“ESTIMATION OF AMINOTRANSFERASE LEVELS IN NORMAL INDIVIDUALS IN RELATION TO BODY MASS INDEX, AGE AND SEX - A CROSS SECTIONAL STUDY”

REG NO. BG0111005

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAUM, KARNATAKA

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ENDORSEMENT

This is to certify that the dissertation entitled **"ESTIMATION OF AMINOTRANSFERASE LEVELS IN NORMAL INDIVIDUALS IN RELATION TO BODY MASS INDEX, AGE AND SEX - A CROSS SECTIONAL STUDY"** is a bonafide research work done by **THE CANDIDATE REG NO. BG0111005**

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LIST OF ABBREVIATIONS USED

ADA	-	American Diabetes Association
AGA	-	American Gastroenterological Association
ALT	-	Alanine aminotransferase
AST	-	Aspartate aminotransferase
AUC	-	Area under curve
BMI	-	Body mass index
CI	-	Confidence interval
e.g.	-	For example
FBS	-	Fasting blood sugar
GGT	-	Gamma-glutamyltransferase
HBsAg	-	Hepatitis B surface antigen
HBV	-	hepatitis B virus
HCV	-	hepatitis C virus
HFE	-	hemochromatosis
i.e.	-	That is
IFCC	-	International Federation of Clinical Chemistry
Kg	-	Kilogram
LDH	-	Lactate dehydrogenase
LDL	-	Low density lipoprotein
m	-	Meter

mg	-	Milligram
n	-	Total number
NAFLD	-	Nonalcoholic fatty liver disease
NASH	-	Nonalcoholic steatohepatitis
NHANES	-	National Health and Nutrition Examination Survey
OR	-	Odds ratio
p	-	Probability
PPBS	-	Post prandial blood sugar
SCE	-	Scandinavian Committee on Enzymes
SD	-	Standard deviation
SGOT	-	Serum glutamic oxaloacetic transaminase
SGPT	-	Serum glutamic pyruvic transaminase
U/L	-	Upper limit
ULN	-	Upper limit of normal
USG	-	Ultrasonography

ABSTRACT

Background and objectives

BMI has a strong influence over aminotransferases levels. Both ALT and ALT, the two variables show the greatest contributions to the total BMI variance. The present study was aimed to examine variation in the aminotransferases levels in relation to body mass index, age and sex.

Methodology

This one year cross sectional study was conducted at the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on a total of 100 healthy individuals attending the blood bank/ executive health check up.

Results

In this study, 55% of individuals were males and 45% were females with male to female ratio of 1.2:1. The 31 to 40 and 41 to 50 years age group was comprised of 34% each and the mean age of the study population was 34.81 ± 8.54 years. Body mass index of < 18.5 and 18.5 to 24.9 Kg/m^2 was noted in 36% of the individuals each. The mean BMI of the study population was 22.69 ± 4.03 Kg/m^2. The mean ALT and AST levels in males were 25.69 ± 9.56 and 27.00 ± 10.00 U/L compared to 21.00 ± 8.75 and 21.38 ± 9.08 U/L in females ($p < 0.050$). It was observed that, the mean ALT levels in participants with BMI <18.5 Kg/m^2 were significantly low (13.75 ± 3.16 U/L) compared those with BMI between 18.5 to 24.99 Kg/m^2 (24.44 ± 3.90 U/L) and with > 25 Kg/m^2 (35.11 ± 5.22 U/L) ($p<0.001$). Also, AST levels were also significantly low (14.00 ± 3.50 U/L) in

participants with BMI < 18.5 Kg/m^2 compared those with BMI between 18.5 to 24.99 Kg/m^2 (25.14 ± 4.04 U/L) and > 25 Kg/m^2 (37.07 ± 3.90 U/L) ($p<0.001$).

Conclusion and interpretation

Overall, the present study showed that, mean ALT and AST levels significantly varied with gender and body mass index but, no significant difference was observed among different age groups. The mean AST levels significantly varied in individuals with different grades of BMI according to age whereas the same was not true with ALT levels.

Keywords

Alanine aminotransferase; Aminotransferases levels; Aspartate aminotransferase; Serum glutamic pyruvic transaminase; Serum glutamic pyruvic transaminase.

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Chapter 1

Introduction

INTRODUCTION

Aminotransferases are enzymes produced mainly in the liver. When serum activity is measured, it provides a marker of hepatic disease. Cut-off levels that define abnormality are rather arbitrary and this decreases the specificity of the test in apparently healthy patients. A small, but important, group of patients with aminotransferases abnormality have underlying liver disease that may be treatable. Most can be diagnosed based on history, physical examination, and biochemical-serological profiles.[1]

Alanine aminotransferase was originally referred to as serum glutamic pyruvic transaminase (SGPT). Normally, a low level of ALT exists in the serum. ALT is increased with liver damage and is used to screen for and/or monitor liver disease. It is usually measured concurrently with AST as part of a liver function panel to determine the source of organ damage.

Conditions associated with very high levels of aminotransferases are liver damage (acute viral hepatitis, toxins/drugs including acetaminophen overdose, acute fulminant hepatitis) and tumor necrosis. Moderately high levels of aminotransferases is seen in chronic liver disease, alcohol abuse, cholestasis, heart damage (heart attack, heart failure), kidney damage, muscle injury, hemolysis, heatstroke (level dependent on extent of tissue damage) and high consumption of vitamin A. While mild to moderate levels of aminotransferases can be seen in fatty change in the liver, alcohol abuse, cirrhosis, mononucleosis and drugs intake of statins, aspirin, barbiturates, HIV medication, herbs.[3]

Alanine aminotransferase is usually measured concurrently with AST as part of a liver function panel to determine the source of organ damage. ALT is more specific for liver damage since it is found primarily in the liver and has a longer half-life, whereas AST is found in many other organs. Liver diseases in which AST is higher than ALT include alcohol-induced liver damage, cirrhosis, and liver tumors. ALT catalyzes the transfer of an amino group from alanine to a-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate, that is, glutamate + pyruvate ⇌ α-ketoglutarate + alanine. The synthesis of ALT is dependent on vitamin B6 (pyridoxal phosphate) and will be decreased in the setting of low vitamin B6 and cirrhosis.[1]

Ethanol intake, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection and drug consumption are known risk factors for elevated ALT.[4] Cigarette smoking has also been proposed as risk factor for elevated ALT. On the contrary, coffee may play a protective role for elevated ALT, especially in subjects drinking ethanol.[5]

Obesity is considered a major problem for public health in the 21st century. It is a major factor influencing economy not only because of correlated disease burden, but also through obesity limiting capacity of some individuals to work. Obesity is commonly considered to be a result of the lifestyle of overnutrition and under exercise. Based on this concept, measures aimed at limiting the increase in adiposity have failed to reverse the trend. Normal biological variation in nutritional physiology and thus in individual responses to food intake and energy expenditure is largely ignored. It can be argued that

obesity has two sets of causes: 1) purely lifestyle-related and 2) biological variations.[6]

There is natural variation among individuals in metabolic processes such as caloric requirements, energy expenditure, nutrient absorption, processing of nutrients and appetite regulation in response to the variation in neurohormonal regulation of the central nervous system. Moreover, certain metabolic anomalies, such as diabetes type II, predispose to adiposity. The prevalence of individuals with metabolic problems may be increasing as the variation in human biological characteristics is increasing under conditions of relaxed selection. This process has been documented for anatomical variations and anomalies,[7,8] while with respect to metabolic variation it remains hypothetical.[6]

Strategies of providing public with general advice on how to control body weight treat all people the same way and due to varying levels of comprehension of messages and poor compliance with diets and exercise regimes, largely fail. It seems that targeting groups-at-risk, and giving them individual attention, may be more effective.[9] Thus, to introduce such individualized strategies, it is imperative to understand what individually variable factors increase risk of obesity.[6]

One of possible approaches to the study of individual variation in the obesity risk is to investigate co-variation of adiposity with a number of biological characters while controlling for cultural and socioeconomic differences in a large sample of young people of the same age.[6]

It is postulated that, liver function indicators influence strong variation in BMI. Both ALT and cholesterol, the two variables show the greatest

contributions to the total BMI variance, and are related to the liver function including links to metabolic pathways of gluconeogenesis and glycolysis and thus to the energy processing in the body.[6]

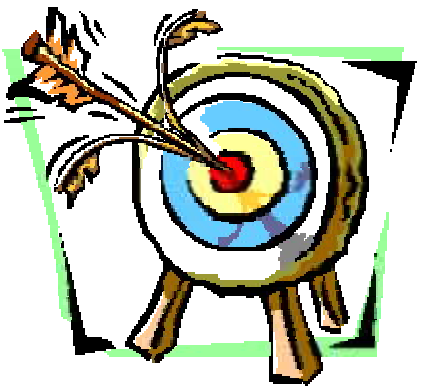
Obesity and overweight have been found to be important risk factors for elevated serum ALT and related to nonalcoholic fatty liver. Recent studies suggest the association between insulin resistance and ALT value.[10] A high ALT value is also believed to be a risk factor for diabetes.[11] Men are more likely to experience an abnormal serum ALT value than women and studies show that the proportion declines in older patients.[12,13]

However, many recent studies also argue that obesity is not all that bad, which is also known as "obesity paradox". For example, some studies suggest that obese people with chronic heart failure tend to have a higher survival rate and a lower chance of cardiovascular diseases.[14] Some ongoing studies also claim that uncomplicated obesity does not compromise the health of the heart.[15]

So, the levels of aminotransferases need to be updated in view of the above mentioned variables. Several studies have proved the variations in Aminotransferases levels in relation to BMI. Owing to little discussion about the relationship between obesity and ALT available in literature,[16] variation in the ALT levels from race to race and region to region[17, 18] and lack of relevant data in Indian subjects this study was proposed to examine variation in the aminotransferases levels in relation to body mass index, age and sex.

Chapter 2

Objectives



OBJECTIVES

The objectives of the study were to compare the variation in the aminotransferases levels in relation to body mass index, age and sex.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

The liver is a major intra abdominal organ and play a vital role in body's metabolism. The functions of liver can be divided as synthetic, metabolic, and detoxification. The synthetic functions are mainly synthesis of bile and major protiens in the body. The metabolic functions are maintaining normal glucose concentrations during fasting and postprandial periods. The processes of glycogenesis, glycogenolysis, glyconeogenesis, lipid metabolism, and insulin degradation all take place within the liver and lastly detoxicifation of drugs and toxic metabolites. Any insult to the liver by pathogenic, toxic or metabolic factors causes impairment in its function. Any injury thus leads to loss of hepatocytes and thus release of the intracellular enzymes and products into the blood. Thus measurement of these enzymes in the blood hints towards hepatocellular injury.[19]

The enzymes most commonly associated with liver injury are aminotransferases, gamma glutamyl transferase and lactate dehydrogenase, 5-nucleotidase. Among these, serum aminotransferases are most sensitive markers of hepatocellular injury. Aspartate aminotransferase (AST, or serum glutamic oxaloacetic transaminase [SGOT]) and alanine aminotransferase (ALT, or serum glutamic pyruvic transaminase [SGPT]) are categorized as the aminotransferases. The aminotransferases catalyze the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to form oxaloacetic acid and pyruvic acid, respectively, during gluconeogenesis. ALT is localized primarily in the liver and is confined to the cytoplasm, whereas AST can be isolated from the liver and a wide variety of extrahepatic sites, including myocardium, skeletal muscle,

kidney, brain, pancreas, and blood cells, in both cytoplasm and mitochondria. Normal serum ALT and AST levels correlate with body mass index.[19]

Elevated serum aminotransferase levels indicate hepatocyte injury or hepatocellular necrosis. The leakage of aminotransferases from hepatocytes as a result of hepatocellular injury can be triggered by various types of liver diseases, including viral hepatitis, ischemic injury, and toxin- or drug-induced hepatotoxicity. It is important to confirm the hepatic origin of an isolated serum AST elevation by checking the serum ALT level. In patients with isolated or disproportionate elevation of serum AST, an extrahepatic origin of AST, such as myocardial or skeletal muscle injury, may have to be excluded. The serum AST level can be raised several-fold by vigorous physical activity, including hiking, marathon running, weight lifting, and wrestling. Muscle-related diseases, such as rhabdomyolysis, muscular dystrophy, and polymyositis, can be associated with isolated elevation of the serum AST level. Rarely, patients with severe rhabdomyolysis can present with elevations of both serum AST and ALT. Serum aminotransferase levels can be within the normal range in patients with advanced fibrosis or cirrhosis. Patients with azotemia may have a falsely lowered serum AST level. Rarely, AST may form a macro-enzyme complex with albumin that results in persistent elevation of the serum AST level.[19]

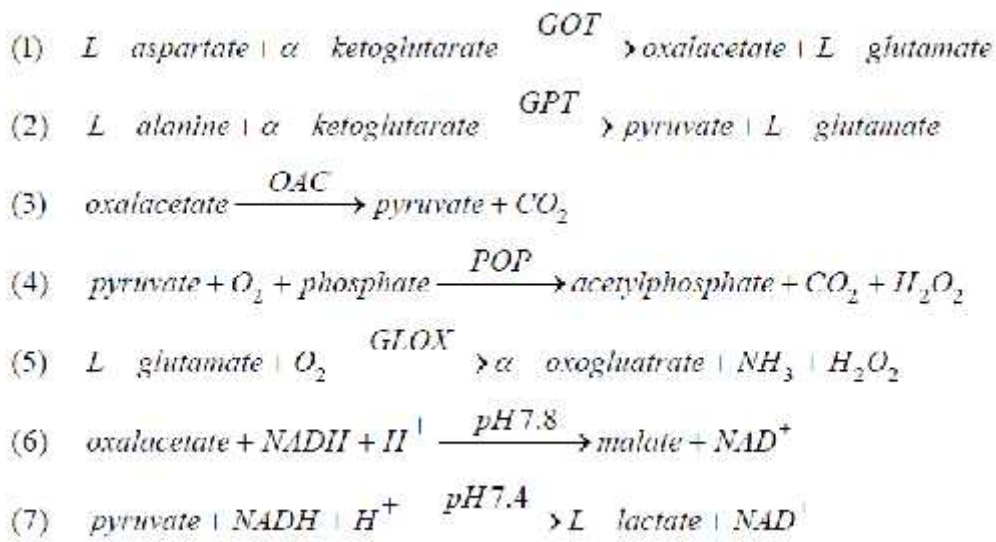
The degree of serum aminotransferase elevations can provide important diagnostic clues. Although there is no universal consensus as to how the degree of aminotransferase elevations should be categorized, a working definition has been proposed that categorizes the abnormalities as mild, moderate, and marked. In general, the majority of liver diseases are characterized by mild to moderate

elevations in serum aminotransferase levels (<500 U/L). In the Western world, nonalcoholic fatty liver disease (NAFLD) is the most common cause of mildly elevated serum aminotransferase values. Typically, serum aminotransferase levels are less than 300 U/L in patients with alcoholic hepatitis or biliary obstruction, although marked aminotransferase elevations can be transiently noted immediately after acute biliary ductal obstruction. The most common cause of marked aminotransferase elevations is acute viral hepatitis, followed in frequency by acetaminophen-induced hepatotoxicity; both disorders can result in aminotransferase levels higher than 1000 U/L.[19]

