EVALUATION OF SERUM KALLISTATIN LEVELS IN CASES OF ALCOHOLIC LIVER DISEASE

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "**EVALUATION OF SERUM KALLISTATIN LEVELS IN CASES OF ALCOHOLIC LIVER DISEASE"** is the original bonafide work done by **DR. G. CHITRA SIVA SANKARI**, Post Graduate Student, Institute of Biochemistry, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai under our direct supervision and guidance.

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DECLARATION

I, **Dr.G.CHITRA SIVA SANKARI**, Post Graduate, Institute of Biochemistry, Madras Medical College, solemnly declare that the dissertation titled "**EVALUATION OF SERUM KALLISTATIN LEVELS IN CASES OF ALCOHOLIC LIVER DISEASE"** is the bonafide work done by me at Institute of Biochemistry, Madras Medical College under the expert guidance and supervision of **Prof. Dr. R.CHITHRAA,** M.D., Professor, Institute of Biochemistry, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch XIII) in Biochemistry.

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ABBREVIATIONS

1. DALY-Disabilty Adjusted Life Year.
2. NAD-Nicotinamide Adenine Dinucleotide
3. TPIC- **T**OLERANCE, **P**HYSICAL DEPENDENCE, **I**MPAIRED CONTROL AND **C**RAVING
4. CETP- Cholesterol Ester Transfer Protein.
5. AIDS- Acquired Immunodeficiency Syndrome.
6. RES- Reticulo -endothelial system.
7. ROS- Reactive Oxygen Species
8. TNF-α - Tumour Necrosis Factor- α
9. IL-1- Interleukin-1
10. IL-6- Interleukin-6
11. MTTP- Microsomal Triglyceride Transfer Protein
12. TAG -Triacyl glycerol
13. VLDL-Very Low Density Lipoprotein
14. LDL_c- Low Density Lipoprotein
15. HDL_c- High Density Lipoprotein
16. PHT-Portal Hypertension
17. MCV-Mean Corpuscular Volume
18. TX A2-Thromboxane A2
19. ADH-Antidiuretic hormone.
20. SAAG-Serum Ascitic fluid Albumin Gradient.
21. HRS-Hepato Renal Syndrome
22. GFR-Glomerular Filtration Rate

23. HCC-Hepatocellular carcinoma.
24. WHO-World Health Organisation.
25. ALD-Alcoholic Liver Disease
26. SERPIN-Serine Protease Inhibitor.
27. PHE-Phenyl alanine
28. ARG-Arginine
29. CDT-Carbohydrate Deficient Transferrin
30. GGT -Gamma Glutamyl Transferase
31. AST- Aspartate Aminotransferases.
32. ALT- Alanine Aminotransferases
33. ALP-Alkaline Phosphatase
34. SGOT – Serum Glutamate Aminotransferase
35. SGPT-Serum Glutamate Pyruvate transferase
36. PICP- C terminal propeptide of procollagen Type -1
37. PINP- N terminal propeptide of procollagen Type -1
38. ICTP-Carboxy Terminal Pyridinoline Cross linked Telopeptide of Type -1 Collagen
39. 5-HIAA- 5-Hydroxy Indole Acetic Acid
40. 5-HTOL - 5- Hydroxy Tryptophol
41. EDTA-Ethylene Diamine Tetra acetic acid
42. OD-Optical Density
43. IFCC-International Federation of Clinical Chemistry
44. LDH-Lactate Dehydrogenase
45. MDH-Malate Dehydrogenase

46. HEDTA- N-2 Hydroxyethyl enediamine triacetic acid.

47. DHBS-3,5 Dichlioro 2-Hydroxy Benzene Sulphonate

48. ANOVA-Analysis of Variance.

49. ROC-Receiver Operating Characteristic

50. AUC-Area Under Curve.

CONTENTS

INTRODUCTION

INTRODUCTION

Alcoholism is one of the most common causes of liver disease all over the world. Liver injury begins with simple steatosis due to alcoholism, followed by alcoholic hepatitis, fibrosis of liver, ending eventually in cirrhosis [1, 2]. Risk factors for liver damage are chronic alcohol intake, obesity, genetic factors and viral hepatitis[3]. Liver injury occurs based on the quantity, duration of alcohol intake and drinking pattern [4].

Alcoholism is a major socioeconomic problem often diagnosed by reporting of the patient himself, but a physician should consider this with high suspicion. Mostly, alcoholics mimic healthy persons, when they reach the physician. At a later phase when the person started with complaints due to alcohol or other reasons, organ damage would have occurred due to alcoholism[5]. Excessive intake of alcohol accounts for 15-20% of visits of patients in primary health care centres and 20-30% of hospitalised patients[6].

EPIDEMIOLOGY:

Alcoholics are safer to be identified at an early stage so that the deleterious effects of alcohol can be prevented earlier. Further therapeutic intervention along with prognosis will also be better in alcoholic liver disease[7,8]. A study showed 14% rise in cirrhosis patients for each 1000 mL alcohol intake irrespective of the beverage consumed[9]. WHO showed an estimate of morbidity and mortality as a consequence of alcoholic liver disease being higher in developed countries (9.2%

of DALY) than developing countries[10,11.] Average consumption of alcoholics in our country is 17% .Sikkim shows a highest average of 51% and after alcohol withdrawal the relapse on an average is 35%[12].

Alcoholic liver disease presents features similar to diseases like viral hepatitis and hepatoma[3]. Alcoholism with hepatitis B and C worsens morbidity leading to chronic viral hepatitis, cirrhosis and death due to hepatocellular malignancy[13,14,15]. In alcoholic liver disease, liver damage shows hepatocellular necrosis and fibrinogenesis in stellate cells due to oxidative stress[16]. Liver damage occurs due to cellular immune response against hepatocytes affected by viral infection[17]. Malnutrition and depression causes high mortality in patients with alcoholic liver disease[18].

However, if problems due to alcohol intake are diagnosed earlier, a physician can easily prevent further progression to alcoholic liver disease. Recently, Biomarkers play an important role in

1. Detecting alcohol induced liver disease at an early stage.
2. Motivating patient for abstinence from alcohol.
3. Monitor progression while patient is on treatment[19].

A human serine proteinase inhibitor namely Kallistatin is one of the newly identified inhibitor of tissue kallikrein found in blood cells, body fluids and tissues. Kallistatin functions as a protective agent against inflammation and oxidative stress. Liver synthesize and secretes kallistatin[20].kallistatin levels decreases as the liver damage increases in alcoholics ,indicating the progression

of cirrhosis. Therefore decreased kallistatin levels is considered as an alcoholic liver disease risk factor.

Kallistatin is a plasma protein which binds with tissue kallikrein and decreases in its levels as liver damage progresses due to alcoholism[21]. Kallistatin is a unique serine proteinase inhibitor which opposes inflammatory action. Kallistatin plays an important role against apoptosis[20]. It has anti angiogenic, antitumor and anti oxidant properties[21].

Kallistatin levels declines as alcoholic liver damage progresses. Therefore this study was undertaken to evaluate the role of kallistatin as a non invasive marker in diagnosing alcoholic liver disease and assessing the severity in liver disease among patients admitted in Rajiv Gandhi Government General Hospital (RGGGH), Chennai.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

LIVER DISEASE AND ALCOHOL DEPENDENCE:

Since liver is the most important site where metabolism of alcohol occurs, it is damaged enormously due to alcoholism[22]. Liver disease can be differentiated into three main types on the morphological basis[23].

- Parenchymal.
- Hepatobiliary
- Vascular

Alcohol acts as a toxin causing damage to parenchyma in liver.

Drinkers who are dependent on alcohol have alcohol use disorders[22,23,24]. Drinkers who experience negative consequences due to alcohol, like separation from family and job are known as alcohol abusers[25, 26]. Drinkers who drink regularly and roughly the same amount of alcohol everyday are alcohol dependent. They develop adaptation to that particular limit initially but later become highly intolerant. Stages of alcohol withdrawal occur if they reduce or stop alcohol intake but suffer from toxic effects of alcohol[5]. Liver has no time to recover from damage if alcohol is consumed on a regular basis everyday .So liver injury depends on the style of intake and not on beverage type[27].

RECOMMENDED SAFE LIMITS:

Safe limits differ among drinkers. It differs on the basis of susceptibility of every individual to liver damage. Royal college of physicians advised a guideline,

recommended a safe limit of 21 units that approximates 168 g of alcohol on a weekly basis[28]:

CALORIFIC VALUE OF ALCOHOL:

Table 1 shows Energy density or calorific value of alcohol[29]

Nutrient	Energy yield Kcal/g	Energy yield KJ/g	Oxygen consumed L/g	Carbon dioxide produced L/g	Respiratory quotient	Energy yield KJ/L of Oxygen
Alcohol	7	29	1429	0.966	0.66	20

SOCIAL DRINKERS, HEAVY DRINKERS AND PROBLEM DRINKERS:

Drinkers are generally classified into three types.

- Social drinkers
- Heavy drinkers
- Problem drinkers[5].

Social drinkers drink less than 2-3 units of alcohol per day .They do not harm themselves or others by drinking, since it is non toxic level. Risk of developing coronary artery disease is also reduced by 40%[5].

Heavy drinkers consume not less than 6 units per day but they do not have immediate toxic effects.[5]

Problem drinkers consume more than 50 units per week in men. They cross recommended safe limits and experience negative effects of drinking[5].

PATHOGENESIS OF ALCOHOLIC LIVER DISEASE:

Discussed under three stages[27].

- Alcoholic Fatty Liver
- Alcoholic Hepatitis
- Cirrhosis

Pathological features begins with simple steatosis .After chronic alcohol intake, there will be a collection of fatty acids in hepatic cells. These can be visualised as large vesicles in microscopy[30]. Excess of fatty acid in hepatic cells leads to triglyceride accumulation triggering fatty liver.

Table 2 outlines important pathological features in alcoholic liver disease in various stages.

Table 2 : Pathogenesis of Alcoholic Liver Disease

ALCOHOLIC FATTY LIVER	ALCOHOLIC HEPATITIS	CIRRHOSIS
1. Enlarged liver. 2. Hepatic cells are distended with macro vesicular cytoplasmic fat vesicles.	1. Degeneration of hepatocytes and necrosis with ballooned cells. Central hyaline necrosis seen. 2. Infiltration of lymphocytes and leucocytes with Mallory bodies seen.	1. Fibrosis is diffuse with regeneration of nodules and terminates with scar formation[31].

Collagen accumulation in liver is the first and foremost event occurring after Chronic alcohol consumption leading to fibrosis followed by scar formation. The pathological events occurring from fibrosis terminating in cirrhosis is still under research[32]. Micro nodular Cirrhosis occurs after two years of onset of alcoholic liver disease[31]. Appropriate therapy and withdrawal of alcohol halts further liver damage and allows the liver to regain its functions[33].

PHARMACOLOGICAL ACTION OF ETHYL ALCOHOL:

Distribution of alcohol is dependent upon the flow of blood across organs which are vascular such as brain that immediately balance with plasma levels. Alcoholic effects depend upon the amount of alcohol consumed per unit body mass. Concentration of ethanol in blood is expressed in milligrams of alcohol per decilitre[34].

ETHANOL ABSORPTION IN INTESTINE:

Alcohol is readily absorbed by duodenum and jejunum especially upper part. Ethanol after ingestion takes about five minutes to reach the blood with peak concentration reaching at thirty minutes to two hours[32]. Delayed absorption occurs after a heavy meal. Absorption is rapid in an empty stomach. Alcohol is slowly absorbed in the absence of lipids, amino acids and glucose. Intake of alcohol for a longer time leads to malabsorption (thiamine and cobalamin deficiency), metabolic alteration (sodium, calcium, ATPase), intestinal motility disorders, low levels of disaccharadases and cell destruction[35, 36].

Alcohol can neither be stored nor can be utilised for repairing the damaged tissue in our body.

METABOLIC PATHWAYS OF ETHYL ALCOHOL:

Ethanol consumed is eliminated from our body through the lungs, kidneys and sweat but majority is catabolised in cytosol of liver hepatocytes by three pathways.

1. Alcohol dehydrogenase pathway.
2. Microsomal ethanol oxidising system pathway.
3. Catalase pathway

Alcohol dehydrogenase pathway:

Alcohol dehydrogenase catabolise alcohol to acetaldehyde by using NAD as a cofactor. As a result, NADH/NAD ratio is increased. This is responsible for the toxic effects of alcohol immediately after consumption[37].

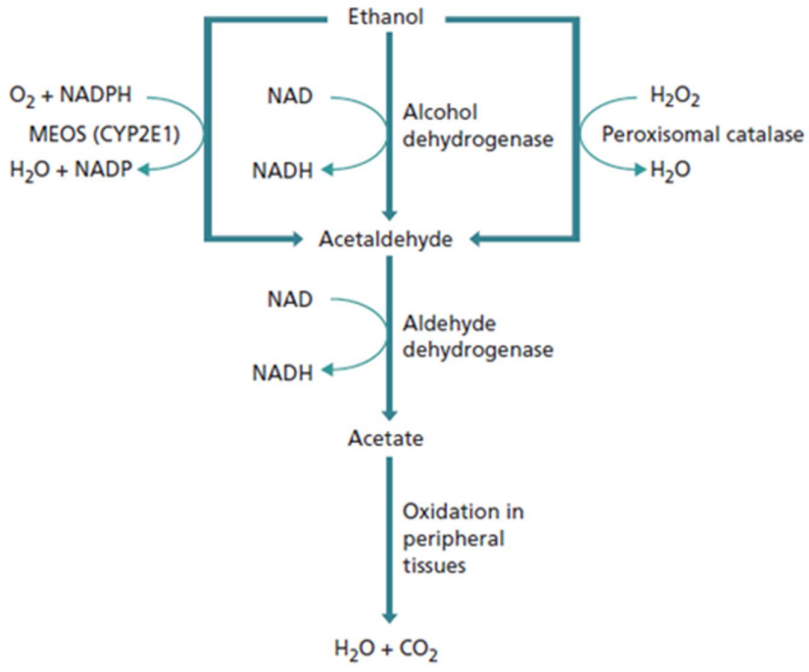
Microsomal ethanol oxidising system pathway:

Frequent alcohol exposure induces ethanol oxidising system in microsomes present in smooth endoplasmic reticulum[32]. Acetaldehyde formed by these pathways gets converted into acetate by aldehyde dehydrogenase in liver which enters citric acid cycle and forms carbon dioxide and water. Acetaldehyde is responsible for flushing in alcoholics[29].

Catalase pathway:

Catalase pathway oxidises small amount of ethanol .Water and oxygen are formed by the catabolism of hydrogen peroxide using peroxisome enzyme .Since formation of hydrogen peroxide is low under normal conditions, catalase pathway play a minor role in oxidation of ethanol .A study performed by Bradford et al proved that kuffer cells occupy a vital part in inducing catalase pathway for metabolising ethanol by regulating the supply of hydrogen peroxide [38]. Figure 1 outlines the three pathways of alcohol oxidation.

Figure-1 : Pathways of alcohol oxidation



Courtesy: Sherlock's disease of liver and biliary system

MEOS: Microsomal Ethanol Oxidising System

CYP2E1; Cytochrome p450 2E1

BIOCHEMICAL CHANGES DUE TO ALCOHOL CONSUMPTION:

1. Lactic Acidosis:

Alcohol metabolism
↓
High NADH : NAD ratio
↓
Pyruvate
↓
Lactate
↓
Lactic acidosis

2. Hyperuricemia:

Lactic acidosis
↓
Hampers uric acid excretion
↓
Hyperuricemia
↓
GOUT

3. Hypoglycemia:

Pyruvate deficiency
↓
Oxaloacetate formation affected
↓
Impaired gluconeogenesis
↓
Hypoglycaemia.

Other biochemical changes includes fatty liver,cirrhosis of liver, neurodegenerative changes and wernicke korsakoff syndrome which will be discussed in later part of the study.

"TPIC" CHARACTERISTICS OF ALCOHOLISM:

Alcohol damages liver depending on amount and period of ethanol consumption. Diagnostic features of alcoholism includes "**T**OLERANCE, **P**HYSICAL DEPENDENCE, **I**MPAIRED CONTROL AND **C**RAVING**"**[39,40,41]**.** Variation in neurotransmitter level and raised membrane fluidity of nerve cells are seen after alcoholism. After chronic alcohol exposure, body adapts to changes by tolerating increased ethanol levels by three mechanisms.

- Daily alcohol intake for a smaller period of time increases metabolism of ethanol by thirty percent but it is transient.(metabolic tolerance)
- Cell membrane changes (pharmacologic tolerance)
- Behavioural adaptation in order to function better under the influence of drugs.

Final stage ends with Physical addiction, that is, ethanol becomes mandatory for the better functioning of neurons[35].

FACTORS AFFECTING ABSORPTION AND METABOLISM OF ETHANOL:

Smoking delays gastric emptying prolonging the period of absorption of ethanol[42].

Factors affecting absorption and metabolism of ethanol are given below

- Environmental conditions
- Genetic factors
- Age in years

- Gender
- Quantity of food present in stomach
- Lifestyle conditions.

Table 3 outlines the clinical features and effects of various alcohol concentrations.

Table 3 : Clinical features and effects of various alcohol concentrations

BLOOD ALCOHOL CONCENTRATION(mg/100mL)	**CLINICAL FEATURES**
20	Euphoria
30	Increased likelihood of having an accident
40	Disinhibited
80	Impaired co-ordination, legal limit for driving.
150	Loss of self control, slurred speech, drowsiness, amnesia.
300	Stupor, coma.
500	Coma, death possible.
600	Death certain.

Courtesy: Annals of clinical biochemistry (2001:38)

IMMUNOLOGICAL EFFECTS OF ALCOHOLISM:

Consumption of ethanol affects cells of the immune system leading to liver damage. Macrophages and monocytes are damaged. Humoral and cellular immunity are disturbed leading to the invasion of infectious agents inside human organ system[43.]Studies performed by cook,1998 proved that levels of immunoglobulin-A are usually elevated in alcoholics and they are more prone for infections like tuberculosis , Acquired ImmunoDeficiency Syndrome and pneumonia[44].

BENEFICIAL EFFECTS OF ALCOHOLISM:

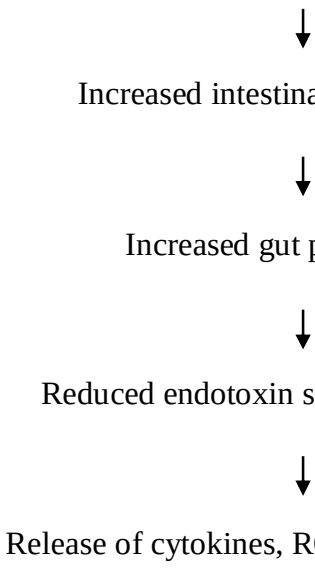
Consumption of less than 70 mL of alcohol per day prevents cardiovascular risks including heart attacks. Apo-A1 synthesis is increased and HDL levels will be elevated. Cholesterol ester transfer protein (CETP) activity will be enhanced that transfers cholesterol from tissues to HDL.

TOXIC EFFECTS OF ALCOHOL:

Alcohol intake causes endotoxic , cytotoxic and hepatotoxic effects in humans.

Figure 2 shows sequence of events showing endotoxic and cytotoxic effects of alcohol.

Figure-2 : Endotoxic and cytotoxic effects of alcohol



RES -ReticuloEndothelial System

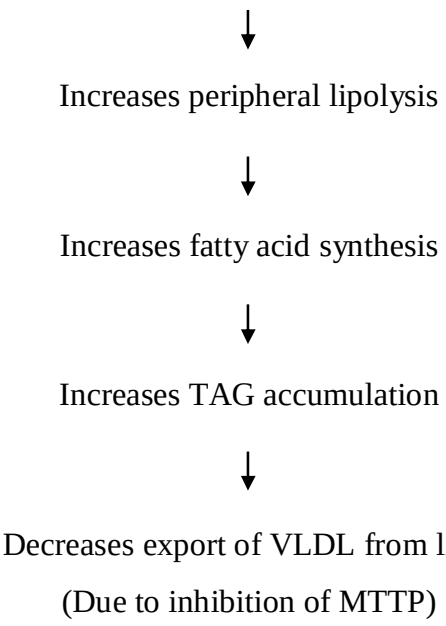
TNFα -TumourNecrosisFactor.

ROS - Reactive Oxygen Species.

IL-6 –Interleukin-6

Figure 3 shows hepatotoxic effects of alcohol.

Figure-3 : Hepatotoxic effects of alcohol



MTTP - Microsomal TriglycerideTransfer Protein.

TAG - Triacyl glycerol.

VLDL - Very Low Density Lipoprotein.

NUTRITIONAL DEFICIENCIES IN ALCOHOLISM:

Folate, niacin, vitamin-B12, Vitamin-B6 and vitamin-A Deficiency commonly occur in alcoholism. Deficiency of thiamine causes Korsakoff and Wernicke's encephalopathy.

Table 4 shows the mineral deficiencies and their toxic effects on alcoholics.

Table No.4 : Mineral deficiencies and their toxic effects on alcoholics

MINERAL DEFICIENCY	TOXIC EFFECTS
Potassium	Periodic muscle paralysis and areflexia
Magnesium	Neurological manifestations.
Calcium	Tetany and muscle palsy
Phosphate	Muscle damage, heart failure, cerebral dysfunction and platelet abnormalities.

CONDITIONS MIMICKING ALCOHOLIC LIVER DISEASE:

Non-alcoholic liver disease, hereditary hemochromatosis and Budd-Chiari syndrome mimics alcoholic liver disease. In non-alcoholic fatty liver disease, features of metabolic syndrome, peripheral insulin resistance, high blood pressure, overweight and hyperlipidemia will be seen. In hereditary hemochromatosis , atrophy of testis with enlarged heart and liver due to excess iron deposits in addition to intolerance to glucose. Budd Chiari Syndrome shows evidence of hypertrophy of caudate lobe, enlargement of liver in addition to engorged hepatic veins in Doppler Ultrasound[33].

CONDITIONS AGGRAVATING ALCOHOLIC LIVER DISEASE:

Chronic Hepatitis C, Smoking, drug abuse and obesity associated with heavy drinking N worsens alcoholic liver disease .They are the high risk groups for developing malignancy.

ETHONOL AFFECTING BODY SYSTEMS:

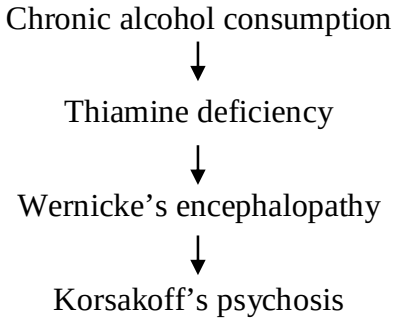
Acute or chronic consumption of alcohol affects human body in a different manner. Acute alcoholism leads to stroke embolic in nature, road traffic accidents and alcohol poisoning[91]. Chronic alcoholism leads to hyperuricemia, inflammation of pancreas, disease of liver and central nervous system[45].Several studies have shown that there is a linear relationship between ethanol intake and liver disease[46].

CENTRAL NERVOUS SYSTEM:

Alcoholic blackouts usually occurs after heavy drinking, i.e., forgetting some events that occurred while drinking[47].

Figure 4 shows sequence of effects of alcohol on central nervous system.

Figure-4 : Alcohol on Central Nervous System



Deficiency of thiamine causes peripheral neuropathy in chronic alcoholics with complaints of numbness in both the limbs, paraesthesia and tingling sensation especially in distal limbs[32].Wernicke's encephalopathy presents with ophthalmoplegia, ataxia and confusion that completely recovers with thiamine therapy. Korsakoff's psychosis characterised by amnestic disorder, anterograde in

nature with minimal retrograde amnesia but the brain skills are normal. Fifty percent of chronic alcoholics have huge brain ventricles and sulci of cerebrum but it is reversible on withdrawal of alcohol at least for a year[48].

