ASSESSMENT OF THE VALUE OF SERUM CHOLINESTERASE AS A LIVER FUNCTION TEST IN PATIENT WITH LIVER DISEASES

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MAY 2022

CERTIFICATE FROM THE DEAN

This is to certify that this dissertation entitled **"ASSESSMENT OF THE VALUE OF SERUM CHOLINESTERASE AS A LIVER FUNCTION TEST IN PATIENT WITH LIVER DISEASES"** is the bonafide work of **Dr. K. PRAVEEN RAJ** in partial fulfillment of the university regulations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, for M.D General Medicine Branch I examination to be held in **MAY 2022.**

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I, Dr. K. PRAVEEN RAJ solemnly declare that, this dissertation entitled **"ASSESSMENT OF THE VALUE OF SERUM CHOLINESTERASE AS A LIVER FUNCTION TEST IN PATIENT WITH LIVER DISEASES"** is a bonafide record of work done by me at Department of General Medicine, Madurai Medical College and Government Rajaji Hospital, Madurai during Mar 2021 – Aug 2021 under the guidance and supervision of **Prof. Dr. S.C. VIVEKANANTHAN M.D., DTCD.** I also declare that this bonafide work or part of this work was not submitted by me or any others for any award, degree, diploma to any other University, Board either in India or abroad. This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfillment of the rules and regulations for the award of Degree of Doctor of Medicine (M.D.), General Medicine Branch -I- examination to be held in May 2022.

Place : Madurai

Date : **Dr. K. PRAVEEN RAJ**

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CONTENTS

INTRODUCTION

INTRODUCTION

Liver is one of the largest vital organs in the human body which has wide range of functions and it accounts for about 2% to 3% of the average body weight. Diseases which involves the hepatobiliary system is one among the highest causes of mortality and morbidity in developing country like India and also worldwide. Liver has two lobes. Right and the left lobe. It is located in the right upper abdominal cavity below the diaphragm. Liver is protected by rib cage and it is maintained in its position by its peritoneal reflections called as ligamentous attachments. Though not all are true ligaments, these attachments are avascular and they are in continuity with glisson capsule or equivalent of visceral peritoneum of liver. The falciform ligament is a ligamentous attachment which arises from at or near the umbilicus and continuous to the anterior aspect of the liver parenchyma in continuity with the umbilical fissure. Liver receives dual blood supply. About 75% of the blood supply is by portal vein which is formed by joining of superior mesenteric vein and the splenic vein. Portal vein belongs to portal circulation which carries blood from the spleen, intestine and its associated organs. Hepatic artery supplies remaining 25% of the blood to the liver. Hepatic artery belongs to systemic circulation. Hepatic artery is a high pressure circulation

whereas portal vein belongs to low pressure circulation. Half of the liver's oxygen demand is met by systemic circulation whereas other half of the liver's oxygen demand is met by portal circulation. The hepatic artery has both alpha adrenergic receptors and beta adrenergic receptors. Hence flow through the hepatic artery is also controlled by the splanchnic nerves of the autonomic nervous system. Biochemical tests to assess the liver functions includes assessment of values of Serum total bilirubin, conjugated and unconjugated bilirubin, total serum protein along with serum albumin, serum globulin, serum glutamic oxaloacetic transaminase otherwise known as aspartate aminotransferase (SGOT otherwise called AST), serum glutamic pyruvic transaminase also known as Alanine aminotransferase (SGPT otherwise called as ALT) and serum Alkaline phosphatase (ALP). These liver function tests most commonly reveals alteration in patients suffering from diseases other than hepatic affection. However, Liver function tests which we are using day by day have several shortcomings:

- PT INR – values will be altered due to vitamin K deficiency, treatments with anticoagulants, deficiency of clotting factors since birth and its values are altered if the patient is treated with fresh frozen plasma.
- Serum bilirubin may be raised in hemolytic anemia of any cause rather than hepatic clearance.

- Alkaline phosphatase is also present in bone, kidney, placenta, intestine and pancreas. Hence disorders of placenta, intestinal mucosa, bone, kidney and pancreas may leads to elevation of ALP in the serum.
- LDH and Aminotransferase levels may be abnormal if there is damage to cell membrane.
- Serum albumin – may be altered due to non-hepatic causes like malnutrition, mal-absorption and diseases of kidneys. Albumin is major part of plasma proteins are produced by the liver polyribosomes which are bound to rough endoplasmic reticulum and are then secreted into the plasma. The Half- life of serum albumin is 21 to 22 days. Reduction in serum albumin reflects reduced synthesis by the hepatocytes, although serum albumin level changes depends upon the plasma volume as well as losses, for instance in to gut or urine, may responsible for development of hypoalbuminemia.

Therefore, there comes a need for a specific and sensitive test for liver disease. Serum cholinesterase has been studying for long duration as a biomarker to assess the synthetic, excretory and metabolic functions of liver for decades. Production of Serum cholinesterase is from the hepatocytes and hence it reflects the function of the liver. Serum cholinesterase values are not affected by transfusion of albumin or transfusion of blood products such as fresh frozen plasma. Previous large

multi centered Studies have proved that serum cholinesterase might be helpful in diagnosing the diseases of the liver and also helps in the assessment of its severity and prognosis of liver diseases.

The enzyme cholinesterase belongs to the enzymatic group which helps in breakdown of the esters of choline and thiocholine. Serum cholinesterases are synthesized by hepatic parenchyma and are released into the circulation. In humans, there are two types of cholinesterase. RBC cholinesterase (true cholinesterase) present in the membrane of the cell and plasma cholinesterase (pseudo cholinesterase). Plasma cholinesterase is also known as butyryl cholinesterase. Multiple studies in the past have proved that it shows excellent correlation with the currently available routine liver function tests such as serum albumin, PT INR, Bilirubin and aminotransferases. This cross sectional study was conducted mainly to measure the serum cholinesterase levels in liver disease patients and to compare the levels of serum cholinesterase with available liver function tests at present in patients with chronic liver diseases.

AIM & OBJECTIVES

AIMS & OBJECTIVES OF THE STUDY

- To measure the serum cholinesterase levels and liver function tests in patients with liver disease and comparing them with prevailing standardized normal values of serum cholinesterase.
- To compare the correlation between levels of serum cholinesterase with prevailing liver function tests such as serum bilirubin, serum proteins, INR, SGOT and SGPT in patients suffering from chronic liver diseases.
- To grade the severity of liver dysfunction by measuring the level of cholinesterase in the serum of those patients.

REVIEW OF LITERATURE

LITERATURE REVIEW

The critical purpose of the present study is to assess the cholinesterase activity in serum as a test of synthetic function of hepatocytes. This test has considerable significance, because it seems to be a test of synthetic function of the hepatocytes.

HISTORY:

The evaluation of cholinesterase found in serum was first proposed by McArdle in 1940, which helps the treating physician for classifying jaundice due to prehepatic, hepatic etiology or post hepatic such as biliary etiology[1]

In 1946, Brauer et al[2]. and in 1947 Ellis et al[3]. demonstrated that level of Serum cholinesterase was lowered in rat whenever the liver of the rat was injured by hepatotoxic agent such as carbon tetrachloride.

Brauer & Root[4] also identified, whenever carbon tetrachloride was administered to dogs, there was an increase in plasma cholinesterase levels at the earlier stages followed by fall in serum cholinesterase levels below the normal range after one to four hours.

The reduction in serum cholinesterase levels after administration of carbon tetrachloride can be due to excessive production of serum

cholinesterase from the hepatocytes, which results in increased levels in serum.

There was no significant association between serum cholinesterase levels in respect to age of the patients, gender, dietary or eating habits, pulse or heart rate, systemic blood pressure, muscle mass or body mass index. Hall & Lucas[5] was the first person to put forth this study.

Croft & Richter[6] studied in detail regarding serum cholinesterase levels in association with exercise and identified that serum cholinesterase rises during strenuous exercise.

Serum cholinesterase levels were normal in obstructive jaundice and reduced in liver diseases. This was found by Bauer[7] and many others[8, 9, 10, 11, 12, 13, 14, 15, 16].

Disease which leads on to cachexia such as cardiac, cancer etc. has decreased serum cholinesterase level and the same was identified by the study conducted by Antopol, Schifrin and Tuchman[10, 13].

McArdle[1] was the person who identified normal cholinesterase activity in serum of patients suffering with diabetes and hyperthyroidism.

In the study conducted by Faber[17, 18] observed that reduction in the levels of serum albumin was associated with low Cholinesterase levels.

LIVER DISEASE:

Liver is the largest organ in human body and weighs about 1.5 kilograms and it forms about 5% of total body weight in a new born. The most common liver diseases encountered includes Alcoholic liver disease, hepatotrophic viral infection, Autoimmune hepatitis, Toxins, Jaundice due to biliary obstruction, Liver abscess, storage disorders, primary or secondary malignancies of the liver, Congestive hepatitis and so on. Understanding the physiology, biochemical functions of the liver and also histology of the liver is crucial in diagnosing and treating the liver pathologies.

FUNCTION OF LIVER:

1. Metabolism of Carbohydrate such as glycolysis, gluconeogenesis, glycogenolysis and glyconeogenesis will takes place in the liver.
2. Metabolism of fat such as lipolysis and lipogenesis.
3. Metabolism of ketones.
4. It helps in regulating levels of amino acids in the blood circulation, which are basic elements for protein synthesis.
5. Production and secretion of bile salts and bile acids.
6. Major organ for Glycogen storage.
7. Synthesis of Amino acids and Proteins.
8. Production of clotting factors and complements.

9. Erythropoiesis (in fetus)
10. Detoxification of toxins
11. Storage organ for vitamins such as vitamin D, vitamin B12, vitamin E, Iron, copper, zinc, cobalt and vitamin A.
12. Functions as an organ for phagocytosis.
13. Synthesizes angiotensinogen, a hormone responsible for raising the blood pressure.
14. Functions as an organ for metabolism of free radicals.
15. Function as a blood reservoir. Almost 10% of total blood volume is in liver sinuses.
16. Responsible for breakdown of insulin and other hormones.
17. Organ for drug metabolism.
18. Organ for all first pass metabolism.

Whenever the liver has broken down the harmful substances, the waste products are eliminated with the help of the bile or released into the blood circulation. This bile along with the waste products reaches the intestine through the biliary tree and excreted out of the body. The waste products which entered the blood circulation are then enters the kidneys and filtered out by renal system and then leave the body via the urine.

HISTOLOGICAL STRUCTURE OF THE LIVER:

The liver stroma consists of:

1. Connective tissue capsule
2. Trabeculae
3. Reticular fibers.

CONNECTIVE TISSUE CAPSULE:

- Serous layer: Visceral peritoneum
- Fibrous layer: Glisson's capsule

TRABECULAE:

- The Glisson's capsule also extends into the inferior surface of the liver as numerous branching trabeculae and the septa.

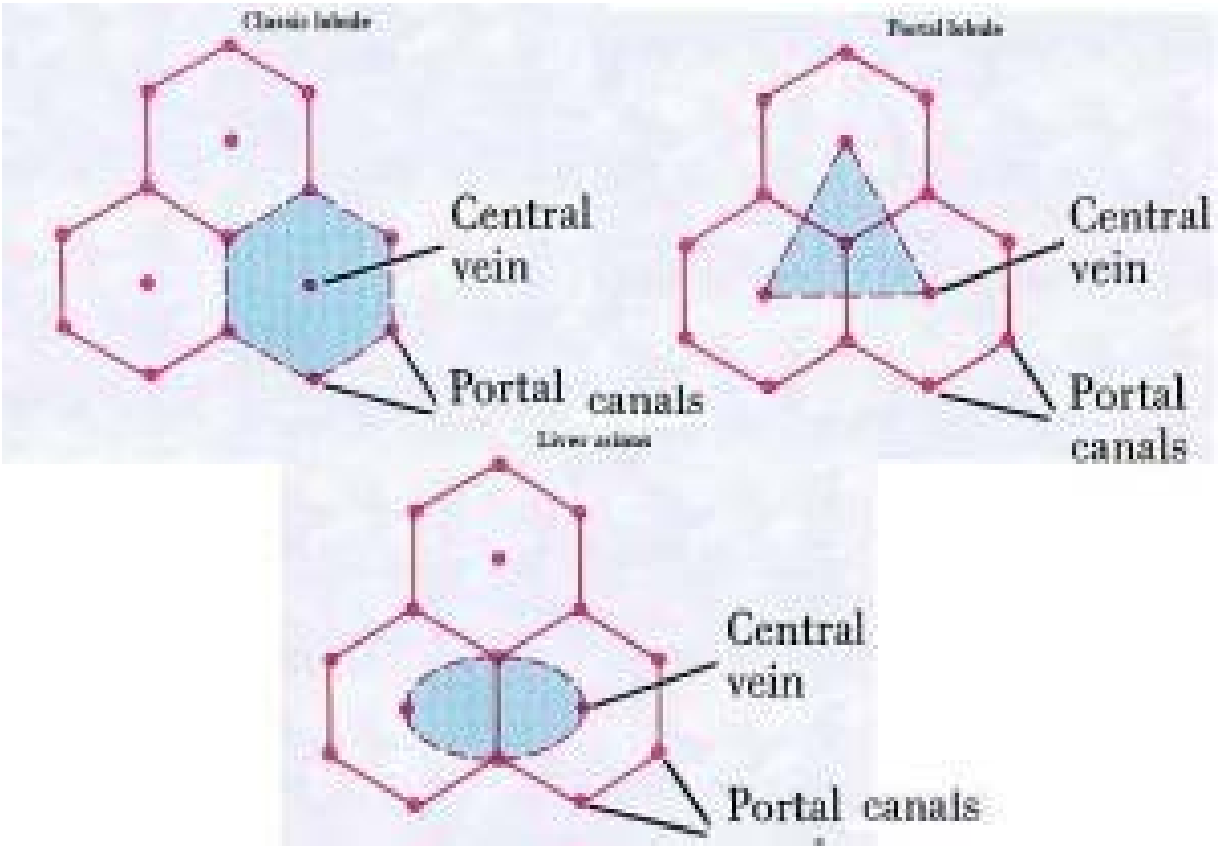
RETICULAR FIBRES:

- It is the supporting connective tissue fibers of the liver
- It lines the sinusoids, also supports the endothelial cells and it forms a denser network of the reticular fibers in wall of central vein.
- These fibers also merge with the collagen fibers in interlobular septum, thereby surrounding the portal vein and bile duct.