Patients with mild to moderate elevation of serum aminotransferases can be asymptomatic for long periods and thus chronic hepatocellular injury can be missed easily in such patients. This clinical scenario is common in patients with non alcoholic fatty liver disease (NAFLD). NAFLD is a liver pathology seen in patients with metabolic syndrome characterized by obesity, type 2 diabetes mellitus, hypertriglyceridemia.[19]

Quantitative analysis of liver enzymes is a sensitive method to detect hepatocellular injury in the absence of clinical symptoms and signs of liver disease; such an approach has emerged as a routinely used indicator of NAFLD. Also, ALT, AST, and gamma-glutamyltransferase (GGT) have been characterized as predictors of type 2 diabetes due to insulin resistance.[19]

AST and ALT are also biological catalyst. Therefore, the assay of AST and ALT activity all based on the following enzyme reactions included original [Eqs.(1) and (2)] and succeeding [Eqs.(3)-(7)] reactions.[20-22]



Where GLOX is glutamate oxidase, POP is pyruvate oxidase, and OAC is oxalacetate decarboxylase. Due to the clinical importance of AST/GOT and ALT/GPT in monitoring patients with liver diseases, AST/GOT and ALT/GPT detection have been researched by a number of scientists all over the world as well as International Federation of Clinical Chemistry (IFCC) and The Scandinavian Committee on Enzymes (SCE).[23]

According to the American Gastroenterological Association (AGA), 1 to 4 percent of the asymptomatic population may have elevated serum liver chemistries.[24] This is consistent with the usual definition of an elevated transaminase level of the top 2.5 percent of the population range. Although one study of 19,877 asymptomatic young Air Force trainees found that only 0.5 percent had elevated ALT levels, physicians who have more patients with obesity, diabetes, and hyperlipidemia will have to address this issue more often.[25]

Given the frequency of this problem, physicians should develop an informed approach to the investigation of transaminase elevations. An audit of

primary care practices found that these abnormalities are not always investigated appropriately and that opportunities to intervene in treatable cases sometimes are missed.[26]

No controlled clinical trials have compared approaches to the management of abnormal transaminase levels. However, the AGA recently published a technical review and a position statement on the evaluation of liver chemistry tests.[26]

ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. Their levels can be elevated in a variety of hepatic disorders. Of the two, ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver and in low concentrations elsewhere. AST has cytosolic and mitochondrial forms and is present in tissues of the liver, heart, skeletal muscle, kidneys, brain, pancreas, and lungs, and in white and red blood cells. AST is less commonly referred to as serum glutamic oxaloacetic transaminase and ALT as serum glutamic pyruvic transaminase.[26]

Although levels of ALT and AST can be extremely elevated (exceeding 2,000 U per L in cases of hepatocyte injury and necrosis related to drugs, toxins, ischemia, and hepatitis), elevations less than five times the upper limit of normal (i.e., about 250 U per L and below) are much more common in primary care medicine. The range of possible etiologies at this level of transaminase elevation is broader and the tests less specific. It also is important to recall that patients with normal ALT and AST levels can have significant liver disease in the setting of chronic hepatocyte injury (e.g., cirrhosis, hepatitis C).[26]

The ratio of AST to ALT has some clinical utility, but has important limitations. In many forms of acute and chronic liver injury or steatosis (fatty infiltration of the liver), the ratio is less than or equal to 1. This is particularly true in patients with hepatitis C. However, an AST/ALT ratio greater than 2 characteristically is present in alcoholic hepatitis.[26]

A recent study[27] of 140 patients with nonalcoholic steatohepatitis (NASH; confirmed by liver biopsy) or alcoholic liver disease found a mean AST/ALT ratio of 0.9 in patients with NASH and 2.6 in patients with alcoholic liver disease. Within the population studied, 87 percent of patients with an AST/ALT ratio of 1.3 or less had NASH (87 percent sensitivity, 84 percent specificity). The severity of NASH as measured by the degree of fibrosis increased, as did the AST/ALT ratio. A mean ratio of 1.4 was found in patients with cirrhosis related to NASH. Wilson's disease, a rare problem, can cause the AST/ALT ratio to exceed 4.While these ratios are suggestive of certain conditions, there is too much overlap between groups to rely on them exclusively when making a diagnosis.

Lactate dehydrogenase (LDH) is a less specific marker of hepatocellular necrosis and usually does not add diagnostic information to that obtained with ALT and AST testing. An exception to this is the transient but massive rise of LDH in cases of ischemic hepatitis and its sustained elevation that, along with elevated alkaline phosphatase levels, suggests malignant infiltration of the liver.[28]

Elevations of ALT and AST are not exclusive to liver pathology. Hyperthyroidism has been found in several studies to increase serum levels of

liver enzymes including ALT and AST.[29] Genetic influences on the level of ALT also are possible.

A study[30] of Danish twins showed that genetic factors accounted for 33 to 66 percent of the variation in ALT, gamma glutamyl transpeptidase, LDH, and bilirubin in patients 73 to 94 years of age. The AGA technical review[24] states that serum ALT has diurnal variation, may vary day to day, and may be affected by exercise. It also notes that serum AST may be 15 percent higher in black men than white men.

Another cause of elevated liver transaminase levels is muscle injury. Strenuous exercise or myopathy can cause elevations (especially of AST) without causing any other symptoms. A creatine kinase or other muscle marker can be obtained to confirm or exclude such a process.[26]

Annual screening of healthy, asymptomatic patients for liver disease using ALT and AST levels is not useful. A Japanese study[31] assessed the accuracy of ALT and AST for detecting hepatitis C, excess alcohol use, and fatty liver disease in male bank employees and found the positive predictive value of the test to be low. Only 3.9 percent of the men with an abnormal ALT level had hepatitis C; 8 percent were excessive users of alcohol; and 35.7 percent had fatty liver.

Aminotransferase levels are sensitive indicators of liver-cell injury and are helpful in recognizing hepatocellular diseases such as hepatitis. Both aminotransferases are normally present in serum at low levels, usually less than 30 to 40 U per liter. The normal range varies widely among laboratories. Some

researchers recommend adjusting aminotransferase values for sex and body-mass index, but these adjustments are rarely made.[4]

In fact, there is poor correlation between the degree of liver-cell damage and the level of the aminotransferases.[32]

The first step in the evaluation is to obtain a complete history in an effort to identify the most common causes of elevated aminotransferase levels: alcohol-related liver injury, chronic hepatitis B and C, autoimmune hepatitis, hepatic steatosis (fatty infiltration of the liver), nonalcoholic steatohepatitis, hemochromatosis, Wilson's disease, alpha1-antitrypsin deficiency, and a recently recognized cause, celiac sprue.[4]

It is more efficient to order all the blood tests in the first group initially, unless the history strongly suggests a definite diagnosis, such as alcohol abuse. The cause of the aminotransferase elevation can usually be identified on assessment of the pattern of the results of liver-enzyme tests and additional testing. The cause of an elevated alanine aminotransferase level varies greatly depending on the population studied. Among 19,877 Air Force trainees who volunteered to donate blood, 99 (0.5 percent) had elevated alanine aminotransferase levels.[25]

A cause for the elevation was found in only 12: 4 had hepatitis B, 4 had hepatitis C, 2 had autoimmune hepatitis, 1 had cholelithiasis, and 1 had acute appendicitis. In a group of 100 consecutive blood donors with elevated alanine aminotransferase levels, 48 percent had changes related to alcohol use, 22% had

fatty liver, 17% had hepatitis C, 4% had another identified problem, and in the remaining 9%, no specific diagnosis was made.[33]

In another study of 149 asymptomatic patients with elevated alanine aminotransferase levels who underwent liver biopsy, 56% had fatty liver, 20% had non-A, non-B hepatitis, 11% had changes related to alcohol use, 3% had hepatitis B, 8% had other causes, and in 2%, no cause was identified.[34]

A recent study assessed 1124 consecutive patients who were referred for chronic elevations in aminotransferase levels. Eighty-one of these patients had no definable cause of the elevation and underwent a liver biopsy. Of these 81 patients, 41 had steatosis, 26 had steatohepatitis, 4 had fibrosis, 2 had cirrhosis, and 8 had normal histologic findings. The patients with histologic evidence of fibrosis and cirrhosis also had evidence of fatty metamorphosis. None of the biopsies yielded a specific diagnosis except those showing steatosis and steatohepatitis.[35]

CAUSES OF ELEVATED AMINOTRANSFERASE LEVELS[4]

Etiology of ALT or AST Elevations When Less Than Five Times Normal

Common hepatic causes

Alcohol

Cirrhosis

Hepatitis B (chronic)

Hepatitis C (chronic)

Steatosis/steatohepatitis

Medications/toxins

Acute viral hepatitis

Less common hepatic causes

Autoimmune hepatitis

Hemochromatosis

$Alpha_1$-antitrypsin deficiency

Wilson's disease

Nonhepatic causes

Celiac disease

Hemolysis

Myopathy

Hyperthyroidism

Strenuous exercise

Macro-AST

Common Agents That Can Cause Liver Transaminase Elevations[26]

Medications	Herbal supplements/vitamins
Acetaminophen	Chaparral leaf
Amiodarone (Cordarone)	Ephedra
Amoxicillin-clavulanic acid	Gentian
Carbamazepine (Tegretol)	Germander
Fluconazole (Diflucan)	Jin bu huan
Glyburide (Micronase)	Kava
Heparin	Scutellaria (skullcap)

Clues in the Evaluation of Mildly Elevated Liver Transaminase Levels[26]

Clinical clue	Suggested diagnosis
Longstanding alcohol abuse	Cirrhosis
Intravenous drug use, history of blood product transfusions, nonsterile needle exposure, AST/ALT ratio < 1.0	Hepatitis B or C
Obesity, diabetes, hyperlipidemia, AST/ALT ratio < 1.0	Steatosis/steatohepatitis
AST/ALT ratio > 2.0	Alcoholic liver disease, Wilson's disease
Increased iron levels	Hemochromatosis
Polypharmacy, illicit drug use, or certain herbal supplement use	Substance/medication-induced
Frequent, strenuous exercise	Exercise-induced
Intestinal bloating; oily, bulky stools	Celiac sprue
Hypergammaglobulinemia	Autoimmune hepatitis
Reduced ceruloplasmin levels, Kayser-Fleischer ring	Wilson's disease
Depressed thyroid-stimulating hormone levels	Hyperthyroidism

Alcohol Abuse

The diagnosis of alcohol abuse can be difficult because many patients conceal information about their alcohol use. The diagnosis is supported by the finding of a ratio of aspartate aminotransferase to alanine aminotransferase of at least 2:1. In a study of hundreds of patients who had histologically confirmed liver disorders, more than 90 percent of the patients who had an aspartate aminotransferase:alanine aminotransferase ratio of at least 2:1 had alcoholic liver disease.[36]

The percentage increased to more than 96% when the ratio was greater than 3:1. The increased ratio reflects primarily the low serum activity of alanine aminotransferase in patients with alcoholic liver disease. This decrease is due to an alcoholrelated deficiency of pyridoxal 5-phosphate.

Measurement of *g*-glutamyltransferase may also be helpful in diagnosing alcohol abuse. A *g*-glutamyltransferase level that is twice the normal level in patients with an aspartate aminotransferase:alanine aminotransferase ratio of at least 2:1 strongly suggests the diagnosis of alcohol abuse. However, the lack of specificity of the *g*-glutamyltransferase level precludes its use as a single test to diagnose alcohol abuse. The degree of elevation of aminotransferase levels may also be helpful in identifying alcohol abuse. It is rare for the aspartate aminotransferase level to be more than eight times the normal value in patients with alcohol abuse, and it is even less common for the alanine aminotransferase level to be more than five times the normal value in such patients.37

In fact, the alanine aminotransferase level may be normal, even in patients with severe alcoholic liver disease.

