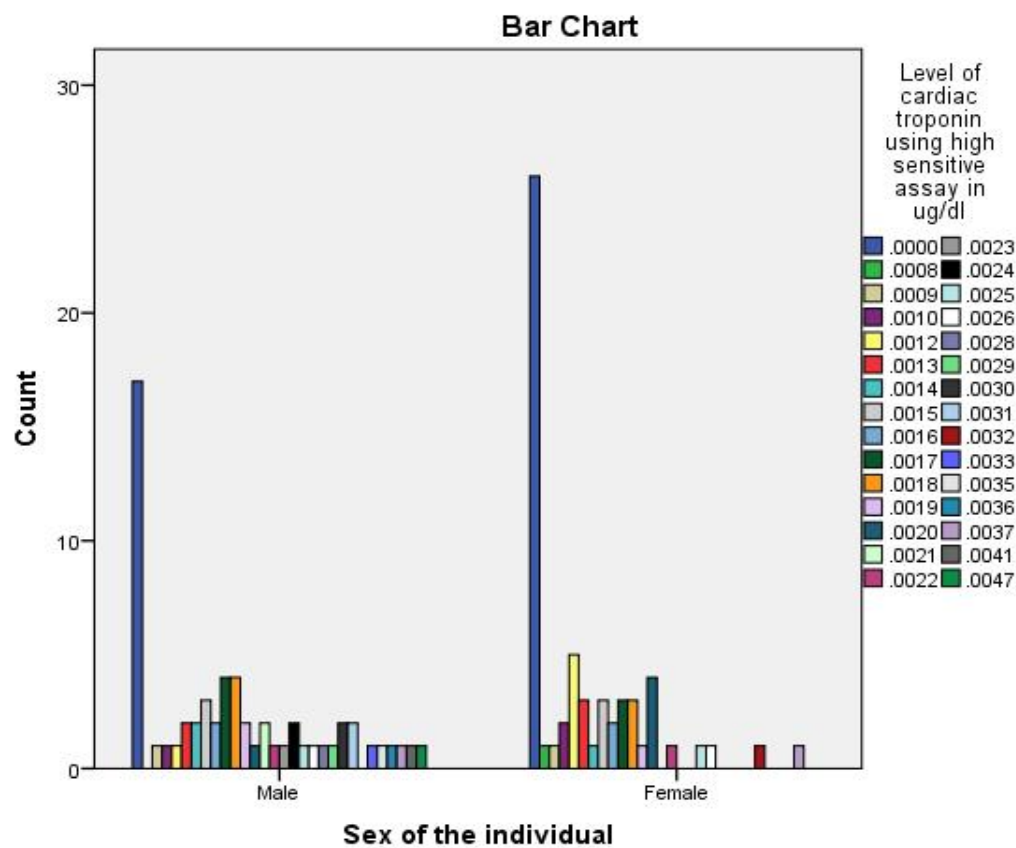


**Figure 6-2. Bar graph showing distribution of hs-cTn level with age categorized in decades, Addis Ababa, Ethiopia July 2022.**