ALCOHOL ON GASTRO INTESTINAL SYSTEM

ESOPHAGUS:

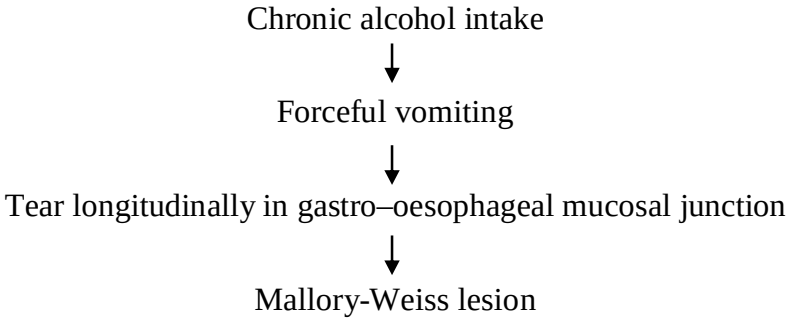
Due to regurgitation of contents of stomach into oesophagus during acute alcoholism, it undergoes inflammatory changes but it is reversible[49].

STOMACH:

As a result of damage to mucosal barrier of stomach, inflammation of gastric mucosa occurs but it is also reversible.

Figure 5 shows the sequence of effects of alcohol on gastro intestinal system.

Figure - 5 : Alcohol on gastro-intestinal system



The two irreversible complications of alcoholism of chronic duration are Portal hypertension induced by cirrhosis and Gastric cell atrophy leading to oesophageal varices[50,89].

PANCREAS:

Acinar cells of pancreas metabolise alcohol. So the toxic effects of alcohol consumption falls directly on acinar cells leading to inflammation of pancreas, insulin resistance and malignancy of pancreas[50].

LIVER:

Ethanol causes accumulation of NADH and prevents gluconeogenesis to occur which leads to decreased availability of glucose resulting in hypoglycaemia. Increased formation of lactate with decrease in oxidation of fatty acids leads to enormous accumulation of fat in hepatic cells leading to fatty liver[51].

ALCOHOL ON CARDIOVASCULAR SYSTEM:

Drinking in small amounts proved to be useful in protecting against cardiac diseases because ethanol raises HDL_c and also causes alteration in mechanism of clotting.

Binge drinking by alcoholics causes dilatation of heart and deleterious effects[52].

ALCOHOL ON CIRCULATORY SYSTEM:

Ethanol causes enlarged red blood cells (Mean Corpuscular Volume) in the absence of anaemia confirming stem cell effect[54]. Drinking for a longer period increases leukocyte count, impaired mobility of granulocytes and suppresses type-4 hypersensitivity reactions to foreign bodies resulting in increased susceptibility to infections, hepatic damage and malignancy in alcoholics. Alcohol

inhibits aggregation of platelets and arrests thromboxane A2 release but falls back to normal once the patient is in abstinence[53].

ALCOHOL ON GENITO-URINARY SYSTEM:

Ethanol causes diuresis due to temporary suppression of Anti diuretic hormone release from posterior pituitary region[32].Excretion of ammonia increases in urine resulting in metabolic acidosis. Alcohol action on respiratory centre often leads to respiratory acidosis.

ALCOHOL ON SEXUAL DYSFUNCTION:

Alcohol decreases sexual activity in men. Testosterone needs NAD+ for its synthesis. Since ethanol consumption decreases NAD+/NADH ratio in testes, testosterone production decreases. Alcoholics for a longer period will have atrophy of testes, seminiferous tubule shrinkage and minimal sperm cells.

ALCOHOLIC LIVER DISEASE-AN OVERVIEW OF STAGING FOR BETTER PROGNOSIS:

Cirrhosis can be categorised into two phases[55].

1) Compensated phase or asymptomatic phase
2) Decompensated phase or progressive phase

COMPENSATED PHASE:

Patients will be mostly without symptoms in this asymptomatic phase except for varices in oesophagus[56,90]. They will have the usual complaints like pain and swelling in abdominal region,vomiting but without dyspnoea. Pressure

in portal vein will be normal. Most alcoholics with damaged liver are identified only in the latter half of this phase[86]. This stage is reversible and has a better prognosis[37].

DECOMPENSATED PHASE:

The disease will be progressive in this stage with the following features

- Fluid collection in abdomen
- Portal hypertension
- Bleeding varices
- Yellowish discolouration of sclera and mucous membrane
- Portosystemic encephalopathy
- Nutritional deficiencies
- Metabolic abnormalities
- Altered kidney function.

This irreversible stage has a poor prognosis when it is further complicated by hepato-renal syndrome, spontaneous bacterial peritonitis, hepato-pulmonary syndrome and hepatocellular malignancy [57].

ASCITES:

A condition characterised by collection of fluid in abdominal cavity often leads to spontaneous bacterial peritonitis. It occurs due to increased hydrostatic pressure and decreased colloidal oncotic pressure [30].Ascites being a less severe complication often presents with breathing difficulty[53]. Difficulty in breathing is due to upper shift of diaphragm due to fluid collection and lung compression

leading to dyspnoea[58]. Portal hypertension causing ascites has to be differentiated from ascites of other aetiology. This is achieved by ascitic fluid analysis. Serum Ascites albumin Gradient more than 1.1 g/dL reveals ascites as a result of portal hypertension[59]. Ascites responds well for therapeutic treatment in some alcoholics while in others, it becomes refractory triggering hepatorenal syndrome[60].

SPONTANEOUS BACTERIAL PERITONITIS:

Infection of ascitic fluid by gram negative bacteria without involvement of bowel. Patient complaints of tenderness in abdominal region, high temperature and lab finding reveals raised neutrophil counts[30].

HEPATIC ENCEHALOPATHY:

Hepatic failure in advanced stages leads to disturbances in levels of consciousness, steady state disturbances, loss of brain function leading to fulminating death.

Levels of ammonia may be normal. Presence of bacteria in intestine and loads of protein in bowel tract confirms Hepatic encephalopathy[61].

HEPATORENAL SYNDROME:

In patients with decompensated liver disease, renal failure occurs in association with portal hypertension and ascites[62].Major criteria includes low glomerular filtration rate without shock, loss of protein in urine should be less

than 500 mg /day. There should be no evidence of obstruction in renal tract or any pathology in kidney on ultrasound[30].

HEPATOCELLULAR CARCINOMA:

Hepatocellular carcinoma is the advanced stage in decompensated liver disease with worst prognosis. This malignancy is associated with infections like Hepatitis-B and Hepatitis-C[63].

SCREENING OF ALCOHOL DEPENDENTS:

The primary responsibility of a physician is to identify alcoholics earlier by screening and provide necessary treatment at an earlier stage for a better prognosis [64]. while screening ,we have to collect information regarding pattern, type and amount of drink consumed everyday and the impact of drinking on social and psychological aspect of his life following accidents, bone fracture and prolonged treatment and frequent admissions in hospitals for emergency care[65]. Tests done biochemically are not so useful and sensitive than screening by questionnaire model[66,67]. It is more helpful for finding out relapse cases also[68].questionnaires for screening dependents of alcohol are shown in table 5.

Table 5 : Questionnaires for screening alcohol dependents

- CAGE (Cut down, Annoyed, Guilty, Eye opener of drinking)[75]
- MAST (Michigan Alcoholism Screening Test)
- AUDIT (Alcohol Use Disorders Identification Test)

WHO designed AUDIT QUESTIONNAIRE which consists of ten questions. This questionnaire has the highest sensitivity and specificity when compared to questionnaires of shorter format[69].

DIAGNOSING ALCOHOLIC LIVER DISEASE:

Several studies have proposed that alcoholic liver disease is best studied by

- Collecting information on alcohol consumption directly from the patient and document it.
- Alcoholic liver disease confirmed by evidence[70].

EXAMINATION OF ALCOHOLIC LIVER DISEASE PATIENT:

Many studies proved that sensitivity is low but specificity is high in detecting patients with alcoholic liver disease[71]. Absence of cirrhosis does not exclude alcoholic liver disease[72, 73]. Some features like swelling in parotid region, Dupuytren's contracture, collection of fluid in abdomen, presence of venous engorgements over the upper chest region, feminizing features, swelling of face and both lower limbs suggest alcoholic liver disease[74].

CLINICAL PROGRESSION OF COMPENSATED LIVER DISEASE:

Most of the studies reveal that cirrhosis is not an advanced stage of liver disease[76]. About 50% of patients are still in stage of compensation even if cirrhosis is present. Once the stage of compensation is identified, mortality rate in 95% of diseased patient is one year of survival and for 90% of patients, it is two years[77,85]. Varices in oesophagus are a common feature in compensated liver disease along with asymptomatic complaints[78]. As the disease progresses into

decompensation, hepatic fibrosis occurs[86]. Pressure in portal vein raises and enlarges in size leading to ascites. Now the liver goes out of compensation[79].

Death rate in compensated liver disease is very low unless it is associated with bleeding oesophageal varices or it has decompensated features in addition leading to hepatic failure[80].

CLINICAL EVALUATION OF DECOMPENSATED LIVER DISEASE:

There are several complications in decompensated liver disease which are outlined in table 6.

Table 6 : Complications in decompensated liver disease

1. Varices characterised by blood vomiting,
2. Portal hypertension with spider naevi ,
3. Fluid collection in abdominal cavity complicated by dyspnoea and spontaneous bacterial peritonitis
4. Porto systemic encephalopathy with slurred speech and flapping tremor,
5. Hepatorenal syndrome,
6. Malignancy,
7. Altered sensorium and
8. Death.

FIVE STAGE STUDY TO OUTLINE THE EFFECTS OF CIRRHOSIS:

Phases in cirrhosis were divided into five stages to study the outcome and prognosis of cirrhosis[81,] .

- Two stages in compensated phase
- Three stages in decompensated phase

Table 7 outlines the stages in compensated phase.

Table 7 : Compensated Phase

Stage-1	Absence of oesophageal varices. Decompensation marked by the development of ascites.
Stage-2	Presence of oesophageal varices with compensated cirrhosis. Patients enter decompensation with ascites and bleeding.

Table-8 outlines the stages in decompensated phase.

Table 8 : DECOMPENSATED PHASE

Stage-3	Upper digestive tract bleeding.
Stage-4	Ascites, Jaundice, Encephalopathy.
Stage-5	Hepatocellular malignancy.

TREATMENT OF ALCOHOLIC LIVER DISEASE:

Alcohol abstinence is the mainstay of treatment of ALD[82].Nutrition therapy is the second line of management. Feeding at shorter interval is usually advised[83,84]. Protein rich diet and vitamin A supplements can be given. Associated complications should be treated. Liver transplantation is done in end stage liver disease[87,88].

KALLISTATIN:

Kallistatin is a kallikrein binding protein present in humans encoded by serpinA4 gene. It is a medium sized protein belonging to serpin family. The word **SERPIN** indicates Serine Protease inhibitor but it is a misnomer . All proteins of serpin family are not serine protease inhibitors and vice versa[92]. It is a negative acute phase reactant. Kallistatin combines with tissue kallikrein to form a covalent bond complex and decreases during sepsis[93] .

Table 9 shows the nomenclature of kallistatin

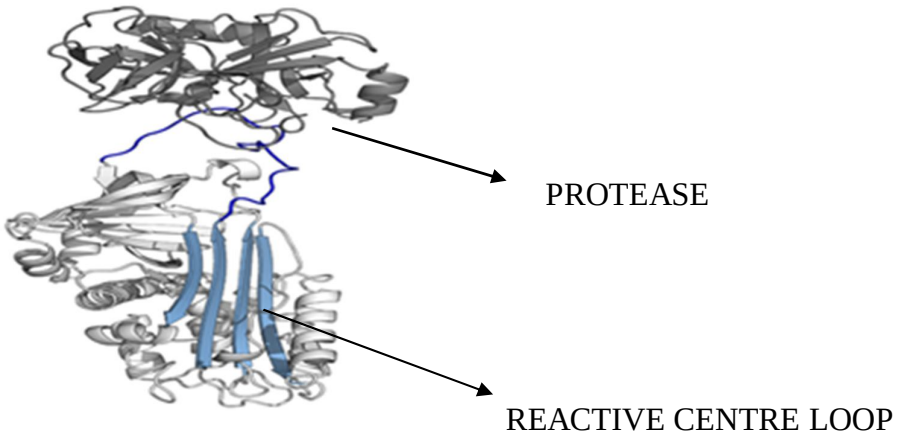
Table-9 : Kallistatin - Nomenclature

Gene name	Serpin A4
Common name	Kallistatin
Localisation	Extracellular
Protein structure	Tertiary
Chromosomal location	14q32.1
Functional activity	Inhibitor of kallikrein. Regulator of vascular function.
Effect of deficiency	Depletion-renal or vascular injury.

STRUCTURE OF SERPIN:

Serpins are spread among plants and viruses. Serpins may be extracellular or intracellular. Till now, thirty six serpins were discovered. Among them, twenty seven are serine or cysteine proteases and nine serpins do not have inhibitory action. A serpin has alpha helices, nine in number, beta sheets - A, B and C and a reaction centre loop seen outside as shown in figure-6. In this figure-6, serpin having a reactive center loop links with protease, undergoes inhibition and cannot reverse in backward direction .Kallistatin is an extracellular serpin which inhibits tissue kallikrein[92].

Figure 6 : SERPIN-A SERINE PROTEASE INHIBITOR



Courtesy: PDB-1K90

BIOCHEMISTRY AND REGULATION OF KALLISTATIN:

Kallistatin present in human plasma is a glycoprotein. It is acidic in nature. The iso-electric pH of kallistatin is 4.6 with a molecular mass of 58 KDa[94]. Kallistatin plays a balancing role in tissue kallikrein – kinin system. This system includes kinins, kininogens, and kallikrein, kallistatin and bradykinin receptors .

Initially, tissue kallikrein is found to be inactive. On reaching tissues and plasma, it becomes active. The activity of kallikrein is controlled by kallistatin[95]. Synthesis of kinins are regulated by kallikrein, but its production and utility is limited by kallistatin. Since kallistatin is produced and secreted throughout the plasma and tissues, it has widespread functions all over the body[96].

KALLISTATIN –FUNCTIONS:

The functions of kallistatin includes

1. Safeguard our body against inflammation.
2. Dilatation of blood vessels to reduce blood pressure.
3. Transport of ions.
4. Contraction and relaxation of smooth muscles.
5. Protects organs from fibrosis.
6. Prevents cell damage by oxidative stess.
7. Activation of complement.
8. Extracellular matrix remodelling.
9. Suppression of tumours.

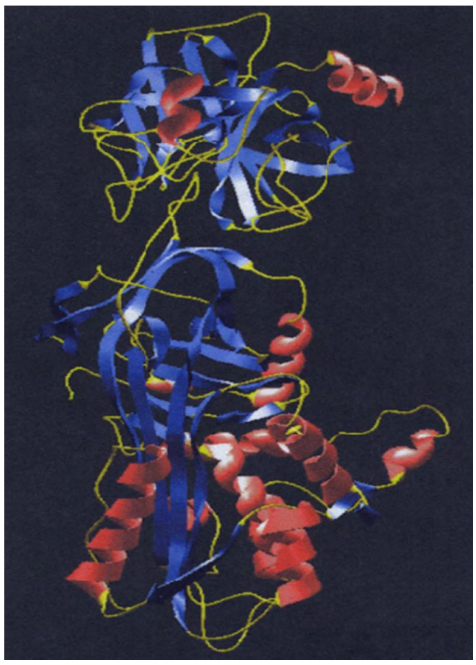
KALLISTATIN SYNTHESIS:

Kallistatin is produced enormously by liver and in minimal amounts by kidney, pancreas, heart, lung and colon. It is released by retina and vessels of blood into plasma.[95].

KALLISTATIN STRUCTURE:

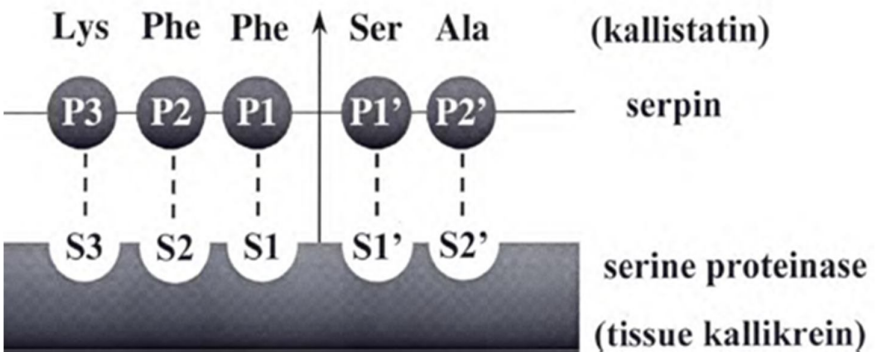
Kallistatin, an extracellular serpin presents with three beta sheets, nine alpha helices and a special reactive centre loop that splits at phe-phe-ser at P2-P1-P1' positions. It has a heparin binding site in its loop . When heparin binds with kallistatin, its role in binding with tissue kallikrein is inhibited[97]. Kallistatin attaches avidly to tissue kallikrein and loosely to elastase and chymotrypsin[98]. Tertiary structure of kallistatin terminates in a reactive loop beginning at P15 extending till P5'at C-terminal prone for damage by proteinases[99].Split sites on either side of scissile links are shown as P1 and P1'aminoterminal ends, and then, are shown as P2,P3...Pn. Carboxy terminals are shown as P2',P3',....Pn. Figure 7 shows a molecular model of kallistatin-tissue kallikrein complex inferring tissue kallikrein lying over kallistatin and Figure 8 shows specific determinants of kallistatin.

Figure-7 : Structural study of kallistatin



Courtesy: Structural and functional study of Human Kallistatin - Chang - yi Chen

Figure 8 : Specificity determinants of a serpin



Courtesy: Structural and functional study ofhuman kallistatin by Chang-yi Chen

KALLISTATIN AND ITS TYPES:

Kallistatin occur in two main forms

1. Wild form (P1 PHE)
2. Mutant form. (P1 ARG)

Wild form has a narrow zone of activity. It acts only by forming a complex with tissue kallikrein. The mutant form has a wide range of activity. It shows inhibitory effect to all members of serpin family[94].

ROLE OF KALLISTATIN IN ALCOHOLIC LIVER DISEASE:

Kallistatin can be used as a pivotal biomarker in diagnosing alcoholic liver disease early when compensated disease sets in. Liver diseases are usually silent and asymptomatic in early stages and burst out with terrible complications when it enters symptomatic decompensated stage[100]. Patient will have poor prognosis ending with portosystemic encephalopathy, bleeding in variceael region and hepatocellular carcinoma.

The diagnostic challenge lies in predicting alcoholic liver disease in early compensated stage, initiate therapy, advise lifestyle modifications and to monitor the efficacy of measures.

Liver biopsy is found to be reliable in diagnosing alcoholic liver disease, assessment of severity of liver disease by grading and evaluating fibrosis. But it has some demerits[101].

Table 10 shows the demerits of liver biopsy.

Table 10 : Demerits of liver biopsy

1. Invasive Procedure.
2. Performance-size dependent.
3. Bleeding complications.
4. Inter and Intra-observer variations.
5. Chance of misdiagnosis.
6. Patient acceptability.
7. Difficulty in repeating the procedure.

Several new non- invasive biomarkers for determining the staging of alcoholic liver disease and to know disease severity came into existence. Among them, serum Kallistatin proved to be an ultimate biomarker with great clinical significance.

Since it is a negative acute phase reactant, there is an inverse relationship between serum kallistatin levels and disease severity from early stage.

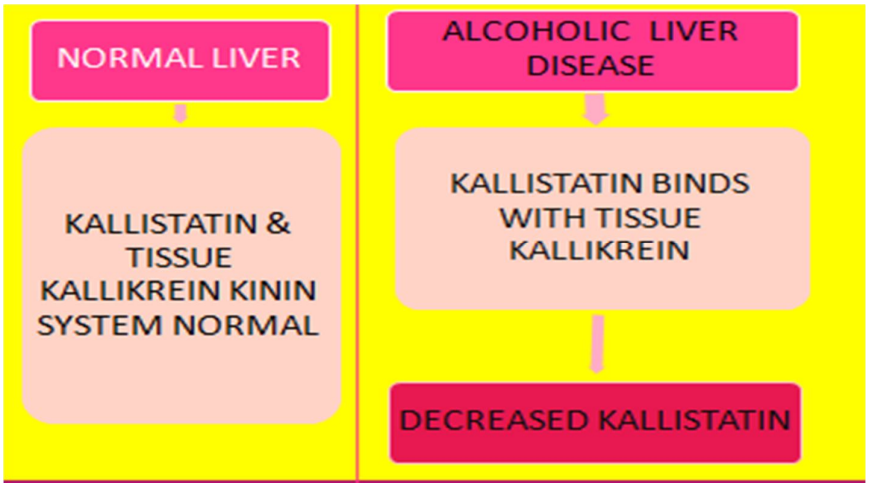
Serum kallistatin decreases in early compensated stage of liver disease, when the patient is asymptomatic and helps to identify early hepatic damage so that therapeutic intervention can be started earlier for a better prognosis[21].

CORRELATION BETWEEN SERUM KALLISTATIN AND ALCOHOLIC LIVER DISEASE:

In a liver with normal morphology, kallistatin and tissue kallikrein-kinin system is normal. Alcohol consumption at a minimal level causes kallistatin to bind with tissue kallikrein and its level declines. Early detection of fall in kallistatin levels helps in detecting alcoholic damage on hepato-biliary system for a better prognosis[21].

Table 11 shows a comparison of role of kallistatin in normal and alcoholic liver disease.

Table 11 : Role of kallistatin in normal and alcoholic liver



ANALYSIS OF KALLISTATIN:

For a routine laboratory analysis of serum kallistatin , separation and in vitro quantitative measurement of human serum kallistatin is done by Enzyme Linked Immunosorbent Assay (Bioassay Technology Laboratory, Shangai , China.), a Sandwich Immunoassay Technology using antibodies.

OTHER BIOMARKERS OF ALCOHOLIC LIVER DISEASE:

CARBOHYDRATE DEFICIENT TRANSFERRIN:

These are glycoproteins bound with iron containing 6% of carbohydrate. It is a beta1 globulin with a molecular mass of 80 kDa present as single chain with small amount of sialic acid [103].CDT is useful for clear segregation of alcoholic from non alcoholic liver disease.CDT returns to normal in 10 days after cessation of drinking.

GAMMA GLUTAMYL TRANSFERASE:

Cell membranes especially endothelium contains a glycoenzyme gamma glutamyl transferase[104]. Two important functions of GGT include peptide transport and glutathione metabolism . Liver is the centre for action of GGT[105]. Half life of GGT is eighteen hours[106]. GGT levels are elevated in hepatic malignancies, alcohol, drug and viral hepatitis.

Levels of gamma glutamyl transferase enzyme is not only elevated in alcoholic liver disease showing good sensitivity, but it is also elevated in non alcoholic liver disease[107]. Extra hepatic conditions like heart failure, chronic kidney disease and inflammation of pancreas also raises GGT[108].GGT levels start

raising in blood as soon as the patient consumes alcohol whether he has liver disease or not. GGT has high sensitivity but lacks specificity .So GGT cannot be used as a marker for screening hepatic disorder due to alcoholism but they can be used in alcohol suspicion.

TRANSAMINASES:

Aminotranferases are of two types .They are

1. Alanine aminotransferases
2. Aspartate aminotransferases.

ALT has cytoplasmic form only. It is specific for liver. AST has cytoplasmic and mitochondrial forms present in cardiac, renal, hepatic and musculoskeletal systems [109]. It is nonspecific for liver. Increased values of ALT and AST often indicate liver disease .Mostly ALT:AST ratio will be less than one. AST:ALT ratio greater than two indicates alcoholic liver disease [110.]