Medication

A careful history-taking and meticulous review of laboratory data are critical for identifying a medication as the cause of elevated aminotransferase levels. A drug effect is a possibility if the increase in liver enzyme levels was associated with the initiation of a medication. Almost any medication can cause an elevation in liver enzyme levels. Common ones include nonsteroidal antiinflammatory drugs, antibiotics, antiepileptic drugs, inhibitors of hydroxymethylglutaryl–coenzyme A reductase, and antituberculosis drugs. In addition to medications, herbal preparations and illicit drugs or substances may cause elevations in liver enzyme levels.[4]

The easiest way to determine whether a medication is responsible for the elevation is to stop treatment and see whether the test results return to normal. If the identified medication is essential to the patient's well-being and no suitable substitute is available, the physician needs to make a risk–benefit analysis to determine whether the drug should be continued despite the elevation in aminotransferase levels. Often, consultation with a hepatologist is necessary. Occasionally, a liver biopsy is necessary to determine the nature and severity of liver injury.[4]

Chronic viral Hepatitis

Chronic hepatitis C is very common in the United States. Approximately 3.9 million Americans are positive for antibodies against hepatitis C, and an estimated 2.7 million people are considered to be chronically infected on the basis of the presence of hepatitis C virus RNA in serum.[38]

The risk of chronic infection is highest in patients with a history of parenteral exposure to the virus (e.g., because of blood transfusions, intravenous drug use, or work-related duties), cocaine use, tattoos, body piercing, and high-risk sexual behavior. The initial test for hepatitis C infection is serologic testing for the hepatitis C antibody. The testing has a sensitivity of 92 to 97 percent, depending on the assay.[39]

A positive test in a patient with risk factors for infection is sufficient to make the diagnosis, but the diagnosis is usually confirmed by measurement of serum levels of hepatitis C virus RNA with use of the reverse-transcriptase polymerase chain reaction. This approach is currently the gold standard for detecting hepatitis C infection.[39]

A positive test should prompt consideration of a liver biopsy to assess the severity of damage. Patients with chronic hepatitis C and evidence of fibrosis are usually treated.[4]

Hepatits B is another common cause of chronic viral hepatits. Initial tests for hepatitis B infection include serologic tests for hepatitis B surface antigen, hepatitis B surface antibody, and hepatitis B core antibody. A positive test for

hepatitis B surface antibody and core antibody indicates the presence of immunity to hepatitis B, and another cause for the elevated aminotransferase levels should be sought. A positive test for hepatitis B surface antigen and core antibody indicates the presence of infection. Tests to determine whether there is viral replication, including serologic tests for hepatitis B e antigen, hepatitis B e antibody, and hepatitis B virus DNA, should be undertaken. In patients with positive tests for hepatitis B virus DNA and hepatitis B e antigen, liver biopsy and treatment should be considered.[4]

Autoimmune Hepatitis

Autoimmune hepatitis occurs primarily in young to- middle-aged women.[40] The ratio of female patients to male patients is 4:1. The diagnosis is based on the presence of elevated aminotransferase levels, the absence of other causes of chronic hepatitis, and serologic and pathological features suggestive of the disease.[41]

A useful screening test is serum protein electrophoresis. More than 80% of patients with autoimmune hepatitis have hypergammaglobulinemia. However, a finding of more than twice the normal level of polyclonal immunoglobulins is most suggestive of the diagnosis. Additional tests that are commonly ordered include serologic tests for antinuclear antibodies, antibodies against smooth muscle, and liver–kidney microsomal antibodies. The first two tests have reported sensitivities of 28% and 40%, respectively.[42]

The third test is rarely positive among patients in the United States, Australia, and Japan.[41] The routine use of these three tests for the diagnosis of

autoimmune hepatitis is not recommended. A liver biopsy is essential to confirm the diagnosis.

Hepatic Steatosis and Nonalcoholic Steatohepatitis

The only clinical evidence of hepatic steatosis and a condition that may be associated with it, nonalcoholic steatohepatitis, may be mild elevations in aminotransferase levels. The levels are usually less than four times the normal value.[43,44]

In contrast to patients with alcohol-related liver disease, patients with nonalcoholic steatohepatitis usually have an aspartate aminotransferase:alanine aminotransferase ratio that is less than 1:1.[44,45]

Fatty infiltration of the liver can be identified by ultrasonography or computed tomography. Ultrasonography should be part of the evaluation of patients with chronically elevated aminotransferase levels. The diagnosis of nonalcoholic steatohepatitis requires a liver biopsy. In addition to fatty infiltration, the histologic findings in patients with nonalcoholic steatohepatitis include pericentral fibrosis, inflammation, liver-cell necrosis, and hyaline cytoplasmic inclusions in hepatocytes that are identical to Mallory's bodies, which are characteristic of alcoholic liver disease.[43]

The two conditions have different natural histories: steatosis appears to have a benign course, whereas nonalcoholic steatohepatitis can progress to cirrhosis.[46]

Liver failure as a result of nonalcoholic steatohepatitis is uncommon. Weight loss is the cornerstone of treatment in patients who are obese.[47]

Other treatments for nonalcoholic steatohepatitis that are being studied include vitamin E and ursodiol. Vitamin E was associated with decreases in alanine aminotransferase and aspartate aminotransferase levels and in histologic abnormalities in two pilot studies.[48,49]

Ursodiol decreased alanine aminotransferase and aspartate aminotransferase levels but not the histologic abnormalities in another pilot study.[50]

Hemochromatosis

Hereditary hemochromatosis is a common genetic disorder. Cost-effective screening starts with the measurement of serum iron and total iron-binding capacity. A transferrin-saturation value (obtained by dividing the serum iron level by the total iron-binding capacity) of more than 45% is suggestive of hemochromatosis.[51]

Measurement of serum ferritin provides less specific information, because it is an acute-phase reactant. If screening tests suggest the presence of iron overload, a liver biopsy should be performed to assess hepatic iron levels and the severity of liver damage. A hepatic iron index (the hepatic iron level in micromoles per gram of dry weight divided by the patient's age) of more than 1.9 is consistent with the presence of homozygous hereditary hemochromatosis.[51]

Genetic testing is now available to identify the mutation in the hemochromatosis (*HFE*) gene that causes the majority of cases. A liver biopsy is not necessary for patients with hereditary hemochromatosis who are younger than 40 years of age and who have normal liver function.[4]

Wilson's Disease

Wilson's disease, a genetic disorder of biliary copper excretion, may cause elevated aminotransferase levels in patients with no other symptoms of the disease. The clinical onset is usually between the ages of 5 and 25 years, but the diagnosis should be considered in patients up to the age of 40 years. The initial screening test for Wilson's disease is measurement of serum ceruloplasmin. The levels will be reduced in approximately 85 percent of affected patients. Patients should also be examined by an ophthalmologist for Kayser–Fleischer rings. If the ceruloplasmin level is normal and Kayser–Fleischer rings are absent, but the physician still suspects that Wilson's disease may be present, the next test is a 24-hour urine collection for a quantitative assessment of copper excretion. Excretion of more than 100 μg of copper per day is suggestive of Wilson's disease. The diagnosis is usually confirmed by liver biopsy to measure hepatic copper levels. Patients with Wilson's disease have hepatic copper levels of more than 250 μg per gram of liver, dry weight. Although the gene responsible for Wilson's disease has been identified, the number of disease-specific mutations is so great that molecular diagnosis is not yet feasible.[4]

Alpha 1-Antitrypsin Deficiency

Alpha1-antitrypsin deficiency is an uncommon cause of chronic liver disease in adults. Decreased levels of alpha1-antitrypsin can be detected either by direct measurement of serum levels or by the lack of a peak in *a*-globulin bands on serum protein electrophoresis. In affected patients, however, serum levels of alpha1- antitrypsin may be increased in response to inflammation, causing a false negative result. The diagnosis is best established by phenotype determination.[4]

Nonhepatic Causes

In a recent study, occult celiac sprue was the cause of chronically elevated aminotransferase levels in 13 of 140 asymptomatic patients who were referred for this reason to a liver clinic.[53] The diagnosis was made by measuring serum levels of antigliadin and antiendomysial antibodies. None of these patients had primary biliary cirrhosis, a liver disease that is occasionally found in patients with celiac sprue. On the basis of this study, we recommend testing for occult celiac sprue if other, more common causes of elevated aminotransferase levels have been ruled out.

Elevated serum aminotransferase levels, especially aspartate aminotransferase levels, may be caused by disorders that affect organs or tissues other than the liver, with the most common being striated muscle. Conditions or activities that can cause such elevations include subclinical inborn errors of muscle metabolism; acquired muscle disorders, such as polymyositis; and strenuous exercise, such as long-distance running. If striated muscle is the source of increased aminotransferase levels, serum levels of creatine kinase and aldolase

will be elevated to the same degree or to an even higher degree. Creatine kinase or aldolase levels should be measured if other, more common hepatic conditions have been ruled out.[4]

If, despite comprehensive testing, the cause of the elevation in aminotransferase levels remains unidentified, then a percutaneous liver biopsy may be indicated. If the alanine aminotransferase and aspartate aminotransferase levels are less than twice the normal value and no chronic liver condition has been identified, we recommend observation alone. Supporting this position are the results of two recent studies. The first study suggested that close clinical follow-up is the most cost-effective strategy for asymptomatic patients with negative tests for viral, metabolic, and autoimmune markers of liver disease and chronically elevated aminotransferase levels.[53]

The second study examined 36 patients with a chronic elevation (at least 50 percent above normal) of alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase levels.[54] Patients with strong evidence of a particular liver disease were excluded. All patients underwent liver biopsy. The results of liver biopsy led to a change in the diagnosis in only five patients and to a change in treatment in two patients.

If the alanine aminotransferase and aspartate aminotransferase levels are persistently more than twice the normal value, we recommend a biopsy. Although the results of the biopsy are unlikely to lead to a diagnosis or to changes in management, they often provide reassurance to the patient and the physician that no serious disorder is present.[4]

Variation of serum aminotransferase levels with body mass index, age and sex

Normal serum ALT in a selected population varies according to age, sex, and ethnic origin. Similar to other biochemical parameters, the normal level of serum ALT was determined in the 1950s and was then adjusted by biochemical laboratories based on testing the healthy population of both sexes.[55]

Previous studies have demonstrated that liver enzyme levels increase progressively with increasing BMI.[56]

In 2002, Prati et al.[57] in a study from Italy defined new normal levels for serum ALT in a healthy population by testing 6835 healthy blood donors, but these results have only been widely accepted over the past few years. In that study the serum ALT level were found to be directly related to sex and body mass index (BMI).

A study show that the increase is progressive from BMI values within the normal weight range and that risk for abnormal values is greatly increased for overweight and obese persons. In the United States, on the basis of analyses of the Third (1998 to 1994) National Health and Nutrition Examination Survey (NHANES), Ruhl and Everhart[58] and Clark et al[59] reported that BMI was strongly associated with the prevalence of abnormal ALT levels.

In NHANES data collected from 1999 to 2002, the prevalence of elevated ALT levels was more than double that of previously available estimates, whereas the positive association between BMI and elevated ALT was similar.[60]

Bedogni et al.[61] reported in the Dionysos study of 6315 adults in Northern Italy that overweight and obese participants had elevated ALT levels compared with those having BMI <24.9, OR=2.0 (95% CI=1.4-2.7), and OR=3.1 (95% CI=2.1-4.7), respectively. Among British women aged 60 to 79 years, Lawlor et al[62] found a linear association of BMI with ALT.

Previous studies[63-65] have found ALT levels to be more closely associated with BMI than AST levels.

In the Western New York Health Study, ALT was more highly correlated with BMI than AST, particularly among men and postmenopausal women.[64]

Burns et al[65] carried out 2 cross-sectional analyses in working men and women and also found a greater effect of BMI on ALT. For those gaining weight in the approximately 2 years between examinations, ALT rose slightly whereas AST did not.

In a population of Korean working men followed over 4 years, ALT was more strongly associated than AST with BMI at baseline, and change in BMI was more strongly associated with having an abnormal ALT on follow-up.[63]

Pattern of hepatic abnormalities associated with obesity in which ALT is greater than AST, which differs from the pattern of alcoholic liver disease.[4] Mechanisms have been proposed for this differential pattern of hepatic enzyme abnormalities, arising from obesity and alcohol consumption.[66]

Alcohol consumption is both a potential confounder and a modifier of the relationship between BMI and liver function abnormalities. Alcohol and obesity

are thought to cause liver enzyme elevations through different mechanisms, and effect modification is plausible.[66]

When Ruhl and Everhart[58] assessed interaction between alcohol consumption and body weight in determining serum ALT using NHANES III data, they found positive and significant interaction in an analysis adjusted for sex.

Bellentani et al[67] also reported findings consistent with effect modification by alcohol, but did not formally test for interaction.

The association between AST level and BMI is stronger in women than in men, even in nondrinkers.

In the Western New York Health Study, Stranges et al[64] found that the effect of BMI on ALT was greatest in premenopausal women, intermediate in men, and lowest in postmenopausal women.

Association between BMI and aminotransferase level varies according to age, with less evidence of an effect on aminotransferase levels among obese people over the age of 65 years.

In a 2005 study, Elinav et al[68] reported that ALT levels were not associated with BMI in older age groups. They suggested a nonlinear relationship of ALT levels with age, with a peak at 40 to 55 years of age.

Stranges et al[64] also reported that AST was associated with BMI only among premenopausal women, not among postmenopausal women. Our evidence provides support for the modification of obesity's effect by age.

In considering the applicability of the KCPS findings to other populations, we recognize that Asian populations generally have a higher percentage of body fat than do Western populations at the same BMI level. Asians generally have a slighter body build than whites and slighter people tend to have less muscle mass and connective tissue. Additionally, visceral adiposity tends to be relatively greater at a particular BMI among Asians compared with whites. The metabolic consequences of this fat distribution pattern may increase risk for complications of obesity, perhaps including hepatic injury. The findings of a study comparing levels of adiponectin in American and Japanese men support the hypothesis of differing risk of metabolic consequences of obesity in Asians versus non-Asians.[69-72]

Kadowak et al[72] examined levels of adiponectin in American (n=98) and Japanese (n=92) men, aged 40 to 49 years. Contrary to expectations, the researchers found that the American men had higher levels of adiponectin than the Japanese men, despite having a higher level of obesity. Their findings could reflect smaller amounts of visceral adipose tissue in American compared with the Japanese men. Because our population included only Koreans, we could not address whether obesity affects liver function to a greater degree than in whites.

These findings add to the growing evidence linking obesity and overweight to liver injury.

A study aimed to assess the range of value of serum alanine aminotransferase in healthy population and to assess the relationship between ALT level and body mass index (BMI), age and gender analyzed a large population of healthy blood donors--all of them were screened for ALT, weight and height. Patients were divided into four groups: I--patients with underweight, II--patients with normal weight, III--patients with overweight, IV--obese patients. In the studied population 862 persons were taken into account (820 men and 42 women), 19-62 years of age. The ALT level varied from 6 to 77 U/L, mean 27.39 U/L. Inadequate BMI was found in 12 persons, normal BMI in 497 persons, overweight in 270 persons and obesity in 83 persons. ALT and BMI are statistically significantly higher in men than in women. In general population and in men group we found correlations between ALT and BMI ($p = 0.0000$), between ALT and age ($p = 0.0000$). In women we did not find those dependences. ALT level was statistically significantly higher in groups with higher BMI: ALT level in group II was higher than in group I ($p < 0.024$), ALT level in group III was higher than in group III ($p = 0.0000$). We did not find any differences in ALT level between group III and IV. ALT level strongly correlates with body mass, age and gender. We suggest the necessity of taking into consideration those parameters in a clinical interpretation of ALT level.[73]

A study was conducted to identify the correlates of ALT activity among healthy medical students of Army Medical College, National University of Sciences and Technology, aged 18–22 years. The population included was 143 volunteer students (93 men and 50 women) selected on the basis of negative answers to a detailed medical questionnaire including past medical history, drug

and alcohol consumption, on the absence of clinical signs of liver disease, on the negativity of serological testing for Hepatitis B and C virus. The mean ALT level of the entire population was 28.7 IU/L. A major sex-difference in ALT value was observed, the mean ALT value being higher in men than in women (32.1±21.7 vs. 22.6±9.7 IU/L, $p<0.004$). According to WHO criteria for Asians, normal BMI was taken from 18.5–23.0 Kg/m2. There was a positive significant correlation between serum ALT level and BMI ($p<0.002$). ALT level strongly correlates with body mass index and gender. There was no significant variation in ALT levels among Punjabis and Sindhis, Balochis, Pathans, and Kashmiris. Authors suggested the need of taking into account these parameters in a clinical interpretation of ALT level.[74]

In 1995, Ramesh et al.[75] from India also showed lower levels of serum ALT compared to the reference Upper limit of normal (ULN) in normal blood donors and emphasized the importance of BMI-adjusted ALT levels among blood donors. In that study, four BMI categories were used for both sexes.