LIVER LOBULES CONCEPT:.

- Classical hepatic lobule
- Portal lobule
- Hepatic acinus of Rappaport

HEPATIC LOBULES:



CLASSIC LOBULE:

- Classic lobule is the Structure and functional unit of the liver.
- It is hexagonal mass of hepatocytes.
- Classic lobule has a vein at the center of the lobule, which is called as central vein and portal tracts in the periphery of the lobule.
- Central axis is occupied by the central vein.
- It is an independent venous unit.
- Spaces between the hepatic lamina are known as hepatic lacuna, which is occupied by hepatic sinusoids.

- Hepatic sinusoids are wide diameter capillaries and the walls of the hepatic sinusoids are made up of multiple pores called as fenestrations.
- The walls are made up of flattened endothelial cells. Pit cells and kupffer cells are attached to endothelial surface.
- Hepatic sinusoids receive blood from portal vein and hepatic artery from the adjacent portal area.
- The blood flows to the central vein → sub lobular vein → hepatic vein → Inferior venacava.

THE PORTAL AREA:

Peripherally, each of the lobule has 3 to 6 portal areas along with more connective tissue fibers, each of which consists of interlobular structures that comprises of portal triad. The portal triad includes:

- A venous tributary of the portal vein with blood rich in nutrients but low in oxygen.
- An arteriole which is a branch of the hepatic artery which supplies oxygenated blood to the hepatocytes.
- One to two small ductules made up of cuboidal epithelium, which are branches of bile conducting system.

BILE CANALICULI:

- It is formed by the spaces present between the plasma membrane and the adjacent hepatocytes.
- It forms a hexagonal network around the hepatocytes.
- Borders around these biliary canaliculi are sealed by tight junctions thereby forming blood bile barrier.
- The biliary canaliculi pass to the periphery of hepatic lobules where they form the intra lobular canal of Herring, which finally empties into the interlobular duct of the portal area.
- Biliary canaliculi are intra laminar and centrifugal in its direction of the flow.

DISSE SPACE OR PERISINUSOIDAL SPACE:

- It is the potential space which lies between the sinusoidal walls and the lamina of the hepatocytes.
- It is always filled with the blood plasma and also by the chylomicrons that percolate through the sinusoidal walls.
- Ito cells are present in this space.

ITO CELLS:

- These cells are irregular in outline.
- It consists of numerous lipid vesicles.

- These cells secrete collagenous matrix, synthesis growth factors for hepatocytes regeneration if it is injured.
- It is the storage cells for Vitamin A which stores the vitamin in its lipid vesicles.

SPACE OF MALL:

- It is the potential space which lies between the hepatic plates of cells and the Glisson's capsule of the portal area.
- Lymphatics of the liver arise from the Space of Mall.

THE PORTAL LOBULE:

- It is the territory of the hepatocyte which is centered around the portal triad.
- It is drawn by connecting the central veins of the three adjacent lobules.
- It is the nutritional lobule of the liver.

HEPATIC ACINUS (ACINUS OF RAPPAPORT):

- It is the diamond shaped area of the hepatic parenchyma.
- It forms the structural and metabolic functions of the liver.
- Concentric region of liver parenchyma with surrounding distributing artery in the center is called as hepatic acinus.

The acinus is divided into 3 zones based on blood supply gradient:

Zone 1:

It lies around the vascular backbone and it is well oxygenated zone.

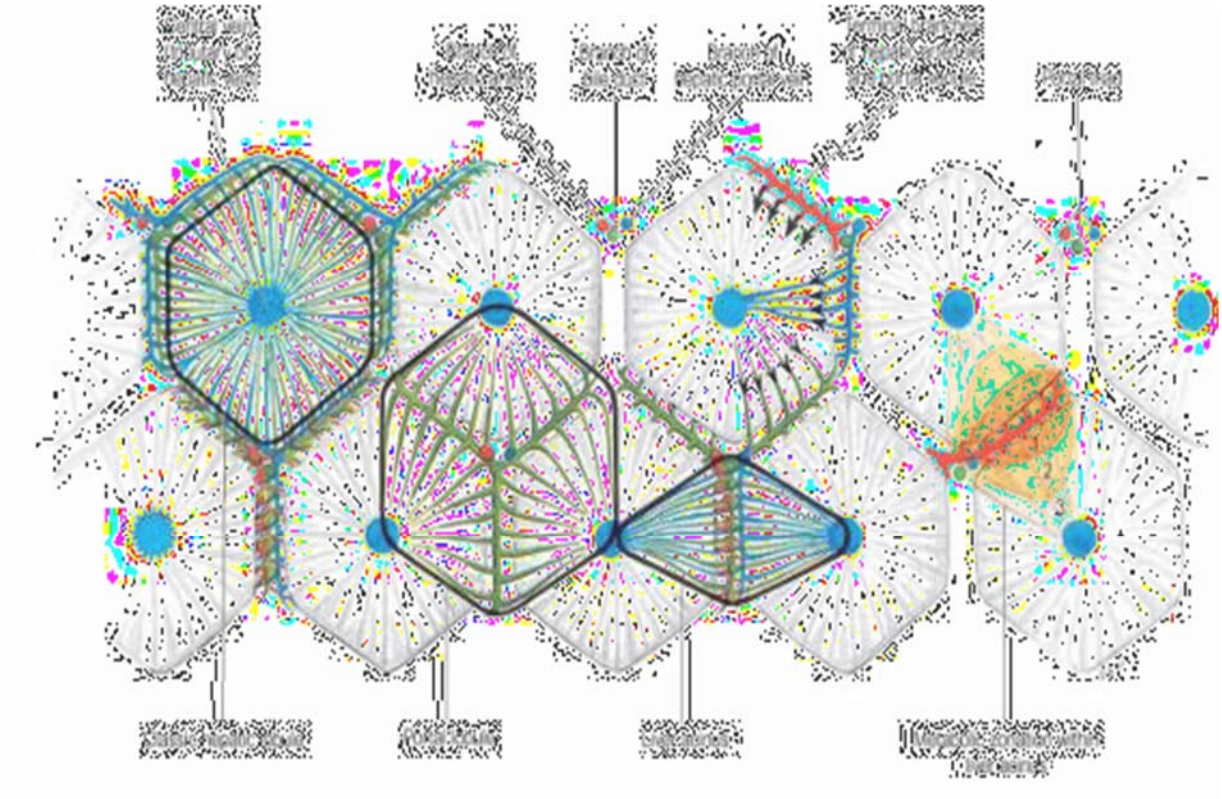
Zone 2:

It is an intermediate zone and it is moderately oxygenated.

Zone 3:

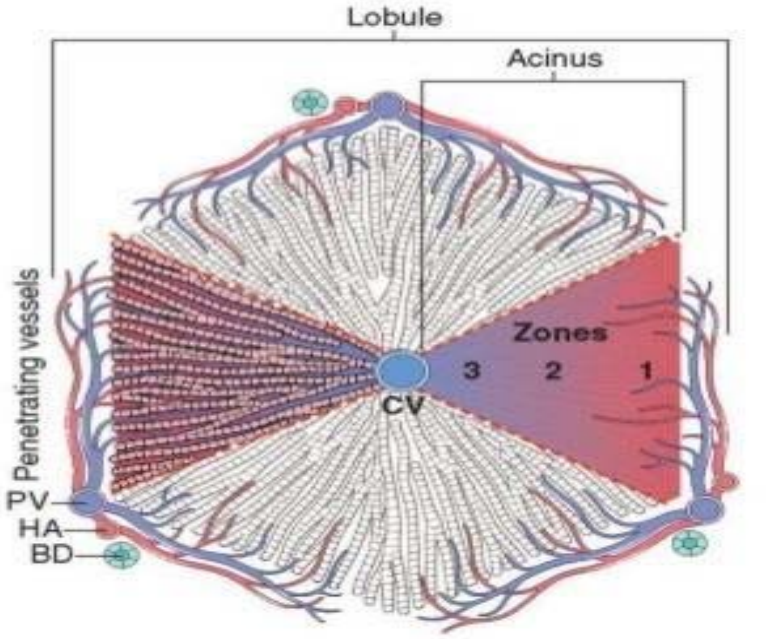
It is close to central vein and it is least oxygenated. It is most vulnerable to anoxic injury.

The below diagram depicts the histological structure of Liver:



HEPATOCYTES:

- These are large cuboidal cells or otherwise polyhedral cells
- These cells have large round central nuclei with eosinophilic cytoplasm which is rich in mitochondria.



Zone 1: close to the blood vessels
Zone 2: intermediat zone
Zone 3: adjacent to central vein

ALCOHOLIC LIVER DISEASE:

INCIDENCE:

Alcoholic liver disease is the term which comprises of liver manifestations which occurs due to alcohol over consumption. Death in alcohol associated liver disease is approximately calculated to be around 1.5lakh/year around the globe. 90-100% of the patients with alcohol over

consumption will ultimately land in hepatic steatosis within time span of 10 years. In patients with overuse of alcohol, around 10-30% will land up in steatohepatitis whereas around 8-20% develops cirrhosis in the same time span.

Among heavy alcohol consumers, 6 to 14% of people those who consumes alcohol more than 60 to 80 grams per day daily for male and exceeding 20 gram per day for females will develop cirrhosis of liver.

From the above statistics, 10% of persons who withdraws alcohol completely will have normalization of microscopic structure of the liver and biochemical parameters.

ALCOHOLIC BEVERAGES AND THEIR ALCOHOL CONTENT:

1. 10 g of alcohol will be present in 250ml of Beer
2. 10 g of alcohol will be available in 100ml of Wine
3. 10g of alcohol will be present in 30 ml of whisky
4. 10 g of alcohol will be present in 45ml of COUNTRY LIQUOR
5. One standard drink stands for 8g of alcohol in United Kingdom and 19.75g of alcohol in Japan.

PATHOGENESIS:

HYPOXIA IN CENTRILOBULAR AREAS:

Increased susceptibility of the pericentral region to hypoxemic or hypoxic injury in hepatic parenchyma occurs due to competitive oxygen

consumption due to metabolism of alcohol which occurs at the same time in the patients with chronic alcohol consumption.

INFILTRATION OF NEUTROPHILS AND ITS ACTIVATION:

- The pathologic hallmark of alcohol hepatitis is determined by the infiltration of neutrophils.
- IL-8 and metabolites of arachidonic acid acts as a neutrophil chemo attractant which signals the neutrophils to enter into the liver and produces inflammation.
- Kupffer cell which secrets inflammatory mediators also have a role in ethanol induced injury to the hepatocytes.

ANTIGENIC ADDUCT FORMATION:

Hydroxyl ethyl radicals and acetaldehyde binds covalently to proteins present in liver cells, thereby causing formation of adducts that are antigenic to the immune system.

INJURIOUS CYTOKINES AND ITS ACTION:

- Multiple studies have concluded that increase in levels of pro inflammatory cytokines such as Tumor Necrosis factor alpha and Interleukin – 6 in the circulation of the patients with alcoholic hepatitis which leads on to the inflammatory processes.

COFACTORS THAT POTENTIATE THE ALCOHOL LIVER DISEASE DEVELOPMENT

AGE:

Alcohol consumption in young age is highly associated with alcoholism in later part of life.

GENDER:

- Prevalence is higher in men when comparing to women.
- For the given amount of alcohol consumption, women are more prone for alcohol related liver injury and fibrosis than men.