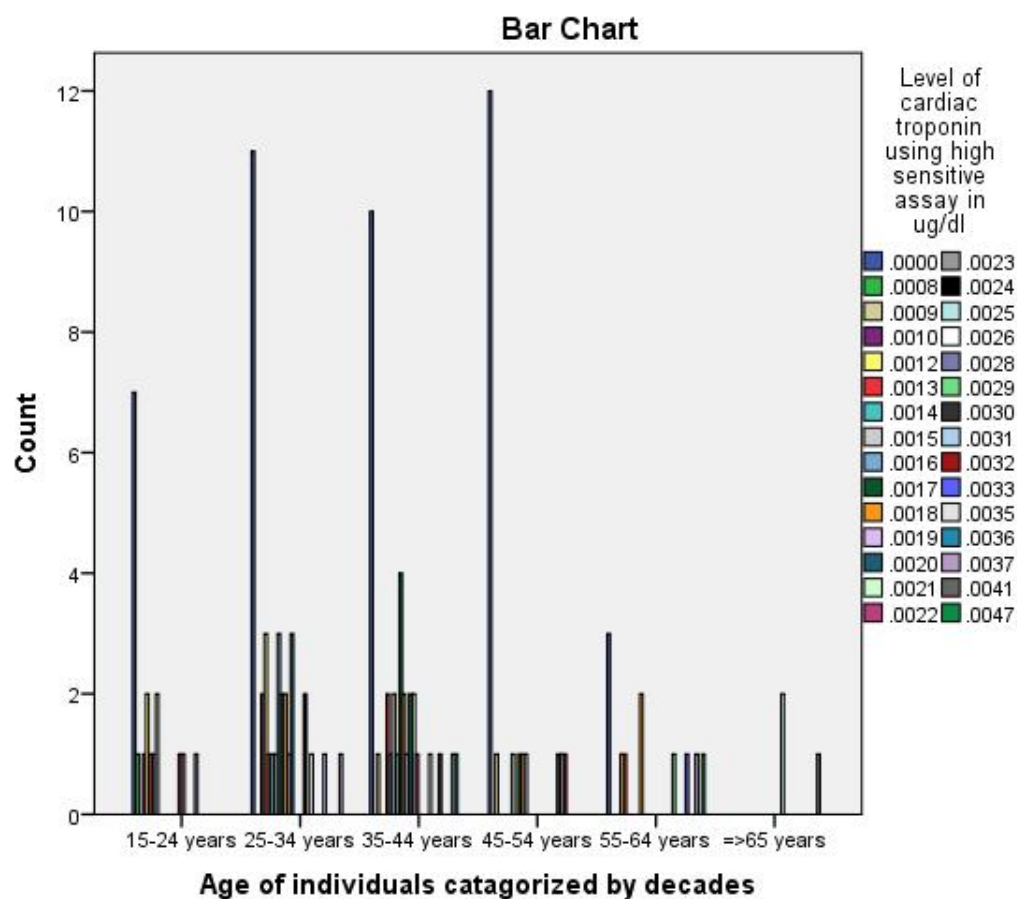


*Figure 6-3. Bar graph showing distribution of hs-cTn level with age categorized in decades, Addis Ababa, Ethiopia, July 2022.*

## 7 Discussions

This prospective study to determine the 99<sup>th</sup> percentile level of cardiac troponin using high sensitive assay in healthy adults for the developing reference in diagnosis and management of acute coronary syndrome in Ethiopian setting and sub-Saharan Africa is the first of its kind research done.

In the study selection of different age range and comparable sex distributions were maintained and the distribution of factors among the population was comprehensively evaluated. The goal of study aims in determining the 99<sup>th</sup> percentile values for cardiac troponins using high sensitivity assay that will optimize the clinical diagnosis of ACS and prognostication of ACS patients in Ethiopian settings and determination of cardiovascular risk factors in community and application of the burden of risk factors identified in mitigating the development of cardiovascular diseases particularly ischemic heart diseases with its untoward effect like death, disability, hospitalization.

In many studies conducted abroad the value of the 99<sup>th</sup> percentile was higher in males as compared to females. A study published on 2018 by Tar-Choon Aw et al. entitled High-Sensitivity cardiac Troponins in Cardio-Healthy Subjects: A Cardiovascular Magnetic Resonance Imaging Study showed that the 99<sup>th</sup> percentile value was 34.3 pg/ml and 15.6 pg/ml in men and women respectively.(27) Another study published on 2012 by Fred S. Apple et al. entitled Determination of 19 Cardiac Troponin I and T Assay 99<sup>th</sup> Percentile Values from a Common Presumably Healthy Population all high sensitivity cardiac troponin assays had 99<sup>th</sup> percentiles that were 1.2–2.4-fold higher in males than females. (31) The 99<sup>th</sup> percentile value was 29.40 pg/ml and was 19.46 pg/ml for male and women group with statistically significant ( $P<0.01$ ) difference in cardiac troponin value in Multi-Center Study of the 99<sup>th</sup> Percentile Value of High Sensitivity Troponin I in A Normal Healthy Chinese Population by Renqian Zhong et al.(24)

In our study the findings were consistent with statistically significant difference in the 99<sup>th</sup> percentile of male and female ( $p=0.002$ ) with mean of 15.9 pg/ml and 0.6 pg/ml for male and female respectively.

