"INVESTIGATION OF EPIDEMIOLOGY AND ETIOLOGY OF LIVER DISEASES AND CHARACTERIZATION OF ITS ASSOCIATION WITH VARIOUS FACTORS"

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BY

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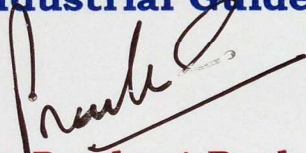**Senior Consultant, M.D. Gastroenterology**

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CERTIFICATE

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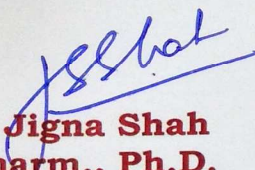
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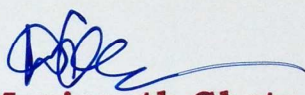
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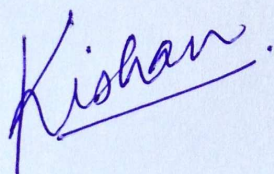Department of Pharmacology,
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ABSTRACT

BACKGROUND AND OBJECTIVE:

The population of Gujarat is at higher risk of facing non-alcohol related liver diseases. It becomes important therefore to be well aware of the exact scenario of burden of liver diseases in the population, and to find out its association and causes. Therefore the objective of the study is to examine etiology and epidemiology of liver diseases at a tertiary care trust hospital in Vadodara district and to characterize its association to various factors.

METHODOLOGY:

A tertiary care trust hospital in the city was chosen to ensure population from all sections of the society for variations and patients from every strata of disease progression. All patients having liver diseases are identified. Various patient details like social background, demography, family history, social and medical history were reported. Causes of developing the condition are tried to be found out and various complications associated with the disease are looked upon. The treatment given and the laboratory reports of various tests at different times during admission periods are also noted down and clinically co-related with patient conditions.

RESULTS:

137 cases of liver diseases were reported. Most of these cases were cases of non- alcoholic liver diseases with alcoholic cases comprising of 25% of total cases. Around 20% of all cases are due to viral infection. Majority of cases were male (77.77%). More than 80% patients are above the age of 40. Urban populations are more susceptible to liver diseases. Monsoon is related to higher incidences of viral diseases. A general rise in liver diseases was observed in festive months is observed. Majority of patients admitted during the study period were of liver cirrhosis (33% cases), followed by Liver Parenchymal Diseases (LPD) which is inclusive of Alcoholic Liver Disease (ALD), Chronic Liver Disease (CLD) and fatty liver (nearly 25%). Diabetes and CVS disorders are most correlated comorbidities with 24% and 19.3% prevalence respectively.

GGT (gamma glutamyl transferase) levels were significantly ($p< 0.001$) high in case of alcoholics while ALP (alkaline phosphatase) values were significantly high($p<0.001$) in viral manifestations. The values of total bilirubin and direct bilirubin were significantly high for hepatitis as well as alcoholic cases, especially in case of non-chronic ALD ($p< 0.05$). HCT (hematocrit) and RDW (red cell distribution width) values seem to correlate well with severity of liver diseases. ALT/AST is greater than 1 in case of viral diseases that lead to chronic conditions, obstruction disorders and FLD. AST/ALT ratios are greater than 1 for alcoholic patients, Cirrhosis patients. The ratio is greater than 2 for ALD.

The DF score (discriminant factor) correlates to alcoholism, ALD, end stage cirrhosis, diabetes mellitus and decompensated kidney functions. The MELD (Model for End-Stage Liver Disease) score relates better to decompensated cirrhosis, Hepato- Cellular Carcinoma (HCC) and Acute over Chronic Liver Failure (ACLF), i.e. decompensating in case of chronic ALD and to ascites and hepato-portal or gall bladder obstruction other than DM. The Child Pugh (CP) score correlates extremely well to alcoholism and presence of varices. All the three scores correlate well to mortality, CP correlating the best though and MELD the least. 65% of the patients with cirrhosis have class C CP score (score > 9). Around 30% of population of patients is above the grade 4 MELD (score > 25).The mean DF value for all tested patients is 47.1.

CONCLUSION:

Liver Diseases are widespread and rampant in the district of Vadodara. Liver diseases correlate well to the male gender, increased age, and urban population and to metabolic disorders. The pattern of change in Liver Function Tests (LFTs) is typical for various diseases and can be instrumental in identification and differential diagnosis of liver diseases. As in most of the developing world, viral hepatitis continues to be the major cause of liver diseases. Prognosis in most of these cases is poor. A descriptive study has been therefore performed at length to assess the current situation, and need has been found for further investigations.

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LIST OF ABBREVIATIONS

ALT	Alanine transaminase
AST	Aspartate transaminase
ALD	Alcoholic Liver Diseases
CLD	Chronic liver disease
CLF	Chronic liver failure
CVS	Cardio vascular system
CP	Child Pug
DALY	Disability adjusted life-year
DF	Maddrey's discriminant fraction
DM	Diabetes Mellitus
FLD	Fatty liver disease
GGT	Gamma-glutamyl transpeptidase
Hb	Haemoglobin
HBsAg	Hepatitis B serum Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCT	Haematocrit
HCV	Hepatitis C Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INR	Internationalized Normal Ratio
LPD	Liver parenchymal disease
LFT	Liver function test
MELD	Model for End-Stage Liver Disease

NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
SMR	Standardised Mortality Ratio
RBC	Red blood cells
RDW	Red cell Distribution Width
WBC	White blood cells
WHO	World Health Organization

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1. <u>INTRODUCTION</u>

The liver is a marvelously resilient and vital organ that plays an indispensable role in nurturing and protecting the body everyday with clockwork precision. The liver supports almost every organ in the body and is vital for survival. It has several key functions to perform. It helps filter and dispose off toxic materials from the blood, feeds the body the energy it needs to function, wards off viruses and infections, produces blood-clotting factors, and regulates sex hormones, cholesterol levels and vitamin and mineral supplies in the body. In fact, the liver performs over 500 functions, far more than any other organ in the body (Rang et al., 2003)

Everything we eat, drink, and breathe, including all the medicines we take: Over-the-counter medicines, Prescription medicines, Vitamins, Dietary supplements, Alternative medicines are processed by the liver and hence affect the Liver. Condition of the liver also affects the way drug responds to our body: Compromised liver metabolizes slower.

Liver disease can prevent the liver from performing its numerous, vital functions. Although alcohol or drugs are the major blames for liver diseases, there are over 100 known forms of liver disease caused by a variety of factors and affecting everyone from infants to older adults. For example, Hepatitis A, Hepatitis B and Hepatitis C are caused by viral attack to the liver. Still other set of liver diseases are caused by drug abuse, exposure to poisons or excessive consumption of alcohol. (Rang et al., 2003)

The disease can be majorly categorized as either of:

- Infections and jaundice

- CLD & fatty liver

- Liver parenchymal disease and cirrhosis

- Failure & carcinoma

- Other diseases

- It is further categorized as Primary or secondary.

It is estimated that liver diseases are among the top ten killer diseases in India, causing lakhs of deaths every year. Besides, there are those who suffer from chronic liver problems, needing recurrent hospitalization and prolonged medical attention, which leaves them physically, mentally, emotionally and financially devastated. While there have been major advances in treating liver diseases, there are no cures. Studies show that 70% of Hepatitis patients get to know of symptoms only at a later stage. One in 12 people worldwide are suffering from chronic hepatitis B or C and yet majority of those infected are unaware. Hepatitis is a dreaded disease that's more rampant than HIV and asthma. Hepatitis kills more than one million people every year. (WHO, 2013)

However, while alcohol causes liver problems in 10-20 percent of the population, which leads to fatty food which leads to obesity and problems in 25 percent of population. The prevalence of Non-Alcohol Related Fatty Liver Disease (NAFLD) among urban Indians is fast catching up with their counterparts in Western countries and accounts for almost 50 per cent of cirrhosis of liver cases. The prevalence of NAFLD in Indian population is 20-30 per cent in the urban areas while it is 36-45 per cent in the West. The rural areas were better off with a prevalence rate of 8.7 per cent. While alcohol is the most common cause for accumulation of fat in the liver, non-alcohol related fatty liver disease has surpassed it. NAFLD is mainly increasing due to wrong dietary habits and high intake of fats, carbohydrates and less consumption of proteins. Vegetarians are at an increased risk as carbohydrates ultimately get converted into fat. Many a time, fat accumulation in internal organs might be higher due to wrong dietary habits even though one is thin. (Walsh and Alexander G, 2006; Dr. Bangar, 2013)

The importance can be understood by the fact that since 1979, the Research Awards Program by the American Liver Foundation has provided more than $24 million in research funding. Over 800 qualified scientists and physicians have pursued careers in liver disease research and treatment as a result of receiving these grants early in their careers. (ncbi.org)

Thus, the population of Gujarat- a majorly vegetarian, foodie province with various snacks on the platter is posed with a higher risk of facing Non- Alcohol related Liver diseases. Sadly however, not many epidemiological studies are made to identify Liver diseases in Gujarat, since it is a state where alcohol is legally banned. The fact is however, that Liver diseases are on a rise owing to life style changes. High fat diet, junk food, sedentary life-style, smoking, pollution,

rampant use of medications, and by and large vegetarian culture of the state has shown a large rise of non- alcoholic liver diseases in the already genetically predisposed population of Gujarat. Increase in cases Diabetes Mellitus and other metabolic disorders have also predisposed the population to a greater risk of liver diseases

It becomes important therefore to be well aware of the exact scenario of burden of liver diseases in the population and to stratify it accurately and thus obtain its exact class wise prevalence. Identification and classification are the first steps towards efficient management of any problem.

An urgent need for an analytical study is indicated so as to highlight the pit holes in the issue and initiate further research and ultimately leading to better prognosis. A descriptive study has been therefore performed at length to assess the current situation, and need has been found for further investigations. The objectives of the thesis are as under:

1. To study the burden if liver diseases in Vadodara district in the given period based on extrapolation of data obtained.

2. To study correlation of demographics, complications, comorbidities and various other parameters to severity of liver diseases.

3. To calculate and find correlation among various scores those define liver diseases.

4. To check correlation among various parameters and between parameters and scores those define severity and prognosis of liver diseases.

2. <u>LITERATURE REVIEW</u>

2.1 <u>THE LIVER</u>

The liver is a reddish brown organ with four lobes of unequal size and shape. A human liver normally weighs 1.44– 1.66 kg and is a soft triangular organ. It is the largest gland in the human body and is exocrine in nature. It is located in the right upper quadrant of the abdominal cavity, just below diaphragm, right of the stomach and over the gallbladder. It is connected to the hepatic artery and the portal vein. The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into capillaries, which then lead to a lobule, the functional unit of liver. Each lobule is made up of millions of specialized hepatic cells (hepatocytes). They constitute the parenchymal cells and are the basic metabolic cells. They There over 300 billion specialized cells (hepatocytes) in the liver that are connected by a well-organized system of bile ducts and blood vessels known as the biliary system. Non-parenchymal cells constitute 40% of the total number of liver cells but only 6.5% of its volume. Sinusoidal hepatic endothelial cells, Kupffer cells and hepatic stellate cells are some of the non-parenchymal cells that line the liver sinusoid. (Kmieć, 2001).

This gland plays a major role in metabolism and has a no. of functions in the body including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and detoxification. The liver produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver is completely surrounded by a peritoneal membrane known as the Glisson's capsule. The liver is the only human internal organ capable of natural regeneration of lost tissue; as little as 25% of a liver can regenerate into a whole liver. Regeneration is very rapid. The liver will return to a normal size in 1 to 2 weeks following the removal of greater than 50% of the liver by mass. (Romer et al, 1977)

The various functions of the liver are carried out by the liver cells or hepatocytes. They take up glucose, minerals, and vitamins from portal and systemic blood and store them. Many important substances such as blood clotting factors, transporter proteins, cholesterol, and bile components are synthesized by the hepatocytes. The hepatocytes also regulate blood levels of substances such as cholesterol and glucose, the liver helps maintain body homeostasis. Currently, there is no

artificial organ or device capable of emulating all the functions of the liver. Some functions can be emulated by liver dialysis, an experimental treatment for liver failure. The liver is thought to be responsible for up to 500 separate functions, usually in combination with other systems and organs. (Rang et al., 2003)

2.2 LIVER DISEASES

2.2.1 Definition

Liver disease covers a broad spectrum of diseases including acute and chronic liver diseases. The International Classification of Diseases (ICD-10) includes within the specific section for liver disease (K70-K77) alcoholic liver disease (K70), toxic liver disease (K71), hepatic failure (K72), chronic hepatitis (K73), fibrosis and cirrhosis of liver (K74), inflammatory liver diseases (K75) and other diseases of liver (K76 including fatty liver K76.0). In addition, viral hepatitis (B15-19) and neoplasms of the liver and intrahepatic bile ducts (C22) are classed as liver diseases.

For the intended study, Liver Diseases was defined as any of the above or otherwise, leading to altered liver structure and/ or function/s

2.2.2 Etiology and Classification

Due to its strategic location and multidimensional functions, the liver is also prone to many diseases. The most common include: Infections such as hepatitis A, B, C, D, E, alcohol damage, fatty liver, cirrhosis, cancer, and drug damage by drugs like paracetamol.

Many types of liver disease still have unknown causes but the most frequent liver diseases are generally caused by one of the following factors (NLF, 2013):

Viral hepatitis

Caused by viruses that attack the liver, viral hepatitis comes in many forms. The most common forms world-wide are hepatitis A, B and C. Although hepatitis A and B can be prevented by vaccine, there is no vaccine for hepatitis C.

Obesity

The leading cause of liver disease is fatty liver disease linked to obesity

Alcohol

Factors such as gender, age, nationality, weight and health can affect how a person's liver metabolizes alcohol. When the liver has too much alcohol to handle, normal liver function may be interrupted leading to a chemical imbalance. If the liver is required to detoxify alcohol continuously, liver cells may be destroyed or altered resulting in fat deposits (fatty liver) and more seriously, either inflammation (alcoholic hepatitis) and/or permanent scarring (cirrhosis). Liver cancer can also result from alcohol induced liver disease.

Genetics

Several forms of liver disease are caused or thought to be caused, by defective genes. These forms of liver disease may be diagnosed in infancy or may not show up until later in life. Examples include hemochromatosis, Wilson disease, tyrosinemia, alpha 1 antitrypsin deficiency and Glycogen Storage disease.

Autoimmune disorders

Sometimes a body's immune system may begin to attack the liver or bile ducts causing inflammation and scarring which leads to a progressive form of liver disease. Examples of liver diseases believed to be caused by the immune system are primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis.

Drugs and toxins

The liver is responsible for processing most of the chemicals and medications that enter your body – this leaves it vulnerable to acute or chronic liver disease caused by chemicals. In some cases, this is a predictable consequence of overexposure or over-consumption of certain chemicals such as acetaminophen or industrial toxins like polyvinyl chloride or carbon tetrachloride. In other cases, chemicals can cause an unpredictable reaction.

Cancer

Although primary liver cancer is relatively uncommon, many other forms of cancer often metastasize in the liver. Because the liver filters a high volume of blood which may be carrying cancer cells, it is susceptible to developing a form of secondary cancer. If cancer originates in the

liver, it is often caused by hepatitis B, hepatitis C or it can develop in cases of advanced liver disease when cirrhosis is present.

