# THE RELATIONSHIP BETWEEN ALCOHOL CONSUMPTION, LIVER ENZYMES AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL


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**MAY, 2016**

## DECLARATION

I hereby declare that work embodied in this thesis was carried out by me under the direct supervision of **Dr Indira R Samal**, Associate professor of Biochemistry department, Punjab Institute of Medical Sciences, Punjab and Co- supervision **Dr. Ekta Chitkara** Associate professor Lovely Professor University, Punjab. This work has not been submitted in part or in full to any other university for any degree or diploma.

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## CERTIFICATE

This is to certify that the present thesis entitled **"The relationship between alcohol consumption, liver enzymes and high density lipoprotein"** is the outcome of the bonafide work carried out by **Mr. Rohit Rana (Registration No: 11400019)** himself under my guidance and the contents of his thesis did not form a basis of the award of any previous degree to him and to the best of my knowledge to anybody else also. The thesis has not been submitted by the candidate for any research degree in any other University.

The dissertation is fit for submission to the partial fulfillment of the conditions for the award of M.Sc. in Clinical Biochemistry. Further certified that the candidate bears a good moral character and nothing adverse has been found against him.

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**TO WHOM IT MAY CONCERN**

This is to certify that **Mr. Rohit Rana** has done practical training in Punjab Institute of Medical Sciences (Hospital and Medical College) from January 01, 2016 to April 30, 2016 in the department of Biochemistry.

During the training period his performance was very good. We wish him all the best for his future endeavours.

**Barinder M Singh**
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## Abstract

Health problems related to lifestyle and behavior are increasingly common in modern societies.Recent studies have indicated that the common liver enzymes, gamma-gutamyltransferase (GGT)and alanine aminotransferase (ALT),asparatate aminotransferase (AST), Alkaline phosphatase (ALP) are increased in alcoholics. In this study we analyzed blood sample of chronic alcoholics. Alcohol consumption, smoking, coffee drinking, income, education, food habits were analyzed using detailed questionnaires. The mean age of study group was 41.95 ± 8.45 years. Serum AST was 36.92 ± 26.35 U/L. Serum ALT was 53.59 ± 31.24 U/L, serum ALP was 102.47 ± 29.03 U/L, serum GGT was 66.63 ± 30.96 U/L and Serum HDL-Cholesterol was 44.68 ± 11.66 mg/dl. The result shows that alcoholics had increased serum liver enzymes and decreased serum HDL- Cholesterol.

**Keywords**: -Alcohol, Alcohol liver disease, Liver enzymes, High density lipoprotein.

# INTRODUCTION

# 1.ALCOHOL

In chemical terminology alcohol is a large group of organic compounds, which are derived from hydrocarbons and contain one or more hydroxyl (-OH) groups. Ethanol (C2H50H ethyl alcohol) is one of this class of compounds, and is the main psychoactive ingredient in alcoholic beverages [1]. Alcohol or ethanol is an intoxicating ingredient found in beer, wine and liquor. Alcohol is produced by fermentation of yeast, sugar and starches. It is formed when yeast ferments sugar in different foods for example: - wine is made from grapes, beer from malted barley, cedar from apples, and vodka from potatoes [2].

## 1.1 ALCOHOLISM

Alcoholism is the dependence on excessive amounts of alcohol, associated with a cumulative pattern of deviant behaviors. Alcoholism is a chronic illness with a slow, insidious onset, which may occur at any age. The cause is unknown, but genetic, cultural and psychosocial factors are suspect and families of alcoholics have a higher incidence of the disease [3]. Chronic alcoholism and its related disorders are one of the major problems in the world. Chronic alcohol abuse will cause the drinker to lose control of his or her drinking. There may be plan to have only a few drinks, but in the end, the drinker loses control over this very valid plan and drinks much more than intended. Chronic alcohol abuse can lead to feelings of guilt and shame as well as to broken relationships and broken f due to family's lack of control over the alcoholintake[4].

## 1.2 LIVER ENZYMES

Prolonged alcohol consumption affects the liver enzymes. Four enzymes are measured in the laboratory to evaluate function of the liver. These enzymes include Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Gamma Glutamyl-Transferase (GGT). The first two are known together as transminases and second two are known together as cholestatic liver enzymes. Elevation in any of these enzymes can indicate the presence of liver disease. Elevation of the transminases can occur with alcoholic liver disease and fatty liver, conditions that can result from excessive alcohol intake. Elevation of the cholestatic liver enzymes can also occur with alcoholic liver disease [5].

### 1.2.1 Aspartate amino transferase (AST)

AST catalysetransmination reaction. AST exists in two different isoenzyme forms, which are genetically distinct, the mitochondrial and cytoplasmic form. AST is found in highest concentration in heart compared with other tissues of the body such as liver, skeletal muscle and kidney [6]. Normallevelof serum AST is 0-40U/L. Elevated mitochondrial AST is seen in extensive tissue necrosis during myocardial infarction and also seen in chronic liver disease.

About 80% of AST activity of the liver disease is contributed by mitochondrial isoenzyme [7]. However the ratio of mitochondrial AST to total AST activity has diagnostic importance in identifying the liver cell necrotic type condition and alcoholic hepatitis [8].

### 1.2.2 Alanine amino transferase (ALT)

ALT is found in kidney, heart, muscle and highest concentration in liver compared with other tissues of the body. ALT is purely cytoplasmic catalyzing the transminase reaction [6]. Normal level of serum ALT is 0-40U/L. Any type of cell injury can increase ALT levels. Marked elevations of ALT levels greater than 500 U/L are observed most often in persons with diseases that affect primarily hepatocytes such as hepatitis, ischemic liver injury and non toxic-induced liver damage [9].Viral hepatitis like A, B, C, D, and E may be responsible for a marked increase in aminotransferase levels. The increase in ALT associated with hepatitis C infection tends to be more than associated with hepatitis A or B [10]. In a recent study it was found that the hepatic fat accumulation in childhood obesity and nonalcoholic fatty liver disease causes serum ALT elevation. Moreover increased ALT level was associated with reduced insulin sensitivity, adiponectin and glucose tolerance as well as increased free fatty acids and triglycerides [11].

### 1.2.3 Gamma-glutamyl-transferase (GGT)

E.C.2.3.2.2, gamma-glutamyl-transferase, catalyzes the transfer of the gamma-glutamyl group from peptides and compounds that contain it to the same accepters [12]. The gamma-glutamyl accepter is the substrate itself, some amino acid or peptides or even water in which case simple hydrolysis takes place. Even though renal tissue have the highest concentration of GGT, the enzyme present in serum appears to originate primarily from the hepatobiliarysystem.GGT is a sensitive indicator of the presence of hepatobiliary disease, being elevated in most subjects regardless of cause [13].

### 1.2.4 Alkaline phosphatase (ALP)

ALP (orthophosphoric monoester phosphohydrolase) catalyzes the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates. ALP activity is present in most organs of the body and is especially associated with membrane and cell surfaces of small intestine, proximal convulated tubules of the kidney, in the bone, liver and placenta. Elevations in serum ALP activity commonly originate from one or more of the sources: the liver and bone. The response of the liver to any form of biliary tree obstruction induces the synthesis of ALP by hepatocytes. Some of the newly formed enzymes enter the circulation to increase the enzyme activity in serum [14]. The elevation tends to be more notable in extra hepatic obstruction than in hepatic obstruction. Liver diseases that principally affect parenchymal cells such as infectious hepatitis, typically show only moderate increase or even normal serum ALP activity [15].

## 1.3 HIGH DENSITY LIPOPROTEIN CHOLESTEROL

High-density lipoproteins cholesterol (HDL-C) is one of the five major groups of lipoproteins. Lipoproteins are complex particles composed of lipids and proteins in varying proportions, which transport lipids from one tissue to the other through plasma[16].HDL-C serves as carrier of cholesterol from peripheral tissues to liver for its degradation and excretion (Scavenger action). HDL contains apoproteins AI and AII. There is continuous exchange of apoproteinsapoCIIandapo E between HDL-C and other lipoproteins like chylomicrons and VLDL [17].

# REVIEW OF LITERATURE

# 2.0 ALCOHOL CONSUMPTION AND HEALTH

## 2.0.1 General aspects

Excessive alcohol drinking is a global problem which compromises both individual and social well-being. Almost all tissues in the body are affected, and consequently it has been shown to be closely related to more than 60 distinct medical conditions, including a wide array of both physiological and mental problems [18, 19]. The impact of ethanol intake on diseases and various types of tissue injury is dependent on two separate but related dimensions of drinking, namely the amount of alcohol consumed and the pattern of drinking. A chronic heavy drinking pattern generates different types of health effects from those created by acute (binge) drinking, while moderate drinking has even been thought to have some beneficial effects on cardiovascular health [20,21,22].

