ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF NURSING AND MIDWIFERY

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of Serum Gamma Glutamyl Transferase Enzyme Activities among Patients with Cardiovascular Diseases Attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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A Research Thesis Submitted to the Department of Medical Laboratory Sciences, School of Nursing and Midwifery, College of Health Sciences, Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Clinical Laboratory Sciences (Clinical Chemistry)

November, 2018

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

School of Nursing and Midwifery

Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Kiyar Jemal entitled "**Assessment of Serum Gamma Glutamyl Transferase Enzyme Activities among Patients with Cardiovascular Diseases Attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**" submitted in partial fulfillment of the requirements of the Degree of Masters of Sciences in Clinical Laboratory Sciences (Clinical chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Background: Gamma-Glutamyl Transferase (GGT) is an enzyme located on the external surface of membranes of various cells. The most important physiological function of GGT is for the extracellular catabolism of glutathione. These catabolism produces the reactive thiol cysteinyl-glycine moiety which causes the reduction of Fe^{3+} to Fe^{2+}, thus starting an Fe dependent redox-cycling process resulting in the production of the reactive oxygen species particularly O_2* and H_2O_2, both capable of stimulating pro-oxidant reactions which cause atherosclerosis. Cardiovascular diseases are a group of disorders that include diseases of the heart and blood vessels or both.

Objective: To assess serum gamma glutamyl transferase enzyme activities among patients with cardiovascular diseases attending Tikur Anbessa specialized hospital, Addis Ababa Ethiopia.

Methodology: A comparative cross-sectional study was conducted on 103 participants from Feb. –May 2018 at TASH cardiology unit. The sociodemographic data were collected using well-structured questionnaire. Other additional information, records, and history was taken from participant medical records on working checklist. Venous blood specimens were collected from the participant according to standard guidelines and used to measure serum level of GGT using spectrophotometric techniques. Descriptive analysis, correlation, and chi square, independent sample T-test, one way ANOVA analysis were used for this study. All continuous data's were expressed in mean ± SD.

Results: Among the total study participants 50.5% were male and within age range of 20-78 years. The mean GGT level among CVDs patients (36.2 ± 24.34 U/L) was found statistically and significantly higher than control subjects (26.5 ± 12.9 U/L) with P value of 0.013. GGT level had statistically significant strong and positive correlation with FBS ($r=0.206$ $p = 0.037$) and TG ($r=0.351$ $p<0.001$). GGT was further positively correlated with TC ($r = 0.017$), LDL-C ($r = 0.121$), DBP ($r = 0.09$), SBP ($r = 0.16$). Reversely, serum GGT was negatively correlated ($r=-0.057$) to HDL-C level.

Conclusion: Higher level of serum GGT in the CVDs patients was noted. Participants with atherosclerotic risk factors such as diabetes, hypertension and dyslipidemia had significant higher serum GGT level compared to those without these factors.

Acknowledgment

My greatest and foremost indebtedness goes to the Almighty Allah who gives me the strength throughout my life.

I would like to acknowledge department of medical laboratory, College of health sciences, Addis Ababa University for giving me a golden opportunity to conduct such an educating research. This special thanks also concerns Arbaminch University which sponsored me to continue my MSc, Ethiopian Public health institute which provided me huge laboratory support, Tikur Anbessa Specialized Hospital and International Clinical Laboratory for their hospitality, warm cooperativeness and support for data collection.

Then my heartfelt gratitude goes to my advisors Mr. Samuel Kinde (Biochemist in department of medical laboratory), Gobena Dedefo (Biochemist in Department of medical laboratory), and Dr. Senbeta Guteta (Cardiologist in Tikur Anbessa Specialized Hospital) for their continuous constructive comments, mentoring, suggestion and guide. The contribution of Mrs Genet Ashebir, staff of clinical chemistry laboratory of EPHI, is also great for this job accomplishment. The contribution of my study participants is also really acknowledged for their kind participation

Lastly my special thanks goes to my colleagues and friends who are always beside of me in my whole project procedures, especially TASH cardiac unit nurses Sr. Lidiya and Sr. Alem, laboratory staff.

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List of Abbreviations

AHF	Acute Heart Failure
BMI	Body Mass Index
CAD	Coronary Artery Disease
CHF	Congestive Heart Failure
CK-MB	Creatinine Kinase- MB
CRP	C reactive protein
CVD	Cardiovascular Disease
EC	Enzyme Committee
FBS	Fasting Blood Sugar
GGT	Gamma Glutamyl Transferase
GSH	Reduced Glutathione
HDL	High Density Lipoprotein
IHD	Ischemic Heart Disease
kDa	Kilo-Dalton
LDL	Low Density Lipoprotein
NSTEMI	Non-ST segment Elevation Myocardial Infarction
STEMI	ST-segment Elevation Myocardial Infarction
TC	Total Cholesterol
TG	Triglyceride
UA	Unstable Angina

1. Introduction

1.1.Background

1.1.1. Gamma Glutamyl Transferase

Gamma-glutamyl transferase (GGT; EC2.3.2.2.) is an enzyme located on the external surface membranes of various cells. Mammalian GGT is a dimeric glycoprotein with a molecular weight of 68 kDa consisting of 2 subunits: a 46 kDa large subunit and a 22 kDa small subunit. However, depending on the degree of glycosylation, the molecular weight has been reported to vary between 38 to 72 kDa for the large and 20 to 66 kDa for the small GGT subunit (1) .

GGT protein produced as a single polypeptide with heavy (large) and light (small) chain. The heavy chain has an intracellular N-terminal sequence, a transmembrane hydrophobic domain, and an extracellular domain and is responsible to secure light chain to the cell membrane and modify its catalytic activity. The light chain harbors the enzyme active center and by its own has protease activity to digest heavy chain (2).

There are more than seven genes that express for GGT in human; nevertheless only one (which is found on chromosome number 22q11) gives rise to complete and fully functional protein. The rest are the pseudogenes. GGT does not have isoform in terms of amino acid sequence, but there is considerable variety of isoform in terms of glycosylation (2, 3).

GGT is found on all cells except erythrocyte, but its activity is reported high in tissues with secretory and absorptive function such as liver, kidney (proximal tubule), biliary cells and bile canaliculi, pancreatic acinar cells, seminal fluid and capillaries of brain and spinal cord (3-5).

Gel filtration chromatography based research identified that there are 4 fractions of GGT with different molecular weight: big (b-GGT), medium (m-GGT), small (s-GGT) and free (f-GGT) (6). Recent studies have suggested that b-GGT consists of membrane micro vesicles and it may serve as a precursor for smaller fractions (m-GGT and s-GGT) whereas f-GGT represents free soluble form of the enzyme (5, 6).

Serum Gamma-glutamyl transferase (GGT) also known as gamma glutamyl transpeptidase is an alternative hepatic function test which has been widely used as a diagnostic index of liver

dysfunction, alcohol consumption and abuse. The most important physiological function of GGT is for the extracellular catabolism of glutathione (GSH), the chief antioxidant in mammalian cells (7, 8).

Glutathione is a tripeptide consisting of glutamic acid, cysteine and glycine amino acid (9, 10). It is synthesized in the cytoplasm of cells and transported out of the cell to be degraded by GGT into glutamyl moiety and dipeptide cysteinyl-glycine which is further degraded by dipeptidase into free cysteine and glycine. This increases the availability of cysteine which is taken up by the cells and used as an essential precursor for the intracellular synthesis of glutathione and proteins (5, 10-13).

1.1.2. Cardiovascular Disease

Cardiovascular diseases are a group of disorders that include diseases of the heart and blood vessels or both (14, 15). There are different types of CVD. Of which Ischemic Heart Disease (IHD) or coronary artery disease (CAD) (heart attack), cerebrovascular disease (stroke), disease of aorta and arteries, congenital heart disease, rheumatic heart disease, cardiomyopathies and cardiac arrhythmias are some of the major CVD (15). Among these CVD, IHD or CAD and Congestive Heart Failure (CHF) are the most common. Acute coronary syndrome is IHD ranges from stable angina to AMI. AMI is a condition when there is an imbalance between supply and demand for oxygen in the myocardium resulting in injury to and the eventual death of myocytes. It is the most serious form of IHD (16).

IHD and cerebrovascular diseases are usually acute events and are mainly caused by atherosclerosis (14). Atherosclerosis is a complex pathological process in the walls of blood vessels that develop over many years. In atherosclerosis fatty materials and cholesterol are deposited in the inner wall lumen of medium and large sized blood vessels. These deposits cause the inner surface of blood vessels to become irregular and narrow, making it harder for blood to flow through. Eventually, the plaque will rupture and trigger the formation of blood clot. Thus if the blood clot develops in the coronary artery and brain, it can cause IHD and cerebrovascular disease respectively (14-16).

Outside the clinical use as a test for hepatobiliary disease and alcohol abuse (17), GGT has acquired large interest for its association with CVD (7, 11, 18-22), diabetes (23), metabolic syndrome (13, 24) and cancer (25).

Cardiovascular epidemiology has recently highlighted a clear link between serum GGT and risk for stroke, infarction and cardiovascular death, associated with the evolution of atherosclerosis related conditions, such as coronary artery and cerebrovascular disease (5, 26, 27). Thus, although the clear mechanism of association between serum GGT elevations and unfavorable prognosis remain unknown, serum GGT might be recognized as future promising cardiovascular prognostic marker.

1.2. Statement of problem

Globally cardiovascular diseases are the number one cause of death and more people die from CVD than any other causes. An estimated of 17.7 million people died from CVD in 2016, which represent 31% of all global death. Of these deaths an estimated of 7.4 million (46%) were due to coronary heart disease and 6.7 million (34%) were due to cerebrovascular disease (stroke) (14, 28). Over ¾ of CVD deaths are takes place in developing countries. Out of the 17.7 million premature deaths (under age of 70) due to non-communicable diseases in 2016, 82% are in developing countries, and 37% are caused by CVDs (28). People with cardiovascular disease or who are at high cardiovascular risk need early detection and management using counselling and medicines (15).

In the United States, approximately 700,000 patients every year suffer a first AMI, and another 500,000 people who had suffered an AMI in the past suffer another one. About 1.7 million patients are hospitalized with ACS each year. The yearly economic burden of CAD is in excess of $133.2 billion, more than a third of the total of $368.4 billion due to CVD overall. The National Heart, Lung, Blood Institute estimates that the current prevalence of CHF in America is 4.9 million individuals with an annual incidence of approximately 400,000 new cases each year. It is the leading cause of hospitalization in individuals 65 years and older (29).

According to the global burden of disease estimates, 68% of the 751 million years living with disability worldwide is attributable to non-communicable, and 84% of this burden arises in developing countries. Heart disease is one of the five leading contributors of years living with disability in elderly people in developing countries. Stroke is reported as leading cause of disability in developing countries second to dementia. CVD is responsible for 151,377 million disability adjusted living years, of which 41% are due to CHD and 31% are due to cerebrovascular diseases (28).

A comprehensive review by many scholars described GGT in its traditional role as a sensitive marker of liver diseases, bile duct conditions, and alcohol consumption. However newly emerging epidemiological findings extended that description to include elevated GGT in association with risk of cardiovascular diseases, inflammatory state, metabolic syndrome, and cancer (5, 7, 13, 21). A series of epidemiological studies has concluded that a moderate elevation of serum GGT activity is positively associated with cardiovascular mortality in patients

affected by coronary artery disease, congestive heart failure and hemorrhagic or ischemic stroke (22, 24, 30, 31). This indicates that GGT shows a promise as a marker for subclinical or early stage disease (7, 19).

Serum GGT actually correlates with several known cardiovascular risk factors. However, the predictive value of GGT has been repeatedly proven to be independent from confounding factors and alcohol-related liver injury (32). Several putative explanations may be offered to explain the association of elevated GGT with the risk for CVD or CVD-related mortality (2, 20).

Nowadays, use of cardiac specific serum biomarkers has become a contributory tool for diagnosis and management of patients with cardiovascular disease. Of these cardiac markers cardiac troponins (Troponin-I and Troponin-T), creatine kinase-MB (CK-MB), myoglobin are common. These cardiac markers are released into the serum during cardiac damage, but most of these markers are not detected earlier before the major cardiac event and complication occurs. Thus there is considerable on going interest and effort towards the identification of cardiac biomarkers which could be used for screening of individuals at risk of developing CVD or predict the prognosis of the disease (8).

The progress and prognosis of cardiovascular disease may be predicted by increasing GGT levels, a tool preferable to other biochemical indicators such as analysis of blood lipid levels. The prognostic significance is dose-dependent, and a stronger relationship between serum GGT and cardiovascular risk was observed (27).

