THE PREVALENCE OF ELEVATED AMINOTRANSFERASES LEVELS AND THEIR RELATIONSHIP WITH RISK FACTORS FOR CHRONIC NONCOMMUNICABLE DISEASES AMONG ADULT AT GILGEL GIBE FLIED RESEARCH CENTER, SOUTH WEST ETHIOPIA

BY: BEGASHEW ZEWDIE (BSc.)

begashewz@gmail.com

RESEARCH PAPER SUBMITTED TO COLLEGE OF PUBLILC HEALTH AND MEDICAL SCIENCES, DEPARTMENT OF EPIDEMIOLOGY JIMMA UNIVERSITY; IN PARTIAL FULFILLMENT FOR THE REQUIRMENT FOR MASTERS OF PUBLIC HEALTH IN EPIDEMIOLOGY

THE PREVALENCE OF ELEVATED AMINOTRANSFERASES LEVELS AND THEIR RELATIONSHIP WITH RISK FACTORS FOR CHRONIC NONCOMMUNICABLE DISEASES AMONG ADULT AT GILGEL GIBE FLIED RESEARCH CENTER, SOUTH WEST ETHIOPIA

BY: BEGASHEW ZEWDIE (BSc)

ADVISORS:
FASIL TESSEMA (MSc.)
DESALEGN MASSA (MPH)

May/2013
Jimma, Ethiopia

Abstract

Background: Liver enzymes such as alanine aminotransferase and aspartate aminotransferase are considered indicators of hepatocellular health in general population. Elevated aminotransferases levels are be considered as an alarming sign for chronic disease development; they might enhance the ability to quickly identify people with the most prevalent risk factors for type 2 diabetes and cardiovascular disease and may be useful in strategies for the prevention of these disorders.

Objective: To determine prevalence of elevated aminotransferases levels and to assess their relationship with major risk factors for chronic noncommunicable diseases among adults residing in Gilgel Gibe Field Research Center.

Methods: Data from the community based cross sectional study conducted in 2008-09 by Jimma University in Gilgel Gibe Field Research Center using WHO STEPs wise approach was used. The data for the analysis came from a randomly selected sample of 1626 (802 males and 824 females) individuals aged 15 years and over to establish biochemical reference ranges.

Results: The overall prevalence of elevated ALT, AST, and both ALT and AST were 37.8 %, 41.1%, and 27.7% respectively. The prevalence of elevated serum ALT (p=0.009), and AST (p= 0.002) level revealed a statistically significant decrease with increasing age . In multivariable analysis factors independently associated with elevated ALT were high body mass index (BMI), AOR= 2.10, 95% CI1.14-3.89, waist to hip ratio (WHR) AOR= 1.77, 95% CI 1.41-2.22, high total cholesterol AOR=1.48, 95% CI: 1.09 -1.99, and raised triglyceride AOR=1.36, 95% CI: 1.05-1.77. On the other hand, high BMI AOR=1.94, 95% CI: 1.03-3.65, WHR AOR= 1.28, 95% CI: 1.03-1.59, high total cholesterol, AOR=1.95, 95% CI: 1.43-2.65 and raised triglyceride AOR=1.43, 95% CI: 1.10 -1.87 were independently and significantly associated with elevated level of AST.

Conclusion: Higher BMI, WHR, total cholesterol, and raised triglyceride were independently associated with elevated ALT, and AST.

ACKNOWLEDGEMENT

First of all, I would like thank my God for his giving me insight through all my life, all I am, all I have it's because of you.

I would like to express my deepest gratitude to my research advisors Mr. Fasil Tessema and Mr. Desalegn Massa for their unreserved encouragements and provision of constructive comments and guidance throughout this research work.

Lastly, my deep acknowledgements extend to Gilgel Gibe Field Research Center for providing the data for this analysis.

Table of Contents

List of Figures

List of Tables

List of abbreviation and acronyms

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

BMI: Body Mass Index

GGFRC: Gilgel Gibe Field Research Center

JU: Jimma University

JUSH: Jimma University Specialized Hospital

NAFLD: Non-Alcoholic Fatty Liver Disease

WC: Waist Circumference

WHR: Waist to Hip Ratio

NHANES: National Health and Nutritional Examination Survey

WHO: World Health Organization

CHAPTER ONE: INTRODUCTION

1.2 Background

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It maintains a stable blood glucose level by taking up and storing glucose as glycogen by the process known as glycogenesis, breaking this down to glucose when needed by the process known as glycogenolysis and forming glucose from non-carbohydrate sources such as amino acids by the process known as gluconeogenesis. Liver function tests including liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs [1].

Normal ranges of these enzymes are based on distributions from "healthy" reference population. The upper limit of normal (ULN) is defined as the mean $\pm$ 2 standard deviation (SD), which implies that 2.5% of the liver tests from these healthy population exceed the ULN [2]. Aminotransferases (ALT and AST), are intracellular liver enzymes and participate in gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid respectively. They have leaked into the circulation and their serum levels reflect the amount of hepatocellular injury [1].

Aminotransferases (and predominantly AST) are not only found in hepatocytes but also in other tissues (heart and skeletal muscles, kidney, brain, pancreas, and lung). The liver contains 400 international unit of ALT AST per gram of protein (mainly cytoplasmic) and 500 international unit of AST per gram of protein (> 80% contained in mitochondria and endoplasmic reticulum). The extent of elevation is related to the particular disease and often to the stage of the disease. Damage to one gram of liver tissue (or the membranes of 171 million hepatocytes) results in a significant increase in the serum ALT activity AST responds in the same fashion, especially following liver cell necrosis and destruction of mitochondria and endoplasmic reticulum [3].

Alanine aminotransferase is more sensitive and specific markers of liver function and its diagnostic sensitivity and specificity for the detection of liver disease is around 83 % [3, 4]. The diagnostic sensitivity of AST is significantly lower (70%) and less specific to the liver [5] .The study of the AST: ALT ratio can yield some additional information but specific etiologic diagnosis cannot usually be based on these routine tests or ratio's. In alcoholic liver disease the AST: ALT ratio is greater than 2:1, due to alcohol-related deficiency of pyridoxal 5-phosphate (B6) [5]. An isolated rise in AST is not uncommon in patients with end-stage alcoholic cirrhosis. In contrast, patients with non-alcoholic fatty liver disease (NAFLD) the AST: ALT ratio is less than one [6].

Although, in clinical practice the serum levels of alanine or aspartate aminotransferase (ALT and AST) represent the most commonly used tests for qualitative assessment of liver disease. However, elevated AST, and ALT levels have been reported as a predictors of chronic diseases risk particularly type 2 diabetes and cardiovascular disease**,** and they might enhance the ability to quickly identify people with the most prevalent risk factors for type 2 diabetes and cardiovascular disease and may be useful in strategies for the prevention of these disorders [7, 8]. Therefore it is important to provide information on prevalence of elevated aminotransferases levels and their relationship with major risk factors for chronic non communicable diseases from population based surveys. The aim of this study is to determine the prevalence of elevated aminotransferases levels and to assess their relationship with major risk factors for chronic non communicable diseases in the general population.

1.2 Statement of the Problem

Aminotransferases may be involved in several critical processes that affect the risk of developing conditions such as diabetes and cardiovascular disease**.** Increased concentrations of these enzymes associated with increased levels of liver fat and an increased risk of developing diabetes and the cardiovascular disease [9, 10]. An analysis of the Third National Health and Nutrition Examination Survey (NHANES), data those with elevated ALT activity had a higher 10-year calculated risk of coronary heart disease than those with normal ALT activity, and the risk progressively increased also for ALT within the normal range[11]. In a review of medical records elevated aspartate aminotransferase (AST), alanine aminotransferase were identified, as potential factors associated with natural death. The adjusted proportional hazard ratios for natural death were 6.75 in the group with markedly elevated AST (>80 U/L) and 2.66 in the group with mildly elevated AST (40–80 U/L), compared to the reference AST (<40 U/L). Similarly the adjusted hazard ratios were 5.41 and 1.44 in the case of ALT levels [12].

Any confirmed rise in serum aminotransferases activity (especially ALT) warrants additional investigation for the detection of underlying liver disease. The cause of an elevated aminotransferase level varies greatly by geography and the population studied. Several carefully conducted clinical studies, which included liver biopsy in all patients, have shown that nonalcoholic fatty liver disease(NAFLD) was the most cause of persistent liver tests abnormalities among asymptomatic subjects[13, 14]. Furthermore, it has been observed that fatty liver disease is associated with major risk factors for chronic non communicable diseases (such as overweight, obesity, excessive alcohol intake, physical inactivity, raised triglyceride, and high total cholesterol) and could therefore be responsible for the increased incidence of type 2 diabetes and cardiovascular disease in peoples with these risk factors [15-17].

A beneficial effect of light-to-moderate alcohol consumption has been well documented particularly in regard to cardiovascular function [18, 19]. In contrast, excessive alcohol consumption is a risk factor for global burden of disease and the effect of excessive alcohol consumption on health is estimated at 3.8% of all global deaths [20]. Alcohol is the most common cause of ALT elevation. Study done on health blood donors has shown that excessive alcohol consumption was the most likely cause of an elevation of ALT which account 50%, followed by obesity which account 22%, while the rest was due to hepatitis C positive [21].

Cigarette smoking is another modifiable risk factors for the development several chronic noncommunicable diseases and the cause for 6 million people's death each year globally [20]. It has been reported that although smoking does not damage hepatocytes directly, it may change the effect of alcohol drinking on AST and ALT activities via the actions of numerous ingredients that alter the activities of these enzymes found in the liver [22]. Conversely, two studies one from Japan and the other from Korea did not show a strong relationship between tobacco smoking and serum liver enzymes; specifically, AST and ALT [23, 24].

Regular physical activity reduces abdominal adipose tissue and improves insulin resistance and increases plasma high-density lipoprotein (HDL) cholesterol levels and reduces triglyceride levels and blood pressure; hence regular physical activity has positive effects on the incidence of coronary artery disease, type 2 diabetes mellitus, hypertension and obesity [25]. Conversely, low level of physical activity independent risk factor for death caused by non-communicable chronic diseases, and estimated that, each year approximately 3.2 million people die due to low level of physical activity [20]. Low level of physical activity also associated with increased risk of developing nonalcoholic fatty liver disease [26]. The results of one study demonstrated a significant reduction in aminotransferase levels in patients with nonalcoholic fatty liver disease who adhered to an aerobic exercise program, regardless of weight loss [27]. The association of obesity and overweight with chronic diseases in adults, particular type 2 diabetes and cardiovascular disease is well known, and are the leading risk for deaths globally. According to WHO global estimates from 2008, at least 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and

obesity [20]. Excess body weight also has an adverse effect on the liver, can be an independent predictor of hepatic steatosis (fatty liver), a common clinical and histological condition frequently associated with excessive body weight in the absence of excessive alcohol consumption [28, 29]. Increased levels of aminotransferases (ALT and AST) are appearing to be the most sensitive biochemical indicators of the presence of nonalcoholic fatty liver disease in obese people [30, 31]. Strong association of these measures of obesity (body mass index and waist to hip ratio) and elevated ALT and AST levels has also been reported [32, 33].