Ethanol is a simple water-soluble molecule. Upon ingestion, it is absorbed rapidly throughout the gastrointestinal tract, mainly in the small intestine [23]. It is freely distributed in the body, especially to organs with a rich blood supply such as the brain and lungs. Exposure to alcohol is greatest in the liver, since blood is received directly from the stomach and small bowel via the hepatic portal vein [24]. As ethanol is mostly metabolized in the liver, this organ is a major target for ethanol toxicity [19]. Alcoholic liver disease is currently a highly prevalent group of hepatic diseases in Finland, causing about 1,000 deaths per year [25]. In addition, there are numerous health problems related to chronic alcohol effects on extra hepatic tissues. A significant proportion of the alcohol-related disease burden is also due to acute alcohol intake, including unintentional and intentional injuries, road traffic accidents, violence and suicides [18, 20].

# 2.1 EPIDEMIOLOGY

In 2014, the World Health Organization released its Global Status report on Alcohol and Health. According to the report, about 38.3 percent of the world's populations consume alcohol regularly. On an average an individual consumption amounts to 6.2 liters of alcohol every year. According to the WHO report published in 2010, 30 percent of India's population, (just less than a third of the country's population) consumed alcohol regularly. Some 11 percent are moderate to heavy drinkers. The average Indian consumes about 4.3 liters of alcohol per annum. The rural average is much higher at about 11.4 liters a year [26]. According to Organization for Economic Cooperation and Development (OECD) report released in May 2015, alcoholism increased by

about 55 percent between 1992 and 2012. It is a continuously rising and is a cause of concern among the youth of the World [27].

Excessive alcohol consumption is ranked as one of the top five risk factors for death and disability worldwide [28]. 2.5 million deaths and 69.4 million annual disability adjusted life years were attributed to harmful alcohol abuse [29]. Almost 9% of adults in the United State meet the criteria for an alcohol-use disorder [30]. Annually about $223.5 billion is spent in treatment of alcohol use disorders [31].There is a strong correlation between the prevalence of alcoholic liver disease(ALD), specifically cirrhosis, and a country's annual per capita alcohol consumption. Eastern European countries have the highest annual per capita consumption (15.7 L per person), while North Africa and the Middle East have the lowest annual per capita consumption (1.0 L per person) [32]. In the United States, the estimated annual per capita consumption of alcohol is 8.4 L per person [33].Countries with higher per capita consumption have the highest rates of ALD.ALD caused nearly five lakh deaths worldwide in 2010, and 14.5 million disability adjusted life years with alcoholic cirrhosis comprising 47.9% of all liver cirrhosis death [32]. In the United State alone, nearly thirty five thousand adults died from liver cirrhosis in 2009 out of which 48.2% of deaths were due to alcohol [34].

## 2.2 METABOLISM OF ALCOHOL

Almost 90% of alcohol ingested is absorbed in the upper gastrointestinal tract and diffuses throughout the body [35, 36]. Studies have shown that ethanol can disrupt the epithelial barrier of the gastrointestinal tract resulting in increased intestinal luminal permeability and enhanced absorption of luminal antigens such as bacteria and endotoxins into the portal circulation [37, 38]. These endotoxins and bacteria cause activation of Kupffer cells in the liver and release cytokines, which may consequently result in liver injury and ALD [39, 40]. Liver being the main organ involved in detoxification is more prone to damages caused by chronic alcohol consumption. Alcohol is metabolized in the liver by both oxidative and non-oxidative pathways [41].

### 2.2.1 Oxidative pathway

The oxidative pathway of alcohol metabolism involves three enzymes, viz: Alcohol dehydrogenase (ADH) in the cytosol, Cytochrome P450 and Catalase in the microsomes [42]. In the cytosol ADH converts alcohol to acetaldehyde and other metabolites. Oxidation of ethanol by alcohol dehydrogenase leads to excess production ofreducednicotinamide adenine dinucleotide $NADH^{+}$. The $NADH^{+}$ generated competes with reducing equivalents from other substrates, including fatty acids, for the respiratory chain, inhibiting their oxidation and causing increased esterification of fatty acids to form triacylglycerol, resulting in the fatty liver. Some metabolism of ethanol takes place via a cytochrome P450-dependent microsomal ethanol oxidizing system

(MEOS) involving NADPH and $O_2$. This system increase in activity in chronic alcoholism and may account for the increased metabolic clearance in this condition [43].

Alcohol oxidizes into acetaldehyde and water by hydrogen peroxide ($H_20_2$) in the presence of catalase enzyme. Acetaldehyde is highly reactive toxic by- product in hepatocytes that may promote glutathione depletion, lipid peroxidation and mitochondrial damage [42]. It also contributes to the changes in the redox state of the cell and the formation of reactive oxygen species (ROS).The product of acetaldehyde breakdown is rapidly removed from the liver and is metabolized into CO2 via theTricarboxylic acid cycle(TCA) in the heart, skeletal muscle and brain. Genetic variation in Alcohol dehydrogenase (ADH) and Aldehyde dehydrogenase (ALDH)influence susceptibility of developing alcoholism and alcohol related liver injury [41].(Figure no 1).

## 2.2.2 Non-oxidative pathway

The non-oxidative pathway is minor in normal conditions and leads to the formation of fatty acid ethyl ester (FAEE) and phosphatidyl ethanol (PEth). Both PEth and FAEE are poorly metabolized; they accumulate in the liver and interfere with cell signaling. Because of the intermediate half life and tendency to accumulate, non-oxidative ethanol metabolites can be used as biomarkers for alcohol consumption [44]. A second non-oxidative pathway occurs at high circulating levels of alcohol and involves phopholipase D (PLD), which convertsphophatidylcholine to generate phosphatidic acid (PA) and subsequently phosphatidyl ethanol. Phosphatidyl ethanol is poorly metabolized and its effects on the cell are unknown, however it might interfere with production of phosphatidic acid and disrupt cell signaling [42]. (Figure no 2).

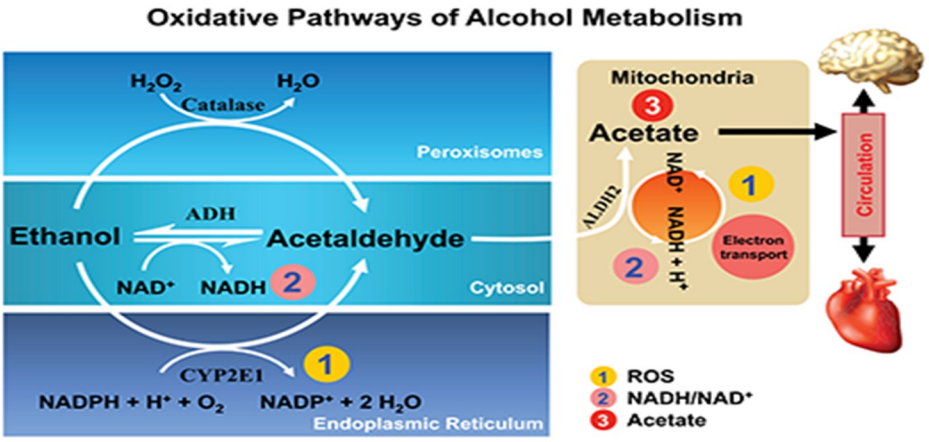


Figure 1.Oxidative pathways of alcohol metabolism. Alcohol is metabolized mainly in the cytosol by alcohol dehydrogenase (ADH) to produce acetaldehyde. Pubs.nia.nih.gov.

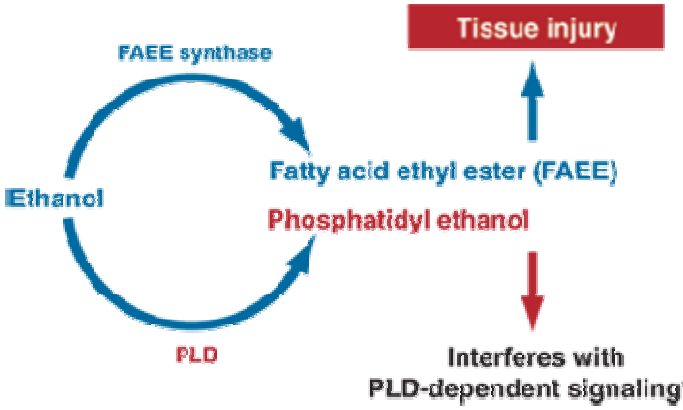


Figure 2.Ethanol is non-oxidatively metabolized by two pathways. Pubs.nia.nih.gov.

## 2.3EFFECT OF ALCOHOL ON LIVER

Liver is one of the largest and most complex organs in the body. It stores vital energy and nutrients, manufactures proteins and enzymes necessary for good health. It also protects the body from disease and helps remove harmful toxins like alcohol from the body hence is highly vulnerable to alcohol related injury.

### 2.3.1 Alcoholic liver disease.

Alcohol abuse and alcohol-induced liver diseases (ALD) areamajor public health problems, both in the US and worldwide. ALD is probably the main cause of death among people with severe alcohol abuse and is responsible for about 3.8% of global mortality [45].Fatty liver (hepatic steatosis) is an early manifestation, can progress to alcoholic liver disease (ALD), on continued alcohol ingestion.