Although liver disease is stereotypically linked to alcohol or drugs, the truth is that there are over 100 known forms of liver disease caused by a variety of factors and affecting everyone from infants to older adults. A list of liver diseases is given as under:

- Alagille Syndrome
- Alpha 1 Anti-Trypsin Deficiency
- Autoimmune Hepatitis
- Biliary Atresia
- Cirrhosis
- Cystic Disease of the Liver
- Fatty Liver Disease
- Galactosemia
- Gallstones
- Gilbert's Syndrome
- Hemochromatosis
- Liver Cancer
- Liver disease in pregnancy
- Neonatal Hepatitis
- Primary Biliary Cirrhosis
- Primary Sclerosing Cholangitis
- Porphyria
- Reye's Syndrome
- Sarcoidosis
- Toxic Hepatitis
- Type 1 Glycogen Storage Disease
- Tyrosinemia
- Viral Hepatitis A, B, C
- Wilson Disease

Classification of Liver diseases

Basis of classifying liver diseases:

1) Juvenile onset and hereditary or Adult- onset and acquired.

2) Inflammatory and non – inflammatory: i.e. according to histological findings.

3) Acute toxic liver disease & neoplastic liver disease

4) Extra-hepatic biliary obstructive disease (cholelithiasis or pancreatitis)& vascular disease (congenital protosystemic shunts)

5) Primary and secondary

6) Alcoholic and Non alcoholic and/ or drug induced, i.e., based on etiology.

However practically, the liver diseases are broadly classified broadly as:

1. Viral Hepatitis

2. Liver Parenchymal Disease

    a. Fatty liver (FLD)

    b. Alcoholic Liver diseases (ALD)

    c. Nonalcoholic liver diseases

        i. Nonalcoholic steatohepatitis (NASH)

        ii. Nonalcoholic fatty liver disease (NAFLD)

    d. Chronic Liver Disease (CLD)

3. Liver Cirrhosis

4. Liver Failure

    a. Acute

    b. Chronic

    c. Acute over chronic (ACLF)

5. Hepato- cellular carcinoma

6. Obstructive Disorders

    a. Gall bladder calculi

    b. Cholelithiasis

    c. Common biliary duct stones

    d. Portal hypertension

2.2.4 Pathophysiology and Progression

Staging of liver disease is needed because it is progressive, most of the time asymptomatic and potentially fatal. An accurate characterization of the disease is difficult but crucial to prevent its evolution and avoid irreversible pathologies such as the hepatocellular carcinoma.

Fatty liver infiltration (steatosis) is the earliest stage of the liver disease and occurs when the fat content in hepatocytes significantly increase. It is asymptomatic and the progress of the hepatic injury to other conditions, more severe, is common e.g., Fibrosis. Pathologically, fibrosis appears during the course of tissue injury or organ damage and its progression rate strongly depends on the cause of liver disease, such as chronic hepatitis.

Cirrhosis is the end-stage of every chronic liver disease. It is characterized by an asymptomatic stage, known as compensated cirrhosis, followed by a rapidly progressive phase where liver dysfunction occurs, decompensated cirrhosis. The most severe evolution condition of cirrhosis is the hepatocellular carcinoma, also called primary liver cancer. (Cotran et al., 2005)

2.2.5 Diagnosis

(NLF, 2013)

The diagnosis of liver function is made by blood tests. Liver function tests can readily pinpoint the extent of liver damage. If infection is suspected, then other serological tests are done. Sometimes, one may require an ultrasound or a CT scan to produce an image of the liver. Physical examination of the liver is not accurate in determining the extent of liver damage. It can only reveal presence of tenderness or the size of liver, but in all cases, some type of radiological study is required to examine it. Damage to the liver is sometimes determined with a biopsy, particularly when the cause of liver damage is unknown. In a biopsy, a needle is inserted into the skin just below the rib cage and a tissue sample obtained. The tissue is sent for histological testing

Hepatitis:

"Hepatitis" literally means "inflammation of the liver." Chronic inflammation of the liver may result in liver damage or failure if left untreated. "Hepatitis" can be caused by many

different things - drinking too much alcohol, traumatic injury, autoimmune disorders, an adverse drug reaction, or a virus such as the hepatitis B virus.

Non Alcoholic Liver Diseases:

Any of the liver diseases that cannot be attributed to either consumption of alcohol or virus is generally referred to as Nonalcoholic liver disease. It is generally secondary to a morbid condition or idiopathic. Drug induced liver damage is not considered under this category. Commonly it is either of NASH and NAFLD.

Cirrhosis

Cirrhosis is a chronic liver disease defined anatomically as a diffuse process with fibrosis and nodule formation. Its causes are myriad and it is considered the end point of most chronic liver diseases. Principle aetiologies are viral hepatitis, alcohol, autoimmune disorders such as autoimmune hepatitis and primary biliary cirrhosis, and metabolic disorders including haemochromatosis and Wilson‟s disease. Recent studies have suggested the progression of non-alcoholic fatty liver disease (NAFLD) to steatohepatitis, fibrosis and cirrhosis. Frequently the aetiology of cirrhosis is unknown with such cases being commonly referred to as „cryptogenic‟ cirrhosis. The aetiology of cirrhosis differs across the world, with alcohol representing a more common cause of cirrhosis in much of the Western world. (Harrison et al; 2007; Lefton et al., 2009)

Diagnosis of cirrhosis

The diagnosis of cirrhosis is often based on a combination of clinical, ultrasound, biochemical and histological findings but liver biopsy is still considered the gold-standard for the diagnosis of cirrhosis. A liver biopsy has certain procedural risks, like bleeding, discomfort for the patient and even a small risk of death, so several other non-invasive methods for diagnosis are employed. These include transient elastography (Fibroscan), magnetic resonance elastography and combinations of USG, CT and MRI.

<u>Disease progression and treatment</u>

Cirrhosis in the absence of complications is referred to as compensated cirrhosis but following the appearance of ascites, oesophageal variceal bleeding, encephalopathy or jaundice is considered to be decompensated.

Cirrhosis has until recently been considered irreversible, but this concept is no longer absolute as regression of fibrosis can be seen and „reversal‟ of cirrhosis has been reported. The principle aim for therapies in patients with cirrhosis is to slow the rate of progression which would lead eventually to liver failure or death. Treatments are few and are mostly focussed on the removal of the aetiologic agent(s), the suppression of hepatic inflammation, inhibition of hepatic stellate cell activation and then therapeutic strategies for the common sequelae of cirrhosis including the early detection of hepatocellular failure, hepatocellular carcinoma, fluid retention, encephalopathy and prevention or treatment of oesophageal varices and oesophageal bleeding.

2.3 LIVER FUNCTION TESTS

Several markers of liver function are included as part of standard laboratory blood tests which in the UK are frequently requested both in primary and secondary care. Standard blood tests which may be bracketed under the nomenclature of „liver function tests‟ (LFTs) include serum bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin, prothrombin time (INR) and gamma-glutamyl transpeptidase (GGT).

(Pratt and Kaplan, 2003)

Aminotransferases (ALT and AST)

ALT is a cytosolic enzyme present in the liver, and to a lesser extent in the heart and skeletal muscles. AST is a mitochondrial enzyme which is present in large quantities in the heart, skeletal muscle and kidney and also in the liver.

Variations in the serum prevalence of ALT and AST are indicative of the early stages of viral hepatitis, as well as other liver insults or injuries including alcohol abuse, autoimmune hepatitis, non-alcoholic steatohepatitis, haemochromatosis, Wilson‟s disease and alpha-1 antitrypsin deficiency.

Non-hepatic causes of elevations in ALT or AST include coeliac disease, striated muscle disorders, some endocrine diseases (hyperthyroidism and Addison"s disease) as well as glycogen storage diseases. Often the ratio of the two enzymes is used in the diagnosis of alcoholic hepatitis and cirrhosis.

Alkaline phosphatase (ALP)

Alkaline phosphatase is an enzyme present in the liver and bile ducts. Elevations in ALP are common in cholestatic diseases (such as primary sclerosing cholangitis and primary biliary cirrhosis) but as ALP is also particularly concentrated in some other tissues (such as bones, placental tissue and in the kidneys) the specificity of ALP as a „liver function test" is not very high. Elevations of ALP can occur in a wide variety of other diseases including Paget"s disease and other bone disorders, with malignant tumours, in renal disease as well as natural elevations being seen during pregnancy. A simultaneous elevation in GGT would suggest a problem of liver origin.

Bilirubin

Bilirubin is a product of haemoglobin catabolism. An increase in serum bilirubin can be as a result of additional bilirubin production, decreased hepatic uptake or decreased conjugation (occurring within the liver). Elevations in unconjugated bilirubin can indicate haemolysis or familial abnormalities of bilirubin metabolism such as Gilbert"s syndrome. Elevations in conjugated bilirubin are more indicative of congenital hyperbilirubinaemias such as Dubin-Johnson syndrome and Rotor"s syndrome. (Limdi, Hyde, 2003)

Although theseliver function test are performed frequently in both primary and secondary care the prevalence and consequences of these standard markers of liver disease have been ill-described in the UK making the interpretation of them difficult, particularly in the case of an isolated elevation in one of these tests.

Gamma Glutamyl Transferase

Although reasonably specific to the liver and a more sensitive marker for cholestatic damage than ALP, Gamma glutamyl transpeptidase (GGT) may be elevated with even minor, sub-clinical levels of liver dysfunction. It can also be helpful in identifying the cause of an isolated elevation in ALP. GGT is raised in alcohol toxicity (acute and chronic). GT is often elevated in those who usealcohol or other liver toxic substances to

excess. GGT is also induced by many drugs, including alcohol, therefore often when the AP is normal a raised GGT can often (but not always) indicate alcohol use. Raised GGT can often be seen in cases of fatty liver and also where the patient consumes large amounts of Aspartame (artificial Sweetener) in diet drinks for example.

2.3.1 Altered LFTs and correlation to Liver Diseases

(Dr. Thomas, 2010)

Interpreting abnormal liver function tests (LFTs) and trying to diagnose any underlying liver disease is a common scenario in Primary Care. Abnormal LFTs may be asymptomatic, and are often inadequately investigated - which may miss an early opportunity of identifying and treating chronic liver disease. (Sherwood et al, 2000)

The primary problem may be the liver, or the abnormal results can be secondary to other problems elsewhere in the body. (Limdi and Hyde, 2003)

Traditionally 'normal' values are defined as being within ± 2 standard deviations meaning that 2.5% of a healthy population will have LFTs outside the normal range. However, as liver disease is frequently asymptomatic, such a 'healthy' population may have significant numbers of people with undiagnosed liver disease, and thus this argument should not be used as an excuse for inadequate investigation.

<u>Common liver investigations</u>

Liver function tests (LFTs) are readily available and are often included as a baseline investigation for a large number of different presentations. They usually consist of:

Bilirubin:

Bilirubin is derived from the breakdown of haem in the red blood cells within the reticuloendothelial system.

The unconjugated bilirubin then binds albumin and is taken up by the liver.

In the liver it is conjugated which then makes it water-soluble and thus allows it to be excreted into the urine.

Normally total serum bilirubin is measured; however, the unconjugated and conjugated portions can be determined by measures of the fractions of indirect bilirubin and direct bilirubin respectively. (Giannini et al., 2005)

Albumin

It is a sensitive marker of hepatic function, but not useful in the acute stages as it has a long half-life (20 days).

Transferases

Usually either alanine aminotransferase (ALT) or aspartate aminotransferase (AST); rarely does a laboratory routinely provide both:

These enzymes normally reside inside cells (in cytoplasm) so raised levels usually represent hepatocellular damage. ALT is more specific to the liver, as AST is also found in cardiac and skeletal muscle and red blood cells.

Very high levels (>1000 IU/L) suggest drug-induced hepatitis (eg paracetamol), acute viral hepatitis (A or B) ischaemic, or rarely, autoimmune hepatitis.

The ratio of AST to ALT can give some extra clues as to the cause:

In chronic liver disease ALT >AST, once cirrhosis established AST >ALT. The extremes of the ratio of AST: ALT can also be helpful: >2 suggests alcoholic liver disease, and a ratio of <1.0 suggests nonalcoholic liver disease.

Gamma-glutamyl transferase (GGT)

It is also related to the bile ducts. Typically elevated in cholestasis (with elevated ALP) but, if ALP normal, suggests induction of hepatic metabolic enzymes (e.g alcohol or enzyme-inducing drugs).

Alkaline phosphatase (ALP)

It comes mainly from the cells lining bile ducts but also in bone. Marked elevation is typical of cholestasis (often with elevated GGT) or bone disorders (usually normal GGT).

Isoenzyme analysis may help identify source. It is physiologically increased when there is increased bone turnover (eg adolescence) and is elevated in the third trimester of pregnancy (produced by the placenta).

When basic LFTs are abnormal, ensure a full history and examination is performed:

Prothrombin (INR) - sensitive marker of hepatic synthetic function.

Viral serology, eg hepatitis B and C, cytomegalovirus (CMV), Epstein-Barr virus and possibly HIV.

Autoantibody screen, eg antimitochondrial antibody, anti-smooth muscle antibody and antinuclear antibody.

Immunoglobulins (if not available, raised immunoglobulins may be suggested by a raised globulin fraction (total protein minus albumin)).

Rise in bilirubin alone: need to know if unconjugated hyperbilirubinaemia or conjugated hyperbilirubinaemia. Usually due to defects of hepatic excretion. It can be detected by measuring the direct bilirubin component of the total bilirubin (>50% confirms the presence of conjugated hyperbilirubinaemia).

*Unconjugated***:**

- Haemolysis - check reticulocyte count, blood film, haptoglobins, LDH and may need direct Coombs' test. Liaise with haematologist.
- Drugs.
- Gilbert's syndrome.
- Crigler-Najjar syndrome.

*Conjugated***:**

- Dubin-Johnson syndrome.
- Rotor syndrome.
- Chronic liver disease, (usually associated with other liver function test (LFT) abnormalities).

Obstructive picture or cholestasis: rise in ALP and GGT more than AST and ALT. This may be intrahepatic or extrahepatic (bilirubin will also be raised).

- ➢ *Intrahepatic*:
- Primary biliary cirrhosis.
- Drugs.
- ➢ *Extrahepatic*:
- Gallstone in common bile duct.
- Head of pancreas neoplasm.
- Drugs, eg erythromycin, tricyclic antidepressants, flucloxacillin, oral contraceptive pill and anabolic steroids.
- Cardiac failure - improves with treatment.
- Primary biliary cirrhosis - more common in women and the first sign is a rise in ALP.

- Primary sclerosing cholangitis.

- Neoplasm - primary (rarely) and secondaries.

- Familial (benign).

Hepatitic picture: rise in AST and ALT more than ALP and GGT:

- Alcohol - fatty infiltration and acute alcoholic hepatitis (usually associated with markedly deranged liver function).

- Cirrhosis of any cause - alcohol being one of the most common.

- Medications, e.g. phenytoin, carbamazepine, isoniazid, statins, methotrexate, paracetamol overdose, amiodarone. (Transaminases may be >1000 IU/L).

- Chronic hepatitis B and C.

- Acute viral hepatitis, e.g. hepatitis A, B and C and CMV infection.

- Autoimmune hepatitis.

- Neoplasms - primary or secondaries.

- Haemochromatosis.

- Metabolic - glycogen storage disorders, Wilson's disease.

- Ischaemic liver injury, eg severe hypotension,

- Fatty liver disease (mild elevation in transaminases <100 IU/L).

- Non-hepatic causes: coeliac disease, haemolysis and hyperthyroidism.

Isolated rise in individual enzymes: for example, ALP and GGT:

➢ Isolated rise in GGT:

- This is most commonly due to alcohol abuse, or enzyme-inducing drugs.

- An isolated rise can occur even if there is no major liver disease.

- The rise is not related to the amount of alcohol intake.

- Also, many heavy alcohol users may have normal GGT.

- Stopping alcohol for 4 weeks should rectify the abnormality.

➢ Isolated rise in ALP:

- Third trimester of pregnancy (comes from the placenta - a normal finding).

- If isolated rise in ALP, consider other sources, eg bone or kidney.