High cholesterol level, raised triglyceride level, and raised fasting blood glucose level) have been well recognized metabolic disorder associated with several chronic no communicable diseases [34]. Each year approximately, 2.6 million deaths due to raised cholesterol; which in turn increases the risks of heart disease and stroke [20]. These disorders also associated with non-alcoholic fatty liver disease; an elevated aminotransferase levels are useful indicators of non-alcoholic fatty liver disease in people with these disorders [33,35]. A 6-year follow up study of metabolic syndrome in 1097 men and women who were free of the metabolic syndrome at baseline found that 20.6% had developed the metabolic syndrome and an OR of 2.25 elevated ALT above the upper limit of normal compared to that of below upper limit of normal. In this study ALT was significantly associated only with fasting plasma glucose at follow-up [7].

Although in Ethiopia, no national data available on the prevalence major risk factors for chronic non communicable diseases. The population based survey conducted by Jimma University in GGFRC revealed that the prevalence of smoking was 9.3%, alcohol consumption was 7.3% .Similarly, the prevalence of physical risk factors (such as: hypertension, overweight and central obesity) were 9.3%, 2.6% and 33.3% respectively whereas the prevalence of metabolic disorders (such as: high total cholesterol and raised triglyceride) were 10.7% and 7.7% respectively [36]. However up to our knowledge there is no population based study done that addressed the prevalence of elevated aminotransferases and their relationship with lifestyle, and biological risk factors in the general population.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of elevated aminotransferases levels and chronic no communicable diseases risk factors.

2.1.1 elevated aminotransferases levels

Normal aminotransferases values are defined as the mean of the distribution ± 2 standard deviations of the normal population. Normal aminotransferases values are defined as the mean of the distribution ± 2 standard deviations of the normal population which, implies that 2.5% of the liver tests from these healthy persons exceed the upper limit of normal (ULN) [2]. The level of aminotransferases, elevation that is considered abnormal varies widely and has recently been brought into question. There is controversy concerning the need for evaluation of people with slightly increased aminotransferase activity, but still within the normal range. The results of an Italian retrospective cohort study in 6835 first-time blood donors suggested that normal values of ALT were based on a reference population which possibly included patients with subclinical hepatitis C infection (HCV) and nonalcoholic liver disease (NAFLD) [37]. According to the authors, suggestion lowering of the upper limit of normal (ULN) for ALT is advisable in patients with chronic hepatitis C infection or nonalcoholic fatty liver disease. The study and conclusions were criticized by others because lowering the ULN of ALT would create an overwhelming number of false-positive results [38]. However, a recent Korean study showed a positive association between high-normal serum ALT (35-40 IU/L) levels and mortality from liver disease, even after adjustment for alcohol consumption, obesity, plasma glucose, serum lipids [39]. It may therefore be justified to lower the ULN for ALT in populations with a high prevalence of liver diseases. I was unable to get global report on estimate of prevalence of elevated aminotransferases and its consequence. I was summarized below few of studies done in different countries regarding these issues.

One population-based study conducted in Casanova, a southern Italy on a total 10,600 inhabitants study participants of 12 years of age and over to determine prevalence of elevated ALT, and AST defined as ALT (>50 IU/L) and AST (>45 IU/L) . The finding of this study showed the prevalence of elevated ALT was found 22.0% for males and 6.6% for females; similarly the prevalence of elevated AST was 7.6% for male and 1.6% for females. In this study as this figure indicate there was great difference of prevalence of elevated ALT, and AST between male and female. The study concluded that the gender difference of prevalence of elevated ALT and AST were due to the difference in distribution of alcohol consumption which was found 55.5% for males and only 15.5% for females [40]. Another community-based epidemiological study of elevated serum alanine aminotransferase levels which was conducted in Kinmen, Taiwan on a large population age 30 years and over. The study found the overall prevalence of elevated ALT defined a serum ALT level ≥ 40 U/L was 7.2% and sex specific prevalence were 9.4% for male and 5.3% for female. In this study it was also reported the prevalence of elevated ALT decrease as population age increase. This study found the prevalence of elevated was 13.1 % for those between age of 30 and 39 years and 5.5% in the participants aged 70 and over [41]. Data from the Third National Health and Nutrition Examination Survey, which was conducted in the United States from 1999 to 2002, to estimate prevalence of elevated aminotransferases, defined as serum ALT levels greater than 43 IU/L and AST greater than 40 IU/L as "elevated" for both men and women indicated that the overall prevalence of elevated ALT, AST, or either ALT or AST were 8.9%, 4.9%, and 9.8%, respectively, in the entire population. In this survey the prevalence of elevated ALT, AST, or either ALT or AST were 7.3%, 3.6%, and 8.1%, respectively when participants who tested positive for hepatitis C virus (HCV) antibody or reported excessive alcohol consumption were excluded from the estimation [35].

An analysis nested case-control study derived from the cohort through a review of medical records conducted in northern Taiwan, to explore the potential factors associated with natural death. The finding of this study showed that AST, alanine aminotransferase were identified, as potential factors associated with natural death. The adjusted proportional hazard ratios for natural death were 6.75 in the group with markedly elevated AST (>80 U/L) and 2.66 in the

group with mildly elevated AST (40–80 U/L), compared to the reference AST (<40 U/L). Similarly the adjusted hazard ratios were 5.41 and 1.44 in the case of ALT levels [12].

I was unable to get published data about the prevalence of elevated aminotransferases in the general population in the Ethiopia. Only I found one study conducted 1995, using 555 subjects' data obtained from records of the clinical chemistry laboratory of the Ethiopian Health and Nutrition Research Institute to establish ranges of clinical normal limits and comparison with adopted limits for adult population. In this study adopted cutoff values used for ALT were > 30 IU/l for males and > 26 IU/l for females; whereas in the case of AST, 42 IU/l for males and 27 IU/l for females. Using these cutoff values the fund that elevated ALT was 25.3% for males and 22.0% for females; whereas elevated AST was 18.8% for males and 19.5% for females [42].

2.1.2 Burden of major risk factors for chronic non communicable diseases

In the World Health Organization, 2002 report, tobacco use, unhealthy diet, excessive alcohol consumption, physical inactivity, overweight, obesity, raised blood pressure, high total cholesterol level, raised triglyceride level and raised blood glucose level) are identified as major risk factors for chronic no communicable diseases such as diabetes, heart disease, asthma such as heart disease, stroke, cancer, chronic respiratory diseases, and diabetes, and included in steps wise approach for chronic disease risk factor surveillance[34].

In 2008, the global burden of major risk factors for chronic non communicable diseases reported that each year approximately 6 million people die from tobacco use (both from direct tobacco use and second-hand smoke),3.2 million people die due to physical inactivity, 2.3 million from the harmful use of alcohol which accounts 3.8% of all deaths in the world, 7.5 million deaths due to raised blood pressure which accounts about 12.8% of all deaths, 2.8 million people die each year as a result of being overweight or obese and 2.6 million deaths due to raised cholesterol ; it increases the risks of heart disease and stroke [20].

Although in Ethiopia, no national data on the magnitude of chronic non-communicable diseases risk factors, the population based survey conducted by Jimma University in GGFRC revealed the

prevalence of smoking was 9.3%, alcohol consumption was 7.3%, and low level physical activity was 16.9%. Similarly, the prevalence of physical risk factors (such as: hypertension, overweight and central obesity) were 9.3%, 2.6% and 33.3% respectively whereas the prevalence of metabolic disorders (such as: high total cholesterol and raised triglyceride) were 10.7% and 7.7% respectively [36].

2.2 Factors related with elevated aminotransferase levels

Knowledge of the lifestyle factors, obesity and metabolic disorders that are associated with elevations in aminotransferases is crucial for appropriate evaluation of patients who may present with an elevated aminotransferases and also for probably interpretation of these enzymes tests when used as screening tools. The relationship between these factors and aminotransferases was discussed in detail in the rest part of this review.

2.2.1 Relationship between lifestyle factors and elevated aminotransferase levels

Tobacco smoking is one of the major risk factors for chronic noncommunicable disease. There is controversy concerning association between smoking cigarette and aminotransferase levels. Across sectional study done in France among blood donors found smoking was negatively associated with serum ALT levels [43]. Conversely, two studies did not show a strong relationship between tobacco smoking and serum liver enzymes; specifically, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [22, 23].On other hand across sectional study done in china, found that the odds of having elevated aminotransferases levels (ALT and AST) was twice among smoker compared to nonsmoker independent of other factors[44].

The association between alcohol consumption and elevated serum aminotransferase levels intensively studied. Study done in Finland on a large population consists of heavy drinkers, moderate drinkers and abstainers found that in heavy drinkers, serum AST and ALT were significantly higher than in moderate drinkers or abstainers [45].

On other hand a recent publication of Khawaja S his colleagues has reported serum ALT but not AST activities in moderate drinkers was higher than those in abstainers [46]. Loomba R and colleagues have reported joint effects of excessive alcohol consuming and obesity raised the odds of elevated ALT by 9 and AST by 21-fold [47].

Physical activity level is another lifestyle factor associated with aminotransferases levels. The analysis of data from a population-based cohort study, the Shanghai men's health study revealed that total physical activity (which included leisure time, daily living, and commuting to work physical activity) were inversely related to elevated ALT, and AST, but the association was attenuated after adjustment for body mass index (BMI) and waist to hip ratio (WHR) [48]. The authors suggested that regular physical activity reduce body weight thereby accumulation fatty in the liver which influence liver enzymes values.

A follow up study conducted in Australia to evaluate effect of a lifestyle intervention (physical activities) on 152 patients with abnormal liver enzymes and metabolic risk factors by randomizing subjects to a moderate (6 sessions/10 weeks), low intensity (3 sessions/4 weeks) lifestyle counseling intervention and control group(no physical activity intervention) [49] . The results shows that likelihood of elevated ALT levels in both the moderate and low-intensity groups was reduced by over 70% compared to controls. The authors have concluded that moderate and even low-intensity lifestyle counseling interventions targeting improvement in physical activity is a practical and effective method for improving the health of patients with elevated liver enzymes.

2.2.2 Relationship between measure of obesity and elevated aminotransferase levels

Under this subheading the relationship between measure of obesity such as body mass index (BMI) and waist to hip ratio (WHR) and elevated alanine aminotransferases (ALT) and aspartate aminotransferase (AST) was reviewed.

Obesity which is a major public health problem worldwide and risk factor for several chronic noncommunicable disease is also has an adverse effect on the liver, can be an independent predictor of hepatic steatosis (fatty liver), a common clinical and histological condition frequently associated with excessive body weight in the absence of excessive alcohol consumption [28, 29]. A prospective cohort study was conducted in Korea in 2002, on a total

of 5237 healthy men without diagnosed non-alcoholic fatty liver disease (NAFLD) and without increases of either ALT (>35 U/L) to examine the association between ALT within its reference interval and risk for subsequent development of NAFLD. The results show that increased ALT concentration, even within the reference interval, was an independent predictor of incident NAFLD [31]. Increasing evidence shows that abdominal adiposity has a direct influence on health, and waist circumference is a reliable indicator of visceral fat and central adiposity, which correlates with health risks to a greater extent than does adipose tissue in other regions of the body because of high association between the visceral fat and insulin resistance [50, 51]. An analysis of data consisted of 6315 adults from Dionysos study which was conducted in Northern Italy, to investigate relationship between BMI and serum alanine aminotransferase .The results shows that BMI was independent of (sex, age, ethanol intake, HBV and HCV infection, coffee and drug consumption, and cigarette smoking) associated with elevated ALT in the general population [52]. The authors concluded that BMI is stronger predictor of elevated ALT than alcohol intake. One of the limitations of this study the effect of physical activity on the enzyme activity was not mentioned.