There is evidence that GGT may be useful as a biochemical marker of the preclinical development of atherosclerosis. GGT was detected in atheromatous plaques in the carotid and coronary arteries, where it triggers the oxidation of low-density lipoproteins, a pathological process occurring during atherosclerotic lesion progression (26). Therefore, it is possible that circulating GGT participates in the pathogenesis of atherosclerotic CVD and its associated complications (7, 11).

1.3. Significance of the study

A major challenge in cardiovascular medicine is the identification and prediction of patients prone to acute, life-threatening events. Hence this study is intended to provide information regarding association of GGT with CVD which help mainly cardiovascular disease patients to be early detected and got treatment in advance of complication. These data will be useful also for physicians who treat patients with CVD in the cardiology department and may aid in diagnosis, treatment, and follow-up. This study again will help as a baseline data for further investigation of involvement of GGT in pathophysiology of CVD at molecular level.

2. Literature Review

2.1. Mechanism of Association of serum GGT with CVD

Several population-based studies and scientific review have investigated the association of GGT with CVD or CVD risk factors. Accordingly, although the mechanism of association between serum GGT and CVD is clearly unknown, the possible way is through catabolism of glutathione. The reactive thiol of cysteinyl-glycine moiety originated during catabolism of glutathione may cause the reduction of ferric (Fe3+) to ferrous (Fe2+) ion, thus starting an iron dependent redox-cycling process resulting in the production of the reactive oxygen species particularly superoxide anion and hydrogen peroxide, both capable of stimulating pro-oxidant reactions. GGT mediated pro-oxidant reactions catalyze the oxidation of LDL lipoproteins (lipid peroxidation), which contributes to the formation of inflammatory atheroma within the vascular endothelial wall (5, 7, 21, 26, 27, 33).

There is evidence that these reactions occur within atherosclerotic plaques and they present the most accepted putative mechanism of a direct participation of GGT in the pathophysiology of atherosclerosis leading to promotion of atherosclerotic process, plaque instability and coronary ischemic events (11, 21) (*See fig1)*

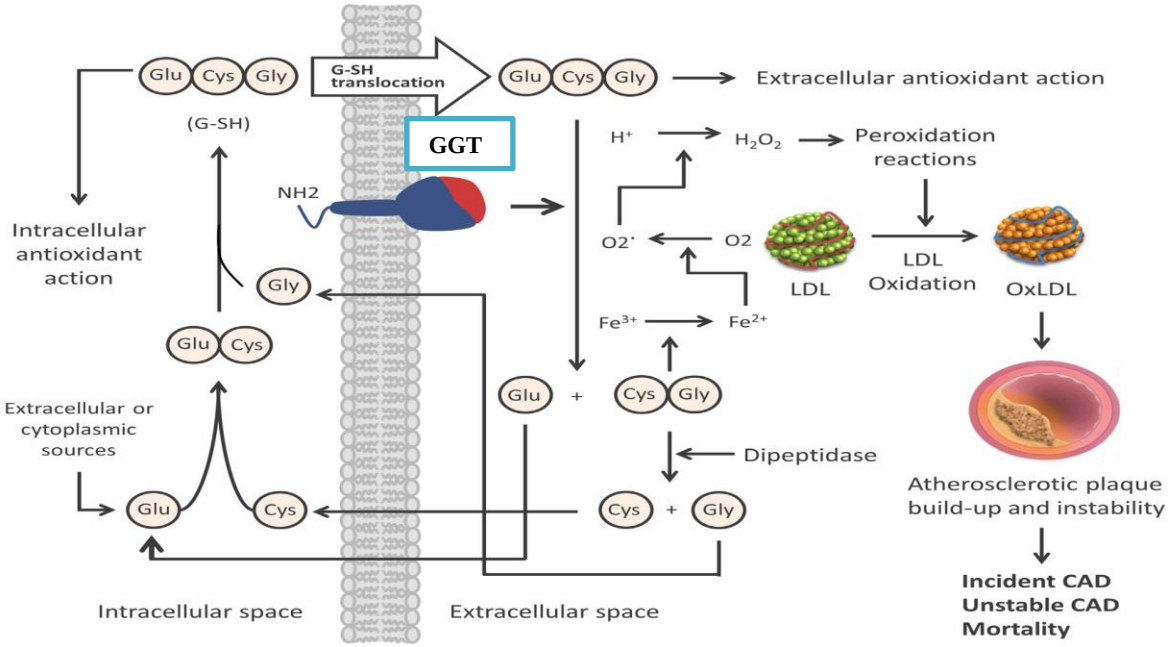


Figure 1. Gamma-glutamyl transferase (GGT) reaction and the proposed mechanism of related pro-oxidant and atherogenic activity (obtained from Ndrepepa G. et al 2016)

2.2. Association of serum GGT with different types of CVD

Ndrepepa G. et al. systematic review is one of the major scientific review assessed the association of GGT with cardiovascular risk and disease. Based on various evidences from large epidemiological studies, they concluded that, the existence of an association of elevated GGT activity with CVD, CHD, arterial hypertension, congestive heart failure, cardiac arrhythmias and CVD related mortality (5). Another systematic review done by Mason JE and his colleagues also concluded that, GGT is an independent risk marker for the development of cardiovascular disease, with an 18% per quartile increase in risk (7). Koenig along with his co-worker reviewed several large population-based studies from Austria, Finland, Korea, and United States and end up with a significant increased disease and mortality risk linked to elevated GGT levels, with statistically significant links to all types CVD (13).

A large occupational association based cohort study done by Sung K.C.*et al.* indicates that, there are similar hazards ratio for all-cause and CVD mortality for people in the highest GGT quartile, adjusting for fatty liver assessed by either ultrasound or fatty liver index (32). Increasing GGT levels were strongly associated with all-cause mortality, particularly in patients with ischemic heart disease (20). Jiang S. and his co-workers also reviewed various literatures and summarized as serum GGT has strong association with different CVD (hypertension, CAD, CHF and others CVD types) and involved in cardiovascular disease mechanisms (27). GGT is associated with all-cause mortality, coronary artery disease, and incident HF (22).

2.2.1. Association of serum GGT with CAD or ACS

Prospective case control study done in India by Kumar NS. et al. on 151 patients (100 ACS patients and 51 control subjects) showed that, the mean GGT levels of ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina (UA) subgroups are 93.86, 87.87 and 29.27 U/L respectively, which showed statistical significant difference ($p<0.001$) when compared with control subjects 21.99 U/L (8).

In similar manner, cross sectional study conducted in 2014 by Jyoti J. *et al*. in India on 323 subjects indicates, GGT activity is increased in subjects with ACS. High levels of serum GGT on admission were associated with the burden of atherosclerosis in patients with ACS. The magnitude of raised GGT among ACS was 41.80%. The magnitude of raised GGT in non ST elevated myocardial infarction (NSTEMI), ST elevated myocardial infarction (STEMI) and unstable angina (UA) was 57.32%, 37.85% and 32.81% respectively (34).

In 2013, study done on 180 coronary angiography underwent ACS patients; serum GGT levels are associated with increased burden of atherosclerosis (35). In prospective study conducted on 469 patients with angiographic documented CAD; GGT showed an independent prognostic value beyond known established risk factors in subgroup of 262 patients with previous MI (36). Similarly, Bharani V. *et al*. conducted their study on 200 coronary angiography patients and concluded that; highest GGT quartile has a significant association with severity of disease (37).

Higher serum GGT within its reference range is significantly correlated with CHD risk prediction (38). Elevation of GGT related with higher prevalence of family history of CAD (39). Demerilli S. *et al*. study also demonstrated higher serum GGT activity is significantly predictive for patients with ACS (40). A case control study done in 2014 on 60 patients with acute ischemic stroke and 44 control subjects indicates that; the mean GGT levels are significantly higher in patients compare to control subjects (12). In patients with CAD, elevated activity of circulating GGT is associated with an increased risk of all causes, cardiac and non-cardiac mortality (30).

2.2.2. Association of serum GGT with CHF

Although the mechanisms underlying this association remain largely unknown, hepatic congestion/ischemia is an obvious mechanistic explanation for the elevation of GGT in heart failure. A potential involvement of GGT in pathogenesis of atherogenesis cause CAD and MI; which are generally regarded as number one cause of CHF and metabolic syndrome is an established risk factor for CHF (31, 41).

Study conducted in Czech Republic from 2004-2012 indicates, among patients with acute heart failure 44% have abnormal/elevated GGT levels. Acute heart failure patients without ACS had significantly higher GGT levels (42). Another study done in America by Poelzl *et al.* from 2000-2007 on outdoor patients with CHF shows high prevalence of elevated GGT among patient with CHF. Prevalence of elevated GGT is 42.9% in men (GGT >65 IU/L) and 50.2% in women (GGT >38 IU/L), which is higher than healthy subjects (18.6% in men and 19.2% in women). The GGT levels are associated with disease severity (31).

In prospective study of large population based study, participant with higher serum GGT concentration within its normal range are associated with greater risk of heart failure and incrementally improved prediction of HF risk. Participant with serum GGT >median had 1.71 fold risk of HF compared to individuals with GGT < median (33). Based on study done on 200

samples (120 from patient with HF and 80 from healthy control subjects), there is a significant increase in the activity of GGT enzyme in HF patient (66.9±1.7 IU/L) in comparison with control subjects (12.07± 0.6 IU/L) (43). Liver function test abnormalities are strongly associated with HF severity and clinical manifestation (31, 41, 42).

2.3. Association of serum GGT with CVD associated risk factors

A systematic review and meta-analysis, GGT level is positively associated with development of hypertension (35, 44, 45). Population based data show a positive association of GGT and traditional cardiovascular risk factors including old age, male gender, BMI, smoking, lack of exercise, hypertension, hyper-cholesterolemia, hypertriglyceridemia, low HDL and high fasting glucose (2, 5, 14). Elevation of GGT persists over time and related to higher prevalence of obesity, dyslipidemia, hypertension, and metabolic syndrome and parental history of diabetes (39).

GGT is positively associated with triglyceride and VLDL cholesterol in coronary angiography documented patients. An increase in total cholesterol and waist circumference from GGT quartile I to quartile IV also noted. No trend is observed in LDL and HDL cholesterol across various GGT quartiles (11). There is a significant difference in the incidence of dyslipidemia, hypertension and smoking habit among ACS patient and controls subjects (8).

An epidemiological investigation done by Ruttmann E. *et al.* in a cohort of 163,944 Austrian adults observed that, the strongest age and sex adjusted correlation between GGT and triglyceride. GGT also positively correlated with uric acid, body mass index, cholesterol, blood pressure, glucose, and smoking; and negatively correlated with HDL cholesterol, physical activity, and education (22). Increased mean GGT levels are found in hypertensive, high LDL cholesterol, and triglyceride levels (12).

Strong positive correlation of GGT with body mass index, total cholesterol, and diabetes mellitus is noted in British Regional Heart Study by Wannamethee reported in October of 1995. A lesser correlation is seen in relation to blood pressure, heart rate, and cigarette smoking (18). Higher quartiles of GGT are well correlated with higher BMI, waist circumference, BP, lipid levels and glucose. Participant with higher GGT are older and had more CHD risk factors such as hypertension, dyslipidemia, more alcohol consumption and current smoker (38).

Coffee is a widely consumed beverage, which is rich source of antioxidants and other bioactive compounds. Several studies have reported negative associations between coffee consumption and GGT enzyme activity (46, 47). Among that, the large national cross-sectional health survey conducted in Finland by Danielsson J. *et al*. data on coffee, alcohol consumption and serum GGT activities indicated that, the highest GGT levels are found in heavy drinkers consuming no coffee. In the heavy drinkers reporting ≥5 cups of coffee per day, the activities are significantly lower than in the corresponding group with no coffee consumption ($P < 0.05$) for those consuming 5–6 cups, $P < 0.01$ for those consuming >6 cups) (46).

2.4.Conceptual framework

Based on the literature review serum GGT level is associated with different variables positively or negatively accordingly. Of which sociodemographic characteristics of the individuals, common CVD associated risk factors, presence or absence of other metabolic condition and confounding factors such as different medication, dietary contents.