Nilgun Isiksacan and colleagues; concluded that the cut-off levels of hsTnT for the diagnosis of AMI were found to be significantly higher in men over 40 years of age than in men less than 40 years of age. (53) M. Odette Gore et al. The 99th percentile values for the hs-cTnT assay in DHS, ARIC, and CHS were 18, 22, and 36 ng/l (subcohort 1) and 14, 21, and 28 ng/l (subcohort 2), respectively. These differences in 99th percentile values paralleled age differences across cohorts. Analyses within sex/age strata yielded similar results between cohorts. Within each cohort, 99th percentile values increased with age and were higher in men.(54) The result in our study revealed same finding with statistically significant ( $P=0.012$ ) difference among age group categorized in decades with those  $\geq 65$  years old having higher value (mean=30.33 pg/dl) and between age group of 15-24 years old having cTn mean value of 9.56 pg/dl.

## 8 Conclusion

This study established the 99<sup>th</sup> percentile of adult healthy population in Addis Ababa, Ethiopia which is 45.74 pg/ml, 44.97 pg/ml & 31.93 pg/ml for male and female respectively ;that should be employed to improve the timely and optimal management of patient with ACS.

The evidences that are brought in the result will be used as bases in developing national cut of value of the 99<sup>th</sup> percentile cTn level in Ethiopia and in large for Sub-Saharan Africa.

## 9 Recommendations

The study recommends the use of the 99<sup>th</sup> percentile value of 45.74 pg/ml cut of value for general adult population and 44.97 pg/ml & 31.93 pg/ml for male and female respectively ; lower value of 31.93 pg/ml for women at least in Addis Ababa population for diagnosis and management of ACS.

The author also recommends continuation of such ground breaking and cross cutting research to be undertaken in collaboration with various stakeholders particularly, ministry of Health, Ethiopia, Ethiopian Medical Associations, Ethiopian cardiac professional associations, SPHMMC, ALERT center and other universities so that national data's will be available which benefits in optimizing patient's care as well encourage health professionals in developing primary data in their own settings.

The author recommends development of national guidelines for management of ACS patient using in country data as this one research can be used as milestone input.

## 10 Strength and Limitation of study

### **The strengths of this research are;**

1. Prospective nature of study design
2. Use of multiple accredited screening tools to identify healthy population from selected apparently healthy samples.
3. Use of accredited laboratory services to have reliable results.
4. Use of various specialized health professionals in collecting the data and skilled analyzer of the collected data's.
5. A paradigm shift in undertaking research to develop in country reference data.

### **The identified limitations of this research are;**

1. The primary limitation of the study was scarcity of financial support.
2. The other limitation was problems encountered in collection of data timely as consequence of COVID-19 pandemic.
3. To make inference to the general population of Ethiopia was not possible as the study was not adequately powered with sample size of minimum 600 individuals and other regions as well rural parts of Ethiopia which represent more than 85% of population were not included.

## **11 Competing and Conflicting Interests; Research funding; Employment or leadership; Honorarium**

The authors stated that they have no conflict of interest regarding the publication of this article.

The funding organizations played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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The principal investigator is employee of ALERT center and conducted the research in completion of adult cardiology fellowship program at SPHMMC.

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## 13 Consent Form and questionnaire

### Consent form for study participants

Saint Paul's hospital millennium medical college.

Questionnaire for assessing the level of cardiac troponin and associated risk factors among adult individual living in Addis Ababa, Ethiopia, February 2022 to April, 2022.

#### Consent form

##### A).INFORMATION TO THE PARTICIPANT

###### Self-introduction and consent

Greeting: - Good morning/afternoon.

My name is \_\_\_\_\_. I am a Nurse/laboratory technician. We are conducting a scientific research on the level of cardiac troponin and associated risk factors among adult individual living in Addis Ababa, Ethiopia. Therefore, I would like to inform you that you are one of the potential participants in this study. This study requires you to participate so that important information can be obtained regarding your health. Your participation is entirely based on your willingness and your refusal doesn't affect the service you get from us in any way. If you are willing to participate in the study, we will collect 20 cc of blood to determine the cardiac troponin, RFT and lipid profiles from the serum, which will not impose any risk to you.