The spectrum of disease ranges from fatty liver (steatosis) to alcoholic steatohepatitis (ASH), hepatic fibrosis and cirrhosis [46, 47].Steatosis is the earliest stage of alcoholic liver disease and the most common alcohol-induced liver disorder. This condition can be reversed, however, when the alcohol intake stops [48].Alcoholic Steatohepatitis(ASH) is comprises of the presence of fatty liver, an inflammatory infiltrate (which mainly consists of polymorph nuclear leukocytes) and hepatocellular damage [49, 50]. Patients with ASH can develop progressive fibrosis. In livers affected by ALD, the fibrotic tissue is typically located in pericentral and perisinusoidal areas, collagen deposits are evident and bridging fibrosis develops [51]. Cirrhosis involves replacement of the normal hepatic parenchyma with extensive thick bands of fibrous tissue and regenerative nodules, which results in the clinical manifestations of portal hypertension and liver failure [52,53].The prevalence of alcoholic liver disease is influenced by many factors, including genetic factors (e.g., predilection to alcohol abuse, sex) and environmental factors (e.g., availability of alcohol, social acceptability of alcohol use, concomitant hepatotoxic insults), which make this disease, difficult to define [54]. In general, however, the risk of liver disease increases with the quantity and duration of an alcohol intake. Although necessary, excessive alcohol use is not sufficient to promote alcoholic liver disease. Only 1 in 5 heavy drinkers, develops alcoholic hepatitis, and 1 in 4 develops cirrhosis indicating that pathogenesis of ALD is multifactorial and includes several overlapping events. The two-hit hypothesis postulates that the steatosis liver is the first hit, and this steatosis liver is susceptible to secondary insults including a vulnerability to reactive oxygen species (ROS), gut-derived endotoxins, and adipocytokines such as, tumor necrosis factor (TNF-α) and other cytokines. All of these hits are widely believed to be major contributors to alcohol-induced liver injury and may compound an initial steatosis. An evolving concept that is gaining acceptance is that certain accumulated fatty acids are toxic to the liver. Thus, ethanol-elicited hepatic lipid accumulation, as well as that caused by dietary sources,

has prompted renewed interest as a cornerstone of liver toxicity as it may not only initiate but enhance the progression of alcoholic liver disease [55, 56, 57].(Figure no 3.)

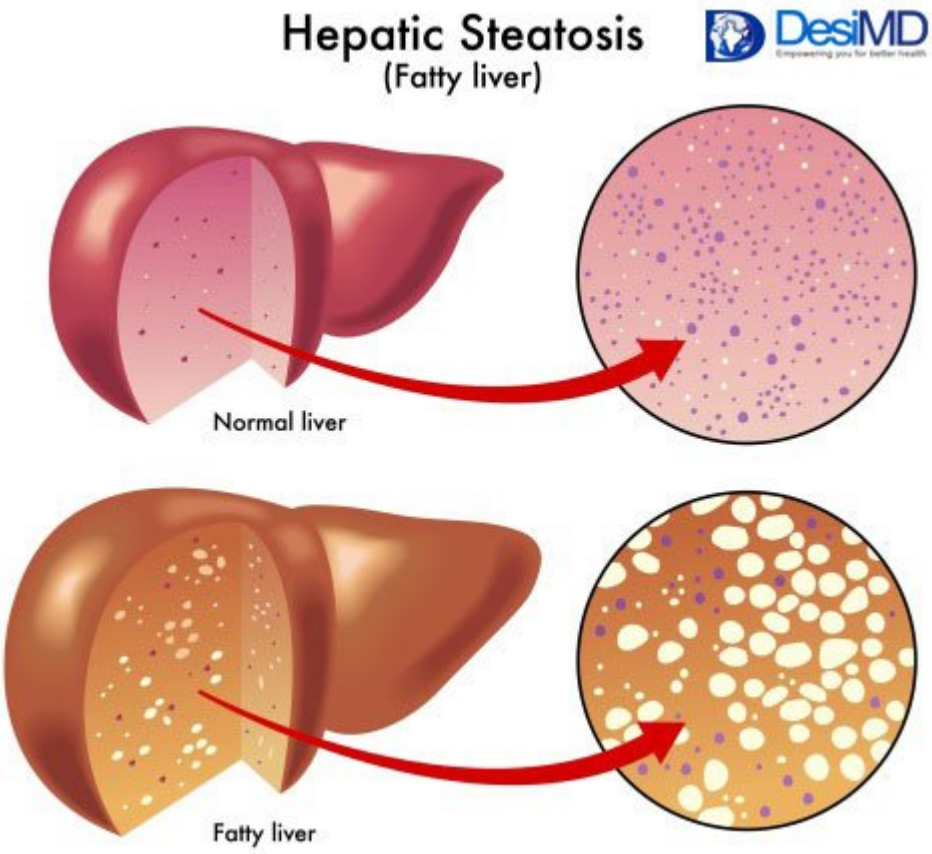


Figure no 3 Fatty liver.homeremediesforlife.com

## 2.4OTHER EFFECTS OF ALCOHOL

### 2.4.1 Short term effects

The short term effects of alcohol consumption (beer, wine, or other alcoholic beverages), range from a decrease in anxiety and motor skills at lower doses to intoxication (drunkenness), unconsciousness, anterograde amnesia (memory blackouts) and central nervous system depression at higher doses. Cell membrane is highly permeable to alcohol which diffuse into nearly every cell from the blood. The concentration of alcohol in blood is measured by blood alcohol content. The amount and circumstances of alcohol consumption play a large part in determining the extent of intoxication, for example, if a person eats a heavy meal before alcohol consumption, alcohol is absorbed more slowly [58]. Large amounts of alcohol consumption can lead to alcohol poisoning and death [59]. Alcohol can greatly exacerbate sleep problems. During alcohol abstinence, residual disruptions in sleep regularity and sleep patterns are the greatest predictors of relapse of alcohol intake [60].

### 2.4.2 Long term effects

Consumption of low to moderate amounts of alcohol for a long period of time is associated with cardioprotective effect [61,62]. Health effects associated with alcohol intake in large amounts include an increased risk of alcoholism, malnutrition, chronic pancreatitis, alcoholic liver disease and cancer. In addition damage to the central nervous system and peripheral nervous system can occur from chronic alcohol abuse [63, 64]. The long term use of alcohol is capable of damaging nearly everyorgan and system in the body [65]. Chronic alcohol abuse has serious effects on physical and mental health. It can lead to wide range of neuropsychiatric or neurological impairment, cardiovascular disease, and malignant neoplasms [66].

The effects of alcohol abuse on the ageing process includes hypertension, cardiac arrhythmia, cancers, gastrointestinal disorders, bone loss and emotional disturbances especially depression [67].

### 2.5.3 Psychiatric effects

Long term misuse of alcohol can cause a wide range of mental health problems. Severe cognitive problems are common; approximately 10 percent of all dementia cases are related to alcohol consumption, making it the second leading cause of dementia [68]. Excessive alcohol use causes damage to brain function and psychological health [69]. (Figure no 4).

Psychiatric disorders are common in alcoholics with as many as 25 percent suffering from severe psychiatric disturbances. The most prevalent psychiatric symptoms are anxiety and depressive disorders [70]. Psychiatric disorders differ depending on gender. Women who have alcohol abuse disorder often have a co-occurring psychiatric diagnosis such as major depression, anxiety, panic disorder, bulimia, and border line personality disorder, whereas men have a co-occurring diagnosis of antisocial personality disorder, bipolar disorder, schizophrenia, impulse disorder [71]. (Figure no 5).

### 2.6.4 Social effects

The social problems arising from alcoholism are serious and caused by the pathological changes in the brain and intoxication effects of alcohol [72]. Alcohol abuse is associated with increased risk of committing criminal offences, including child abuse, domestic violence, rape, burglary, and assault [73]. Alcoholism is associated with loss of employment which can lead to financial problems [74].Drinking at inappropriate times and behavioral changes caused by reduced judgment, can lead to legal consequences, such as criminal charges for drunk driving or civil penalties leading to criminal sentences [75].