A population-based cohort study conducted in china found that both BMI and WHR were positively associated with elevated ALT and AST levels [48]. On other hand in a community-based epidemiological study of elevated serum alanine aminotransferase levels done Kinmen, Taiwan, gender-related differences regards to the association between measure of obesity and elevated ALT was revealed that obesity (high waist circumference) significantly related to elevated serum ALT levels only for males [41]. Ruhl and colleagues studied the joint effect of body weight and alcohol on elevated serum aminotransferases using the third US National Health and Nutrition Examination Survey of 1988–1994 by excluding participants with hepatitis B or C.

They found that overweight persons, consumption of >2 standard drinks per day increased the risk of elevated aminotransferase levels, and among the obese, >1standard drink per day was associated with a higher risk elevated aminotransferase levels [53]. The authors conclude synergistic effect of obesity and alcohol drinking on aminotransferases activity.

2.2.3 Relationship between biochemical tests and elevated aminotransferase levels

High cholesterol level, raised triglyceride level, and raised fasting blood glucose level) have been well recognized components of metabolic syndrome (a cluster of metabolic disorders) associated with several chronic noncommunicable disease [34]. These disorders also associated with non-alcoholic fatty liver disease; an elevated aminotransferase levels (are useful indicators of non-alcoholic fatty liver disease in people with these disorders [33, 35]. A cross-sectional study conducted in Iran among adult army personnel found, elevated serum alanine aminotransferase have significant association with the components of metabolic syndrome such as raised triglyceride level (≥150 mg/dl), and raised fasting blood glucose level (≥110 mg/dl) and raised total cholesterol (≥240 mg/dl0 [54]. On other hand a cross sectional study done in china found that positive association of raised triglyceride level and fasting blood glucose level with elevated ALT and AST levels . However in the multivariable analysis of this study no association between high total cholesterol level and elevated ALT and AST levels was observed [55].

In summary**,** cigarette smoking, alcohol consumption, level of physical activity, measure of obesity such as (high BMI and WHR) and biochemical tests such as (high total cholesterol, total triglyceride and) and raised fasting blood glucose) are factors that association with elevated serum alanine aminotransferase and aspartate aminotransferase levels. Few of the reviewed literatures were used; small sample size. In almost all studies different definition of elevated (abnormal) serum alanine aminotransferase and aspartate aminotransferase levels i.e. cutoff values were used either to determine prevalence of elevated aminotransferases or to

determining the prevalence of elevated serum aminotransferases levels and identifying factors associated with elevated serum aminotransferases levels is important for prevention of condition related to hepatocellular damage and for proper interpretation of these enzymes at clinical setting ; nevertheless, In Ethiopia up to our knowledge data no study available that address prevalence of elevated serum alanine aminotransferase and aspartate aminotransferase levels and their relationship with lifestyle factors, measure of obesity and biochemical tests at population level. |Therefore this study is designed to fill this gap.

CHAPTER THREE: SIGNIFICNACE OF THE STUDY

To the best of our knowledge there are no community-based studies that, addressed the prevalence an elevated serum ALT and AST levels and associated factors for the general population of Ethiopia. Therefore the aim of this study is to determine prevalence of elevated aminotransferase levels(alanine aminotransferase and aspartate aminotransferase) and to assess their relationships with lifestyle factors (such as tobacco smoking, alcohol consumption, level of physical activity), and biological factors (such as body mass index, waist to hip ratio, total cholesterol, total triglyceride, and fasting blood glucose levels) among adults residing in GGFR by using data of study participants randomly selected for biochemical determination in Gilgel Gibe Field Research Center (GGFRC) by Jimma University. Because, of strong relationship between an elevated aminotransferases levels and chronic disease development particularly diabetes mellitus and cardiovascular disease; it is important to provide information on prevalence

of elevated aminotransferases levels along with lifestyle and biological risk factors from population based survey which might be useful for physicians caring for patients with liver disease and for chronic noncommunicable disease prevention and control strategies.

CHAPTER FOUR: OBJECTIVES AND HYPOTHESIS

4.1 General objective

To determine prevalence of elevated aminotransferases and their relationship with major risk factors for chronic noncommunicable diseases among adults residing in Gilgel Gibe Field Research Center.

4.2 Specific Objectives

1. Describe the demographic, lifestyle, anthropometrics and biochemical characteristics of adult aged 15 and over.

2. Determine prevalence of elevated serum alanine aminotransferase and aspartate aminotransferase levels in adult aged 15 and over.
3. Examine the relationship between lifestyle risk factors and elevated serum aminotransferases levels.
4. Examine the relationship between biological risk factors and elevated serum aminotransferases levels.

4.3 Research question

Are lifestyle and biological risk factors associated with elevated serum alanine aminotransferase and aspartate aminotransferase levels in apparently healthy population?

CHAPTER FIVE: MATERIALS AND METHODS

5.1 Study area

The current analysis was based on data collected from late September 2008 to the end of January 2009 at Gilgel Gibe Field Research Center (GGFRC) by Jimma University. GGFRC is located in Southwestern Ethiopia, Jimma zone, around Gilgel Gibe Hydroelectric dam, 260 kilometers southwest of Addis Ababa and 55 km Northeast of Jimma town. The center serves as health and demographic surveillance site for the University and comprises of 8 rural and 2 urban kebeles (the lowest administrative unit in Ethiopia). The study area comprised of about 11,000 households with a total population of 50,000. Out of the total population, age range of 15 to 64 years comprised about 49%. Majority of the residents live on subsistence agriculture producing

mainly food crops. The center has one heath center and four health posts in the study area. There are two trained health extension workers in each kebele. The location map of the study area of initial survey is illustrated by figure 1 below. Data was extracted from January 22/07/2005 – 26/07/2005 E.C

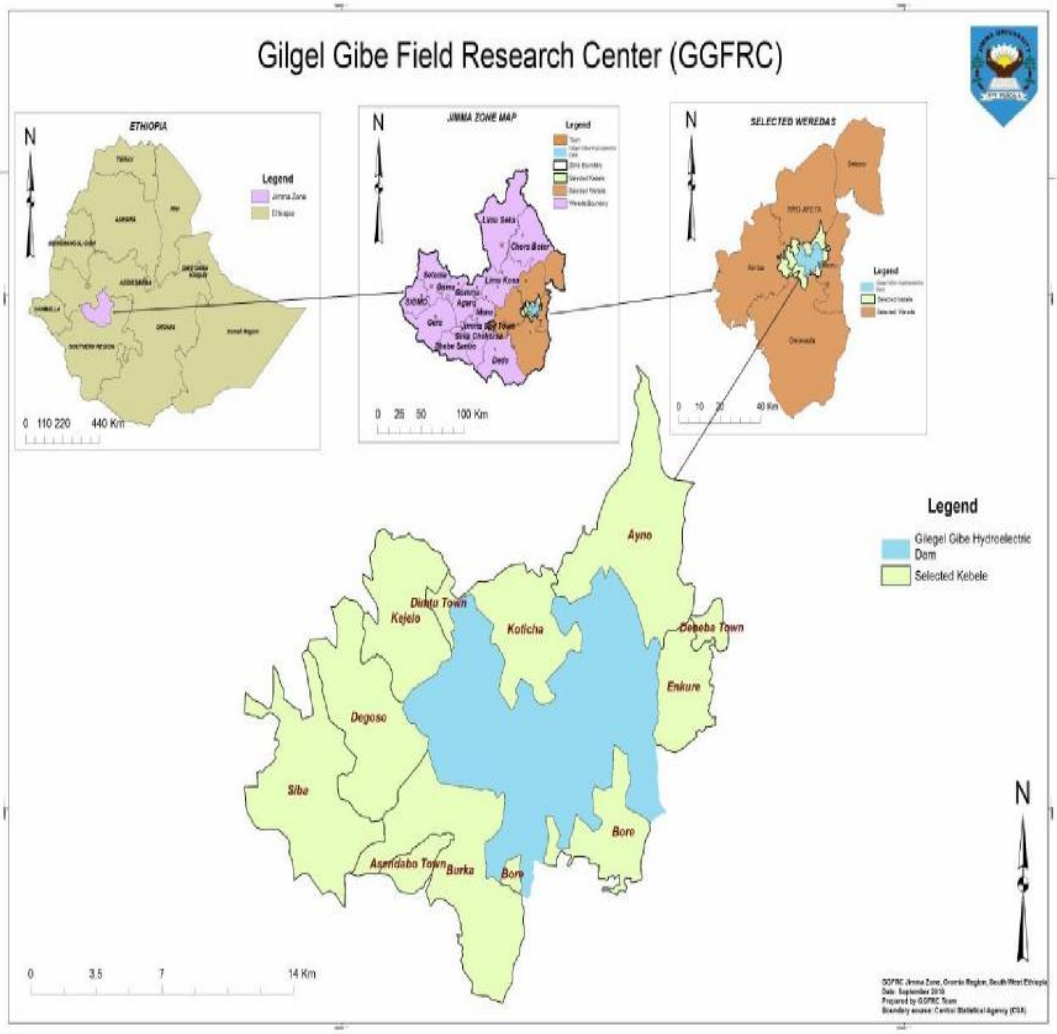

Figure 1: Location map of the study area: Gilgel Gibe Field Research Center [Source: Ref.56].

5.2 Study design

Community based cross sectional

5.3 Population

5.3.1 Source population

- All adults in the age group of 15 years and above residing in the GGFRC site.

5.3.2 Study population

- Subjects in this study consisted of sample of adult population in the age group of 15 years and above in GGFRC site recruited for biochemical reference intervals determination.

5.4 Sample size determination and Sampling techniques

5.4.1 Sample size determination

The sample size was determined, based on recommendations in the WHO.STEP wise surveillance manual, in each stratum of age, sex and residence. For both sexes, 250 individuals were taken from each age stratum giving a sample size of 2500. Further stratification of the study population into urban and rural within age and sex strata, the sample size was doubled to 5,000 and taking 10% non-response rate, the total sample size became 5,500 for step I and II. For step three (biochemical determination), 60% (3,300) of the sampled individuals for step I and II were selected by simple random sampling and included for blood sample collection as per WHO. STEPS manual recommendation (REFERENCE).

Although the initial survey was planned to include a total of 3,300 study participants in step III for biochemical determination, only a sample of 1861 blood samples was managed. Out of 1,861 participants aged 15 years or older, five adults with no serum aminotransferase tests , 210 with fasting blood glucose and 40 with no triglyceride measurements were excluded that left 1626 adults for analysis.

5.4.2 Sampling procedure

In the initial survey study participants were selected using the 2008 updated census list of the population and households of the ten kebeles were used as sampling frame. The sample size was allotted to urban and rural strata proportional to their size in a ratio of 25% to 75%, respectively. Furthermore, equal sample were allocated into each sex and age strata. Age was grouped to five strata, with an interval of ten years. Individual study subjects were then selected using simple random sampling technique [56].