- In the elderly consider:

- Fractures

- Paget's disease of bone

- Osteomalacia

- Bony metastases

ALP is not usually raised in myeloma or osteoporosis (without a fracture).

Occasionally, the liver enzymes, eg ALP, GGT, AST or ALT may all be similarly elevated making it difficult to determine whether it is a cholestatic or hepatitic picture.

2.4 EPIDEMIOLOGY OF LIVER DISEASES

Not much work has been done on Epidemiological studies of Liver Diseases in the state of Gujarat or in India. None of the studies available were analytical or comprehensive and descriptive enough. However internationally, some good studies have been conducted under observation of recognized institutions or bodies. Some of the other studies are a good analytical picture published as a paper or as part of the Thesis work. Various studies are available for Europe, Canada, UK and USA. Studies are also available for Pakistan and China.

Blachier et al., *performed a study on the burden* of liver diseases in Europe in 2013 as a review of available data. The reviewed several epidemiological studies over the period of 30 years and classified them disease wise for countries in the primary structure. The study summarizes the currently-available data on the incidence and prevalence of the main liver diseases in Europe. They concluded that data in the literature is scarce and that more efforts should be made to fully understand the extent of the burden of liver disease in Europe.

Mann et al., performed a study on The Epidemiology of Alcoholic liver disease in USA in 2003. This article describes the various forms of alcoholic liver disease (ALD), with particular emphasis on cirrhosis. The study states that gender and ethnic differences also account for some important variations in rates of liver disease. Mortality rates from cirrhosis have declined in the United States and some other countries since the 1970s. A number of factors may have contributed to this decline, including increased participation in treatment for alcohol problems and Alcoholics Anonymous membership, decreases in

alcohol consumption, and changes in the consumption of certain types of alcoholic beverages.

Kim et al, Summary of a workshop on Burden of liver disease in the United States in 2003 have combined in their study sevral short diseasewise studies of liver epidemiology to form a comprehensive one. And has good collection of data from history till date.

Srivatanakul et al., in their mini review on Epidemiology of Liver Cancer: An Overview, 2004 have done global study of liver epidemiology and distributed their data continent wise based on the causes. In the study they showed that the incidence of liver cancer varies widely throughout the world, with high rates in sub – Saharan Africa, eastern and southeastern Asia, and Melanesia and a low incidence in Northern and Western Europe and the Americas. Several important risk factors have been demonstrated, including chronic infection with hepatitis B and C viruses and other environmental factors, such as exposure to aflatoxin, consumption of alcohol, and cigarette smoking. By contrast, cholangiocarcinoma is less common, accounting for only 7.7% of malignant tumors of the liver in the United States. However, in parts of Southeast Asia, cholangiocarcinoma occurs more frequently; it is responsible for more than 60% of liver tumors in northeastern Thailand. The geographic distribution worldwide coincides with endemic areas of the liver flukes, Opisthorchis viverrini and Clonorchis sinensis. The interaction between genes and the environment and the interplay of environmental factors, which include diet and other lifestyle parameters, illustrate the complexity underlying susceptibility.

Fedeli et al., performed a detailed study on Descriptive epidemiology of chronic liver disease in northeastern Italy: an analysis of multiple causes of death, 2013. In the study, The crude mortality rate of all C LD was close to 40 per 100,000 residents. In middle ages (35 to 74 years) C LD was mentioned in about 10% and 6% of all deaths in males and females, respectively. Etiology was unspecified in about half of C LD deaths. In females and males, respectively, HC V was mentioned in 44% and 21% and alcohol in 11% and 26% of overall CLD deaths. A bimodal distribution with age was observed for HC V-related proportional mortality among females, reflecting the available seroprevalence data.

Zhou ET AL., performed Prevalence of fatty liver disease and its risk factors in the population of South China IN 2007. Among the subjects they studied, the prevalence of confirmed alcoholic liver disease (ALD), suspected ALD and nonalcoholic fatty liver disease (NAFLD) were 0.4%, 1.8%, and 15.0%, respectively. The prevalence rate (23.0%) was significantly higher in urban areas than (12.9%) in rural areas. After adjustment for age, gender and residency, the standardized prevalence of FLD in adults was 14.5%. Among them, confirmed ALD, suspected ALD and NAFLD were 0.5%, 2.3%, and 11.7%, respectively, in adults and 1.3% (all NAFLD) in children at the age of 7-18 years. The overall prevalence of FLD increased with age in both genders to the peak of 27.4% in the group of subjects at the age of 60-70 years. The prevalence rate was significantly higher in men than in women under the age of 50 years (22.4% vs 7.1%, $P < 0.001$). However, the opposite phenomenon was found over the age of 50 years (20.6% vs 27.6%, $P < 0.05$).

Perz et al., studied the contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide in 2006 under the WHO. They found that globally, 57% of cirrhosis was attributable to either HBV (30%) or HCV (27%) and 78% of HCC was attributable to HBV (53%) or HCV (25%). Regionally, these infections usually accounted for >50% of HCC and cirrhosis. These fractions represent 929,000 deaths due to chronic HBV and HCV infections, including 446,000 cirrhosis deaths (HBV: $n = 235,000$; HCV: $n = 211,000$) and 483,000 liver cancer deaths (HBV: $n = 328,000$; HCV: $n = 155,000$). Country wise data are represented in charts and the data obtained from the study in India closely resembles the infection pattern in our study.

Irshad et al., studied the Prevalence of viral HBV and HCV among different group patients in Gujarat Pakistan. The study population was divided into 4 age groups each comprising of 100 individual patients and analyzed for different parameters for the presence of HBV and HCV in comparison with positive and negative controls. The prevalence of HBV and HCV was higher in groups 2 (22%) and 4 (39%) respectively. Assay profile revealed that the incidence of HCV was higher in fe- male patients as compare to the male patients. The present study indicates that more than 60% of the cirrhosis and hepatocellular carcinoma in the Region is attributable to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection.

Kalra et al., performed study for of Prevalence of Nonalcoholic Fatty Liver Disease (NAFLD) in Type 2 Diabetes Patients in India (SPRINT) for India in 2006

Prevalence of the disease was found to be higher in females (60%) than in males (54.3%) T2DM patients; with prevalence of NAFLD varying from 44.1% in western India to 72.4% in northern states. In our study the prevalence of NAFLD increased with increasing age, with 239(45.8%) identified patients in age group of 25-50 years and 283(54.2%) among those aged 51 years the study reinforced the well established clinical association of NAFLD with elements of metabolic syndrome (MetS) including dyslipidemia, hypertension and obesity; as T2DM population with these co-morbid conditions had 38%, 17% and 14% higher risk respectively, for NAFLD.

The mean AST and ALT levels were 54.8 ± 36.1 IU/L and 55.6 ± 39.8 IU/L, respectively in NAFLD population and highest in age group of 25-40 years and lowest in 71-84 years age group. Mean ALT levels were found to be higher than mean AST levels across all age groups in identified T2DM NAFLD cohort, with 340(65.3%) patients having elevation of both AST and ALT levels.

Shah and Mulla performed a study on PREVALENCE OF HEPATITIS D VIRUS (HDV) IN SOUTH GUJARAT. Out of 141 HbsAg positive patients 12 patients were positive for anti-HDV ELISA. High prevalence rate was found in middle aged man. HBV-HDV infection togather cause more severe liver damage HDV infection was more associated with blood tranfusion.

3. <u>METHODOLOGY</u>

3.1 <u>STUDY DESIGN</u>

The epidemiological study performed is <u>*Prevalence*</u> study performed in a limited set up to study the trends in Liver diseases in a manner comprehensive enough so that can be extrapolated to the population of the city. In nature, it is a <u>*Descriptive Observational*</u> study that is presented more or less in a manner of <u>*Case Series*</u> where detailed description of the patient was filled in as a questionnaire from the available patient data. It is a <u>*Cross-Sectional*</u> study lasting a period of approximately a little less than one academic year. (Approx. 7 months).

Thus, to classify, the given study is a Descriptive Observational Prevalence Study in form of a Case Report Series and is performed as a Cross-sectional study.

All incidents of hospitalization due to liver diseases were studied. In other words, all the Patients admitted to the Hospital for Gastro-enterological problems or those admitted under Dr. Prashant Buch during the period of study (august 2013- February 2014) formed the Sample set and were reported as a part of the study. The cases of liver disease among them being identified and studied in detail as cases for possible interpretations. A total of over 200 GI patients with around 150 liver patients were studied.

Patient age, gender, background, history, chief complaint, condition on arrival, during stay and at discharge, patient reports and medical sheets at various stages during hospital stay were studied for all patients.

3.2 <u>MATERIALS AN REQUIREMENTS</u>

Permissions for following were obtained:

- ➢ Access to case files of all the patients admitted for GI problems in the hospital between the time-span of August 2013 to May 2014.
- ➢ Access to wards and admitted patients during the period of study.
- ➢ Review of study protocol ethics committee or a review board.

Required pattern of data entry and questionnaire was prepared

Patient privacy and confidentiality shall be maintained at all levels and patient name, address or contacts shall not be revealed at any stage during the study.

3.3 <u>SETTINGS AND LOCATIONS</u>

A tertiary care trust hospital in the city was chosen for the purpose. A trust hospital would ensure population from all sections of the society so that a wide range of variations is obtained. A tertiary care unit would ensure patients from every strata of disease progression.

3.4 <u>PARAMETERS</u>

Following parameters for all patients were considered

    3.4.1   Age, gender,

    3.4.2   demography, sociology, family history, social history

    3.4.3   disease- hepatic- non hepatic, specific, associated complications,

    3.4.4   medications,

    3.4.5   serologic reports

        3.4.5.1 LFT: SGOT, SGPT, Alk. PO_4 , GGT, S. Bilirubin, S. protein analysis

        3.4.5.2 Blood: Hb, RBC, Plt, TC, DC, HCT, MCV, MCH, MCHC, RDW

        3.4.5.3 Electrolytes: Na^+ , K^+ ; Creatinin, urea,

        3.4.5.4 Lipid profile, CRP, AFP

As was necessary.

3.5 <u>METHOD</u>

The general working methodology consisted of following steps:

3.5.1 Sample- size selection

For all calculations of incidence and prevalence the ideal scenario is a truly population-based approach but this is seldom achieved. However, it may be possible to conduct specific studies on a representative sample of a population, which was chosen to be all number of hospitalizations due to liver diseases in the given period of study, in the given set- up.

3.5.2 Defining the disease

Several methods may be employed which in isolation or combination lead to the diagnosis of a particular disease, or indeed the assertion that a particular disease is not present in an individual. These include the identification of histological changes in cell or tissue architecture, the presence or absence of clinical signs and symptoms, and the use of test results, such as laboratory diagnostic tests including blood serology. Frequently such signs, symptoms and tests in isolation are indicative of a disease but are not sufficient to warrant a formal diagnosis. As such the presence of signs and symptoms or positive tests can often be used as a measure of undiagnosed disease. In almost all circumstances to acquire a diagnosis a person must be seen by a health care professional of some sort.

Liver disease covers a broad spectrum of diseases including acute and chronic liver diseases. The International Classification of Diseases (ICD-10) includes within the specific section for liver disease (K70-K77) alcoholic liver disease (K70), toxic liver disease (K71), hepatic failure (K72), chronic hepatitis (K73), fibrosis and cirrhosis of liver (K74), inflammatory liver diseases (K75) and other diseases of liver (K76 including fatty liver K76.0). In addition, viral hepatitis (B15-19) and neoplasms of the liver and intrahepatic bile ducts (C22) are classed as liver diseases.

For the intended study, Liver Diseases was defined as any of the above or otherwise, leading to altered liver structure and/ or function/s.

3.5.3 Identification of person with disease

The diagnosis was done by the specialist doctor; and all cases identified as liver disease were reported.

Date of onset

Frequently in epidemiological studies the identification of cases of disease is a pragmatic one where the date on which a subject acquires a diagnosis of a particular disease is considered the date of disease onset.

Diagnosis

The diagnosis of liver diseases is often based on a combination of clinical, ultrasound, biochemical and histological findings but liver biopsy is still considered the „gold-standard‟ for the diagnosis of cirrhosis.

Other than signs and symptoms,

1) various serological tests: LFTs, routine blood tests, antibodies test (IgG; IgM; HBsAg etc.) and

2) Imaging tests: USG; CT; MRI; endoscopy etc. are used.

3.5.4 Reporting the cases

Various patient details like age, gender, social background, demography, family history, social and medical history, etc. are reported.

Identity

Since patient confidentiality must be maintained at all times, patients IP number was used as identity.

Age

The age of the patient in whole years at the time of admission.

The patients were classified in three major age groups in the study for the purpose of relevance, namely,

1) < 40 years

2) 40- 60 years

3) > 60 years

Sociology/ socio- economic status:

Patients were divided into four categories based on their socio- economic status.

1) Urban
2) Semi- urban
3) Semi- rural
4) Rural

The decision regarding the matter was made based on one or more of the following:

1) Direct interaction with the patient.

2) Questioning the patient; looking at the patient

3) Considering the ward of admission.

Alcoholic

Any past consumption of alcohol, habitual or otherwise; chronic or occasional, in how much ever quantity and any such history, irrespective of the present status. Drinkers who have given up drinking also fall in this criterion.

3.5.5 Compiling cases

Each case was prepared by entering all available details in a fixed proforma. All such cases were compiled and filed and thus a case file was created. This compilation was then transferred to a tabulated EXCEL sheet and analysis was eventually performed. Subsequently data on patients with cirrhosis and other Liver diseases were extracted and all of the data management and analysis in the studies were performed, that included in this thesis. I also conceived various ideas on determining prognosis of disease based on available data, using available data and forming new relationships between them; and also the idea of relating various scores determining prognosis of disease to each other and relevant parameters. The attempt also was to look for new markers of defining the disease, new associations, which was successful as well.

3.5.6 Classifying the cases

All the GI patients amongst those admitted in the given time span were identified and categorized as:

1) Those with liver diseases and

2) Those without liver diseases

The patients with hepatic diseases thus identified were studied in detail on day to day basis, from admission to discharge. The case file is looked into for necessary details on above mentioned parameters.

The disease can be identified and categorized as either of:

1.) Infections and jaundice

2.) CLD & fatty liver

3.) Liver parenchymal disease and cirrhosis

4.) Failure & carcinoma

5.) Other diseases

It is further categorized as *Primary* or *secondary.*

3.5.7 Co-relating parameters

Causes of developing the condition were tried to be found out and various complications associated with the disease are looked upon. For example: associated DM, HTN, hepato-renal syndrome, ascites, varices and hepatic encephalopathy.

Co-relation and inter dependence of various complications with the diseases are studied.

The treatment given and the laboratory reports of various tests at different times during admission periods are also noted down and clinically co-related with patient conditions.

All the relevant details are noted down in a fixed preformat as data compilation. For patients of pre-date and those who were admitted and discharged between timespan of consecutive visits, files and case summaries (discharge/death summaries) are looked into.

ALT/AST and AST/ALT Ratios:

From the literature, it is known that if ALT > AST, chronic liver diseases are indicated. However, AST > ALT indicates progression of the disease to Cirrhosis. When AST is twice as much as ALT or more, it indicates prolonged liver damage or acute liver damage

out together with high GGT values. AST/ ALT and ALT/AST values were calculated and assessed. Based on the outcomes following conclusions were made.

ALT/AST > 1, Chronic liver diseases.

AST/ALT> 1, Acute onset disease or progression to Cirrhosis

AST/ALT> 2, Alcohol abuse

AST/ALT< 1, non-alcoholic disease.

AST+ALT/ALP+GGT

When AST and ALT are more altered compared to ALP and GGT, it presents the hepatic picture of the diseases and signifies:

- Alcohol - fatty infiltration and acute alcoholic hepatitis (usually associated with markedly deranged liver function).