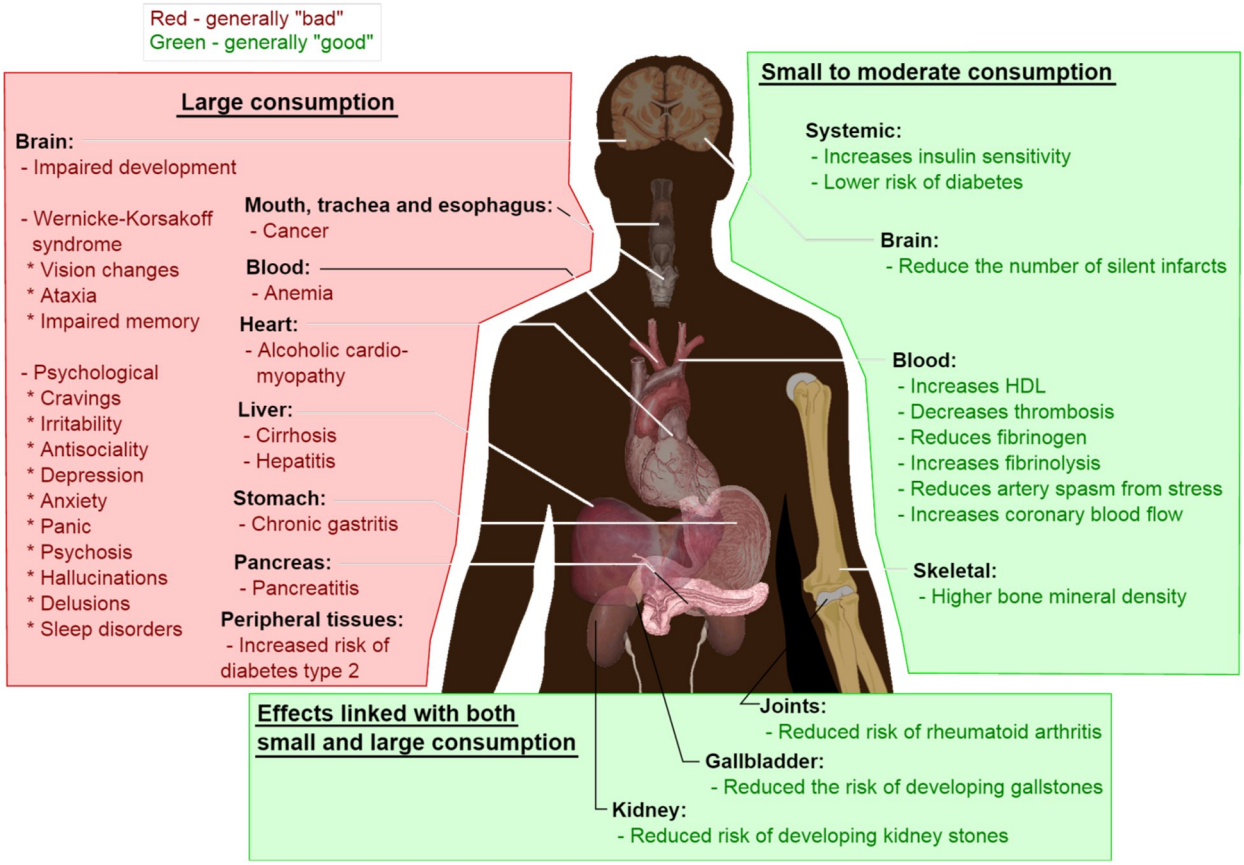


Figure 4.Effect of alcohol consumption.www.Protofcall.com.

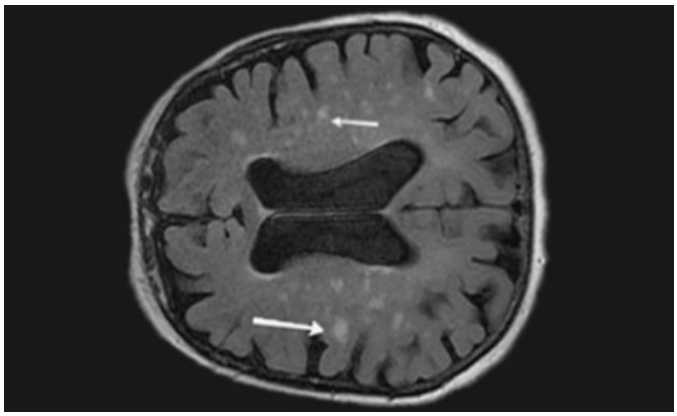

Figure5: Alcoholic Dementia. www.healthcareglobal.com.

## 2.5 GENETIC ASPECTS OF ALCOHOL-RELATED HEALTH PROBLEMS

Unlike many other chronic liver diseases, alcoholic liver disease is potentially avoidable, since excess alcohol consumption is a prerequisite for its development. However, alcohol consumption per se may not be the only explanation, since alcoholic liver disease progression is sometimes seen only in a minority of heavy drinkers. Although liver steatosis evolves in most heavy drinkers, only about 30% develop significant necroinflammation and fibrosis, out of which 10% progress to cirrhosis [76]. This variability in the natural course is believed to result from a complex interplay between environmental, individually acquired and inherent modifying factors.

A recent genome-wide association study identified a genetic polymorphism of the patatinlikephospholipase-3 I148M variant (rs738409 C>G)encoding for an isoleucinetomethionine substitution, a gene coding for a transmembrane protein most prominently expressed in the hepatocytes [77]. This variant leadsto*in vivo* triglyceride accumulation in hepatocytes, thus appearing to be the strongest determinant of human steatosis [78]. This variation was also found to be associated with elevated levels of liver enzymes in healthy subjects and with disease severity, especially steatosis and fibrosis, in non-alcoholic fatty liver disease [79, 80]. It also confers a higher risk of cirrhosis and liver damage in alcoholic liver disease [81, 82].

Several other genetic variants have been suggested to contribute to individual susceptibility to ALD. These include variants of alcohol-metabolizing enzymes, genes involved in regulating oxidative stress and those involved in endotoxin-mediated inflammation [76]. Polymorphisms in the alcohol dehydrogenase and acetaldehyde dehydrogenase (ALDH) genes may affect the rates of acetaldehyde generation and metabolism and thereby influencing acetaldehyde toxicity. Some enzyme variants may metabolize ethanol at a faster rate, or may be unable to process acetaldehyde. Accumulation of acetaldehyde, even after small amounts of ethanol, causes a condition known as Oriental flushing syndrome, with clinical manifestations that include flushing, sweating, tachycardia, nausea and vomiting [83]. Gene variants underlying this syndrome are commonly found in Asians but rarely in Caucasians [84]. In addition, a low ALDH activity phenotype increases the risk of upper digestive, head and neck cancers, and the risk of malignancies in mainly Japanese population [85, 86]. In addition, there may be racial differences in the sensitivity of the liver to different toxic stimuli, e.g. alcohol-induced acetaldehyde accumulation, or to the risk of alcohol consumers gaining weight [87]. Variations in genes that encode antioxidant enzymes, cytokines and other inflammatory mediators may also have a role in disease susceptibility [88, 89].

## 2.6 EFFECTS OF ALCOHOL ON LIVER ENZYMES

### 2.6.1 Gamma-gutamyltransferase (GGT)

Gamma-gutamyltransferase(GGT)is a membrane-bound glycoprotein enzyme derived mainly from the hepatocytes and biliary epithelial cells, renal tubules, pancreas and intestine [90]. Increased serum GGT activity has long been used in clinical practice as a marker of both liver dysfunction and excessive alcohol intake [91]. GGT is known to increase in all forms of liver disease, especially in cases of biliary obstruction, with small increases (2–5 times normal) observed in connection with fatty liver, so that GGT is of limited value for the purpose of screening alcohol consumption per se in patients with non-alcoholic liver diseases or in hospitalized patients, for instance [92,93].

### 2.6.2 Serum aminotransferase (ALT, AST)

Hepatocellular injury, whether acute or chronic, results in an increase in serum concentrations of aminotransferases. Alanine aminotransferase(ALT)originates primarily from the hepatocytes, whereas Aspartate aminotransferase(AST)is found additionally in the heart, skeletal muscle tissue, kidney and brain. As a consequence, serum AST may also increase in response to pathological processes in the heart or skeletal muscle, while serum ALT is considered a fairly specific marker of liver disease. Elevated serum aminotransferase levels can be found in asymptomatic patients for a variety of reasons, e.g. excessive alcohol intake, overweight, viral or autoimmune hepatitis, hemochromatosis, Wilson's disease, alpha 1-antitrypsin deficiency, celiac disease, genetic disorders in muscle metabolism, acquired muscle diseases, or strenuous exercise [94].It has also been suggested that alterations in the relative activities of AST and ALT may be related to the occurrence of hepatic mitochondrial damage and skeletal or cardiac muscle injury (alcoholic myopathy), which are common among alcoholic patients [95]. However, an AST/ALT ratio greater than two characteristically is present in alcoholic hepatitis.

### 2.6.3 Alkaline phosphatase (ALP)

Alkaline phosphatase(ALP)is an enzyme that transports metabolites across cell membranes. Pathological elevations are commonly observed in liver and bone diseases, although the enzyme may originate from several other tissues [96]. The synthesis and release of hepatic ALP is stimulated by cholestasis, and when released, its half-life in the circulation is about 7 days [90]. Since elevated ALP is a somewhat unspecific parameter, it needs to be interpreted in the context of a clinical diagnosis and other laboratory markers.

## 2.7 HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C) AND ALCOHOL

### 2.7.1 General aspects of HDL-Cholesterol

HDL is synthesized in the liver. HDL contains free cholesterol and phospholipids (mostly lecithin) and apoprotein (A, CII, E, etc).Increasing concentrations of HDL cholesterol is strongly associated with decrease in atherosclerosis within the walls of arteries. This is important because atherosclerosis eventually results in sudden plaque ruptures, cardiovascular disease, stroke and other vascular diseases. HDL cholesterol is sometimes referred to as "good cholesterol" because it can transport fat molecules out of artery walls, reduce macrophage accumulation, and thus help prevent or even regress atherosclerosis (97).