In 1998, Piton et al.[76] from France studied 1033 male and female blood donors and reported similar findings, but in that study the ULN was defined for females according to BMI as 32 U/L and 45 U/L for BMI <23 kg/m2 and ≥23 kg/m2, respectively, and for males according to BMI as 43 U/L and 66 U/L for BMI <23 kg/m2 and ≥23 kg/m2, respectively.

In 1999, Leclercq et al.[77] reported a significant correlation between serum ALT levels to BMI, sex, and age, with a mean ALT level of 21.8 U/L.

In the most recently published study by Kariv et al.[78] in which 17 496 normal people were evaluated in 2006, the mean serum ALT level was 37.5 U/L compared to a reference ULN of 52. Kariv et al.[78] found a significant effect of obesity and sex on ALT level.

A study carried out by Ramesh et al,[75] on 1,028 voluntary blood donors to see how body mass index (BMI) correlated with the serum alanine amino transferase (ALT) activity. The mean ALT (U/1) values were 19.35, 27.63, 40.79 and 54.41 in the four BMI categories of ≤20, 20.1–25, 25.1–30 and >30, respectively. This study showed that the mean serum ALT level of obese subjects (BMI > 30kg/m^2), compared with the two categories of normal subjects (i.e. BMI ≤ 20 and BMI = 20.1–25 kg/m^2), was increased by 2.8 and 1.96 times, respectively. Compared with the BMI group ≤ 20, there was a gradual per cent increase in the mean serum ALT in the three different BMI groups: 20.1–25 (+133%), 25.1–30 (+196%) and >30 kg/m^2 (+280%). This indicates the need to correct ALT values for BMI for blood donor screening, instead of using actual ALT values.

A study was carried out by Prati et al,[57] for updating the aminotransferases levels in healthy individuals, they found out Serum ALT activity was independently related to body mass index and to laboratory indicators of abnormal lipid or carbohydrate metabolism. Updated upper limits (for men, 500 nkat/L [30 U/L]; for women, 317 nkat/L [19 U/L]) were lower than current limits (for men, 667 nkat/L [40 U/L]; for women, 500 nkat/L [30 U/L]).

A study was done by Bedogni et al,[5] to establish the contribution of body mass index (BMI), sex, age, ethanol intake, hepatitis B (HBV) and hepatitis C (HCV) virus infection, coffee and drug consumption, and cigarette smoking to account for an elevated alanine transaminase (ALT) level in the general population. A total of 6315 adult subjects from the Dionysos study were included. Logistic regression was used to quantify the contribution of the variables of interest to elevated ALT, with the exception of coffee and drug consumption, were significant predictors of elevated ALT at univariable analyses. When significant predictors were employed in a multivariable model, age and cigarette smoking were no longer significant. The AUC was 0.77 (95% CI=0.74-0.80) for the multivariable model and 0.64 (95% Cl=0.60-0.68) for the univariable BMI model ($p<0.0001$ for the comparison).

Another study was conducted by Antonino et al,[79] to determine association between body mass index (BMI) and serum liver enzyme activity [GGT, ALT, AST)] in 3167 subjects. The subjects were examined during a program of preventive medicine. Analysis of covariance was used to compare the serum liver enzyme activities of the subjects, who were subdivided according to BMI, while also considering age, alcohol and cigarette consumption, and physical activity. In men, the percentage increase in the geometric mean of liver enzyme activity of the obese subjects compared with that of the normal subjects was 47.7% for GGT, 55.3% for ALT, and 19.7 for AST.

In a study conducted by Miguel et al,[80] on 1,036 consecutive blood donors to determine ALT levels and their association with gender, obesity, and hepatitis virus infection markers. In each donation aspartate- aminotransferase, lactate

dehydrogenase and gamma glutamyl transferase activity were also determined and body mass index (BMI) was calculated. 579 men and 457 women donated blood; ALT activity was 25.3± 14.5 IU/L for men and 16.3±7.9 IU/L for women. The upper normal value for men was 56 IU/L and 34 IU/L for women. On applying this value to the study group 4.8% of the men and 2% of the women had values greater than the cut- off. Among the men with increased ALT levels, 53.5% had a BMI >27, 7.1% also had an increased level of GGT and 7.1% had increased levels of AST and LDH. None of them were HBsAg nor anti-HCV positive. Among the women with increased ALT, 33.3% had BMI >27, 33.3% had increased levels of LDH and AST, and 11.1% were anti-HCV positive (only 1 donor). They concluded that different cutoff values should be considered for men and women. Factors such as obesity, may account for more than 50% of the cases with increased ALT values, indicating the low specificity of the test.

A study conducted by Sull et al.[81] showed across the range of BMI values (<18.5 to >or=32 kg/m) in men, alanine aminotransferase (ALT) was estimated to increase by 18.8 U/L and aspartate aminotransferase (AST) increased by 7.1 U/L. In women, ALT increased by 9.9 U/L, whereas AST increased by 4.5 U/L. In men, interactions between BMI and alcohol consumption were significant (P<0.001) for ALT and AST, but the degree of effect modification was quantitatively minor. However, ALT and AST levels were somewhat higher in heavy alcohol drinkers than in nondrinkers. For women, the relationship of aminotransferase levels with BMI did not vary by alcohol consumption. The relationship of BMI with aminotransferases weakened with increasing age.

Given the increasing prevalence of obesity and metabolic syndromes, BMI-based screening serum of ALT levels is needed. It will help to diagnose possible liver disease early in healthy individuals.

Standardized values for different laboratory methods of testing serum ALT and AST may be an important target for future studies so that different laboratories around the world can reflect similar or identical values.

Chapter 4

Methodology

METHODOLOGY

The present one year cross sectional study was conducted at the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Study design

The study design was a cross sectional study.

Study period and duration

The present study was conducted for the period of one year, from January 2012 to December 2012.

Place

This study was done in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, a teaching hospital attached to Jawaharlal Nehru Medical College, Belgaum.

Source of Data

Healthy individuals attending the blood bank / executive health check up after finding the suitability based on selection criteria were selected for the study.

Sample size

A total of 100 Healthy individuals attending the executive health check up were enrolled.

Sampling procedure

As this was a cross-sectional study, the effect sample size was calculated by using the following formula.

$n = 4 p q / d^2$

Where, n = Sample size

p = Number of healthy blood donors visiting the blood bank annually

q = 100 – p = 50%

d = Absolute error considered as 10%

Based on this formula the sample size was worked out as 100. Every third patient fulfilling the selection criteria was included in the study.

Selection criteria

Inclusion

- Healthy individuals attending executive health check-up.
- Aged between of 18 to 60 years.

Exclusion

- Age < 18 years.
- Alcoholics (>4 drinks/ day or 56gms/days as per National Institute of Alcohol abuse and Alcoholism).
- Diabetics (FBS>126mg/dl, PPBS>200mg/dl as per ADA guidelines 2010).
- Abnormal lipid profile (LDL< 120mg/dl; Cholesterol<200mg/dl).

- Patient with evidence of viral hepatitis.
- Patients with history of consumption of hepatotoxic drugs in past one month.
- Patients with history of hospitalization in past one month.

Ethical clearance

Prior to the commencement, the ethical clearance was obtained from Institutional Ethics and Research Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

Patients fulfilling the selection criteria were briefed about the nature of the study. The patients expressing their willingness to participate in the study were enrolled after obtaining a written informed consent (Annexure I).

Method of collection of data

Demographic data such as age and sex, detailed history was obtained through an interview. A thorough physical examination was conducted followed by systemic examination and the findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

The patients were evaluated for the tests.

- Liver function tests – AST and ALT levels
- Fasting and postprandial blood sugar

- HBsAg
- HCV
- Lipid profile
- USG abdomen

Outcome variables

Body mass index

Body mass index was calculated based on formula;

$$\text{Body Mass Index} = \frac{\text{Weight (Kg)}}{\text{Height}^2 \text{ (m)}}$$

Body mass index in the range of less than 18.5 kg/m^2 were considered as underweight, 18.5 to 24.9 kg/m^2 were considered as normal, 25.0 to 29.9 kg/m^2 were considered as overweight.

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet (Annexure III). The categorical data was expressed as rates, ratios and proportions. The continuous data was expressed as mean ± standard deviation (SD) and median with range (minimum to maximum). The comparison of AST and ALT levels in different sexes was done using unpaired 't' test while one way ANOVA was used to compared three or more mean values. A probability value ('p' value) of less than or equal to 0.050 was considered as statistically significant.

Chapter 5

Results

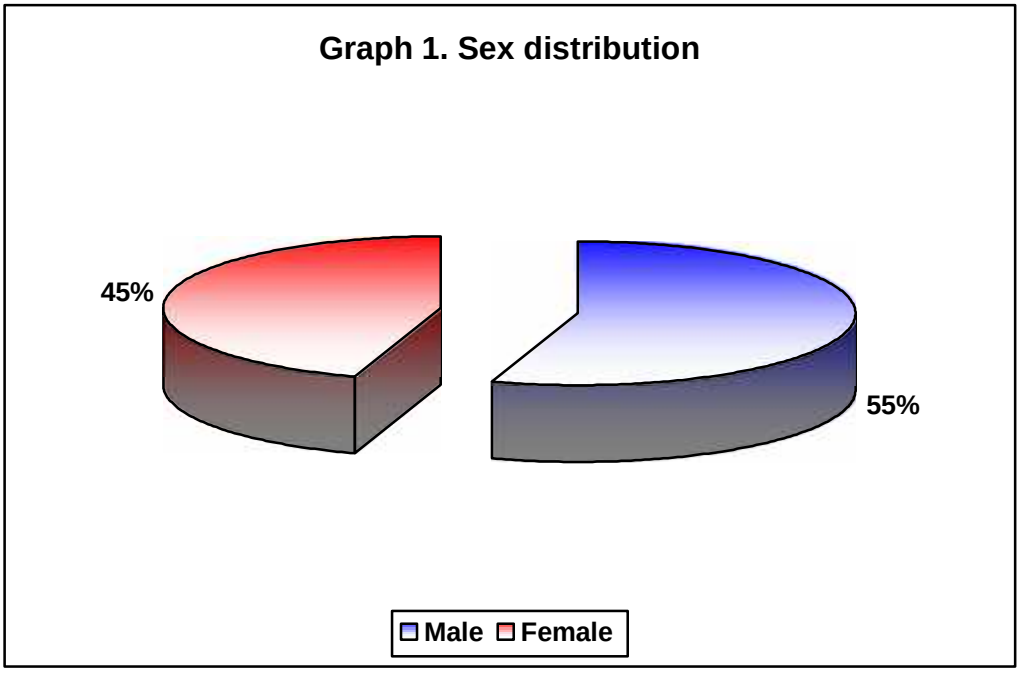
RESULTS

This one year cross sectional study was done on a total of 100 healthy individuals attending the blood bank / executive health check up at the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

The data obtained was coded and entered into Microsoft Excel Worksheet and the data was analysed. The final results and interpretations were tabulated as below.

Table 1. Sex distribution

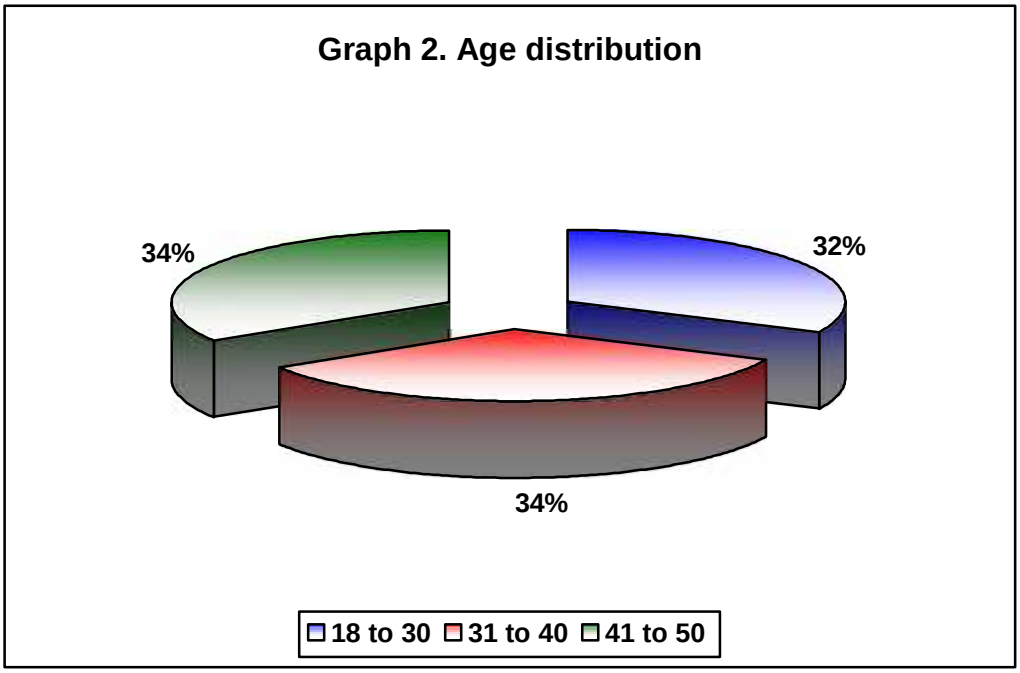
Sex	Distribution (n=100)	
	Number	**Percentage**
Male	55	55.00
Female	45	45.00
Total	**100**	**100.00**



In the present study 55% of individuals were males and 45% were females. The male to female ratio was 1.2:1.