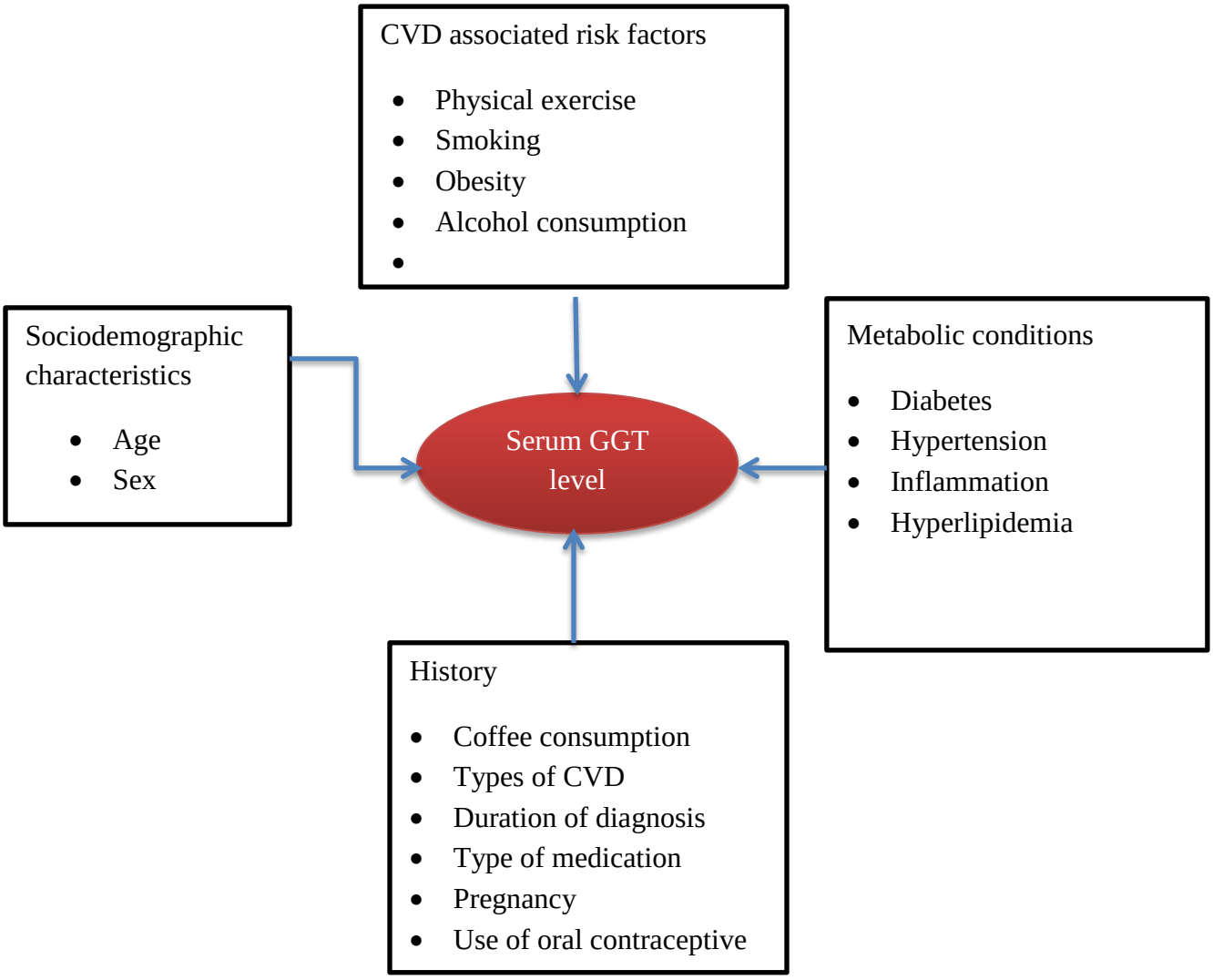


Figure2. Diagram of conceptual framework

3. Objective

3.1.General objective

- To assess serum Gamma Glutamyl Transferase enzyme activities among patients with cardiovascular diseases attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To compare the level of serum GGT among patients with cardiovascular diseases versus control subject
- To evaluate the association of serum GGT level and other cardiovascular disease risk factors (smoking, hypertension, obesity, diabetes, dyslipidemia)
- To assess the level of serum GGT among different types of CVD
- To evaluate the association of serum GGT level and cardiac markers (troponin and creatine kinase- MB) among patients with cardiovascular diseases

4. Hypothesis

H_0: There is no significant mean difference between serum levels of gamma glutamyl transferase enzyme activities of cardiovascular disease patient and control subjects.

5. Materials and methods

5.1. Study Area

Tikur Anbessa specialized hospital is located in the capital Addis Ababa, Ethiopia. It is Ethiopia's largest specialized public tertiary referral Hospital and one of University Hospitals in the country where patients from all over the country get referral service. Tikur Anbessa is a very large referral hospital and sees approximately 370,000- 400,000 patients a year. Among that the cardiology department sees around 80,000 patients a year. The hospital has 800 beds, with 169 specialists, 65 non-teaching doctors. It got eight major operating theatre rooms.

Tikur Anbessa Specialized Hospital is affiliated with the Addis Ababa University's college of health sciences. It is the training center for fellows, postgraduate, undergraduate, medical students, dentists, nurses, Radiographers and medical laboratory technologist (48).

5.2. Study design and periods

A comparative cross-sectional study was conducted from Feb. - May 2018.

5.3. Population

5.3.1. Source population

The source populations of this study were all cardiovascular diseases patients who visited TASH cardiology unit

5.3.2. Study population

The study populations of this study were all cardiovascular diseases patients who visited TASH cardiology unit during study period.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

- Individual ≥18 years old
- Capable of independent communication and give informed consent

5.4.2. Exclusion criteria

- ✓ History of hepatobiliary and kidney disease
- ✓ Previous history of viral marker infection (B&C)
- ✓ Pregnancy and lactation
- ✓ Previous diagnosis of malignancy

5.5.Study variables

5.5.1. Dependent variables

- GGT enzyme activity (IU/L)

5.5.2. Independent variables

- Age
- Sex
- Blood pressure
- Anthropometric indices
- Habit of substance abuse (Cigarette, Alcohol, Khat)
- Coffee consumption
- Strenuous Physical Exercise
- Type of CVD
- Duration of diagnosis
- Current medication follow up status
- FBS
- C-Reactive Protein
- Lipid profiles (TG, TC, LDL, HDL)
- Cardiac marker (CK-MB, Troponin-I)

5.6. Measurement and Data collection

5.6.1. Sample size and sampling techniques

Convenient sampling method was used to select 103 study participants. The required sample size was obtained from all cardiovascular diseases patients attended cardiology unit of the hospital and all customers visited international clinical laboratory for wellness checkup during the study period. Accordingly, 103 participants (50 cases and 53 control subjects) were participated in this study.

5.6.2. Data and sample collection procedures

5.6.2.1. Data collection procedures

After a brief explanation, the patient's consent was asked for their willingness and participation. Then, using well-structured questionaries' the sociodemographic data was collected through face to face interview. Blood pressure (BP) was measured using a standard mercury manometer with the participant in a sitting position for 5 min prior to measurement, where the average measurement was recorded on the checklist prepared for this purpose. In addition, other important clinical record such as type of CVD diagnosed and duration of clinical diagnosis was taken from medical record of the participant. Anthropometric measurement such as weight and height of participant was taken in order to calculate the BMI.

5.6.2.2. Blood sample collection and processing

5ml of venous blood sample was collected from the participant (after 8hr fasting) into 10ml serum separator tubes. Then, the specimen was centrifuged at 1500 rpm for 5 min and the serum was separated. Lipid profiles and FBS were analyzed from serum at TASH clinical chemistry laboratory. Then, the leftover sample was transferred to nunc tube and stored at -20 °C until GGT, CRP and cardiac markers such as troponin and CK-MB were determined at EPHI referral clinical chemistry laboratory. Laboratory methods of each parameter with respective principles are stated in annexure.

5.7. Data quality assurance

- **Questionnaires**

To maintain the quality of data obtained through face to face interview of the participants' the well-prepared questionnaires was translated into Amharic version and cross checked with English version. The questionnaire was pretested in advance of data collection period. There was a well-prepared working checklist for every parameter taken and medical record. The data was collected by experienced nurses of the respective unit of the hospital. Then, all collected data's through questionnaires and working checklists was checked for completeness, cleared and entered into computer Microsoft excel worksheet and then exported to SPSS (version 20.0) software for statistical analysis.

- **Lab parameters**

Pre-analytical: In order to maintain the quality of blood sample, venous blood was collected and processed by experienced laboratory technologist following standard operating procedures at every step. Samples was stored in appropriate refrigerator temperature (-20°C) until analysis in TASH and ICL laboratory. Samples with incomplete information were rejected. Transportation of sample to EPHI laboratory was held under appropriate temperature using ice box.

Analytical: There was quality control sample which was run daily in the morning before the actual sample running to check the performance of clinical chemistry analyzers. The correlation result done for method comparison between automations was referred from leaflet of corresponding analyte.

Post analytical: The results was printed out after checking appropriateness of all the test results with their corresponding SI unit and the data was carefully entered into Microsoft Excel worksheet and saved for statistical analysis

5.8. Data analysis and interpretation

Descriptive analysis, Spearman/Pearson correlation, and chi-square, independent sample T-test, one way ANOVA followed by post hoc analysis were used for this study. Normality distributions of continuous variables were checked using kolmogorov-Smirnov and Shapiro-Wilk tests and analyzed for homogeneity using the Levene tests. The differences between normally distributed numeric variables were evaluated by independent sample t-test or one way

ANOVA, while non-normally distributed variables were analyzed by Mann-Whitney U test or Kruskal-Wallis variance analysis, as appropriate. Chi-square test was employed for the comparison of categorical variables. All continuous data's were expressed in mean ± SD and two tailed P value ≤0.05 was considered as a statistically significant.

5.9. Ethical consideration

The data was collected after ethical clearance was obtained from Medical Laboratory Department research and ethical review committee, and department of internal medicine, College of Health Sciences, Addis Ababa University. Ethical review was also obtained from ICL research committee in order to collect data of control subjects. Furthermore, the consent of each participant was asked after a brief explanation of the objective of the study and their confidentiality was kept by using code instead of names (see annexure).

5.10. Dissemination of the result

The finding of this study will be presented and submitted to Addis Ababa University, School of nursing and midwifery, and Department of Medical Laboratory Science. It will also be presented for scientific community elsewhere and its manuscript will be submitted to peer reviewed national or international journal and it will be presented in relevant workshops, seminars and scientific conferences.

5.11. Operational definition

Acute myocardial infarction (AMI): An acute infarction of the heart muscle occurring during the period when circulation to a region of the heart is obstructed and necrosis is occurring

Congestive heart failure (CHF) : A clinical syndrome due to heart disease, characterized by breathlessness and abnormal sodium and water retention

Atherosclerosis: Any of a group of diseases characterized by thickening and loss of elasticity of arterial walls

Diabetic: individuals with fasting blood glucose level of greater than 126 mg/dl or taking anti-diabetic drugs

Hypertensive: individuals with BP of ≥150/90 or taking anti-hypertensive medication

Dyslipidemia - one or more abnormalities in serum lipids

Strenuous physical exercise: leisure-time exercise resulting in sweating and shortness of breath at least two to three times a week

Elevated GGT: individual with level of serum GGT > 50 U/L for male and >35 U/L for female

Underweight – individual with BMI of < 19 kg/m^2 for male and <18 kg/m^2 for female

Overweight - individual with BMI between 25 and 29.9 kg/m^2.for male and 24-28.9 for female

Obese: individual with BMI ≥30 kg/m^2 for male and ≥29 for female

6. Results

6.1. Socio-demographic characteristics of study participants

A total of 103 serum samples of the participants were statistically analyzed and results are presented as follows.

As summarized in (table1), the study subject's age at the time of presentation varied from 20-78 years with a mean age of 51±13 years, and majority 38 (36.9%) of study subjects belonged to 51-65 years of age. Almost comparable frequency of both gender of participants 52 (50.5%) male and 51 (49.5%) female were participated in the study.

Majority 77 (74.8%) of the participants have no habit of doing strenuous physical exercise both in cases and control subjects. Only 6 (5.8%) of the participants do exercise 2-3 days per week. Based on coffee consumption, 34 (33%) of them drink 2-5 cups of coffee daily and about 29 (28.2%) were not drink coffee completely.

Out of the whole participants none of them have history of current smoking, but 7 (6.8%) of them were former smoker and the rest 96 (93.2%) were reported as they never smoked. Majority 98 (95.1%) of the study subjects has no history of chat chewing.

Study participants were also assessed about their history of alcohol consumption and accordingly, majority 72(69.9%) reported that they were never drunk. But, 6(5.8%) case participants reported that they stopped drinking alcohol and the rest 25(24.3%) were reported that they were drinking alcohol currently.