### 2.7.2 Relation between alcohol consumption and high density lipoprotein cholesterol

Increased high-density lipoprotein cholesterol (HDL-C) levels promote a reverse transport of lipids that stabilizes atherosclerotic plaque. Approximately half of cardiovascular benefits from moderate alcohol consumption are from increased levels of high-density lipoprotein cholesterol (HDL-C), decreased levels of low-density lipoprotein cholesterol (LDL-C), and a lowering of plasma apolipoproteinconcentration [98]. In addition, alcohol consumption may alter the activities of plasma proteins and enzymes involved in lipoprotein metabolism. Alcohol intake also results in modifications of lipoprotein particles; specifically, low sialic acid content in apolipoprotein components of lipoprotein particles and acetaldehyde modification of apolipoprotein. The effects of alcohol on lipoproteins in cholesterol transport, as well as the novel effects of lipoproteins on vascular cell wall, comprise a complex mechanism through which alcohol is cardioprotective [99].Elizabeth R et al in a study showed that alcohol consumption raises HDL cholesterol levels by increasing the transport rate of Apo-lipoproteins A1 and A2. It concluded that alcohol intake increases HDL-C in a dose dependent fashion, associated with and possibly caused by an increase in the transport rate(TR) of HDL Apo-lipoproteins A1 and A2 [100].

Red wine drinking, at 47 g/d, is associated with a 27% increase in HDL cholesterol. Regular wine consumption is associated with 30 to 90% higher levels of polyunsaturated lipids in HDL and with a 27% increase in the cholesterol esterification rate [101]. During three weeks of moderate alcohol consumption, an increase in apo A-I is followed by an increase in HDL cholesterol. The kinetics and sequence of these increases may be an additional mechanism of action underlying the reduced coronary heart disease risk in moderate drinkers [102].

Moderate alcohol intake decreases clot formation by multiple additive mechanisms. Moderate alcohol consumption reduces platelet aggregation, decreases fibrinogen levels,plasma viscosity, von Willebrand factor, and factor VII[103, 104, 105]. Regular moderate alcohol consumption has no significant effect on fibrinolysis, as opposed to higher levels of consumption [106].

Heavy alcohol consumption can adversely affect essentially every organ system [107].There is evidence that chronic consumption of as little as two drinks per day increases the risk of upper respiratory and upper digestive tract malignancies and breast cancer. The relative risk of oral and pharyngeal cancers associated with two drinks per day is 1.75; the same level of alcohol consumption is associated with a relative risk of 1.51 for esophageal cancer[108]. The relative risk of colon cancer associated with two drinks per day is 1.08. In a meta-analysis of 53 studies the relative risk of breast cancer in women was 1.32 for an average intake of 35 to 44 g/d of alcohol per day, and 1.46 for those consuming more than 44 g/d. The concurrent use of alcohol and tobacco conferred no additional risk of breast cancer [109]. In the Nurse's Health Study, the use of post-menopausal hormone replacement therapy for 5 or more years, together with consumption of 20 or more grams of alcohol daily, resulted in a relative risk of breast cancer of 1.99 compared with nondrinking women without hormones replacement therapy [110].

# AIM AND OBJECTIVES

## 3.0 RATIONALE

The prevalence of alcohol consumption in Punjab is very high especially among people of 20-60 years of age. This chronic alcohol consumption leads to several diseases amongst which alcoholic liver disease(ALD) is the commonest. In this study, the change in liver enzymes which is directly related to alcoholic liver damage will be evaluated. Also, HDL-Cknownas good cholesterol will be estimated and it will be studied to see if there is any correlation between it and the consumption of alcohol.

## 3.1 Aim and objectives

1. To investigate the change in liver enzymes (AST, ALT, ALP, GGT) in alcoholic subjects.
2. To correlate between alcohol consumption and change in HDL-C level in serum sample.

# MATERIAL AND METHODS

## 4.1 Subjects

Blood samples were collected from chronic alcoholic subjects visitingthe psychiatry department of Punjab Institute of Medical Sciences(PIMS), Jalandhar. Informed written consent was obtained from all participants. In this survey 132 alcoholic subjects and 108 non-alcoholic subjects were included.

Control: About 108 subjects aged between 20 to 60 years who had come for routine checkup and were non alcoholic, were selected as controls. Informed written consent was also obtained from these subjects.

## 4.2 Inclusion criteria

Subjects between 20 to 60 years taking at least 150 ml of alcohol daily for one year and above were included in the study.

## 4.3 Exclusion criteria

In this study, pregnant women, elderly (above 60 years) and children below 20 were excluded. A subject addicted to any other drug was also excluded from the study. Patients suffering from liver cancer and chronic heart disease were also not included in the study.

## 4.4 Laboratory measurements

The various biochemical parameters were measured in the laboratory of the Punjab Institute of Medical Sciences using standard clinical chemical methods. Serum AST, ALT, ALP and GGTactivities were measured by standard kinetic methods following the recommendations of thetest according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and Serum high-density lipoprotein cholesterol (HDL-C) activity was measured by HDL- C Immuno FS homogeneous method using BS 400 clinical chemistry analyzer(fully automatedMindray Machine).

## 4.5 Statistical methods

Values are expressed as means ± SD or means ± 95% confidence interval (CI), as indicated. Logarithmic transformation of AST, ALT, ALP, GGT and HDL data was used to obtain non-skewed distributions with homogeneity of variance. Differences between the groups were determined withLevene's test for the equality of variance (Indentpent*t* test) using the parameters comparisons. Differences between correlations were analysed with the t-test for comparison between alcoholics and non-alcoholic parameters. The SPSS 24 version, statistical software

packages for Windows was used for the statistical analyses (SPSS Inc., Chicago, IL, USA), p-value of < 0.05 was considered statistically significant.


**Figure 4.6.1 BS 400 CHEMISTRY ANALYZER FULLY AUTOMATED**

**(MINDRAY MACHINE)**

## 4.6 BS 400 CHEMISTRY ANALYZER FULLY AUTOMATED

## (MINDRAY MACHINE)

### Principle:

### Basic operation (BS-400)

- Check all the cans of distilled water, cleaning solution and waste.
- Switch on the UPS and then computer.
- Put on the instrument, water filter and instrument refrigerator.
- Wait for system initialization.
- Click on calibration.
- Select the parameters.
- Click on quality control.
- Select the tests and request.
- Keep at S1, control (Norms and Path) C1,C2 and calibrator at S2.
- Click on the `Run test'.
- Click on `Sample` and put patient ID.
- Select the tests and request.
- Click on `Run test`.
- After completion click `Shut down'.

- Switch off the instrument.
- Shut down the computer, BS 400 analyzer, cover and off the instrument.

## Technical specificity

- 400 test per hour.
- Analysis principle: colorimetry, turbidimetry, ISE method.
- Reaction type: end point, fixed-time and kinetic.
- Sample disk :90 position
- Sample volume: 02-45 µl.
- Reagent disk: 80 position
- Reaction cuvette: 90 cuvette, 5mm,30mm optical path 5mm capacity 750 µl.
- Reaction temp: - 37°C.
- Photometric system: static fibre optics and reversed optics of holographic concave flat- field gratings.
- Light source 12V, tungsten-halogen lamp, 20W.
- Wavelength:12W,length,(example):340nm,380nm,412nm,450nm,505nm,546nm,570 nm,605nm,660nm,700nm,740nm,and 800nm.
- Measuring period: 9 Second.

- Deionised water consumption: 20 L/H.

## 4.7METHOD FOR SGPT USING BS 400 MINDRAY MACHINE CHEMISTRY AUTO ANALYZER

### 4.7.1 SGPT (SERUM GLUTAMATE PYRUVATE TRANSAMINASE) (ALAT, GPT) FS IFCC METHOD WITH/WITHOUT PYXIDOXAL-5-PHOSPHATE.

**Methodology:** Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) [Modified)

**Principle:**

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALAT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDHD}} \text{Lactate} + \text{NAD}^+$$

Addition of pyridoxal-5- Phosphate (P-5-P) stabilizes the activity of transaminase and avoid falsely low values in sample containing insufficient endogenous P-5-P from patients with myocardial infarction, liver disease and intensive care patient [111].