HEPATITIS C INFCETION:

- 14 % to 36% of the individuals suffering from Alcoholic liver disease also have concurrent chronic infection with hepatitis C.
- This concurrent infection with hepatitis C particularly increases the risk of hepatocellular carcinoma.

HEPATITIS B INFECTION:

Data arrived from the previous studies suggests that risk for hepatocellular carcinoma is high in patients with alcohol consumption and chronic HBV co infection.

MEDICATIONS:

Multiple doses of acetaminophens also elevate the risk of hepatocellular injury, due to depletion of glutathione stores in the hepatocytes.

EXCESS IRON:

Excess iron and excess alcohol consumption produces increased amount of reactive oxygen species in the human body. This reactive oxygen species increases lipid peroxidation of membrane of the cell and further damage the lipid bilayer of the cell thereby causing cell lysis.

HEPATITIS CAUSED BY HEPATOTROPHIC VIRUSES:

HEPATITIS A VIRUS INFECTION:

- Sub-acute disease which is distributed globally.
- It affects mainly children and young adults.
- It is often a self-limited disease that almost resolves on its own and does not become chronic disease. Overt illness is present only in 5% of patients those who gets infected.
- The incubation period varies between 2 to 6 weeks.
- The clinical syndrome usually classified into two stages. Prodromal or pre-icteric stage and icteric stage.
- Onset may be acute or insidious characterized by fever, anorexia, malaise, nausea and vomiting and sometimes associated with right

hypochondrial tenderness due to liver enlargement. Subtle jaundice may be present sometimes.

- Recovery period is slow usually varies between 4 weeks to 6 weeks.
- Although rare, fulminant hepatic failure can occur in less than 1 percent of cases.
- Hepatitis A virus is a member of the genus Heparnavirus in the family of picornoviridae. It is most commonly transmitted by faeco oral route.

MORPHOLOGY:

- HAV is a 27 nanometer, non-enveloped RNA virus. This virus is resistant to inactivation by the heat at 60 degree centigrade for an hour. It is also resistant to ether and acidic pH 3 but it is inactivated by boiling for about one minute.
- It is also inactivated by 1:4000formaldehyde at 37 degree centigrade for 72 hours and also by chlorine 1ppm for about 30 minutes. This virus survives at 4 degree centigrade or below.

PATHOGENESIS:

- Natural infection with hepatitis A virus is seen only in humans.
- Once it reaches the intestine it multiplies in the intestinal epithelial cells and reaches the liver via the blood stream. It also shed in the

feces during the late incubation period and also during the prodromal phase of the illness.

- This virus is rarely detectable in the feces once jaundice develops.
- Chronic carriers are not identified yet. virus persistence in the nature is by continuing in apparent infections.
- A brief viremia usually occurs during prodromal phase but it usually ceases by the appearance of the jaundice. Chronic viremia does not occur. Therefore parenteral transmission of this virus is extremely rare.
- Hepatic injury occurs as a result of host immune response to Hepatitis A virus. Viral replication always occurs in the cytoplasm of the hepatocyte. Damage to the liver cells and destruction of the infected liver cells is mediated by human leukocyte antigen (HLA) – restricted, CD8+ T lymphocytes (Cytotoxic T Lymphocytes) which is specific to Hepatitis A virus and NK Cells which is otherwise known as natural killer cells. Interferon- gamma seems to have a central role in facilitating clearance of infected liver cells.

HEPATITIS B VIRUS INFECTION:

Hepatitis B virus is included in the family-hepadnaviridae and genus orthohepadnavirus. Hepatitis B virus is a DNA virus. This virus is double stranded. An estimation all over the globe states that there are

approximately more than 25 crores of people were carriers of Hepatitis B virus, among those people approximately 6 lakhs people dies every year due to Hepatitis B virus related liver disease.

PATHOGENESIS:

Immune mediated mechanism plays a vital role in the pathogenesis involved in HBV related liver injury. This immune mechanism leads on to direct cytotoxic injury to the liver parenchyma.

MECHANISM OF IMMUNE MEDIATED INJURY TO THE HEPATOCYTES:

Cytotoxic T-cell dependent cellular injury is the main pathogenic mechanism behind the hepatocyte injury in Hepatitis B viral infection.

In infection with chronic hepatitis B, patients who had become HBeAg negative in the serum have usually more vigorous cytotoxic T lymphocytes mediated reaction to Hepatitis B virus infected cells whereas patients who tends to have persistent HBeAg positive usually have less cytotoxic T-cell mediated response.

Massive destruction by immunological cells to the infected hepatocytes tends to be the cause for fulminant Hepatitis B.

DIRECT CYTOTOXIC LIVER INJURY:

There is usually no correlation between viral load in a particular patient and the severity of liver injury. Direct cytopathic hepatotoxicity can occur in patients those who are having very high viral load as in fibrosing cholestatic hepatitis.

HEPATITIS C VIRUS INFECTION:

13 crores to 17 crores people around the earth is infected by Hepatitis C infection in the present day. Among infected people, around 75-85% progress to CLD.

During the course of illness, approximately five to twenty percent of people develops cirrhosis. One to five percent of patients with hepatitis C virus infection may progress to develop hepatocellular carcinoma.

Hepatitis C virus is a RNA virus. It is single stranded. Hepacivirus is the genus of this virus and it is included in the family Flaviviridae.

Hepatitis C is one among the virus with very high Spontaneous mutation rate. There are 90 sub types and 11 genotypes for this virus.

PATHOGENESIS:

VIRAL REPLICATION AND CELL INJURY:

High viral replication is associated with genotype 1

More progressive liver disease is associated with genotype 1 b

IMMUNE MEDIATED MECHANISM FOR CYTOTOXIC CELL INJURY:

Cytotoxic T lymphocytes are present in the areas of portal, periportal areas and also in lobular areas in patients suffering from hepatitis C infection which are responsible for the immune mechanism mediated cytotoxic injury.

FACTORS WHICH HELPS IN CIRRHOSIS PROGRESSION IN PATIENTS WITH HCV INFECTION:

Among both genders, Males are more vulnerable.

If the patient is above 40 years of age during viral infection, likelihood of progression to cirrhosis is high.

Patients with excessive alcohol intake

If the patient had associated HIV or Hepatitis B co infection then there is more chances that the patient may progress to cirrhosis.

OBSTRUCTIVE JAUNDICE CLASSIFICATION:

BENJAMIN CLASSIFICATION(1983):

Type 1: complete obstruction of biliary tree

It has classical symptoms of obstructive jaundice with biochemical changes.

- Tumors: Malignancy of head of pancreas
- Surgical ligation of common Bile duet

- cholangiocarcinoma
- Diseases involving liver parenchyma

Type 2: Intermittent obstruction of biliary tree

Symptoms and biochemical abnormalities will be present but jaundice not always present.

- Choledocholithiasis- Biliary stones
- Periampullary carcinoma
- Duodenal diverticulosis
- Parasitic infestations of the biliary tree
- Hemobilia- Blood in the bile
- Choledochal cyst
- Bile duct papillomas

Type 3: chronic incomplete obstruction of biliary tree

Pathological changes are usually present in bile duct and in the liver with or without classical symptoms.

- Strictures involving Common bile duct
- Congenital strictures or stenosis of biliary tree
- Traumatic obstruction
- Sclerosing cholangitis
- Post radio therapy- for malignancy
- Stenosis of biliary enteric anastomosis
- Chronic pancreatitis

Type 4: segmental obstruction to biliary tree

To frame it as segmental obstruction to biliary tree there should be one or more segments of the biliary tree in the intra hepatic part should be obstructed

- Cholangiocarcinoma
- Traumatic lesions causing obstruction
- Sclerosing cholangitis
- Stones occluding the intra hepatic regions of the biliary tree.

HEPATIC FIBROSIS AND CIRRHOSIS:

Hepatic fibrosis is defined as reversible wound healing response to liver injury followed by accumulation of extra cellular matrix or 'scar', it follows chronic liver disease but not self-limited.

Hepatic stellate cells activation is an initiating step in hepatic fibrosis.

'Not only hepatic fibrosis is reversible, but it is also increasingly made clear that cirrhosis may be reversible as well. The exact point at which fibrosis / cirrhosis become irreversible and its biologic determinants are not clearly known[19]

There are around 20 various types of collagens are identified in liver. collagen types 1, 3, 5, 11 are mainly distributed in capsule, blood vessels and in portal triad.

Type 4 collagens are largely distributed in space of Disse as delicate strands.

In fibrosis, deposition of type1 and type 3 collagens results in formation of septa. Blood shunting may occur due to formation of vascular channels in these septa. Irrespective of etiology of liver disease, cirrhosis is initiated by necrosis of hepatocytes followed by deposition of collagen in space of Disse.

There will be significant obliteration in the Sinusoidal cells openings which are also called as fenestrations.

Exchange mechanism happening between plasma and hepatocytes solutes will be affected. Secretory function of liver will also be affected.

Hepatic stellate cells are the main source of growth factor in the liver, but these cells also respond to these growth factors, thereby emphasize the importance of tight regulation of autocrine growth factor activity control within pericellular level.

Cells in the perisinusoidal areas and stellate cells acquire myofibrils thereby causes increase in resistance of the blood vessels in hepatic parenchyma. Healthy hepatocytes which develops into new cells tends to form spherical nodules.

The architectural changes happening in Cirrhosis of the hepatic parenchyma is:

1. The portal tracts and the hepatic veins are bridged by formation of fibrous septa

2. Architectural disruption of liver parenchyma.

3. Regenerating nodules surrounded by the fibrosis – Fibrosis may be micro nodular (<3 mm) or macro nodular.

CAUSES OF FIBROSIS AND LIVER CIRRHOSIS:

CAUSES OF PRESINUSOIDAL FIBROSIS:

I. Idiopathic portal fibrosis

II. Parasitic infestations such as Schistosomiasis

III. Drugs

IV. Vinyl chloride

V. sarcoidosis

PARENCHYMAL CAUSES OF FIBROSIS: (SINUSOIDAL)

DRUGS AND TOXINS:

- Alcohol such as methanol and ethanol
- Anticancer drug such as Methotrexate
- Hypervitaminosis A
- Anti-tubercular agents like Isoniazid
- Antiarrhythmic agents such as Amiodarone
- Anti hypertensives such as α methyldopa

INFECTIONS

- chronic hepatitis B and chronic hepatitis C
- Brucella
- Echinococcous granulosis infestation.

AUTO IMMUNE CAUSES:

- Autoimmune hepatitis

METABOLIC AND GENETIC DISEASE:

- Bile acid disorder
- Wilson's disease
- Hemochromatosis
- Porphyria (Disorders of porphyrin metabolism)
- α-1 antitrypsin deficiency
- Disorders of carbohydrate metabolism
- Disorders of lipid metabolism
- urea cycle disorders
- Disorders of amino acid metabolism

CAUSES OF BILIARY OBSTRUCTION:

- Secondary and primary biliary cirrhosis
- Cystic fibrosis
- Atresia of the biliary tracts
- Neonatal hepatitis.
- Congenital biliary cyst.

IDIOPATHIC/ MISCELLENOUS CAUSES:

- Nonalcoholic steato hepatitis
- Indian childhood cirrhosis
- Granulomatous liver disease such as sarcoidosis
- Polycystic liver disease

POST SINUSOIDAL FIBROSIS CAUSES:

- Sinusoidal obstruction syndromes (veno occulusive diseases)

CLINICAL FEATURES OF A PATIENT WITH CIRRHOSIS:

- Anorexia, easy fatiguability and wasting
- Jaundice and anemia
- Tachycardia and hyperdynamic circulation
- Spider naevi or spider angioma
- Palmar erythema and Dupuytren's contracture
- White nails (Leuconychia)
- Hypogonadism and reduced libido
- Gynaecomastia (Breast enlargement in male)
- Enlargement of parotids (alcoholic)
- Fluid retention
- Bleeding diasthesis
- Flapping tremors
- Splenic enlargement

COMPLICATIONS OF CIRRHOSIS:

1. PORTAL HYPERTENSION

- Gastropathy due to portal hypertension
- Gastric and esophageal varices
- Hemorrhoids
- Caput medusae
- Hypersplenism and cytopenia
- Ascites
- Spontaneous bacterial peritonitis (SBP)
- Hepatorenal syndrome type1 and type 2
- Hepatic encephalopathy
- Porto pulmonary hypertension
- Hepato pulmonary syndrome

COAGULOPATHY :

- Vitamin K dependent clotting factor deficiency
- Cytopenia such as thrombocytopenia

BONE DISEASE :

- Osteopenia, Osteoporosis and osteomalacia

HEMATOLOGIC ABNORMALITIES :

- Hemolysis (Zieve syndrome)
- Anemia (Multifactorial)
- Thrombocytopenia (Due to hypersplenism)
- Leucopenia (Due to hypersplenism).

LIVER FUNCTION TESTS:

1. Serum albumin :

Albumin is the most abundant plasma protein with serum concentration of 3.5 – 5.5 g/dl.