TYPE-1 COLLAGEN DERIVED PEPTIDES:

Patient consuming ethanol shows an elevated levels of type-1 collagen metabolic assays especially carboxy terminal (PICP), amino terminal (PINP) and type-1 collagen degradation products(ICTP).These markers are also used for assessing severity of alcohol damage in liver[111].

ACETALDEHYDE ADDUCTS:

After heavy consumption of ethanol, acetaldehyde adducts are elevated in blood. Social drinkers are usually, detected by this method .False positive results are seen in diabetes, elevated urea level in blood and in cases of endogenous acetaldehyde formation.

5-HYDROXY INDOLE ACETIC ACID:5 -HYDROXY TRYPTOPHOL RATIO:

Serotonin is metabolised to form 5-hydroxy indole acetic acid and 5-hydroxy tryptophol. The ratio of 5HIAA:5HTOL in urine is useful for detecting alcohol consumption within day[112]. These metabolites are present in urine for 6-20 hours greater than after its absence from plasma.

URINE ETHYL GLUCORONIDE:

Urine ethyl glucoronide assay has good sensitivity and specificity. Apart from serum and urine, it can also be assayed in hair and tissues .Though it detects alcohol consumption within a day,results are diluted by water intake[36].

PHOSPHATIDYL ETHANOL:

When a person consumes alcohol, phospholipase-D combines with ethanol to form phosphatidyl ethanol . The results are highly sensitive and specific as it detects ethanol for two weeks after drinking is ceased.

OTHER MARKERS:

Other markers of alcoholic liver disease includes serum mitochondrial AST, Serum Beta Hexosaminidase , Sialic acid, Fatty acid ethyl esters and urinary dolichols. These markers are under clinical evaluation and trials.

REFERENCE RANGE OF SERUM KALLISTATIN:

Reference range is 18.5-25.6µg/mL[113].

AIMS & OBJECTIVES

AIM OF THE STUDY

The aim of the study is:

- To evaluate the role of **kallistatin** as a non invasive marker in the diagnosis of alcoholic liver disease and its usefulness in correlation with disease severity.
- To compare serum kallistatin levels in alcoholic liver disease patients with apparently healthy individuals.

To fulfil the aim of the study, serum kallistatin levels are assessed in control groups and by dividing the subjects into two groups viz., compensated or asymptomatic group and decompensated or progressive group along with other biochemical parameters affected by alcohol consumption .The other parameters would include AST, ALT, ALP, GGT, Serum total and direct bilirubin, total protein and albumin, TGL, HDL_c, Cholesterol and LDL_c .

MATERIALS & METHODS

MATERIALS AND METHODS

This is a case control study and was conducted after getting ethical committee approval. The study is comprised of a total number of 90 human subjects. Controls were recruited from outpatient department during their visit for non hepatic causes. 30 apparently healthy individuals, with history of total abstinence from alcohol and normal ultrasound abdomen were selected. 60 cases were selected from gastroenterology department, Rajiv Gandhi Government General Hospital (RGGGH), Chennai. Consent was taken from all the subjects as well as the controls. Among the 60 cases selected, 30 were compensated alcoholic liver disease and 30 were decompensated alcoholic liver disease cases.

INCLUSION CRITERIA:

- Patients with alcoholic liver disease diagnosed by ultrasound or liver biopsy.

EXCLUSION CRITERIA:

- Patients with non- alcoholic liver disease.
- Viral hepatitis.
- Autoimmune liver diseases.
- Genetic or Metabolic liver diseases like Wilson's disease, Alpha-1 antitrypsin deficiency.
- Hepatocellular carcinoma patients.

- Inflammatory conditions like pneumonia, ulcerative colitis and Crohn's disease.

Patients were grouped into two –based on compensated and decompensated features.

CASES

- GROUP-1: 30 alcoholic liver disease patients with compensated features (with or without varices but no ascites)
- GROUP-2: 30 alcoholic liver disease patients with decompensated features (ascites, jaundice, hepatic encephalopathy)

BLOOD SAMPLE COLLECTION:

- Collection of blood sample done after 8-12 hours of fasting.
- About 5 mL of venous blood was collected from antecubital vein under aseptic precautions and separated into two tubes Investigations performed as per the following table 12.

Table 12 : Blood collection tubes and investigations

Tubes	Anticoagulant	Amount of blood	Investigations
Tube-1	EDTA	2 mL	plasma lipid profile
Tube-2	_	3mL	Serum total and direct bilirubin , AST, ALT, ALP, GGT, Total protein, albumin Kallistatin.

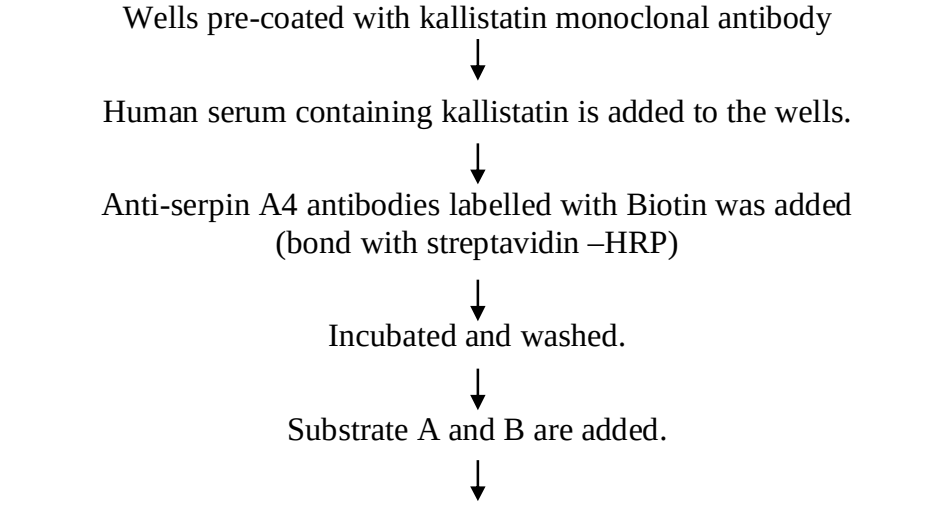
Blood samples were analyzed by the following methodologies.

ESTIMATION OF SERUM KALLISTATIN (ENZYME LINKED IMMUNOSORBENT ASSAY):

Principle:

Human kallistatin levels are quantified by enzyme linked immunosorbent assay by sandwich technique .Figure 9 shows the principle of the test.

Figure 9 : Principle of the test:



Materials provided in kit:

1. Standard solution (240µg/mL)
2. Standard dilution (3mL)
3. Coated ELISA plate.
4. Streptavidin-HRP (6mL)
5. Washing concentrate (30x)
6. Antiserpina 4 Antibodies labelled with biotin (1 mL)

Assay procedure:

As per the steps given in kit insert, the following were done, to dilute the standard solution for the preparation of different concentrations.

120ng/mL	Standard No.5	120µL original standard+120µL standard diluents
60ng/mL	Standard No.4	120µL standard No.5+120µL standard diluents.
30ng/mL	Standard No.3	120µL standard No.4+120µL standard diluents.
15ng/mL	Standard No.2	120µL standard No.3+120µL standard diluents.
7.5ng/mL	Standard No.1	120µL standard No.2+120µL standard diluents.

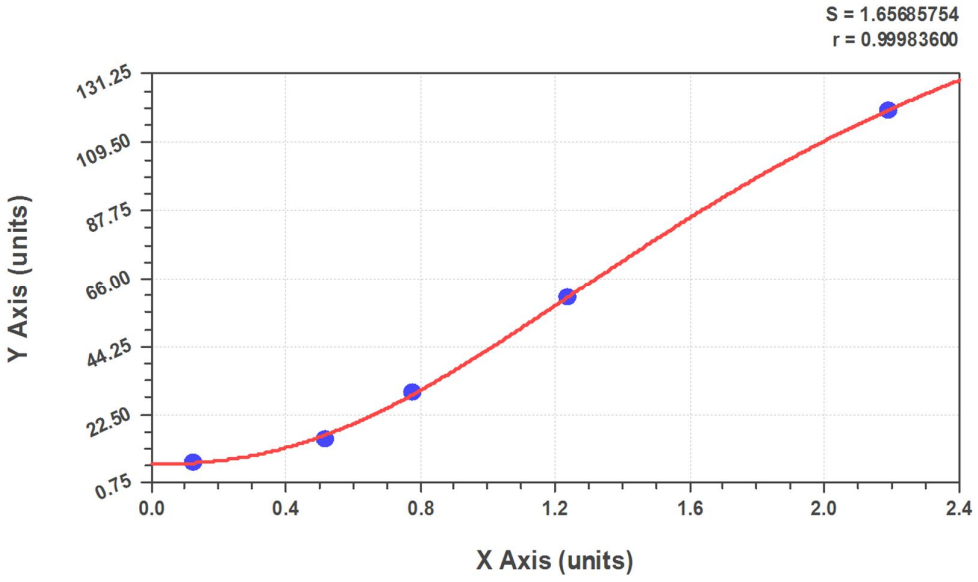
standard	S5	S4	S3	S2	S1
240ng/mL	120ng/mL	60ng/mL	30ng/mL	15ng/mL	7.5ng/mL

Reagents, samples and standards are prepared. A and B chromogen, stop solutions added in blank wells. In Standard solution well, 50µL of standard and 50µL of streptavidin HRP were added. In sample well, sample of about 40µL and antibodies of SERPIN A4, 10µl, streptavidin HRP 50µl were added. Mixed and incubated at 37˚C. Allow to stand for 60 minutes. Solution for washing is prepared by diluting with distilled water. Plate is washed for five times. Chromogen A and B added, sealed with a membrane and incubated at 37˚C. Allow to stand for 10 minutes, followed by addition of stop solution. Colour changes from blue to yellow. Absorbance value read within 10 minutes at 450 nm.

Calculation:

The standard curve is drawn by plotting the absorbance value for each standard solution on the vertical Y-axis versus corresponding kallistatin concentration (7.5, 15, 30, 60,120 and 240µg/mL respectively) along X-axis. Linear regression equation has been calculated for plotting the standard curve. The other OD values are substituted and corresponding concentration of samples were deciphered from the plotted standard graph.

STANDARDS	CONCENTRATION (ng/mL)	OPTICAL DENSITY(OD)
S1	7.5	0.318
S2	15	0.534
S3	30	0.792
S4	60	1.258
S5	120	2.215



Assay range:

Varies between 7.5-53.2 µg/mL of serum kallistatin.

REAGENT STORAGE AND STABILITY:

If unopened, can be stored at 2-8˚C for 6 months and -20˚C for 12 months. The Biochemical parameters undertaken for the study , were analysed by the following methodologies.

ESTIMATION OF LIQUID BILIRUBIN (TOTAL AND DIRECT BILIRUBIN)

Method : "Diazo Method of Pearlman and Lee, is an endpoint method"

Reagent Kits are used

Principle

In acidic medium, the reaction begins with bilirubin and sulphanilic acid(diazotized).Azobilirubin is formed which is pink in colour. Colour is formed rapidly in direct bilirubin since it is soluble in water. A surfactant is used for solubilising the unconjugated bilirubin . Reaction ends with the same colour as that of direct bilirubin.

"Absorbance is directly proportional to bilirubin concentration - Reagents

Reagent 1: Total bilirubin reagent

Surfactant	1.00%
Hydrochloric acid	100mmol/L
sulphanilic acid	5 mmol/L

Reagent 2: Direct bilirubin reagent

Sulphanilic acid	10mmol/l
Hydrochloric acid	100mmol/L

Reagent 3: Sodium nitrite reagent

Sodium nitrite	144 mmol/L

Assay procedure:

Take three test tubes viz., blank, standard and test (B, S & T).

- In Blank, 500µL working reagent+25µL distilled water taken.
- In Standard, 500µL working reagent+25µL standard added.
- In Test, 500µL working reagent+25µL test sample added.
- Mixed and incubated at 37˚ c for 5 minutes. Absorbance read at 546/630nm.

Reference values:

TOTAL BILIRUBIN : 0.1-1.2 mg/dL

DIRECT BILIRUBIN : 0-0.2mg/dL.

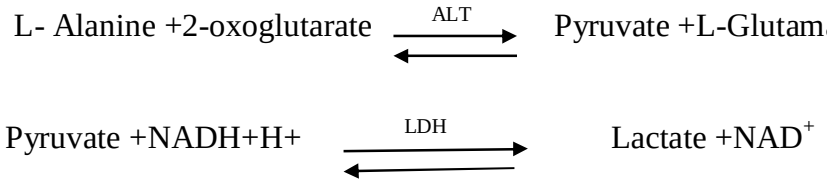
ESTIMATION OF SERUM ALANINE AMINOTRANSFERASE (SGPT)

Method

Dynamic Extended Stability Modified IFCC Method

Kit used

Principle:



Change in absorbance with respect to time, is directly proportional to ALT activity i.e., when NADH is converted to NAD.

ALT-Alanine Aminotransferase

LDH - Lactate dehydrogenase.

Reagent composition

Reagent R1:

Tris buffer	100mmol/L
L-alanine	440mmol/L
LDH	▪ 4U/mL
α Ketoglutarate	13.20mmol/L

Reagent R2:

β NADH	1.52 mmol/L

R1 (four parts) mixed with R2 (one part) in a tube.

PROCEDURE:

Working reagent	500µL
Sample	25µL

Mix and read absorbance.

CALCULATION

- SGPT (ALT) ACTIVITY (IU/L) =ΔA/min*Factor (3376) by A.

Reference range

- Till 42 U/L at 37˚ c

ESTIMATION OF SERUM ASPARTATE AMINOTRANSFERASE (SGOT)

Method

Dynamic Extended Stability Method Modified IFCC Method

Principle

L-Aspartate+2-oxoglutarate $\xrightleftharpoons{AST}$ oxaloacetate +l-glutamate

Oxaloacetate + NADH + H^+ $\xrightleftharpoons{MDH}$ Malate +NAD^+

Change in absorbance with respect to time is directly proportional to AST activity i.e., when NADH is converted to NAD.

- AST-Aspartate AminoTransferase
- MDH-Malate Dehydrogenase
- Reagent composition

Reagent R1:

Tris buffer(ph 7.8)	20 mmol/L
L-Aspartate	230mmol/L
LDH	･ 33.3μKat/L
2-Oxoglutarate	13.21mmol/L
MDH	･ 3.33μKat/L

Reagent R2:

NADH	1.51mmol/L

R1 (four parts) mixed with R2 (one part) in a tube.

Procedure

Working reagent	500μL
Sample	25μL

Mix well and read absorbance.

Calculation

- SGOT (AST) Activity (IU/L) =ΔA/ min* Factor (3376) by A.

Reference range

- 10-40 U/L

ESTIMATION OF SERUM ALKALINE PHOSPHATASE (ALP)

Methodology

IFCC- Method, Kinetic.

Principle

2amino-2-methyl -1-propanol+p-nitrophenyl-O-phosphate+H_2O $\xrightarrow{ALP}$

4-nitrophenol+2-amino-2methyl-1-propanol phosphate.

ALP-Alkaline Phosphatase

Rate of formation of 4-nitrophenol is directly proportional to Alkaline phosphatase activity.

Reagent composition

2-Amino-2-methyl-1-propanol	350mmol/L
Magnesium	2.0mmol/L
Zinc	1.0mmol/L
HEDTA	2.Ommol/L
Para-nitro phenyl phosphate	16.0mmol/L

Procedure

Working reagent	1000µL
Sample	20µL

Mix well and read absorbance

Calculation

ALP activity (IU/L) =ΔA/min* Factor (2764)

Reference range

38-94IU/L

ESTIMATION OF GAMMA GLUTAMYL TRANSFERASE (GGT)

Methodology

IFCC Method kinetic

Principle

L-γ glutamyl 3-carboxy-4-nitroanilide+glycyl glycine $\xrightarrow{GGT}$

L- γ - glutamyl glcyl glycine+5-amino-2p-nitrobenzoate.

The glutamyl moiety is enzymatically transferred from gamma glutamyl p-nitro anilide to glycylglycine releasing p-nitro anilide .Activity of GGT is monitored by increase in absorbance measured based on the principle adopted for a spectrophotometer, at 405 nm by semi autoanalyser.

Reagent composition

Tris buffer - pH 8.2+ 0.1(25 c)	100mM
Glutamyl p-nitroanilide	4mM
Glycylglycine	100mM

3.0 mL of deionised water is added to dissolve the reagent in the vial.

Procedure

working reagent	1000µL
Sample	100µL

Reagent and sample mixed in a cuvette and the results are noted

Reference range

Upto 50U/L.

LIPID PROFILE

The biochemical parameters undertaken for study is analysed by the following methodologies

ESTIMATION OF PLASMA TOTAL CHOLESTEROL

Method

Cholesterol Esterase-Cholesterol Oxidase

Principle

Cholesterol ester+H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol +fatty acids

Cholesterol+o_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholestenone +hydrogen peroxide

$2H_2O_2$ +Phenol+4 Aminoantipyrene $\xrightarrow{\text{peroxidise}}$ red quinine +$4H_2O$

Intensity of red coloured complex is directly proportional to the concentration of cholesterol measured at 505 nm.

Reagents

Goods buffer(Ph-6.4)	100mmol/L
Cholesterol Esterase	≥200u/L
Cholesterol Oxidase	≥100u/L
Peroxidase	≥3000U/L
4-Aminoantipyrene	0.3 mmol/L
Phenol	5mmol/L

Standard (cholesterol: 200 mg/dL)

Cholesterol - 2g/L

Procedure

Reagent was reconstituted to 1mL and 10µL of plasma was added. Reading was taken after incubation for five minutes at 37˚ c.

Reference values

150-260 mg/dL

ESTIMATION OF PLASMA TRIGLYCERIDES

Method

Enzymatic method, End point by colorimetry.

Principle

Triglyceride+H_2O $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol +fatty acid

Glycerol +ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3- phosphate +ADP

Glycerol-3- phosphate $\xrightarrow{\text{Glycerol3 phosphate oxidase}}$ Di hydroxy acetone phosphate+H_2O_2

H_2O_2+4 –aminoantipyrene+3, 5 DHBS $\xrightarrow{\text{peroxidase}}$ red quinine+2H_2O

Intensity of purple coloured complex formed is directly proportional to the concentration of triglycerides.

Reagents

Pipes buffer (ph-7.0)	40mmol/L
lipoprotein lipase	4000U/L
glycerol kinase	1500U/L
glycerol 3 phosphate oxidase	4000U/L
Peroxidase	2200U/L
4-Aminoantipyrene	0.4 mmol/L
ATP	2mmol/L
Magnesium	2.5 mmol/L
DHBS(3,5 Dichlioro 2-Hydroxy Benzene Sulphonate)	0.2mmol/L

Standard (Triglycerides	200 mg/dL)
Glycerol (trig. Equivalent)	2g/L

Procedure

Reagent was reconstituted upto one mL.About 10mL of plasma is added.Incubated at 37°c for 5 minutes .Readings taken at 546 nm.

Reference range:

Males	60-165 mg/dL
Females	40-140mg/dL

ESTIMATION OF HDL CHOLESTEROL

Method

Phosphotungstic acid method, End point assay.

Principle

Precipitation with phosphotungstic acid and magnesium chloride was done to remove HDL from chylomicrons , LDL and VLDL. After centrifugation, cholesterol remained as supernatant in HDL fraction. This cholesterol was assayed by enzymatic cholesterol method using cholesterol esterase ,oxidase , peroxidise and the chromogen 4-aminoantiptyrene/phenol

Reagents

Reagent 1(enzyme/ chromogen)

Cholesterol esterase	≥200U/L
Cholesterol oxidase	≥250U/L
Peroxidase	≥1000U/L
4-aminoantipyrene	0.5mmol/L

Reagent 1A (BUFFER)

Pipes buffer ph 6.90	50 mmol/L
Phenol	24 mmol/L
Sodium cholate	0,5 mmol/L

Reagent 2 (precipitating reagent)

Phosphotungstic acid	24mmol/L
Magnesium chloride	39 mmol/L

Standard (HDL Cholesterol 50 mg/dL)

Cholesterol 0.5g/L

Reconstitution of reagents:

After allowing the reagents to attain room temperature, added the contents of 1 bottle of reagent 1 with 1A. Mixed gently till it was dissolved .Used after 5 minutes.

Procedure:

After attaining room temperature, sample, precipitating reagent 2 and reconstituted reagent were used.

1) Precipitation

Centrifuge tube is dispensed.

Sample	200µL
Precipitating reagent 2	200µL

Centrifuged for 10 minutes at 1000 rpm. Supernatant was separated quickly and cholesterol content is assayed as follows.

2) Cholesterol assay:

Reaction type	End point
Reaction slope	increasing
Wavelength	500nm.
Incubation	5 minutes 37 c
Sample volume(supernatant)	20µL
Reagent volume	1.0 mL

Reference range

LOW	40 mg/dL
HIGH	60 mg/dL

ESTIMATION OF LDL CHOLESTEROL

LDL Cholesterol can be interpreted by "Friedewald's formula" as follows

LDL Cholesterol=Total cholesterol-(HDL_c +Triglycerides/5.0)mg/dL

(Applicable for triglyceride levels lesser than 400 mg/dL)

ESTIMATION OF TOTAL PROTEIN

Method

Biuret method, End point assay

Principle

In alkaline medium, Protein present in the serum reacts with copper salts. purple coloured complex is formed. Intensity of the colour is directly proportional to the concentration of protein in the sample measured spectrophotometrically at 540 nm."

Procedure

Analyser was calibrated with a given protein standard concentration of 6g/dL.

Working reagent	500µL
Sample	10µL

After mixing, incubation done at 15-30˚ c for 10 minutes. Readings are measured against reagent blank at 540 nm.

Calculation

$$\text{Concentration of protein (g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} * \text{concentration of standard}$$

Reference range

6 to 8 g/dL

ESTIMATION OF SERUM ALBUMIN

Methodology

Bromocresol green, Endpoint assay

Principle

Albumin behaves as a positively charged ion and binds to anionic dye bromocresol green at a pH 3.68.Green coloured complex is formed. The intensity of colour is directly proportional to concentration of albumin in the sample. Final colour is measured at an absorbance of 578 nm.

Procedure:

Analyser was calibrated using a given standard concentration of 4g/dl.

Working reagent	1mL
Sample	10µL

Mixed well. Read after five minutes .Absorbance was measured spectrophotometrically at 578 nm.

Calculation

$$\text{Albumin (g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} * \text{concentration of standard}$$

Reference range

3.5 -5 g/dL

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

SPSS (Statistical Package for Social Science) version 16.0 software was used for analysing statistical data.

Various analytes indicating functions of liver including total bilirubin, direct bilirubin ,SGOT, SGPT, ALP, GGT, total protein , albumin ,total cholesterol , triglycerides ,LDL cholesterol and HDL cholesterol are done in patients with alcoholic liver disease and healthy controls.

- Mean and Standard deviation are calculated for each study group. Students' "t" test was done to compare the mean values. **p value** was calculated." A **p value** of less than 0.05 is considered to be significant .A **p value** of less than 0.01 is considered to be highly significant .
- For comparing more than two variables of the same group and between two groups, one way ANOVA was done. This F test was done to compare serum kallistatin levels between compensated and decompensated cases of alcoholic liver disease of one group and with healthy controls. A p value <0.05 is considered to be significant.
- Pearson correlation of parameters namely total bilirubin ,direct bilirubin ,SGOT , SGPT ,ALP ,GGT , total protein and albumin were analysed.
- Spearman rank correlation shows rank correlation of kallistatin with other variables like ALP, AST, Albumin,GGT ,total and direct billirubin.

- Receiver operating characteristic (ROC) Curve was done to assess sensitivity and specificity of kallistatin. Area under ROC curve (AUC) is useful for finding out the expected cases of alcoholic liver disease. Best cut off values are obtained for serum kallistatin level.
- Logistic regression analysis was done to show the association between kallistatin and other parameters in alcoholic liver disease.