- Cirrhosis of any cause - alcohol being one of the most common.

- Medications, eg phenytoin, carbamazepine, isoniazid, statins, methotrexate, paracetamol overdose, amiodarone. (Transaminases may be >1000 IU/L).

- Chronic hepatitis B and C.

- Acute viral hepatitis, eg hepatitis A, B and C and CMV infection.

- Autoimmune hepatitis.

- Neoplasms - primary or secondaries.

- Metabolic - glycogen storage disorders, Wilson's disease.

- Ischaemic liver injury, eg severe hypotension,

- Fatty liver disease (mild elevation in transaminases <100 IU/L).

When ALP and GGT are comparatively more altered it represents the biliary picture and signifies:

- *Intrahepatic disorders:*

- Primary biliary cirrhosis.

- Drugs.

➢ *Extrahepatic disorders*:

- Gallstone in common bile duct.

- Head of pancreas neoplasm.

- Drugs, e.g. erythromycin, tricyclic antidepressants, flucloxacillin, oral contraceptive pill and anabolic steroids.

- Cardiac failure - improves with treatment.

- Primary biliary cirrhosis - more common in women and the first sign is a rise in ALP.

- Primary sclerosing cholangitis.

- Neoplasm - primary (rarely) and secondaries.

- Familial (benign).

Based on this knowledge, a formula was devised as:

$$AST+ALT- 105/ ALP+ GGT- 155$$

Where 105 is the sum of upper limits of ALT and AST (65+ 40)

And 155 is the sum of upper limits of ALP and GGT (100+ 55)

A value between -2 and 2 would define the obstructive picture while mode values greater than 2 would define a hepatic picture in most cases. The tabulated data verifies the same.

SCORE ANALYSES

Various scoring system are used to assess severity of liver diseases and its complications.

Maddrey's Discriminant Factor (DF score):

The Maddrey's discriminant function is used to predict prognosis in alcoholic hepatitis and identify patients suitable for treatment with steroids

It is calculated by a simple formula:

(4.6 X (PT test - control)) + S. Bilirubin in mg/dl

Prospective studies have shown that, it is useful in predicting short term prognosis especially mortality within 30 days. A value more than 32 implies poor outcome with one month mortality ranging from 35% to 45%. (Forest et al., 2005)

In case of DF of 32 or more

- consider liver biopsy to confirm diagnosis

- offer corticosteroid treatment

Child- Pugh's Score:

Used for prognosis of liver cirrhosis to predict mortality as well as the required strength of treatment and the necessity of liver transplantation. The scoring is done as follows.

Table 3.1: CP score calculation

Measure	1 point	2 points	3 points
Total bilirubin, μmol/l (mg/dl)	<34 (<2)	34-50 (2-3)	>50 (>3)
Serum albumin, g/dl	>3.5	2.8-3.5	<2.8
PT INR	<1.7	1.71-2.30	> 2.30
Ascites	None	Mild	Moderate to Severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

The scores are classified as class A, B or C and are interpreted as under:

Table 3.2: Interpretation of CP score

Points	Class	One year survival	Two year survival	Abdo. Surgery Peri- operative mortality	Comments
5-6	A	100%	85%	10%	Life expectancy: 15- 25 years
7-9	B	81%	57%	30%	Transplant evaluation indicated.
10-15	C	45%	35%	82%	Life Expectancy: 1-3 years.

MELD and MELD- Na scores:

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. It was initially developed to predict death within three months of surgery in patients who had undergone a transjugular intrahepatic portosystemic shunt (TIPS) procedure, and was subsequently found to be useful in determining prognosis and prioritizing for receipt of a liver transplant. This score is now used by the United Network for Organ Sharing (UNOS) and Euro transplant for prioritizing allocation of liver transplants instead of the older Child-Pugh score. As defined by UNOS, The Model for End-Stage Liver Disease (MELD) is a numerical scale ranging from 6 (less ill) to 40 (gravely ill), used for liver transplant candidates age 12and older. It gives each person a 'score' (number) based on how urgently he or she needs a liver transplant within the next three months. The number is calculated by a formula using three routine lab test results:

MELD uses the patient's values for serum bilirubin, serum creatinine, and the international normalized ratio for prothrombin time (INR) to predict survival. It is calculated according to the following formula:

MELD = $3.78 \times \ln[$serum bilirubin (mg/dL)$] + 11.2 \times \ln[$INR$] + 9.57 \times \ln[$serum creatinine (mg/dL)$] + 6.43$

Or

MELD Score = (0.957 * ln(Serum Cr) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10 (if hemodialysis, value for Creatinine is automatically set to 4.0)

If the patient has been dialyzed twice within the last 7 days, then the value for serum creatinine used should be 4.0

Any value less than one is given a value of 1 (i.e. if bilirubin is 0.8, a value of 1.0 is used) to prevent the occurrence of scores below 0 (the natural logarithm of 1 is 0, and any value below 1 would yield a negative result).

Patients with a diagnosis of liver cancer will be assigned a MELD score based on how advanced the cancer is.[citation needed]

In interpreting the MELD Score in hospitalized patients, the 3 month mortality is:

40 or more — 71.3% mortality

30–39 — 52.6% mortality

20–29 — 19.6% mortality

10–19 — 6.0% mortality

<9 — 1.9% mortality

A modification in MELD score is the MELD-Na score. It incorporates sodium levels for score calculation and is said to have yield better associating results. It is calculated as follows:

MELD-Na = MELD Score - Na - 0.025*MELD*(140-Na) + 140

All the three scores relate poorly to each other and are only approximate in giving idea of severity and prognosis of liver diseases showing their limitations and need for a better more comprehensive system of scoring.

The study differs from other studies performed across the globe, especially those done for UK; whole of Europe, USA and Canada in that Alcoholism continues to be the greatest cause of Liver diseases in the developed countries. There are several studies linking the prevalence to alcohol consumption pattern and habits in these countries.

3.5.8 Determining burden of the disease

The burden of disease, or the burden of a specific disease, can encompass a wide range of measures. These include:

1) the frequency of disease,

2) the mortality from disease (and consequent years of life lost),

3) the morbidity associated with disease, and

4) Aspects of societal burden such as the financial and service requirements associated with disease.

3.5.9 Data analysis and Application of necessary statistical tool for further analysis

Necessary details were used and the data was organized into the required form. MS Excel tabulation was done and necessary categorizations were obtained.

Necessary statistical tools were to be used and a clinical outcome was obtained regarding prevalence, demography, and causes and co-related parameters in forms of means, frequency, and magnitude and as apt for the data set.

4. <u>RESULTS</u>

In the present study the data shows that, the ratio of liver diseases is significantly high in the city with 135 cases of liver diseases out of 200 GI patients in one hospital of the city in a span of seven months. Considering the expanse of the city, and number of tertiary care hospitals available, such a data from only one hospital amounts to a huge risk of liver diseases in the city. Most of these cases are cases of Non- alcoholic Liver Diseases with alcoholic cases comprising of only 25% of total cases (which is not a small number though). Around 20% of all cases are due to viral infection. The finding follows the general trend with majority of cases being male (77.77%) and only less than one fourth of all cases being females. A major chunk of patients are above the age of 40 (>80%), most belonging to age group of 40- 60 years (about 50%). The mean age for hepatic diseases is 51.69 as compared to 52.17 for all patients with GI diseases. Expectedly, the mean age for males with GI diseases is 50.3 years, less than the 56.81 years for females. Urban populations are more susceptible to liver diseases.

4.1 <u>DISEASE WISE CLASSIFICATIONS</u>

A disease wise demographic classification of the data was made. In general, the diseases observed were: Acute over chronic liver failure (ACLF), Liver parenchymal disease (LPD), and Alcoholic liver disease (ALD), Chronic liver disease (CLD), Liver Cirrhosis, CBD stone, GB calculi, Hepatocellular Carcinoma (HCC), fatty liver, Viral hepatitis and Liver abscess.

Majority of patients admitted during the period were of liver cirrhosis (45), followed by Liver parenchymal diseases which is inclusive of ALD, CLD and fatty liver (35 cases, i.e. approximately 25%). Viral hepatitis accounts for nearly 9 % of the cases (11 cases). On the other hand, CBD stone and ACLF have only 2 cases each. Cirrhosis and ALD and CLD have majority of admissions as males. The mean ages are lowest for ALD and viral hepatitis. Cirrhosis and LPD constitute the highest amount of population above 60. 10 out of 45 cases of cirrhosis is due to viral infection.

A summary of disease wise classification of demographics is tabulated below in tables 4.1.1 and 4.1.2

Table 4.1.1: Disease wise classification of Demographics

DISEASE	Total	ACLF	ALD	CLD	CBDS	Cirrhosis	HCC	fatty liver	LPD	GB	Hepatitis viral	liv. abscess
No.	135	2	9	26	2	45	13	4	35	3	11	7
MALE	105	0	9	23	2	37	10	3	29	1	7	5
FEMALE	30	2	0	3	0	8	3	1	6	2	4	2
BELOW 40	26; 2f	0	5	3	0	7; 1f	1	2	9	1	4; 2f	1
40-60	69; 13f	1	4	14; 3F	0	22; 3f	8; 2f	1	20	0	6; 1f	2; 1f
ABOVE 60	40; 13f	1	0	7; 1f	2	15; 3f	2	1; 1f	8	2; 2f	1f	4; 1f
Alcoholic	34; 4 f	1	9	8	0	17; 2f	1	1		0	0	0
Viral	26; 5f	1	0	3	0	10; 1f (4B; 7C)	4	0		0	8; 2f (2A; 3B; 3C; 3E)	0
mean age	52.17	0	0	0	0	0	0	0	0	0	0	0
mean age for hepatic pts.	51.69	64	40.22	51.82	62.5	53.04	53.81	44.25	51.91	58.25	42.9	53.33
mean age- male	50.3	0	40.22	51.26	62.5	52.25	55	38	50.79	28	41.28	50
m. age- female	56.81	64		49.33	0	54.71	48	63	57.33	68.33	50	60

Table 4.1.2: Association of Socio-Economic Status to Liver Diseases

DISEASE	Total	ACLF	ALD	CLD	CBDS	Cirrhosis	HCC	fatty liver	LPD	GB	Hepatitis viral	liv. abscess
No.	135	2	9	26	2	45	13	4	35	3	11	7
Urban	32; 11f	1	3	3	1	9; 1f	5; 2f	3; 1f		1; 1f	3; 3f	0
Rural	21; 6f	0	0	3	0	6; 2f	1	0		1; 1f	2; 1f	3
Semi Urban	41; 6f	1	5	8	1	14; 2f	4	1		1	4	3; 1f
Semi Rural	22; 3f	0	1	5	0	8; 1f	1	0		0	2	2; 1f
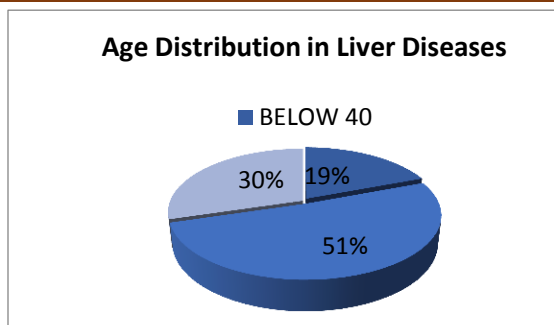
Fig. 4.1 Age Distribution in Liver Disease
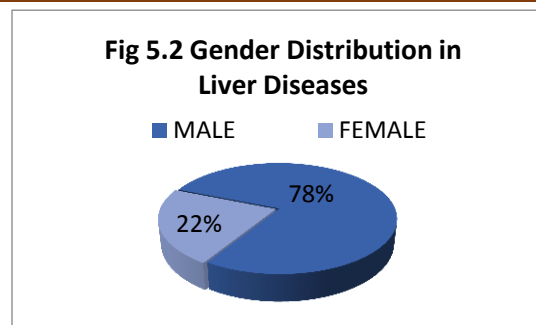
Fig. 4.2 Gender Distribution in Liver Diseases
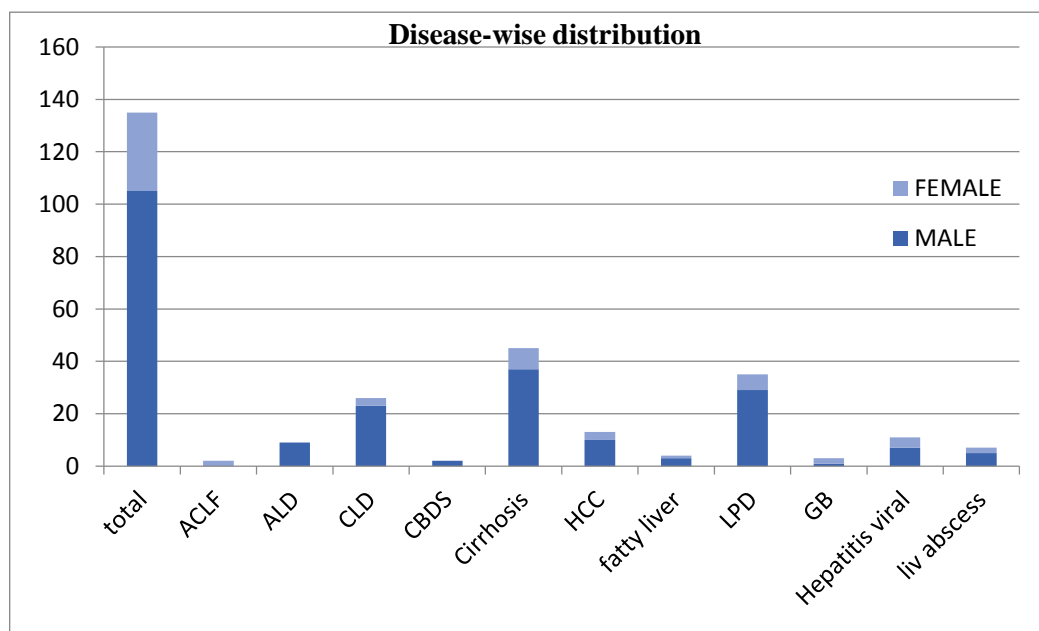
Fig. 4.3 Disease-wise Classification

4.2 ASSOCIATION OF VARIOUS CO- MORBIDITIES TO LIVER DISEASES

Various co-morbidities associated with liver diseases are tabulated as follows in Table 4.2. Expectedly, Diabetes and CVS disorders are most correlated comorbidities with 32 and 26 cases respectively (24% and 19.25%). Most co-morbidities are seen over the age of 40. Various articles also show correlations with thyroid disorders as is confirmed in the study. The correlation is almost equal in both males and females. However the mean age at correlation is very high, i.e. 64.89 years. Carcinoma is also linked to failure if liver with mean age being as low as 49.7 years and is equally correlated in both males and females. Infection, sepsis or SBP are associated comorbidities in patients of comparatively lower

age. CVS is co-morbidity in patients at mean age of 62 years, whereas DM is associated at a relatively lower age of 59 years. Maximum correlation in females is observed for CVS disorders.