Table 1: Socio-demographic characteristics of CVD patients attending cardiac clinic of TASH from February-May 2018 (n=103)

Variables	Categories	Cases	Controls	Total
		No (%)	No (%)	No (%)
Sex	M	26 (52)	26 (49.1)	52 (50.5)
	F	24 (48)	27 (50.9)	51 (49.5)
Age (yrs)	19-34	5 (10)	10 (18.9)	15 (14.6)
	35-50	16 (32)	19 (35.8)	35 (34)
	51-65	19 (38)	19 (35.8)	38 (36.9)
	>65	10 (20)	5 (9.4)	15 (14.6)
Physical Exercise	No	35 (70)	42 (79.2)	77 (74.8)
	Once/week	5 (10)	5 (9.4)	10 (9.7)
	2-3 days/week	4 (8)	2 (3.8)	6 (5.8)
	Everyday	6 (12)	4 (7.5)	10 (9.7)
Coffee consumption	No	13 (26)	16 (30.2)	29 (28.2)
	1 cup/day	10 (20)	17 (32.1)	27 (26.2)
	2-5 cups/day	16 (32)	18 (34)	34 (33)
	>5 cups/day	11 (22)	2 (3.8)	13 (12.6)
Habit of cigarette smoking	Never	45 (90)	51 (96.2)	96 (93.2)
	Former	5 (10)	2 (3.8)	7 (6.8)
	Current	-	-	-
Habit of chat chewing	No	46 (92)	52 (98.1)	98 (95.1)
	Yes	4 (8)	1 (1.9)	5 (4.9)
Habit of alcohol consumption	Never	26 (52)	46 (86.8)	72 (69.9)
	Former	6 (12)	-	6 (5.8)
	Current	18 (36)	7 (13.2)	25 (24.3)

6.2. Normality distribution of continuous data

All continuous variables found in this study were checked for normality distributions. Accordingly age, BMI, SBP, DBP, duration of clinical diagnosis, duration of current medication, GGT, FBS, TC, HDL-C, LDL-C, TG, hsCRP, CK-MB and Troponin were checked. Among these variables; Age, TC and LDL-C were normally distributed without being logarithmically transformed. However, BMI, DBP, HDL-C, TG, and CRP were continuous variables those approximately normally distributed after logarithmically transformed. But, SBP, duration of clinical diagnosis, duration of current medication, GGT, FBS, CK-MB and Troponin were failed to be normally distributed even after they were logarithmically transformed. Below the normality check result and histogram of normally distributed continuous data listed (Table 2). (Refer figure 3&4 for normality distribution)

Table 2: Results of normality test, skewness and kurtosis of normally distributed continuous data of study participants

No	Normality test				
	Variables	**Kolmogorov -Smirnov[a] (P value)**	**Shapiro-Wilk (P value)**	**Skewness**	**Kurtosis**
1	Age	0.200*	0.06	-0.322	-0.546
2	TC	0.200*	0.116	0.493	0.911
3	LDL-C	0.200*	0.53	0.121	-0.106
4	BMI	0.022	0.004	0.711	0.955
5	Log (BMI)	0.091	0.363	0.077	0.259
6	DBP	0.002	0.012	0.120	0.187
7	Log (DBP)	0.091	0.363	0.077	0.259
8	HDL	0.003	<0.001	1.303	3.318
9	Log (HDL)	0.200*	0.331	0.243	-0.195
10	TG	0.001	<0.001	1.482	3.32
11	Log (TG)	0.200*	0.930	0.121	-0.091
12	hsCRP	<0.001	<0.001	9.667	96.182
13	Log (hsCRP)	0.200*	0.186	0.112	0.129

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

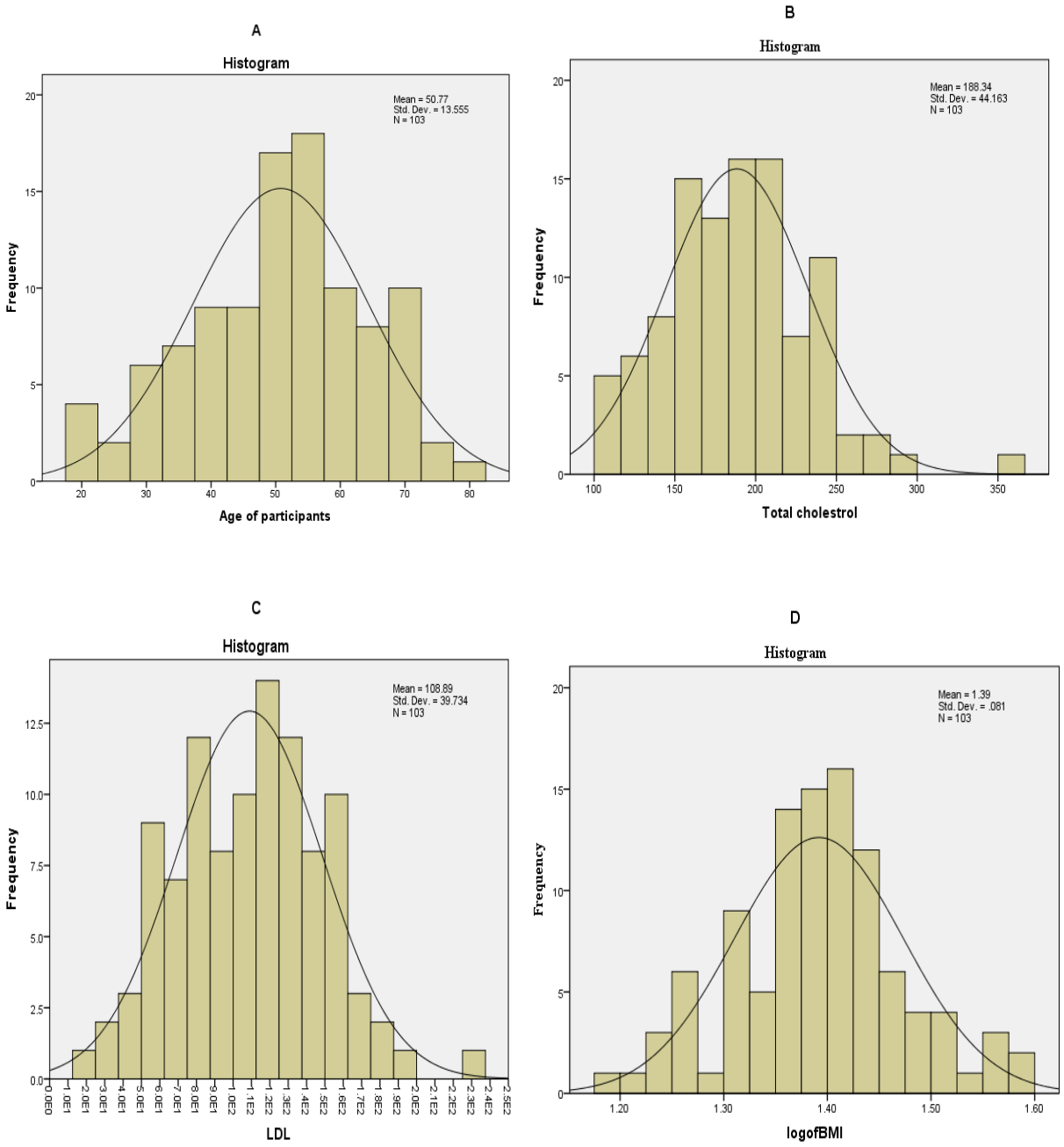


Figure 3: Histograms of approximately normal distribution of Age (A), TC (B), LDL-C(C) and logarithmically transformed BMI (D)

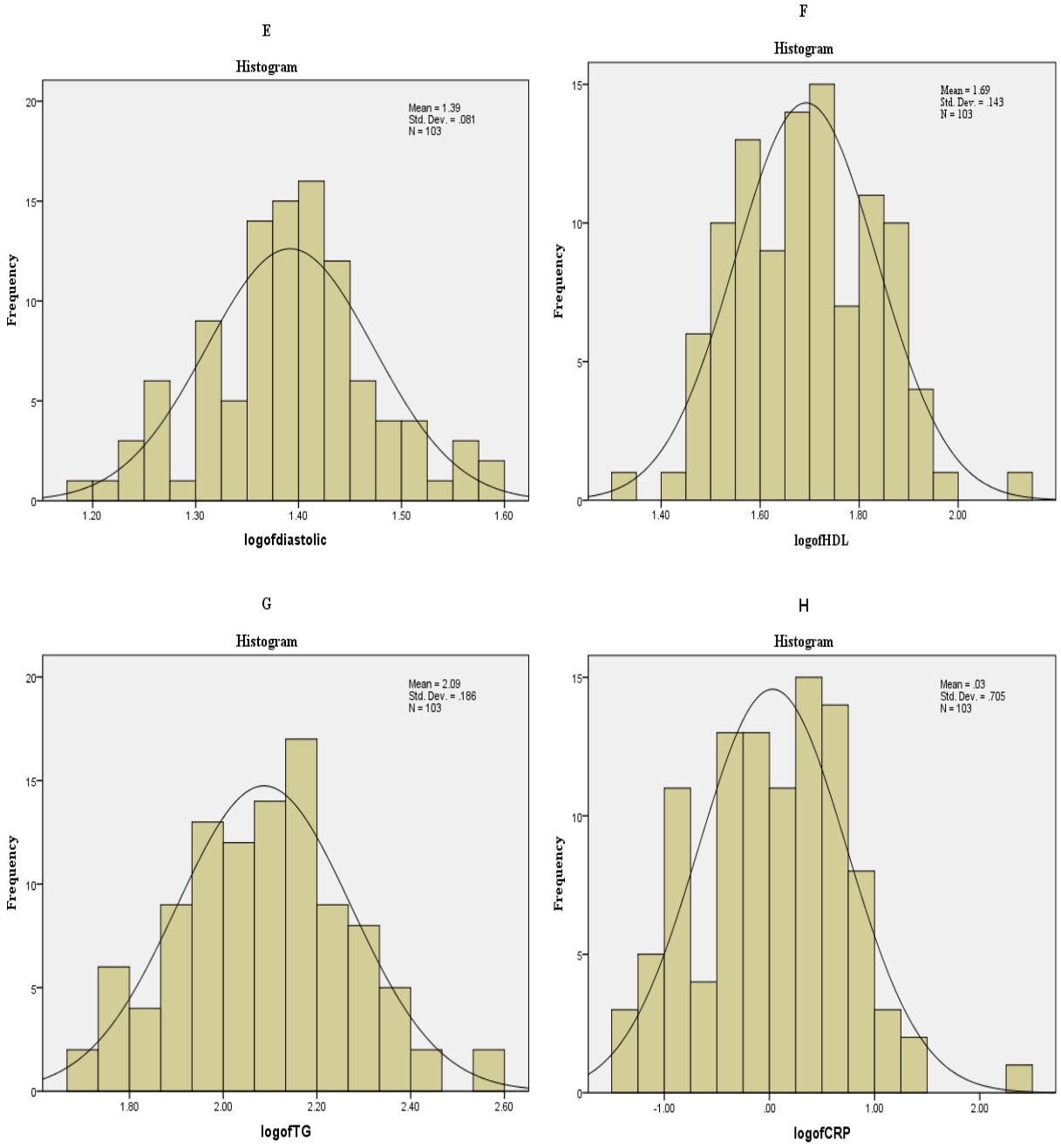


Figure 4: Histograms of approximately normal distribution of logarithmically transformed DBP (E), logarithmically transformed HDL-C (F), logarithmically transformed TG (G) and logarithmically transformed CRP (H)

6.3.Clinical and biochemical characteristics of study participants

Normal BMI was found in majority 41 (39.8) of the participants. The minimum and maximum BMI were 15 and 39.7 respectively with the mean of 25 Kg/m^2 and standard deviation of 4.8 Kg/m^2. The overweight and obese range of BMI constitutes 39 (37.9%) and 14 (13.6%) respectively. Only 9 (8.7%) of participants were underweight.

Systolic and diastolic blood pressure of study participants were taken to assess whether participants were hypertensive or not and further whether it is associated with elevation of GGT. Accordingly it was taken two times and the average was used for analysis. The first was taken by physician during investigation and the second by experienced nurses (data collector) just for research purpose. This is done intentionally due to the study participants were cardiac patients which includes hypertensive ones. Based on this, the mean ± SD of DBP was 81.74 ±10.3mmHg, which is approximately in the range of normal BP. The minimum and maximum value was 60 and 115 mmHg respectively. Though the majority 63 (61.2%) of the participants had DBP of less than 85 mmHg as a general, more than half 29 (58%) of the case subjects had DBP greater than 85mmHg.

Majority 79 (76.7%) of the participants had systolic blood pressure (SBP) of less than 130 mmHg with the median of 122. The minimum and maximum range of SBP of the study participants were 60 and 200 mmHg respectively. About 85% of the control subjects had SBP of less than 130 mmHg, whereas the case participants were about 68%. This is one of the indication of hypertension is common in cardiac cases than any others.