5.5 Inclusion and exclusion criteria

5.5.1. Inclusion criteria

- In the initial survey participants who were apparently health, permanent resident of the study area were included.
- In the current analysis both male and female age 15years or older for whom biochemical tests available were included.

5.5.2. Exclusion criteria

- In the initial survey participants who were age below 15 years and above 64 years and acutely ill and disabled at the time of collection of blood samples were excluded.

Comment [FT1]: May not be necessary as this are expected to be available in the main document

5.6 Variables

5.6.1 Dependent variables

- Serum alanine aminotransferase
- Serum aspartate aminotransferase

5.6.2. Independent variables/ Main exposure variables of interest:

- Lifestyle factors such as :
  - Alcohol consumption
  - Cigarette smoking

- Level of physical activity (including intensity, duration, and frequency) such as;:
  - ⇨ Work related vigorous intensity activities causes breathe much harder than normal such as: carrying wood, sawing hardwood, cutting crops (sugar cane) and heavy gardening (digging)
  - ⇨ Work related moderate intensity activities causes breathe somewhat harder than normal such as : washing clothes by hand, milking cows, cooking, planting, harvesting crops, digging dry soil (with spade) and mixing cement
  - ⇨ Leisure/ spare time related moderate intensity activities causes breathe somewhat harder than normal such as: cycling, jogging, dancing, and horse-riding
  - ⇨ Leisure/ spare time related vigorous intensity activities causes breathe much harder than normal such as: soccer, rugby, tennis and fast swimming

- Physical measurements such as: weight, height, and waist, hip circumferences, systolic and diastolic blood pressure
- Biochemical parameters such as:
  - Total serum triglyceride
  - Total serum cholesterol
  - Serum fasting blood glucose

5.6.3 Other Covariates:

- Demographic variables: age, sex , residence
- Dietary habit:
  - Raw green leafy vegetables
  - Fruit such as : apple, banana, orange
- Medical history of :
  - Diabetes
  - Use of anti-diabetes agents
  - Family history diabetes mellitus
  - Heart attack (angina) diseases
  - Hypertension and use antihypertensive medications

5.7 Data collection Tools and Procedure

5.7.1 Data collection Tools

Interviewer administered structured questionnaires adapted from WHO STEPS manual was used to collect data. All study instruments were translated into local languages (Amharic and Afaan Oromo) by native speakers and then back translated to English by two other competent persons. The questionnaire for step I comprised questions about socioeconomic and demographic variables and questions for assessing behavioral/lifestyle risk factors for CNCDs including (cigarette smoking, alcohol drinking, dietary habit, khat chewing, and level of physical activity) and symptoms and history of CNCDs(such as diabetes, ischemic heart diseases etc.). The recording formats were used to record physical measurement values of Step II such as blood pressure (BP), pulse rate, weight, height, waist and hip circumference and standardized measuring instruments were used for physical measurements and standard laboratory equipment's and procedures were used for biochemical determination (such as fasting blood glucose level, total cholesterol level, total triglycerides,alanine aminotransferase, aspartate aminotransferase activity and other biochemical analytes). A detail of this issue was presented in the initial survey [36, 57].

Electronic Data was accessed after having the written permission from Jimma university ethical clearance committee and from the advisors after approval of the proposal from GGFRCs team . Data extraction template was used to extract data on socio-demographic variables, lifestyle/behavioral factors such as (smoking habits, alcohol consumption habit, and level physical activity), anthropometric data included height and weight, waist and hip circumferences, blood pressure (systolic and diastolic blood pressure), laboratory tests such as (alanine transaminase, aspartate transaminase, total cholesterol, total triglyceride and fasting blood glucose), medical history of diabetic mellitus, use of anti-diabetes agents, medical history ischemic heart diseases, medical history hypertension and use antihypertensive medications.

5.7.2 Data collection procedure

WHO Step I:

Face to face interview was conducted at home level after the interviewers explained the purpose of the study and obtained the participant's signed consent to participate in the study. Eligible respondents were declared unavailable if they were not found on three separate visits Socio-demographic variables and information on behavioral risk for CNCD were collected.

WHO Step II:

After completion of face to face interview, all respondents were given appointment for physical measurements. On the next morning physical measurements including blood pressure, pulse rate, weight, height, and waist and hip circumferences were measured following standard procedures. Three blood pressure (BP) readings were taken at a minimum of three minutes interval after the participant rested for 30 minutes. BP was measured in sitting position with the arm placed at the level of the heart mostly on right upper arm in mild flexion using the WHO recommended automatic BP monitor (Omron$^{(R)}$ HEM-711DLX intelliSense Bannockburn, Illinois, USA). Values of the three BP and pulse rate readings were recorded on respective recording formats. Height was measured using a Stadiometer (INVICTA Plastics Limited, England, Model 2007246) to the nearest 0.1cms while the participant stood still bare footed. Weight was measured to the nearest 0.1 kg with a calibrated portable digital weight scale (model 770; Seca, Germany) while the participant lightly clothed and shoes off. Waist and hip circumferences were measured to the nearest 0.1cms using measuring tapes. Waist circumference was measured in centimeters at the midpoint between the bottom of the ribs and the top of the iliac crest. Hip circumference was also measured in centimeters at the largest posterior extension of the buttocks.

WHO Step III:

Additionally those respondents who were selected for biochemical tests were given instructions for overnight fasting (not to eat or drink after 8:00 pm) and early morning appointment given. Whole venous blood sample was collected in the morning (8:00am to 12:00 noon) after cleaning the cubital area by 70% alcohol and stored in 3 ml vacutainer tubes. Then the sample was placed in ice-box and transported to the Jimma University specialized Hospital (JUSH) laboratory where the laboratory procedures are performed. Fasting blood glucose was determined on site immediately after sample collection using Glucometer (Sensocard, Hungary). Serum total cholesterol, triglycerides, alanine aminotransferase and aspartate aminotransferase were determined using Human star 80 (Gesellschaft fur Biochemica und Diagnostica, Germany) with specific reagents for each biochemical values as per the manufacturer's instructions. Six laboratory technicians trained on the purpose of the study, laboratory procedures and analysis, format completion and repository storage did the laboratory work within 12 hours of blood sample collection at Jimma University Specialized Hospital Laboratory. A detail of this issue was presented in the initial survey [36, 57]. For the current study analysis data was extracted by principal investigator using prepared data extraction template.

5.8 Data quality Assurance

Data were collected as per the WHO STEP protocol recommendation using standard pretested questionnaires and instruments. Data collectors and supervisors were trained on issues related to data collection techniques. Data collectors checked for data completeness and consistency before leaving each house.

Field supervisors also checked the completeness and consistency of the data on daily basis and they returned to interviewers if the data were incomplete and inconsistent. Interviewers re- administered the questionnaire to the respondent under supervision by the supervisors. Data were double entered by trained data clerks using EpiDdata version 3.1. Incomplete and inconsistent data identified during data entry were returned to the data collectors for rectification. Moreover, data were checked for completeness, inconsistency and outliers by looking at their distribution. Data were properly filed and stored both in electronic copies with back up and hard copies. Supervisors checked laboratory procedures randomly and each completed formats. During daily operation of

the Human star 80 clinical chemistry analyzer, blood chemistry control with normal and high values were run before actual sample analysis according to the manufacture's instruction. Moreover, standard operating procedures were followed for all laboratory procedures.

5.9. Data Management

Data on Step I, II and III was received by rewritable-CD. The files of each participant (questionnaire, body measurements, and biochemistry tests) were then merged using the participant identity number. After merging, data was cleaned, participants for whom biochemical test available were filtered. Lifestyle /behavioral factors, and biochemical tests were categorized as per the operational definition of the present study.

5.10. Data Analysis

The data extracted from the main study database that was entered into EpiData entry II was exported to SPSS for analysis. SPSS version 20 and Stata version 11 was used for data analysis.

Descriptive measures were computed as medians and inters- quartile ranges for continuous variables and proportions for Categorical variables. Separate analyses was conducted for ALT and AST levels.

Elevated aminotransferase levels `were defined as enzymes activity above the upper limit of normal (ULN), accordingly an elevated ALT was defined as ALT activity > 28 (IU/l) and elevated AST was defined as AST activity >31(IU/l [57]. Prevalence of elevated ALT and AST were calculated and 95% CIs were calculated using the binomial distribution. Chi-square test for trend was used to compare prevalence of elevated ALT and AST levels across age group.

Unavailable analysis: Binary logistic regression was used to evaluate relationship between lifestyle factors and biological factors and an elevated ALT and AST levels. Multiple logistic regression analysis was done to control confounding effects and to investigate factors independently associated with an elevated serum ALT and AST levels. To build the multivariable logistic regression model; variables that had statistically significant association with elevated ALT, and AST levels on univariable analysis at ($p < 0.25$) including the main exposure variables for study and a other covariates were included in the multiple binary logistic regression model. Before fitting multiple logistic regression correlation among main exposure variables was checked, none of the independent variables in this analysis were perfectly correlated; hence multicollinearity was not a problem. The association of a particular variable was expressed as odds ratio (OR) with a 95% confidence interval (CI) and a two tailed p-value < 0.05 was considered statistically significant.

5.11 Ethical Consideration

The proposal was presented to Medical Sciences Faculty research committee for assuring scientific integrity and human subjects' protection. The proposal was then submitted to the University's Research and Publication Office for final ethical clearance. The proposal was approved by Jimma University ethical review committee. Supportive letter was obtained from the university and given to the Jimma Zonal and to the four Woredas administrations. Two written consent formats were developed and used: one for interview and physical measurements and the second one for blood sample collection. Ethical clearance was obtained from Jimma University's Research and Publication Office. Signed informed consent was obtained from study participants before interview, physical measurements and blood sample collection. Individuals who had elevated BP or having indication of any of the CNCDs during the survey were referred to the nearest health center or hospital for further investigation and management. For the current analysis ethical clearance was obtained from the ethical review board of Jimma University College of Public Health and Medical Science to conduct this study. Additionally the secondary data was accessed from GGFRC through legal and official means.

Confidentiality was maintained by the principal investigator only accessing the data. Extracted data was stored in electronic copies with back up.

5.12 Dissemination of the Result

The finding of this study will be communicated to the stakeholders and target population through presentation for Jimma University community members, Jimma university research and publication office, and GGFRC department. Additionally copy of this material will be preserved in health institutions in the study area, Colleges, Jimma university library and publication office, College of Public Health and Medical Science, department of Epidemiology. Finally efforts will be made to publish results in national and international journal for dissemination worldwide.

5.13 Operational definition

Risk factors: refers to any attributable, characteristics or exposure of an individual, which increases the likelihood of developing a chronic non communicable diseases.

Body mass index (BMI): was calculated as weight in Kg divided by height in meter square. High BMI was defined as BMI $\geq$25 kg/m^2 [58].

Waist to hip ration (WHR): Was calculated as waist circumference (cm) dividing by hip circumference (cm). High WHR was defined as WHR >1 for male and >0.85 for female [58].

Hypertension (HTN): was defined as systolic blood pressure greater than or equal to 140mmHg or diastolic blood pressure greater than or equal to 90mmHg or people with normal blood pressure but taking antihypertensive drugs [58].

Alcohol use: participants who reported no alcohol use ever or no alcohol use in the last year were classified as nondrinkers, and served as the referent group. Those who reported any alcohol drink daily in the previous year were classified as daily drinkers.