Table 4.2: Co-morbidities associated with liver diseases

Co-morbidity	Frequency	M	F	<40	40- 60	>60	Avg. Age
Renal	14	11	3	3	8	3	52.14
CVS	26	16	10	0	13	13	61.84
DM	32	25	6	2	16	14	58.93
Hypothyroid	9	5	4	0	4	5	64.89
Pancreatic	9	6	3	3	3	3	52
H. obstr/ portal HT	8	6	2	2	3	3	50.125
Carcinoma	9	5	4	1	6	2	49.75
Infection/ sepsis/ SBP	16	13	3	6	6	4	48
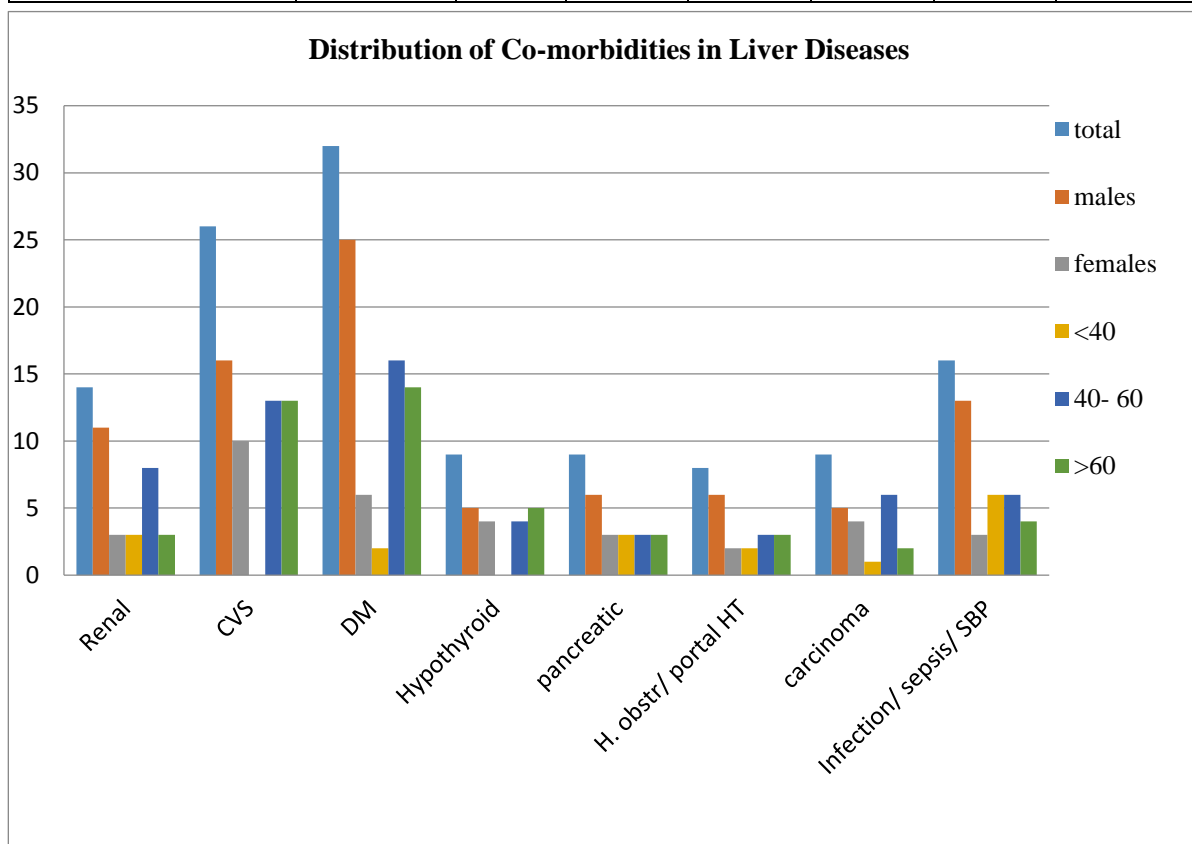
Fig. 4.4 Distribution of Co-morbidities in Liver Diseases

4.3 SEASONAL VARIATION IN LIVER DISEASES

No much correlation with seasonal variation was observed among any of diseases. However monsoon is related to higher incidences of viral diseases. Hepatitis E was observed with a higher rate in the month of September. A general rise in liver diseases was observed in festive months of August, November and December.

Month wise distribution of liver diseases is summarized in Table 4.3

Table 4.3: Month wise distribution of liver diseases

MONTH	HEPATIC	Male	female	below 40	40- 60	above 60	ALD	CLD	Cirrhosis	HCC	Hepetitis	FLD	Abscess	LPD	Obs/calculi	Other
July	5	4	1	1	3	1	0	0	4	0	0	0	0	0	0	1
Aug	27	20	7	6	16	5	0	2	8	1	3	1	1	2	2	7
Sept	15	12	3	3	8	4	1	1	6	1	2	2	0	4	0	0
Oct	12	8	4	2	8	2	0	2	4	3	1	0	0	2	1	1
Nov	21	16	5	2	13	6	2	7	6	4	1	0	0	9	1	1
Dec	20	15	5	5	9	6	0	4	5	1	3	1	3	7	2	0
Jan	15	14	1	3	8	4	3	1	6	2	1	0	1	4	1	0
Feb	15	13	2	5	4	6	0	5	5	0	2	0	1	8	2	0

4.4 COMPLICATIONS ASSOCIATED WITH LIVER DISAESES

Varices, ascites and encephalopathy are major complications of liver diseases. More than half the admitted patients have either one or the other of these complications. Alcohol consumption is related to decrease in mean age of onset of these complications. Death occurred in 5 cases. Complications are tabulated in brief in Table 4.4

Table 4.4: Complications of Liver Diseases

Complications	No.	M	F	<40	40-60	>60	Avg. Age	ALC	Alc.Mean Age	Viral
Ascites	55	43	12	11	31	13	51.82	19	44.47	11
Varices	31	27	4	8	17	6	51	17	44.71	6
both A+V	15	12	3	4	10	1	49.6	10	45.6	4
Encephalopathy	13	9	4	1	8	4	56.15	3	50.66	2
Death	5	2	3	1	3	1	52.4	2	45	1
Infection/ sepsis/ SBP	16	13	3	6	6	4	48	6	47.8	4

4.5 CORRELATION TO BIOCHEMICAL PARAMETERS AND ITS ANALYSIS

4.5.1 Parameters traditionally included in LFTs

The parameters that are generally correlated to liver diseases include ALT (SGPT), AST (SGOT), ALP, GGT, Bilirubin levels. Generally a more than two times altered value compared to the normal range is associated significant pathology. As a general observation, all these parameters are abnormal in all liver disease cases.

Considering patients with alcoholic history, as can be observed, specifically the GGT levels are significantly high (mean: 181.7) with highest value reaching as high as 611 U/L. The normal values of GGT being 5- 55 U/L. The same mean value for non-drinkers was found to be around 79. High values of GGT thus indicate correlation to high alcohol intake. Higher the levels of liver damage due to alcohol consumption, higher the GGT values. An even further shot up levels of GGT (> 180 U/L) would indicate ALD. This confirmed by the finding of mean GGT values of ALD (mean= 223.05). Another point to be noted is that the ALP levels in alcoholics, however, although high, the mean value is not twice as much as the normal upper range.

On the contrary, considering the cases of viral manifestations in liver disease, ALP values are extremely high (mean: 242.32); whereas the GGT value, although high (mean= 98.79), is considerably lesser than in alcoholic cases (even without deducting alcoholic cases in those infected by the virus).

Another point of distinction lies in the ALT and AST levels of the two groups. Although ALT and AST levels are significantly high in both groups, as can be seen from the table (Table 4.6), these levels are at least two folds higher in cases of viral diseases. Also, looking at the data of ALD and CLD, the values tend to be higher in CLD as compared to acute onset ALD. The values tend to be as high as 2425 IU/L (mean value: 623.3) for ALT and 1204 IU/L (mean value: 316) for AST in cases of hepatitis. This would thus confirm affirmatively and amply that ALT and AST would be basically excellent markers for liver inflammation.

Looking at the bilirubin levels, they are markedly high in all liver conditions and serve as a good marker of liver function; the highest mean values being 15.89 for total bilirubin and 12.89 for direct bilirubin, both for hepatitis. In both, viral conditions and alcohol history, the bilirubin levels are significantly high, more so in case of non-chronic ALD. The basic difference however lies in that the proportion of direct bilirubin is much more elevated in viral diseases as compared to alcoholic disease. This is even more eminent in obstruction diseases.

Having a look at the obstruction diseases, the only parameters significantly high are the bilirubin levels, more specifically the direct bilirubin, thus making direct bilirubin a major marker for obstructions in the hepatic system, may it be due to GB calculi, CBD stones, other blockage, IBHR or CHD dilations, increased pressure, etc.

Looking at cirrhosis, all parameters tend to be more or less normal with no major abnormal values, same being the case with fatty liver diseases.

Table 4.5: Normal values of relevant parameters

HCT	RDW CV	RDW SD	Billi T	Billi D	Billi I	SGPT (ALT)	SGOT (AST)	ALP	GGT
(40- 50)	(11.6- 14)	(39- 46)	(0- 1)	(0- 0.3)	(0- 0.7)	(30- 65)	(0- 40)	(25- 100)	(5- 55)

Table 4.6.1: Correlation of Liver Disease to LFT

PARAME-TERS	Billi. T	Billi. D	SGPT (ALT)	SGOT (AST)	ALP	GGT
Total hepatic	6.82 (0.91)	4.98 (0.75)	107.73 (17.52)	156.4 (20.11)	177.3 (14.69)	106.84 (15.46)
Alc.	8.43 (1.62)	6.15 (1.37)	111.96 (23.90)	196.45 (15.27)	187.79 (24.65)	181.7 (47.42)
Viral history	8.07 (2.46)	6.33 (2.46)	285.73 (2.05)	205.93 (16.71)	242.316 (26.87)	98.79 (26.49)
Cirrhosis	5.43 (1.27)	3.29 (0.82)	56.39 (7.92)	84.98 (11.77)	122.604 (11.79)	90.47 (34.91)
Hepatitis	15.89 (2.61)	12.89 (1.80)	89.6 (58.87)	139.13 (28.36)	284.6 (26.25)	126.8 (31.90)
Obstruction	2.3 (1.31)	2.125 (1.30)	58.6 (20.58)	35 (9.66)	197 (42.67)	37.33 (7.08)
ALD	11.195 (2.75)	8.36 (2.08)	46.7 (100.11)	126.1 (31.27)	168.85 (38.29)	223.05 (51.50)
CLD	4.55 (1.51)	3.49 (0.96)	86.29 (6.02)	86.9 (19.52)	213.2 (47.02)	120.9 (10.58)
LPD	6.08 (1.34557)	4.14 (1.07669)	70.96 (5.74661)	86.55 (15.5932)	195.2 (38.4804)	119.3 (27.401)
fatty liver	0.45 (0.25)	0.25 (0.05)	58.5 (22.5)	29 (14)	114.5 (1.5)	78 (3.0)

Differential diagnosis using LFTs can be done as under:

Table 4.6.2 correlation of parameters to diseases for differential diagnosis

PARAMETERS	GGT	ALP	AST	ALT	BILLI T.	BILLI. D
ALD	↑↑	↑	↑	↑	↑↑	↑↑
Viral	↑	↑↑	↑↑	↑↑	↑	↑↑
CLD	↑↑	↑	↑↑	↑↑	↑	↑

4.5.2 Correlation of Liver Diseases to other biochemical parameters

Other than Hb, INR, Na^+ levels, A:G ratio, and the usual LFTs, the parameters that seemed to associate quite well to the deterioration of the liver functions were the Hematocrit (HCT) levels and the Red cell Distribution width (RDW- CV and RDW- SD) levels.

The hematocrit, also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF) is the volume percentage (%) of red blood cells in blood. This measurement depends on the number of red blood cells and the size of red blood cells. It is normally about 45% for men and 40% for women. The HCT levels as can be observed are low in most liver disorders. The levels are specifically low in case of Cirrhosis and viral infections. Greater the progression of disease more altered the HCT values (see table below).

The red blood cell distribution width (RDW or RCDW) is a measure of the variation of red blood cell (RBC) volume that is reported as part of a standard complete blood count. Usually red blood cells are a standard size of about 6-8μm. Certain disorders, however, cause a significant variation in cell size. Higher RDW values indicate greater variation in size. Normal reference range in human red blood cells is 11.5-14.5%. If anemia is observed, RDW test results are often used together with mean corpuscular volume (MCV) results to determine the possible causes of the anemia.RDW can be reported statistically as coefficient of variation (CV) and/or standard deviation (SD), RDW-CV and/or RDW-SD, respectively. (Kjeldsberg et al., 2005)

RDW-SD (express in fL) is an actual measurement of the width of the RBC size distribution histogram (see the first image below) and is measured by calculating the width (in fL) at the 20% height level of the RBC size distribution histogram and is therefore not influenced by the average RBC size (mean corpuscular volume,

MCV).RDW-CV (express in %) is calculated from standard deviation and MCV mathematically is therefore affected by the average RBC size (MCV). (Purves et al., 2004)

Similar to HCT values, RDW values are altered in most liver disorders. These values are highly elevated in case of liver cirrhosis, denoting highly uneven distribution. RDW- SD values are however, more strongly related to alcoholism and ALD whereas RDW- CV values are more associated to the viral onset of diseases.

Table 4.6.3: Correlation of Liver Diseases to other biochemical parameters

PARAMETERS	HCT	RDW CV	RDW SD
Total hepatic	30.9 (1.37855)	18.5 (0.604752)	51.66 (1.55892)
Alcoholics	34.3 (4.09865)	18.54 (1.08203)	52.92 (3.73514)
Viral history	29.8 (1.822)	18.6 (0.9348)	49.76 (3.061)
Cirrhosis	29.23 (3.74398)	19.71 (1.04941)	56.51 (2.58757)
Hepatitis	34.4 (1.8715)	18.45 (1.50423)	47.45 (4.23527)
Obstruction	33.5 (1.16952)	14.7 (0.439093)	44.3 (2.04315)
ALD	30.96 (1.40095)	17.85 (2.13099)	61.5 (11.6289)
CLD	31.7 (2.08531)	15.17 (0.309707)	50.6 (1.12561)
LPD	33.52 (1.35989)	16.14 (0.91049)	48.33 (2.63481)
fatty liver	38.6 (3.75411)	14.1 (0.8)	40.95 (1.65)