Table 2. Age distribution

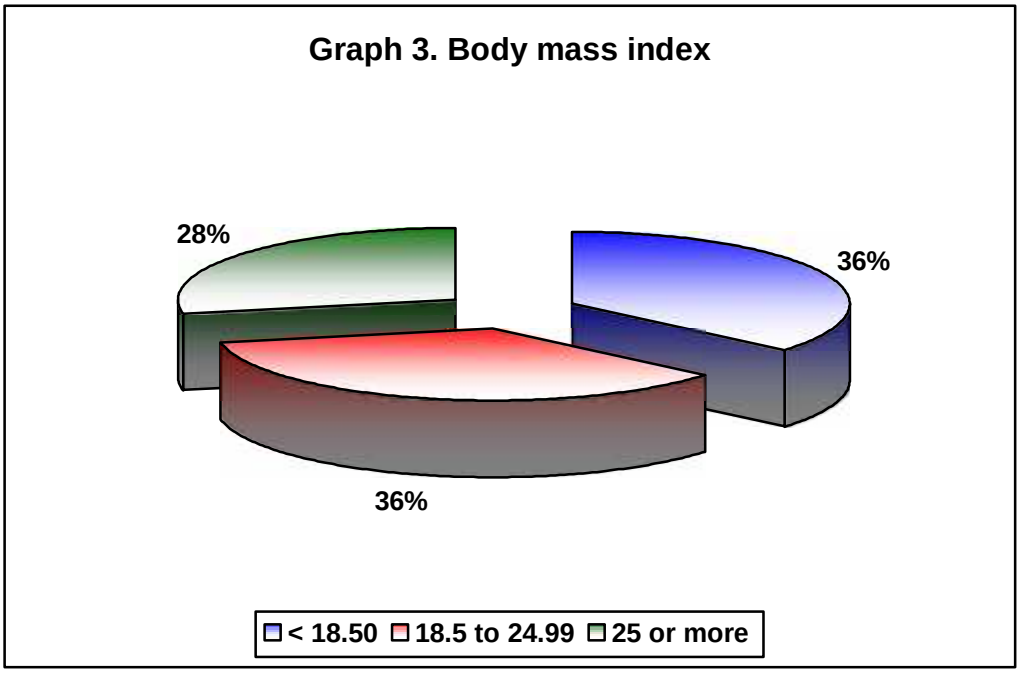
Age group (Years)	Distribution (n=100)	
	Number	**Percentage**
18 to 30	32	32.00
31 to 40	34	34.00
41 to 50	34	34.00
Total	**100**	**100.00**



In this study 34% of the participants were in the age group of 31 to 40 and 41 to 50 years each, while remaining 32% were aged 18 to 30 years. The mean age of the study population was 34.81 ± 8.54 years.

Table 3. Body mass index

Body mass index (Kg/m2)	Distribution (n=100)	
	Number	**Percentage**
< 18.50	36	36.00
18.5 to 24.99	36	36.00
25 or more	28	28.00
Total	**100**	**100.00**



In the present study 36% of the individuals each had body mass index < 18.5 and 18.5 to 24.9 Kg/m^2. However, 28% of the participants had BMI > 25 Kg/m^2. The mean BMI of the study population was 22.69 ± 4.03 Kg/m^2.

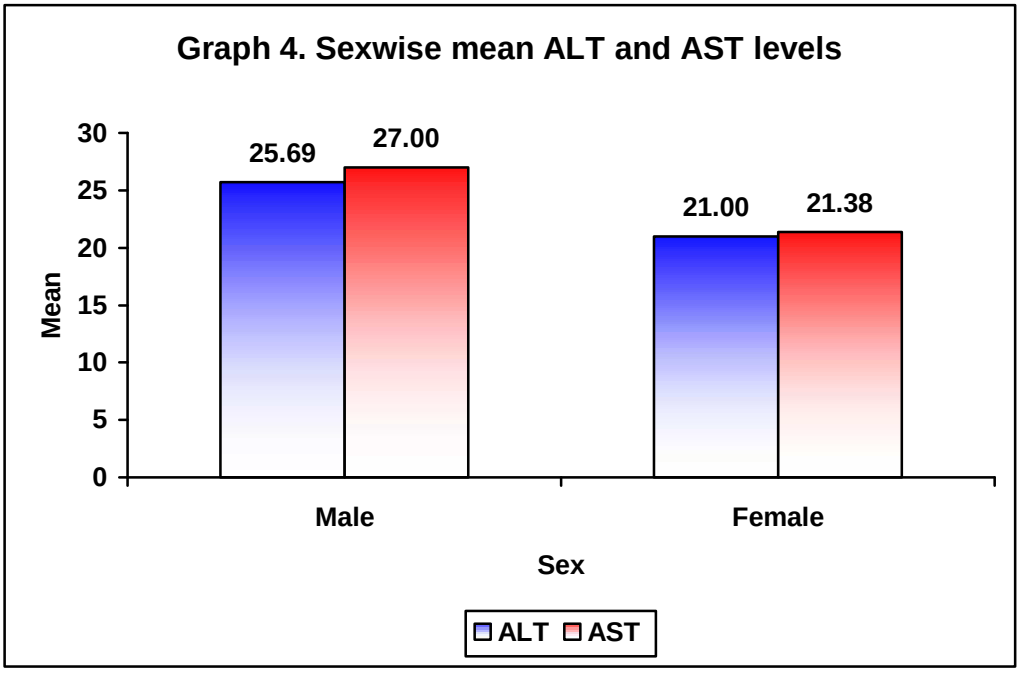
Table 4. Vitals

Variables	Distribution (n=100)	
	Mean	**SD**
Pulse rate (/min)	80.55	8.52
Systolic blood pressure (mm Hg)	127.74	10.05
Diastolic blood pressure (mm Hg)	76.02	5.43
Respiratory rate (/min)	14.45	1.82

Table 4 shows the mean pulse rate, systolic blood pressure, diastolic blood pressure and respiratory rate.

Table 5. Sexwise mean ALT and AST levels (U/L)

Sex	ALT (n=100)		AST (n=100)	
	Mean	**SD**	**Mean**	**SD**
Male	25.69	9.56	27.00	10.00
Female	21.00	8.75	21.38	9.08
p value	**0.013**		**0.004**	



In this study the mean ALT and AST levels in males were 25.69 ± 9.56 and 27.00 ± 10.00 U/L compared to 21.00 ± 8.75 and 21.38 ± 9.08 U/L in females ($p < 0.050$).

Table 6. Sexwise median ALT and AST levels (U/L)

Sex	ALT (n=100)			AST (n=100)		
	Median	Range		Median	Range	
		Min	Max		Min	Max
Male	24.00	12.00	41	26.00	12	42
Female	22.00	10.00	40	22.00	10	41

In the present study median ALT levels in males were 24 U/L with range 12 U/L being minimum and 41 U/L being maximum and the median AST levels were 26 U/L with range 12 U/L being minimum to 42 U/L being maximum. Among females, the same were found to be 22 U/L with 10 U/L to 40 U/L and 22 U/L with 10 U/L to 41 U/L respectively.

Table 7. Agewise mean ALT and AST levels (U/L)

Age group (years)	ALT (n=100)		AST (n=100)	
	Mean	**SD**	**Mean**	**SD**
18 to 30	24.44	10.76	25.19	10.68
31 to 40	23.74	8.69	24.41	9.04
41 to 50	22.62	9.06	23.85	10.38
F value	3.090		0.146	
p value	0.567		0.864	

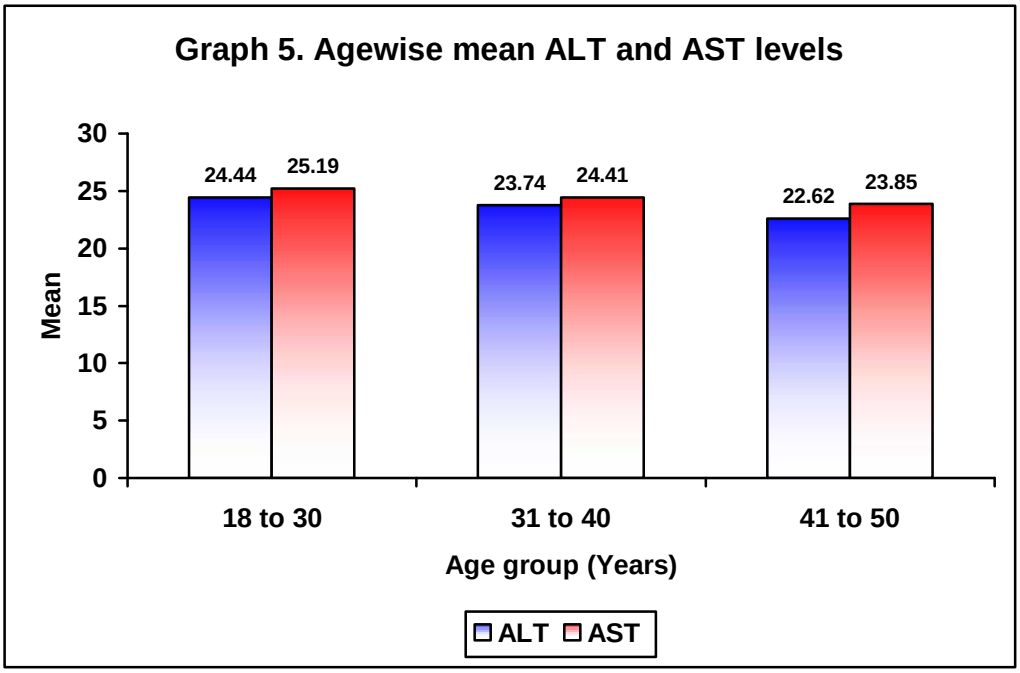


Table 7 and graph 5 shows agewise mean ALT and AST levels. No statistically significant difference was observed in ALT and AST levels among different age groups (p>0.050).

Table 8. Agewise median ALT and AST levels (U/L)

Age group (years)	ALT (n=100)			AST (n=100)		
	Median	Range		Median	Range	
		Min	Max		Min	Max
18 to 30	22.00	10.00	41	22.50	10	42
31 to 40	23.00	10.00	41	22.00	10	42
41 to 50	23.00	10.00	39	24.00	10	40

In this study the median ALT levels among the participants aged between 18 to 30 were 22 U/L and in those with age between 31 to 40 and 41 to 50 years the ALT levels were 23 U/L. The AST levels among the participants aged between 18 to 30, 31 to 40 and 41 to 50 years were 22.5, 22 and 24 U/L.

Table 9. Body mass index and mean ALT and AST levels (U/L)

Body mass index (Kg/m^2)	ALT (n=100)		AST (n=100)	
	Mean	**SD**	**Mean**	**SD**
< 18.5	13.75	3.16	14.00	3.50
18.5 to 24.99	24.44	3.90	25.14	4.04
25 or more	35.11	5.22	37.07	3.90
p value	**<0.001**		**<0.001**	

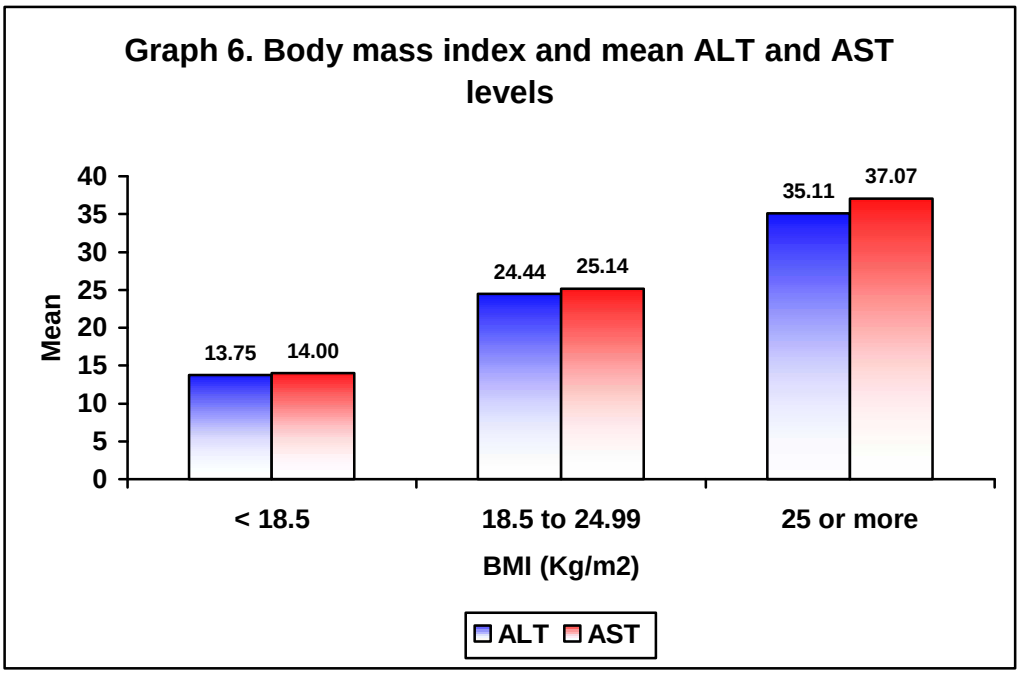


Table 9 and graph 6 shows variations in mean ALT and AST levels with reference to body mass index. It was observed that, ALT and AST levels significantly increased with increase in BMI ($p<0.001$).

Table 10. Body mass index and median ALT and AST levels (U/L)

Body mass index (Kg/m^2)	ALT (n=100)			ALT (n=100)		
	Median	Range		Median	Range	
		Min	Max		Min	Max
< 18.5	14.00	10	22	14.00	10	22
18.5 to 24.99	24.00	21	37.	24.00	21	38
25 or more	36.00	19	41	38.00	24	42

In this study the median ALT levels in individuals with BMI < 18.5, 18.5 to 24.9 and ≥ 25 Kg/m^2 were 14, 24 and 36 U/L and the AST levels among the same participants were 14, 24 and 38 U/L respectively.

Table 11. Age and sexwise ALT levels (U/L)

Age group (Years)	Sex			
	Male (n=55)		Female (n=45)	
	Mean	SD	Mean	SD
18 to 30	26.28	10.52	22.07	11.00
31 to 40	25.18	9.34	22.29	8.01
41 to 50	25.60	9.32	18.36	6.93
F value	0.057		0.927	
p value	0.944		0.403	

Table 11 and graph 7 shows variations in mean ALT levels with reference to age and sex. It was observed that, the mean ALT levels in males and females were comparable in all the age groups (p>0.050).