Smoking Status: On the basis of self-reported history of smoking status, subjects were categories as

- ✓ **Current smoker**: was reported current smoking at the time of the survey
- ✓ **Past smoker**: was reported previous history of cigarette smoking but quit now
- ✓ **Nonsmoker:** has no history of smoking cigarettes [58].

Dietary habit: One serving of vegetable is considered to be 1 cup of raw green leafy vegetables, ½ cup of other vegetables (cooked or chopped raw) or ½ cup of vegetable juice. One serving of fruit was considered to be 1 medium size piece of apple, banana or orange, ½ cup of chopped, cooked, canned fruit or ½ cup of fruit juice, not artificially flavoured. One standard serving equal to 80 grams [58]. The following indicators will be used:

•Low fruit and vegetable intake: participants' consuming less than 400 grams of vegetables and fruits per day OR Five servings of 80 grams each.

•Adequate fruit and vegetable intake: participants' consuming less than 400 grams of vegetables and fruits per day OR Five servings of 80 grams each

Level of physical activity: In accordance to WHO MET (Metabolic Equivalent) was used the analysis of physical activity. MET is defined as the ratio of the work metabolic rate the resting metabolic rate. One MET is defined as 1 kcal/kg/hour. Total physical activity (total METs) was calculated by combining the vigorous physical activities and moderate physical activities, 4 METs get assigned to the time spent in moderate activities, and 8 METs to the time spent in vigorous activities [59]. The companied METS was categorized as follow: Low: 0 – 600METS/week, medium: 600 – 2999 METS/week and high: ≥ 3000 METS/week.

Fasting blood glucose (FBG): Blood glucose estimation obtained from a subject who has undergone an overnight fast for at least (8-12) hours from any food or drink [58].

For the purpose of this study the following FBG categories were used:

- Normal if FBG <110 mg/dl
- Raised if FBG ≥ 110mg/dL

Diagnosis of diabetes: having history of physician diagnosed of diabetes or those who are on treatment during the survey or subjects their FBS≥126 mg/dL [60].

Cardiac disease: Individuals classified as having Cardiac disease **(**angina) were those who had a history of chest pain, set off by physical exertion, forcing them to stop or slow down when they were walking with subsequent relief, and located in the sternum or the left anterior chest and left arm reported during the last 12 months identified by the survey or having history of physician diagnosed [61].

Elevated aminotransferase levels: were defined as the result being above the upper limit of normal (ULN), accordingly elevated ALT was defined as ALT activity > 28 (IU/l) and elevated AST was defined as AST activity >31(IU/l) [57].

High total cholesterol: was defined as total cholesterol level ≥ 200 mg/dl and triglyceride levels were defined as ≥150 mg/dl [58].

CHAPTER FIVE: RESULT

5.1 Demographic, lifestyle, and biological characteristics of study participants

.

Among the 1626 participants, 824 (50.7%) were females and 802 (49.3%) were males. Of all respondents about 1531 (94.2%) were rural dwellers while the rest were urban. The median age was 40 years (SD=????): The highest proportion 405 (24.9%) was within the age group of 35-44 with the least proportion 249 (15.3%) being 15-24 years.

Overall 96.6% were classified as nondrinkers and only fifth three (3.3%) participants were reported current daily alcohol drinkers with males 34(4.3%) and that of females 19(2.4%). With respect to any tobacco product smoking, 85.7% study participants were classified as non smokers and among those who had the experience of smoking (224 (13.8%),), 158 (70.5%) were current smokers and 66(29.5%) were ex-smokers. The higher proportion of current smokers 146 (18.6%) and ex-smoker 54(6.9%) were observed among males with 142(19.0%) among rural males.

Majority of the study participant 978 (60.1%) were classified as having high level of physical activity, i.e., greater or equal to 3000MET-minute per week. Only 241 (14.8%) classified as low level of physical activity, i.e., less than 600MET-minute per week. With respect to body

mass index, vast majority, 1580 (97.2%) of the participants, had BMI <25Kg/m2 and with high BMI ≥ 25 Kg/m2, level, 30 (3.6%) of females and 16 (2.0%) males were categorized as having high BMI level (table 2).

Table 12 :The lifestyle, biological, and clinical characteristics among subjects aged 15 and above by residence and gender, in GGFRC, 2008/2009

Variables	***Urban***		***Rural***		***Total***		
	Males	Females	Males	Females	Males	Females	Total
	№(%)	№(%)	№(%)	№(%)	№(%)	№(%)	№(%)
Alcohol consumption							
No	30 (88.2)	35(87.5)	719(94.2)	726(98.1)	749(95.7)	761(97.6)	1510(96.6)
Yes	4(11.8)	5(12.5)	30(3.9)	14(1.9)	34(4.3)	19(2.4)	53 (3.3)
Smoking status							
Never	27(79.4)	36(90.0)	556(74.2)	720(93.8)	583(74.5)	756(96.9)	1339(85.7)
Ex-smoker	3(8.8)	3(7.5)	51(6.8)	9(1.2)	54(6.9)	12(1.5)	66(4.2)
Current smoker	4(11.8)	1(2.5)	142(19.0)	11(1.4)	146 (18.6)	12(1.5)	158(9.7)
Levels of physical activity£							
Low	6(15.4)	14(25.0)	63(8.3)	158(20.6)	69(8.6)	172(20.9)	241(14.8)
Moderate	5(12.8)	9(16.1)	127(16.6)	266(34.6)	132(16.5)	275(33.4)	407(25.0)
High	28(71.8)	33(58.9)	573(75.1)	344(44.8)	601(74.9)	377(45.8)	978(60.1)
Low fruits and vegetables intake							
No	3(8.8)	4(7.1)	116(15.6)	88(12.1)	119(15.3)	92(12.0)	211(13.7)
Yes	31(91.2)	36(64.3)	627(84.4)	639(87.9)	658(84.7)	675(88.0)	1333(86.3)
High BMI							
No	35(89.7)	54(96.4)	751(98.4)	740(96.4)	786(98)	794 (96.4)	1580(97.2)
Yes	4(10.3)	2(3.6)	12(1.6)	28(3.6)	16 (2)	30 (3.6)	46(2.8)

High WHR							
No	30(76.9)	16(28.6)	717 (94.0)	334(43.5)	747(93.1)	350(42.5)	1097(67.5)
Yes	9(23.1)	40(71.4)	46 (6.0)	434(56.5)	55(6.9)	474(57.5)	529(32.5)
Cholesterol level(mg/gl)							
<200	35(89.7)	43(76.6)	647(84.8)	681(88.7)	682(85.0)	724(87.9)	1405(86.5)
≥200	4(10.3)	13(23.2	116(15.2)	87(11.3)	120(15.0)	100(12.1)	220(13.5)
Triglyceride level(mg/dl)							
<150	35(89.7)	41(73.2)	624(81.8)	614(79.9)	659(82.2)	655(79.5)	1314(80.8)
≥150	4(10.3)	15(26.8)	139(18.2)	154(20.1)	143(17.8)	169(20.5)	312(19.2)
Fasting blood glucose level(mg/dl)							
<110	36(92.3)	52(92.9)	659(86.4)	675(87.9)	695(86.7)	727(88.9)	1430(87.9)
≥110	3(7.7)	4(7.1)	104(13.6)	93(12.1)	107(13.3)	97(11.8)	196(12.1)
Hypertension							
No	34(87.2)	49(87.5)	690(90.4)	703(91.5)	724(90.3)	752(91.3)	1476(90.8)
Yes	5(12.8)	7(12.5)	73(9.6)	65(8.5)	78(9.7)	72(8.7)	150(9.2)
Diagnosed for cardiac disease							
No	34(100)	39(97.5)	742(99.1)	726(98.1)	776(99.1)	765(98.1)	1541(98.6)
Yes	0(0)	1(2.5)	7(0.9)	14(1.9)	7(0.9)	23(2.8)	22(1.4)
Diagnosed for diabetes							
No	38(97.4)	55(98.2)	730(95.7)	746(97.1)	768(95.8)	801(97.2)	1571(96.6)
Yes	1(2.6)	1(1.8)	33(4.3)	22(2.9)	34(4.2)	23(2.8)	55(3.4)

1

[1] Abbreviation: GGFRC, Gilgel Gibe Field Research center, METs= Metabolic Equivalents, FBG= Fasting Blood Glucose, High BMI was defined as BMI ≥ 25 Kg/m2; High WHR was defined as WHR ≥1 for male and WHR ≥ 1 for female. **£**, Low level of physical activity (< 600 MET-minute/week), moderate level of physical activity (600 - 2999 MET-minute/week), high level of physical activity (≥3000MET-minute/week).

5.2 Prevalence of elevated alanine transaminase (ALT), and aspartate transaminase (AST) levels

The overall prevalence and 95% confidence interval of ALT, AST and both ALT and AST levels for the study participants were 37.8% (95%CI: 35% - 40%), 41.1% (95% CI: 39%, 43%), and 27.7% (95% CI: 25 %, 30%) respectively. The majority of subjects with an elevated measurements had a test result less than or equal to 2 times the upper limit of normal (ULN): 94.3% ALT; 95.7% AST (see figure 3 and 4 below).

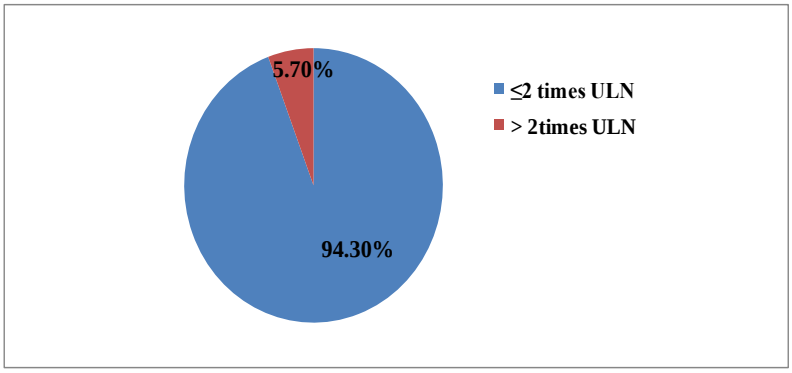

Figure 3: Shows pattern of elevation (in percentage) of alanine aminotransferase in study participants with elevated test result. Elevation indicate, ALT levels less than or equal to 2 times and greater than 2 times the upper limit of normal (ULN), in GGFRC, 2008/2009.