Histogram for Cirrhosis of liver

Fig. 4.5 Histogram for Cirrhosis of Liver
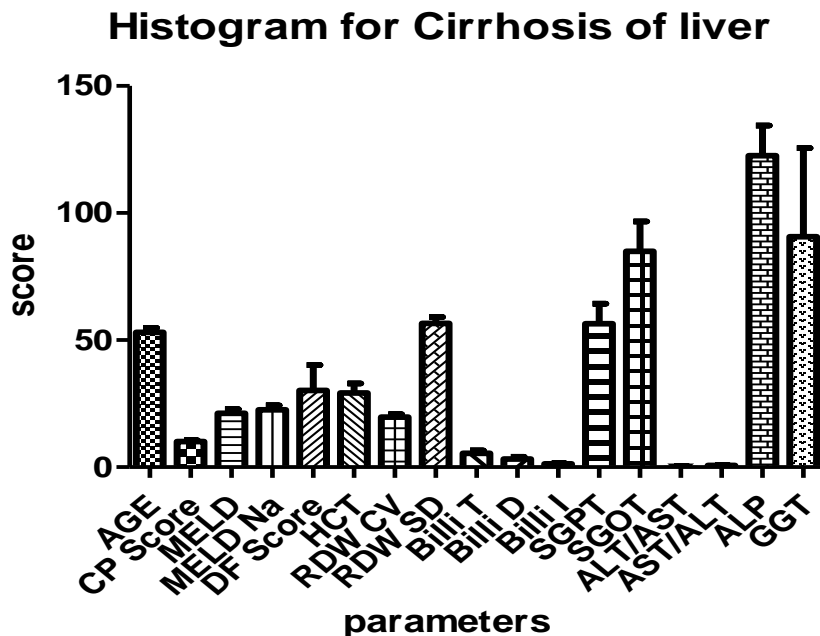
Histogram for Obstructive disorders
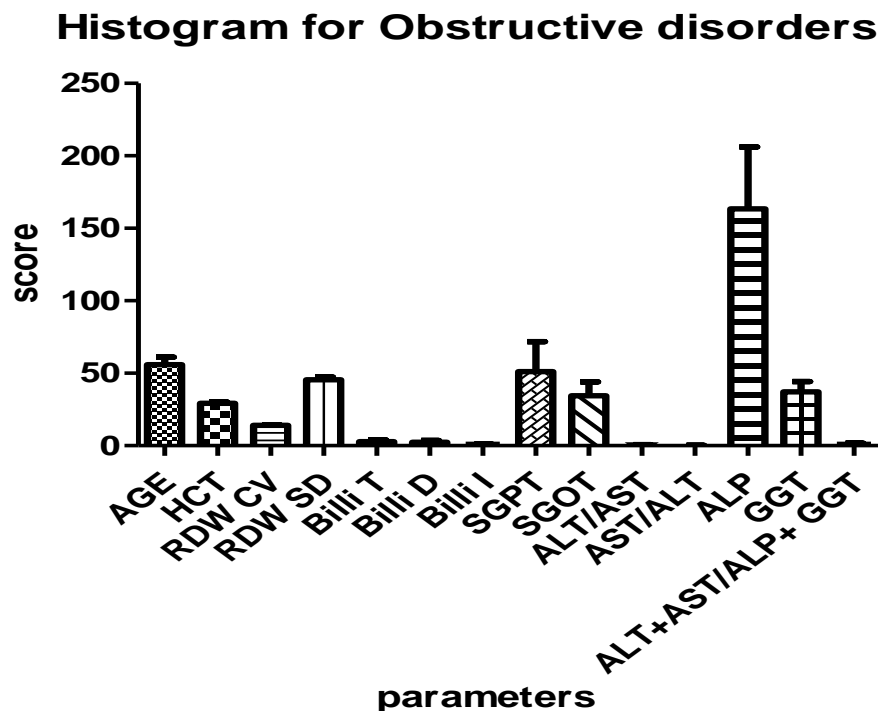
Fig. 4.6 Histogram for Obstructive Disorders
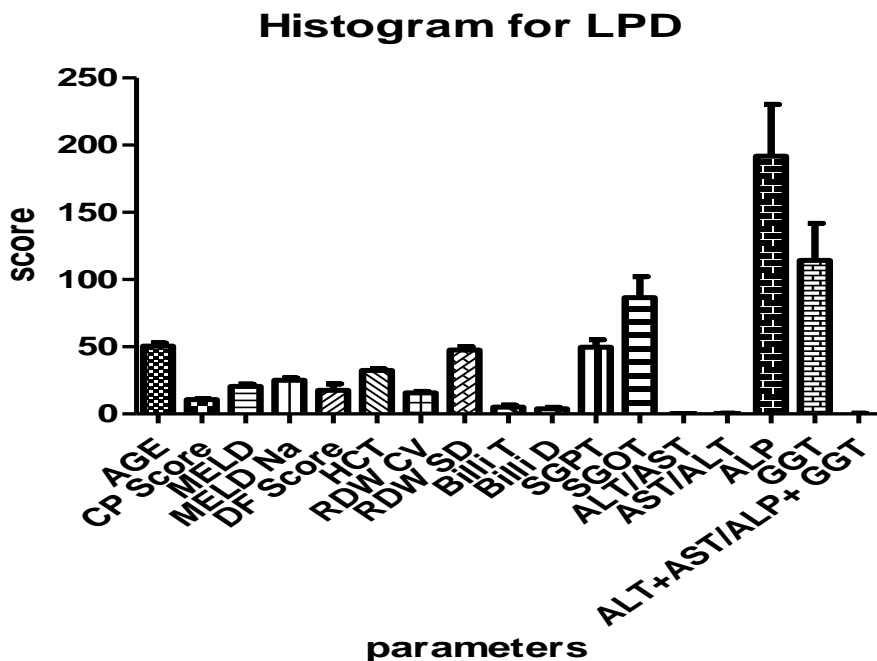
Fig. 4.7 Histogram for LPD
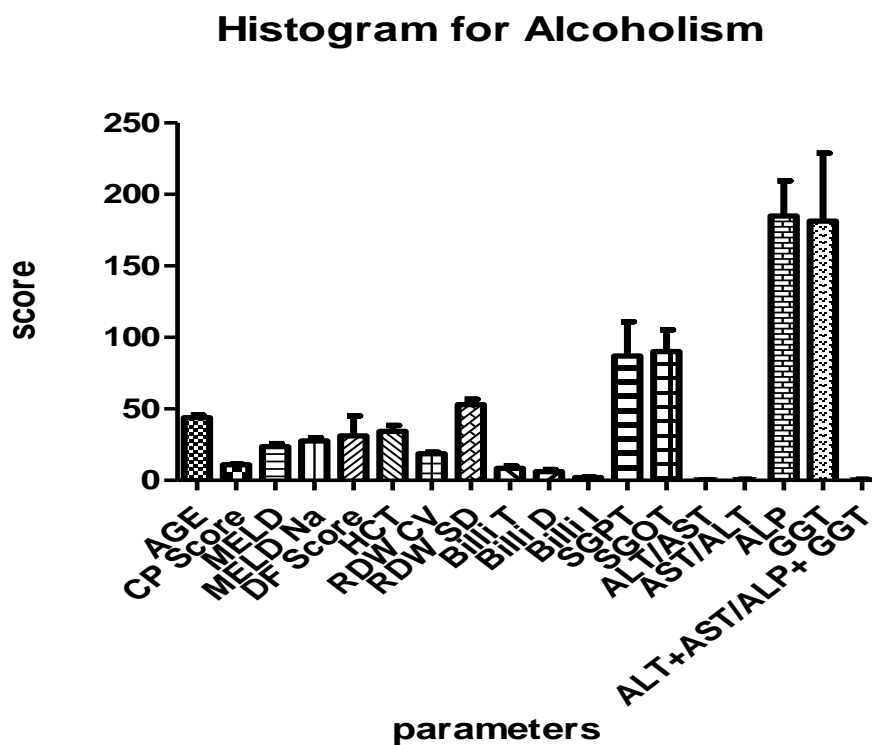
Fig. 4.8 Histogram for Alcoholism

Histogram for viral hepatitis
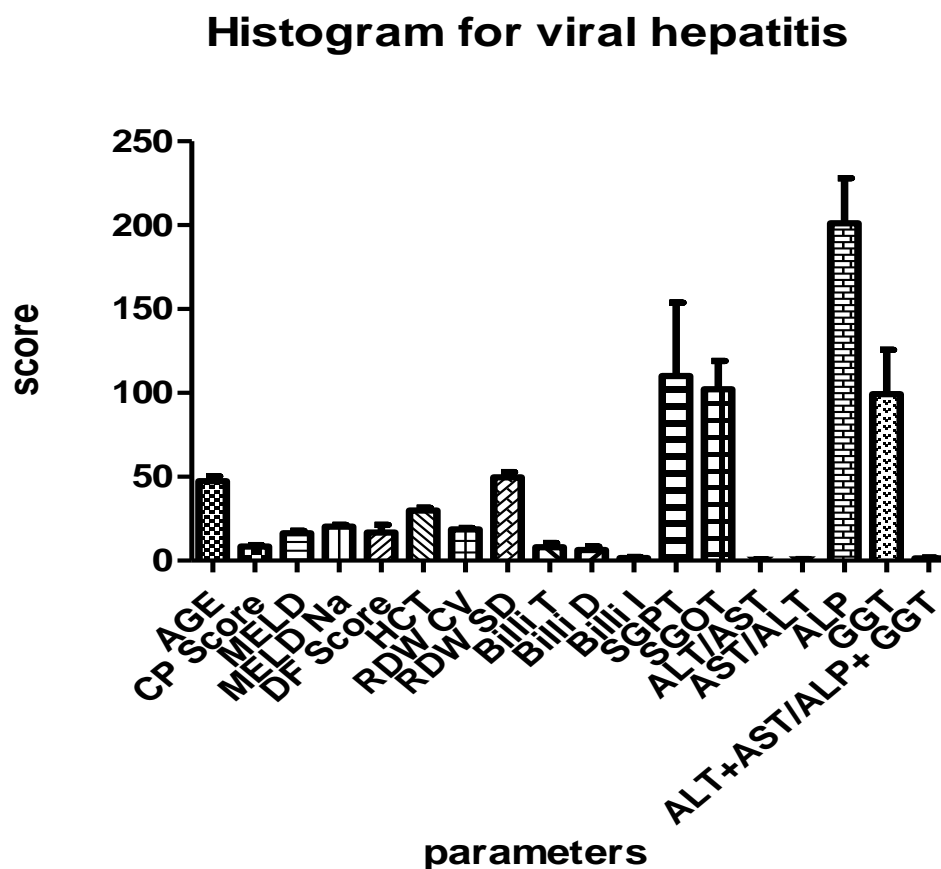
Fig. 4.9 Histogram for Viral Hepatitis

4.5.3 ALT/AST and AST/ALT Ratios

As can be inferred from the studies, the general understanding is that if ALT > AST, chronic liver diseases are indicated. However, AST > ALT indicates progression of the disease to Cirrhosis. When AST is twice as much as ALT or more, it indicates prolonged liver damage or acute liver damage out together with high GGT values.

That is, ALT/AST > 1 it indicates Chronic liver diseases, AST/ALT > 1 indicates Acute onset disease or progression to Cirrhosis, AST/ALT > 2 shows Alcohol abuse and AST/ALT< 1 signifies non-alcoholic disease.

As can be observed from the tabulated data, the values of ALT/AST are greater than 1 in case of viral diseases that lead to chronic conditions, chronic hepatitis, obstruction

disorders and fatty liver diseases since all of them are chronic and lack alcohol as the primary cause.

AST/ALT ratios are greater than 1 for alcoholic patients, Cirrhosis patients. The values are also greater than but close to 1 for viral diseases and hepatitis, accounting for acute hepatitis and newly detected or acute viral hepatitis and related conditions. The ratio is greater than 2 for ALD. It is also greater than 2 for CLD and close to 2 for LPD, implying their onset in major portion of population is alcohol based. The same value for Fatty liver diseases is <1, denying alcohol as its cause of onset in majority of the cases.

4.5.4 AST+ALT/ALP+GGT

Based on the formula:

$$AST+ALT- 105/ ALP+ GGT- 155$$

Where 105 is the sum of upper limits of ALT and AST (65+ 40)

And 155 is the sum of upper limits of ALP and GGT (100+ 55)

A value between -2 and 2 would define the obstructive picture while mode values greater than 2 would define a hepatic picture in most cases. The tabulated data verifies the same.

The outcomes are tabulated as under:

Table 4.6.4: Correlation to parameter ratios and report analysis

PARAMETERS	ALT/AST	AST/ALT	ALT+AST/ALP+ GGT
Total hepatic	1.01 (0.0617376)	1.55 (0.111761)	3.4 (0.717859)
Alc.	0.78 (0.150253)	1.68 (0.209137)	0.655 (0.307359)
Viral history	1.07 (0.2200)	1.38 (0.2226)	4.15 (0.5548)
Cirrhosis	0.761 (0.0945116)	1.69 (0.184458)	1.33 (0.428836)
Hepatitis	1.45 (0.343698)	1.015 (0.311943)	7.42 (0.982625)
Obstruction	1.7 (0.244316)	0.67 (0.105143)	0.3 (0.689489)
ALD	0.51 (0.113486)	2.29 (0.378938)	0.57 (3.88912)
CLD	0.72 (0.107643)	2.34 (0.216293)	0.9 (0.540621)
LPD	0.96 (0.08; 2.7)	1.86 (0.37; 11.47)	0.32 (-7.2; 11.35)
fatty liver	2.14 (0.627219)	0.47 (0.138737)	-0.5 (0.5304)

4.6 <u>SCORE ANALYSES</u>

Various scoring system are used to assess severity and prognosis of liver diseases and its complications.

Following scores were calculated as applicable for patients based on availability of data.

1) Maddrey's Discriminant factor (DF score): for alcoholic hepatitis and chronic liver diseases.

2) Child Pugh score for liver cirrhosis

3) MELD and MELD-Na score for liver cirrhosis.

First and foremost, as can be observed from table 5.7, the three scores badly correlate to each other. The general observation of data is that, the DF score is consistently correlated with Alcoholism, ALD, and End Stage Cirrhosis. It also correlates well to DM and decompensated kidney functions as co-morbidity (table 5.8).The MELD score relates better to decompensated cirrhosis, HCC and ACLF, i.e. decompensating in case of chronic ALD. It also correlates well to ascites and hepato-portal or GB obstruction other than DM.

The CP score also correlates extremely well to alcoholism and presence of varices. All the three scores correlate well to mortality, CP correlating the best though to mortality and MELD the least.

Considering the CP score, the average score for patients suffering with cirrhosis is 10.10; which amounts to a high score (class C) indicating decompensation and a decreases life expectancy of only 1- 3 years. 65% of the patients with cirrhosis have class C CP score (score > 9). The average age of these 65% patients is only 46 years, implying low age populations being the victim of most severe form of cirrhosis, which is significantly alarming. Only 5% of the patients have a class A score (<7), i.e. a life expectancy of 10-15 years.

Considering MELD score, the average score for the population is around 21, i.e. pretty severe (mid-range grade 3 score). Considering the MELD-Na values, this average value reaches an alarming 25. Around 30% of population of patients is above the grade 4 (score

> 25) and need to consider transplant urgently. The average age of such patients is 50.69, which is again, really low. Only 2% of patients have a score of 9 or less (safe value).

Considering the DF value, the mean value for all tested patients is 47.1 which is way above the high risk value of 32, with the highest value reaching as high as 109. 61% patients have a value of DF greater than 32 and need steroid for controlled prognosis. The average age of such patients is as low as 50.47 years.

MELD- Na scores seem to relate better to the morbidity as compared to MELD scores, esp. in case of ascites and encephalopathy.

The CP score fairly correlates to the HCT and RDW values. RDW-SD values show complete correlation to the CP score. HCT values show a correlation of 75% to the CP score.

The DF score shows 85.7% correlation to HCT. The RDW-SD values show an approximate correlation to the DF score with a little variations in between, but the general trend to severity of the score is followed.

The MELD values do not correlate well to the HCT values, but are very well correlated to the RDW-CV values.

Thus, RDW and HCT values in general, are in good correlation to the various scores determining severity of liver diseases.