RESULTS

MASTER CHART OF CONTROL GROUPS

CASES	AGE	GENDER	HEIGHT (cms)	WEIGHT (kg)	BMI (kg/m2)	DURATION (years)	USG ABDOMEN	T.BILI (mg/dL)	D.BILI (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	AST/ALT	SAP (IU/L)	T.PROTEIN (g/dL)	ALBUMIN (g/dL)	GGT (IU/L)	T.CHOL (mg/dl)	TGL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	KALLIS (µg/mL)
1	53	M	145	62	29.5	NO	normal	0.3	0.02	18	18	1	72	7.4	4.4	20	163	160	96	33	24.76
2	45	M	148	61	27.8	NO	normal	0.7	0.4	14	20	0.7	69	7.8	3.6	19	122	55	77	34	24.33
3	21	M	146	65	30.5	NO	normal	0.5	0.2	22	28	0.8	67	7	4.4	12	140	45	87	46	31.9
4	67	M	142	52	25.8	NO	normal	0.7	0.3	17	24	0.7	72	7.6	4	14	121	65	70	38	25.67
5	25	M	146	61	28.6	NO	normal	0.8	0.4	16	21	0.8	67	7.1	4.5	12	149	82	95	38	24.67
6	25	M	141	64	32.2	NO	normal	0.6	0.2	29	27	1.1	90	7	4.5	27	194	135	118	49	23.89
7	72	M	141	62	31.2	NO	normal	0.3	0.1	16	21	0.8	50	6.1	4	16	178	93	80	79	28.89
8	25	M	175	56	18.3	NO	normal	0.5	0.2	26	21	1.2	53	7.4	4.5	14	155	74	87	53	23.98
9	50	M	164	56	20.8	NO	normal	1.1	0.5	15	25	0.6	79	6.1	4.3	202	117	105	57	42	20.56
10	32	M	146	54	25.3	NO	normal	0.7	0.3	19	24	0.8	90	7.2	4.8	54	162	161	91	39	23.47
11	34	M	145	52	24.7	NO	normal	0.7	0.3	38	34	1.1	55	6.9	4.5	17	170	71	108	48	18.36
12	65	M	146	54	25.3	NO	normal	0.9	0.4	28	34	0.8	65	7.7	4.8	9	133	97	71	43	25.22
13	63	M	147	56	25.9	NO	normal	0.8	0.5	29	28	1	69	6.7	4.3	16	104	67	41	50	20.53
14	35	M	143	54	26.4	NO	normal	1	0.5	34	27	1.3	68	7.2	4.7	15	144	82	81	47	19.57
15	57	M	167	72	25.8	NO	normal	1.2	0.6	24	26	0.9	62	7.7	4.7	34	161	118	78	59	23.89
16	29	M	179	54	16.9	NO	normal	1	0.4	20	27	0.7	83	7.2	4.8	49	183	156	105	47	19.98
17	50	M	179	72	22.5	NO	normal	0.8	0.4	29	32	0.9	66	7.5	4.3	54	172	81	98	58	21.67
18	30	M	171	63	21.5	NO	normal	2.8	0.9	29	21	1.4	92	7	4.7	54	158	194	82	37	18.87
19	27	M	185	58	16.9	NO	normal	0.7	0.3	18	24	0.8	87	7.4	4.6	4	184	93	117	48	19.35
20	49	M	160	56	21.9	NO	normal	0.6	0.2	16	23	0.7	84	7.2	4.4	144	204	141	136.8	39	20.17
21	62	M	164	56	20.8	NO	normal	1.7	1.2	25	27	0.9	85	6.6	3.5	32	155	76	117.8	22	26.78
22	70	M	162	61	23.2	NO	normal	0.7	0.2	24	18	1.3	75	6.2	3.8	17	130	81	81.8	32	26.89
23	50	M	145	54	25.7	NO	normal	5	2.9	32	21	1.5	85	6.5	3.5	33	97	71	67.8	15	26.45
24	43	M	152	47	20.3	NO	normal	0.6	0.2	22	23	1	68	6.4	3.8	19	150	62	115.6	22	25.78
25	25	M	158	61	24.4	NO	normal	0.6	0.1	24	28	0.9	54	6.1	4.5	19	130	49	95.2	25	27.76
26	32	M	149	61	27.5	NO	normal	0.5	0.2	24	27	0.9	87	6.5	4.1	20	185	58	159.4	14	29.78
27	42	M	146	61	28.6	NO	normal	0.3	0.1	23	26	0.9	67	6.1	3.9	19	171	74	139.2	17	25.76
28	25	M	149	56	25.2	NO	normal	0.3	0.1	33	35	0.9	68	6.2	3.9	36	181	65	132	36	27.78
29	24	M	150	61	27.1	NO	normal	0.4	0.1	16	19	0.8	65	6.3	4.5	54	152	53	95.4	46	23.12
30	23	M	152	53	22.9	NO	normal	0.7	0.1	19	21	0.9	68	6.3	4.2	11	189	68	144.4	31	24.89

MASTER CHART OF ALCOHOLIC LIVER DISEASE PATIENTS

CASES	AGE	GENDER	HEIGHT (cms)	WEIGHT (kg)	BMI (kg/m2)	DURATION (years)	USG ABDOMEN	T.BILI (mg/dL)	D.BILI (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	AST/ALT	SAP (IU/L)	T.PROTEIN (g/dL)	ALBUMIN (g/dL)	GGT (IU/L)	T.CHOL (mg/dl)	TGL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	KALLIS (μg/ml)
1	45	M	160	54	21.1	10	FATTY LIVER	0.6	0.3	42	32	1.3	64	6.7	4.7	42	234	181	156.8	41	13.12
2	43	M	145	54	25.7	8	FATTY LIVER	0.7	0.3	39	28	1.4	75	6.8	4.5	35	180	163	108.4	39	18.31
3	45	M	148	61	27.8	8	FATTY LIVER	0.5	6.3	36	24	1.5	72	6.7	4.5	35	144	126	82.8	36	11.55
4	58	M	149	62	27.9	7	FATTY LIVER	0.7	0.3	43	33	1.3	64	6.8	4.4	35	133	154	65.2	37	14.46
5	59	M	148	61	27.8	7	FATTY LIVER	0.6	0.3	39	32	1.2	60	7	4.6	30	158	143	77.4	52	15.15
6	64	M	168	85	30.1	8	FATTY LIVER	0.4	0.2	39	28	1.4	62	6.6	4.3	18	145	132	39.6	79	18.7
7	46	M	164	62	23.1	7	FATTY LIVER	0.7	0.3	44	69	0.6	68	6.4	4.4	20	139	143	72.4	38	12.21
8	49	M	162	56	21.3	8	FATTY LIVER	1.4	0.6	63	23	2.7	63	7.6	4.9	52	166	134	89.2	50	19.11
9	48	M	161	49	18.9	9	CLD	1.3	0.7	49	34	1.4	129	7.5	3.1	88	72	124	28.2	19	15.17
10	46	M	162	51	19.4	9	CLD	1.4	0.7	39	28	1.4	102	5.8	3.6	35	109	145	54	26	16.47
11	46	M	161	62	23.9	9	CLD	1.5	0.9	23	22	1.0	67	7.5	4.5	58	84	112	28.6	33	20.27
12	36	M	164	75	27.9	5	FATTY LIVER	1.4	0.7	37	24	1.5	64	6.6	3.1	45	83	118	28.4	31	13.06
13	36	M	165	72	26.4	6	FATTY LIVER	0.9	0.4	46	29	1.6	65	6.5	3.2	28	89	108	35.4	32	14.09
14	44	M	171	73	25.0	7	FATTY LIVER	1.2	0.9	56	37	1.5	76	6.2	3.2	66	84	109	29.2	33	12.14
15	44	M	169	72	25.2	8	FATTY LIVER	0.7	0.3	47	19	2.5	65	7	3.7	20	163	153	82.4	50	12.28
16	40	M	144	64	30.9	10	CLD	3.1	0.7	69	37	1.9	62	7	3.6	14	143	121	67.8	51	12.87
17	36	M	149	62	27.9	15	FATTY LIVER	2.1	1.6	94	22	4.3	78	5.8	2.3	551	170	154	89.2	50	14.56
18	39	M	144	62	29.9	10	CLD	4.5	2.1	56	33	1.7	64	6	2.8	42	180	163	121.4	26	14.87
19	61	M	146	61	28.6	10	FATTY LIVER	0.6	0.3	56	35	1.6	72	7.3	4.6	45	167	142	95.6	43	19.89
20	43	M	143	62	30.3	7	FATTY LIVER	0.7	0.3	23	21	1.1	81	6.9	4.2	28	170	156	100.8	38	15.01
21	51	M	143	61	29.8	7	FATTY LIVER	0.4	0.2	38	28	1.4	69	7.9	4.7	92	317	261	213.8	51	13.56
22	43	M	167	75	26.9	8	FATTY LIVER	0.5	0.2	39	13	3.0	63	6.8	4.6	26	159	153	87.4	41	17.75
23	47	M	162	54	20.6	8	FATTY LIVER	0.5	0.3	34	24	1.4	61	6.2	4.2	76	115	90	41	56	17.91
24	41	M	161	49	18.9	7	FATTY LIVER	0.5	0.2	38	33	1.2	67	7	4.6	45	129	137	68.6	33	12.31
25	57	M	162	52	19.8	6	FATTY LIVER	0.5	0.2	25	17	1.5	75	6.6	4.4	13	177	154	110.2	36	12.11
26	63	M	168	93.9	33.3	7	FATTY LIVER	0.5	0.2	41	35	1.2	64	6.7	4.4	27	238	187	151.6	49	16.44
27	40	M	161	52	20.1	20	CLD	3.1	1.3	133	72	1.8	103	6.7	3.4	282	140	127	96.6	18	12.01
28	40	M	164	56	20.8	8	CLD	4.5	2.2	65	34	1.9	186	3.5	2.4	58	142	172	84.6	23	9.334
29	37	M	162	57	21.7	10	CLD	20.5	11.5	129	30	4.3	112	4.9	2.2	275	151	166	45.8	72	9.27
30	47	M	161	64	24.7	20	CLD	2.9	1.3	1270	348	3.6	286	5.6	2.3	87	206	165	142	31	11.12
31	53	M	148	62	28.3	5	DCLD+ASCITES	3.4	1.4	97	28	3.5	124	4.6	2.9	87	147	156	84.8	31	13.18
32	52	M	152	61	26.4	20	DCLD+ASCITES	7.9	5.2	83	32	2.6	132	5.4	2.1	89	143	132	99.6	17	14.82
33	36	M	151	60	26.3	15	DCLD+ASCITES	2.3	1.4	64	46	1.4	117	6	2.2	33	102	132	48.6	27	9.78
34	35	M	149	64	28.8	10	DCLD+ASCITES	8.5	4.5	142	67	2.1	126	4.9	2.3	71	112	153	62.4	19	9.48
35	52	M	148	62	28.3	5	DCLD+ASCITES	2.1	0.9	99	38	2.6	157	5.7	2.8	294	110	152	53.6	26	12.13
36	54	M	161	66	25.5	8	DCLD+ASCITES	1.4	0.7	78	35	2.2	113	5.4	3.1	79	154	145	110	15	10.45
37	50	M	160	62	24.2	8	DCLD+ASCITES	1.9	0.8	53	20	2.7	131	5.1	2.4	32	102	132	60.6	15	13.12
38	44	M	161	58	22.4	20	DCLD+ASCITES	2.3	1.1	413	112	3.7	147	5.9	3.1	74	110	143	63.4	18	12.57
39	60	M	160	52	20.3	20	DCLD+ASCITES	5.3	1.5	67	36	1.9	126	5.9	2.2	74	132	165	82	17	15.8
40	52	M	161	58	22.4	8	DCLD+ASCITES	0.7	0.3	56	32	1.8	132	5.7	3.2	76	163	143	84.4	50	11.5

CASES	AGE	GENDER	HEIGHT (cms)	WEIGHT (kg)	BMI (kg/m2)	DURATION (years)	USG ABDOMEN	T.BILI (mg/dL)	D.BILI (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	AST/ALT	SAP (IU/L)	T.PROTEIN (g/dL)	ALBUMIN (g/dL)	GGT (IU/L)	T.CHOL (mg/dl)	TGL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	KALLIS (µg/ml)
41	42	M	163	61	23.0	7	DCLD+ASCITES	0.4	0.6	54	31	1.7	134	6.3	2.9	32	226	341	129.8	28	13.49
42	48	M	165	62	22.8	6	DCLD+ASCITES	1.5	0.6	45	36	1.3	129	5.6	3.4	38	128	153	62.4	35	19.14
43	41	M	168	64	22.7	6	DCLD+ASCITES	0.6	0.2	45	24	1.9	134	5.3	3.3	84	195	175	120	40	8.65
44	46	M	169	81	28.4	7	DCLD+ASCITES	0.9	0.2	45	32	1.4	129	5.9	3.2	64	202	330	119	17	13.64
45	41	M	171	62	21.2	8	DCLD+ASCITES	0.5	0.2	36	31	1.2	132	6.1	3.4	87	142	165	74	35	17.61
46	55	M	160	62	24.2	25	DCLD+ASCITES	9.3	4.7	73	31	2.4	137	5.5	2.2	75	132	165	82	17	7.11
47	40	M	156	72	29.6	10	DCLD+ASCITES	13.7	11	59	23	2.6	177	5.8	2.9	76	134	143	92.4	13	8.34
48	49	M	158	70	28.0	10	DCLD+ASCITES	7.3	2.7	92	28	3.3	132	6	2.2	89	143	164	89.2	21	13.71
49	43	M	157	68	27.6	10	DCLD+ASCITES	15.8	13.5	140	46	3.0	123	5.1	2.5	95	152	162	106.6	13	13.89
50	54	M	152	64	27.7	10	DCLD+ASCITES	5	2.4	254	251	1.0	145	3.8	2.7	81	132	142	90.6	13	7.12
51	50	M	151	61	26.8	9	DCLD+ASCITES	3.2	1.4	78	33	2.4	115	6.2	3.4	86	134	141	90.8	15	11.67
52	40	M	154	66	27.8	10	DCLD+ASCITES	25	12.3	149	45	3.3	146	5.8	3	73	136	163	94.4	9	17.22
53	56	M	154	62	26.1	10	DCLD+ASCITES	10	4.3	89	35	2.5	134	4.98	2	82	131	132	95.6	9	15.12
54	58	M	152	62	26.8	8	DCLD+ASCITES	7.9	4.1	109	39	2.8	173	6.2	2.2	362	78	115	34	21	15.56
55	35	M	156	61	25.1	8	DCLD+ASCITES	4.9	1.5	160	28	5.7	134	5.2	2.1	79	143	171	96.8	12	10.29
56	63	M	154	62	26.1	8	DCLD+ASCITES	6.7	3	177	53	3.3	141	3.9	3	73	137	153	75.4	31	13.42
57	65	M	152	63	27.3	10	DCLD+ASCITES	4.3	1.6	78	28	2.8	176	5.4	2.1	81	121	156	69.8	20	12.55
58	56	M	151	62	27.2	9	DCLD+ASCITES	0.8	0.3	88	38	2.3	138	3.2	3	72	229	187	175.6	16	12.56
59	60	M	152	63	27.3	40	DCLD+ASCITES	2.2	1.8	62	35	1.8	139	5.1	2.6	61	99	163	37.4	29	7.23
60	60	M	162	61	23.2	5	DCLD+ASCITES	2.1	1.7	41	25	1.6	142	5.4	2.5	70	121	161	67.8	21	9.13

RESULTS

Table 13 : Comparison of various analytes between alcoholic liver disease patients and healthy controls

	ALCOHOLIC LIVER DISEASE	N	MEAN	STANDARD DEVIATION	p VALUE
Total billirubin(mg/dL)	Cases	60	3.61	4.91	0.004-S
	Controls	30	0.92	0.91	
Direct billirubin(mg/dL)	cases	60	2.03	3.05	0.01-S
	controls	30	0.41	0.53	
AST(U/L)	cases	60	96.30	165.98	0.02-S
	controls	30	23.20	6.38	
ALT(U/L)	cases	60	43.03	45.12	0.01-S
	controls	30	25.0	4.65	
AST/ALT RATIO	cases	60	2.12	0.97	<0.001**-HS
	controls	30	0.94	0.22	
ALP(U/L)	cases	60	110.90	43.35	<0.001**-HS
	controls	30	72.07	11.76	
GGT(U/L)	cases	60	82.28	90.79	0.01-S
	controls	30	34.87	41.06	
Total protein(g/dL)	Cases	60	5.97	0.99	<0.001**-HS
	controls	30	6.88	0.56	
Albumin(g/dL)	cases	60	3.27	0.89	<0.001**-HS
	controls	30	4.28	0.39	
Total cholesterol(mg/dL)	cases	60	146.28	44.11	0.32-NS
	controls	30	155.13	27.25	
TGL(mg/dL)	cases	60	155.47	41.80	<0.001**-HS
	controls	30	91.07	31.45	
HDLc(mg/dL)	cases	60	31.07	15.20	0.01-S
	controls	30	39.57	14.18	
LDLc(mg/dL)	cases	60	84.12	36.75	0.08-NS
	controls	30	97.48	27.25	
Kallistatin(µg/mL)	cases	60	13.42	3.24	<0.001**-HS
	controls	30	24.16	3.44	

S-SIGNIFICANT

HS-HIGHLY SIGNIFICANT

NS-NOT SIGNIFICANT

** SIGNIFICANCE AT 1% LEVEL (HIGHLY SIGNIFICANT)

Table 13 shows datas of total bilirubin ,direct bilirubin , SGOT , SGPT, ALP , Total protein, albumin, GGT, total cholesterol, TGL, LDLc, HDLc and kallistatin among alcoholic liver disease and non-alcoholic healthy controls.

An insignificant p value was obtained for variables like total cholesterol and LDLc.

A Significant p value is obtained for total bilirubin, direct bilirubin, SGOT, SGPT, GGT and HDLc.

A highly significant p value was obtained for AST/ALT ratio, total protein, albumin, SAP, TGL and kallistatin.

In this present study, serum kallistatin levels in alcoholic liver disease cases showed a mean of 13.42$\pm$ 3.24µg/mL and non alcoholic healthy controls a mean of 24.16$\pm$ 3.44µg/mL. Highly significant p value was obtained (p value< 0.001).

Table 14 : Box and Whisker plot for Kallistatin

Statistics	Control	Compensated	Decompensated
Number	**30**	**30**	**30**
Mean	24.16	14.50	12.34
Sd	3.44	3.00	3.15
Standard Error of Mean	0.63	0.55	0.58
F-Value	115.80		
P-value	<0.001		
Significant	Highly Significant		

Figure 10 : Box and whisker plot showing the distribution of kallistatin among the three study groups

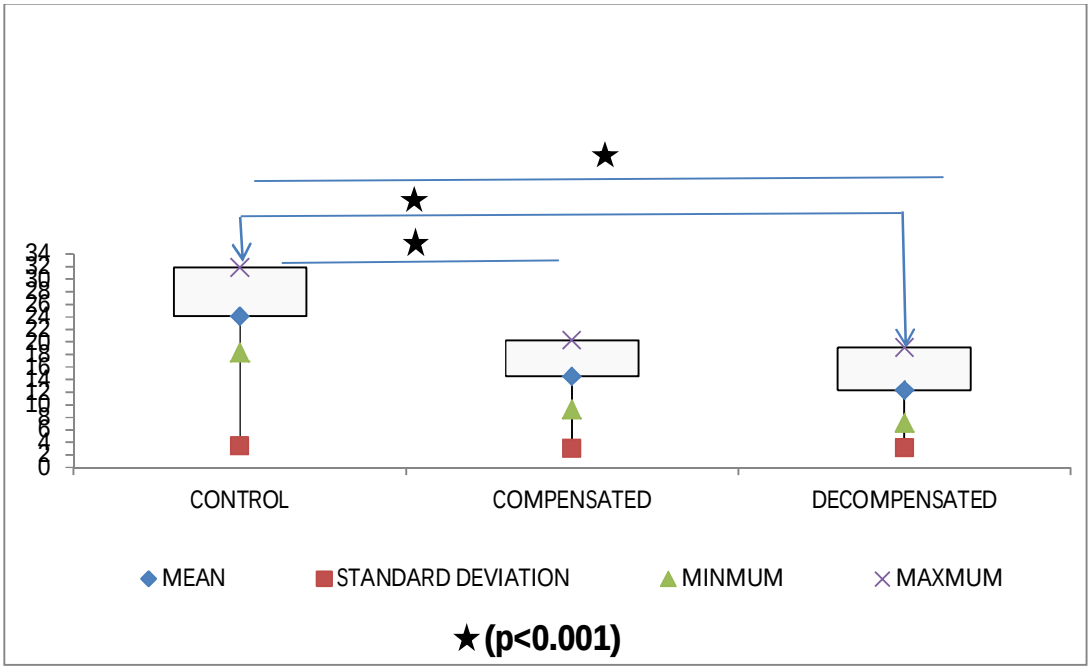


Figure 10 shows the mean concentration of kallistatin among the study groups. In this study, concentration of kallistatin is markedly decreased in compensated cases as 14.5±3.0µg/mL compared to healthy controls as 24.16±3.44µg/mL which will be helpful for early identification of hepatic damage and suggest prompt intervention.

Table 15 : ANOVA to compare the values of various biochemical parameters among three study groups.

Variable	Control			Compensated			Decompensated			p- value
	N=30			N=30			N=30			
	Mean	SD	SEM	Mean	SD	SEM	Mean	SD	SEM	
Kallistatin(µg/mL)	24.16	3.44	0.63	14.50	3.00	0.55	12.34	3.15	0.58	<0.001**-HS
Total bilirubin (mg/dL)	0.92	0.91	0.17	1.96	3.69	0.67	5.26	5.46	0.99	<0.001**-HS
Direct bilirubin (mg/dL)	0.41	0.53	0.10	1.19	2.27	0.41	2.86	3.52	0.64	0.001-S
Total protein (g/dL)	6.88	0.56	0.10	6.55	0.85	0.16	5.38	0.73	0.13	<0.001**-HS
Albumin(g/dL)	4.28	0.39	0.07	3.85	0.85	0.15	2.70	0.46	0.08	<0.001**-HS
SGOT(U/L)	23.30	6.38	1.17	91.73	224.06	40.91	100.87	76.17	13.91	0.06*-NS
SGPT(U/L)	25.00	4.65	0.85	41.47	59.20	10.81	44.60	42.51	7.76	0.16*-NS
AST/ALT	0.94	0.22	0.04	1.81	0.91	0.17	2.43	0.95	0.17	<0.001**-HS
GGT(U/L)	34.87	41.06	7.50	75.60	109.94	20.07	88.97	67.75	12.37	0.02-S

**HIGHLY SIGNIFICANT AT 1% LEVEL.

*NOT SIGNIFICANT

S-SIGNIFICANT

HS-HIGHLY SIGNIFICANT

NS-NOT SIGNIFICANT

TABLE 15 shows ANOVA to compare the values of various biochemical parameters among three study groups. Statistically significant p values are obtained for kallistatin, total and direct bilirubin, total protein and albumin, AST/ALT ratio and GGT .Insignificant p values are obtained for SGOT and

SGPT .In this study, there is a marked decrease in mean concentration of serum kallistatin levels in compensated group of 14.50±3.0µg/mL and decompensated group of 12.34±3.15µg/mL, when compared to controls as 24.16±3.4µg/mL showing a highly significant p value of <0.001.