Based on GGT categories, study participants with value less than 40 IU/L constitutes about 76.7%. The median was 25 IU/L with minimum and maximum values of 10 and 99 IU/L respectively. Even though the value of median was within the normal range for both male and female, about 36% of the cardiac patients participated in this study had GGT value greater than 40 IU/L.

Below the table indicates the baseline of clinical and biochemical data with their mean and standard deviation (SD) for normally distributed. A median was reported for data with a skewed distribution.

Table 3: Baseline Clinical and biochemical parameters of CVDs patients attending TASH from February to May, 2018 (N=103)

Variables	Categories	Cases	Controls	Total	Mean ±SD	Range	Median
		No (%)	No (%)	No (%)			
BMI (Kg/m^2)	Underweight (<18.5)	5 (10)	4 (7.5)	9 (8.7)	25±4.8	15-39.7	-
	Normal (18.5-24.5)	27 (54)	14 (26.4)	41 (39.8)			
	Overweight (24.6-29.9)	12 (24)	27 (50.9)	39 (37.9)			
	Obese (≥30)	6 (12)	8 (15.1)	14 (13.6)			
DBP (mmHg)	≤85	21 (42)	42 (79.2)	63 (61.2)	81.74 ±10.3	60-115	-
	>85	29 (58)	11 (20.8)	40 (38.8)			
SBP (mmHg)	≤130	34 (68)	45 (84.9)	79 (76.7)	-	60-200	122
	>130	16 (32)	8 (15.1)	24 (23.3)			
GGT (IU/L)	≤40	32 (64)	47 (88.7)	79 (76.7)		10-99	25
	>40	18 (36)	6 (11.3)	24 (23.3)			
FBS (mg/dl)	≤110	36 (72)	42 (79.2)	78 (75.7)		34-265	97
	>110	14 (28)	11 (20.8)	25 (24.3)			
TC (mg/dl)	≤200	34 (68)	29 (54.7)	63 (61.2)	188.34 ± 44.12	104-353	
	>200	16 (32)	24 (45.3)	40 (38.8)			
HDL-C (mg/dl)	≤40	3 (6)	30 (56.6)	33 (32)	52 ± 18	22-137	
	>40	47 (94)	23 (43.4)	70 (68)			
LDL-C (mg/dl)	≤ 130	41 (82)	29 (54.7)	70 (68)	108.9 ± 39.7	14-232	
	>130	9 (18)	24 (45.3)	33 (32)			
TG (mg/dl)	≤150	34 (68)	40 (75.5)	74 (71.4)	134 ± 61.2	47-389	
	>150	16 (32)	13 (24.5)	29 (28.2)			
hsCRP (mg/L)	≤3.0	23 (46)	51 (96.2)	74 (71.8)	4.98 ± 23.6	0.04-238	
	>3.0	27 (54)	2 (3.8)	29 (28.2)			
CK-MB (U/L)	≤24	42 (84)	-	42 (84)		0.7-41	11.5
	>24	8 (16)	-	8 (16)			
Troponin (ng/L)	≤14	38 (76)	-	38 (76)		3-405.3	4.5
	>14	12 (24)	-	12 (24)			

Fasting blood glucose of the study participants were also determined to assess their diabetic status and whether diabetes mellitus is a risk factor for elevation of GGT in cardiovascular disease. During data collection period some of the case participants had comorbidity of cardiac and diabetic disease and taking anti diabetic drug follow up which affect the result. However, majority 78 (75%) of the participants had FBS results of less than 110 mg/dl with median of 97 mg/dl. The minimum and maximum values were 34 and 265 mg/dl respectively.

Lipid profiles of study participants were also determined to assess dyslipidemia which is the common risk factors for CVD and GGT elevation. Based on this, the mean TC of participants was found 188 mg/dl with SD of 44 mg/dl. About 63 (61.2%) of the participants had TC value less than 200mg/dl with minimum and maximum value of 104 and 353 mg/dl respectively.

HDL-C is also one of the lipid profiles that determined in this study. The mean value of HDL-C of study participants was 52 mg/dl with SD of 18 mg/dl. Its minimum and maximum values were 22 and 137 mg/dl respectively. About 70 (68%) of the study participants had higher HDL-C value greater than 40mg/dl.

Elevated LDL-C is a major risk factor that plays a great role in atherosclerosis formation process through its oxidation. The mean level of LDL-C among the study participant was 108.9 mg/dl with standard deviation of 39.7 mg/dl. The minimum and the maximum values among these patients were 14 and 232 mg/dl respectively. Majority of study participant's 70 (68%) especially case participants had LDL-C value less than 130mg/dl.

Triglyceride is another lipid profiles that determined in this study. The mean value of this analyte was 134 mg/dl with standard deviation of 61.2 mg/dl. This mean value is desirable result within normal range. About 74 (71.4%) of the participants had low value of TG which is less than 150mg/dl. The minimum and maximum values were 47 and 389 respectively.

High sensitive C-reactive protein (hsCRP) is acute phase reactant that released into blood stream during inflammatory state. Highly sensitive measurement of CRP may also be used as an aid in the assessment of the risk of future coronary heart disease. It may also be an additional

independent indicator of recurrent event prognosis in patients with stable coronary disease or acute coronary syndrome. Thus, it is also one of the analyte that assessed among study participants. Accordingly, its mean value was 4.98 mg/l with standard deviation of 23.6 mg/l. Majority 51 (96%) of control subjects had low CRP value of <3 mg/l. Of which even most of control subjects had value of less than 1 mg/l. In contrast, most of the case participants had elevated value of greater than 3 mg/l. The minimum and maximum values were 0.04 and 238 mg/l respectively.

Another analytes that detected in this study were cardiac biomarkers such as troponin and CK-MB. Cardiac troponin is specific and highly sensitive marker for myocardial damage. Cardiac troponin increases rapidly after myocardial infarction (AMI). These cardiac markers were performed only for case participants just to evaluate the use of GGT as cardiac marker relatively. Accordingly, the median value for troponin was 4.5 ng/l with a range of 3-405.3 ng/l. About 12 (24%) of case participants had higher troponin value >14 ng/l.

CK-MB is one of the isoenzymes of creatine kinase that appreciably found in the myocardial tissue and support in the diagnosis of myocardial infarction. About 42 (84%) of the case participants had normal value of CK-MB less than 24 U/L with median of 11.5 U/L. The minimum and maximum values were 0.7 and 41 U/L respectively.

6.4. Comparison of clinical and biochemical parameters among cases and controls

All continuous clinical and biochemical parameters were checked weather there were statistically significant mean difference (P <0.05) between control and case participants. As indicated on the table below, among parametric variables there were statistically significant mean differences of BMI, DBP, HDL-C, LDL-C and GGT between case and control group. Of which the mean of GGT, DBP and HDL-C were significantly higher in case group than controls.

Table 4: Comparison of clinical and biochemical characteristics of study participants between case and control (N=103)

No	Parametric variables	Mean ± Standard deviation		P value
		Cases (N=50)	Controls (N=53)	
1	Age (yrs)	53.3 ± 13.4	48.4 ± 13.4	0.070[a]
2	BMI (Kg/m^2)	24 ± 4.65	26.1 ± 4.73	0.027* [a]
3	DBP (mmHg)	85.3 ± 11.35	78.4 ± 7.83	<0.001* [a]
4	TC (mg/dl)	184.6 ± 49.3	191.9 ± 38.83	0.41 [a]
5	HDL-C (mg/dl)	63.1 ± 17.6	41.7 ± 11.6	<0.001* [a]
6	LDL-C (mg/dl)	92.4 ± 40.99	124.5 ± 31.7	<0.001* [a]
7	TG (mg/dl)	138.8 ± 61.4	129.4 ± 61.2	0.436 [a]
8	GGT (IU/L)	36.2 ± 24.34	26.5 ± 12.9	0.013* [a]
Non-parametric variables				
No	Variables	Median		P value
		Cases (N=50)	Controls (N=53)	
1	SBP (mmHg)	125	133	0.876[b]
2	FBS (mg/dl)	104	90	<0.001* [b]
3	hsCRP(mg/l)	6.94	0.26	<0.001* [b]
([a]) : Independent sample T test				
([b]): Mann-Whitney U test				
(*) : Statistically significant				

The mean of GGT was higher in cases (36.2 ± 24.34 IU/L) than control (26.5 ± 12.9 IU/L) with t (101) = 2.54 and p=0.013. Similarly the mean of DBP (85.3 ± 11.35 mmHg) and HDL-C (63.1 ± 17.6 mg/dl) were higher in case group than control group (78.4 ± 7.83, 41.7 ± 11.6) respectively.

However, the mean of BMI was significantly lower in case group (24 ± 4.65 Kg/m^2) than control group (26.1 ± 4.73 Kg/m^2) with t (101) = -2.24 and p= 0.027. In similar manner the mean of LDL-C was also significantly lower in case group (92.4 ± 40.99 mg/dl) than control group (124.5 ± 31.7) with t (101) = -4.43 and p<0.001. However, case and control group had no significant mean difference on age, TC and TG with p= 0.07, 0.41, 0.436 respectively.

For non- parametric data, a Mann-Whitney U test indicated that FBS level was significantly higher in case group with median of 104mg/dl than in control group with median of 90 mg/dl, U=731.5, p <0.001. Similarly, hsCRP was significantly elevated among case group with median of 6.94mg/l than in control ones with median of 0.26 mg/l, U=202.5 and p<0.001. However, there was no statistically significant difference in the SBP (p=0.876) between the two groups.

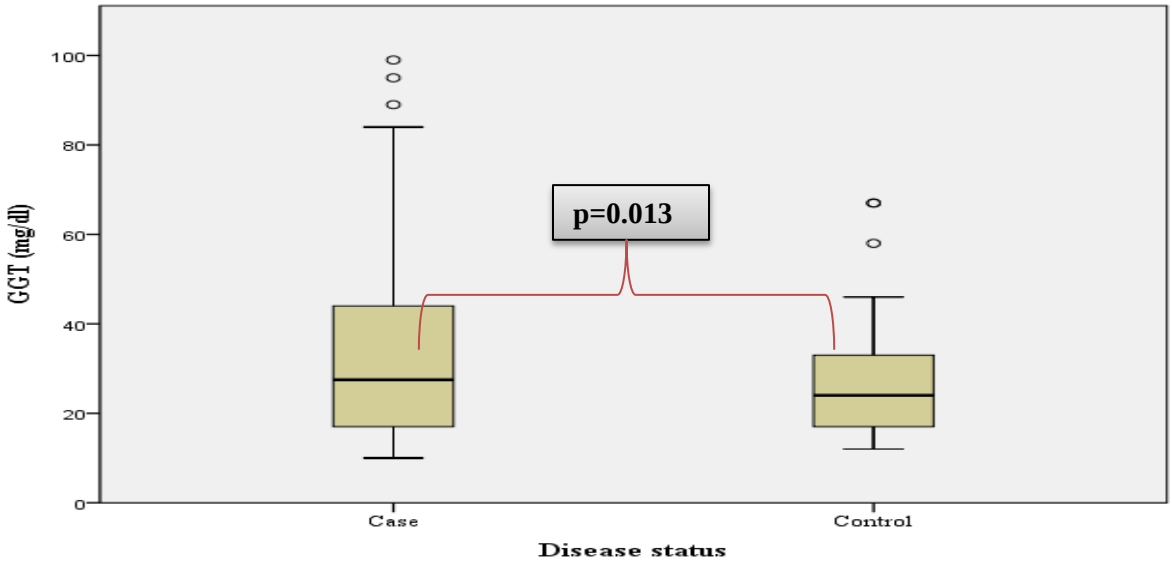


Figure 5: Box and whisker plots of serum GGT level in case and control group of study participants

6.5. Comparison of sociodemographic and clinical data among case subjects

Comparison of mean difference of serum GGT was assessed between cases participants groups based on different independent variables. Accordingly, the mean of serum GGT was higher in male participants (34.5±21.6 IU/L) relative to female (27.9±17.6 IU/L) but, it is statistically non-significant. Similarly, the higher the levels of serum GGT the older the age of participants noted.

There was no statistically significant difference in the mean of serum GGT between groups of physical exercise and coffee consumption. Almost comparable mean of GGT was noted between non-smoker (31±20.5 IU/L) and former smoker **(**32.7±8 IU/L). Chat chewer participants had significantly higher mean GGT (48.8±30.8 IU/L) than those with no history of chewing (30.3±19 IU/L) with p value of 0.041. In similar manner, statistically significant mean difference was observed between groups of alcohol consumption in which current drinker had higher mean of GGT with p value of 0.022. Participants with higher value of BMI had higher mean of serum GGT but, it was not statistically significant.