## Reagents

## Components and concentration

| | | |
|---|---|---|
| **R1** TRISPH 7.15 | 140 mmol/L | |
| L-Alanine700 mmol/L | | |
| LDH (Lactate dehydrogenase) | | ≥ 2300 U/L |
| **R2** 2- Oxoglutarate | | 85 mmol/L |
| NADH | | 1 mmol/L |
| **Pyridoxal-5- phosphate FS** | | |
| Good's buffer | PH 9.6 | 100 mmol/L |
| Pyridoxal-5- phosphate | | 13 mmol/L |

| |
|---|
| **Specimen** |
| Serum, Heparin plasma or EDTA plasma |
| **Stability** |

| |
|---|
| 3 days at 20-25 ℃ |
| 7 days at 4-8 ℃ |
| 3 months at -20 ℃ |
| Only freeze once! Discard contaminated specimens![112]. |

**PROCEDURE:**

**Steps:**

1. An aliquot of serum is placed in the sample cup.
2. The sample cup containing the serum is then placed in the Sample disk (BS 400 chemistry analyzer machine).
3. Using the computer key board, the sample information is fed in to the machine and givecommand to start.
4. The sample probe aspirates 20µl of serum sample and dispenses into the cuvette.
5. Then reagent probe1 aspirate 200µl of reagent 1 and probe 2 aspirate 50µl reagent 2 and dispenses into the same cuvette.
6. The optical density of the reacting solution is measured at 340nm.
7. The SGPT activity is then calculated, automatically.

**Normal Value**
SGPT 0-40 U/L

## 4.7.2 SGOT (SERUM GLUTAMATE OXALOACETATE TRANSAMINASE) (AST) FS IFCC METHOD WITH/WITHOUT PYXIDOXAL-5-PHOSPHATE.

**Methodology:**Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

## Principle:

$$\text{L- Aspartate + 2- Oxoglutarate} \xrightarrow{\text{ASAT}} \text{L- Glutamate + Oxalacetate.}$$

$$\text{Oxalacetate + NADH+ } H^{+} \xrightarrow{\text{MDH}} \text{ L- Malate + } NAD^{+}$$

Additions of pyridoxal-5- Phosphate (P-5-P) stabilizes the activity of transaminase and avoid falsely low values in sample containing insufficient endogenous P-5-P from patients with myocardial infarction, liver disease and intensive care patient (111).

## Reagents:

## Components and concentrations:-

| | | |
|---|---|---|
| **R1:** TRIS | PH 7.65 | 110mmol/L |
| L- Aspartate | 320 mmol/L | |
| MDH (Malate dehydrogenase) | | 800 mmol/L |
| LDH (Lactate Dehydrogenase) | | >1200 U/L |
| **R2:** 2- Oxoglutarate | 65mmol/L | |
| NADH | 1 mmol/L | |
| **Pyridoxal-5- Phosphate FS** | | |
| Good's Buffer | PH 9.6 | 100 mmol/L |
| Pyridoxal-5-phosphate | | 13 mmol/L |

| **Specimen** |
|---|
| Serum, Heparin plasma or EDTA plasma |

| **Stability** | | |
|---|---|---|
| 4 days | at | 20-25 ℃ |
| 7 days | at | 4-8 ℃ |
| 3 months | at | -20 ℃ |
| Discard contaminated specimens. Only freeze once!(112). | | |

## PROCEDURE:

### Steps:

1. An aliquot of serum is placed in the sample cup.
2. The sample cup containing the serum is then placed in the Sample disk (BS 400 chemistry analyzer machine).
3. Using the computer key board, the sample information is fed in to the machine and givecommand to start.
4. The sample probe aspirates 20µl of serum sample and dispenses into the cuvette.
5. Then reagent probe1 aspirate 200µl of reagent 1 and probe 2 aspirate 50µl reagent2 and dispenses into the same cuvette.
6. The optical density of the reacting solution is measured at 340nm.
7. The SGOT activity is then calculated, automatically.

## Normal Value

SGOT 0-40U/L

### 4.7.3 ALKALINE PHOSPHATASE (ALP) (FS), IFCC METHOD 37 C

**Methodology:** Kinetic photometric test, according to the international federation of clinical chemistry and laboratory medicine (IFCC).

## Principle:

2- amino-2-methyl-1-propanol + P- Nitrophenylphosphate+ $H_2O$ $\xrightarrow{ALP}$ 2-amino-2-methyl-1-propanolphosphate + 4- Nitrophenol.

| **Reagents:** | | |
|---|---|---|
| **Components and concentrations:** | | |
| **R1:** 2 Amino-2- methyl-1-propanol | PH 10.4 | 1.1 mmol/L |
| Magnesium acetate | 2 mmol/L | |
| Zinc Sulphate 0.5 mmol/L | | |
| HEDTA | 2.5 mmol/L | |
| **R2:** p- Nitrophenylphosphate | | 80 mmol/L |

| **Specimen** | | |
|---|---|---|
| Serum or Heparin plasma. Do not use hemolytic samples | | |
| **Stability:** 3 days | at | 20-25 ℃ |
| 7 days | at | 4-8 ℃ |
| 2 months | at | -20 ℃ |
| Only freeze once! Discard contaminated specimens![112]. | | |

## PROCEDURE:

### Steps:

1. An aliquot of serum is placed in the sample cup.
2. The sample cup containing the serum is then placed in the Sample disk (BS 400 chemistry analyzer machine).
3. Using the computer key board, the sample information is fed in to the machineandgive command to start.
4. The sample probe aspirates 4µl of serum sample and dispenses into the cuvette.
5. Then reagent probe1 aspirate 200µl of reagent 1 and probe 2 aspirate 50µl reagent2 and dispenses into the same cuvette.
6. The optical density of the reacting solution is measured at 340nm.
7. The ALP activity is then calculated, automatically.

## Normal Values

ALP 25-90 U/L

### 4.7.4 GAMMA –GT FS* SZASZ MOD./ IFCC STAND

### Methodology:

Kinetic photometric test according to Szasz/Persijn. The test has also been standardized to the method according to IFCC (International Federation of Clinical Chemistry). Result according to IFCC is obtained using a special factor or in case a calibrator (TruCal U) is used, by use of the calibrator value given for the IFCC method (113).

### Principle:

GAMMA-GT catalyzes the transfer of glutamic acid to acceptors like glycylglycine in this case. This process release 5-amino-2-nitrobenzoate which can be measured at 405

nm. The increase in absorbance at this wavelength is directly related to the activity of gamma-GT.

L-Gamma-glutamyl-3-carboxy-4-nitranilide + Glycylglycine

Gamma-GT

Gamma- glutamyl-glycylglycine+ 5-Amino-2-nitrobenzoate.

## Reagents:

## Components and concentrations:

| | | | |
|---|---|---|---|
| **R1:** TRIS | | PH 8.28 | 135mmol/L |
| Glycylglycine | 135 mmol/L | | |
| **R2:** L- Gamma-glutamyl-3- carboxy- | | | |
| 4-nitronilide | | PH 6.00 | 22 mmol/L |

## Stability

| |
|---|
| Serum, Heparin plasma |
| Stability: at least 1 week between -20 ℃ and + 25 ℃ |
| Only freeze once! Discard contaminated specimens [112]. |

# PROCEDURE:

**Steps:**

1. An aliquot of serum is placed in the sample cup.
2. The sample cup containing the serum is then placed in the Sample disk (BS 400 chemistry analyzer machine).

3. Using the computer key board, the sample information is fed in to the machine and givecommand to start.
4. The sample probe aspirates 20µl of serum sample and dispenses into the cuvette.
5. Then reagent probe1 aspirate 200µl of reagent 1 and probe 2 aspirate 50µl reagent2 and dispenses into the same cuvette.
6. The optical density of the reacting solution is measured at 340nm.
7. The GGT activity is then calculated, automatically.

## Normal Value

GGT 11-50 U/L

### 4.8.5 HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL- C) IMMUNO FS*

### Methodology:

Previous HDL- cholesterol determinations were performed by time consuming precipitation methods. HDL-C Immune FS is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins are used to complex with LDL, VLDL and chylomicrons in a way that only HDL- cholesterol is selectively determined by an enzymatic cholesterol measurement (114).

### Principle:

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Anti-human B-lipoprotein antibodies}}$ Antigen-antibody complexes + HDL.

HDL- cholesterol + $H_2O$ + $O_2$ $\xrightarrow{\text{CHE \& CHO}}$ Choles-4-en-3-one + fatty acid + $H_2O_2$

$H_2O_2$ + F-DAOS + 4-Aminoantipyrine $\xrightarrow{\text{POD}}$ Blue complex + $H_2O$

## Reagents:

## Components and concentrations:

| | | |
|---|---|---|
| **R1**: Good's buffer | PH 7.0 | 25mmol/L |
| 4-Aminoantipyrine | | 0.75mmol/L |
| Peroxidase | (POD) | 2000U/L |
| Ascorbate oxidase | | 2250U/L |
| Anti-human B-lipoprotein | | |
| Antibody (Sheep) | | |
| **R2:** Good's buffer | PH 7.0 | 30mmol/L |
| Cholesterol esterase | (CHE) 4000 U/L | |
| Cholesterol oxidase | (CHO) 20000 U/L | |
| N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-0.8mmol/L | | |
| 3, 5- dimethoxy-4- fluoroaniline | | |
| Sodium salt | (F-DAOS) | |

| **Specimen** |
|---|
| Serum or Heparin plasma. |
| **Stability:** 2 days at 20-25 ℃ |

| |
|---|
| 7 days at 4-8 ℃ |
| 3 months at -20 ℃ |
| Only freeze once! Discard contaminated specimens! [112]. |

# PROCEDURE:

## Steps:

1. An aliquot of serum is placed in the sample cup.
2. The sample cup containing the serum is then placed in the Sample disk (BS 400 chemistry analyzer machine).
3. Using the computer key board, the sample information is fed in to the machine and givecommand to start.
4. The sample probe aspirates 3µl of serum sample and dispenses into the cuvette.
5. Then reagent probe1 aspirate 240µl of reagent 1 and probe 2 aspirate 60µl reagent2 and dispenses into the same cuvette.
6. The optical density of the reacting solution is measured at 340nm.
7. The HDL-C activity is then calculated, automatically.