Table 16

COMPARISON OF VARIOUS PARAMETERS IN ALCOHOLIC LIVER DISEASE (COMPENSATED AND DECOMPENSATED) CASES AND HEALTHY CONTROLS

PARAMETER	**CONTROLS(n=30)**	**COMPENSATED LD(n=30)**	**DECOMPENSATED LD(n=30)**
Kallistatin(µg/mL)	24.16±3.44	14.50±3.00	12.34±3.15
Total bilirubin(mg/dL)	0.92±091	1.96±3.69	5.26±5.46
Direct bilirubin(mg/dL)	0.41±0.53	1.19±2.27	2.86±3.52
SGOT(U/L)	23.2±6.38	91.73±224.06	100.87±76.17
SGPT(U/L)	25±4.65	41.47±59.20	44.60±42.51
AST/ALT RATIO	0.94±0.22	1.81±0.91	2.43±0.95
ALP(U/L)	72.07±11.76	84.63±46.23	137.17±16.05
GGT(U/L)	34.87±41.06	75.60±109.94	88.97±67.75
Total protein(g/dL)	6.88±0.56	6.55±0.85	5.38±7.34
Albumin(g/dL)	4.28±0.39	3.85±0.85	2.70±0.46
Total cholesterol(mg/dL)	155.13±27.25	152.90±51.46	139.67±34.92
Triglycerides(mg/dL)	91.07±38.45	146.43±31.48	164.50±48.93
LDLc(mg/dL)	97.48±27.25	83.15±43.64	85.10±29.01
HDLc(mg/dL)	39.57±14.18	40.47±13.90	21.67±9.7

LD-liver disease

Table 16 showed (mean± standard deviation) for all the analytes of liver function tests taken for study in three groups which includes healthy controls, compensated and decompensated liver disease patients. Datas are shown for parameters like total and direct bilirubin ,SGOT,SGPT,AST/ALT ratio, GGT, ALP, total protein and albumin, total cholesterol ,TGL, LDLc, HDLc and kallistatin. After comparing all parameters, this study infers that serum kallistatin levels are markedly decreased in compensated as14.50±3.0µg/mL and decompensated as 12.34±3.15µg/mL liver disease than healthy controls as 24.16±3.44µg/mL.

TABLE 17

MEAN CONCENTRATION OF KALLISTATIN BETWEEN STUDY GROUPS

Variable	Control			Compensated			Decompensated			p-value
	N=30			N=30			N=30			
	Mean	Sd	SEM	Mean	Sd	SEM	Mean	Sd	SEM	
Kallistatin(µg/mL)	24.16	3.44	0.63	14.50	3.00	0.55	12.34	3.15	0.58	<0.001

FIGURE 11

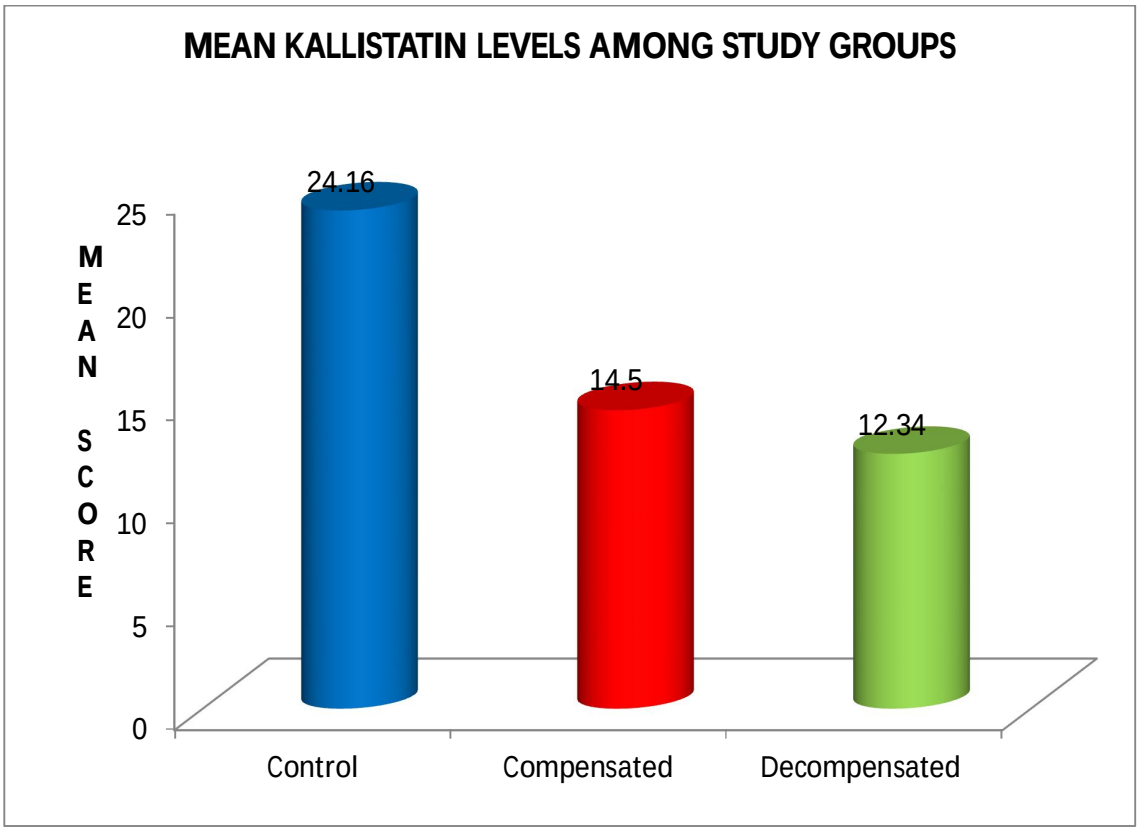


TABLE 18

MEAN CONCENTRATION OF TOTAL AND DIRECT BILIRUBIN BETWEEN STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROLS	p VALUE
Total bilirubin(mg/dL)	1.96	5.26	0.92	0.000
Direct bilirubin(mg/dL)	1.19	2.86	0.41	0.001

Figure 12

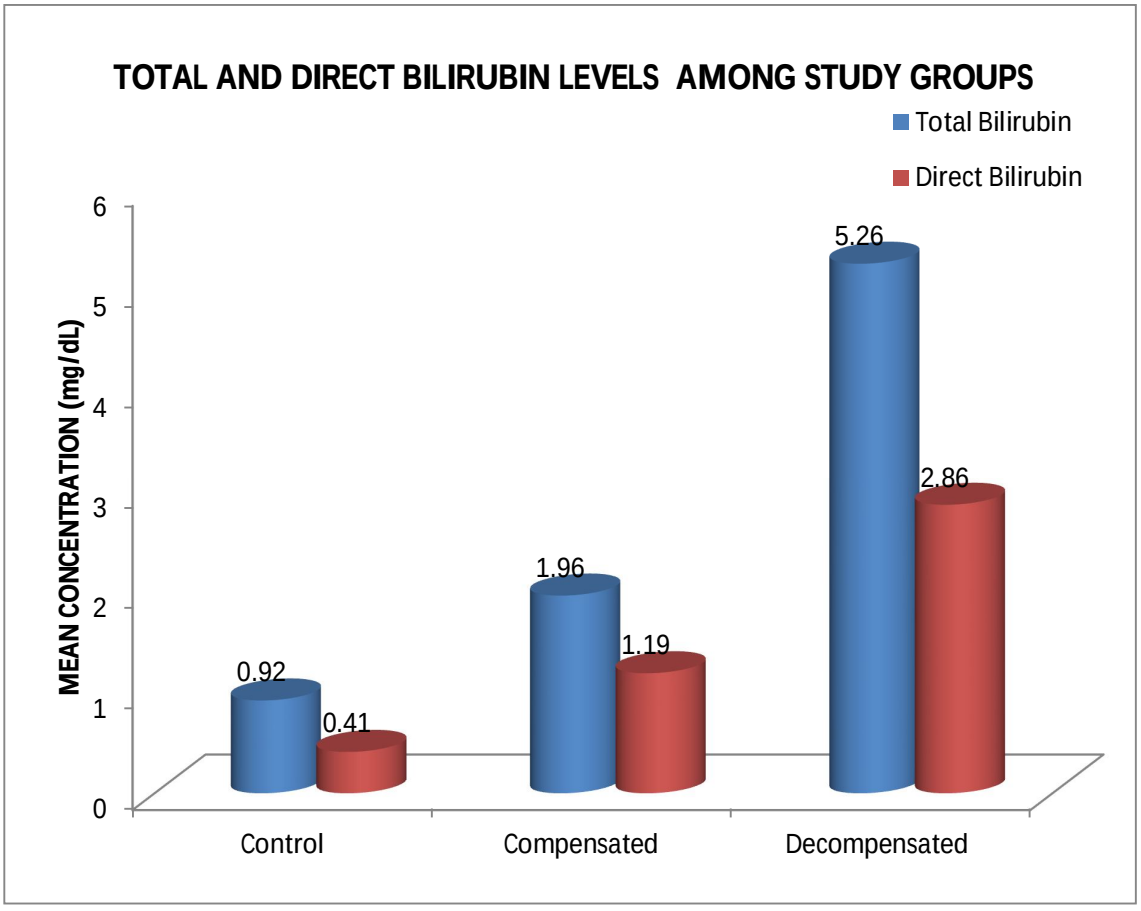


TABLE 19

MEAN CONCENTRATION OF TOTAL PROTEIN AND ALBUMIN BETWEEN STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROL	P VALUE
Total protein(g/dL)	6.55	5.38	6.88	0.000
Albumin(g/dL)	3.85	2.7	4.28	0.000

Figure 13

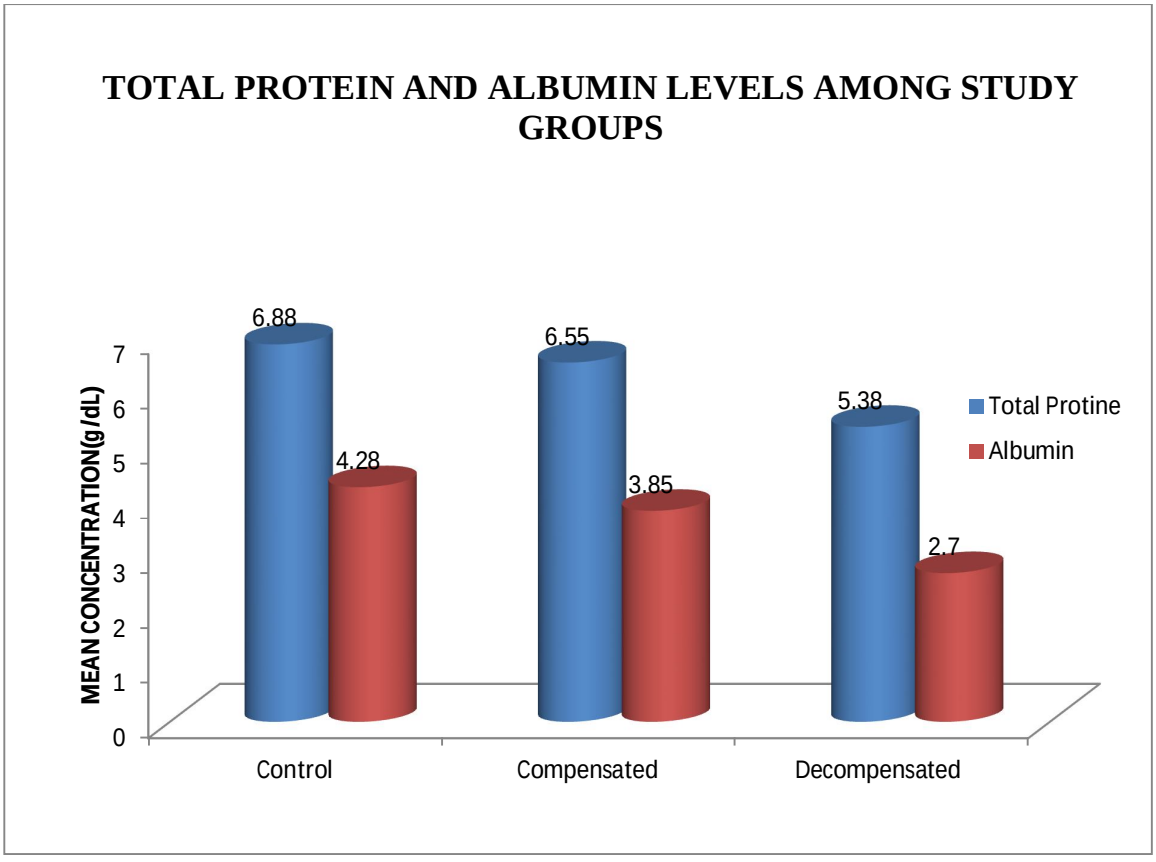


TABLE 20

MEAN CONCENTRATIONS OF SGOT, SGPT AND AST/ALT RATIO BETWEEN STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROLS	p VALUE
SGOT(U/L)	91.73	100.87	23.2	0.06
SGPT(U/L)	41.47	44.6	25	0.16
AST/ALT	1.81	2.43	0.94	0.00

FIGURE 14

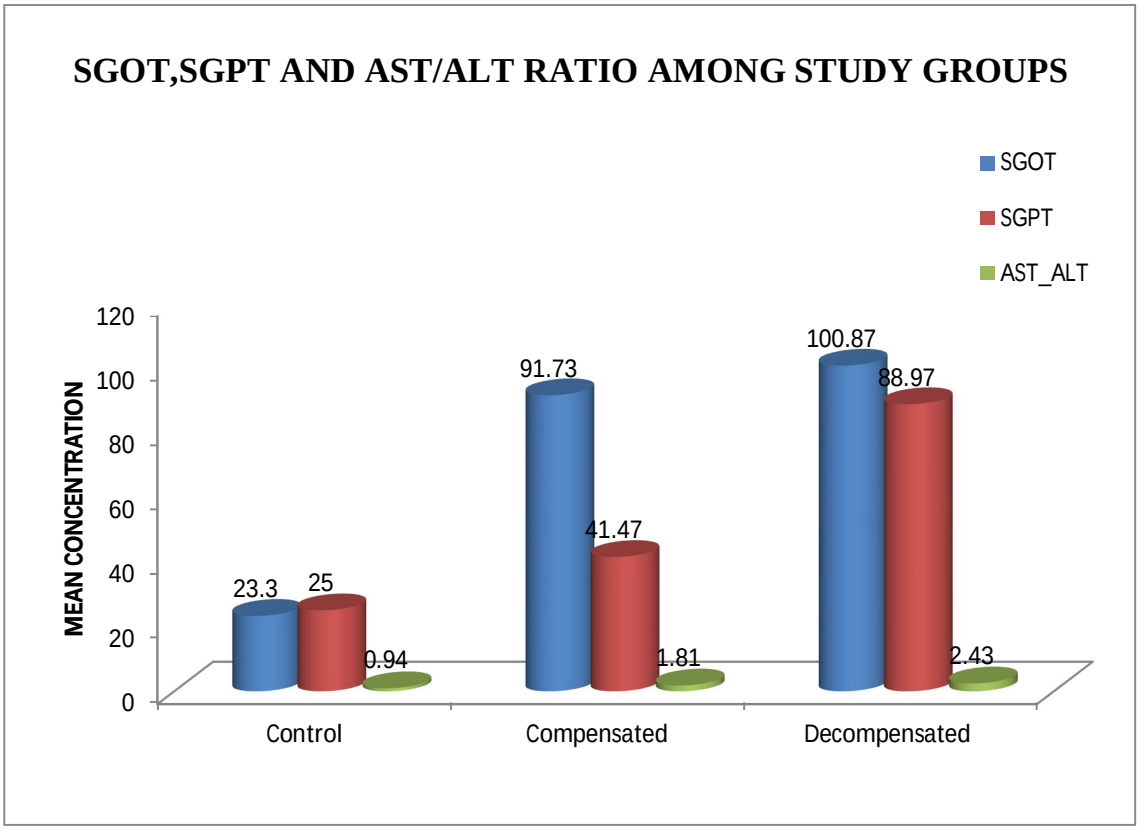


TABLE 21

MEAN CONCENTRATION OF GGT AMONG STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROLS	p VALUE
GGT(U/L)	75.6	88.97	34.87	0.02

FIGURE 15

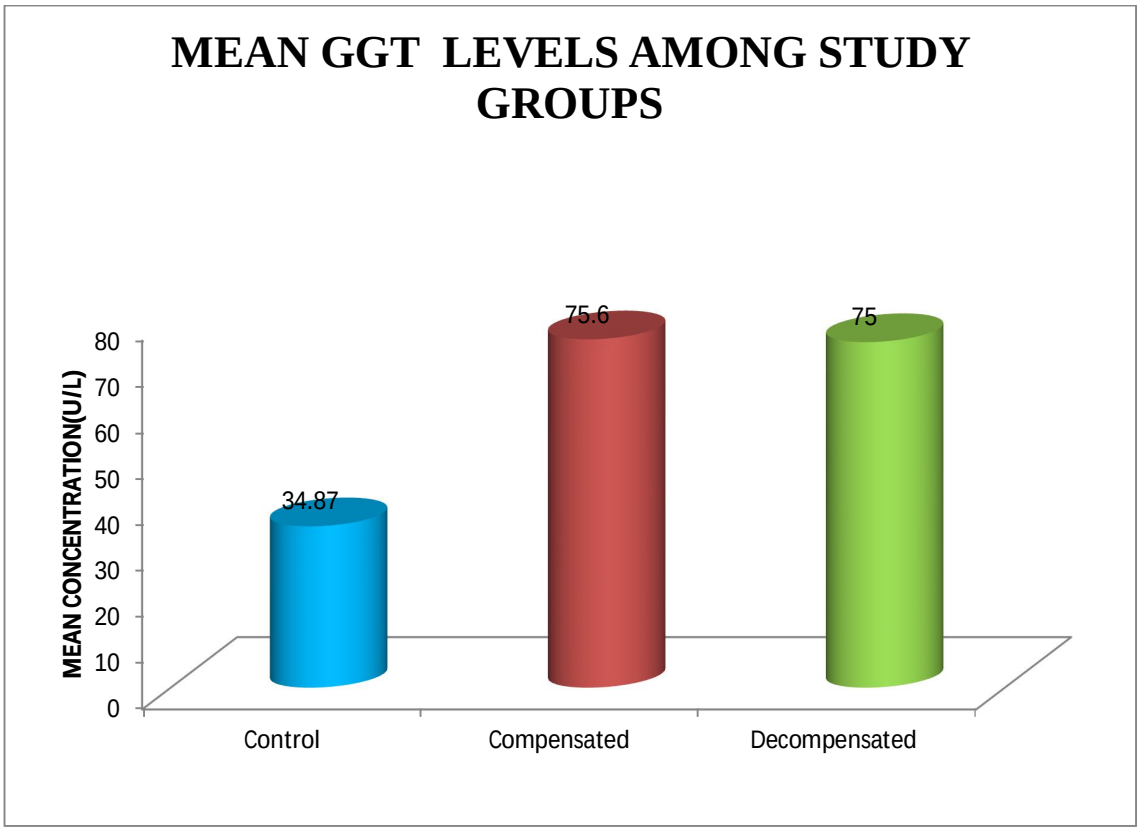


TABLE 22:

COMPARISON OF VARIOUS ANALYTES BETWEEN HEALTHY CONTROLS AND COMPENSATED LIVER DISEASE PATIENTS.

	Controls N=30		**Compensated N=30**		**Statistics**			
	MEAN	**SD**	**MEAN**	**SD**	**"t"-value**	**df**	**p-value**	**Significant**
Total bilirubin (mg/dL)	0.92	0.91	1.96	3.69	1.51	58	0.14	NS
Direct bilirubin (mg/dL)	0.41	0.53	1.19	2.27	1.84	58	0.07	NS
SGOT(U/L)	23.20	6.38	91.73	224.06	1.67	58	0.10	NS
SGPT(U/L)	25.00	4.65	41.47	59.20	1.52	58	0.13	NS
AST_ALT	0.94	0.22	1.81	0.91	5.09	58	<0.001**	Highly Significant
ALP(U/L)	72.07	11.76	84.63	46.23	1.44	58	0.15	NS
T-PROTEIN (g/dL)	6.88	0.56	6.55	0.85	1.75	58	0.09	NS
ALBUMIN (g/dL)	4.28	0.39	3.85	0.85	2.57	58	0.01	Significant
GGT(U/L)	34.87	41.06	75.60	109.94	1.90	58	0.06	NS
T. Cholesterol (mg/dL)	155.13	27.25	152.90	51.46	0.21	58	0.83	NS
TGL (mg/dL)	91.07	38.45	146.43	31.48	6.10	58	<0.001**	Highly Significant
LDLc (mg/dL)	97.48	27.25	83.15	43.64	1.53	58	0.13	NS
HDLc(mg/dL)	39.57	14.18	40.47	13.90	0.25	58	0.81	NS
Kallistatin (µg/mL)	24.16	3.44	14.50	3.00	11.59	58	<0.001**	Highly Significant

TABLE 22 shows the comparison of various analytes between control and compensated alcoholic liver disease patients .p value was found to be highly significant for albumin(0.01), AST/ALT ratio(<0.001) and kallistatin (<0.001). Insignificant p values are obtained for total and direct bilirubin ,SGOT, SGPT, ALP, GGT, LDLc, HDLc and total cholesterol.

TABLE 23:

COMPARISON OF VARIOUS ANALYTES BETWEEN COMPENSATED AND DECOMPENSATED LIVER DISEASE PATIENTS.

	COMPENSATED		DECOMPENSATED		STATISTICS			
	MEAN	Sd	MEAN	Sd	t-value	df	p-value	Significant
Total bilirubin (mg/dL)	1.96	3.69	5.26	5.46	2.74	58	0.01	Significant
Direct bilirubin (mg/dL)	1.19	2.27	2.86	3.52	2.19	58	0.03	Significant
SGOT (U/L)	91.73	224.06	100.87	76.17	0.21	58	0.83	NS
SGPT (U/L)	41.47	59.20	44.60	42.51	0.24	58	0.82	NS
AST_ALT	1.81	0.91	2.43	0.95	2.58	58	0.01	Significant
ALP (U/L)	84.63	46.23	137.17	16.05	5.88	58	$<0.001^{**}$	Highly Significant
T-PROTEIN (g/dL)	6.55	0.85	5.38	7.34	5.71	58	$<0.001^{**}$	Highly Significant
ALBUMIN (g/dL)	3.85	0.85	2.70	0.46	6.53	58	$<0.001^{**}$	Highly Significant
GGT (U/L)	75.60	109.94	88.97	67.75	0.57	58	0.57	NS
T. Cholesterol (mg/dL)	152.90	51.46	139.67	34.92	1.17	58	0.25	NS
TGL (mg/dL)	146.43	31.48	164.50	48.93	1.70	58	0.09	NS
LDLc (mg/dL)	83.15	43.64	85.10	29.01	0.20	58	0.84	NS
HDLc(mg/dL)	40.47	13.90	21.67	9.70	6.08	58	$<0.001^{**}$	Highly significant
Kallistatin (µg/mL)	14.50	3.00	12.34	3.15	2.72	58	0.01	Significant

**Denotes significance at 1% level (highly significant)

NS- Not significant at 5% level.

Table 23 shows the comparison of various analytes between compensated and decompensated liver disease patients. Significant p value is obtained for serum kallistatin levels (p Value 0.01) among the two study groups (compensated and decompensated liver disease cases) inferring that they are comparable.