Table 12. Age and sexwise AST levels

Age group (Years)	Sex			
	Male (n=55)		Female (n=45)	
	Mean	SD	Mean	SD
18 to 30	27.28	10.27	22.50	10.98
31 to 40	26.24	9.16	22.59	8.80
41 to 50	27.40	10.90	18.79	7.27
F value	0.070		0.821	
p value	0.932		0.446	

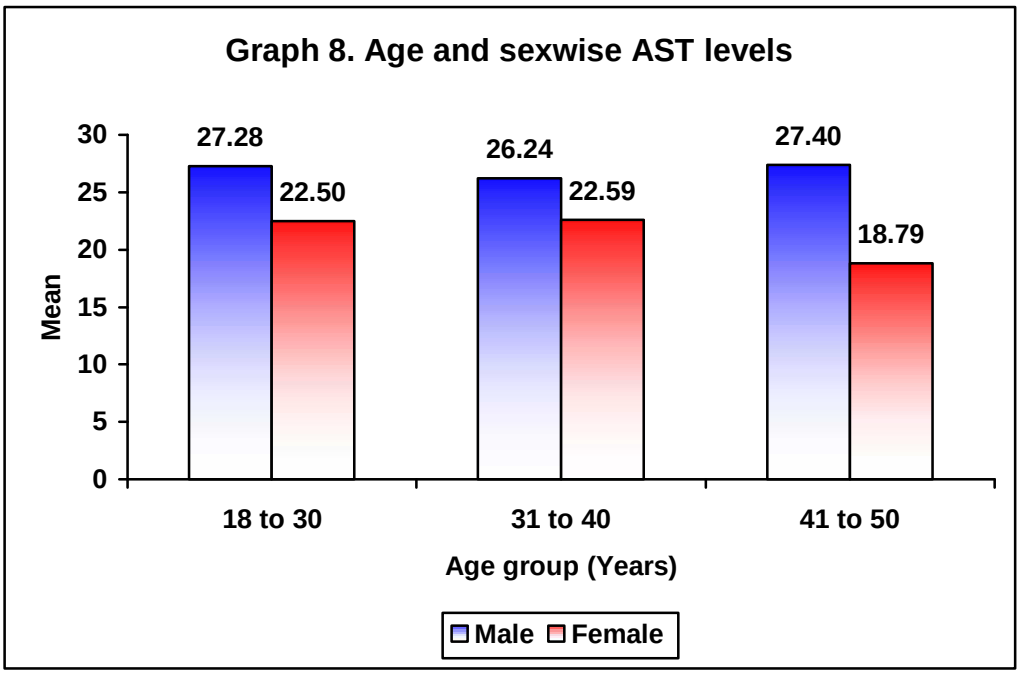


Table 12 and graph 8 shows variations in mean AST levels with reference to age and sex. The mean AST levels in males and females were comparable in all the age groups (p>0.050).

Table 13. BMI and agewise ALT levels

Age group (Years)	Body mass index (Kg/m^2)					
	< 18.50		18.5 to 24.99		25 or more	
	Mean	SD	Mean	SD	Mean	SD
18 to 30	12.40	2.07	22.82	2.60	37.00	5.22
31 to 40	14.75	3.55	24.08	4.08	34.10	3.73
41 to 50	13.86	3.30	26.15	4.22	33.57	6.75
F value	1.575		2.440		1.231	
p value	0.222		0.103		0.309	

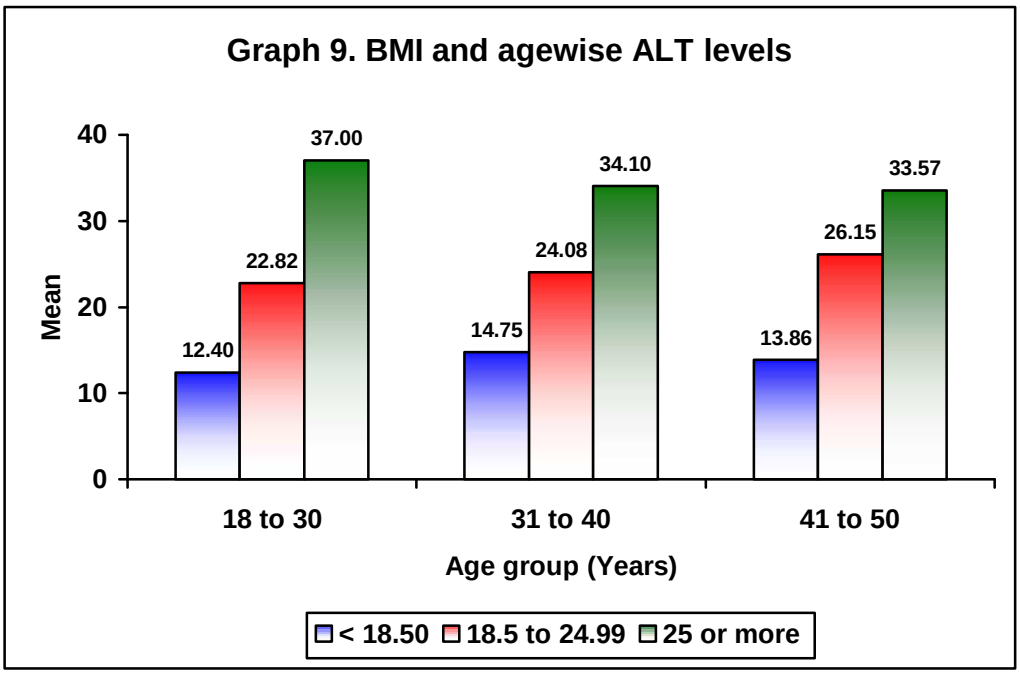


Table 13 and graph 9 shows variations in mean ALT levels with reference to age and body mass index. No statistically significant difference was noted in mean ALT levels among the participants with different obesity grades and age groups (p>0.050).

Table 14. BMI and agewise AST levels

Age group (Years)	Body mass index (Kg/m2)					
	< 18.50		18.5 to 24.99		25 or more	
	Mean	SD	Mean	SD	Mean	SD
18 to 30	13.40	2.95	23.27	2.05	37.82	4.83
31 to 40	15.25	4.00	24.58	4.60	35.20	3.55
41 to 50	13.36	3.34	27.23	4.04	38.57	0.98
F value	1.161		3.462		2.015	
p value	0.326		0.043		0.154	

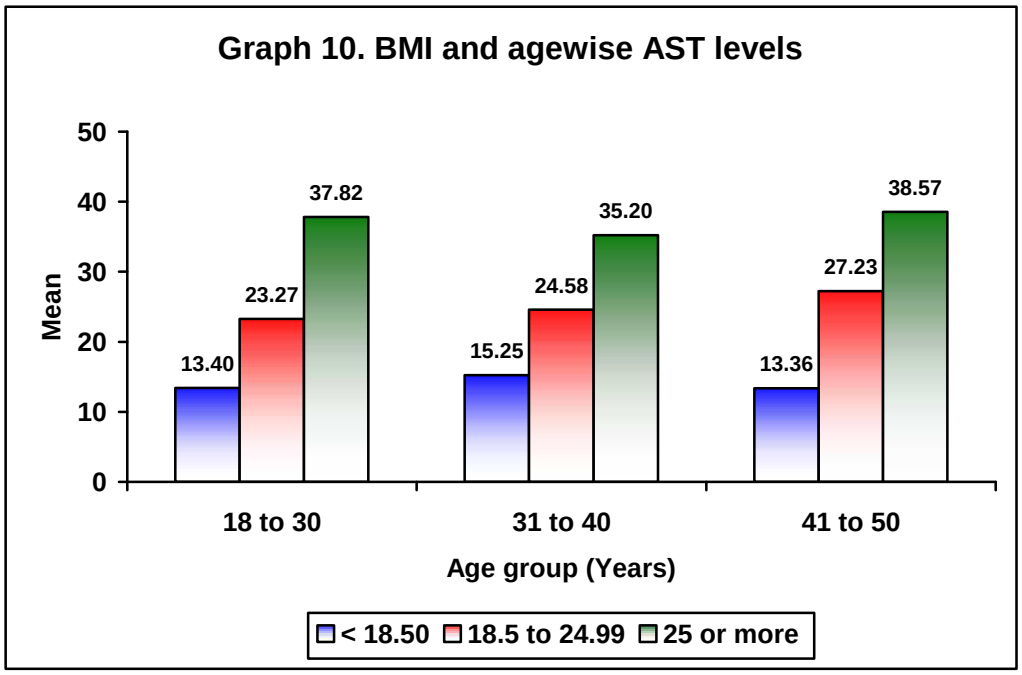


Table 14 and graph 10 shows variations in mean AST levels with reference to age and body mass index. Statistically significant age wise difference was noted in AST levels among the participants who had BMI between 18.5 to 24.9 Kg/m^2 (p=0.043).

Chapter 6

Discussion

DISCUSSION

A sedentary lifestyle coupled with an increased intake of energy dense food is contributing to the increased prevalence of obesity worldwide.[82] Obesity is associated with a variety of health risks, including an increased incidence of cardiovascular events and diabetes mellitus.[82,83] Obesity increases mortality and risk of morbidity from cardiovascular disease, dyslipidemia, hypertension, stroke, osteoarthritis, sleep apnea, type 2 diabetes mellitus and several types of cancer. The hepatic manifestation of metabolic syndrome is non-alcoholic fatty liver disease reflected by deposition of fat in the hepatocytes as seen in liver biopsy causing injury to the hepatocytes and thus elevating serum liver enzymes.

It has been suggested that there is a variation in the serum aminotransferases levels with respect to BMI. As the BMI increases there is a steady rises in the aminotransferases levels. Hence there is a need to redefine the normal range of aminotransferases levels in relation with BMI. Liver function indicators influence strong variation in BMI. It is also observed that men are more likely to experience an abnormal serum aminotransferases levels than women and studies show that the proportion declines in older patients.

However, there is little data about the relationship between BMI, age, sex and aminotransferases levels available in literature, especially in Indian context. Hence, this study was planned to examine variation in the aminotransferases levels in relation to body mass index, age and sex.

The present cross sectional study was conducted on a total of 100 healthy individuals attending the executive health check up at the Department of

Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Sex and aminotransferases levels

In the present study 55% of individuals were males and 45% were females. The male to female ratio was 1.2:1. The mean ALT and AST levels in males were significantly high (25.69 ± 9.56 and 27.00 ± 10.00 U/L) compared to in females (21.00 ± 8.75 and 21.38 ± 9.08 U/L; p=0.013 and p=0.004). Normal serum ALT and AST in a selected population varies according to age, sex, and ethnic origin. Findings of the present study showing gender differences in ALT levels were consistent with the several other previous studies.

A study aimed to assess the range of value of serum alanine aminotransferase in healthy population and to assess the relationship between ALT level and body mass index (BMI), age and gender reported strong correlation between ALT levels and gender.[73] A study[74] from India to identify ALT activity among healthy medical students of Army Medical College, National University of Sciences and Technology, aged 18–22 years reported strong correlation between ALT levels and gender. Leclercq et al.[77] reported a significant correlation between serum ALT levels to BMI, sex, and age, with a mean ALT level of 21.8 U/L. Kariv et al.[78] found a significant effect of sex on ALT level. In a study conducted by Miguel et al, on 1,036 consecutive blood donors to determine ALT levels and their association with gender concluded that different cutoff values should be considered for men and women.

Age and aminotransferases levels

In this study most of the patients were aged between 31 to 40 and 41 to 50 years (34% each) and 32% were aged 18 to 30 years. The mean age of the study population was 34.81 ± 8.54 years. No statistically significant difference was observed in ALT and AST levels among different age groups ($p>0.050$). The mean ALT and AST levels in males and females were comparable in all the age groups ($p>0.050$). In contrast, a study aimed to assess the range of value of serum alanine aminotransferase in healthy population and to assess the relationship between ALT level and body mass index (BMI), age and gender reported strong correlation between ALT levels and age.[73]

BMI and aminotransferases levels

Previous studies have demonstrated that liver enzyme levels increase progressively with increasing BMI. In the present study 36% of the individuals each had body mass index < 18.5 and 18.5 to 24.9 Kg/m^2 and 28% of the participants had BMI > 25 Kg/m^2. The mean BMI of the study population was 22.69 ± 4.03 Kg/m^2. It was observed that, the mean ALT levels in participants with BMI <18.5 Kg/m^2 were significantly low (13.75 ± 3.16 U/L) compared those with BMI between 18.5 to 24.99 Kg/m^2 (24.44 ± 3.90 U/L) and with > 25 Kg/m^2 (35.11 ± 5.22 U/L) ($p<0.001$). Similarly, AST levels were also significantly low (14.00 ± 3.50 U/L) in participants with BMI < 18.5 Kg/m^2 compared those with BMI between 18.5 to 24.99 Kg/m^2 (25.14 ± 4.04 U/L) and > 25 Kg/m^2 (37.07 ± 3.90 U/L) ($p<0.001$).

In 2002, Prati et al.[57] in a study from Italy defined new normal levels for serum ALT in a healthy population by testing 6835 healthy blood donors, In that study the serum ALT level were found to be directly related to sex and BMI. A study showed that the increase is progressive from BMI values within the normal weight range and that risk for abnormal values is greatly increased for overweight and obese persons. In the United States, on the basis of analyses of the Third (1998 to 1994) National Health and Nutrition Examination Survey (NHANES), Ruhl and Everhart[58] and Clark et al[59] reported that BMI was strongly associated with the prevalence of abnormal ALT levels. In NHANES data collected from 1999 to 2002, the prevalence of elevated ALT levels was more than double that of previously available estimates, whereas the positive association between BMI and elevated ALT was similar.

Bedogni et al[5] reported that overweight and obese participants had elevated ALT levels compared with those having BMI <24.9, OR=2.0 (95% CI=1.4-2.7), and OR=3.1 (95% CI=2.1-4.7), respectively in the Dionysos study of 6315 adults in Northern Italy. Lawlor et al[62] found a linear association of BMI with ALT. Leclercq et al.[77] reported a significant correlation between serum ALT levels to BMI. Kariv et al.[78] found a significant effect of obesity and sex on ALT level.