The information gathered will be used for writing a proposal for partial fulfillment of a subspecialty certificate in adult cardiology. Your participation is only determined by you. Here, I want to assure you that any information obtained from you and your medical records will remain confidential indefinitely. The participant won't be asked any fee during the study. You can dropout any time during the study and also you have full right to ask us questions. If, at any time, you have questions about the study, you may contact the principal investigators;

1. Dr. Daniel Azmeraw (+251-932289827) or

Do you wish to participate in the study?

If the participant agrees to participate in the study, proceed with interview and Draw blood for the cardiac troponin, RFT and lipid profiles after the patient has signed the consent.

I, \_\_\_\_\_ have been told of the contents of this research form and I have adequate information about the research and understood it; and I do agree to participate in this Research study.

Name of Participant ..... Signature \_\_\_\_\_ Date \_\_\_\_\_

If the participant says "No, I don't want to participate in the study", thank him (her) and stop.

Thank you!

Name of data collector's \_\_\_\_\_ Date \_\_\_\_/\_\_\_\_/\_\_\_\_

Name of Supervisor's \_\_\_\_\_ Date \_\_\_\_/\_\_\_\_/\_\_\_\_

I. Interview code no \_\_\_\_\_

II. Sociodemographic variables

1.1 Age \_\_\_\_\_ ( Years) \_

1.2 Sex \_\_ A. Male B. Female \_\_

1.3 Marital status a. single b. Married C. divorced D. separated E. Widowed

1.4 Educational status A. Illiterate B. read and write C. Primary D. secondary E. tertiary

1.5 Occupational status A. Unemployed C. employed

1.6 Annual income per birr \_\_\_\_\_

1.7 Religion A. Christian B. Muslim C. Others \_\_\_\_\_

III. Associated risk factors

3.1 Exercise A. regularly B. Occasionally C. No

3.2 Weight \_\_\_\_\_ ( Kg)

3.3 Height \_\_\_\_\_ ( cm)

3.4 Waist circumference \_\_\_\_\_ -( cm)

3.5 BP sitting right hand A. \_\_\_\_\_, B.. \_\_\_\_\_

3.6 BP sitting left hand A. \_\_\_\_\_, B.. \_\_\_\_\_

3.7 BP standing A. \_\_\_\_\_, B. \_\_\_\_\_

3.8 Smoking A. Current smoker \_\_\_\_ ( Pack/year) B. Past smoker \_\_\_\_ (months since last smoke) C. Passive smoker D. Never

3.9 Alcohol use A. Yes \_\_\_\_\_ (ethanol mg/day) B. NO

3.10 Salt intake A. Yes B. Occasionally C. No

3.11 Staple diet; \_\_\_\_\_

3.12 Any cardiac disease A. Yes B. No C. Never checked

- 3.13 If yes specify Diagnosis-----
- 3.14
- 3.15 Hypertension A. Yes B. No C. Never checked
- 3.16 Diabetes mellitus A. Yes B. No C. Never checked
- 3.17 Any kidney disease A. Yes B. No C. Never checked
- 3.18 Family history of cardiac disease A. Yes B. No C. Never checked
- 3.19 If yes specify relation\_\_\_\_Diagnosis-----
- 3.20 Does the individual have illicit drug use? A. Yes\_\_\_\_\_B. No
- 3.21 If yes, specify \_\_\_\_\_
- 3.22 Any other Known diagnosed problems? A. Yes \_\_\_\_\_ B. No

IV. ECHO results\_\_\_\_\_

V. Laboratory tests

5.1 Cardiac troponin\_\_\_\_\_

5.2 Total cholesterol\_\_\_\_\_

5.3 Triglycerides\_\_\_\_\_

5.4 HDL\_\_\_\_\_

5.5 LDL\_\_\_\_\_

5.6 Creatinine \_\_\_\_\_

5.7 Urea\_\_\_\_\_

5.8 HbA1C\_\_\_\_\_