TABLE 24

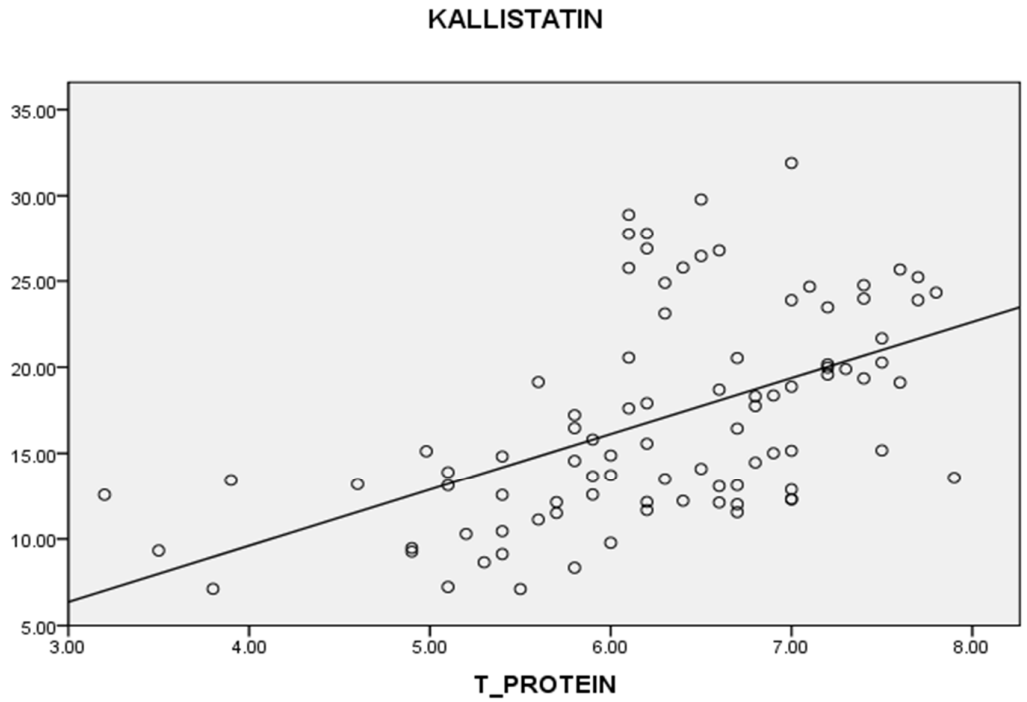
PEARSON CORRELATION (r) AND SPEARMAN RANK CORRELATION (p) BETWEEN VARIOUS PARAMETERS OF ALCOHOLIC LIVER DISEASE AND SERUM KALLISTATIN LEVELS

Parameter	**Pearson correlation**		**Spearman rank correlation**	
	r	**p**	**r**	**p**
Total bilirubin(mg/dL)	-0.28	<0.001**	-0.47	<0.001**
Direct bilirubin(mg/dL)	-0.29	<0.001**	-0.54	<0.001**
AST(U/L)	-0.25	0.002	-0.67	<0.001**
ALT(U/L)	-0.26	0.001		
AST/ALT ratio	-0.47	<0.001**		
ALP(U/L)	-0.51	<0.001**	-0.53	<0.001**
Total protein(g/dL)	0.51	<0.001**		
Albumin(g/dL)	0.56	<0.001**	0.55	<0.001**
GGT(U/L)	-0.24	0.003	-0.53	<0.001**

Table 24 shows Pearson and Spearman relationship between serum kallistatin levels and other liver function tests. Patients with alcoholic liver disease showed a marked positive correlation between serum kallistatin levels and serum albumin, total protein. We can also find a marked negative correlation present between serum kallistatin levels and total bilirubin, direct bilirubin, AST, ALT, AST/ALT ratio, ALP and GGT.

FIGURE16:

CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND TOTAL PROTEIN LEVELS IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

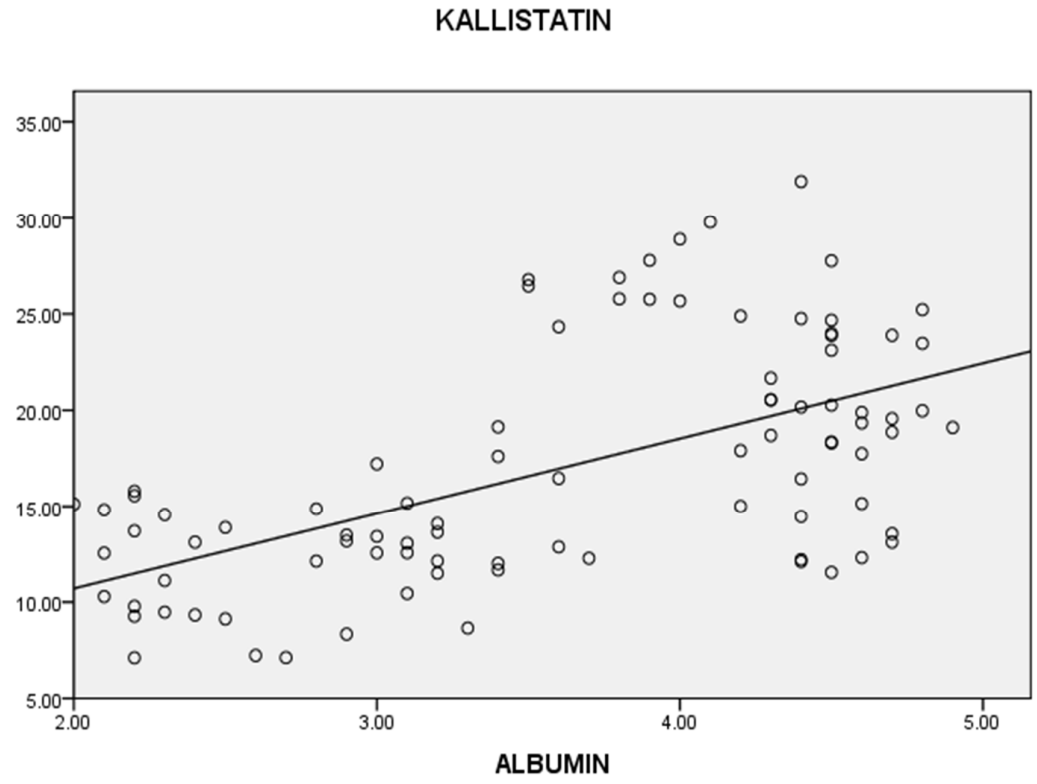


r=0.51	P< 0.001

Figure 16 reveals a positive correlation between serum kallistatin levels and total protein with an r value of 0.51 p value highly significant (<0.001)

Figure 17

CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND ALBUMIN LEVELS IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

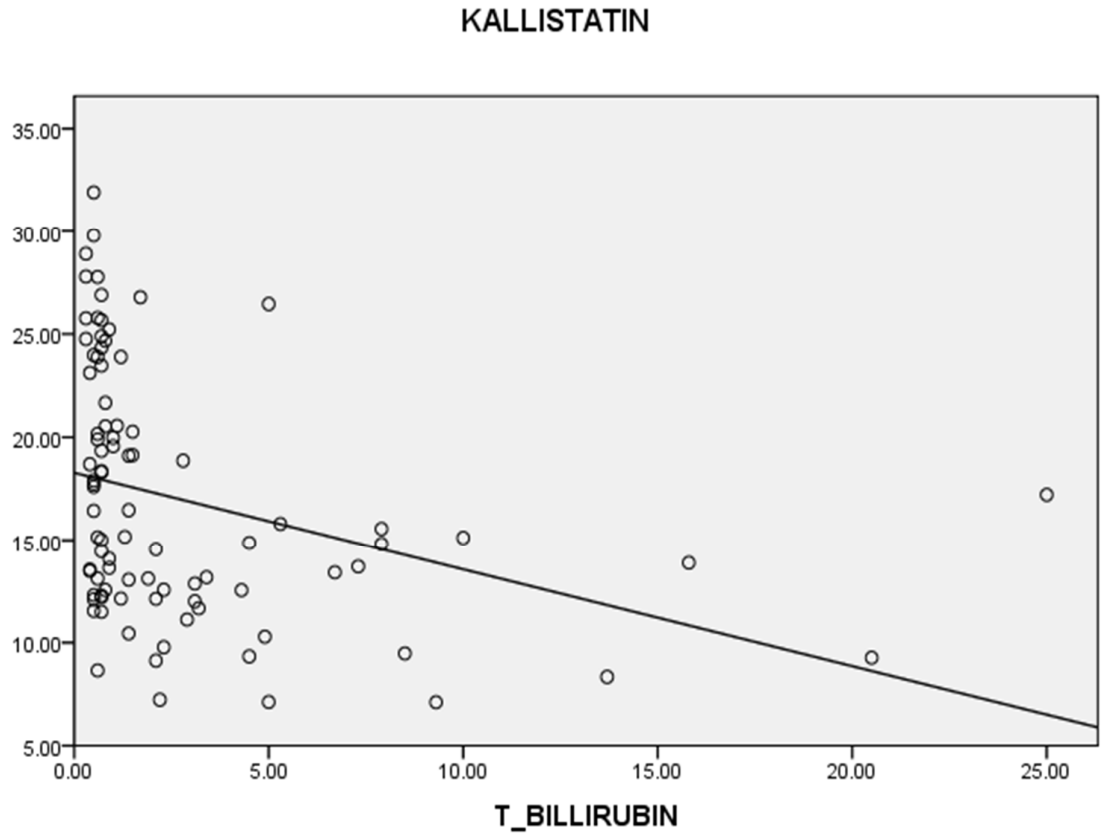


r=0.56	P <0.001

Figure 17 shows a marked positive correlation between serum kallistatin levels with an r value of 0.56 and p value of <0.001 (highly significant).

Figure 18

CORRELATION BETWEEN SERUM KALLISTATIN AND TOTAL BILIRUBIN IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

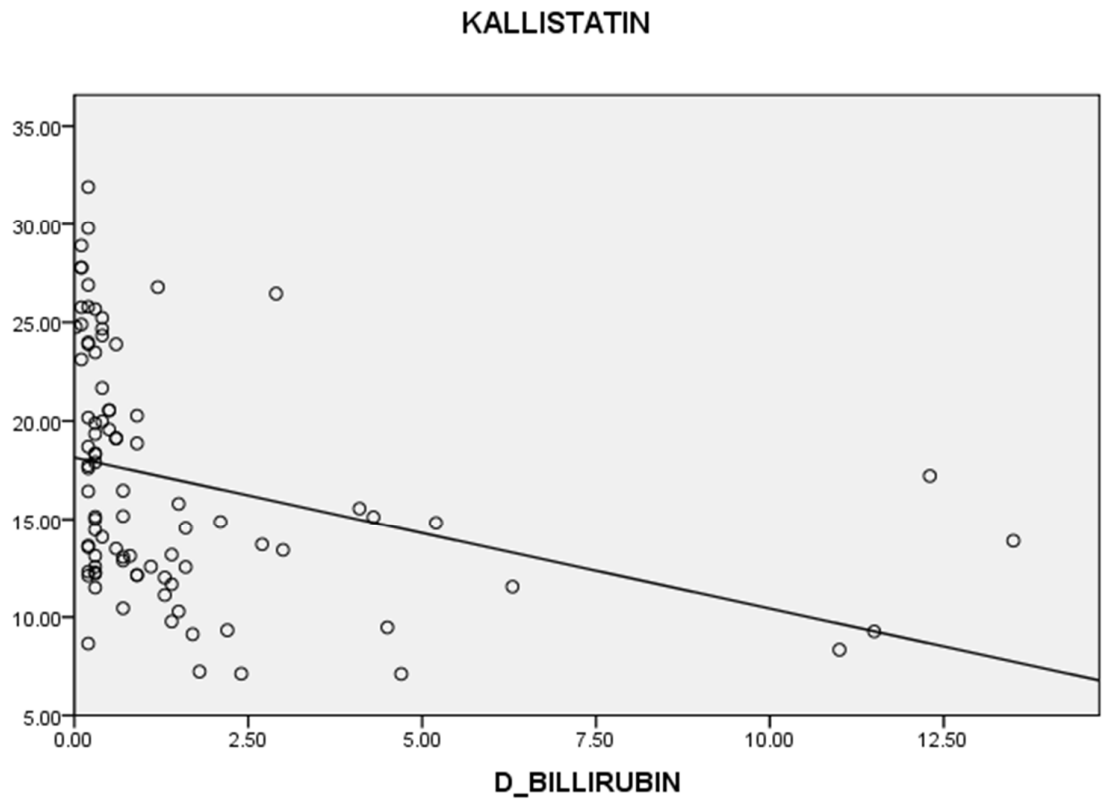


r=-0.28	P< 0.001

Figure 18 shows a negative correlation between serum kallistatin levels and total bilirubin levels with an r value of 0.28 and p<0.001(highly significant)

FIGURE 19

CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND DIRECT BILIRUBIN IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

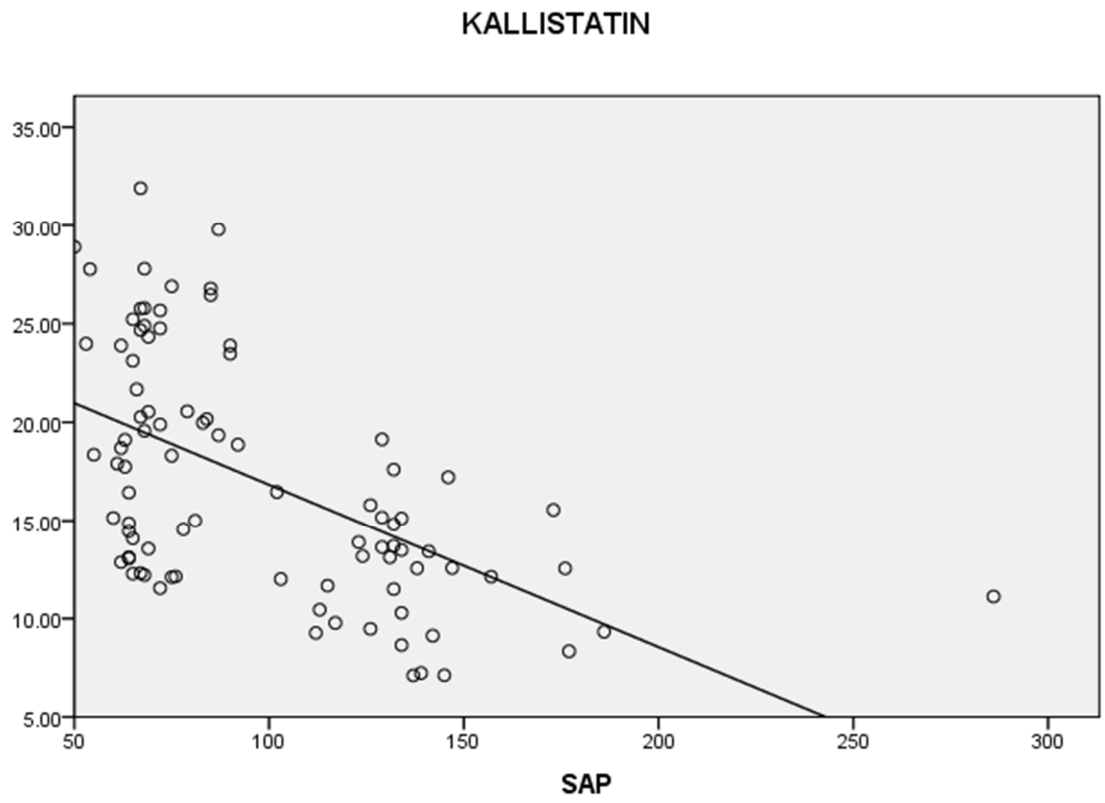


r=-0.29	p<0.001

Figure 19 shows a negative correlation between serum kallistatin levels and direct billirubin levels with an r value of 0.29 and p value of <0.001(highly significant)

FIGURE 20

CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND SAP IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

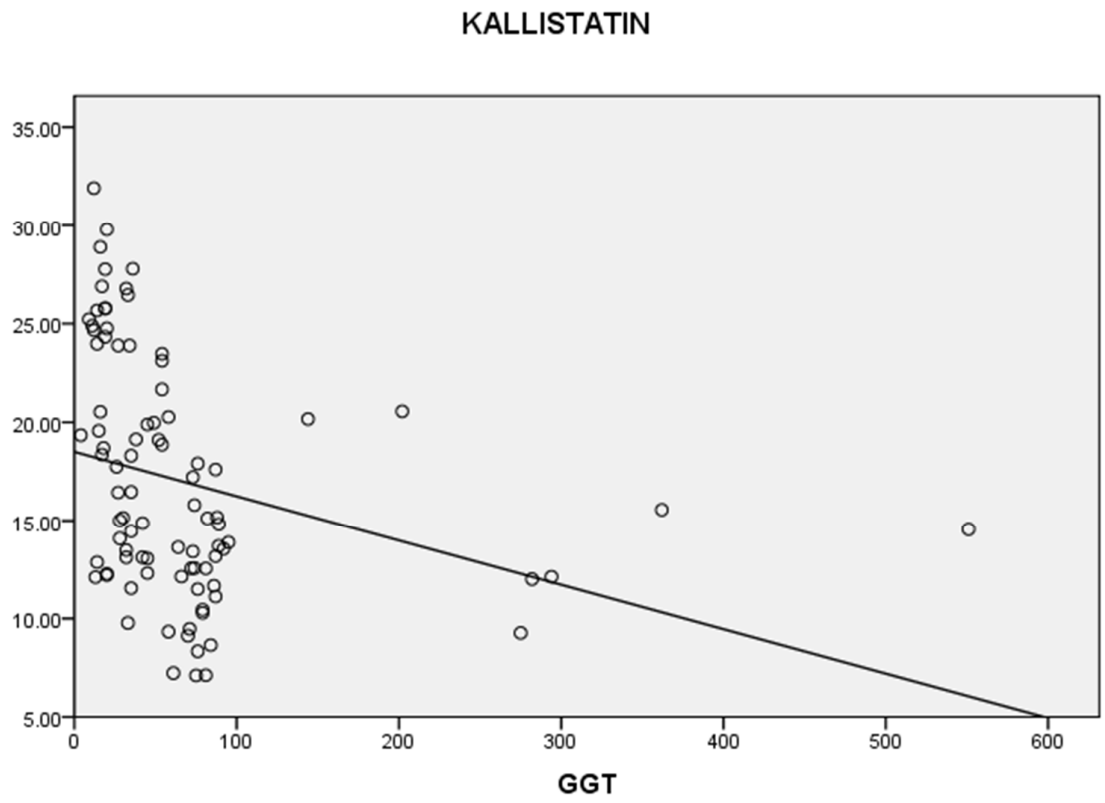


r=-0.51	P<0.001

Figure 20 shows a negative correlation between serum kallistatin levels and alkaline phosphatase levels with an r value of 0.51 and pvalue <0.001(highly significant)

FIGURE 21

CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND GGT LEVELS IN PATIENTS WITH ALCOHOLIC LIVER DISEASE



r=0.24	P=0.003

Figure 21 shows a negative correlation between serum kallistatin levels and GGT levels with an r value of 0.24 and p value of 0.003 (significant)

TABLE 25 : LOGISTIC REGRESSION ANALYSIS

Variable	Unadjusted	Unadjusted OR			Final Model	
		95 % CI	P-VALUE	Unadjusted OR	95% CI	P-VALUE
BMI	3.79	0.997 – 2.329	0.052			
T-BILLIRUBIN (mg/dL)	0.00	0.107 – 8.581	0.969			
D-BILLIRUBIN (mg/dL)	0.24	0.029 - 8.398	0.625			
SGOT (U/L)	0.46	0.716 – 1.176	0.496			
SGPT (U/L)	0.02	0.652 – 1.442	0.878			
AST, ALT	0.02	0.005 – 42.38	0.884			
SAP (U/L)	3.54	0.991 – 1.551	0.050	3.79	3.32	0.005
ALBUMIN (g/dL)	4.31	1.638 - 2.698	0.038	4.92	4.68	0.001
T-CHOLESTEROL (mg/dL)	0.16	0.154 - 16.69	0.693			
TGL(mg/dL)	0.64	0.484 – 1.357	0.423			
LDLc(mg/dL)	0.17	0.058 – 6.436	0.681			
HDLc(mg/dL)	0.15	0.061 – 6.493	0.697			

TABLE 25 shows a logistic regression analysis interpreting the association of each biochemical parameter (levels of kallistatin levels and other analytes) with alcoholic liver disease (unadjusted odd's ratios).This analysis infers that SAP and albumin are significant variables at levels of 0.005 and 0.001 respectively .Also ,we infer that albumin found to be a highly determining variable in relation to serum kallistatin levels with a p value of 0.001 than SAP levels with a p value of 0.005 in diagnosing alcoholic liver disease. Out of these two variables, we know that, for 1 µg/mL rise in serum kallistatin levels, the odds, of liver cirrhosis decreases by (1-0.492)*100=50.8% after bringing down the effects of all other analytes.

TABLE 26 : Based on ROC

VARIABLE	
Area Under Curve (AUC)	0.922
Optimal cut of value	20.22 µg/mL
Sensitivity	93.65 %
Specificity	96.30 %
Positive Predictive value	98.33 %
Negative Predictive value	86.67 %

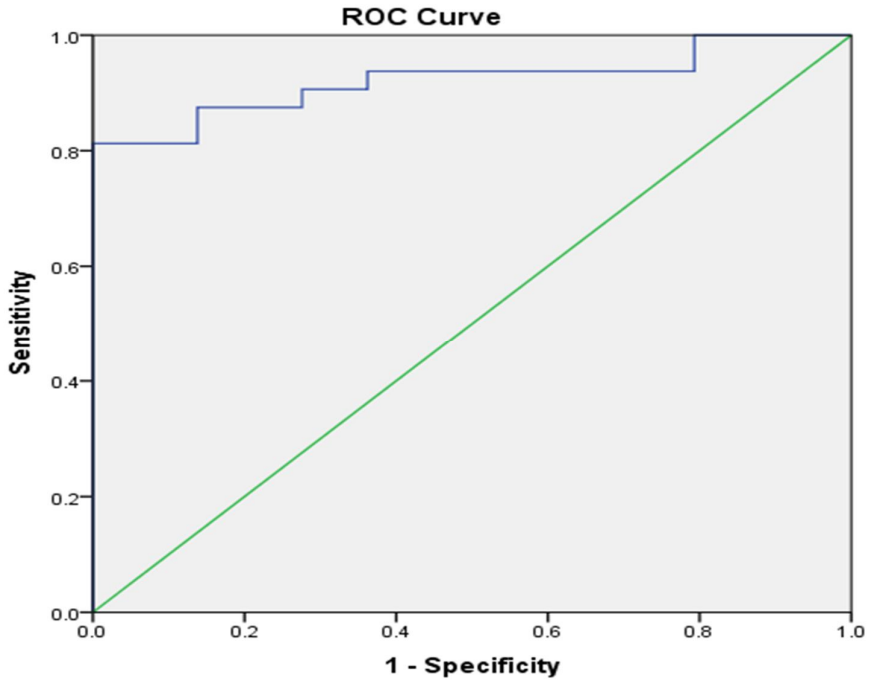


Table 26 shows a Receiver Operating Characteristic curve with x-axis showing specificity and y-axis showing sensitivity. Area under curve was 0.922 with an optimal cut off value 20.22µg/mL which proves that kallistatin can be a good non-invasive biomarker for diagnosing alcoholic liver disease .sensitivity, specificity, positive and negative predictive values are 93.65%, 96.30%, 98.33% and 86.67% respectively.

DISCUSSION

DISCUSSION

Alcoholism, a common worldwide social stigma, wherein the consumption of alcohol within safer limits, seems to be normal. When the drinking habit becomes regular and frequent, organ damage starts. Liver is commonly affected by alcoholism. Since, the liver has a large reserve capacity, patient has no symptoms and seems to be healthy, but as the liver injury progresses, its functions are impaired obviously, indicated by a decrease in total protein and albumin synthesis[114].

In a review study, classification of liver damage on the basis of ultrasound, proved to be better than liver biopsy. They achieved a detection of 81.48% of compensated liver disease and 91.7% of de-compensated liver disease [115,116]. Nearly 50% of patients with liver disease are identified in compensated stage itself[117].They can live for a period of ten to twelve years with compensated features. Patients with compensated features may even present with esophageal varices but once de-compensated features viz., portal hypertension ,ascites and encephalopathy sets in , mortality is reduced to one year. Decompensated liver disease is considered to be a precursor of hepatocellular malignancy[118].