A study by Prati et al[57] for updating the aminotransferases levels in healthy individuals, found that, serum ALT activity was independently related to body mass index and to laboratory indicators of abnormal lipid or carbohydrate metabolism.

Age, BMI and aminotransferases levels

In this study, no statistically significant difference was noted in mean ALT levels among the participants with different BMI grades and age groups ($p>0.050$). In contrast, previous studies have found ALT levels to be more closely associated with BMI than AST levels. In the Western New York Health Study,[64] ALT was more highly correlated with BMI than AST, particularly among men and postmenopausal women. Burns et al[65] carried out 2 cross-sectional analyses in working men and women and also found a greater effect of BMI on ALT. For those gaining weight in the approximately 2 years between examinations, ALT rose slightly whereas AST did not. In a population of Korean working men followed over 4 years, ALT was more strongly associated than AST with BMI at baseline, and change in BMI was more strongly associated with having an abnormal ALT on follow-up.[65]

In this study, the comparison of mean AST levels showed statistically significant age wise difference among the participants who had BMI between 18.5 to 24.9 Kg/m^2 that is, the mean AST levels in individuals aged between 18 to 30 years were 23.27 ± 2.05 compared to 24.58 ± 4.60 in those who were aged 31 to 40 years and 27.23 ± 4.04 with age between 41 to 50 years ($p=0.043$). However no statistically significant difference was observed among the individuals with BMI less than 18.5 and > 25 Kg/m^2.

Overall, these results indicate BMI-based screening serum of ALT and AST levels as due to the increasing prevalence of obesity and metabolic

syndromes which will help diagnosing a possible liver disease early in healthy individuals.

Chapter 7

Conclusion

CONCLUSION

The present study mean ALT and AST levels significantly varied with gender and different grades of BMI. However, no statistically significant difference was observed in ALT and AST levels among different age groups. Further the mean AST levels significantly varied in individuals with different grades of BMI according to age whereas the same was not true with ALT levels.

Chapter 8

Summary

SUMMARY

BMI has a strong influence over aminotransferases levels. Both ALT and ALT, the two variables show the greatest contributions to the total BMI variance. The present study was aimed to examine variation in the aminotransferases levels in relation to body mass index, age and sex.

This one year cross sectional study was conducted at the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on a total of 100 healthy individuals attending the blood bank/ executive health check up.

In this study, 55% of individuals were males and 45% were females with male to female ratio of 1.2:1. The 31 to 40 and 41 to 50 years age group was comprised of 34% each, while remaining 32% were aged 18 to 30 years and the mean age of the study population was 34.81 ± 8.54 years. Body mass index of < 18.5 and 18.5 to 24.9 Kg/m^2 was noted in 36% of the individuals each. However, 28% of the participants had BMI > 25 Kg/m^2. The mean BMI of the study population was 22.69 ± 4.03 Kg/m^2. The mean ALT and AST levels in males were 25.69 ± 9.56 and 27.00 ± 10.00 U/L compared to 21.00 ± 8.75 and 21.38 ± 9.08 U/L in females ($p < 0.050$). It was observed that, the mean ALT levels in participants with BMI <18.5 Kg/m^2 were significantly low (13.75 ± 3.16 U/L) compared those with BMI between 18.5 to 24.99 Kg/m^2 (24.44 ± 3.90 U/L) and with > 25 Kg/m^2 (35.11 ± 5.22 U/L) ($p<0.001$). Also, AST levels were also significantly low (14.00 ± 3.50 U/L) in participants with BMI < 18.5 Kg/m^2

compared those with BMI between 18.5 to 24.99 Kg/m^2 (25.14 ± 4.04 U/L) and > 25 Kg/m^2 (37.07 ± 3.90 U/L) ($p<0.001$).

Overall, the present study showed that, mean ALT and AST levels significantly varied with gender and body mass index but, no significant difference was observed among different age groups. The mean AST levels significantly varied in individuals with different grades of BMI according to age whereas the same was not true with ALT levels.

Chapter 9

Bibliography

BIBLIOGRAPHY

1. Sherman KE. Alanine aminotransferase in clinical practice. A review. Arch Intern Med 1991;151(2):260-5.

2. McPherson RA, Matthew R, Pincus MR. Henry's Clinical Diagnosis and Management by Laboratory Methods. 22nd ed. Philadelphia: Elsevier Saunders; 2011.

3. Devraj S, Wheeler TM. Aspartate Aminotransferase. Available from: URL:http://emedicine.medscape.com/article/2087224-overview#aw2aab6b3 Access Date 18.08.2013.

4. Pratt DS, Kaplan MS. Evaluation of abnormal enzyme results in asymptomatic patients. New Engl J Med 2000;342:1266–71

5. 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. Dig Liver Dis 2003;35(9):648-52.

6. Henneberg M, Rühli FJ, Gruber P, Woitek U. Alanine Transaminase Individual Variation Is a Better Marker than Socio-Cultural Factors for Body Mass Increase in Healthy Males. Food and Nutrition Sciences 2011; 2:1054-62.

7. Bhatia K, Ghabriel M, Henneberg M. Anatomical Variations in the Branches of the Human Arch of the Aorta: A Possible Increase in Recent Times? Folia Morphologica 2005;64(3):217-23.

8. Rühli FJ, Henneberg M. Clinical Perspectives on Secular Trends of Intervertebral Foramen Diameters in an Industrialized European Society. European Spine Journal 2004;13:733-9.

9. Henneberg M, Ulijaszek SJ. Body Frame Dimen-sions Can Predict Obesity: Body Mass Index, Body Frame and Fatness. Am J Human Biol 2010;22(1):83-91.

10. Hanley AJ, Wiliams K, Festa A, Wagenknecht LE, Wagenknecht LE, Agostino RB, et al. Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 2004;53: 2623-32.

11. Vozarova B, Stefan N, Lindsay RS, Saremia A, Pratley RE. High alanine aminotransfetase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002;51;1889-5

12. Liu CM, Tung TH, Liu JH, Chen VT, Lin CH, Hsu CT, et al. A community-based epidemiological study of elevated serum alanine aminotransferase levels in Kinmen, Taiwan. World J Gastroenterol 2005; 11:1616-22.

13. Hermos JA, Cohen SA, Hall R, Gagnon DR, Brophy MT, Fiore LD. Association of elevated alanine aminotransferase with BMI and diabetes in older veteran outpatients.Diabetes Res Clin Pract 2008;80(1):153-8.

14. Oreopoulos A, Padwal R, Kalautar-Zadeh, Fonarow GC, Norris CM, McAliser FA. Body mass index and mortality in heart failure: a metaanalysis. Am heart J 2008;156:13-22.

15. Iacobellis G, Sharma AM. Obesity and the heart: redefinition of the relationship. Obes Rev 2007;8:35-9.

16. Gianluca I, Antonio M, Maria CR, Alessandra Z, Concetta VI, Frida L. Normal serum alanini aminotranferace activity in uncomplicated obesity. World J Gastroenterol 2005;11;6018-21.

17. Stevens J, Plankey MW, Williamson D. The body mass index-mortality relationship in white and African American women. Obes Res 1998;6:268-77.

18. Cossrow N, Falkner B. Race/ethenic issues in obesity and obesity-related comorbidities. J Clin Endocrinol Metab 2004;89:2590-4.

19. Feldman M, Friedman LS, Brandt LJ. Sleisenger and Fordtran's Gastrointestinal and Liver Disease: pathophysiology, diagnosis, management. 8th ed., Philadelphia, PA: Saunders; 2006

20. Wang JY. Biological Chemistry, 3rd ed. Higher Education Press, Beijing 2002.

21. Bergmeyer HU, Bernst E. Glutamate-oxaloacetate transaminase: UV-assay manual method. In: Methods of Enzymatic Analysis, Bergmeyer HU eds. 2nd English Ed. New York; Academic Press: 1963. p. 727-33.

22. Bergmeyer HU Bernst, E. Glutamate-pyruvate transaminase: UV-assay manual method, In: Methods of Enzymatic Analysis, Bergmeyer HU eds. 2nd English Ed. New York; Academic Press: 1963. p. 752-8.

23. Huang XJ, Choi YK, Im HS, Yarimaga O, Yoon E, Kim HS. Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques Sensors 2006;6:756-82

24. Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterology 2002;123:1367–84.

25. Kundrotas LW, Clement DJ. Serum alanine aminotransferase (ALT) elevation in asymptomatic US Air Force basic trainee blood donors. Dig Dis Sci 1993;38:2145–50.

26. Gibony PT. Mildly Elevated Liver Transaminase Levels in the Asymptomatic Patient. Am Fam Physician 2005 ;71(6):1105-10.

27. Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol. 1999;94:1018–22.

28. Davern TJ, Scharschmidt BF. Biochemical liver tests. In: Sleisenger & Fordtran's Gastrointestinal and liver disease: pathophysiology, diagnosis, management, Feldman M, Friedman LS, Sleisenger MH, eds. 7th ed. Philadelphia: Saunders; 2002 p. 1227–38.

29. Bayraktar M, Van Thiel DH. Abnormalities in measures of liver function and injury in thyroid disorders. Hepatogastroenterology 1997;44:1614–8.

30. Bathum L, Petersen HC, Rosholm JU, Hyltoft PP, Vaupel J, Christensen K. Evidence for a substantial genetic influence on biochemical liver function tests: results from a population-based Danish twin study. Clin Chem 2001;47:81–7.

31. Yano E, Tagawa K, Yamaoka K, Mori M. Test validity of periodic liver function tests in a population of Japanese male bank employees. J Clin Epidemiol 2001;54:945–51.

32. Pratt DS, Kaplan MM. Laboratory tests. In: Schiff ER, Sorrell MF, Maddrey WC, eds. Schiff's diseases of the liver. 8th ed., Philadelphia: Lippincott–Raven; 1999 p. 205-44.

33. Katkov WN, Friedman LS, Cody H, Evans A, Kuo G, Choo QL, et al. Elevated serum alanine aminotransferase levels in blood donors: the contribution of hepatitis C virus. Ann Intern Med 1991;115:882-4.

34. Hultcrantz R, Glaumann H, Lindberg G, Nilsson LH. Liver investigation in 149 asymptomatic patients with moderately elevated activities of serum aminotransferases. Scand J Gastroenterol 1986;21:109-13.

35. 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; 94:3010-4.

36. Cohen JA, Kaplan MM. The SGOT/SGPT ratio an indicator of alcoholic liver disease. Dig Dis Sci 1979;24:835-8.

37. Diehl AM, Potter J, Boitnott J, Van Duyn MA, Herlong HF, Mezey E. Relationship between pyridoxal 5'-phosphate deficiency and aminotransferase levels in alcoholic hepatitis. Gastroenterology 1984;86:632-6.

38. Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med 1999;341:556-62.

39. Schiff ER, de Medina M, Kahn RS. New perspectives in the diagnosis of hepatitis C. Semin Liver Dis 1999;19(1):3-15.

40. Krawitt EL. Autoimmune hepatitis. N Engl J Med 1996;334:897-903.

41. Manns MP. Autoimmune hepatitis. In: Schiff's diseases of the liver, Schiff ER, Sorrell MF, Maddrey WC, eds. 8th ed. Philadelphia: Lippincott–Raven; 1999. p. 919-35.

42. Czaja AJ. Natural history, clinical features, and treatment of autoimmune hepatitis. Semin Liver Dis 1984;4:1-12.

43. Diehl AM, Goodman Z, Ishak KG. Alcohollike liver disease in nonalcoholics: a clinical and histologic comparison with alcohol-induced liver injury. Gastroenterology 1988;95:1056-62.

44. Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology 1994;107:1103-9.

45. Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol 1999; 94:1018-22.

46. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, Mc-Cullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-9.

47. Eriksson S, Eriksson KF, Bondesson L. Nonalcoholic steatohepatitis in obesity: a reversible condition. Acta Med Scand 1986;220:83-8.

48. Hasegawa T, Yoneda M, Nakamura K, et al. Diagnostic significance of measurement of serum transforming growth factor b1 (TGF-b1) level and effect of a-tocopherol in patients with nonalcoholic steatohepatitis (NASH). Gastroenterology 1997;112:Suppl 1:A1278.

49. Lavine JE. Treatment of obesity-induced steatohepatitis with vitamin E. Gastroenterology 1998;114:A1284.

50. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, et al. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 1996;23:1464-7

51. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, et al. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 1996;23:1464-7.

52. Bardella MT, Vecchi M, Conte D, Del Ninno E, Fraquelli M, Pacchetti S, et al. Chronic unexplained hypertransaminasemia may be caused by occult celiac disease. Hepatology 1999; 29:654-7.

53. Das A, Post AB. Should liver biopsy be done in asymptomatic patients with chronically elevated transaminases: a cost-utility analysis. Gastroenterology 1998;114:A9.

54. Sorbi D, McGill DB, Thistle JL, Henry JJ, Therneau TM, Lindor KD. An assessment of the role of liver biopsies in asymptomatic patients with chronic liver test abnormalities. Hepatology 1999;30:477A.

55. Akbar HO, Fallatah HI. Serum ALT levels in cohort of healthy blood donors and volunteers from Saudi Arabia: The influence of sex and body mass index. Ann Gastroenterol Hepatol 2010;1(2):12-8.

56. Chen ZW, Chen LY, Dai HL, Chen JH, Fang LZ. Relationship between alanine aminotransferase levels and metabolic syndrome in nonalcoholic fatty liver disease. J Zhejiang Univ Sci B. 2008;9(8):616-22.

57. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002;137(1):1-10.

58. Ruhl CE, Everhart JE. Joint effects of body weight and alcohol on elevated serum alanine aminotransferase in the United States population. Clin Gastroenterol Hepatol 2005;3:1260–8.

59. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol. 2003;98:960–7.

60. Hsiao TJ, Chen JC, Wang JD. Insulin resistance and ferritin as major determinants of nonalcoholic fatty liver disease in apparently healthy obese patients. Int J Obes Relat Metab Disord 2004;28:167–72.

61. Bedogni G, Miglioli L, Masutti F, Castiglione A, Tiribelli C, Bellentani S. Accuracy of body mass index in detecting an elevated alanine aminotransferase level in adolescents. Ann Hum Biol 2004;31:570–577.

62. Lawlor DA, Sattar N, Smith GD, et al. The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. Am J Epidemiol 2005;161:1081–8.