It is encoded by ALB Gene.

Molecular weight-65000 Daltons.

When albumin is ionized in water at pH 7.4, as found in body it is negatively charged.

Synthesized in the liver as preproalbumin which in turn cleaved by golgi apparatus and released as albumin

12g albumin is synthesized per day by the hepatic parenchyma which accounts for about 25 percent of gross protein synthesis by the hepatocytes.

ALBUMIN FUNCTIONS:

1. Important modulator of colloidal osmotic pressure
2. Carrier protein for bilirubin, fatty acids and ions.
3. Nutrition
4. Buffering action

Hypoalbuminemia occurs when there is injury to liver cells thereby decreasing the ability to produce albumin. However other cause of hypoalbuminemia includes:

- Protein losing enteropathy
- Protein energy malnutrition
- Renal diseases such as Nephrotic syndrome
- Chronic illness

Half-life of albumin is 21 days. Therefore albumin could not be taken as a marker of liver dysfunction in patients with acute liver diseases.

It is used as a marker in assessing the prognosis in patients with chronic liver disease. Pre albumin, which is also produced by the hepatocytes, has shorter half-life than albumin.

PROTHROMBIN TIME:

It is a measure of time taken for the development of thrombin from the pro-thrombin. In other words, Pro-thrombin Time (PT) measures the time (In seconds) for the plasma to clot after addition of thromboplastin which is a mixture of tissue factor, phospholipid and calcium to the patient's plasma sample.

Many preparations of thromboplastin reagents are available in the market. Hence each thromboplastin preparation will give different results. Hence WHO (World Health Organization) came up with INR(International Normalized Ratio) standard reporting format for Pro-thrombin Test results.

INR has been calculated by ratio of patient's PT by control PT value which is obtained by using international reference thromboplastin reagent which was developed by WHO.

PT is dependent upon factor II, factor VII, factor IX and factor X as well as extrinsic pathway factors and common pathway factors. Thus, pro-thrombin time is a measure of synthetic function of the liver.

Normal value: 10.9 – 12.5seconds Pro-thrombin time can also be increased by

i. Use of antagonists to vitamin K
ii. vitamin K deficiency states
iii. Disseminated intravascular coagulation
iv. Deficiency of clotting factor
v. Anti-phospholipid antibodies
vi. Factor X deficiency associated with systemic amyloidosis
vii. Massive hemorrhage
viii. Specific inhibitors to common pathway factors (Rare cause)
ix. Collection and processing error.

INR (international normalized ratio) has also been used to standardize anticoagulation therapy monitoring and is also used in CHILD PUGH scoring system as well as in MELD scoring system

TESTS FOR LIVER CELL DAMAGE AND EXCRETORY FUNCTION OF HEPATOCYTES:

I. AST (SGOT) – Aspartate aminotransferase(Glutamic-Oxaloacetic transaminase)

II. ALT (SGPT) – Alanine aminotransferase(Glutamic-pyruvic transaminase)

ALT is cytosolic enzyme found in the hepatocyte cytoplasm.

But, AST is present in mitochondrial and cytosolic isoenzymes and is also found in organs like skeletal muscle, kidney, brain, cardiac musculature, RBC's and pancreas.

Both AST and ALT may be higher in normal males and females. They are also higher in patients in high BMI (Body Mass Index).

NORMAL VALUES OF LIVER ENZYMES:

ALT – 10-55U/L

AST- 10-40 U/L

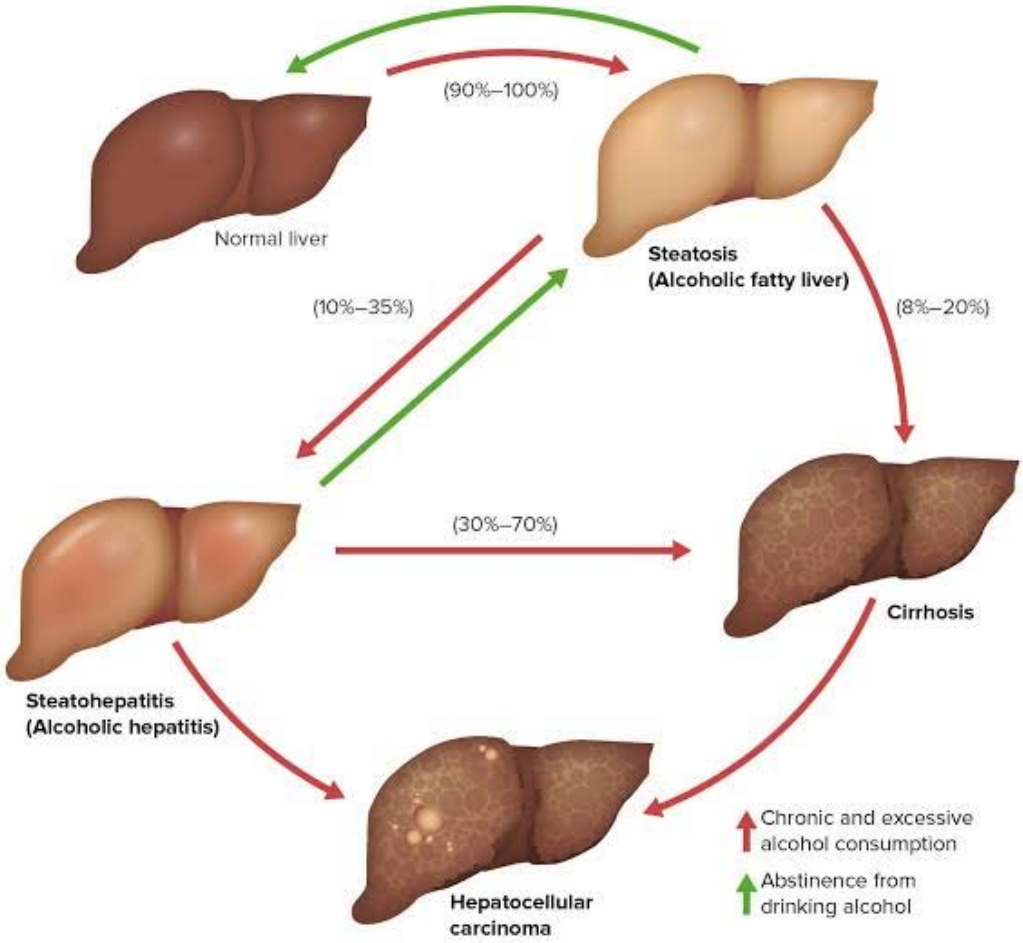
In patients with raised AST levels which is disproportionate to ALT levels, origin of this enzyme other than liver must be kept in mind before arrive at the diagnosis. In severe rhabdomyolysis, both enzymes are elevated.

In early phases of liver injury, enzymes do elevate, but it correlates poorly with the severity of hepatocellular injury.

Hence, it becomes poor predictor of the outcome in early phases of liver injury. Uremia may leads to a falsely low serum AST levels.

AST/ALT ratio CORRELATION:

1. In Alcoholic hepatitis - ≥ 2
2. In patients with Cirrhosis - > 1
3. Patients with Nonalcoholic steatohepatitis - ≤ 1
4. In Wilson's disease- ratio may even be greater than 4 in severe cases.



MARKERS OF CHOLESTASIS:

ALKALINE PHOSPHATASE (ALP):

It is a membrane bound Glycoprotein that helps in catalyzes the hydrolase enzyme which is responsible for removing phosphate groups, from numerous types of molecules such as nucleotides, alkaloids and proteins.

It is present in several tissues, mainly concentrated in the liver, bile duct, kidney, bones, placenta, and intestinal mucosa.

Four Isoenzymes of ALP which is classified depends upon the site of expression.

- Intestinal ALP
- Placental ALP
- Tissue nonspecific ALP or liver/bone/kidney ALP or (L/B/K) ALP
- Germ cell ALP

Half-life of ALP is 5 to 7 days and Normal value ranges from 35 to 130U/L.

CONDITIONS WITH RAISED ALP LEVELS:

1. Bile pathway obstruction such as primary biliary cirrhosis, primary sclerosing cholangitis, stones, benign strictures and tumors.

2. Liver metastasis
3. Osteoblast bone tumor
4. Liver injury such as Alcoholic hepatitis and viral hepatitis
5. Osteomalacia
6. Leukemia and Lymphoma
7. Celiac disease
8. Paget's disease of bone
9. Infiltrative diseases like Amyloid and Sarcoid
10. Seminomas
11. Hyperthyroidism and Hyperparathyroidism
12. Acute coronary syndrome
13. Chronic renal failure
14. Pregnancy

CONDITIONS WITH REDUCED ALP LEVELS:

1. Hypophosphatasia
2. Postmenopausal women receiving estrogen therapy
3. Malnutrition
4. Magnesium deficiency
5. Severe anemia
6. Men with recent heart surgery

Wilson's disease may be associated with very low levels of alkaline phosphatase.

"BY STANDER PHENOMENON": ALP Values may be increased due to nonspecific hepatitis in Hodgkin disease and malignancies of kidney, but there will be no direct liver involvement.

"REGAN ISOENZYME": ALP levels are raised in patients as a result of malignancy but without bone/liver involvement

GAMMA GLUTAMYL TRANSPEPTIDASE (GGTP):

It is an established serum marker for alcoholic liver disease.

Elevated GGT has been linked to be an increased risk for multiple diseases such as diabetes, cardiovascular disease and metabolic syndrome.

It is a microsomal enzyme, which is formed in hepatocytes, epithelial lining of biliary system, kidneys, heart, lung, pancreas, brain, and spleen. Normal value: 0-30 U/L

ALP Values are raised in cholestasis. GGT/ALP > 2.5 is suggestive of alcoholic liver disease.

5' NUCLEOTIDASE:

Its activity is first demonstrated 60 years ago in heart and skeletal muscles. This enzyme catalyzes the hydrolysis of phosphate esterified at carbon 5' of the ribose and deoxyribose portions of nucleotide molecules.

It is synthesized in hepatocytes, pancreas, brain, blood vessels and myocardium. It is found predominantly in the biliary canalicular and also in the sinusoidal plasma membrane in hepatobiliary system.

Normal value of 5'Nucleotidase: 0-11U/L.

Even though, 5'Nuclotidase is elevated in cholestasis it lags behind elevations of ALP and GGT.

Normal value of 5'Nuclotidase is < 1.1 g/dl

SERUM BILIRUBIN:

Bilirubin is divided into direct bilirubin and indirect bilirubin

DIRECT BILIRUBIN:

1.It is also known as conjugated bilirubin

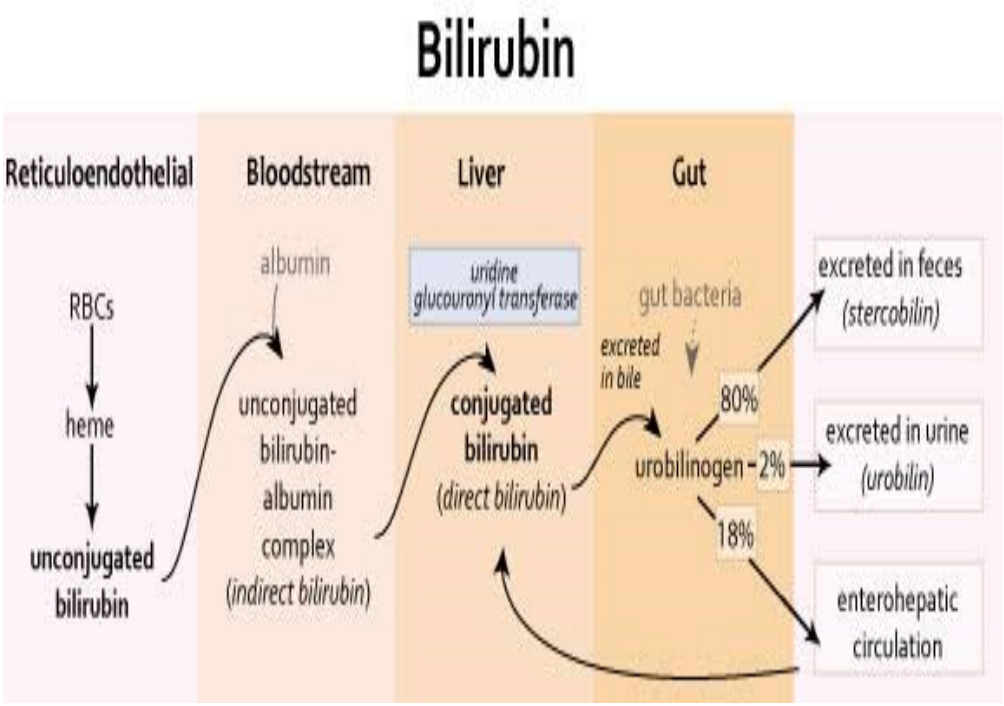
2.It is water soluble in nature.