As a general case participants, the majority 37 (74%) had no hypertension. About 13 (26%) participants were with hypertension. Hypertension has statistically significant association with the level of serum GGT in which hypertensive participants had higher mean of GGT (38.7±25.6 IU/L) than normal ones (30.9±18.6 IU/L) with P value of 0.034. But, SBP and DBP had no significance mean of GGT difference between their groups

Diabetes mellitus is one of the major comorbidity found among cardiac patients; accordingly participants with diabetes and without diabetes were 24% and 76% respectively. This status was assessed by level of fasting blood glucose and history of taking antidiabetic drugs (which accessed from medical record of the patients). There was statistically significant difference between groups of FBS, in which those with higher FBS (>110mg/dl) had higher mean GGT 38.8±21.2mg/dl relative to those with lower FBS (<110mg/dl) 28.8±18.9 mg/dl.

Of total study participants, about 12 (24%) were with dyslipidemia. Both Diabetes mellitus and dyslipidemia were also had statistically significant association with the level of serum GGT in this study. Participants with diabetes (39.7±23.2 IU/L) and dyslipidemia (40.5±21.5 IU/L) had higher mean of GGT in the serum than normal ones (29.4±18.7, 30.2±19.3 IU/L) respectively. Among all lipid profiles, TG and LDL-C show statistically significant mean difference between

their groups in which those with higher TG (>150mg/dl) had higher mean GGT 38.2±21.2 IU/L relative to those lower TG (≤150mg/dl) 28.5±18.4 IU/L with p value of 0.03. Similarly, participants with higher LDL-C (≤130 mg/dl) had higher mean GGT 34±21.3 IU/L than those with lower LDL-C (>130 mg/dl) 25±14.8 U/L with p value of 0.04.

IHD was the major 24 (48%) type of CVD among case participants followed by CAD 17 (34%). There was no significant mean GGT difference seen between types of CVD. The duration of cardiovascular disease diagnosis of most of the cardiac patients participated in this study were less than 1 year. Only 3 (6%) of the participants had duration of > 10 years with CVD. The median of the duration was 2.5 years with range of 1 month-15 years. There was statistically significant mean difference of GGT between duration of diagnosis of case participant in which those of <1 year of diagnosis had higher than that of others.

During the data collection period majority 36 (72%) of cardiac patients participated in this study were following their treatment based on the types of diagnosis. Among the drugs, antihypertensive, lipid lowering, diuretics, antidiabetic drugs were the frequently observed medication from medical records of the participants. Most of them were following these drugs in combination for duration of less than 1 year with median of 1 year and range of 2 month-6 years. Significant mean GGT difference was not seen between groups of duration and status of treatment follow up.

Table 5: Clinical characteristics of CVD patients attending cardiac clinic of TASH from February-May 2018 (N=50)

Variables	Categories	No (%)	P Value
Hypertension	Yes	13 (26)	0.034*
	No	37 (74)	
DM	Yes	12 (24)	<0.001*
	No	38 (76)	
Dyslipidemia	Yes	10 (20)	<0.001*
	No	40 (80)	
Type of clinical diagnosis (CVDs)	IHD	24 (48)	0.59
	CAD	17 (34)	
	CHF	6 (12)	
	Stroke	3 (6)	
Duration of diagnosis (in yrs.)	<1	20 (40)	0.04*
	1-5	22 (44)	
	6-10	5 (10)	
	11-15	3 (6)	
Treatment follow up currently	Yes	36 (72)	0.544
	No	14 (28)	
Duration of Rx follow up (in yrs.)	<1	33 (66)	0.505
	1-5	16 (32)	
	>5	1 (2)	

6.6. Correlation analysis result of independent variables with serum GGT level

Based on scale of measurement of each independent variable, different types of correlation were used to assess the strength of association between independent variables and level of serum GGT.

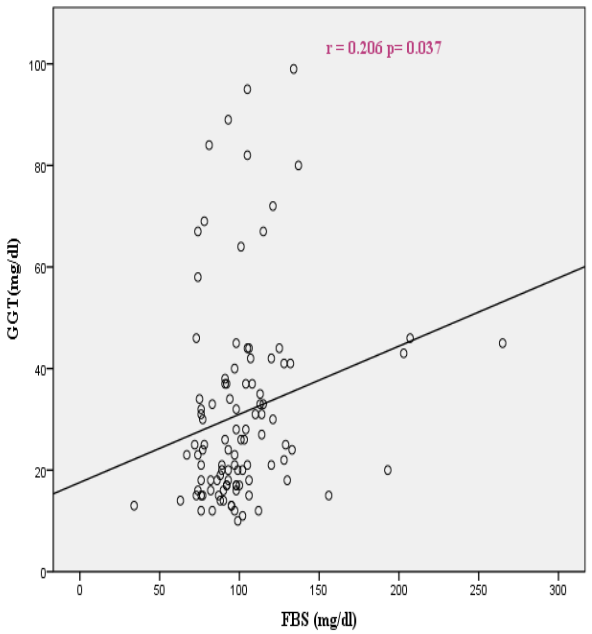
Table 6: Correlation analysis result of independent variables with serum GGT level among study participant

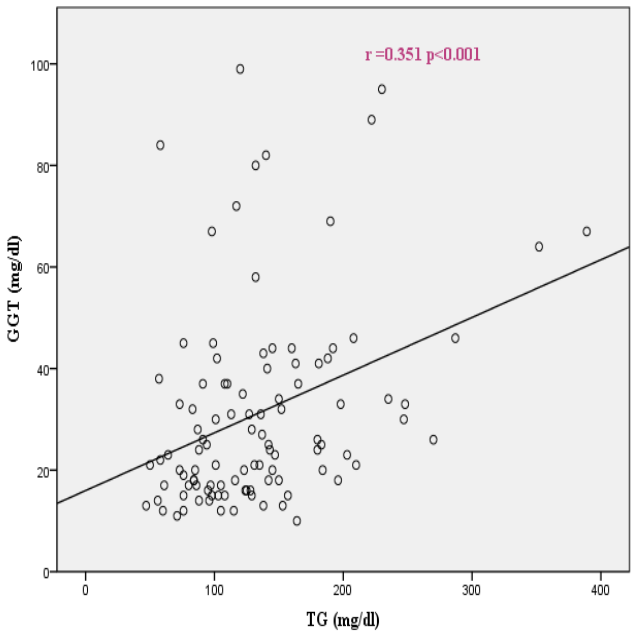
No	Variables	X^2	P value
1	Sex	2.998	0.083 [a]
2	Cigarette smoking	6.6	0.01*[a]
3	Alcohol consumption	6.372	0.041*[a]
4	Types of CVDs	2.62	0.624 [a]
5	Current medication follow up status	0.651	0.41[a]
		r (rho)	
1	Coffee consumption	0.09	0.097[b]
2	BMI group	0.167	0.092[b]
3	SBP	0.161	0.105[c]
4	DBP	0.09	0.367 [c]
5	Duration of diagnosis	-0.173	0.23 [c]
6	Duration of treatment follow up	-0.171	0.235 [c]
7	FBS	0.206	0.037*[c]
8	TC	0.017	0.862 [c]
9	HDL-C	-0.057	0.565 [c]
10	LDL-C	0.121	0.222 [c]
11	TG	0.351	<0.001 [c]*
12	CRP	0.264	0.007*[c]
13	CK-MB	-0.094	0.517 [c]
14	Troponin	0.24	0.094 [c]
([a]): chi-square			
([b]) : spearman ranks correlation			
([c]): bivariate Pearson correlation			
(*): Statistically significant			

Independent variables such as sex, physical exercise, cigarette smoking, chewing chat, alcohol consumption, types of CVDs and current medication follow up status were assessed by using chi-square method weather and to some extent do they associate with serum GGT level. Of which physical exercise and chat chewing habit doesn't fit chi-square assumption because there were cells with expected count less than five. The rest were listed in table 6 with their chi-square and p value.

Smoking cigarette and consuming alcohol had statistically significant association with level of GGT in the serum with p value of 0.01 and 0.041 respectively. However, gender, types of CVDs and status of medication follow up were associated with serum GGT level but not statistically significant.

Fasting blood sugar (P = 0.037), triglyceride (P<0.001) and C-reactive protein (P=0.007) had significantly positive correlation with serum GGT level. Though it is not statistically significant, duration of diagnosis, duration of treatment follow up, HDL-C and CK-MB had inversely correlated with GGT. The negative correlation of CK-MB was unexpected finding of this study. Scatter plot of statistically correlated independent variables were depicted in the following figures.





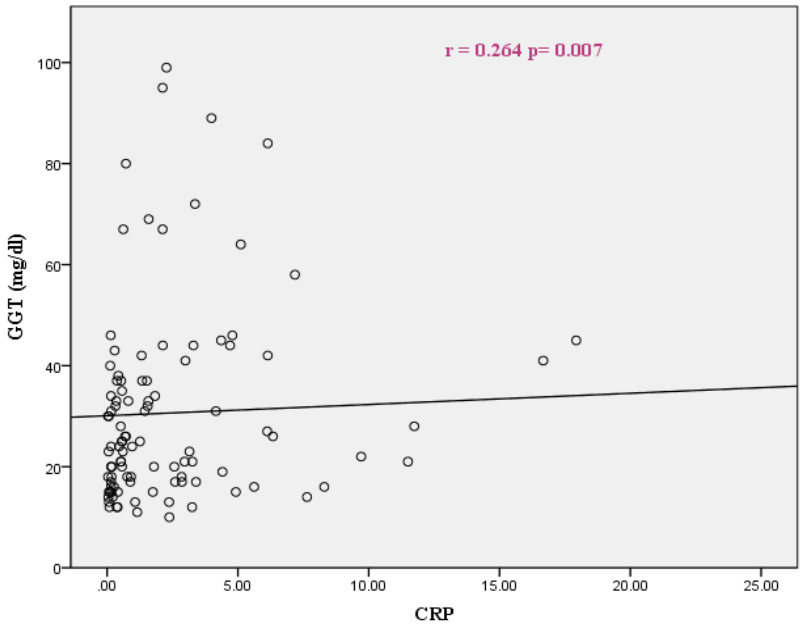


Figure 6: Scatter plots diagram that show relationship between GGT and FBS (top left), GGT and TG (top right) and GGT and CRP (bottom).

7. Discussion

This study was intended to assess the level of serum gamma glutamyl transferase among different types of CVDs patients in comparison with control subjects. We also assessed the mean difference of each independent variable between case and control subjects. Because all study done previously reports separately as there is association between different types of CVDs and level of serum GGT, we incorporated all types CVDs that we got during data collection period. Accordingly, ischemic heart disease, coronary artery disease, and congestive heart failure and stroke were cases that we observed during our data collection period. Besides their types, there was no significant mean GGT level difference noted between CVDs in our study.

Increased serum GGT level has been traditionally understood as a marker of alcohol abuse and/or liver damage. However, a large number of studies suggested that serum GGT is not only a marker for oxidative stress but also a risk factor of cardiovascular disease and metabolic syndrome (5, 7, 8, 13, 20, 22, 27, 31, 32, 40).

Despite the fact that serum GGT activity which reflects the risk of CVDs is not completely understood, there are several possible mechanisms that support the hypothesis. The first probable mechanism is that GGT is regarded as a biomarker for oxidative stress. Oxidative stress could be a crucial factor in the pathophysiology of cardiovascular disease and GGT has an important role in maintaining intracellular glutathione transport into most types of cells. (38)

The second plausible mechanism is that GGT is related with subclinical chronic inflammation. Inflammation is an important mechanism of atherosclerotic cardiovascular disease. Furthermore, excess reactive oxygen species and superoxide which is generated by oxidative stress and low-grade inflammation recapitulate not only endothelial dysfunction but also cardiovascular dysfunction (24, 26). This concept is emphasized in this study because serum GGT level had moderate and positive correlation ($r = 0.264$, p value = 0.007) with CRP which is first acute phase reactant secreted in to the systemic circulation during inflammation.

In our study, there was statistically significant mean difference of GGT between duration of diagnosis of case participant in which those of <1 year of diagnosis had higher than that of

others. In contrast to this study, elevated serum GGT indicates that patients with CVD had severe CAD and had higher risk acute coronary events due to increased burden of atherosclerosis. High levels of GGT can be used for predicting of high risk patients (35). This difference might be due to, in our study most of the participants were had a duration of diagnosis less than 1 year.

This study was also assessed the effect of different medication taken for cardiac problems on the level of serum GGT, but this was difficult because patients were taking multiple drugs simultaneously. Instead the level was assessed depend on the status whether they was taking/ following the treatment or not and duration of treatment was taken. Accordingly, significant difference was not seen between groups of duration and status of treatment follow up. There was no previous data to discuss this finding.