63. Lee DH, Ha MH, Christiani DC. Body weight, alcohol consumption and liver enzyme activity—a 4-year follow-up study. Int J Epidemiol. 2001;30:766–70.

64. Stranges S, Dorn JM, Muti P, Freudenheim JL, Farinaro E, Russell M, et al. Body fat distribution, relative weight, and liver enzyme levels: a population-based study. Hepatology 2004;39:754–63.

65. Burns CJ, Boswell JM, Olsen GW. Liver enzyme activity and body mass index. J Occup Environ Med 1996;38:1248–52.

66. Mukai M, Ozasa K, Hayashi K, Kawai K. Various S-GOT/S-GPT ratios in nonviral liver disorders and related physical conditions and life-style. Dig Dis Sci. 2002;47:549–55.

67. Bellentani S, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med. 2000;132:112–117.

68. Elinav E, Ben Dov IZ, Ackerman E, Kiderman A, Glikberg F, Shapira Y, et al. Correlation between serum alanine aminotransferase activity and age: an inverted U curve pattern. Am J Gastroenterol. 2005;100:2201–4.

69. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363:157–63.

70. Deurenberg P, Deurenberg YM, Wang J, Lin FP, Schmidt G. The impact of body build on the relationship between body mass index and percent body fat. Int J Obes Relat Metab Disord. 1999;23:537–42.

71. Sniderman AD, Bhopal R, Prabhakaran D, Sarrafzadegan N, Tchernof A. et al. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. Int J Epidemiol. 2007;36:220–5.

72. Kadowaki T, Sekikawa A, Okamura T, Takamiya T, Kashiwagi A, Zaky WR, et al. Higher levels of adiponectin in American than in Japanese men despite obesity. Metabolism. 2006;55:1561–3.

73. Morańska I, Jurczyk K, Kłosińska E, Wiśniewski K, Karpińska E, Wawrzynowicz-Syczewska M, et al. Relationship of serum alanine aminotransferase (ALT) to body weight, age and sex in blood donors population. Wiad Lek. 2004;57(9-10):427-30.

74. Bilal M, Tariq A, Khan S, Quratulain, Tariq A, Shahid MF, et al. Influence of gender, BMI, and ethnicity on serum ALT levels of healthy students of a medical school. J Ayub Med Coll Abbottabad 2011;23(1): 70-2.

75. Ramesh V, Saraswat S, Choudhury N, Gupta RK. Relationship of serum alanine transaminases to Body mass Index in blood donors: a need to correct ALT to BMI in healthy blood donor. Transfus Med. 1995;5(4): 273-4.

76. 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;27:1213–9.

77. Leclercq I, Horsmans Y, De Bruyere M, Geubel AP. Influence of body mass index, sex and age on serum alanine aminotransferase (ALT) level in healthy blood donors. Acta Gastroenterol Belg. 1999;62:16-20.

78. Kariv R, Leshno M, Beth-Or A, Strul H, Blendis L, Kokia E, et al. Re-evaluation of serum alanine aminotransferase upper normal limit and its modulating factors in a large-scale population study. Liver Int. 2006;26: 445-50.

79. Salvaggio A, Periti M, Miano L, Tavanelli M, Marzorati D. Body Mass Index and liver enzyme activity in serum. Clin Chem 1991;37(5):720-3.

80. Lozano M, Cid J, Bedini JL, Mazzara R, Gimenez N, Mas E, Ballesta A, et al. Study of serum Alanine transferases in blood donors in Spain. Haematologica 1998;83:237-39.

81. Sull JW, Yun JE, Lee SY, Ohrr H, Jee SH, Guallar E, et al. Body Mass Index and serum aminotransferases levels in Korean men and women. J Clin Gastroenterol 2009 Oct;43(9): 869-75.

82. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell 2001;104:531–43.

83. Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 2008;93:S9–30.

Annexures

Annexure I

ANNEXURE I – CONSENT FORM

"ESTIMATION OF AMINOTRANSFERASE LEVELS IN NORMAL INDIVIDUALS IN RELATION TO BODY MASS INDEX, AGE AND SEX- A CROSS SECTIONAL STUDY"

Objective and purpose of the study:

This research is intended to estimate the aminotransferases levels in relation to Body Mass Index (BMI), Age and Sex.

Procedure:

If you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood and urine samples for the necessary investigations.

Risk and Benefits:

The only risk and possible discomfort you might get is while taking blood from my arm for the investigations. It may cause swelling, pain, redness, bruising or infection (rarely happens) at the site from where the blood is drawn.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study, or if I decide to take part I can later change my mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MS degree, review and publishing.

If you have any questions about your rights as a participant, you may call:

1. Dr. ***** ******
   Investigator, PG in General Medicine, JNMC,
   Phone No.: ***** ****

2. Dr. **** *****
   Professor, in General Medicine, JNMC,
   Phone No.: ***** ****,

3. Dr. ***** *****
   Guide & HOD,
   Department of Medicine, JNMC,
   Phone No: ***** ****
   Extn: ***** *****

4. Dr. P.V. Patil, Chairman,
   J.N.M.C Ethical Committee for Human Research,
   Phone number: *******.
   Extn: *****

Consent Statement

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form, and have had all my questions answered.

Name of the Participant : _____ Signature / Thumb print _____

Name of the Witness: _____ Signature / Thumb print _____

Investigator Name: _____ Signature / Thumb print _____

Date: Place:

Annexures

Annexure II

ANNEXURE II – PROFORMA

Case No:

NAME: AGE/SEX:

IP No. ADDRESS:

OCCUPATION:

COMPLAINTS AT PRESENTATION:

HISTORY :

k/c/o Type 2 diabetes mellitus yes ☐ no ☐

If yes,

Duration of illness -

Details of treatment –

H/o Alcohol- yes ☐ no ☐

Quantity-

H/o Dyslipidemia yes ☐ no ☐

If yes- type-

Treatment

H/o Viral Hepatitis yes ☐ no ☐

If yes- type

H/o Hepatotoxic drug yes ☐ no ☐

If yes- which drug

H/o hospitalization in past 1 month yes no

If yes- reason for admission

ON EXAMINATION :

- NUTRITION :

  HEIGHT :

  WEIGHT:

  BMI :

- <u>BLOOD PRESSURE :</u>

INVESTIGATIONS :

- FBS –
- PPBS
- TOTAL TRIGLYCERIDES –
- TOTAL CHOLESTEROL-
- HBsAg and HCV
- ALT and AST levels–
- USG abdomen

Annexures

Annexure III

ANNEXURE III – MASTER CHART

Serial number	Out patient number	Sex	Age (Years)	General physical examination							Investigations	
				Height (Cms)	Weight (Kgs)	Body mass index (Kg/m^2)	Vitals				ALT	AST
							Pulse rate (bpm)	BP (mm Hg)		Respiratory rate		
								Systolic	Diastolic			
1	1914994	M	34	172	54	18.25	86	120	80	14	15	16
2	1915790	M	43	163	46	17.31	62	126	82	16	15	12
3	1916646	M	36	167	50	17.93	71	120	80	16	20	22
4	1915007	F	44	155	44	18.31	78	120	80	18	21	20
5	1916315	M	19	168	52	18.42	64	110	70	12	12	14
6	1916079	F	42	161	63	24.3	82	112	76	14	32	32
7	1916468	F	31	158	60	24.03	86	110	80	14	26	24
8	1915882	F	20	156	45	18.49	90	128	80	12	10	14
9	1915996	F	42	165	50	18.37	94	122	80	16	14	14
10	1915836	F	34	168	78	27.64	60	130	72	12	30	32
11	1916194	M	25	165	50	18.37	76	128	80	13	14	17
12	1916359	M	41	174	86	28.41	74	126	80	14	19	40
13	1916403	M	39	177	82	26.17	78	120	80	17	34	36
14	1916235	M	44	168	86	30.47	77	110	70	18	39	40
15	1917433	M	26	172	60	20.28	81	112	72	12	22	22
16	1916397	F	42	161	63	24.3	87	108	60	16	24	24
17	1917390	F	27	158	61	24.44	86	110	80	13	22	21
18	1918784	M	36	168	52	18.42	88	126	80	16	14	16
19	1919271	M	18	164	64	23.8	92	130	78	15	22	23
20	1919208	F	38	174	78	25.76	76	132	82	18	30	32
21	1919154	M	49	177	57	18.19	78	140	70	12	14	12
22	1918854	M	29	166	50	18.14	84	122	80	18	14	13
23	1918874	F	37	181	88	26.86	72	126	80	12	32	32
24	1920792	M	48	156	74	30.41	86	122	80	12	37	38
25	1918780	F	26	160	62	24.22	77	126	80	16	28	27
26	1918839	F	47	155	44	18.31	84	130	72	14	20	21
27	1918890	F	39	162	62	23.62	88	130	70	13	22	22
28	1920931	M	28	167	62	22.23	78	140	70	11	22	24
29	1921682	M	44	166	74	26.85	79	112	80	12	36	38
30	1921692	F	31	168	79	27.99	100	106	60	14	30	32
31	1921644	M	22	172	68	22.99	96	140	72	14	21	22
32	1922045	M	43	177	68	21.71	87	120	80	16	24	24
33	1921687	F	43	167	51	18.29	88	126	72	14	10	11
34	1923455	F	28	158	45	18.03	82	122	80	18	10	12
35	1922330	M	46	162	48	18.29	83	120	80	16	15	14
36	1923529	F	36	168	65	23.03	68	124	82	13	22	22
37	1925272	F	42	170	66	22.84	82	122	82	15	22	24
38	1925297	F	27	158	68	27.24	66	120	70	15	22	24
39	1923668	M	34	177	69	22.02	71	110	70	16	24	22

40	1923778	M	33	180	78	24.07	80	110	70	18	24	24
41	1923867	M	31	172	54	18.25	88	120	80	14	14	15
42	1923972	M	46	161	47	18.13	87	134	80	14	14	12
43	1925148	M	20	162	63	24.01	88	136	70	16	28	27
44	1925215	M	34	158	72	28.84	86	132	80	15	34	36
45	1925476	M	44	172	68	22.99	72	128	78	15	28	28
46	1925425	F	24	162	46	17.53	71	126	72	14	10	10
47	1925565	F	36	158	46	18.43	82	140	80	11	12	10
48	1926506	F	28	166	78	28.31	88	136	80	15	40	40
49	1927804	F	47	156	58	23.83	90	120	80	16	22	24
50	1925502	M	23	172	54	18.25	72	132	82	17	14	16
51	1925544	M	36	170	78	26.99	61	120	80	18	36	34
52	1924985	M	46	162	63	24.01	68	110	76	14	27	28
53	1924990	M	26	166	76	27.58	100	100	70	14	39	40
54	1924991	M	37	157	74	30.02	96	116	70	15	36	36
55	1926515	M	41	182	75	22.64	82	120	70	16	26	28
56	1927868	F	36	160	47	18.36	84	112	80	16	12	11
57	1928193	F	25	158	46	18.43	88	140	70	14	12	10
58	1928243	F	46	162	63	24.01	88	138	80	14	24	26
59	1977546	F	32	161	60	23.15	82	136	72	12	21	22
60	1977586	F	26	156	60	24.65	81	120	80	12	22	24
61	1977695	F	44	161	47	18.13	84	112	72	11	11	10
62	1926537	M	28	162	76	28.96	76	110	70	13	38	38
63	1926925	M	39	168	76	26.93	82	130	80	14	41	42
64	1926946	M	42	170	53	18.34	61	122	72	12	13	14
65	1927801	M	21	170	78	26.99	66	120	72	13	38	37
66	1927925	M	46	167	66	23.67	78	124	80	13	26	28
67	1928137	M	26	182	90	27.17	88	126	80	14	36	38
68	1961154	F	34	155	44	18.31	89	126	80	15	22	20
69	1962204	F	41	161	63	24.3	85	124	60	15	24	24
70	1963635	F	42	163	49	18.44	84	126	72	16	11	12
71	1927771	F	28	174	64	21.14	86	120	80	16	21	22
72	1928802	M	36	178	75	23.67	72	120	80	14	36	38
73	1980331	F	21	165	75	27.55	77	136	72	14	40	41
74	1928137	M	48	176	65	20.98	82	140	70	13	37	38
75	1935255	F	37	157	58	23.53	88	132	80	14	21	21
76	1939537	F	21	156	59	24.24	76	140	80	16	22	22
77	1936102	F	44	157	45	18.26	77	140	80	14	10	11
78	1928424	M	37	167	59	21.16	82	132	82	16	24	26
79	1995183	M	28	178	92	29.04	82	126	80	17	36	38
80	1995445	M	38	180	76	23.46	84	130	70	16	24	26
81	1996777	M	38	173	55	18.38	88	120	80	12	14	15
82	1996884	M	22	172	60	20.28	86	134	70	12	21	22
83	1996966	M	46	171	80	27.36	81	130	70	14	32	38
84	1996910	F	37	161	47	18.13	71	128	82	11	18	20
85	1996994	M	24	180	59	18.21	72	122	80	12	16	18
86	2027074	M	41	167	75	26.89	74	126	80	15	36	38
87	2027093	M	32	178	58	18.31	76	140	80	15	14	16
88	2029812	M	42	176	65	20.98	88	134	72	16	24	26
89	2027119	F	26	162	48	18.29	82	130	70	16	12	10

90	2030014	M	46	164	72	26.77	84	122	80	15	36	38
91	2027241	F	31	161	47	18.13	82	120	76	14	10	10
92	2030225	M	18	168	80	28.34	81	140	70	15	39	40
93	2027190	F	34	156	44	18.08	60	130	72	16	12	12
94	2030059	M	42	168	52	18.42	66	122	80	16	14	14
95	2030173	F	19	152	72	31.16	82	126	70	14	38	38
96	2030236	F	38	167	80	28.69	82	124	80	14	38	40
97	2030103	F	44	166	50	18.14	84	130	70	13	12	10
98	2029778	M	28	168	78	27.64	88	130	70	12	41	42
99	2029971	F	37	155	58	24.14	78	120	82	14	21	22
100	2031074	M	38	170	64	22.15	80	126	84	16	24	26

ANNEXURE III – KEY TO MASTER CHART

ALT	-	Alanine aminotransferase
AST	-	Aspartate aminotransferase
BP	-	Blood pressure
bpm	-	Beats per minute
Cms	-	Centimeters
F	-	Female
Kg	-	Kilogram
M	-	Male
m	-	Meter
mm Hg	-	Millimeter of mercury