3.It makes up less than 10% of total bilirubin

4.It is excreted in urine as it is conjugated form of bilirubin.

INDIRECT BILIRUBIN:

1. Also known as Unconjugated bilirubin.
2. It is lipid soluble and water insoluble.
3. It makes up more than 90% of total bilirubin in the serum.
4. It is excreted by the biliary system.



CONDITIONS CAUSING OF INCREASED INDIRECT BILIRUBIN:

1. Lysis of Red blood cells
2. Ineffective erythropoiesis as in case of vitamin B12 deficiency
3. Resolution of hematoma
4. Rhabdomyolysis

CONDITIONS CAUSING OF INCREASED DIRECT BILIRUBIN:

1. Hepatitis due to any cause
2. Obstructive jaundice
3. Liver cirrhosis
4. Liver metastasis

Conjugated hyperbilirubinemia is not useful for differentiating hepatocellular liver injury from obstruction to biliary system.

Since, serum bilirubin levels indicates prognosis in case of liver cirrhosis, acute liver failure, alcoholic hepatitis and in primary biliary cirrhosis, it is incorporated in the CHILD PUGH and MELD scoring system.

ACETYLCHOLINESTERASE (AchE):

It is a cholinergic enzyme predominantly found in postsynaptic neuromuscular junctions mainly in muscles and nerves. Acetylcholinesterases are a family of enzymes which are known to catalyze the hydrolysis of acetylcholine into acetic acid and choline, thereby inhibits the neurotransmitter ability to signal conduction – Thereby causing skeletal muscles incapable of relaxation[20].

AchE(Acetylcholinesterase) is structurally determined to have narrower substrate than that of BchE (Butyrl cholinesterase), as they binds specifically to Ach.

The presence of big aromatic residue from the volume of the AchE aromatic gorge produces a narrow pathway, which allows more selectivity of the enzyme at its active site[21].

This enzyme might require high positively charged substrate or an inhibitor than BchE.

SITES:

Red blood cells, Central nervous system, Peripheral nervous system and in muscles

MAJOR PROPERTIES:

- High turnover rate
- It has higher affinity for acetylcholine
- Lower affinity for non-cholinesters

SERUM CHOLINESTERASE (PSEUDO OR BUTYRL CHOLINESTERASE):

BChE (Butyrl cholinesterase) more commonly distributed in liver and serum of the humans[22].

BChE is found to improve the hydrolysis rate of the cocaine. It protects mice from the cocaine's toxic effects and also protects the human body from organophosphorous compounds(OPC), highly suggestive of detoxification role in the human body[23, 24, 25].

BChE has a wide variety of active site than AchE, it also has catalytic gorge with lesser aromatic residues lining and therefore it is more voluminous and also easily accomodative for various substrates, particularly for butyrlcholine[26].

Half-life of serum cholinesterase is 12 days.

ROLE OF SERUM CHOLINESTERSE IN VARIOUS PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS:

Many investigators[18, 27, 28, 29] have studied and found that serum cholinesterase in healthy people in detail and have stated that there is a wide range of variation in enzyme concentration from one person to another person.

The difference in enzyme activity does not correlate with age, gender, weight, height, BMI or body surface area.

Level of cholinesterase is usually constant for each healthy, well-nourished individual, from time to time.

LOW SERUM CHOLINESTERASE LEVELS:

1. LIVER DISEASES AND BILIARY SYSTEM DISEASES:

When liver parenchyma is injured, the serum cholinesterase level decreases, almost invariably[27].

Reduction in the level of serum cholinesterase is more marked in patients with CLD than in patients with acute liver injury such as acute viral hepatitis, ascending cholangitis etc.

Multiple serial studies of serum cholineterase in both acute and in chronic disease have proved that, changes in its levels in serum closely reflect changes in hepatocellular function.

When compared with the other liver function tests such as serum albumin, serum total bilirubin, serum direct bilirubin, serum indirect bilirubin, SGPT, SGOT, total proteins) none had appeared to mirror the altering status of the liver parenchymal function as sensitively as did by the serum cholinesterase.

Serum cholinesterase activity was found to be normal in patients suffering with obstructive jaundice, unless it is complicated by considerable liver parenchymal involvement.

1. **EFFECT OF MALNUTRITION AND OTHER CHRONIC DEBILITATING DISEASES:**

Reduction in the level of serum cholinesterase had been found in blood of patients who were malnourished, due to anorexia, starvation or chronic inflammatory disease.

MILHORAT[30] has been observed that when patients suffering from debilitating diseases are treated and then rehabilitated, there is a rise in their serum cholinesterase activity was noted.

2. **ANEMIA**

Many authors have commented upon the reduced level of serum cholinesterase in patients suffering from various types of anemia such as pernicious anemia, anemia due to malabsorption syndromes, Thalassemia, anemia due to blood loss and anemia of chronic diseases.

3. Organophosphorous compound poisoning and the drugs acting through enzyme inhibition also cause reduction in serum cholinesterase levels.
4. Various drugs such as caffeine, theophylline, morphine, phenothiazine derivatives, codeine, procaine hydrochloride, etc., are also well known to reduce the cholinesterase activities in serum[31, 6, 32, 33, 34] The exact mechanism is unknown.
5. Many European studies had shown that there is a prevalence of 3- 4% of congenital serum cholinesterase deficiency in general population[35].
6. Serum cholinesterase is decreased in some community people in southern part of India[44]. Possible explanation is genetic basis.

Disease states in which serum cholinesterase levels is normal to high:

- Myasthenia gravis[36]
- Bronchial asthma[37, 38]
- Epilepsy[28, 30]
- Hyperthyroidism[38, 29, 30]
- Diabetes mellitus[28, 30]

CONDITIONS IN WHICH SERUM CHOLINESTERASE ACTIVITY IN SERUM IS HIGH:

1. **Nephrotic syndrome[38, 18]:**

Increased levels of serum cholinesterase in patients with nephrotic syndrome had been found by several studies. One of the theories is that the

increased level of serum cholinesterase in nephrotic syndrome reflects on increased production of hepatocytes to synthesis new proteins.

Since the cholinesterase molecule is over double the size of the albumin molecule, it cannot pass through the glomerular basement membrane and not excreted in the urine.

2. Vigorous exercise:

Croft and Richter[6] analyzed and reported, that the vigorous muscular exercise to the body for a short duration causes a transient elevation in serum cholinesterase levels, which returns to normal after a period of rest.

MATERIALS AND METHODS:

SOURCE OF DATA:

50 chronic liver disease patients - both out patients and in patients - in Government Rajaji Hospital Madurai during the study period of 6 months from March 2021 to August 2021.

SAMPLE SIZE - 50

DESIGN OF THE STUDY:

Hospital based cross sectional observational study

STUDY DURATION: 6 months (Mar 2021 – Aug 2021)

INCLUSION CRITERIA:

- Patients with chronic liver disease proven by both imaging and clinical diagnosis of liver dysfunction in whom atleast four out of five liver function test comprising serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase(ALP), Total Bilirubin and Serum Albumin were abnormal who attended Medicine and Medical Gastroenterology outpatient clinic and inpatients in Medical ward.

EXCLUSION CRITERIA:

- Patients with age <30 or > 60 years
- Acute abdominal diseases
- Anemia
- Protein energy malnutrition
- Chronic infection
- Chronic illness such as Thyroid disorders, Diabetes, Bronchial asthma, Myasthenia Gravis.
- Seizure disorder
- Postoperative subjects
- Organophosphorous poisoning
- Pregnancy
- Patients on oral contraceptive pills, MAO inhibitors, cyclophosphamide, caffeine, theophylline, morphine, phenothiazine derivatives, codeine and procaine hydrochloride
- Malignancy
- Extensive burns
- Chronic debilitating illness

DATA COLLECTION AND METHODS:

After selection of cases, thorough history taking and clinical examination was performed and following Investigations were done using the method mentioned below:

1. Serum cholinesterase was performed using DGKC method
2. Total and direct bilirubin was estimated using DMSO method
3. NADH, Kinetic UV method, IFCC was used for estimation of Alanine aminotransferase
4. Aspartate aminotransferase was measured using NADH, kinetic UV method, IFCC
5. Total protein estimation done by means of Biuret method
6. Alkaline phosphatase by p- Nitrophenylphosphate, kinetic method DGKC
7. Serum albumin was measured using Bromocresol Green method
8. Serum Globulin
9. Prothrombin time and INR
10. Ultrasonography of abdomen
11. Upper GI endoscopy
12. Complete blood count using automated hematology analyzer
13. Erythrocyte sedimentation rate by Westergren method
14. C-Reactive protein by latex enhanced nephelometry.

METHOD OF SERUM CHOLINESTERASE ANALYSIS:

METHOD**: Kinetic test**

PRINCIPLE:

Cholinesterase is an enzyme which hydrolyses butyrylthiocholine upon release of butyric acid and thiocholine. Thiocholine, which reduces yellow color potassium hexacyanoferrate(III) to a colorless potassium hexacyanoferrate(II). The decrease of absorbance is particularly measured at 405nm.

REACTION:

$$\text{Butyrlthiocholine} + H_2O \xrightarrow[\text{Cholinesterase}]{} \text{Thiocholine} + \text{butyrate}$$

$$2\ \text{Thiocholine} + 2[\ \text{fe}\ (\ CN)6]^{3-} + H_2O \longrightarrow \text{choline} + 2[\text{fe}\ (CN)6]4- + H_2O$$

REAGENTS:

Components and concentration:

R1: Pyrophosphate pH 7.6	95mmol/L
Potassium hexacyanoferrate(III)	2.5mmol/L
R2: Butrylcholine	75mmol/L

Fresh and non-hemolysed serum was used for cholinesterase assay

Sample of about 20 microlitres was used with 1ml of the reagent and the absorbance was first read at 15 seconds and then again read at 45 seconds and results were calculated by using the formula

Activity in U/L = Absorbance/ 30seconds * factor Factor =

[TV* 1000*2] ÷ [14.64 * SV*P]

TV-Total reaction volume in ml

SV-sample volume in ml

Recommendation of the German society of clinical chemistry (DGKC)

14.64 = millimoles absorption

Coefficient of 5thio-2nitrobenzoic acid at 405nm.

P- Cuvette pathlength in cm

2 = At 37 degree centigrade, conversion from the absorbance per second to absorbance per minute.

Men – [4620-11500 U/L]

Women- [3930-10800 U/L]

STATISTICAL METHODS USED:

The collected data was analyzed by SPSS software. Pearson correlation coefficient and the P value were calculated to find the correlation and the statistical significance of the study respectively.

P< 0.05- Significant.

P> 0.05 – not significant

P < 0.0001 – highly significant

OBSERVATION

AND

RESULTS

OBSERVATION AND RESULTS

Table 1: AGE DISTRIBUTION

Age	Frequency	Male	Female
20-30	1	1	0
31-40	21	19	2
41-50	20	16	4
51-60	8	6	2
Total	**50**	**42**	**8**

- From the above age distribution table, it is evident that in our study population chronic liver diseases are mostly occurring in patients in the age group of 31 to 40 and 41 to 50 with slight increase in incidence in the age group of 31 to 40 which the productive age group of the country rather than 41 to 50.
- In our study population chronic liver diseases are less common in patients in less than 30 years of age and also more than 50 years of age.
- Among the gender distribution, females are more commonly affected than their male counterpart.
- Slight rise in incidence in females in their age group of 41 to 50 years whereas 31 to 40 years and 51 to 60 years having equal incidence of chronic liver disease.

Table 2: SEX DISTRIBUTION OF THE STUDY POPULATION

Sex	Frequency	Percentage
MALE	42	84%
FEMALE	8	16%
Total	50	100%

Among 50 patients participated in our study, 84% of male patients were suffering from chronic liver disease and 16% of female patients were suffering from chronic liver disease.