The level of GGT among CVD case and control: In our study the mean GGT level among CVDs patients was found statistically and significantly higher (36.2 ± 24.34 IU/L) than control subjects (26.5 ± 12.9 IU/L) with P value of 0.013. This finding is higher when compared with case control study on acute ischemic stroke group (23.3 ± 11.8 IU/L) vs control subjects (15± 5.7 IU/L) (12). Another case control study done on patient with congestive heart failure indicates higher value than in ours in which GGT activity among patients was higher (66.9 ± 1.7 IU/L) in comparison with controls (12.07±0.6 IU/L) (43). The statistically significant mean difference of GGT was noted between ACS cases (69.7 IU/L) when compared to controls (21.9 IU/L). This indicates higher level of circulating GGT was found compared to those with healthy control subjects (8). This difference might be due to difference in study subject and sample size.

The level of serum GGT with different CVD risk factors: Another aim of this study was to assess the association of serum GGT level with different clinical covariates including age, sex, systolic and diastolic BP, body mass index (BMI), hypertension, diabetes, smoking status, alcohol and coffee consumption, total, LDL and HDL cholesterol, serum triglycerides, and fasting blood glucose, and cardiac markers. This is because the third plausible mechanism of association of GGT with CVD was due to a strong correlation of GGT with various atherosclerotic risk factors. Previous studies reported that serum GGT concentrations were related with hypertension, dyslipidemia, and diabetes (22, 24, 35, 39). Serum GGT level was also found positively correlated with risk factors of atherosclerotic CVD and inflammation such as male gender, obesity, smoking, lack of

exercise, CRP, hyper-cholesterolemia and hypertriglyceridemia, and high fasting glucose (2, 5, 14, 22).

In similar manner, in our study participants with these factors had statistically significant higher serum GGT level compared to those without these factors. The mean GGT level among hypertensive (57.7±18.4), diabetic (62.5±15.5) and with dyslipidemia (59.5±20.6) was higher than normal ones (48.2 ±20.5, 52.7±16.6, 46.5±19.7) respectively (8). Our finding is consistent with this study, but it is lower.

Additionally, the above concept was supported clinically with the reading of SBP and DBP and laboratory result of FBS and lipid profiles. Similarly our finding indicates, GGT level had statistically significant strong and positive correlation of with FBS ($r=0.206$ $p = 0.037$) and TG ($r=0.351$ $p<0.001$). GGT was further positively correlated with total cholesterol ($r = 0.017$), LDL-C ($r = 0.121$), DBP ($r = 0.09$), SBP ($r = 0.16$). Reversely, as also shown in this study, serum GGT was negatively correlated ($r=-0.057$) to HDL-cholesterol level which is a well-known negative risk factor of CVD. This finding is also consistent with many studies (12, 18, 22, 38).

The mean of serum GGT was higher in male participants (34.5±21.6) relative to female (27.9±17.6) but, it is statistically non-significant. Possible explanation for higher values in males could be higher prevalence of risk factors like smoking and alcohol in men and seminal vesicles as an extra source of GGT production in men. This finding is consistent with the finding of study done on 200 coronary angiography underwent patients in which mean GGT level of men was 29.2 U/L compared with women 26.3 U/L (37). On other studies done previously, significant mean difference and correlation was also seen between gender groups (12, 34). Reversely, mean GGT level did not differ between male and female in study done by Gurbuzer (12).

In this study, there is weak and positive correlation ($r= 0.167$) between BMI and serum GGT levels in which insignificantly participants with higher value of BMI had higher GGT level (38). This study also assessed the effect of smoking, coffee and alcohol consumption, and chat chewing on level of serum GGT. These substances are known by having different constituent and bioactive compounds and results in physiological effects. There is no significant mean GGT

difference noted between groups of smoking habit and coffee consumption. But significant mean GGT difference was seen between the groups of chat chewing and alcohol consumption. However smoking habit and alcohol consumption has statistically significant association with the level of GGT in the serum.

Association of cardiac markers with GGT was also one concern of our study. Hence, troponin was positively correlated with GGT (r=0.24). This finding was consistent with study done on 403 ACS in which moderate and positive correlation (r=0.23) demonstrated between serum GGT activity and troponin. In the same study CK-MB was also weakly and positively correlated (r=0.156) with GGT (40). But unexpectedly in this study CK-MB had negative and weak correlation (r= -0.094) with GGT. This might be due to small sample size or study population difference.

8. Strength and limitation of the study

8.1.Strength

The major strength of this study is, it is the only study in Ethiopia which assessed association of GGT with CVDs and it doesn't conducted only on specific types of CVDs like other study done in other countries. It incorporated all types of CVDs and compared in between. This study also included association of GGT with consumption of anti-oxidant agent containing substances such as coffee and khat.

The study also incorporated major clinical data from measurements, medical records (although it is secondary data), using questioners and extensive laboratory measurements to evaluate risk factors associated with CVDs and elevation of GGT. Measurements of height, and weight to calculate BMI, were taken by professional nurses just for this purpose.

Moreover, laboratory parameters were determined in accredited referral clinical chemistry of Ethiopian Public Health Institute

8.2. Limitation

The study was cross-sectional and therefore shows no definitive cause-and-effect relationships between parameters. Also the size of the population of individuals studied was small. Some of the data of this study participants like clinical characteristics and participants' behaviors (e.g. alcohol consumption and khat chewing) were subjectively obtained from medical records and patients. It was difficult to assess the effect of medication on the level of serum GGT because patients were taking multiple drugs at once

9. Conclusion and Recommendation

9.1.Conclusion

In conclusion, this cross-sectional analysis indicates the higher level of serum GGT in the CVDs patients. Participants with atherosclerotic risk factors such as diabetes, hypertension and dyslipidemia had significant higher serum GGT level compared to those without these factors. GGT was strongly and positively correlated with CRP. This study demonstrated the significant and positive correlation of GGT with FBS and TG. GGT was positively correlated with total cholesterol, LDL-C, DBP, SBP, BMI, troponin, alcohol consumption and smoking. However GGT was negatively correlated to HDL-cholesterol level.

9.2. Recommendation

Measurement of serum GGT activities is a cost effective and simple marker of CVDs risk factors. Therefore routine measurement of serum GGT activities should be complimented with others biomarkers of CVD risk factors (CK-MB, Troponin, lipid profiles and others) in the diagnosis of high risk patients of CVDs in clinical practice. Further interventional and longitudinal studies with larger numbers of patients should be conducted to provide more informative data on this subject and to conclude about the influence of CVD risk factors on GGT levels in the circulation.

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11. ANNEXES

Annex I

Information sheet in English Version

Title of the Research Project: assessment of gamma glutamyl transferase enzyme activities among patient with cardiovascular disease at Black Lion specialized hospital, Addis Ababa Ethiopia.

Principal Investigator: Kiyar Jemal (BSc, MSc candidate)

Name of the Organization: Addis Ababa University College of Health Sciences School of Allied Health Science Department of medical laboratory.

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, three advisors; two from Addis Ababa University, Medical Laboratory department and one from cardiology unit. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to compare gamma glutamyl transferase activities among cardiovascular patients so that we will suggest best strategy for diagnosis option of cardiovascular diseases.

Procedures and what will be expected from you for participation

In order to perform the indicated study black lion specialized hospital, Addis Ababa, Ethiopia, you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by a principal investigator, nurses of cardiology unit and laboratory technologist of the hospital laboratory. Then, you are requested to give your consent to the sample collector. After consent, 3ml blood specimen will be collected from you by specimen collector and face to face interview for additional questions.

Potential risks and Discomforts

There will be minor discomfort during blood specimen collection. During collection of specimen from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. But based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and managing of cardiovascular disease. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result for free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

PI: Kiyar Jemal

Phone: 0921463254

E-mail: kiyar3405@gmail.com

Information sheet in Amharic version

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

መግቢያ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

የጥናቱ ርዕስ፡ assessment of gamma glutamyl transferase enzyme activities among patient with cardiovascular disease at Black Lion specialized hospital, Addis Ababa Ethiopia.

የጥናቱ ባለቤት፡ ኪያር ጀማል (BSc, MSc candidate)

የጥናቱ አላማ፡ የልብ ህመምተኞችን ላይ በሽታውን ለመመርመር የሚጠቅም ወይም የሚያስችል አዲስ ጥናት ነው፡፡
እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቀሚና ምቹ ሆነው ተመርጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቹን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡ ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል፡፡ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም፡፡

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡፡ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ::

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት፡፡ የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን የልብ ህመም ለመመርመር ና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ::ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት፡፡የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡ ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡

ኪያር ጀማል

ሞባይል፡ +251-921463254

ኢሜይል፡ kiyar3405@gmail.com

Informed consent form in English version

Code number…..……

Name of principal investigator: Kiyar Jemal, Department of medical laboratory; AAU

Advisors/Co-investigators: Mr. Samuel Kinde, and Mr. Gobena Dedefo, Department of medical laboratory; AAU and Dr. Senbeta, Black lion specialized hospital, AAU

Name of institute: AAU and BLSH

Funded by: AAU

Reviewed by: Departmental Research and Ethics Review Committee (AAU),

Research Title: Assessment of gamma glutamyl transferase enzyme activity among cardiovascular disease patients attending black lion specialized hospital, Addis Ababa, Ethiopia

I had been informed that the objective of this study is. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosis and treatment of cardiovascular disease in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood sample and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Signature_____ Date_____

Thank you for consenting to take part in the study

Informed consent form in Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “assessment of gamma glutamyl transferase enzyme activity among cardiovascular disease patients attending black lion specialized hospital, Addis Ababa, Ethiopia” ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎችም ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም ---------- ፊርማ ----------

ቀን----------

ANNEX II

QUESTIONNAIRE

ADDIS ABABAUNIVERSITY, COLLEGE OF HEALTH SCIENCE, SCHOOL OF ALLIED HEALTH SCIENCES, DEPARTEMENT OF MEDICAL LABORATORY

SCIENCES

Instruction: Please try to complete all information

Part I: Socio-demographic characteristics

1. Card no._____

2. Age (in yrs.) _____

3. Gender: Male☐ Female☐

Part II: Questions to assess risk factors for cardiovascular disease and GGT level

1. Do you perform strenuous physical exercise? A. Yes B. No
2. If Yes, how often per week?
   A. Everyday B. Every 2-3 days C. Once per week D. Other_____ (Specify)
3. Do you drink coffee? A. Yes B. No
4. If yes, how much/day
   A. 1 cup B. 2-5 cup C. >5 cup D. Other _____ (Specify)
5. Have you ever smoked cigarette? A. Yes B. No, Never C. Stopped
6. If yes, how much/day?
   A. 1 cigar B. 2-5 C. 6-10 D. 1 packet E. Other____(Specify)
7. If yes for Q5, for how long? _____ (yrs.)
8. Do you chew khat? A. Yes B. No
9. Have you ever consumed alcohol products?
   A. Yes B. No, Never C. Stopped
10. If yes for Q9, how much /day? _____ (Cup/Bottle.)

Thank you for your time and cooperativeness

Part III: Data collection checklist

IIIA: Checklist to record anthropometric data and blood pressure

S. No	Variables	Value	Remark
1.	Weight (Kg)		
2	Height (m)		
3	BMI (Kg/m^2)		
4	Blood pressure		

IIIB: Checklist to gather medical history and current medication from medical records (only for case participants)

S. No	Parameters	Records	Remark
1	Type of clinical diagnosis		
2	Date of diagnosis		
3	Type of current medication		
4	Date of Rx started		

IIIC: Checklist to record laboratory findings

S. No.	Tests	Results	Remark
1	GGT		
2	FBS		
3	Total cholesterol		
4	HDL- cholesterol		
5	LDL- cholesterol		
6	Triglyceride		
7	CK-MB		
8	Troponin		
9	CRP		

Amharic version of Questionnaire

መጠይቅ

በአዲስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ፤ የህክምና ላቦራቶሪ ት/ክፍል

መመሪያ፡ እባክዎ ሁሉንም መረጃ ለመምላት ይሞክሩ

I: የሻል መረጃ

1. የካርድ ቁጥር _____
2. ዕድሜዎት _____
3. ጸታ ወ ☐ ሴ ☐

II: ከልብ ህመምና ከGGT ኢንዛይም መጠን ጋር ተያያዥነት ያላቸው ጥያቄዎች

1. ጉልበት የሚያስፈልግ የሰዉነት እንቅሥቃሤ ያደርጋሉ? ሀ. አዎ ለ. አይ፣ አላደርግም
2. መልስዎ አዎ ከሆነ፣ በሳምንት ምን ያህል ጊዜ ?