Table 4.7: Association between Score

IP no.	AGE	SEX	DISEASE	CP Score	MELD	DF Score
IP/14/1020	41	M	liver parenchymal disease		23	9.86
IP/13/6537	67	M	Cirrhosis	CP 7,B	9	14.5
IP/14/754	69	M	CLD		15	16.74
IP/14/754	69	M	CLD; SBP; ARF		21	16.74
IP/13/8514	67	M	Cirrhosis	CP 6,A	13	19.18
IP/14/663	62	M	HCC; decompansated liver		26	20.56
IP/13/5645	54	F	ACLF		19	25.06
IP/13/8536	48	M	ALD (vasc. Malformation- rt eye lid bleed)		33	25.78
IP/13/9224	53	M	CLD		20	26.6
IP/13/9493	55	M	Cirrhosis	CP 10,C	22	28.32
IP/13/6085	52	M	Ch. Alc. Hepatitis		28	28.43
IP/13/7457	72	F	Cirrhosis	CP 9,B	16	36.12
IP/14/159	57	M	Cirrhosis	CP 9,B	21	37.44
IP/14/774	42	M	cirrhosis+ SBP	CP 11,C	14	42.19
IP/14/983	36	M	cirrhosis; CLD; GB calculi	CP 12,C	29	47.76
0	65	F	Cirrhosis	CP 7,B	18	49.6
IP/14/5349	33	M	cirrhosis; LPD	CP 10,C	33	50.72
IP/14/739	30	M	alcoholic hepatitis		14	51.25
IP/13/8843	52	M	Cirrhosis	CP 13,C	31	51.33
IP/14/590	42	M	Cirrhosis	CP 11,C	28	54.08
IP/13/8257	63	M	CLD		30	56.5
IP/13/9078	42	M	Cirrhosis	CP 11,C	19	60.66
IP/13/8490	74	F	ACLF		14	73.6
IP/13/9074	45	F	Cirrhosis	CP 12,C	24	78.43
IP/14/479	60	M	Cirrhosis	CP 11,C	23	84.96
IP/13/7197	61	M	Cirrhosis	CP 12,C	28	96.86
IP/14/585	48	M	ALD		17	105.56
IP/13/8944	36	M	alc. Hepatitis		35	109.02
IP/13/7550	50	F	CLD		23	
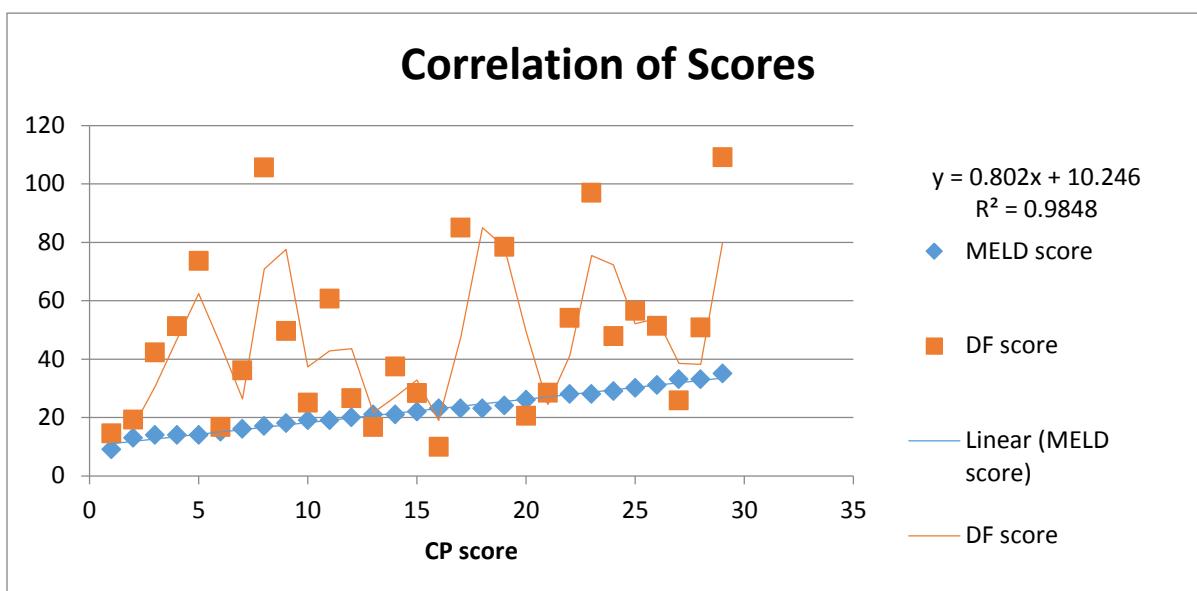
Fig. 4.10 Correlation of Scores

Table 4.8: Scores and their association with Co-morbidity and complications

AGE	GENDER	DISEASE	COMOR BIDITY	Alcohol	Ascites	Varices	Encephal	CP Score	MELD	DF Score
67	M	Cirrhosis	HTN; HCV					CP 7,B	9	14.5
67	M	Cirrhosis				Y		CP 6,A	13	19.18
42	M	cirrhosis+ SBP	Gr.II fatty liver	Y	Y			CP 11,C	14	42.19
30	M	alcoholic hepatitis	Tachycardia	Y					14	51.25
74	F	ACLF	cirrhosis; HTN; DM				Y		14	73.6
69	M	CLD	ARF+CLD+SBP						15	16.74
72	F	Cirrhosis	HTN; asthma					CP 9,B	16	36.12
48	M	ALD	DM		Y				17	105.5
65	F	Cirrhosis				Y		CP 7,B	18	49.6
54	F	ACLF	GI bleed; hyperkalaemia	Y	Y	Y			19	25.06
42	M	Cirrhosis	CLD; kidney stone					CP 11,C	19	60.66
53	M	CLD	umbelical hernia		Y				20	26.6
69	M	CLD; SBP;	lung RA; ARF;						21	16.74

		ARF	Restricted disease							
57	M	Cirrhosis	IHD; DM; PHT		Y	Y		CP 9,B	21	37.44
55	M	Cirrhosis	ALD	Y	Y	Y	Y	CP 10,C	22	28.32
41	M	LPD	DM; HTN						23	9.86
60	M	Cirrhosis	DM		Y	N	Y	CP 11,C	23	84.96
50	F	CLD	AKI; SBP;URTI; cholecystitis				Y		23	
45	F	Cirrhosis	ALD; hypotension	Y	Y	Y		CP 12,C	24	78.43
62	M	HCC; decompans-ated liver	DM; HTN; IHD; Pul. HTN	Y			Y		26	20.56
52	M	Chronic alc Hepatitis	DM: 12 yrs+ HTN: < 1yr	Y	N	N	N		28	28.43
42	M	Cirrhosis			Y		Y	CP 11,C	28	54.08
61	M	Cirrhosis	squamous cell Ca. pelvis		Y			CP 12,C	28	96.86
36	M	cirrhosis; CLD; GB calculi	CLD; GB calculi; SOJ	Y	Y	Y		CP 12,C	29	47.76
63	M	CLD	DM; Cld 10 yrs			Y			30	56.5
52	M	Cirrhosis	HRS		Y	Y		CP 13,C	31	51.33
48	M	ALD	ALD (vasc. Malformation-right eye lid bleed)	Y	Y	N			33	25.78
33	M	cirrhosis; LPD		Y	Y			CP 10,C	33	50.72
36	M	alc. Hepatitis		Y	N	Y			35	109.0

4.7 LINE OF TREATMENT

The general line of treatment includes:

1) Antibiotics: Rifagut (rifamycin 550), is the antibiotic of choice in the given power as it also known to delay hepatic encephalopathy in case of decompensation. Antibiotics are given in case of ascites and SBP to avoid secondary infections, or when encephalopathy is anticipated, or in case of co-infections, or when viral hepatitis occurs.

   Terlyz, pipracillin, amoxicillin and augmentin or ciplox are other choice of antibiotics. Meropenam is indicated for severe co-infections.

2) Nutritive fluids and electrolyte balance: Isolyte- M, DNS, Albumen, etc are given

3) Anti-viral: In case of newly diagnosed viral hepatitis

4) Vitamins: Folvite, B-complex and B-12 are recommended as morbidity diluters.

5) Steroids: in case DF factor is above 32 and for heavy drinkers. Prednisone is usually used.

6) Di-uretics: in case of ascites or SBp or varices, to get free of fluid. Spiranolactone (aldactone) or Lasix (frusemide) is used.

7) Co-agulators and blood modifiers: Vit. K, FPP, PCV as required, in case of blood-loss or altered INR.

8) Supportives: IVF, enemas (looz enema), antacid etc. as required.

5. __DISCUSSION__

Liver diseases are associated with significant morbidity and mortality. They are on a rise in the country and are prevalent at a significantly high rate. The study is aimed at examining etiology and epidemiology of liver diseases at a tertiary care trust hospital in Vadodara district and to characterize its association to various factors so as to lay the founding brick to its better management and alleviation. In the present study we observed that about 75% of all cases detected are not due to alcohol consumption. The high prevalence can be attributed altered lifestyle, poor dietary habits and increase in access to alcohol over the years in the state. There is a significant rise in the risk factors like Type 2 DM, Obesity, pollution and smoking that show high association with liver diseases. Another factor contributing to high prevalence of liver diseases is the genetic predisposal. The population of India is genetically pre-disposed to liver diseases and catches the disease faster and at lower risks. This is due to presence of a gene called APOC3 (Petersen et al., 2010). Study reveals that around 20% of all cases are due to viral infection. A study done in south- Gujarat showed high prevalence of hepatitis infection and co- infection in the state (Shah and Mulla, 2012). This could further contribute to rise in liver diseases as can be inferred from the study. It is observed that the risk of a liver disease increases with age, especially above the age of 40 till the age of 60. Males are found to be in general more susceptible to liver diseases over all age groups in contrast to some of the studies done in China and India, where proportion of females surpasses those of males in post 60 years of age (Kalra et al., 2013; Yong-Jian Zhou et al., 2007; Neuschwander-Tetri, Caldwell, 2003). However, it is in consistency in the manner that the proportion of females to male has risen in age group 60 and above as compared to that in below 40 and 40- 60 age groups. The prevalence of FLD in males increased stably with age, and steadily from 50-60 years of age in females. The peak prevalence was observed in females in the age group of 55 and above, which was about 7 years later than that in males, which might be due to the menopausal status and lack of physical exercise in this period of time (Kalra et al., 2013).

Findings of the present study correlates to a study performed in china, among patients diagnosed having FLD (18.0% males, 16.7% female, P > 0.05), a significantly large data set. Among the prevalence rate was significantly higher in urban areas than in rural areas. The prevalence rate was significantly higher in men than in women under the age of 50 years. All results correlate well with the study performed herewith. However, the opposite phenomenon was found over the age of 50 years. (Yong-Jian Zhou et al., 2007).

There are conflicting results regarding the relation of NAFLD to age and gender (Neuschwander‑Tetri and Caldwell, 2003). Bedogni *et al* found that the prevalence of NAFLD increases with age in both genders and then significantly decreases over 66 years of age. The variations between studies can be attributed to the differences in social, cultural and environmental backgrounds among the target subjects.

In the present investigation, we found more prevalence of liver diseases in urban population. More susceptibility in urban areas has multifaceted possibilities. The study indicates that urban residency, low education are the risk factors for FLD. Theoretically, a convincing argument could be greater exposure to risk factors in urban areas. Sedentary lifestyle leading to metabolic disorders like DM, obesity and others, high exposure to population, poor dietary habits etc. Another more convincing and perhaps more practical explanation to it are lack of awareness among the rural people and lack of access to facilities. The diagnosis rate generally tends to be low in rural population. Higher number of cases during festive season can be attributed to poor dietary habits, junk consumption and increased alcohol consumption during festivals and celebrations.

The present study reveals that among all the diseases, cirrhosis, the most severe one seems to be causing the highest trouble. Its occurrence is the highest and at the least mean age of the population (< 50 years) which is very alarming. However, its higher prevalence compared to fatty liver disease may because of only inpatients being considered. Cirrhosis and LPD constitute the highest amount of study population, above 60. The mean ages are lowest for ALD and viral hepatitis. This may be due to premature deterioration of liver due excess burden to the liver by the risk factors- alcohol and hepatitis virus respectively.

In our study, we found 20% cases are of viral diseases, which is in close correlation to a study done on "The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide" at Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Viral Hepatitis, Epidemiology Branch, Atlanta, USA and at World Health Organization, Department of Blood Safety and Clinical Technology, Geneva, Switzerland reveals that globally, 57% of cirrhosis was attributable to either HBV (30%) or HCV (27%) and 78% of HCC was attributable to HBV (53%) or HCV (25%). Regionally, these infections usually accounted for >50% of HCC and cirrhosis. The data obtained for India in this study correlates to those obtained in our study. (Perz et al, 2006).

In the present study, it was found that liver diseases are correlated to various comorbidities with most co-morbidities occuring over the age of 40. Multiple organ failure (HRS, CHF) and metabolic disorders (DM, thyroid) lead to increased mortality and morbidity and decreased life expectancy by adding extra burden to liver. These may be secondary to, or may have a causal role in liver diseases and have a role in poor prognosis. Liver diseases correlate the most to diabetes and CVS disorders. The development of chronic liver disease may lead to glucose intolerance and occasionally diabetes. It remains unclear whether DM precedes the development of significant chronic liver disease. Among men with diabetes, the risk of CNLD and HCC is doubled. This increase in risk is independent of alcoholic liver disease, viral hepatitis, or demographic features (El- Seraq et al., 2003). Patients with NAFLD are at high risk for coronary atherosclerosis regardless of classical cardiovascular risk factors, especially visceral adiposity. Many theories are suggested for the same, but no definitive mechanism is proven to explain the phenomenon. (Kim et al., 2012) Various studies have different outcomes regarding association of thyroid disorders to Liver diseases. (Vilaret al., 2013; Malik 2002; Khemichian and Fong 2011) An association is found in the given study. However, No strong argument on specific cause for association of thyroid disorders to liver diseases is available. Most co-morbidity is seen over the age of 40.

The results from various studies compared to the current study reinforces the well-established clinical association of NAFLD with elements of metabolic syndrome (MetS)

including dyslipidemia, hypertension and obesity across all age groups in identified T2DM NAFLD patients. (Kalra et al., 2013)

Also, the occurrence of Alcohol related diseases (25%) is higher than what could be expected for a dry state.

Varices, ascites and encephalopathy are major complications of liver diseases. More than half the admitted patients have either one or the other of these complications. These complications are markers of disease progression and depict diseases severity in its respective order. Alcohol consumption is related to decrease in mean age of onset of these complications. Death occurred in 5 cases.

In the given study, the overall mean for AST and ALT levels are 156.4 and 107.3 respectively, which is very high compared to those reported in some of the other studies (Kalra et al., 2013; Yong-Jian Zhou et al., 2007; Neuschwander-Tetri, Caldwell, 2003). Average ALP and GGT values are also very high. In general, ALP levels and AST levels show highest and most significant correlation to severity, morbidity and prognosis of liver diseases. Considering patients with alcoholic history, the GGT levels are significantly high (mean: 181.7) with highest value reaching as high as 611 U/L. GGT is a non- specific marker for liver damage. It has been reported that alcohol produces hepatocyte damage which causes increase in GGT levels. An even further shot up levels of GGT (> 180 U/L) would indicate ALD. This confirmed by the finding of mean GGT values of ALD (mean= 223.05). The chart also confirms affirmatively and amply that ALT and AST would be basically excellent markers for liver inflammation, going by its distribution. The bilirubin levels are significantly high for alcoholics, more so in case of non-chronic ALD. The proportion of direct bilirubin is much more elevated in viral diseases as compared to alcoholic disease. This is even more eminent in obstruction diseases. The AST : ALT ratio and the formula derived therein tells a lot about liver disease, its nature, progression and severity and can be used as testing parameter in diagnosis, differentiation and choosing line of treatment. It confirms the general scheme of diagnosis.

As an outcome of the study, HCT and RDW relate well to severity of liver diseases. This may be due to altered hematopoiesis. Splenic sequestration and destruction of platelets,

white blood cells (WBCs) and red blood cells (RBCs) occurs in the portal hypertension-induced enlarged spleen. In patients with cirrhosis, there is a redistribution of platelets, with up to 90% of the circulating platelet mass located in the enlarged spleen. Similarly, splenic sequestration of RBCs contributes to the anemia of liver disease. (Qamar and Grace, 2009)

While Milić et al., show a negative correlation to RDW to liver diseases, several other studies have found that RDW positively correlates to progression of liver diseases (Milić et al., 2011; Mustafa et al., 2013). A study co-relates RDW to fibrotic score in NASH and has found good correlations. They hypothesized that the relationship between RDW and NASH may be a result of an effect of an inflammatory process that suppresses mature erythrocytes and secretes young erythrocytes into the circulation, leading to anisocytosis and high RDW values. (Mustafa et al., 2013)

In the present study we observed that all the three scores namely CP, DF, MELD relate poorly to each other and are only approximate in giving idea of severity and prognosis of liver diseases showing their limitations and need for a better more comprehensive system of scoring.