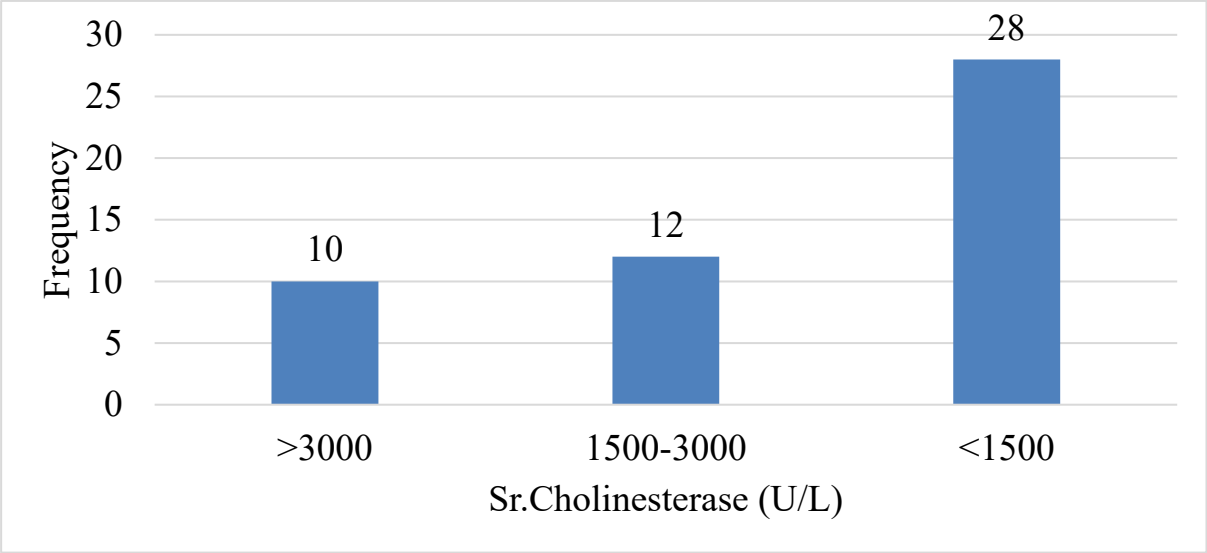
Table 3: ETIOLOGY OF CHRONIC LIVER DISEASE:

Etiology	Frequency	Percentage
Alcohol	35	70
HBS Ag positive	9	18
HCV positive	6	12
Total	100	100

In our study population, 70% of patients developed chronic liver disease due to alcohol intake and 18% of patients developed chronic liver disease due to Hepatitis B virus infection and 12% of patients developed chronic liver disease due to Hepatitis C virus infection. This frequency table shows that in our study population, the most common etiology for chronic liver disease is consumption of alcohol.

Table 3: FREQUENCY DISTRIBUTION OF SERUM CHOLINESTERASE (U/L)

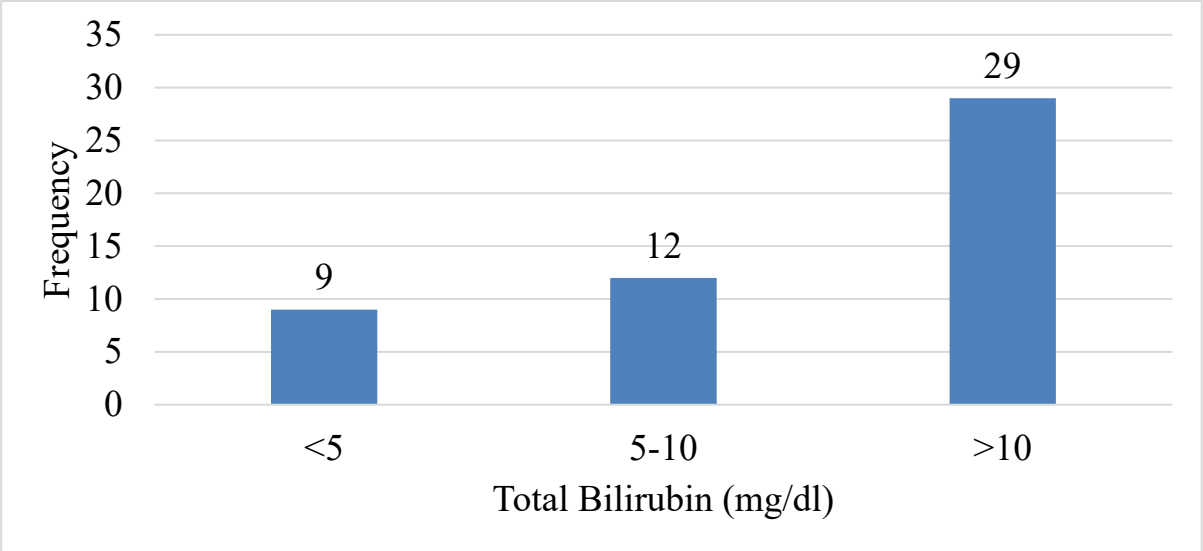
	Frequency	Percentage (%)
>3000	10	20.0
1500-3000	12	24.0
<1500	28	56.0
Total	50	100.0



The above frequency distribution table and bar chart reveals that in our study, 10 patients (20% of the study population) had serum cholinesterase levels more than 3000 units per liter and 12 patients (24% of study population) had serum cholinesterase levels between 1500 units per liter to 3000 units per liter and 28 patients (56%) had serum cholinesterase levels less than 1500 units per liter.

Table 4: FREQUENCY DISTRIBUTION OF SERUM TOTAL BILIRUBIN (mg/dl)

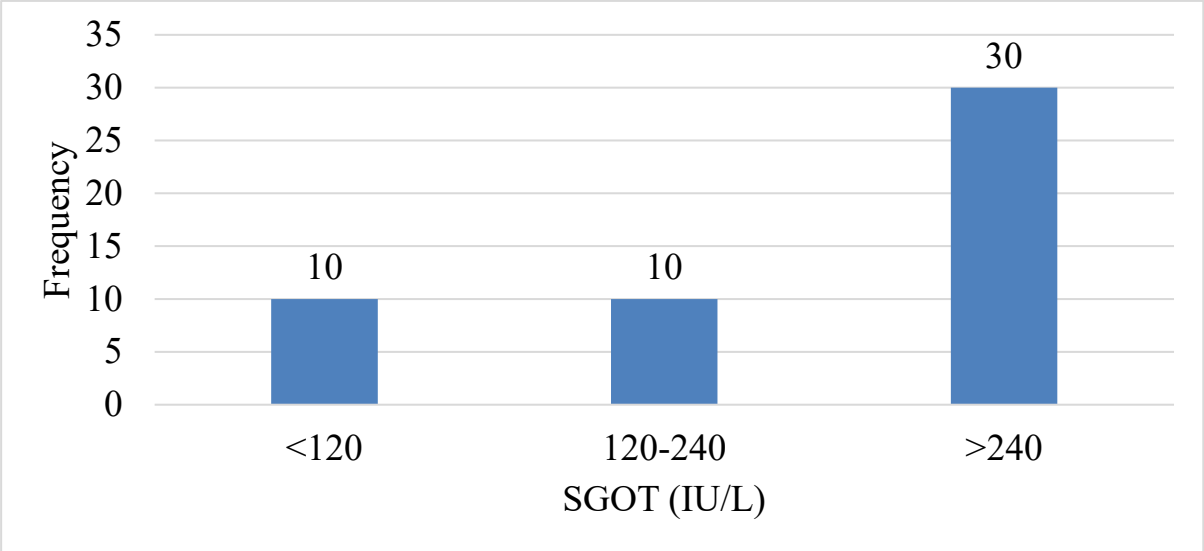
	Frequency	Percentage (%)
<5	9	18.0
5-10	12	24.0
>10	29	58.0
Total	50	100.0



The frequency distribution table and bar chart above depicts that about 9 patients (18%) in the study population had serum total bilirubin value less than 5mg/dl and 12 patient in our study population (24%) had serum total bilirubin values between 5 to 10mg/dl and 29 patients (58%) in our study population had serum total bilirubin values above 10mg/dl.

Table 5: FREQUENCY DISTRIBUTION OF SGOT LEVELS (IU/L)

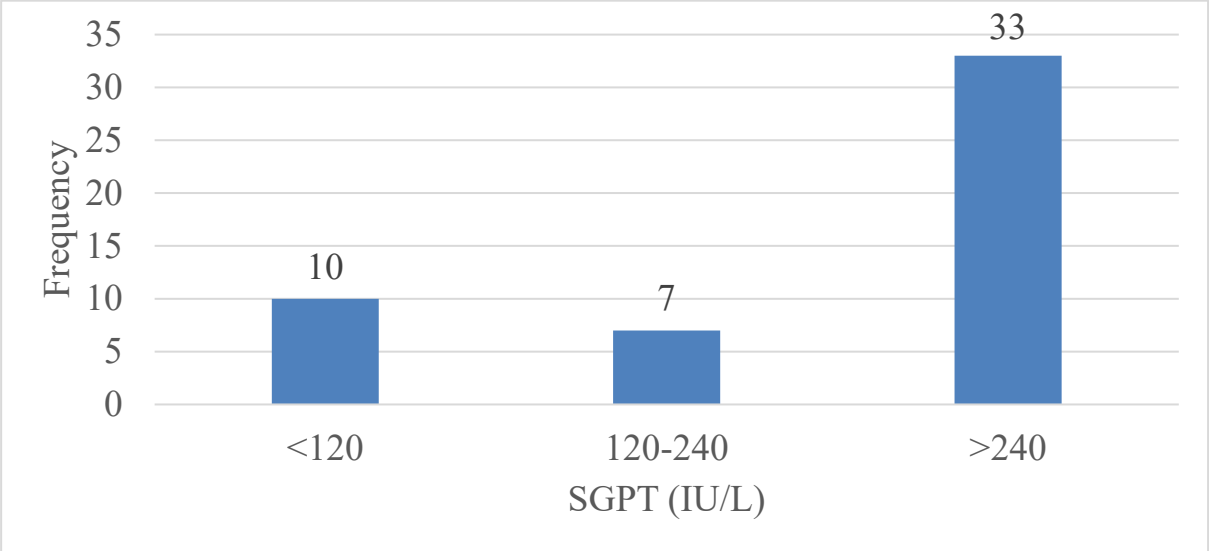
	Frequency	Percentage (%)
<120	10	20.0
120-240	10	20.0
>240	30	60.0
Total	50	100.0



The above bar chart and the frequency distribution table shows that, the patients with SGOT levels below 120IU/L was 10 in number (20%) and the patients with SGOT levels between 120 to 240U/L were 10 (20% of total study population) and the patients with SGOT levels more than 240 was 30 in number (60%).

Table 6: FREQUENCY DISTRIBUTION OF SGPT (IU/L) LEVELS

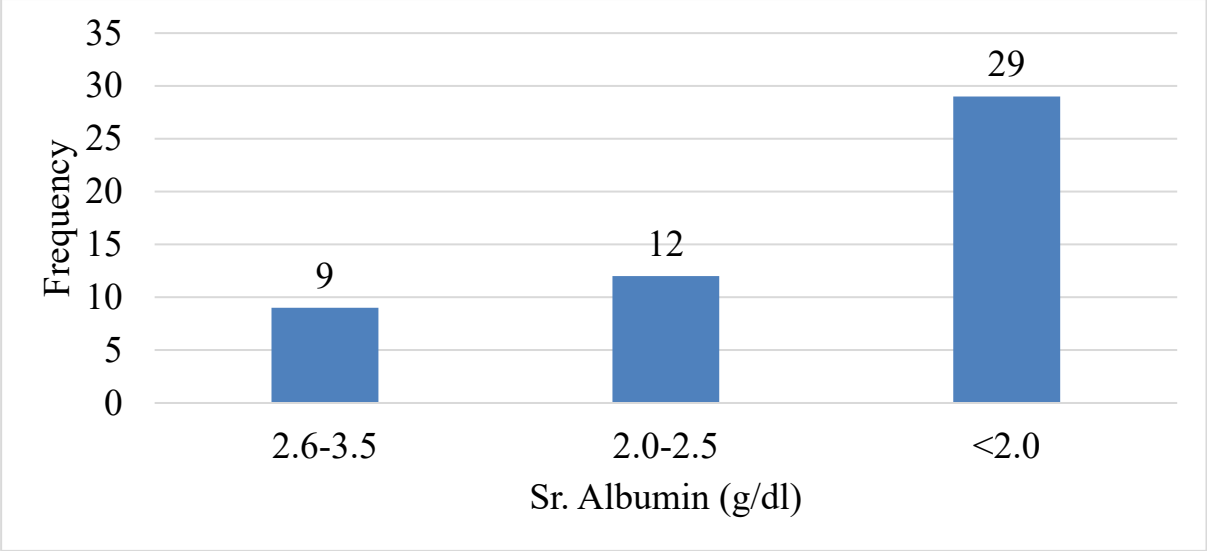
	Frequency	Percentage (%)
<120	10	20.0
120-240	7	14.0
>240	33	66.0
Total	50	100.0



The frequency distribution table and bar chart depicting SGPT (IU/L) levels in our study population were shown above. This frequency distribution shows that in our study population patients with SGPT levels less than 120 IU/L were 20% (10 patients) and there were 7 patients with SGPT levels between 120IU/L to 240IU/L which accounts for about 14% of the total study population and there were 33 patients with SGPT levels more than 240IU/L which accounts for about 66% of the total study population.