   ሀ. በየቀኑ ለ. 2-3 ቀን ሐ. ነ ቀን መ. ሌላ ካለ_____

3. ቡና ይጠጣሉ? ሀ. አዎ ለ. አልጠጣም
4. መልስዎ አዎ ከሆነ፣ በቀን ምን ያህል ይጠጣሉ?

   ሀ. ነ ሲኒ ለ. 2-5 ሲኒ ሐ. >5 ሲኒ መ. ሌላ ካለ_____

5. ሲጋራ ያጨሳሉ? ሀ. አዎ ለ. አላጨስም ሐ. አቁሜያለሁ
6. መልስዎ አዎ ከሆነ፣ በቀን ምን ያህል ሲጋራ ያጨሳሉ?

   ሀ. 1 ፊሬ ለ. 2-5 ፊሬ ሐ. 6-10 ፊሬ መ. ነ ፓኬት ሠ. ሌላ ካለ_____

7. ለ5ኛ ጥያቄ መልስዎ አዎ ከሆነ፣ ለምን ያህል ጊዜ ይሆናል ያጭሱት? _____(በዓመት)
8. ጫት ይቅማሉ? ሀ. አዎ ለ. አልቅምም
9. የአልኮል ውጤቶች (አረቂ, ቢራ, ጠጅ, ጠላ) ይጠጣሉ? ሀ. አዎ ለ. አልጠጣም ሐ. አቁሜያለሁ
10. ለ9ኛ ጥያቄ መልስዎ አዎ ከሆነ፣ በቀን ምን ያህል ይጠጣሉ? _____(በመለኪያ/ጠርሙስ)

ለትብብርዎ እናመሰግናለን

ANNEX III

Standard operating procedure (SOP)

A. Pre-analytical

Sample collection

Fasting blood samples will be taken from the anti-cubital vein of the arm by using syringes after proper antisepsis with alcohol and sterile cotton swabs in the morning before 10am. Then the blood from each participant will be transferred to serum separator tube and allowed to stand for 30 minutes. Serum will be separated by centrifugation at 1500 rpm. All the medical equipment used for blood collections will be safe and sterile.

Procedure for serum separation

1. 5 ml whole blood will be drawn into serum separator tube containing no anticoagulant.
2. It will be kept in upright position at room temperature for 30-45 min to allow clotting.
3. It will be centrifuged for 5 min at manufacturer's recommended speed 1500 rpm.
4. The serum carefully aspirated at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. A clean pipette for each tube will be used.
5. Serum will be inspected for turbidity. Turbid samples will be centrifuged and aspirated again to remove remaining insoluble matter.
6. Aliquot into nunc tubes and stored at –20 °C. The nunc tubes will be labeled with patient identification number.

B. Analytical

Gamma glutamyl transferase test

Clinical Significance:

Gamma glutamyl transferase (GGT) is used in the diagnosis and monitoring of hepatobiliary diseases. Enzymatic activity of GGT is often the only parameter with increased values when testing for such diseases, and is one of the most sensitive indicators known. GGT is also a sensitive screening test for occult alcoholism. Elevated GGT activities are found in the serum of patients requiring long-term medication with phenobarbital and phenytoin.

Principle:

Enzymatic colorimetric assay: γ-glutamyltransferase (GGT) transfers the γ-glutamyl group of L-γ-glutamyl -3-carboxy-4-nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzoate

liberated is proportional to the GGT activity in the sample. It is determined by measuring the increase in absorbance photometrically (49).

Glucose

Clinical significance:

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas. The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure, and liver disease.

Test Method: Glucose oxidase

Test principle

Glucose oxidase (GOD) converts the sample Glucose into gluconate. The Hydrogen peroxide (H2O2) produced in the reaction is degraded by peroxidase (POD) and gives a colored product Phenol and 4-Aminoantipyrine which is measurable using Trinder indicator reaction at 505 nm. The increase in absorbance correlates with the glucose concentration of the sample (50).

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinone} + 4H_2O$$

Specimen: Serum

Procedure

	Blank	Sample
Reagent 1	240 µL	240 µL
Distilled water	3 µL	-
Sample	-	3 µL
Mix, incubate at 37 ℃ for 5 min., and read the blank absorbance, then add:		
Reagent 2	60 µL	60 µL
Mix thoroughly 37 ℃, and read the absorbance again 5-10 min. later.		
ΔA = [ΔA sample]- [ΔA blank]		

Lipid profile tests

A. Total cholesterol test

Clinical significance

Cholesterol is a main component of cell membranes and lipoprotein and it is the precursor for steroid hormones and bile acids synthesizing. Cholesterol is transported in plasma by low density lipoprotein. The level of the individual's total cholesterol is used in screening early atherosclerosis and monitoring the clinical effect of drugs or low-fat diet.

Test principle

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyzes cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows (51).

Cholesteryl ester + H_2O $\xrightarrow{\textit{cholesteryl ester hydrolase}}$ cholesterol + fatty acid

Cholesterol + O_2 $\xrightarrow{\textit{Cholesterol oxidase}}$ cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4-aminophenazone + phenol $\xrightarrow{\textit{Peroxidase}}$ 4-(p-benzoquinone-monoimino)-phenazone + 4H2

Procedure

	Blank	Sample
Reagent	1000 µL	1000 µL
Distilled water	10 µL	-
Sample	-	10 µL
Mix thoroughly 37 ℃, and read the absorbance 10 min. later.		
ΔA = [ΔA sample]- [ΔA blank]		

B. Triglycerides test

Clinical significance

Triglycerides are the most abundant naturally lipids. It consists of three fatty acids and one glycerol, and is transported in plasma combing with apolipoproteins. Measurement of triglycerides is used for detecting early atherosclerotic risks, classing hyper-lipoproteinemia and

monitoring the clinical effect of drugs or low-fat diet. High triglyceride levels often lead to liver or kidneys disease, diabetes and pancreas disease.

Test principle

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows (52).

Triglycerides + 3H2O $\xrightarrow{Lipase}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{Glycerokinase}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O2 $\xrightarrow{Glycerophosphate\ oxidase}$ dihydroxyacetone phosphate + H2O2

H2O2 + 4-aminophenazone + 4-chlorophenol $\xrightarrow{Peroxidase}$ 4-(p-benzoquinone-monoimino)-phenazone + 2H2O + HCl

Test procedure

	Blank	Sample
Reagent	1000 µL	1000 µL
Distilled water	10 µL	-
Sample	-	10 µL
Mix thoroughly 37 ℃, and read the absorbance 10 min. later.		
ΔA = [ΔA sample]- [ΔA blank]		

C. HDL- C test

Clinical significance

HDL cholesterol is inversely related to the risk of developing coronary artery disease. A low HDL/LDL cholesterol ratio is directly related to the risk of developing coronary artery disease. High HDL cholesterol is associated with the "longevity" syndrome.

Test principle

The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL chol is detected under the assay conditions (53).

The reactions are as follows

ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 ⟶ soluble nonreactive complexes with apoB-containing lipoproteins

HDL-cholesteryl esters —*PEG-cholesteryl esterase*→ HDL-unesterified cholesterol + fatty acid

Un-esterified chol + O2 —*PEG-cholesterol oxidase*→ cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N-succinyl ethylene diamine

Peroxidase + H2O + H^+ ⟶ qunoneimine dye + H2O

Test procedure

	Blank	Sample
Reagent 1	900 μL	900 μL
Distilled water	12 μL	-
Sample	-	12 μL
Mix and incubate at 37 ℃ for 5 min., then add:		
Reagent 2	300 μL	300 μL
Mix thoroughly and incubate at 37 ℃ for 5 min. and then read the absorbance change value.		
ΔA = [ΔA sample]- [ΔA blank]		

D. LDL-C test

Clinical significance

LDL-Cholesterol is directly related to the risk of developing coronary heart disease. A low HDL/LDL-Cholesterol ratio is directly related to the risk of developing coronary artery disease. Elevated LDL-Cholesterol is the primary target of cholesterol-lowering therapy.

Test principle

（1） HDL, VLDL, Chylomicrons ⇌ Cholestenone + H_2O_2

$2H_2O_2$ —*Catalase*→ $2H_2O + O_2$

（2） LDL ⟶ Cholestenone + H_2O_2

H_2O_2 + TOOS + 4-aminoantipyrin —*POD*→ Quinonimine

The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the LDL-cholesterol concentration (54).

Test procedure

	Blank	Sample
Reagent 1	900 µL	900 µL
Distilled water	12 µL	-
Sample	-	12 µL
Mix and incubate at 37 ℃ for 5 min., then add:		
Reagent 2	300 µL	300 µL
Mix thoroughly and incubate at 37 ℃ for 5 min. and then read the absorbance change value.		
ΔA = [ΔA sample]- [ΔA blank]		

CK-MB Test

Clinical significance

Measurements of creatine kinase and its isoenzymes are used in the diagnosis and treatment of myocardial infarction and muscle diseases such as progressive, Duchenne-type muscular dystrophy.

Principle

The CK-MB assay is a two-step assay to determine the presence of the MB isoenzyme of creatine kinase (CK-MB) in human serum and plasma using chemiluminescent immunoassay technology with flexible assay protocols. In the first step, sample and anti-CK-MB coated paramagnetic microparticles are combined. CK-MB present in the sample binds to the anti-CK-MB coated microparticles. After incubation and washing, anti-CK-MB acridinium-labeled conjugate is added in the second step.

Following another incubation and wash, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of CK-MB in the sample and the RLUs detected by the ARCHITECT immunoassay System optics (55).

C - reactive protein test

Clinical significance:

C-reactive protein is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-membered ring having a molecular weight of 105000 daltons. CRP is the most sensitive of the acute phase

reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the classical complement pathway. The CRP response frequently precedes clinical symptoms, including fever. In normal healthy individuals CRP is a trace protein with a range up to 0.5 mg/dL. After onset of an acute phase response the serum CRP concentration rises rapidly and extensively. The increase begins within 6 to 12 hours and the peak value is reached within 24 to 48 hours. Levels above 100 mg/L are associated with severe stimuli such as major trauma and severe infection (sepsis). CRP response may be less pronounced in patients suffering from liver disease. CRP assays are used to detect systemic inflammatory processes; to assess treatment of bacterial infections with antibiotics; to detect intrauterine infections with concomitant premature amniorrhexis; to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia; and to distinguish between infection and bone marrow rejection. Postoperative monitoring of CRP levels of patients can aid in the recognition of unexpected complications (persisting high or increasing levels). Measuring changes in the concentration of CRP provides useful diagnostic information about how acute and how serious a disease is. It also allows judgments about the disease genesis. Persistence of a high serum CRP concentration is usually a grave prognostic sign which generally indicates the presence of an uncontrolled infection

Principle:

Particle enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically (56).

Troponin

Clinical significance: Troponin I (TnI) is a key regulatory protein of the striated musculature. Although its function in the contractile apparatus is the same in all striated muscles, TnI originating from the myocardium (cardiac TnI) clearly differs from skeletal muscle TnI. Due to this high tissue-specificity, cardiac troponin I (cTnI) is a highly sensitive marker for myocardial damage. Cardiac TnI allows differentiation between skeletal muscle lesions (e.g. rhabdomyolysis, polytraumatism) and myocardial injury. In cases of acute myocardial infarction (AMI), cTnI levels in serum rise about 3-6 hours after the onset of cardiac symptoms, peak at 12-

16 hours, and can remain elevated for 4-9 days. Elevated cTnI levels have also been reported in cases of unstable angina pectoris (UAP) and congestive heart failure (CHF). Cardiac TnI is a well-established prognostic marker which can predict the near-, mid- and even long-term outcome of patients with acute coronary syndrome (ACS).

Test principles and procedures:

Test principle - Competitive immunoassay with analyte liberation was used applied.

1st incubation (9 minutes): 30 µL of sample, two biotinylated monoclonal anti-cardiac troponin I antibodies, and two monoclonal anti-cardiac troponin I antibodies labeled with a ruthenium complex react to form a sandwich complex.

2nd incubation (9 minutes): After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Measurement method: Electrochemiluminscent

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument- specifically generated by 2-point calibration and a master curve provided via the reagent barcode (57).

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Kiyar Jemal (B.Sc.)
Signature: _____
Date of submission: _____

This proposal has been submitted with our approval as advisors.
Advisor: Samuel Kinde (MSc, PhD fellow)
Signature: _____
Date: _____
Place: Addis Ababa, Ethiopia.
Advisor: Gobena Dedefo (MSc, PhD candidate)
Signature: _____
Date: _____
Place: Addis Ababa, Ethiopia.