## Normal value

HDL 35-70 (mg/dl)

# RESULTS

## 5.0 DESCRIPTION OF STUDY POPULATION

One hundred and thirty two alcoholics were enrolled in this study. The mean age of the alcoholics was 41.95 ± 4.45 years .All the subjects were male. The longest duration of alcohol abuse was 15 years while the shortest duration of abuse was 2 years. Majority of the study population ingested at least 150 ml of alcohol daily. Majority of the study population had studied uptoclassXII. Few subjects of the study population were cigarette smokers. Average income of the study population was above 30,000 rupee per month. Majority of study population had no physical activity. Around 50% of the alcoholic subjects hadhypertension.More than 25% of the study population had either parent suffering from diabetics.

Hundred and eight males of age group 20 to 60 years acted as controls. They had no history of alcoholism. The liver enzymes, (AST, ALT, GGT, ALP) and HDL-C concentration in the control subjects was within the normal reference range.

| **GROUP STATISTICS** | | | | |
|---|---|---|---|---|
| | **GROUP** | **N** | **Mean** | **Std. Deviation** |
| AGE | ALCOHOLIC | 132 | 41.95 | 8.45 |
| | CONTOL | 108 | 41.03 | 9.44 |
| AST | ALCOHOLIC | 132 | 36.92 | 26.35 |
| | CONTOL | 108 | 24 | 4.62 |
| ALT | ALCOHOLIC | 132 | 53.59 | 31.24 |
| | CONTOL | 108 | 27.52 | 7.22 |
| ALP | ALCOHOLIC | 132 | 102.47 | 29.03 |
| | CONTOL | 108 | 71.95 | 9.93 |
| GGT | ALCOHOLIC | 132 | 66.63 | 30.96 |
| | CONTOL | 108 | 29.42 | 7.38 |
| HDL | ALCOHOLIC | 132 | 44.68 | 11.66 |
| | CONTOL | 108 | 50.9 | 7.26 |

Table5.1. Data has been reported as mean ± SD in group.

**Independent Samples Test**

| | | Levene's test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | Df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| AGE | Equal variances assumed | 1.317 | 0.252 | 0.769 | 238 | 0.442 | 0.890 | 1.156 | -1.388 | 3.168 |
| | Equal variances not assumed | | | 0.761 | 217.156 | 0.447 | 0.890 | 1.169 | -1.415 | 3.194 |
| SGOT | Equal variances assumed | 38.348 | 0.000 | 5.027 | 238 | 0.000 | 12.915 | 2.569 | 7.854 | 17.976 |
| | Equal variances not assumed | | | 5.526 | 140.819 | 0.000 | 12.915 | 2.337 | 8.295 | 17.535 |
| SGPT | Equal variances assumed | 71.507 | 0.000 | 8.485 | 238 | 0.000 | 26.071 | 3.073 | 20.018 | 32.124 |
| | Equal variances not assumed | | | 9.288 | 147.889 | 0.000 | 26.071 | 2.807 | 20.524 | 31.617 |
| ALP | Equal variances assumed | 60.687 | 0.000 | 10.414 | 238 | 0.000 | 30.524 | 2.931 | 24.749 | 36.298 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Equal variances not assumed | | | 11.277 | 166.827 | 0.000 | 30.524 | 2.707 | 25.180 | 35.868 |
| GGT | Equal variances assumed | 71.193 | 0.000 | 10.356 | 238 | 0.000 | 32.157 | 3.105 | 26.040 | 38.274 |
| | Equal variances not assumed | | | 11.334 | 148.293 | 0.000 | 32.157 | 2.837 | 26.551 | 37.764 |
| HDL | Equal variances assumed | 11.608 | 0.001 | -4.830 | 238 | 0.000 | -6.226 | 1.289 | -8.765 | -3.686 |
| | Equal variances not assumed | | | -5.049 | 223.189 | 0.000 | -6.226 | 1.233 | -8.655 | -3.796 |

Table5.2 Data has been reported as mean ± SD in group.

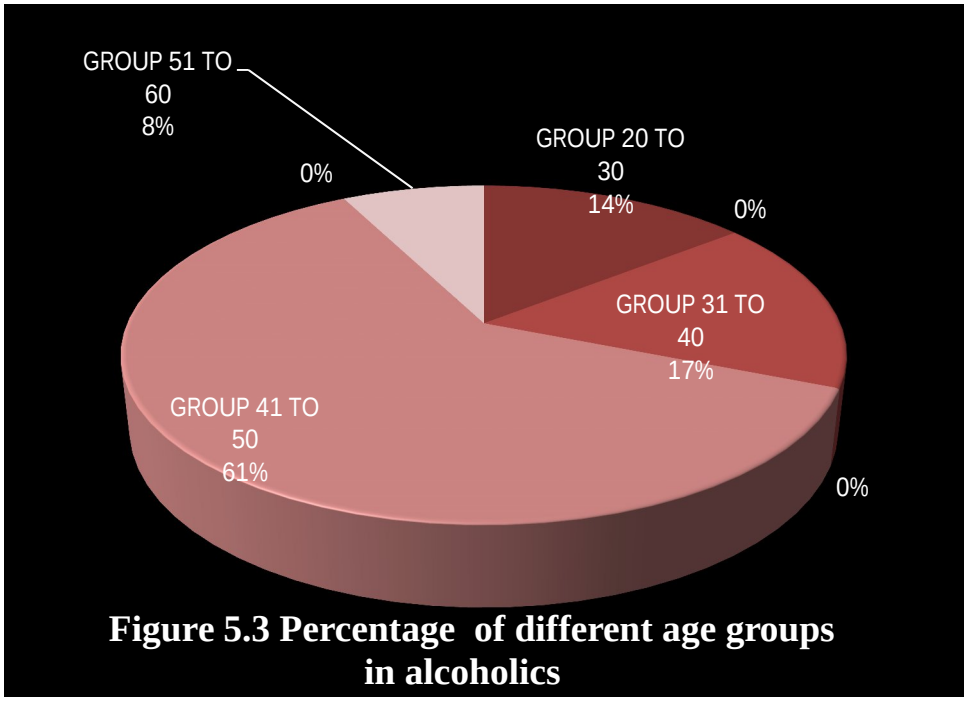


**Figure 5.3 Percentage of different age groups in alcoholics**

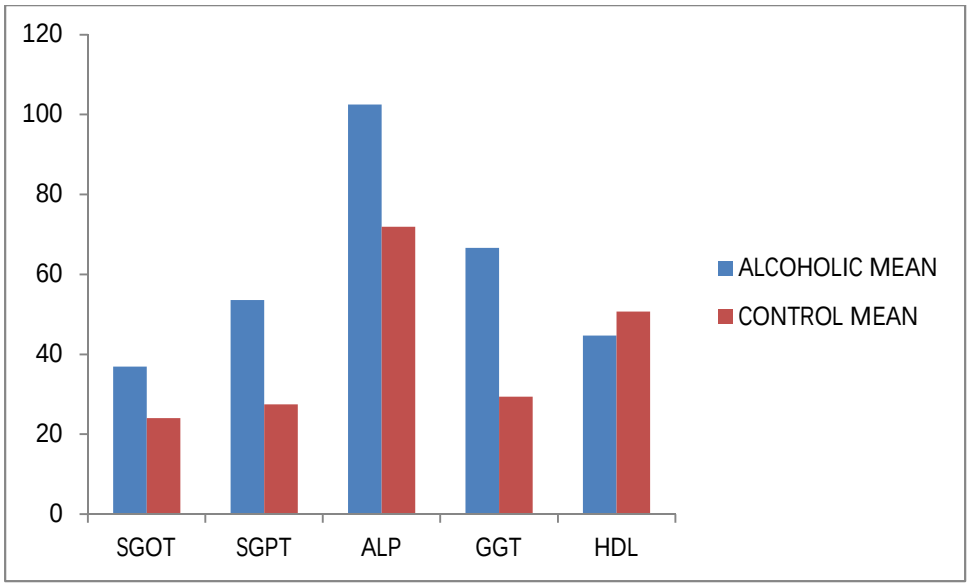


Figure 5.4Graphs shows that alcoholic mean and control mean.

The mean age of patients was 41.95 ± 8.45 years while that of control was 41.03 ± 9.44years t value = 0.44. There was no statistical significant difference between the age of alcoholics and control subjects considering $p = <0.05$.