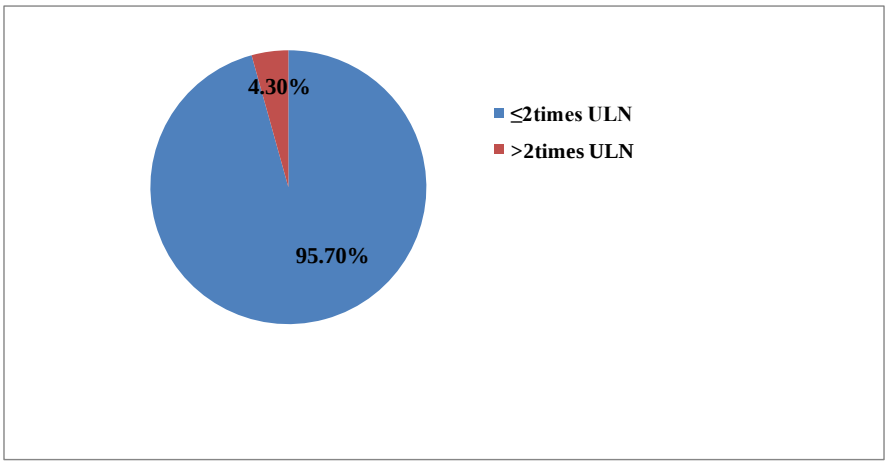

Figure 4: Shows pattern of elevation (in percentage) of aspirate aminotransferase in study participants with elevated test result. Elevation indicate, ALT levels less than or equal to 2 times and greater than 2 times the upper limit of normal (ULN), in GGFRC, 2008/2009

The sex specific prevalence of an elevated serum ALT, and AST levels were not different between males and females (36.9% vs. 38.6%, p-value for x^2 test = 0.484) and (40.0% vs. 42.1%, p-value for x^2 test = 0.393) respectively. Age specific prevalence of an elevated serum ALT, and AST level revealing a statistically significant decrease with increasing study-subject age by means of the x^2 trend test (p=0.009) and(p= 0.002) respectively. In addition, after stratifying data by age into one of five broad (age) groups, and sex ; the age-specified prevalence of an elevated serum ALT level revealed a significant inverse relationship with age when applying the x^2trend test (p= 0.035) for females study subjects but not so for male (p = 0.279); whereas the age-specified prevalence of an elevated serum AST level revealed a significant inverse relationship with age for both males and females (p-value for x^2 trend test = 0.032 and 0.023) respectively(see table3 and 4).

Table 2~~3~~: Gender- and age-specific prevalence of elevated serum alanine aminotransferase (ALT) level among subjects aged 15 and above in GGFRC, 2008/2009

ALT(IU/L)												
	Male				Female				Total			
Age	Valid test No	Elevated No (%)	95%CI for Prevalence of elevated	P-value £	Valid test No	Elevated No (%)	95%CI for Prevalence of elevated	P-value	Valid test No	Elevated No (%)	95%CI for Prevalence of elevated	P-value £
15-24	124	51(41.1)	(32.4,49.8)	0.279	125	57(45.6)	(36.8, 54.4)		249	108(43.4)	(37.2, 49.5)	0.009
25-34	152	58(38.2)	(30.4, 45.9)		163	65(39.9)	(32.3, 47.4)	0.035	315	123(39.0)	(33.6, 44.4)	
35-44	197	79(40.1)	(33.2, 46.9)		208	78(37.5)	(30.9, 44.1)		405	157(38.8)	(34.0, 43.5)	
45-54	146	44(30.1)	(22.7, 37.6)		164	66(40.2)	(32.7, 47.8)		310	110(35.5)	(30.1, 40.8)	
≥55	183	64(35.0)	(28.0, 41.9)		164	52(31.7)	(24.6, 8.9)		347	116(33.4)	(28.5, 38.4)	
Total	802	296(37)	(33.6, 40.3)		824	318(38.6)	(35.3, 41.9)		1626	614(37.8)	(35.4, 40.1)	

[2]

[2] **Abbreviation:** GGFRC, Gilgel Gibe Field Research center, ALT= Alanine aminotransaminase, IU= International Unit, CI= confidence Interval

Elevated ALT was defined as ALT activity >28 (IU/l)

£, p-value for x^2 test for trend

Table 34: Gender- and age-specific prevalence of elevated serum aspartate aminotransferase (ALT) level among subjects aged 15 and above in GGFRC, 2008/2009

Comment [FT2]: Do same

AST(IU/L)												
	Male(n=802)				Female(n=824)				Total (n=1626)			
Age group	Valid test №	Elevated AST № (%)	95%CI for Prevalence elevated AST	P-value£	Valid test(№)	Elevated AST No (%)	95%CI for Prevalence of elevated AST	P-value£	Valid test(№)	Elevated AST No (%)	95%CI for Prevalence elevated AST	P-value £
15-24	124	52(42)	(33.2, 50.6)	0.032	125	61(48.8)	(39.9, 57.6)	0.023	249	113 (45)	(39.2,51.6)	0.002
25-34	152	73(48)	(40.0, 56.0)		163	69(42.3)	(34.7,49.9)		315	142(45)	(39.6,50.6)	
35-44	197	83(42)	(35.2, 49.0)		208	93(44.7)	(37.9, 51.5)		405	176(43)	(38.6,48.3)	
45-54	146	45(31)	(23.3, 38.3)		164	67(40.9)	(33.3, 48.4)		310	112(36)	(30.8,41.5)	
≥55	183	68(37)	(30.1, 44.2)		164	57(34.8)	(27.4, 42.1)		347	125(36)	(30.9,41.5)	
Total	802	321(40)	(36.6, 43.4)		824	347(42)	(38.7, 45.5)		1626	668(41)	(38.7,43.5)	

1

Abbreviation: GGFRC, Gilgel Gibe Field Research center,AST= Aspartate aminotransferase IU= International Unit CI= confidence Interval

Elevated AST was defined as AST activity >31(IU/l)

£, p-value for x^2 test for trend

5.3 Univariable association of demographic, lifestyle, biological and clinical characteristics with an elevated aminotransferase levels

Association between the main exposure variables; lifestyle factors (such as smoking status, alcohol consumption, level of physical activity),biological factors (such as WHR, BMI, total cholesterol, triglyceride, and fasting blood sugar) levels , other covariates such as dietary habit and clinical factors (such as diabetes, hypertension, and angina) with an elevated ALT, and AST levels were examined using binary logistic regression. Univariable analyses showed smoking status, level physical activity, body mass index(BMI), waist to hip ratio (WHR), total cholesterol, and triglyceride were statistically significant association with an elevated ALT, and AST at $p<0.25$. The other variables did not show statistical significant association with either ALT or AST at p-value < 0.25 (Table 4).

Table 45: Univaraite analysis of associated factors for elevated serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels among subjects aged 15 and over in, GGFRC 2008/2009

		Elevated ALT			Elevated AST		
Variables		No (n=1012)	Yes (n=614)	Crude OR(95%CI)	No (n=958)	Yes (n=668)	Crude OR(95%CI)
Gender	Male	506	296	0.93(0.76 - 1.14)	481	321	0.92(0.75 - 1.12)
	female	506	318	1	477	347	1
Age group	15-24	141	108	1.53(1.09 - 2.13)*	136	113	1.48(1.06 - 2.06)*
	25-34	192	123	1.28(0.93 - 1.75)§	173	142	1.46(1.07 - 1.99)*
	35-44	248	157	1.26(0.93 - 1.70)§	229	176	1.37(1.02- 1.83)*
	45-54	200	110	1.09(0.79 - 1.51)	198	112	1.01(0.73 - 1.38)
	≥55	231	116	1	222	125	1
Smoking status	Never	843	496	1	790	549	1
	Ex-smoker	36	30	1.42(0.86 - 2.33)§	32	34	1.53(0.93 - 2.51)§
	Current smoker	100	58	0.99(0.70 - 1.39)	105	53	0.73(0.51 - 1.03)
Alcohol drinking	No	947	563	1	900	610	1
	yes	32	21	1.10(0.63 - 1.93)	27	26	1.42(1.02 - 2.46)
Levels of physical activity£	Low	130	111	1.51(1.13 -2.01)*	127	114	1.41(1.06 - 1.88)*
	Moderate	258	149	1.22(1.80 - 2.29)*	233	174	1.18(1.93 - 2.49)*
	High	624	354	1	598	380	1
Low fruits and vegetables intake	No	135	76	1	130	81	1
	Yes	831	502	0.92(0.69 - 1.26)	787	546	0.89(0.67 - 1.21)

BMI	< 25	992	588	1	939	641	1
	≥ 25	20	26	2.19(1.21 - 3.96)*	19	27	2.08(1.15 - 3.78)*
WHR	No	652	409	1	628	433	1
	Yes	360	205	1.35(1.09- 1.67)*	330	235	1.77 (1.43- 2.18)*
Cholesterol level(mg/dl)	<200	861	545	1	893	513	1
	≥200	97	123	1.46(1.11-1.97)*	119	101	2.00(1.50- 2.67)*
Triglyceride(mg/dl)	<150	841	473	1	804	510	1
	≥150	171	141	1.47(1.14 – 1.88)*	154	158	1.62(1.26 - 2.07)*
FBG(mg/dl)	< 110	884	538	1	841	581	1
	≥110	128	76	0.98(0.72 - 1.32)	117	87	1.08(0.80 - 1.45)
Diagnosis Diabetes	No	976	593	1	923	646	1
	Yes	36	21	0.96(0.56 - 1.66)	35	22	0.89(0.52 - 1.55)
Hypertension	No	916	560	1	870	606	1
	Yes	96	54	0.92(0.65 - 1.31)	88	62	1.01(0.72 - 1.42)
Cardiac disease	No	964	577	1	913	628	1
	Yes	15	7	0.78(0.32 - 1.92)	14	8	0.83(0.35 - 1.99)

4

Abbreviations: GGFRC, Gilgel Gibe Field Research center ALT=Alanine aminotransferase, AST= Aspartate aminotransferase, FBG= Fasting Blood Glucose, WHR= Waist to Hip Ratio, BMI= Body Mass Index, CI= Confidence Interval, METs= Metabolic Equivalents, OR=Odds Ratio

Elevated ALT was defined as ALT > activity 28 (IU/l) , Elevated AST was defined as AST activity > 31(U/l), High BMI was defined as BMI ≥ 25 Kg/m^2, High WHR was defined as WHR >1 for male and > 0.85 for female **;** £, Low level of physical activity (< 600 MET-minute/week), moderate level of physical activity (600 - 2999 MET-minute/week), high level of physical activity (≥3000MET-minute/week) §,P-value<0.25; ∗, p-value< 0.05

5.4 Multivariate analysis of risk factors for elevated ALT and AST

Upon adjustment for the main variables for the study and other covariate the only factors associated with elevated ALT levels in multivariate analysis were body mass index (BMI), waist to hip ratio (WHR), total cholesterol, and triglyceride. The odds of having an elevated ALT among participants who had high body mass index (BMI≥25kg/m^2) were 2.10 times compared to those with BMI (<25 kg/m2). Participants with high wait to hip ratio (WHR >1 for males and >0.5 for females) were 1.77 time more likely to have an elevated ALT compared to those with normal WHR. Those with high total cholesterol (≥ 200 mg/dl) had a higher tendency to have elevated ALT levels compared to those with < 200 md/dl (AOR= 1.48, with 95% CI:1.09 – 1.99. Raised triglyceride was significantly associated with elevated ALT levels (AOR=1.36, CI: 1.05 – 1.77). For an elevated AST levels body mass index (BM), waist to hip ratio (WHR),, total cholesterol level and triglyceride level were significantly associated with an elevated enzyme activity. Adults with high BMI (≥25kg/m2) had a higher risk of having elevated AST levels compared to those with BMI <25kg/m2; (AOR=1.99, 95% CI: 1.03 – 3.65). Having high WHR was associated with greater risk of elevated AST (AOR=1.28, 95% CI: 1.03 – 1.59) and also high total cholesterol (≥ 200 mg/dl) levels compared to those < 200 mg/dl (AOR=1.95, 95% CI: 1.43 – 2.65) .The odds of having elevated AST among those with raised triglyceride was 1.43 times higher compared to those with normal value (table 5).