Liver performs a wide variety of functions. Functional damage occurs due to chain of disorders of other organ systems. A single test cannot identify liver damage as well as, trace out the progression of liver disease. Minor damage to liver cannot be assessed in early stage as the liver functions appear to be normal

due to compensatory mechanisms. Some tests may be indicative of liver damage but they are not useful in assessing the prognosis of disease and vice versa.

Kallistatin, a recent non-invasive biomarker, is considered in the present study, to prove its diagnostic sensitivity in detecting liver dysfunction at an early stage and to assess the progress as it enters a vulnerable stage which causes irreversible damage to liver. In order to trace the progression of liver damage due to alcohol, with kallistatin levels, alcoholic liver disease has been categorized into compensated liver disease and de-compensated liver disease.

This study includes a control group of 30 and a study group of 60, with age limits in comparable range. The subjects of the control group as well as the study group comprised of only males since females were neither exposed with alcoholic history nor did they present with any obvious findings in ultrasound abdomen, suggestive of alcoholic liver disease. Tradition of Indian females prevents them to come out with alcohol history in social atmosphere. This could not be considered as a limitation of study since, several studies from other countries, conveyed the essence of the findings that, kallistatin levels showed no significant difference among gender and age related groups[119].

After analyzing the values of all the parameters in this study, they were found to be within the reference range published in various reviews or as per the kit insert values for reference range, depending on the method adopted. Serum kallistatin levels in this study for healthy controls showed a mean concentration

of 24.16±3.44 µg/mL which is closer to the reference range of 22.1+3.5µg/mL, quoted by Chao J et al study[120].

At the onset of alcoholic liver disease, sets into the decompensated stage, various analytes like total and direct bilirubin , total protein and albumin , SGOT, SGPT, ALP, GGT and lipid profile levels, are affected.kit methods are used to assay the substrates and enzymes .These methods are based on Modified IFCC procedures, enzymatic and kinetic methods .

TOTAL AND DIRECT BILIRUBIN LEVELS AMONG THE STUDY GROUPS:

Alcoholic liver disease patients show markedly elevated levels of conjugated bilirubin level in blood[121,122]. In this study, similar values are obtained in Table 13 which shows a direct bilirubin of 2.03± 3.05mg/dL and 0.41± 0.53mg/dL in cases and in controls respectively. This increase is about four to five times elevated in alcoholic liver disease than healthy controls. The p value was found to be significant($p<0.01$).Table 15 shows total bilirubin value of 3.61±4.91mg/dL in patients with alcoholic liver disease and controls showed 0.92±0.91mg/dL showing a significant variation of p Value of 0.004. Figure 12 showed a markedly elevated levels of total and direct bilirubin in decompensated liver disease than compensated liver disease .Table 22 shows no significant variation in total biliubin of p value<0.14 and direct bilirubin with a p value<0.07 between control and compensated groups respectively.

TOTAL PROTEIN AND ALBUMIN LEVELS AMONG THE STUDY GROUPS:

Consumption of alcohol decreases the rate of catabolism of proteins in liver. In this study,table13 shows a decrease in mean concentration of serum total protein level of 5.97± 0.99 g/dL in alcoholic liver disease cases than healthy controls of 6.88±0.56 g/dL with a highly significant p value(<0.001). Figure13 shows changes in total protein and albumin levels among the study groups. Table 22 shows mean concentration of total protein of 6.88± 0.56 g/dL in healthy controls and 6.55±0.85 g/dL in compensated liver disease patients inferring that p value of 0.09 is not significant among healthy controls and compensated liver disease patients .Synthesis of albumin decreases in cases of alcoholic liver disease often associated with malnutrition[123-125]. This study also shows a mean concentration of albumin in cases about 3.27±0.89g/dL which is less when compared to healthy controls as 4.28±0.39g/dL with a significant p value of 0.01, as in Table 22.

ENZYME ALTERATION IN ALCOHOLIC LIVER DISEASE:

Serum aspartate amino transferase and serum glutamate oxaloacetate transferase are present in enormous amount in hepatic cells. Whenever there is liver damage due to alcohol or toxins, this enzyme leak out from cells and comes into circulation. Even though they act as good markers of hepatic damage, they lack sensitivity[126].As proposed in previous studies, Table 13 showed that SGPT and SGOT are moderately increased to 43.03±45.12U/L and 96.30±165.98U/L in alcoholic liver disease than healthy controls as 25.0±4.65U/L and 23.20±6.38U/L

with a p value of 0.01 and 0.02, respectively. We can also note that aspartate transaminases levels are markedly raised when compared to alanine transaminases, as in table 13.This is due to the distribution of AST in enormous tissues of our body than ALT[127].Alcoholic liver disease patients commonly presents with elevation of AST/ALT ratio than non alcoholics. This is called De-ritis ratio. Pyridoxal -5'- phosphate is vital for AST/ALT activity[128]. Pyridoxal-5'-phosphate deficiency associated with malnutrition in chronic alcoholics, leads to decreased ALT activity. As a result AST/ALT ratio is raised.

As in Jerold A. Cohen et al. review ,table 1 showed AST/ALT ratio in alcoholic liver disease as 2.12$\pm$0.97 being greater than healthy controls as 0.94$\pm$0.22 [129].

Figure 14 shows changes in AST/ALT ratio being highest as the patient enters de-compensated stage with a mean concentration of 2.43$\pm$0.95. Figure14 shows the changes in SGOT and SGPT among the study groups.

Serum alkaline phosphatase is an enzyme which arises from the outer cell membrane of liver but it has entirely different activities.ALP activity is useful for finding out the etiology and extent of liver damage. In this present study, Table 13 showed serum alkaline phosphatase levels being elevated in alcoholic liver disease as 110.90$\pm$ 43.35U/L than healthy controls as 72.07$\pm$11. 76 U/L.

Gamma glutamyl transferase is an enzyme of biliary canaliculi. Alcohol intake for a longer period of time induces hepatic damage and causes an elevation

of GGT level[130,131]. In a study done by Krastev et al, it was proved that GGT levels do not elevate in the beginning of alcoholic liver disease and patient coming for follow up after treatment[132]. Similar to the previously mentioned study ,this study too, shows similar results in table 22.Datas in Table 22 showed that GGT levels in compensated liver disease shows no statistical significance between control and compensated groups with a p value<0.06 .Figure 15 shows the changes in GGT among the study groups .In Table 13, comparison of GGT levels in alcoholic liver disease and healthy controls showed a significant p value of 0.01as in the study performed by S .Orlowskin et al . This increase may be due to microsomal induction and damage of hepatic cells[133].

KALLISTATIN IN ALCOHOLIC LIVER DISEASE:

After reviewing the available literatures, some markers like AST and ALT lack either sensitivity or specificity or both. Some markers like GGT lack specificity. This study is aimed to evaluate the role of kallistatin as a non-invasive biomarker in the diagnosis of alcoholic liver disease, its usefulness in correlation with disease severity and to compare serum kallistatin levels with apparently healthy individuals.

Table 13 showed a mean concentration of serum kallistatin levels in alcoholic liver disease being markedly decreased to13.42+ 3.24µg/mL, when compared to healthy controls as 24.16+3.44µg/mL. p value of <0.001 is found to be highly significant.

CORRELATION BETWEEN SERUM KALLISTATIN AND OTHER BIOCHEMICAL PARAMETERS:

Correlation tests including Pearson and Spearman rank correlations (in table 24) done to analyse the positive and negative correlation of serum kallistatin levels and other variables included in the study. Datas showed that Serum kallistatin levels positively correlated with total protein with $r=0.51$ and $p<0.001$ in figure 16, but negatively correlated with GGT levels with $r=-0.24$ and p value<0.03 in figure 21.In addition, serum kallistatin is again positively correlated with serum albumin with $r=0.56$ and p value<0.001 in figure 17 and negatively correlated with ALP showing $r=0.51$ and p value<0.001 in figure 20.serum kallistatin also showed negative correlations with total bilirubin showing $r=-0.28$ and p value <0.001 in figure 18 and direct bilirubin with $r=-0.29$ and p value<0.001 in figure 19.

On comparison of the datas of various analytes between controls and compensated groups of alcoholic liver disease in table 22, we can infer that occasional drinkers showed no significant variation in their biochemical parameters for assessing liver function except for kallistatin, TGL, albumin and AST/ALT ratio. Social drinkers may be there in this group .But liver damage might go unnoticed in this stage because of insignificant change in most of the variables indicating normal liver function[134].

Comparing the datas of various analytes between compensated and de-compensated groups in table 23, total protein , albumin ,GGT and ALP shows a

highly significant p value of 0.001 than kallistatin which showed a significant p value of 0.01. Comparison of the same analytes mentioned above in table 22 between healthy controls and cases showed a non significant p value except albumin. Since Serum kallistatin showed a highly significant p value of <0.001 as in the above mentioned table, kallistatin proved to be a better marker when compared to other analytes both in:

- control and compensated groups(0.001) in table 22 and
- compensated and decompensated groups(0.01) as in table 23.

ANOVA study in table 15 also showed a highly significant p value of <0.001 for serum kallistatin assay .we gather from the study that albumin too, proves to be highly determining variable in relation to serum kallistatin levels with a highly significant p value of 0.001 as shown by a logistic regression analysis in table 25.

Receiver operating characteristic curve in table 26 also emphasises that kallistatin can be a good non-invasive biomarker in diagnosing alcoholic liver disease with a high sensitivity of 93.65% and specificity of 96.30%.positive predictive value is 98.33% and negative predictive value is 86.67 .area under curve is 0.922 and optimal cut-off value as 20.22µg/mL.

The major pillars of the study are

1. Simultaneous measurement of most of the analytes of liver panel

- Synthetic function-total protein and albumin
- Excretory function-total and direct bilirubin
- Enzyme profile-SGOT, SGPT, AST/ALT RATIO,ALP and GGT.
- Lipid profile-total cholesterol ,triglycerides ,HDLc and LDLc.

2. Evaluation of the relationship of serum kallistatin levels and other biochemical parameters.

CONCLUSION

CONCLUSION

In this study, sixty cases of alcoholic liver disease proven by ultrasound abdomen and clinical history were taken up. Cases with or without liver biopsy were included, too. Age and gender matched thirty controls that showed normal ultrasound abdomen and no alcohol history was selected. Various parameters like total and direct bilirubin, total protein and albumin, SGOT, SGPT, AST/ALT ratio, ALP, GGT and Lipid profile were done in sixty cases and thirty controls

In this study, we found that

- Serum kallistatin can be used as a parameter for identifying early damage of liver due to alcohol consumption.
- Serum kallistatin levels decreases as the liver damage increases .As a result, mortality due to alcoholic liver disease also increases.
- Serum kallistatin levels were positively correlated with parameters viz.,total protein and albumin levels; and negatively correlated with parameters viz., total and direct bilirubin ,ALP and GGT. These results infer that, serum kallistatin levels can play a vital and protective variable in preventing alcoholic liver disease .This study leads a pathway for therapeutic intervention to be started earlier on the basis of serum kallistatin levels.
- Estimation of serum kallistatin levels can be added in routine investigations for liver function tests in patients with alcoholic liver disease.

LIMITATION OF THE STUDY

LIMITATIONS OF THE STUDY

Small sample size, when collected from a single medical hospital, may deprive us of the exact significant values, which will be more obvious with a larger sample size, if collected from an array of hospitals.

Small subgroups taken for the study hinders to put forward the relationship of some co morbidities associated with alcoholic liver disease

Follow up study could have been done, to strengthen our findings and prove the advantages of using kallistatin, as a new non-invasive biomarker, to detect liver damage and its therapeutic benefits.

SCOPE FOR FURTHER STUDIES

FUTURE PROSPECTS OF THE STUDY

On the basis of culture, living atmosphere and traditional lifestyle, studies can be conducted in various places of India involving large groups of population and interpret a reference range for serum kallistatin levels.

Studies on serum kallistatin levels can be conducted in a group of females and find out the diagnostic efficacy in alcoholism.

This present study can be conducted by comparing serum kallistatin levels with other parameters in various groups like heavy drinkers, problem drinkers and social drinkers.

Molecular basis of alcoholic liver disease can be studied by conducting series of research studies, in various groups and compared to establish the genetic map.

Research at different levels, can be done to throw light on the development of the pathological events in various stages of alcoholic liver disease.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Lefkowitch JH. Morphology of alcoholic liver disease. Clin Liver Disease 2005; 9: 37-53.
2. Mendez-Sanchez N, Meda-Valdes P, Uribe M. Alcoholic liver disease. An update. Ann Hepatol 2005;4: 32-42
3. MacSween RN, Burt AD. Histologic spectrum of alcoholic liver disease. Semin Liver Disease 1986; 6:221-232.
4. Robert S. O'Shea, Srinivasan Dasarathy, Arthur J. McCullough, and the Practice Guideline Committee of the American Association for the Study of Liver Diseases and the Practice Parameters Committee of the American College of Gastroenterology.
5. Peter.c.sharpe .Biochemical detection and monitoring of alcohol abuse and abstinence. Annals of clinical biochemistry 2001:47:1:13-27 (Review).
6. Pamela Bean,Karsten Liegmann,Trond lovli,Christina westby and Erling sundrehagen:semiautomated procedures for evaluation of CDT in the diagnosis of alcohol abuse :clinical chemistry:1997:43:6;983-989.
7. Chick J, Erickson CK. Conference summary: Consensus Conference on Alcohol Dependence and the Role of Pharmacotherapy in its Treatment. Alcohol Clin Exp Res 1996;20:391-402.
8. Kitchens JM. Does this patient have an alcohol problem? JAMA 1994; 272:1782-1787.
9. Corrao G, Ferrari P, Zambon A, Torchio P. Are the recent trends in liver cirrhosis mortality affected by the changes in alcohol consumption? Analysis of latency period in European countries. J Stud Alcohol 1997;58: 486-494.
10. World Health Organization. Global Status Report on Alcohol 2004. Geneva, Switzerland: World Health Organization; 2004.
11. Ezzati M, Lopez A, Rodgers A, Vander Hoorn S, Murray C; the Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. Lancet 2002;360:1347- 1360

12. Gyatso, TR., Bagdas, BB; (1998) In: Health Status In Sikkim. (Dept. of Health and Family Welfare, Govt. of Sikkim).

13. Balasubramanian S, Kowdley KV.Effect of alcohol on viral hepatitis and other forms of liver dysfunction.clin Liver Dis 2005:9:83-101.

14. Schiffer The alcoholic patient with hepatitis c virus infection.Am J Med 1999; 107 (6 suppl 2);95 S-9S.

15. Wong LL,LimmWM,TsaiNK,et al Hepatitis B and alcohol affect survival of Hepatocellular carcinoma patients.World J Gastro enterol 2005;11:3491-7.

16. Lieber CS. Metabolism of alcohol.Clin Liver Disease2005;9:1-35.

17. Fernandez-Checa jc.Alcohol induced liver disease:when fat and oxidative stress meet. Ann Hepatol2003;2:69-75.

18. Bertoletti A,Ferrari C.Kinetics of the immune response during HBV and HCV infection.Hepatology2003:38:4-13.

19. Alcoholic Liver Disease:Clinical and Sonographic features.Sien-Sing Yang.J Med Ultrasound 2008;16(2):140-149

20. Chao JL,SchmaierA ,Chen L M, Yang Z R,Chao L. Kallistatin a novel human tissue kallikrein inhibitor : levels in body fluids, blood cells,and tissues in health and disease. J LabClinMed 1996;127 :612–20

21. Original Article Kallistatin, a new and reliable biomarker for the diagnosis of liver cirrhosis Zhiyun Chenga ,b, YinghuiLva ,b, SuqiuPanga,b, RuyuBaia,b, Mingxi Wanga,b, ShuyuLina,b, TianwenXuc, DuncanSpaldingd, Nagy Habibd, RuianXu a,b,n.

22. Diagnosis and Treatment of Alcoholic Liver Disease and Its Complications Luis S. Marsano, M.D., Christian Mendez, M.D., Daniell Hill, M.D., Shirish Barve, Ph.D., and Craig J. McClain, M.D

23. Hasin D, Paykin A, Meydan J, Grant B. Withdrawal and tolerance: prognostic significance in DSM-IV alcohol dependence. J Stud Alcohol 2000;61(3):431–8.

24. Corrao G, Bagnardi V, Vittadini G, Favilli S. Capture–recapture methods to size alcohol related problems in a population. J Epidemiol Community Health 2000;54(8):603–10.

25. Gordis E. Advances in research on alcoholism and what they promise for future treatment and prevention. Med Health R I 1999;82(4):121

26. Chick J, Erickson CK. Conference summary: consensus conference on alcohol dependence and the role of pharmacotherapy in its treatment. Alcohol Clin Exp Res 1996;20(2):391–402.

27. Sherlock's Diseases of the Liver and Biliary System, Twelfth Edition. Edited by JameSs S. Dooley, Anna S.F. Lok, Andrew K. Burroughs, E. Jenny Heathcote. © 2011 by Blackwell Publishing Ltd. Published 2011 by Blackwell Publishing Ltd. 507

28. Wohl and Good Hart:Modern Nutrition In Health And Disease,1964.pg.318.

29. Das SK, Nayak P, Vasudevan DM (2003)Biochemical markers of alcohol consumption. Ind J Clin Biochem. 18(2), 111-118

30. Inaba, Darryl; Cohen, William B. (2004). Uppers, downers, all arounders: physical and mental effects of psychoactive drugs (5th ed.). Ashland, Or: CNS Publications. ISBN 0-926544-27-6

31. Nobert W.Tietz Ph d.,:Fundamental of clinical Biochemistry.(Ed)1986 pg.1672-1674.

32. Greenwel P,Dominguez-Rosales JA ,Mavi.G,Rivas-Estilla AM and Rojikind M(2000):Hydrogen peroxide:a link betttween formaldehyde elicited alpha (1) collagenase gene regulation and oxidative stress in mouse hepatic stellate cells-Hepatology 31:109-116.

33. Jean s.Wilson et al:Harrison's principle of internal medicine(Ed) International ed.1991(12):pg.1439,1483-1488.

34. Marc A.Schuckit:alcohol and alcoholism :Harrison's Textbook of Internal Medicine:14th Edition :Section 6:2503-2508.

35. Marvin H Sleisenger MD et al: Gastro enterology Pathophysiology Diagnosis Management v (Ed) W.B.Saunder's publications 1993 a vol ii:p.2031,b Vol i :p. 1062.

36. Neville R Pimstone and J Burbige Ethyl alcohol and disease Med Clin North America(1984):p.77-90.

37. Cotram et al: Robbins Pathologic Basis of Diseases (Ed) W.B.Saunders company,1995(4)p-389.

38. Tonnesen H, Rosenberg J, Nielsen HJ, et al. Effect of preoperative abstinence on poor postoperative outcome in alcohol misusers: randomised controlled trial. Br Med J 1999;318(7194):1311–16.

39. Schiff's diseases of the liver. – 11th ed. / edited by Eugene R. Schiff, Willis C. Maddrey, Michael F. Sorrell.p. ; cm.Diseases of the liver Includes bibliographical references and index. ISBN-13: 978-0-470-65468-2 (hardcover : alk. paper) ISBN-10: 0-470-65468-6 (hardcover : alk. paper) 1. Liver –Diseases. I. Schiff, Eugene R.

40. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders 4th edn. Washington, DC: APA, 1994.

41. World Health Organization. World Health Organization. The ICD/10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines, 10th revision. Geneva: WHO.

42. Johnson RD Howittz M,Maddox AF,Wishart JM and Shearmann DJ(1991).cigarette smoking and rate of gastric emptying.effect of alcohol absorption.BMJ 302:20-23

43. Neumann Mg 2003 cytokines –central factorst in alvcoholic ; liver disease alcohol res health27:307-316

44. Cook RT 1998 alcoholim,alcohol abuse and damage to the immune syatem. Alcohol Clin ExpRes 22 1927-194

45. Biomarkers for assessing ethanol consumption and the development of alcoholic liver disease University oftampere medical school, Finland,2007

46. Bellentani S Saccoccio G,Costa G,Tiribelli C,Manenti F, Sodde M, Saveria CL, Sasso F, Pozzatto G, Cristianini G and Brandi G (1997). Drinking habits as cofactorsof risk for alcohol induced liver damage.The Dionysos study group.Gut 41:845-850.

47. Savolainen VT, Liesto K. Männikkö A, et al: Alcohol consumption and alcoholic liver disease: Evidence of a threshold level of effects of ethanol. Alcohol Clin Exp Res 1993;17(5):1112-1117.

48. Sorensen TIA, Orholm M, Bentsen KD, et al: Prospective evaluation of alcohol abuse and alcoholic liver injury in men as predictors of development of cirrhosis. Lancet 1984; 2(8397):241-244.

49. O'Shea R S, Dasarathy S, McCullough A J: Alcoholic Liver Disease. Hepatology 2010;51(1):307-328.

50. Groszmann R, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, et al. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. N Engl J Med. 2005; 353:2254–61.

51. Jepsen P , Ott P, Andersen PK, Sorensen HT, Vilstrup H. The clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. Hepatology. 2010;51:1675–82

52. Pelletier S, Vaucher E, Aider R, Martin S, Perney P, Balmes JL, et al. Wine consumption is not associated with a decreased risk of alcoholic cirrhosis in heavy drinkers. Alcohol Alcohol 2002;37: 618-621

53. Salerno F, Guevara M, Bernardi M, Moreau R, Wong F, Angeli P, et al. Refractory ascites: pathogenesis, definition and therapy of a severe complication in patients with cirrhosis. Liver Int. 2010;1: 937–47.

54. Yokoyoma M.YokoyomaA ,YokoyomaY,Hamana G,Funazu K,Kondo set al, Mean corpuscular volume and aldehyde dehydrogenase-2 in male Japanese workers. Alcohol clin Exp Res 2003:27:1395-401.

55. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol. 2006;44:217–31.

56. Rosalki S (1984) Identifying the alcoholic. In Clinical Biochemistry of Alcoholism, (Ed. RosalkiS) Churchill, Livingstone, Edinburgh 65-92.

57. Gowelock, A.H. (1988) In: Varley's Practical Clinical Biochemistry. 6th edn. Heinemann Professional Publishing, p.519

58. Alcoholic liver disease: morphological manifestations. Review by an international group. Lancet 1981;1 :707-711.

59. Ishak KG, Zimmerman HJ, Ray MB. Alcoholic liver disease: pathologic, pathogenetic and clinical aspects. Alcohol Clin Exp Res 1991; 15:45-66.

60. Benvegnu L ,Gios M , Boccato S , AlbertiA .Natural history of compensated viral cirrhosis:aprospectivestudy on the incidence and hierarchy of major complications . Gut 2004;53:744–9.

61. Lauridsen, M. M., Thiele, M., Kimer, N., & Vilstrup, H. (2013). The continuous reaction times method for diagnosing, grading, and monitoring minimal/covert hepatic encephalopathy. Metabolic Brain Disease, 28(2), 231–4. Doi :10. 1007/s11011-012-9373-z

62. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol. 2006;44: 217–31.

63. Kappus, M. R., & Bajaj, J. S. (2012). Covert hepatic encephalopathy: not as minimal as you might think. Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association, 10(11), 1208–19. doi:10.1016/j.cgh. 2012.05.026 Khanna, D., & Tsevat, J. (2007). REPORTS, (December), 218–223.

64. Prytz H, Melin T. Identification of alcoholic liver disease or hidden alcohol abuse in patients with elevated liver enzymes. J Intern Med 1993;233 :21-26.

65. Moore RD, Bone LR, Geller G, Mamon JA, Stokes EJ, Levine DM. Prevalence, detection, and treatment of alcoholism in hospitalized patients.JAMA 1989;261 : 403-407

66. Girela E, Villanueva E, Hernandez-Cueto C, Luna JD. Comparison of the CAGE questionnaire versus some biochemical markers in the diagnosis of alcoholism. Alcohol Alcohol 1994;29: 337-343.