Independent sample t-test was used to analyze the result of liver enzymes and HDL cholesterol among alcoholics and non- alcoholics of matched aged group. The mean serum AST of alcoholics was 36.92 ± 26.35 U/L, which shows a moderate rise from the normal range, while that of the control group was 24 ± 4.62 U/L, which is typical for a normal population (0-40 I/U). To test the hypothesis that alcoholics and non-alcoholics were associated with derangement in liver enzymes and HDL cholesterol, an independent sample t- test was performed using SPSS statistic software version 24, as can be seen in table 5.0. The alcoholic and non- alcoholic subjectsdistribution was sufficiently normal for conducting a t- test. Additionally, the assumption of homogeneity of variance was tested and satisfied via levene's *F- test, F (38)*, $p=<0.05$. The independent sample t-test was statistically significant $t= 0.00$ and $p= <0.05$ for AST. Among alcohol consumers 26.5% increase in serum AST was observed.

The mean SGPT of alcoholics was 53.59 ± 31.24 U/L (higher than reference range). While that of control group was 27.52 ± 7.22 U/L (within reference range). The t- value of ALT was *0.000* and *p value of <0.05*. This shows that there is a statistically significant difference between the alcoholic group and non- alcoholic group. Among alcohol consumers, 68.1% increase in serum ALT was observed (range 0-40 U/L).

The mean of ALP value of alcoholics was 102.47 ± 29.03 U/L (higher than reference range) and that of control was 71.95 ± 9.93 U/L (which is within in normal range). The t- value was *0.000* at *p =<0.05*. This shows that there is a statistically significant difference between the ALP value of alcoholics and non- alcoholics subjects. Among alcohol consumers, 66% increase in Serum ALP was observed (range 25-90 U/L).

The mean GGT valve of alcoholic patients was 66.63 ± 30.96 U/L, while that of control group was 29.42 ± 7.38 U/L. The t- value was *0.000*, *p= <0.05*, this shows that there is a statistically significant difference between the alcoholics and control group. Among alcohol consumers, 58.3% increase in serum GGT was observed (range 11-50 U/L).

The mean HDL value of alcoholic patients was 44.68 ± 11.66 mg/dl, while that of control group was 50.90 ± 7.26 mg/dl. This shows that the HDL-C value is higher in controls than alcoholic consumers at p value of *<0.05, t = 0.000*, there is significant difference between the control group and alcoholics. This shows that alcohol consumption leads to decrease in HDL cholesterol. Among alcohol consumers 37.80% decrease in HDL cholesterol was observed (range 35-70 mg/dl).

# DISCUSSION

The study shows that alcoholics have higher value of liver enzymes such as AST, ALT, ALP and GGT, when compared with non-alcoholic, age matched subjects. A brief dietary history showed that alcoholics consumed more of dietary fats, had less physical activity leading to positive calorie balance and obesity.

Batic- mujanovic et al shows that cigarette smoking adversely affects HDL-C by lowering its level, further increasing the risk for coronary heart disease [115].According to the Heart UK: the cholesterol charity association, acrolein is a chemical, which is found in cigarettes. This substance decreases plasma HDL cholesterol and thus decreased transport of cholesterol to the liver [116]. In our study 18.9% of subjects were alcoholics and smokers.

There was an increase in the value of AST by 26.5%, ALT by 68.1%, ALP by66% and GGT by 58.3%in alcoholics.Teddy Charles Adias et al in a study found that the value of prothrombin

time, activated partial thromboplastin time, and ALT, AST, GGT were highly elevated in chronic alcoholics [117].

## Many studies have correlated alcohol consumption with liver enzymes:-

Paivikki I Alatalo et al; 2008, examined the links between moderate ethanol consumption, body mass index and liver enzymes. An increase in BMI, which was more striking in moderate drinkers, was associated with increase in ALT, AST and GGT [118].

Lutz P et al; in 2011, found detrimental interaction between smoking and alcohol consumption with respect to GGT, AST and ALT [119].

Excessive alcohol drinking is directly related to an increased risk of stroke and the GGT level is useful for the assessment of risks related to alcohol drinking as found by PekkaJousilahti et al [120].

Several blood tests such as GGT or mean corpuscular volume (MCV) are among the commonly used markers to identify heavy drinking. The study by SuthatLiangpunsakul et al; 2013, examined the relationship between alcohol drinking and the levels of these markers in a nation wide study, composed primarily of light drinkers. It was found out that when these tests were done alone or in combination, the sensitivity and positive predictive values of them were too low to be clinically useful to identify the subjects in the heavy drinking category [121].

In a prospective study performed in 2001,byDuk-Hee Lee et al; to investigate the effects of baseline body mass index (BMI), alcohol consumption and its relationship with liver enzymes, it was found that there was an increase in AST and ALT values with increase in BMI, but not with alcohol consumption.Slight to moderate gain in weight was associated with increase in serum liver enzymes [122].

Sato et al; 2008, compared liver enzymes with alcohol consumption in predicting the risk of type 2 diabetes. The result showed that moderate daily alcohol consumption decreased the risk of type 2 diabetes and higher levels of GGT and ALT increased the risk [123].

The present study shows that there was a significant decrease in serum HDL-C among alcoholics when compared with non-alcoholic subjects. HDL cholesterol value was decreased by 37.80% in alcoholics. This finding is contrary to a study by HidekatsuYanai et al; 2014, which reported an increase in serum HDL-C in moderate alcoholics [124].

A study by Wanju et al; 2013, also reported an increase in the value of serum HDL-C in alcoholics who consumed 450 ml of alcohol daily. This contradiction can be due to difference in the quantity and concentration of alcohol consumed by the subjects under study [125].

In a study examine the relation between alcohol and HDL cholesterol, by Gordon T et al, it was found that HDL cholesterol levels were lower in those who never consumed alcohol than in occasional drinkers [126].

Linn et al; 1993 found an increase in plasma HDL cholesterol levels by 5 mg/dl after daily consumption of moderate amounts of alcohol [127].

A study by Elizabeth R et al, showed that alcohol consumption raises HDL cholesterol levels by increasing the concentration of Apo-lipoproteins A1 and A2. It concluded that alcohol intake increases HDL-C in a dose dependent fashion, associated with, and possibly caused by an increase in the concentration of HDL Apo-lipoproteins A1 and A2[100].

Decrease in HDL-C in the present study could be due to the amount, types of alcohol (i.e. Beer, Rum, Whisky, etc) consumed by the subjects. Dietary factors and physical activity would have also affected the HDL-C levels.

# CONCLUSION

**7.1**From the result of this study, it can be concluded that alcohol has detrimental effects on the liver. It was observed that the liver enzymes (AST, ALT, ALP and GGT), were raised above the reference range in the alcoholic subjects. This rise is due to the deleterious effect of ethanol on hepatocytes, causing leakage of cytosolic enzymes into the blood stream. Also a decrease in high density lipoprotein cholesterol (HDL-C), in sera of alcoholic subjects observed, can lead to higher risk of development of coronary heart disease.

# SUGGESTION

**8.1**The present study could have been done in detail, that is, the amount and the type of alcohol consumed i.e.beer, whisky, rum, couldhave been noted.Dietary history, including intake of type

and amount of fats, dietary fibres such as vegetables and fruits also needs to be included in thestudy. Arising the consciousness about the adverse effects of alcohol in the general population will help in reducing the morbidity and mortality due to alcoholism.

# ADDENDUM

# PATIENT PROFORMA

| | | | | | |
|---|---|---|---|---|---|
| NAME | | MRD No. | | | |
| AGE | | GENDER | | | |
| WEIGHT (KG) | | HEIGHT(CM) | | BMI | |
| EDUCATION | | OCCUPATION | | OIL/FAT CONSUMPTION | |
| COFFEE(YES/NO) | | No. OF TIMES/DAY | | | |
| FOOD HABITS | | | | | |
| VEGETARIAN (YES/NO) | | NON-VEG (YES/NO) | | | |
| NO OF TIMES/DAY | | NO OF FAMILY MEMBERS | | | |
| ADDICTIONS | | | | | |
| ALCOHOL/TYPE (YES/NO) | | AMOUNT/DAY | | No. OF TIMES CONSUMED /WEEK | |
| SMOKING (YES/NO) | | No.OF CIGARETTES SMOKED PER DAY | | No. OF TIMES SMOKED /WEEK | |
| OTHER (YES/NO) | | AMOUNT | | No. OF TIMES CONSUMED /WEEK | |
| ECONOMIC STATUS | | | | | |
| EXERCISE | | | | | |
| RUNNING | | GYM | | YOGA | |
| FAMILY HISTORY | | ALCOHOLISM/DM/HT | | | |
| DIABETES (YES/NO) | | DURATION OF DIABETES | | | |
| TREATMENT OF DIABETES | INSULIN | | | | |
| | ORAL | | | | |
| | AYURVEDIC | | | | |
| | DIET | | | | |
| | HOMEOPATHY | | | | |
| HYPERTENSION (YES/NO) | | DURATION OF HYPERTENSION | | | |
| DRUG NAME | | DURATION | | | |
| ANY OTHER ABNORMALITIES | | | | | |

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