Table 56: Multiple logistic regression analysis of factors associated with elevated Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), among subjects aged 15 and above in GGFRC, 2008/2009

	Elevated ALT		***Elevated AST***	
Variables	AOR(95%CI)£	p-value	AOR(95%CI)£	p-value
Age group				
15-24	1.75 (1.23- 2.49)	0.002	1.75(1.23- 2.49)	0.002
25-34	1.41 (1.01- 1.96)	0.044	1.66(1.19 - 2.31)	0.003
35-44	1.35(0.98 - 1.86)	0.070	1.54(1.12 - 2.12)	0.009
45-54	1.23(0.88 - 1.71)	0.224	1.19(00.86 - 1.67)	0.295
≥55	1		1	
Smoking status				
Never	1		1	
Ex-smoker	1.49(0.89 - 2.48)	0.129	0.71(0.03 - 1.87)	0.140
Current smoker	1.18(0.82 - 1.68)	0.374	0.95(0.66 - 1.37)	0.796
Levels of physical activity				
Low	1.23(0.88 - 1.69)	0.222	1.06(0.77 - 1.48)	0.718
Moderate	0.98(0.76 - 1.26)	0.886	1.07(0.84 - 1.38)	0.572
High	1		1	
High BMI				
No	1		1	
Yes	2.10(1.14- 3.89)	0.018	1.94(1.03- 3.65)	0.041
High WHR				
No	1		1	
Yes	1.77(1.41- 2.22)	0.015	1.28(1.03-1.59)	0.026
Total cholesterol (mg/dl)				
<200 mg/dl	1		1	
≥ 200 mg/dl	1.48(1.09- 1.99)	0.010	1.95(1.43 - 2.65)	0.001
Triglyceride (mg/dl)				
<150 mg/dl	1		1	
≥150 mg/dl	1.36(1.05- 1.77)	0.003	1.43(1.10 - 1.87)	0.006

5

Abbreviations: GGFRC, Gilgel Gibe Field Research center, ALT=Alanine aminotransferase, AST= Aspartate aminotransferase, WHR= Waist to Hip Ratio, BMI= Body Mass Index, CI= Confidence Interval, METs= Metabolic Equivalents, AOR= Adjusted Odds Ratio. **£**, adjusted for all variables in the model. Elevated ALT was defined as ALT > activity 28 (IU/l) , Elevated AST was defined as AST activity > 31(U/l)

CHAPTER SIX: DISCUSSION

The aims of the analysis was to determine the prevalence of elevated ALT, and AST levels and to examine their relationship with lifestyle factors (smoking status, alcohol consumption, level of physical activity), and biological factors (BMI, WHR, total cholesterol levels, triglyceride levels, and fasting blood glucose levels) among adult population. The overall prevalence of ALT, AST, and both AST and ALT were 37%, 41.1% and 27.7% respectively. Sex specific prevalence of elevated ALT was found 38.6 % for female and 36.9% for males; whereas the prevalence of elevated AST was 42.1% for female, and 40.0% for males. Age- specific prevalence of an elevated serum ALT and AST level revealing a statistically significant decrease with increasing study-subject ($p=0.009$ and $p= 0.002$) respectively.

The prevalence of an elevated serum ALT and AST levels appeared to vary according to the results of different studies conducted in different countries [35, 40, 41]. This difference might be largely due to the difference in the distribution of various risk factors, the prevalence of different liver diseases in populations, the definitions of normal and abnormal results, and methodological assay used to determine these enzymes activity. To the best of our knowledge there were no published data that addressed the prevalence of elevated aminotransferase (ALT and AST) in the general population in the Ethiopia; it is hard to directly compare the overall prevalence of elevated aminotransferase in the present study with publication from developed countries.

Among adults aged 15 years and over significant and independent association was observed between the independent factors body mass index, waist to hip ratio, total cholesterol level, triglyceride level and an elevated with the outcome variables ALT and AST. We found an inverse association between physical activity and ALT and AST in univariable analysis .However after adjustment the association was attenuated. An inverse association between physical activity and serum aminotransferase levels has been reported from the Shanghai population-based cohort study among men [48]. George A and his colleagues evaluate effect of a lifestyle

intervention (physical activities) on 152 patients with abnormal liver enzymes and metabolic risk factors by randomizing subjects to a moderate (6 sessions/10 weeks), low intensity (3 sessions/4 weeks) lifestyle counseling intervention and control group (no physical activity intervention found that likelihood of elevated alanine aminotransferase (ALT) levels in both moderate and low-intensity groups was reduced by over 70% compared to controls [49].

Participants with high body mass index (BMI) and waist to hip ratio (WHR) were much more likely to have elevated ALT, and AST compared to those normal (BMI and WHR) in the present study, which was also comparable to results of previous studies in adults [48, 52]. In the present study association between an elevated ALT and BMI and WHR somewhat stronger than the association of an elevated AST and BMI and WHR. Increasing evidence showed that abdominal adiposity has a direct influence on health, and waist circumference and /or waist to hip ratio is a reliable indicator of visceral fat and central adiposity, which correlates with health risks to a greater extent than does adipose tissue in other regions of the body because of high association between the visceral fat and insulin resistance [50, 51]. Visceral fat accumulation related with nonalcoholic fatty liver disease (NAFLD) and an increased ALT concentration which is more liver specific than AST, even within the reference interval, was an independent predictor of incident NAFLD [31]. This finding highlights the importance of greater central or general adiposity as a risk factor for potential liver damage.

High total cholesterol and raised triglyceride were independently associated with elevated ALT, and AST in the present study. Available literature showed elevated aminotransferase levels (ALT and AST) are useful indicators of non-alcoholic fatty liver disease in the group of people with metabolic disorders such as hypercholesterolemia and hypertriglyceridemia [33, 35]. The finding of thisstudy was found to be consistent with a cross-sectional study conducted in Iran among adult army personnel that found, elevated serum alanine aminotransferase have significant association with the components of metabolic syndrome such as raised triglyceride level (≥150 mg/dl), and raised total cholesterol (≥240 mg/dl [57]. And another cross sectional study done in China found positive association between raised triglyceride level and an elevated ALT and AST level [55].

CHAPTER SEVEN: STRENGTH AND LIMITATION OF THE STUDY

The strength of this study is use of large sample size and data was collected using standardized measuring instruments in the initial survey. Although I have been able to examine the associations of several characteristics of study participants with elevated aminotransferase levels in general population there are limitations in the interpretation of these findings resulting from the absence of particular data items from the initially collected data. Particularly the absence of data on, HIV status of the participants CD4 count, serologic tests for viral hepatitis, medical history of liver disease, (all of which may affect liver function) means that observed associations might be confounded by these variables. As with any cross-sectional study it is not possible to infer anything regarding causality from these results.

CHAPTER Eight : CONCLUSION AND RECOMMENDATION

Conclusions

In the present high body mass index (BMI), high waist to hip ratio (WHR), high total cholesterol and raised triglyceride were independently associated with elevated alanine aminotransferase and aspartate aminotransferase.

Recommendations

The effects of body mass index, waist to hip ratio, total cholesterol, and total triglyceride should be taken into consideration in the clinical interpretation of these enzymes. A central issue related to the management of obesity-related chronic diseases concerns laboratory screening and diagnosis. It is also suggested to include liver function tests in routine health checkup and elevated ALT and AST be considered as an alarming sign for development of chronic disease.

REFERENCE

1. Bekele T. Clinical Chemistry II Lecture Note Series. 2004,Jimma, Ethiopia.
2. Limdi JK & Hyde GM. Evaluation of abnormal liver function tests: Postgrad Med J 2003; 79:307–312.
3. Pratt DS & Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med. 2000; 342: 1266–71.
4. Hall Ph, & Cash J. What is the Real Function of the Liver 'Function' Tests? Ulster Medical Journal, 2012; 81(1):30-36.
5. Cohen JA, Kaplan MM. The SGOT/SGPT ratio-an indicator of alcoholic liver disease. Dig Dis Sci. 1979; 24: 835–8.
6. Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating non-alcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol. 1999; 94: 1018–22.
7. Schindhehn RK, Dekker TM, Nijpels G, Stehouwer CD, Bouter LM, Heine R, et al. Alanine aminotransferase and the 6-year risk of the metabolic syndrome in Caucasian men and women: the Hoom Study: Diabetic medicine 2007; 24:430-435.
8. Kim HC, Kang DR, Nam CM, Hur NW, Shim JS, Jee SH, et al. Elevated Serum Aminotransferase Level as a Predictor of Intracerebral Hemorrhage: Journal of Stroke, 2005; 36:1642-1647.
9. Arndt V, Brenner H, Rothenbacher D, Zschenderlein B, Fraisse E. Elevated liver enzyme activity in construction workers: prevalence and impact on early retirement and all-cause mortality: Int Arch Occup Environ Health, 1998; 71: 405 - 412.
10. Herder C, Karakas M, & Koenig W. Biomarkers for the Prediction of Type 2 Diabetes and Cardiovascular Disease: Journal of Clinical Pharmacology & Therapeutics, 2011; 90 (1):52-66.
11. Wakabayashi H, Nishiyama Y, Ushiyama T, Maeba T, Maeta H. Evaluation of the effect of age on functioning hepatocyte mass and liver blood flow using liver scintigraphy in preoperative estimations for surgical patients: comparison with CT volumetry. J Surg Res 2002; 106: 246–53.
12. Kuo C-J, Tsai S-Y, Liao Y-T, Conwell Y, Lee W-C, Huang M-C, et al. Elevated Aspartate and Alanine Aminotransferase Levels and Natural Death among Patients

with Methamphetamine Dependence, 2012, PLoS ONE 7(1): e29325. doi:10.1371/journal.pone.0029325

13. Skelly MM, James PD and Ryder SD. Findings on liver biopsy to investigate abnormal liver function tests in the absence of diagnostic serology. J Hepatol. 2001; 35: 195–199.
14. Daniel S, Ben-Menachem T, Vasudevan G, Ma CK, Blumenkehl M. Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. Am J Gastroenterol. 1999; 94: 3010–3014.
15. Sattar N, McConnachie A, Ford I, Gaw A, Cleland S, Forouhi NG, et al. Serial metabolic measurements and conversion to type 2 diabetes in the west of Scotland coronary prevention study: Specific elevations in alanine aminotransferase and triglycerides suggest hepatic fat accumulation as a potential contributing factor: Diabetes 2007; 56:984-91.
16. Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, Cristanini G, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med. 2000; 132:112–117.
17. Ioannou GN, Weiss NS, Boyko EJ, Kahn SE, Lee SP. Contribution of metabolic factors to alanine aminotransferase activity in persons with other causes of liver disease. Gastroenterology, 2005; 128:627–635.
18. Rehm J, Gmel G, Sempos CT, and Trevisan M Alcohol-related morbidity and mortality: Alcohol Res Health, 2003; 27:39–51.
19. Rimm EB, Williams P, Fosher K, Criqui M, & Stampfer MJ .Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors: BMJ, 1999; 319:1523–1528.
20. World Health Organization. Global status report on no communicable diseases 2010. Geneva, 2011.
21. Katkov W N, Friedman LS, Cody H, Evans A, Kuo G, Choo QL, Houghton M,et al. Elevated serum alanine aminotransferase levels in blood donors: the contribution of hepatitis C virus. Annals of Internal Medicine, 1991; **115**, 882–884.
22. Wannamethee SG, Shaper AG. Cigarette smoking and serum liver enzymes: the role of alcohol and inflammation. Ann Clin Biochem 2010, 47:321–326.