Table 7: FREQUENCY DISTRIBUTION OF SERUM ALBUMIN LEVELS (g/dl)

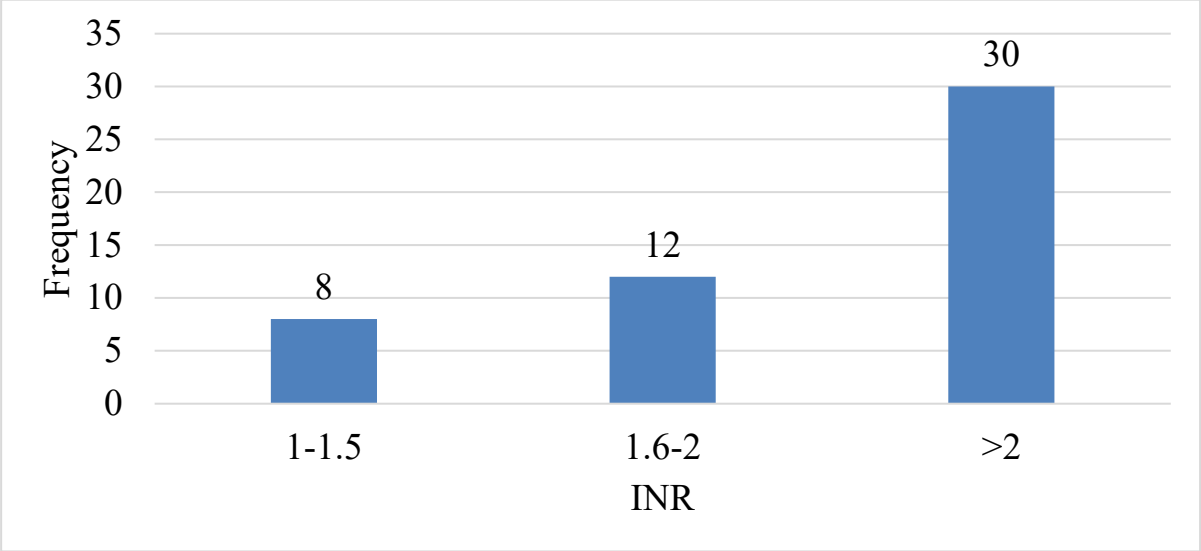
	Frequency	Percentage (%)
2.6-3.5	9	18.0
2.0-2.5	12	24.0
<2.0	29	58.0
Total	50	100.0



The above shown bar diagram and frequency distribution table depicts the frequency distribution of serum albumin values in grams per deciliter in our study population. 9 patients in our study population have serum albumin levels between 2.6 g/dl to 3.5 g/dl which is about 18% of our study population and 12 patients have serum albumin level ranges between 2 to 2.5 g/dl which is 24% of the study population and 29 patients have albumin levels less than 2 g/dl which accounts for 58% of the study population.

Table 8: FREQUENCY DISTRIBUTION OF INR (INTERNATIONAL NORMALIZED RATIO) LEVELS

	Frequency	Percentage (%)
1-1.5	8	16.0
1.6-2	12	24.0
>2	30	60.0
Total	50	100.0



The above frequency distribution of INR values in the form of table and bar diagram shows that patients with INR values ranges from 1 to 1.5 were 8 in numbers and they accounts for 16% of the total study population. Patients with INR ranges between1.6 to 2 were 12 in numbers and they contribute to 24% of the total study population. Patients with INR more than 2 were 30 in number and they accounts for 60% of the total study population.

Table 9: CORRELATION BETWEEN SERUM CHOLINESTERASE AND SGOT (IU/L)

		SGOT (IU/L)			Total
		<120	**120-240**	**>240**	
Sr.Cholinesterase (U/L)	>3000	10	0	0	10
	1500-3000	0	10	2	12
	<1500	0	0	28	28
Total		**10**	**10**	**30**	**50**

Chi-square for Sr. Cholinesterase and SGOT is 88.889 with df=4 at p value <0.0001.

The above correlation table depicts the correlation between serum cholinesterase and SGOT in IU/L. whenever there is a rise in SGOT levels there is a proportionate fall in the serum cholinesterase levels with the p value <0.0001 which shows statistically significant negative correlation between serum cholinesterase and SGOT.

This correlation proves that serum cholinesterase can be taken as an excellent routine cost effective biomarker for chronic liver diseases.

Table 10: CORRELATION BETWEEN SERUM CHOLINESTERASE AND SGPT (IU/L)

		SGPT (IU/L)			Total
		<120	**120-240**	**>240**	
Sr.Cholinesterase (U/L)	>3000	10	0	0	10
	1500-3000	0	7	5	12
	<1500	0	0	28	28
Total		**10**	**7**	**33**	**50**

Chi-square for Sr. Cholinesterase and SGPT is 74.747 with df=4 at p value <0.0001.

The above Chi-square table compares the serum cholinesterase levels with SGPT (IU/L). This tablet depicts that statistically significant negative correlation between serum cholinesterase and SGPT levels in IU/L in patients with chronic liver diseases.

Thereby the above Chi- square proves that serum cholinesterase can be considered as an highly reliable serological marker for the patients with chronic liver diseases to arrive at a diagnosis and also to assess the prognosis of those patients.

Table 11: CORRELATION BETWEEN SERUM CHOLINESTERASE AND SERUM ALBUMIN (g/dl)

		Sr. Albumin (g/dl)			Total
		2.6-3.5	**2.0-2.5**	**<2.0**	
Sr.Cholinesterase (U/L)	>3000	9	1	0	10
	1500-3000	0	11	1	12
	<1500	0	0	28	28
Total		**9**	**12**	**29**	**50**

Chi-square for Sr. Cholinesterase and Albumin is 85.850 with df=4 at p value <0.0001.

Chi-square table above compares serum cholinesterase levels (U/L) with serum albumin in grams per deciliter. This table shows statistically significant positive correlation between serum cholinesterase levels and serum albumin levels in patients with chronic liver diseases

The above table proves that serum cholinesterase can be considered as an alternative biomarker to assess the diagnosis, severity and prognosis of the patients with chronic liver diseases.

Table 12: CORRELATION BETWEEN SERUM CHOLINESTERASE AND INR (INTERNATIONAL NORMALIZED RATIO)

		INR			Total
		1-1.5	**1.6-2**	**>2**	
Sr.Cholinesterase (U/L)	>3000	8	2	0	10
	1500-3000	0	10	2	12
	<1500	0	0	28	28
Total		**8**	**12**	**30**	**50**

Chi-square for Sr. Cholinesterase and INR is 73.611 with df=4 at p value <0.0001.

The above Chi-square table shows statistically significant negative correlation between INR and serum cholinesterase levels with p value <0.0001.

This shows that serum cholinesterase can be taken as an alternative biomarker for patients with chronic liver disease as it reflects the synthetic function of the liver. Serum cholinesterase levels falls proportionately with rise in INR.

Hence serum cholinesterase can be considered as cost effective alternative for INR in patients with liver diseases.

Table 13: CORRELATION BETWEEN SERUM CHOLINESTERASE AND TOTAL BILIRUBIN (mg/dl)

		Total Bilirubin (mg/dl)			Total
		<5	**5-10**	**>10**	
Sr.Cholinesterase (U/L)	>3000	9	1	0	10
	1500-3000	0	11	1	12
	<1500	0	0	28	28
Total		**9**	**12**	**29**	**50**

Chi-square for Sr. Cholinesterase and Total Bilirubin is 85.850 with df=4 at p value <0.0001.

The above table depicts the correlation between total serum bilirubin in milligrams/deciliter values and serum cholinesterase levels in U/L. From the Chi-square test depicted above, the derived p value<0.0001, which is statistically significant and it shows negative correlation.

Hence serum cholinesterase can be considered as biomarker alternative to total serum bilirubin as a liver function tests in patients with chronic liver diseases.

From the above, we can conclude that there is a significant association between Serum Cholinesterase values and liver function test values.

DISCUSSION

DISCUSSION

Our study was mainly conducted to evaluate the level of serum cholinesterase among patients with chronic liver diseases and to compare its levels with other liver function tests such as serum albumin, total serum bilirubin, INR, SGOT and SGPT.

Our study population consisted of 50 chronic liver disease patients diagnosed by both ultrasonography and clinically. Serum cholinesterase levels were measured in all those 50 patients along with routinely performed tests like serum bilirubin, serum albumin, PT INR, SGOT and SGPT.

Analysis was done to study correlation between the levels of serum cholinesterase with liver function tests like serum albumin, serum bilirubin, INR, SGOT and SGPT. The Following observations were made from our study.

Age distribution:

Out of 50 study population, most of the patients were in the 31 – 40 years age group (80%). This showed that chronic liver disease is most commonly seen in 31-40 years of age in our study population.

Sex distribution:

Out of 50 study group population, 42 patients were males (84%) and the remaining 8 patients (16%) were females.

Etiology:

Among 50 patients with chronic liver disease, the most common etiology for chronic liver disease in our study was found to be due to alcohol in 35 patients (70%) which is followed by Hepatitis B virus infection which accounts for about 18% (9 patients) and Hepatitis C virus infection which causes chronic liver disease in 6 patients (12%).

Serum cholinesterase levels:

Among the 50 study population, 10 patients had serum cholinesterase levels more than 3000 U/L which accounts for 20% of the patients. 12 patients (24%) had serum cholinesterase levels between 1500 to 3000 U/L and 28 patients (56%) had serum cholinesterase levels less than 1500 U/L. This shows that majority of our study population had severely reduced cholinesterase levels.

Total bilirubin:

Out of 50 patients in the study population, 9 patients (18%) had total bilirubin less than 5mg/dl, 12 patients (24%) had total bilirubin ranges between 5 to 10 mg/dl and 29 patients (58%) had total bilirubin more than 10 mg/dl.

Coagulopathy:

In our study population, 30 patients (60%) had INR levels more than 2. Thus, majority of our study population had coagulopathy.

SGOT:

In this study, 20% (10 patients) had SGOT levels less than 120 IU/L, 20% (10 patients) had SGOT levels in between 120 to 240 IU/L and 60% (30 patients) had SGOT levels more than 240 IU/L.

SGPT:

In our study, 10 patients (20%) had SGPT levels less than 120 IU/L whereas 7 patients (14%) had SGPT levels between 120 to 240 IU/L and 33 patients (66%) had SGPT levels more than 240 IU/L.

Serum proteins:

9 patients (18%) had serum albumin in the range of 2.6 to 3.5 g/dl. 12 patients (24%) of patients had serum albumin between 2 to 2.5 mg/dl and 29 patients (58%) had serum albumin less than 2 mg/dl. This shows that the majority of the study population had severe hypoalbuminemia.

CORRELATION BETWEEN SERUM ALBUMIN AND SERUM CHOLINESTERASE:

In our study, correlation between serum albumin levels and serum cholinesterase were studied. It shows that they were positively correlated with a p value <0.0001. This was comparable with the conclusion from the study done by Jeyamani Ramachandran et al and Fanping Meng et al.

CORRELATION BETWEEN SERUM TOTAL BILIRUBIN AND SERUM CHOLINESTERASE:

In this study, we found that serum total bilirubin levels are negatively correlated with serum cholinesterase with patients with chronic liver diseases and the p value <0.0001 which was significant statistically. This is comparable with the outcome of study done by Jeyamani Ramachandran et al.

CORRELATION BETWEEN INR AND SERUM CHOLINESTERASE LEVELS:

In our study population, it was identified that INR values were negatively correlated with the serum cholinesterase values and it was also statistically significant with p value <0.0001. This was equivalent to the observations made by Jeyamani Ramachandran et al and Fanping Meng et al studies.

CORRELATION BETWEEN SGOT AND SERUM CHOLINESTERASE LEVELS:

In our study population, it was identified that SGOT values were negatively correlated with the serum cholinesterase values and it was also statistically significant with p value <0.0001.

CORRELATION BETWEEN SGPT AND SERUM CHOLINESTERASE LEVELS:

In our study population, it was identified that SGPT values were negatively correlated with the serum cholinesterase values and it was also statistically significant with p value <0.0001.

LIMITATIONS OF THE STUDY

- Size of the study population is small.
- It is a single centered study.
- All the subjects were from the same centre. Therefore racial, dietary habits and environmental factors may also play a role in causation of liver disease.
- Group of peoples with congenital deficiency of serum cholinesterase in the southern part of India were not included in the exclusion criteria.
- Liver biopsy was not done to identify the pathological cause of chronic liver disease.

CONCLUSION

CONCLUSION

From our study, the following conclusions were made:

- Chronic liver diseases were more commonly seen among 31 to 40 years of age in our study population.
- It is more common in male counterpart in our study population.
- Alcohol is the most commonly observed etiology of chronic liver disease in our study.
- Majority of patients in our study population had bilirubin levels more than 10 mg/dl.
- Most patients in our study had coagulopathy, and their INR levels were more than 2.0.
- There was highly significant positive correlation between the serum albumin levels and the serum cholinesterase levels. Patients with lower serum albumin level also had a lower serum cholinesterase level and the fall in serum cholinesterase was proportional to fall in albumin.
- Statistically significant negative correlation was noted between serum bilirubin levels and serum cholinesterase levels. Study population with higher serum bilirubin levels also had a lower serum cholinesterase levels.

- There was statistically significant negative correlation between the INR values and the serum cholinesterase levels. Patients having coagulopathy also had low serum cholinesterase level.

- Significant correlation had been found between the serum cholinesterase levels and severity of chronic liver disease. The levels were very low in patients with more severe liver disease.