NAFLD has been increasingly recognized as the most common liver disease globally. So far no accurate incidence is available. The prevalent data obtained from clinical series and autopsy studies suggest that 20%-30% of individuals have FLD. While the general trend in outcome is the same, variations amongst prevalence, distribution and related outcomes have been observed among studies performed in same and different geographical regions. The discrepancy between these studies may probably be due to the difference in methods of sample selection, modalities used for diagnosis and diversity of life styles and dietary habits in different areas.

6. <u>**CONCLUSION**</u>

Liver Diseases are widespread and rampant in the district of Vadodara. A large number of interacting factors contribute to an individual's risk for liver diseases: these include environmental exposures, genetic factors, diet, lifestyle, age, and gender. In the given study, liver diseases correlated well to the male gender, increased age, and urban population and to metabolic disorders. Most of these cases are cases of Nonalcoholic Liver Diseases with alcoholic cases comprising of only 25% of total cases. The finding follows the general trend with majority of cases being male. Around 20% of all cases are due to viral infection. As in most of the developing world, viral hepatitis continues to be the major cause of liver diseases. The large differences in the pattern of liver diseases incidence between developed and developing countries imply different priorities for prevention. Prognosis in most of these cases is poor. The pattern of change in Liver Function Tests is typical for various diseases and can be instrumental in identification and differential diagnosis of liver diseases. The study outcomes will be useful in the modeling and cost-effectiveness analyses that could be undertaken to look at the burden of liver disease in the district of Vadodara. There is lack of epidemiological data for liver diseases in the state, and no comprehensive study has been done so far documenting the same. An urgent need for an analytical study is indicated so as to highlight the pit holes in the issue and initiate further research and ultimately leading to better prognosis. A descriptive study has been therefore performed at length to assess the current situation, and need has been found for further investigations. Using a more recent extraction of data it should be possible to think upon further management of diseases and ways to eliminate its socio-economic burden. . In addition, it is probable that certain aspects or parameters have not been addressed in this thesis. Of particular interest clinically is the occurrence of hepatocellular carcinoma. Further exploration of readily available data would inform the current practices for surveillance of hepatocellular carcinoma and potentially identify both high and low risk groups in which to either target or stop surveillance.

There are several methodological limitations in this study. First, that it is a study conducted under premises of only one hospital. The sample size may therefore not be a

true representative of whole district significant enough. To counter this, comparisons have been made with various studies and the data is thus validated. Also, although participants were randomly selected, collection of data from only one hospital may bring about bias to the results due to factors like ease of access, awareness, availability and affordability etc. To eliminate such errors, the hospital was wisely chosen so is to be a true representative of the real data set in in miniature form.

7. <u>**REFRENCES**</u>

1. Anatomy & Physiology of Liver - Functions of the Liver. (www.nlfindia.com/liverZone/functions.asp) National Liver Foundation. India. Retrieved on 2010-08-13.

2. Bambha KM, Biggins SW. Inequities of the Model for End-Stage Liver Disease: an examination of current components and future additions. *Curr Opin Organ Transplant* 2008;13(3):227-33.

3. Bedogni, G., Miglioli, L., Masutti, F., Tiribelli, C., Marchesini, G., & Bellentani, S. (2005). Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology*, *42*(1), 44-52.

4. Blachier, Martin, Henri Leleu, Markus Peck-Radosavljevic, Dominique-Charles Valla, and Françoise Roudot-Thoraval. "The Burden of Liver Disease in Europe: A Review of Available Epidemiological Data." *Journal of Hepatology* 58, no. 3 (March 2013): 593–608. doi:10.1016/j.jhep.2012.12.005.

5. Bosetti C, Levi F, Lucchini F, Zatonski WA, Negri E, La Vecchia C. Worldwide mortality from cirrhosis: An update to 2002. *J Hepatol* 2007;46(5):827-39. 262

6. Browning, J. D., & Horton, J. D. (2004). Molecular mediators of hepatic steatosis and liver injury. *Journal of Clinical Investigation*, *114*(2), 147-152.

7. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999;29(3):

8. Cengiz Mustafa, Burcu Aslan Candır, Güldal Yılmaz, Gülen Akyol, and Seren Ozenirler. "Is Increased Red Cell Distribution Width an Indicating Marker of Nonalcoholic Steatohepatitis and Fibrotic Stage?" *World Journal of Gastroenterology* 19, no. 42 (2013): 7412. doi:10.3748/wjg.v19.i42.7412.

9. Christensen E, Schlichting P, Fauerholdt L, Gluud C, Andersen PK, Juhl E, et al. Prognostic value of Child-Turcotte criteria in medically treated cirrhosis. *Hepatology* 1984;4(3):430-5.

10. Cotran, Ramzi S.; Kumar, Vinay; Fausto, Nelson; Nelso Fausto; Robbins, Stanley L.; Abbas, Abul K. (2005). *Robbins and Cotran pathologic basis of disease* (7th ed.). St. Louis, MO: Elsevier Saunders. p. 878. ISBN 0-7216-0187-1.

11. Denzer UW, Luth S. Non-invasive diagnosis and monitoring of liver fibrosis and cirrhosis. *Best Pract Res Clin Gastroenterol* 2009;23(3):453-60.

12. Dr. Huw Thomas, Current Version: Dr Gurvinder Rull, Abnormal Liver Function Tests. Last Checked: 16/07/2010. Document ID: 610, Version: 23. Patients.co.uk. (http://www.patient.co.uk/doctor/abnormal-liver-function-tests) Egton Medical Information Systems Limited. Registered in England. No 2117205

13. Dr. Manisha Bangar, Non-alcoholic related fatty liver disease rising among urban Indians, Indian Association for Study of Liver Diseases, for The Hindu, article: 4636723

14. EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. *J Hepatol* 1999;30(5):956-61.

15. El-serag, Hashem B., Thomas Tran, and James E. Everhart. "Diabetes Increases the Risk of Chronic Liver Disease and Hepatocellular Carcinoma." *Gastroenterology* 126, no. 2 (February 1, 2004): 460–68. doi:10.1053/j.gastro.2003.10.065.

16. Freeman AJ, Law MG, Kaldor JM, Dore GJ. Predicting progression to cirrhosis in chronic hepatitis C virus infection. *J Viral Hepat* 2003;10(4):285-93.

17. Garceau AJ, Chalmers TC. The natural history of cirrhosis. I. Survival with esophageal varices. *N Engl J Med* 1963;268:469-73. 265

18. Giannini EG, Testa R, Savarino V; Liver enzyme alteration: a guide for clinicians. CMAJ. 2005 Feb 1;172(3):367-79.

19. Giannini EG, Testa R, Savarino V; Liver enzyme alteration: a guide for clinicians. CMAJ. 2005 Feb 1;172(3):367-79.

20. Giboney PT; Mildly elevated liver transaminase levels in the asymptomatic patient. Am Fam Physician. 2005 Mar 15;71(6):1105-10.

21. Giboney PT; Mildly elevated liver transaminase levels in the asymptomatic patient. Am Fam Physician. 2005 Mar 15;71(6):1105-10.

22. Harrison SA, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 2003;98(9):2042-7.

23. Heathcote J; Abnormal liver function found after an unplanned consultation: case outcome. BMJ. 2004 Aug 28;329(7464):500; discussion 500-1.

24. Hultcrantz R, Glaumann H, Lindberg G, Nilsson LH. Liver investigation in 149 asymptomatic patients with moderately elevated activities of serum aminotransferases. *Scand J Gastroenterol* 1986;21(1):109-13.

25. Iredale JP. Cirrhosis: new research provides a basis for rational and targeted treatments. *BMJ* 2003;327(7407):143-7.

26. Jepsen P, Ott P, Andersen PK, Sorensen HT, Vilstrup H. Clinical course of alcoholic liver cirrhosis: A Danish population-based cohort study. *Hepatology* 2009.

27. Joseph F. Perz, Gregory L. Armstrong, Leigh A. Farrington, Yvan J.F. Hutin, Beth P. Bell; The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide; Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Viral Hepatitis, Epidemiology Branch, Atlanta, GA 30333, USA; World Health Organization, Department of Blood Safety and Clinical Technology, Geneva, Switzerland; Journal of Hepatology 45 (2006) 529–538

28. Khemichian, Saro, and Tse-Ling Fong. "Hepatic Dysfunction in Hyperthyroidism." *Gastroenterology & Hepatology* 7, no. 5 (May 2011): 337–39.

29. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. *BMJ* 2004; 328(7446):983.

30. Kim, Donghee, Su-Yeon Choi, Eun Ha Park, Whal Lee, Jin Hwa Kang, Won Kim, Yoon Jun Kim, et al. "Nonalcoholic Fatty Liver Disease Is Associated with Coronary Artery Calcification." *Hepatology* 56, no. 2 (August 1, 2012): 605–13. doi:10.1002/hep.25593.

31. Kjeldsberg, Carl R.; Perkins, Sherrie L.. Practical Diagnosis of Hematologic Disorders. American Society for Clinical Pathology. ISBN 089189571X. 5th edition.

32. Kmieć Z (2001). "Cooperation of liver cells in health and disease". *Adv Anat Embryol Cell Biol* 161: III–XIII, 1–151. PMID 11729749

33. Lee TH, Kim WR, Benson JT, Therneau TM, Melton LJ, 3rd. Serum aminotransferase activity and mortality risk in a United States community. *Hepatology* 2008;47(3):880-7.

34. Limdi JK, Hyde GM. Evaluation of abnormal liver function tests. *Postgrad Med J* 2003;79(932):307-12.

35. Limdi JK, Hyde GM; Evaluation of abnormal liver function tests. Postgrad Med J. 2003 Jun;79(932):307-12.

36. Limdi JK, Hyde GM; Evaluation of abnormal liver function tests. Postgrad Med J. 2003 Jun;79(932):307-12.

37. Liver Disease - Liver Disease Information - Types of Liver Disease | Canadian Liver Foundation mieć Z (2001). "Cooperation of liver cells in health and disease". *Adv Anat Embryol Cell Biol* 161: III–XIII, 1– 151. PMID 11729749

38. Malik, R. "The Relationship between the Thyroid Gland and the Liver." *QJM* 95, no. 9 (September 1, 2002): 559–69. doi:10.1093/qjmed/95.9.559.

39. Mann, Robert E., Reginald G. Smart, and Richard Govoni. "The Epidemiology of Alcoholic Liver Disease." *Alcohol Research* 27, no. 3 (July 1, 2003): 209.

40. Mathers CD, Sadana R, Salomon JA, Murray CJ, Lopez AD. Healthy life expectancy in 191 countries, 1999. *Lancet* 2001;357(9269):1685-91.

41. Myers RP. Noninvasive markers of liver fibrosis: playing the probabilities. *Liver Int* 2008;28(10):1328-31.

42. Neuschwander-Tetri, Brent A, and Stephen H Caldwell. "Nonalcoholic Steatohepatitis: Summary of an AASLD Single Topic Conference." *Hepatology (Baltimore, Md.)* 37, no. 5 (May 2003): 1202–19. doi:10.1053/jhep.2003.50193.

43. Petersen K. F. et al. Apolipoprotein C3 gene variants in non-alcoholic fatty liver disease. N. Engl. J. Med. 362, 1082–1089 (2010).

44. Purves, William K.; David Sadava; Gordon H. Orians; H. Craig Heller (2004). Life: The Science of Biology (7th ed.). Sunderland, Mass: Sinauer Associates. p. 954. ISBN 0-71679856-5.

45. Qamar, A A, and N D Grace. "Abnormal Hematological Indices in Cirrhosis." *Canadian Journal of Gastroenterology = Journal Canadien de Gastroenterologie* 23, no. 6 (June 2009): 441–45.

46. Rang HP, Dale MM, Ritter JM and Moore PK. (2003) Pharmacology, 5th ed, Bath, Churchill Livingstone

47. Rang HP, Dale MM, Ritter JM and Moore PK. (2003) Pharmacology, 5th ed, Bath, Churchill Livingstone

48. Roberts SE, Goldacre MJ, Yeates D. Trends in mortality after hospital admission for liver cirrhosis in an English population from 1968 to 1999. *Gut* 2005;54(11):1615-21.

49. Romer, Alfred Sherwood; Parsons, Thomas S. (1977). *The Vertebrate Body*. Philadelphia, PA: Holt-Saunders International. pp. 354–5. ISBN 0-03-910284-X.

50. Sanjay Kalra, Manoj Vithalani, Gurjeet Gulati, CM Kulkarni, Yogesh Kadam, James Pallivathukkal, Brahmananda Das, Rakesh Sahay, KD Modi; Study of Prevalence of Nonalcoholic Fatty Liver Disease (NAFLD) in Type 2 Diabetes Patients in India (SPRINT). july 2013, Journal of the association of physicians of india ,VOL. 61

51. Shah Latika J, Mulla Summaiya A; prevalence of hepatitis d virus (hdv) in south gujarat. National journal of medical research. Volume 2 Issue 2 Apr – June 2012 print ISSN: 2249 4995

52. Sherlock S, Dooley J. *Diseases of the Liver and Biliary System*: Blackwell Publishing, 2002.

53. Sherwood P, Lyburn I, Brown S, et al: How are abnormal results for liver function tests dealt with in primary care? Audit of yield and impact. BMJ. 2001 Feb 3;322(7281):276-8.

54. Sherwood P, Lyburn I, Brown S, Ryder S. How are abnormal results for liver function tests dealt with in primary care? Audit of yield and impact. *BMJ* 2001;322(7281):276-8.

55. Sherwood P, Lyburn I, Brown S; How are abnormal results for liver function tests dealt with in primary care? Audit of yield and impact. BMJ. 2001 Feb 3;322(7281):276-8.

56. Thomson SJ, Westlake S, Rahman TM, Cowan ML, Majeed A, Maxwell JD, et al. Chronic Liver Disease--An Increasing Problem: A Study of Hospital Admission and Mortality Rates in England, 1979-2005, with Particular Reference to Alcoholic Liver Disease. *Alcohol Alcohol* 2008.

57. Vilar, Consuelo P., Helma P. Cotrim, Gesira S. Florentino, Cibelle P. V. Barreto, André Vinicius A. Florentino, Gerson Bragagnoli, and Paulo A. Schwingel. "Association between Nonalcoholic Fatty Liver Disease and Coronary Artery Disease." *Revista Da Associação Médica Brasileira* 59, no. 3 (May 2013): 290–97. doi:10.1016/j.ramb.2012.11.006.

58. Walsh K, Alexander G; Alcoholic liver disease. Postgrad Med J. 2000 May;76(895):280-6.

59. Walsh K, Alexander G; Alcoholic liver disease. Postgrad Med J. 2000 May;76(895):280-6.

60. Williams J, Roberts S, Ali F, Cheung WY, Cohen D, Demery G, et al. Gastroenterology services in the UK. The burden of disease, and the organisation and delivery of services for gastrointestinal and liver disorders: a review of the evidence. *Gut* 2007;56 Suppl 1:1-113.

61. Wright C, Rivera JC, Baetz JH. Liver function testing in a working population: three strategies to reduce false-positive results. *J Occup Med* 1988;30(9):693-7.

62. Zhou, Y. J., Li, Y. Y., Nie, Y. Q., Ma, J. X., Lu, L. G., Shi, S. L., ... & Hu, P. J. (2007). Prevalence of fatty liver disease and its risk factors in the population of South China. *World journal of gastroenterology*, *13*(47), 6419.

The study design under the Title **'EPIDEMIOLOGY OF LIVER DISEASES IN VADODARA DISTRICT'** have been reviewed by the review board of the Hospital and is found to be appropriate.

The review number is RCL/13/GI/001. The study can be conducted for one academic year starting August 2013, within the Hospital premises.

Date: 12)4)2014

Research head/ Guide

(Dr. Prashant Buch,

Gastroenterologist)

Review Body Head

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Head of Operations)