23. Kaido T, Honda Y & Kitamura K. Association between liver dysfunction and hyperglycemia in Japanese male workers at printing and papermaking plants: Journal of Occupational Health, 2002; 44: 301–306.
24. Jang ES, Jeong SH, Hwang SH, Kim HS, Ahn SY, Lee J, et al. Effects of coffee, smoking, and alcohol on liver function tests: a comprehensive cross-sectional study: Gastroenterology, 2012; 12:145.
25. Rigla M, Sanchez-Quesada JL, Ordonez-Llanos J, et al. Effect of physical exercise on lipoprotein (a) and low-density lipoprotein modifications in type 1 and type 2 diabetic patients. Metabolism. 2000;49: 640-647.
26. Church TS, Kuk JL, Ross R, Priest EL, Biltoft E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. Gastroenterology 2006; 130:2023–2030.
27. Sreenivasa BC, Alexander G, Kalyani B, Pandey R, Rastogi S, Pandey A, et al. Effect of exercise and dietary modification on serum aminotransferase levels in patients with nonalcoholic steatohepatitis. Journal of Gastroenterol Hepatol, 2006; 21:191-198.
28. Marchesini G, Brizi M, &Bianchi G. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome, 2001;Diabetes 50:1844–1850
29. Omagari K, Kadokawa Y, Masuda J, Egawa I, Sawa T, Hazama H, et al. Fatty liver in non-alcoholic non-overweight Japanese adults: Incidence and clinical characteristics: Journal of Gastroenterol Hepatology, 2002;17:1098-1105.
30. Qureshi I, Shabana A & Fareeha A. Effect of overweight and obesity on liver function in a sample from Pakistani population: Pakistan J. Zool. 2006; 38(1): 49-54.
31. Chang Y, Ryu S, Sung E & Jang Y. Higher Concentrations of Alanine Aminotransferase within the Reference Interval Predict Nonalcoholic Fatty Liver Disease: Clinical Chemistry, 2007*; 53*(4): 686–692
32. Olak E, Pap D, Majkic-Singh N, & Obradovic I. The association of obesity and liver enzyme activities in a student population at increased risk for cardiovascular disease: Journal of Medical Biochemistry, 2013; 32: 26–31.
33. Ruhl C E, Everhart J E. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States population : Gastroenterology 2003; 124(1): 71–9.

34. World Health Organization. The world health report 2002: Reducing risks, promoting healthy life. World Health Organization, Geneva, 2002.
35. Fraser A, Longnecker MP, & Lawlor DA. Prevalence of elevated alanine aminotransferase among United State adolescents and associated factors: NHANES 1999-2004: Gastroenterology, 2007; 133(6): 1814–1820.
36. Alemseged F, Haileamlak A, Muluneh TA, Tessema F, Woldemichael K, Asefa M, et al. Risk factors for chronic no communicable diseases in Southwest Ethiopia: A population based study. Ethiop J Health Sci, 2012; 22(special issue): 19-28.
37. Prati D, Taioli E, Zanella A et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002; 137: 1-9.
38. Kaplan MM. Alanine aminotransferase levels: what's normal? Ann Intern Med. 2002; 137: 49-51.
39. Kim HC, Nam CM, Jee SH, Han KH, Oh DY, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver disease: prospective cohort study. BMJ 2004 doi/10.1136/bmj.38050.593634.63.
40. Pendino GM, Mariano A, Surace P, Caserta CA, Fiorillo TM, Amante A, et al. Prevalence and Etiology of Altered Liver Tests: A Population-Based Survey in a Mediterranean Town: Hepatology 2005; 41:1151-1159.
41. Liu C-M, Tung T-H, Liu J-H, Lin C-H Hsu, C-T, Chou P, et al. A community-based epidemiological study of elevated serum alanine aminotransferase levels in Kinmen, Taiwan: World J Gastroenterol 2005; 11(11):1616-1622.
42. Mekonnen Y, Damecha G & Ambaye Ch. Establishing ranges of clinical normal limits and comparison with adopted limits for adult population: Ethiop. J. Health Dev. 1997; 11(2):97-101
43. Piton A, Poynard T, Imbert-Bismut F, Khalil L, Delattre J, Pelissier E, et al. Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. Hepatology 1998; 27(5): 1213–9.
44. Zhang H, Mei He S, Sun J, Wang CY, Jiang YF, Gu Q, et.al. Prevalence and Etiology of Abnormal Liver Tests in an Adult Population in Jilin, China: International journal of medical science, 2011; 8(3):254-262.

45. Alatalo P, Koivisto H, Puukka K, Hietala J, Anttila P, Bloigu R, et al. Biomarkers of liver status in heavy drinkers, moderate drinkers and abstainers. Alcohol, 2009; 44:199-203.
46. Khawaja S, Mahmood K & Munshi A. Risk assessment of alcohol and obesity on liver enzymes: Journal of Health, 2012; 4(7): 436-441.
47. Loomba R, Bettencourt R, Barrett-Connor E. Synergistic association between alcohol intake and body mass index with serum alanine and aspartate aminotransferase levels in older adults: The Rancho Bernardo Study: Aliment Pharmacol Ther. 2009; 30(11-12): 1137–1149.
48. Villegas R, Xiang YB, Elasy T, Cai Q, Xu W, Li H, et al. Liver enzymes, type 2 diabetes mellitus, and metabolic syndrome in middle-Aged, urban Chinese men: Journal of metabolic syndrome and related disorders, 2011; 9(4): Pp. 305-311.
49. George A, Bauman A, Johnston A, Farrell G, Chey T, & George J. Effect of a lifestyle intervention in patients with abnormal liver enzymes and metabolic risk factors: Journal of Gastroenterology and Hepatology, 2009; 24:399–407.
50. Janssen I, Heymsfield SB, Allison DB, et al. Body mass index and waist circumference independently contribute to the prediction of non-abdominal, abdominal subcutaneous and visceral fat. Am J Clin Nutr 2002; 75:683–8.
51. Taylor RW, Jones IE, Williams SM, et al. Evaluation of waist circumference, waist-to-hip ratio, and the conicity index as screening tools for high trunk fat mass, as measured by dual-energy X-ray absorptiometry, in children aged 3–19 y. Am J Clin Nutr 2000;72: 490–5.
52. Bedogni G, Miglioli L, Battistini N, Masutti F, Tiribelli C, & Bellentani S. Body mass index is a good predictor of an elevated alanine transaminase level in the general population: hints from the Dionysos study: Digestive and Liver Disease, 2003; 35:648–652
53. Ruhl CE & Everhart JE. Joint Effects of Body Weight and Alcohol on Elevated Serum Alanine Aminotransferase in the United States Population: Clinical Gastroenterology and Hepatology, 2005; 3:1260–1268.
54. Iravani S, Sabayan B, Sedaghat S, Heydari ST, Javad P, Lankarani KB, et al. The association of elevated serum alanine aminotransferase with metabolic syndrome in a

military population in southern Iran: Iranian Cardiovascular Research Journal, 2010; 4 (2):74-80.

55. Zhang H, Mei He S, Sun J, Wang CY, Jiang YF, Gu Q, et.al. Prevalence and Etiology of Abnormal Liver Tests in an Adult Population in Jilin, China: International journal of medical science, 2011; 8(3):254-262.
56. Jimma University. Overview of the research process, study area and study population. Chronic illnesses survey and biological profile for evidence base practice in medicine and public health: The Gilgel Gibe Field Research center Experience, Southwest Ethiopia: Ethiop Journal of Health Science, 2012; 22(Special Issue):3-5.
57. Woldemichael K, Haileamlak A, Muluneh TA, Alemseged F, Tessema F, Asefa M, et al. Biochemical profile Gilgel Gibe Field Research Center, Southwest Ethiopia. Ethiopia Journal of Health Science, 2012; 22 (special issue): 50-60.
58. World Health Organization. Chronic diseases and health promotion. STEP wise approach to surveillance (STEPS) 2005. STEPS Manual. Available at: http://www.who.int/chp/steps. (accessed, Dec. 2012)
59. World Health Organization. Global Physical Activity Questionnaire (GPAQ) analysis guide. Surveillance and Population-Based Prevention Department of Chronic Diseases and Health Promotion: (WHO), 1211.
60. World health organization. Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO consultation. Part 1. Diagnosis and classification of diabetes mellitus. Geneva, World Health Organization 1999. Available from: http://whqlibdoc.who.int/hq/1 999/WHO_n cd_ncs_99.2.pdf (accessed December 2012)
61. Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina - summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). J Am Coll Cardiol. 2003; 41 (1): 159-68.

Annex (I): Conceptual Frame work for the study

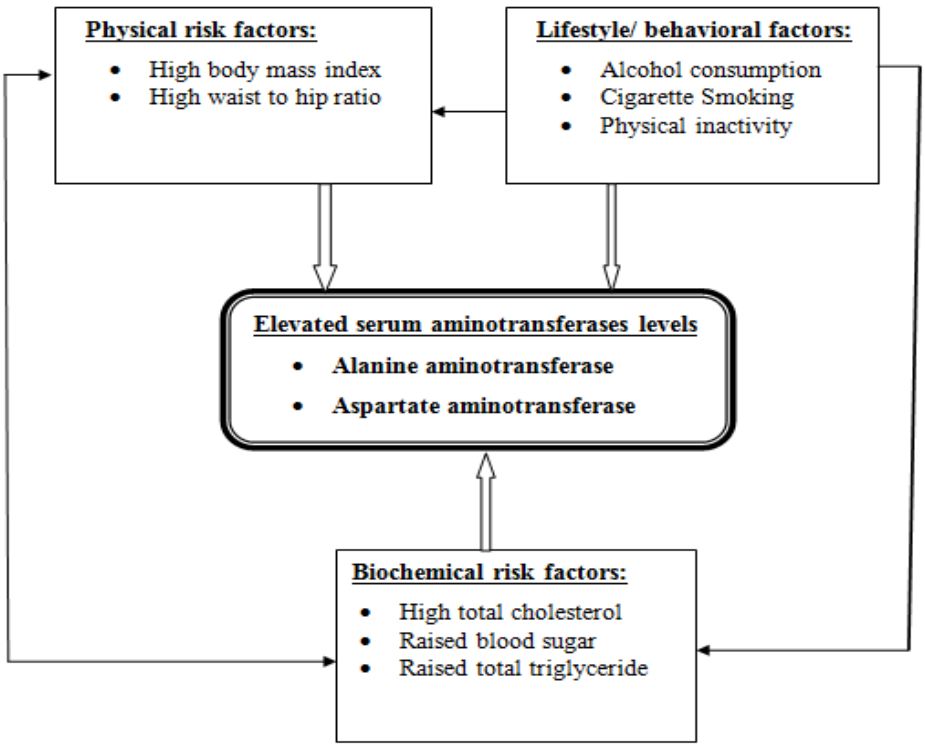

Figure: Shows conceptual framework developed after literature review for the assessment of relationship between major risk factors for chronic noncommunicable diseases and elevated serum aminotransferases levels**.**