- There was significant correlation between serum cholinesterase values and SGOT levels. Patients with reduced serum cholinesterase had elevated SGOT levels. It also has statistically significant negative correlation.

- It was also observed from the study that there was a significant negative correlation between serum cholinesterase levels and SGPT. The SGPT levels were very high in patients with very low serum cholinesterase levels.

  Thus, there was significant correlation between the levels of serum cholinesterase and severity of chronic liver diseases.

SUMMARY

SUMMARY

The estimation of serum cholinesterase levels in patients with chronic liver diseases has several implications in the assessment and management of the patients.

Cholinesterase activity in the serum of patients with chronic liver diseases had shown excellent correlation with routinely performed other liver function tests.

Cholinesterase assessment in serum proves to be more useful in settings where commonly performed tests for liver function had shown abnormal results or altered values.

It is simple and relatively inexpensive and can be easily tested on an outpatient basis.

Not only it is useful in the diagnosis of liver diseases, but it also predicts the severity of liver diseases which helps to assess the prognosis and further management of the patients.

Thus the measurement of serum cholinesterase routinely in patients with liver diseases will prove useful in the diagnosis, treatment and also in assessing severity of liver diseases.

ANNEXURES

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PROFORMA

PATIENT DETAILS:

Name: Age: Sex: IP No.:

ON ADMISSION:

Main Complaints:

- H/o jaundice
- H/o abdominal distension
- H/o pedal oedema and scrotal swelling
- H/o reduced urine output
- H/o breathlessness
- H/o orthopnoea
- H/o Paroxysmal nocturnal dyspnoea
- H/o hemetemesis
- H/o melena and hematochezia
- H/o seizures
- H/o altered sensorium
- H/o altered or disturbed sleep pattern
- H/o chest pain
- H/o abdominal pain
- H/o fever
- H/o constipation
- H/o intake of any drug intake

Co – Morbid Illness:

Significant Past History: CLINICAL EXAMINATION:

Pulse BP:

RR: Temp:

Pallor: Icterus:

CVS: RS:

P/A:

CNS: INVESTIGATIONS:

Complete Hemogram:

Urine Routiene:

Anti HCV antibody:

HBSAg:Antigen

Renal Function Test:

(Including blood urea and serum creatinine)

Bleeding Time

Clotting Time:

PT/INR:

Blood Grouping and Rh typing:

Serum cholinesterase levels:

Electrocardiogram:

Chest XRAY:

Ultrasonogram Abdomen:

Liver Function Tests:

(Including total bilirubin, direct bilirubin, indirect bilirubin, SGOT, SGPT, ALP)

Serum proteins:

(Including serum albumin and serum globulin)

OGD scopy:

ABBREVIATIONS

ABBREVIATIONS

PT INR	– Prothrombin time / International Normalized
Ratio MELD	– Model For End stage Liver Disease
CTP	– Child Turcotte Pugh classification
ALP	– Alkaline phosphatase
LDH	– Lactate dehydrogenase
GIT	– Gastro intestinal tract
EVL	– Endoscopic variceal ligation
TIPS	– Trans jugular intrahepatic portosystemic shunt
SBP	– Spontaneous bacterial peritonitis
UGI	– Upper gastrointestinal tract
HRS	– Hepato renal syndrome
GABA	– Gamma-amino butyric acid
CNS	– Central nervous system
HPS	– Hepato pulmonary syndrome
JVP	– Jugular venous pressure
SVR	– systemic vascular resistance

HR – Heart rate

CO – cardiac output

RAAS – Renin angiotensin aldosterone system

DEXA – Dual energy X ray absorptiometry

AST/SGOT – Aspartate aminotransferase/serum glutamic oxaloacetic transaminase

ALT/SGPT – Alanine aminotransfearse/serum glutamic pyruvic transaminase

GGT – Gamma-Glutamyl transpeptidase

NASH – Nonalcoholic steatohepatitis

5'NT – 5' Nucleotidase

U/L – units/litre

KPa – kilopascal

USG – Ultrasonography

CT – Computed Tomography

Ach – Acetylcholine

ஆராய்ச்சி ஒப்புதல் படிவம்

பெயர்:

வயது:

தேதி:

நோயாளி எண்:

ஆராய்ச்சி சேர்க்கை எண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு விளக்கபட்டது. எனக்கு விளக்கபட்ட விஷயங்களை நான் புரிந்து கொண்டு எனது முழு மனதுடன் சம்மதிக்கிறேன்.இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியில் இருந்து எந்தநேரமும் பின்வாங்கலாம் என்றும் அதனால் எந்தபாதிப்பும் எனக்கு ஏற்படாது என்பதையும் புரிந்துகொண்டேன். நான் என்னுடைய சுயநினைவுடன் மற்றும் முழுசுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் பங்கு கொள்ள சம்மதிக்கிறேன்.

INSTITUTIONAL ETHICS COMMITTEE
MADURAI MEDICAL COLLEGE & GOVT. RAJAJI HOSPITAL, MADURAI
CDSCO:Reg.No.ECR/1365/Inst/TN/2020 &
DHR Reg.No.EC/NEW/INST/2020/484

Study Title	:	Assessment of the value of serum cholinesterase as a liver function test in patients with liver disease
Principle Investigator	:	Dr.Praveen Raj.K
Designation	:	PG in MD., General Medicine (2019 – 2022)
Guide	:	Dr.S.C.Vivekananthan, M.D., DTCD Professor of General Medicine
Department	:	Department of General Medicine, Govt. Rajaji Hospital & Madurai Medical College, Madurai.

The request for an approval from the Institutional Ethics Committee (IEC) was considered on the IEC meeting held on **03.03.2021** at Auditorium, Govt. Rajaji Hospital, Madurai at 10.00 AM.

The Members of the committee, the Secretary and the Chairman are pleased to inform you that your proposed project mentioned above is **Approved.**

You should inform the IEC in case of any changes in study procedure, methodology, sample size investigation, Investigator or guide or any other changes.

1. You should not deviate from the area of work for which you had applied for ethical clearance.

2. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions. If encountered during from study.

3. You should abide to the rules and regulations of the institution(s)

4. You should complete the work within the specific period and if any extension is required, you should apply for the permission again for extension period.

5. You should submit the summary of the work to the ethical committee on completion of the study.

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This is to certify that this dissertation titled "**ASSESSMENT OF THE VALUE OF SERUM CHOLINESTERASE AS A LIVER FUNCTION TEST IN PATIENTS WITH LIVER DISEASES**" of the candidate **Dr. K.PRAVEEN RAJ** with **Registration Number 201911114** for the award of M.D degree in the branch of GENERAL MEDICINE. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file containing from introduction to conclusion pages and result shows 1 percentage of plagiarism in the dissertation.

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MASTER CHART

S. NO	AGE/SEX	MARITAL STATUS	Smoking	Alcohol	Viral markers	Serum Cholinest-erase U/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Indirect Bilirubin mg/dl	SGOT IU/L	SGPT IU/L	ALP IU/L	Total protein g/dl	Serum Albumin g/dl	INR
1	37/M	Married	Yes	Yes		1378	14.5	8.9	5.6	307	332	147	3.8	1.7	2.3
2	48/M	Married	No	Yes		2722	9.7	6.1	3.6	218	190	118	4.1	2	1.7
3	39/M	Married	No	No	HBSAg +	890	21.2	16.7	4.5	536	713	201	3.5	1.7	2.8
4	31/M	Unmarried	Yes	Yes		3937	4.8	3.1	1.7	108	120	114	5.9	2.9	1.1
5	32/M	Married	No	No	HBSAg +	752	34.8	20.4	14.4	817	988	263	3.2	1.5	2.7
6	58/F	Married	No	No		970	18.6	13.9	4.7	364	549	163	3.5	1.5	2.9
7	44/M	Married	Yes	Yes		3699	4.7	2.7	2	111	119	81	5.7	2.8	1.2
8	30/M	Married	Yes	No		2550	9.9	5.5	4.4	245	279	124	4	2	1.6
9	52/M	Married	No	No		998	16.6	12.3	4.3	342	542	150	3.6	1.6	2.9
10	48M	Married	Yes	Yes		1489	13	7.9	5.1	295	337	138	3.9	1.7	2.4
11	43/F	Married	No	No	HCV +	3118	8.3	5.7	2.6	118	111	122	5	1.9	1.6
12	45/M	Married	Yes	Yes		2690	9.9	5.1	4.8	228	240	175	4.1	2.3	1.8
13	38/M	Married	Yes	No		999	18	8.9	9.1	330	501	144	3.7	2	2.8
14	34/M	Unmarried	Yes	No		1439	12.8	8.4	4.4	290	420	156	4	1.7	2.4
15	45/M	Married	No	Yes		3839	4.9	2.5	2.4	117	104	78	5.8	1.9	1.2
16	51/F	Married	No	No	HCV +	3431	5.2	3.1	2.1	106	112	100	5.7	3	1.3
17	42/M	Married	Yes	Yes		3536	6.5	4.3	2.2	115	119	119	5.3	3	1.7

18	37/M	Married	Yes	Yes		3965	4.6	2.9	1.7	101	107	111	6	2.6	1
19	54/M	Married	No	No	HBSAg +	1497	13.6	8.4	5.2	298	512	103	3.8	3	2.6
20	33/M	Married	Yes	No		1356	13	8.6	4.4	292	307	117	4	1.8	1.9
21	36/F	Married	No	No	HBSAg +	1508	12.4	8	4.4	270	413	167	4.1	1.9	2.2
22	39/M	Unmarried	No	Yes		1024	15	11.5	3.5	338	712	155	3.6	1.7	2.5
23	43/M	Married	No	No	HBSAg +	2900	8.8	6.5	2.3	212	294	119	4.2	2.1	1.6
24	31/M	Married	Yes	Yes		865	23.4	17.9	5.5	576	816	212	3.4	1.5	3
25	55/M	Married	No	No	HCV +	2710	7.8	3.2	4.6	201	190	113	4	2.8	2.1
26	48/F	Married	No	No	HBSAg +	725	27.1	19	8.1	672	796	230	3.2	1.5	3.1
27	41/M	Married	Yes	Yes		1110	14.8	9	5.8	325	599	151	3.7	1.8	2.9
28	36/M	Married	No	Yes		1257	12	7.8	4.2	252	418	155	3.5	1.8	2.7
29	53/M	Married	Yes	Yes		1001	15.5	9.8	5.7	349	733	161	3.6	1.6	2.6
30	48/M	Married	No	Yes		1467	13	8	5	290	320	130	4	1.9	2.5
31	39/M	Married	Yes	Yes		2350	7.8	5.3	2.5	233	285	97	4.2	2	1.6
32	44/M	Married	No	No	HCV +	1050	15.2	9.5	5.7	336	651	99	3.7	1.6	2.8
33	42/M	Married	Yes	No		3010	9.1	6.1	3	114	98	81	4.6	2.6	1.4
34	39/F	Married	No	No	HCV +	3344	6.2	3.3	2.9	100	87	65	5.3	3.4	1.1
35	53/M	Married	No	Yes		2877	9.9	6	3.9	218	240	125	4.2	2.1	1.7
36	47/M	Married	Yes	Yes		2374	10	7.3	2.7	234	289	140	4.2	2.2	1.6

37	50/M	Married	Yes	No		878	24.3	17.2	7.1	601	817	208	3.5	1.7	3.1
38	38/M	Married	Yes	Yes		1199	13.7	8.8	4.9	303	569	144	3.8	1.8	2.9
39	44/M	Married	Yes	Yes		1498	12.9	6.9	6	285	500	99	3.8	1.8	2.1
40	40/M	Married	No	No	HBSAg +	2998	9.3	6.4	2.9	204	239	127	4.6	2.2	1.6
41	36/M	Married	No	No	HCV +	1263	14.6	8.7	5.9	319	503	149	3.8	1.7	2.2
42	33/M	Unmarried	Yes	Yes		3437	5.1	3.1	2	101	107	124	5.1	3.1	1.3
43	58/M	Married	Yes	Yes		2221	9.8	6.5	3.3	188	209	160	4.2	2	1.7
44	48/M	Married	No	Yes		1165	14.1	8.4	5.7	312	573	148	3.8	1.8	2.4
45	42/F	Married	No	No	HBSAg +	901	25.5	16	9.5	598	803	200	3.5	1.8	2.8
46	38/M	Married	Yes	Yes		1056	14.9	9.8	5.1	331	693	139	3.6	1.7	2.2
47	33/M	Married	Yes	Yes		1400	12.8	6.5	6.3	286	491	95	3.9	1.9	2.1
48	45/F	Married	No	No	HBSAg +	2581	9.6	5.2	4.4	230	217	122	4	2.2	1.6
49	34/M	Married	Yes	Yes		781	31.3	15.8	15.5	759	947	260	3.3	1.5	3.4
50	48/M	Married	No	Yes		912	25	15	10	591	788	197	3.5	1.8	2.8