67. Levine J. The relative value of consultation, questionnaires and laboratory investigation in the identification of excessive alcohol consumption. Alcohol Alcohol 1990;25:539-553.

68. Helander A, Eriksson CJ. Laboratory tests for acute alcohol consumption: results of the WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence. Alcohol Clin Exp Res 2002;26:1070-1077

69. Fiellin DA, Reid MC, O'Connor PG. Screening for alcohol problems in primary care: a systematic review. Arch Intern Med 2000;160:1977- 1989.

70. Menon KV, Gores GJ, Shah VH. Pathogenesis, diagnosis, and treatment of alcoholic liver disease. Mayo Clin Proc 2001;76: 1021-1029.

71. Cabre E, Rodriguez-Iglesias P, Caballeria J, Quer JC, Sanchez-Lombrana JL, Pares A, et al. Short- and long-term outcome of severe alcohol-induced hepatitis treated with steroids or enteral nutrition: a multicenter randomized trial. Hepatology 2000;32: 36-42.

72. Leung NW, Farrant P, Peters TJ. Liver volume measurement by ultrasonography in normal subjects and alcoholic patients. J Hepatol 1986;2: 157-164.

73. Hamberg KJ, Carstensen B, Sorensen TI, Eghoje K. Accuracy of clinical diagnosis of cirrhosis among alcohol-abusing men. J Clin Epidemiol 1996;49: 1295-1301.

74. Orrego H, Israel Y, Blake JE, Medline A. Assessment of prognostic factors in alcoholic liver disease: toward a global quantitative expression of severity. Hepatology 1983;3: 896-905.

75. Aertgeerts B. Buntinx F, Kester A: The value of the CAGE in screening for alcohol abuse and alcohol dependence in general clinical populations: a diagnostic meta-analysis. J Clin Epidemiol 2004;57: 30-39.

76. Richard O . Duda, Peter E . Hart, and David G. Stork. Pattern Classification (2nd Edition).Wiley-Interscience, 2000.

77. Berzigotti A, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Morillas R, et al. Obesity is an independent risk factor for clinical decompensation in patients with cirrhosis. Hepatology.2011;54: 555–61

78. Zoli M, Merkel C, Magalotti D, Gueli C, Grimaldi M, Gatta A, et al. Natural history of cirrhoticpatients with small esophageal varices: a prospective study. Am J Gastroenterol. 2000;95: 503–8.

79. D'Amico G, Villanueva C, Burroughs AK, Dollinger M, Planas R, Solà R, et al. Clinical stages of cirrhosis a multicenter study of 1858 patients. Hepatology. 2010;52 (S1):329

80. Dr. M.N.Chatterjee and Dr.RKana Shinde "Textbook of Biochemistry1993 (1).pg.828.

81. Evaluation and prognosis of patients with cirrhosis.Module 2 :Evaluation,staging monitoring of chronic Hepatitis C.Lesson-5 Evaluation and prognosis of patients with cirrhosis

82. Orrego H, Israel Y, Blake JE, Medline A. Assessment of prognostic factors in alcoholic liver disease: toward a global quantitative expression of severity. Hepatology 1983;3: 896-905.

83. Pessione F, Ramond MJ, Peters L, et al. Five-year survival predictivefactors in patients with excessive alcohol intake and cirrhosis. Effect of alcoholic hepatitis, smoking and abstinence. Liver Int 2003;23(1):45–53.

84. Tonnesen H, Rosenberg J, Nielsen HJ, et al. Effect of preoperative abstinence on poor postoperative outcome in alcohol misusers: randomised controlled trial. Br Med J 1999;318(7194):1311–16

85. Forman LM, Lucey MR. Predicting the prognosis of chronic liver disease: an evolution from child to MELD. Mayo end-stage liver disease. Hepatology 2001;33:473–475

86. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. Hepatology 2001;33:464–470

87. Lucey MR, Brown KA, Everson GT, et al. Minimal criteria for placement of adults on the liver transplant waiting list: a report of a national conference organized by the American Society of Transplant Physicians and the American Association for the Study of Liver Diseases. Liver Transpl Surg 1997; 3:628-37.

88. Poynard T, Naveau S, Doffoel M, et al. Evaluation of efficacy of liver transplantation in alcoholic cirrhosis using matched and simulated controls: 5-year survival. Multi-centre group. J Hepatol 1999; 30: 1130–7

89. D'Amico G, Pasta L, Madonia S, Tarantino G, Mancuso A, Malizia G, et al. The incidence of esophageal varices in cirrhosis. Gastroenterology. 2001;120:A2.

90. Merli M, Nicolini G, Angeloni S, Rinaldi V, De Santis A, Merkel C, et al. Incidence and natural history of small esophageal varices in cirrhotic patients. J Hepatol. 2003;38:266–72.

91. Garcia-Tsao G, Groszmann RJ, Fisher RL, Conn HO, Atterbury CE, Glickman M. Portal pressure, presence of gastroesophageal varices and variceal bleeding. Hepatology. 1985;5:419–24.

92. Serpins, Serpinopathies, and Conformational Diseaseswww.preprotech.com

93. Chao, J., and L. Chao. Biochemistry, Regulation and potential function of kallistatin. "BioI. Chern. Hoppe-Seyler." 376; 705-713, 1995.

94. Chang-yi Chen structural and functional study of human kallistatin A dissertation subn1itted to the faculty of the Medical University of South Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Graduate StudiesMolecular and Cellular Biology and Pathobiology 2000.

95. Chao, 1., II NI Stallone, Y. M. Liang, L. M. Chen, D. Z. Wang, L. Chao. "Kallistatin is a Potent new vasodilator. " J. Clin. Invest. 100 ; 1-7, 1997.

96. Serum kallistatin is increased in Type 2 Diabetic Patients with microvascular complications and correlates with the presence of retinopathy.Jeffrey D. McBride:Alicia Jenkins:Tim lyons:Jian-Xing Ma.Investigative ophthalmology and visual science. April 2011,VOL.52,1267.

97. Zhou, G. X., L. Chao and J. Chao. "Kallistatin : a novel human tissue kallikrein inhibitor: Purification, characterization, and reactive center sequence." J. Bio!. Chern. 267; 25873 -25880, 1992.

98. Chao, J., A. Schrnaier, L. M. Chen, Z. Yang, and L. Chao. "Kallistatin, a novel human tissue kallikrein inhibitor: expression in blood cells and abnormal plasma level in human liver disease, sepsis and preeclampsia." J. CHn. Lab. Med. 127; 612-620~1996.

99. Huber, R., and R. W. Carrell. "Implications of the three-dimensional structure of a1-antitrypsin for structure and function of serpins." Biochemistry 28; 8951-8966, 1989.

100. Li TK, Hewitt BG, Grant BF. The alcohol dependence syndrome, 30 years later: a commentary. The 2006 H. David Archibald lecture. Addiction 2007;102(10) : 1522–30.

101. Nord HJ. Biopsy diagnosis of cirrhosis : blind percutaneous versus guided direct vision techniques—a review. Gastrointest Endosc 1982;28:102–4.

102. Peni Ponka , et.al.,Function and regulation of Transferrin and Ferritin:seminar in haematology:1998:35:January:35-54.

103. McCormick DB, Greene HL (1998). Vitamin. In:Tietz Textbook of Clinical Chemistry. (Eds. CABurtis and ER Ashwood) W.B. SaundersCompany, USA. p.1025.

104. Krastev Z, Mateva L, Danev S, Nikolov R (1992)Clinical meaning of GGT activity in follow-up ofpatients with alcohol-related liver injury and cholestasis. Ital J Gastroenterol, 24(4), 185-187

105. Nicholas John Pappas:diagnostic enzymology:Clin. In Lab Medicin:1989:p.598,668.

106. Elemer Nemesanzty and John a.Lott:GGT and its isoenzymes:progress and problems Clical chemistry:1985:(31/6):p.797-803.

107. Daeppen JB, Schoenfeld-Smith K, Smith TL,Schuckit MA. (1999) Characteristics of alcoholdependent subjects with very elevated levels ofGamma-Glutamyltransferase (GGT). J Stud Alcohol, 60(5), 589-594

108. Waern AU, Hellsing K (!980)Indices of alcohol intake. Comparison between serum concentrations of alkaline phosphatase and gamma glutamyl transferase in middle-aged men. Ups J Med Sci, 85(2), 159-163.

109. Nalpas B, Vassault A, LeGuillou A et al (1984) Serum activity of mitochondrial aspartate amino transferase: a sensitivity marker of alcoholism with or without alcoholic hepatitis. Hepatology, 4, 893-896.

110. Conigrave KM, Degenhardt LJ, Whitfield JB, Saunders JB, Helander A, Tabakoff B. CDT, GGT, and AST as markers of alcohol use: the WHO/ ISBRA collaborative project. Alcohol Clin Exp Res 2002;26:332-339.

111. Garnero P,Ferreras M,Karsdal MA,Risteli JBorelO ,Qvist PDelmas PD,.The type1 collagen fragments ICTP and CTXreveal certain enzymatic pathways OF bone collagen degradation.JBone Miner Res18:859-867.

112. Helander A, Beck O and Jones AW(1996)Laboratory testing of recent alcohol consumption. comparison of methanol, ethanol and5-hydroxy tryptophol Clin Chem42:618-624.

113. Chao J,Schmaier A,ChenLM,YangZ,Chao L.Kallistatin,a novel human tissue kallikrein inhibitor:levels in body fluds,blood cells and tissues in health and disease.1996jun:127(6)612-20.

114. Alcoholic liver disease :johns Hopkin's medicine © Copyright 2001-2013 | All Rights Reserved. 600 North Wolfe Street, Baltimore, Maryland 21287

115. Richard Allan, Kerry Thoirs, andMaureen Phillips. Accuracy of ultrasound to identify chronic liver disease. World J Gastroenterol, 28(16):3510–3520, July 2010.

116. Classification and Staging of Chronic LiverDisease based on Ultrasound, Laboratorial and Clinical Data Ricardo Ribeiro, Rui T. Marinho, Jasjit S. Suri, and J. Miguel Sanches

117. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol. 2006;44:217–31.

118. Bruno S, Zuin M, Crosignani A, Rossi S, Zadra F, Roffi L, et al. Predicting mortality risk in patients with compensated HCV-induced cirrhosis: a long term prospective study. AmJ Gastroenterol. 2009;104:1147–58.

119. Kallistatin, a new and reliable biomarker for the diagnosis of liver cirrhosis Zhiyun Chenga ,b , YinghuiL va ,b, SuqiuPanga ,b, Ruyu Baia ,b, Mingxi Wanga, b, ShuyuLina, b, Tianwen Xuc, Duncan Spaldingd, Nagy Habibd, Ruian Xua, Received 2 November 2014; received in revised form 1 February 2015; accepted 14 February 2015

120. Kallistatin, a novel tissue kallikrein inhibitor: levels in body fluids, blood cells and tissues in health and disease. Chao J, Schmaier A,Chen LMYang Z,Chao L.Clin Medicine 1996:JUN 127(6):612-20

121. Indian Journal of Clinical Biochemistry, 2005, 20 (1) Indian Journal of Clinical Biochemistry, 2005; 35 biochemical diagnosis of alcoholism. Subir Kumar Das* and D.M.Vasudevan Department of Biochemistry, Amrita Institute of Medical Sciences, Cochin 682 026, Kerala

122. Ahlgren A, Hedenborg G, Norman A, Wisen O.(1988) Serum bilirubin subfractions in patients with alcohol abuse during detoxication. Scand J Clin Lab Invest, 48(4), 319-26

123. Das SK, Nayak P, Vasudevan DM (2003) Biochemical markers of alcohol consumption. IndJ Clin Biochem. 18(2), 111-118

124. Annoni G, Arosio B, Santambrogio D, Gagliano N,Zern MA (1991) Albumin and procollagen type I gene regulation in alcohol and viral-induced human liver disease. Boll Ist Sieroter Milan, 70(1-2), 391-397.

125. Alcohol, amino acids, and albumin synthesis. II.Oratz M, Rothschild MA, Schreiber SS (1976)Alcohol inhibition of albumin synthesis reversedby arginine and spermine. Gastroenterology;71(1), 123-127

126. Schimdt E, Schimdt FW (1979) Enzyme diagnosisin diseases of the liver and biliary system. In:Advances in Clinical Enzymology. Vol.I. (Eds. ESchimdt, FW Schimdt, ITrautschold, R Friedel)Basel: Karger; pp. 232-292.

127. Dennis e freer and Bernard e statland: the effect of ethanol on the activity of selected enzymes in sera of healthy young adults, clinical chemistry, 1977 (23/5) pg. 830-834.

128. Nalpas B, Vassault A, LeGuillou A et al (1984)Serum activity of mitochondrial aspartate aminotransferase: a sensitivity marker of alcoholism with or without alcoholic hepatitis. Hepatology, 4, 893-896.

129. Jerold A.,Gohen MD et al.,SGOT/SGPT ratio an indicator of alcoholic liver disease.Am.j.Diag.Dise.979,24:pg.835-838.

130. Rosalki S (1984) Identifying the alcoholic. In Clinical Biochemistry of Alcoholism, (Ed. Rosalki S) Churchill, Livingstone, Edinburgh 65-92.

131. Daeppen JB, Schoenfeld-Smith K, Smith TL,Schuckit MA. (1999) Characteristics of alcohol dependent subjects with very elevated levels of Gamma-Glutamyltransferase (GGT). J StudAlcohol, 60(5), 589-594

132. Krastev Z, Mateva L, Danev S, Nikolov R (1992) Clinical meaning of GGT activity in follow-up of patients with alcohol-related liver injury andcholestasis. Ital J Gastroenterol, 24(4), 185-187

133. Szczeklik Sorlowsk in et al ,Serum GGT activity in liver disease. gastroenterology, 1961(41):p:353-359

134. Shiela Sherlock james doodley diseases of liver and biliary system (ED) Black Wel Scientific,1993(9).P.370-390

ANNEXURES

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.G.Chitra Siva Sankari
II Year Post Graduate in M.D. Bio-Chemistry
Institute of Bio-Chemistry
Madras Medical College
Chennai 600 003

Dear Dr.G.Chitra Siva Sankari,

The Institutional Ethics Committee has considered your request and approved your study titled **"EVALUATION OF SERUM KALLISTATIN LEVELS IN CASES OF ALCOHOLIC LIVER DISEASE " NO. 39062016.**

The following members of Ethics Committee were present in the meeting hold on **07.06.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.Isaac Christian Moses,MD.Ph.D.Dean(FAC)MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3	: Member
5.Prof.P.Raghumani,MS, Prof. of Surgery,RGGGH,Ch-3	: Member
6. Prof.Md.Ali,MD.,DM.,HOD-MGE, MMC,Ch-3	: Member
7.Prof.Baby Vasumathi, Director, Inst. of O&G,Ch-8	: Member
8.Prof.K.Ramadevi,MD, Director,Inst.of Bio-Chem,MMC,Ch-3	: Member
9.Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3	: Member
10.Prof.Srinivasagalu, Director,Inst.of Int.Med.,MMC,Ch-3	: Member
11.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
12.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
13.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

CHAIRMAN
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

URKUND

Urkund Analysis Result

Analysed Document: DISSERTATION FINAL.docx (D30223952)
Submitted: 8/23/2017 5:55:00 PM
Submitted By: drmcvk7896@gmail.com
Significance: 2 %

Sources included in the report:

new sir copy thesis.docx (D17539271)
Naveen Sharma-PHARMACOLOGICAL AND PHYTOCHEMICAL STUDIES ON ACTINIOPTERIS DICHOTOMA BEDD.docx (D18167709)

Instances where selected sources appear:

4

PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled **"EVALUATION OF SERUM KALLISTATIN LEVELS IN CASES OF ALCOHOLIC LIVER DISEASE "** of the candidate **DR.G.CHITRA SIVA SANKARI** with registration Number **201523002** for the award of **M.D** in the branch of **BIOCHEMISTRY**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **2 percentage** of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.

PROFORMA

Date: Sample Id : _____

Name: Age : Sex : Ht (cm): Wt (kg) :

Waist circumference:

H/O alcohol intake:

H/O jaundice:

H/O Abdominal pain:

H/O nausea or vomiting _____

H/O Blood in vomiting:

H/o weight loss _____

H/O recent gastric surgeries_____

Other autoimmune diseases: _____

(if any duration _____)

Treatment history:

ASSOCIATED DISEASES WITH DURATION :

Diabetes Mellitus □ Hypertension □

Ischemic heart disease □

Hypothyrodism □ PCOD □

Hypercholesterolemia □

Gastric surgeries □ others □

Drug Intake:

Steroids □ Acetaminophen □ Tetracyclin □

Any other medications □

Smoking □ Passive smoking □ Alternative medicine intake □

EXAMINATION

GENERAL EXAMINATION:

PALLOR/ICTERUS/CLUBBING/CYANOSIS/PEDAL EDEMA/LYMPHADENOPATHY.

BP: PR: RR:

SYSTEMIC EXAMINATION:

CVS:

RS:

ABDOMEN:

CNS:

SAMPLE COLLECTION: Date and time:_____

INVESTIGATIONS:

- Serum Fasting Lipid Profile
- Serum liver function tests
- Serum kallistatin

INFORMATION SHEET

Title: Evaluation of Serum kallistatin in cases of alcoholic liver disease.

Investigator : Dr .G.CHITRA SIVA SANKARI,
II Year Post Graduate, MD Biochemistry
Institute of Biochemistry
Madras Medical College
Chennai - 600003

Guide : Prof. Dr. R.CHITHRAA,
Institute of Biochemistry,
Madras Medical College,
Chennai- 600003.

The purpose of the study is to evaluate the use of kallistatin as a non invasive biomarker in the diagnosis alcoholic liver disease.

Hence, I am doing this study titled "Evaluation of serum kallistatin levels in cases of alcoholic liver disease", at Rajiv Gandhi Govt. General Hospital, Chennai. For this study I need 5mL of blood from 60 alcoholic liver disease patients and 30 apparently healthy individuals.

Your identity will be kept confidential throughout the study as well as during publication or presentation of the study, findings in any clinical forum or journals. Participation in this study is purely voluntary. You can withdraw from this study at any time. Your decision will not result in any loss of benefits to which you are entitled. The results of the study will be intimated to you. If you have willingness to participate in this study, kindly sign in this information sheet and the consent form.

Signature of the investigator

Signature of the participant
Thumb impression

Place:

Date:

PATIENT CONSENT FORM

Title of the study**:"Evaluation of serum Kallistatin levels in cases of alcoholic liver disease."**

Name : Date :

Age : OP No:

Sex : Project Patient No :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I felt free to ask questions about the study which were answered. I, hereby, give my consent to be included as a participant in "**Evaluation of serum Kallistatin levels in cases of alcoholic liver disease."**

1. I have read and understood this consent form and the information that was provided.
2. I have had the consent document explained.
3. I have been explained the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have informed the investigator of all the treatments I am taking or have taken for the past -- months/years including any native (alternative) treatment.
6. I have been informed about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____ month(s).

9. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.
10. I am aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.
11. I hereby give permission to the investigator to release the information obtained from me as a result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are out of the interest of the public.
12. I have understood that my identity will be kept confidential even if my data are published.
13. I have had my questions answered to my satisfaction.
14. I have decided to be a participant in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form, I attest that the information given in this document has been clearly explained. I have understood the contents of the consent form..

Name: Signature with date:

ஆராய்ச்சி தகவல் தாள்

தலைப்பு:

"மதுபானம் சார்ந்த கல்லீரல் நோயாளிகளைக் கண்டுபிடிப்பதில் சீரம் காளிஸ்டேட்டினின் பங்கு பற்றிய ஆய்வு".

ஆராய்ச்சியாளர் : **மரு. கா. சித்ரா சிவசங்கரி,**
பட்ட மேற்படிப்பு மருத்துவ மாணவி,
உயிர்வேதியியல் உயர்நிலைத்துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.

ஆராய்ச்சி மேற்பார்வையாளர் : **பேரா. மரு. R.சித்ரா,**
உயிர்வேதியியல் உயர்நிலைத்துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.

மதுபானம் சார்ந்த கல்லீரல் நோய் நாள்பட்ட கல்லீரல் நோயாளிகளின் மத்தியில் பரவலாக ஏற்படும் நோயாகும்.

கல்லீரல் நோய்களைக் கண்டுபிடிப்பதற்கு திசு ஆய்வு தான் தங்கத் தரநிலையாக நிலவி வருகிறது. ஆனால் திசு ஆய்வு ஒரு துளைத்தல் செயல்முறையாகவும், பல பக்க விளைவுகள் உண்டாக்குவதாகவும் இருப்பதால் ஒரு துளைத்தல் இல்லாத செயல்முறை மிகுந்த மருத்துவ முக்கியத்துவமாக விளங்கும்.

காளிஸ்டேட்டின் ஒரு துளைத்தல் இல்லாத செயல்முறையாக மதுபானம் சார்ந்த கல்லீரல் நோயாளிகளுக்கு மிகுந்த பயனுள்ளதாக இருக்கும்.

தங்களிடமிருந்து ஊசியின் மூலம் 5 மி.லி. இரத்தம் எடுப்பதனால் எந்தவிதமான பக்க விளைவுகளும் ஏற்படாது என உறுதி அளிக்கின்றேன்.

தாங்கள் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதில் பங்கு பெறுவதினால் நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் கூடுதல் செலவீனம் ஏற்படாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயர் மற்றும் அடையாளங்கள் வெளியிடப்படாது என்பதை தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களின் விருப்பத்தின் பேரில் தான் இருக்கிறது. எந்நேரமும் இதிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியின் முடிவுகளை தங்களுக்கு அறிவிப்போம் என்பதை தெரிவித்துக் கொள்கிறோம்.

நீங்கள் இந்த ஆராய்ச்சியில் பங்குபெற விருப்பம் இருப்பின் இந்த தகவல்தாள் மற்றும் ஆராய்ச்சி ஒப்புதல் படிவத்திலும் கையொப்பம் இடுமாறு கேட்டுக் கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம்

பங்கேற்பாளர் கையொப்பம்
/இடது கைவிரல் ரேகை

இடம் :
தேதி :

நோயாளியின் ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு:

"மதுபானம் சார்ந்த கல்லீரல் நோயாளிகளைக் கண்டுபிடிப்பதில் சீரம் காளிஸ்டேட்டினின் பங்கு பற்றிய ஆய்வு".

பங்கேற்பாளர் பெயர் : புற / உள் நோயாளி எண்:

வயது : பால் : கைபேசி/தொலைபேசி எண் :

முகவரி : ஆராய்ச்சி சேர்க்கை எண் :

.....ஆகிய நான் மரு. கா. சித்ரா சிவசங்கரி, பட்டமேற்படிப்பு, மருத்துவ மாணவி, உயிர்வேதியியல் உயர்நிலைத் துறை, சென்னை மருத்துவக் கல்லூரி, சென்னை. மேற்கொள்ளும் ஆராய்ச்சியில் பங்கேற்க எந்தவித நிர்பந்தமின்றி, முழு சுதந்திரத்துடன் சுய நினைவுடன் முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

இந்த ஆராய்ச்சி பற்றிய தகவல் தாளை பெற்றுக் கொண்டு அதன் முழு விவரங்களையும், நோக்கங்களையும் மருத்துவரின் மூலம் புரிந்துக்கொண்டு எனது மருத்துவ குறிப்புகள், பரிசோதனை முடிவுகள் மற்றும் இரத்தத்தை பயன்படுத்திக் கொள்ளவும் மேலும் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் எனவும் அச்செயலினால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்துக் கொண்டு முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

எனது பரிசோதனை முடிவுகளை அறிவியல் சார்ந்த அமைப்புகள் மற்றும் மருத்துவ இதழ்களில் வெளியிடுவதற்கு முழு மனதுடன் சம்மதிக்கின்றனே.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்
/இடது கைவிரல் ரேகை

இடம் :

தேதி :