INTRODUCTION

Chapter 1

INTRODUCTION
The normal liver occupies the right upper quadrant, extending from the fifth intercostal space in the mid clavicular line down to the right costal margin. The lower margin descends below the costal margin during inspiration. The average weight of liver is 1800 gm in men and 1400 gm in women (Furbank, 1967). The liver is the second largest and the heaviest organ in the body and serves a key role in critical metabolic pathways and synthetic functions. It is strategically situated to perform these diverse metabolic functions by being the first organ to receive a nutrient enriched blood supply from the portal system. It is first visible in the developing embryo in the fourth week of pregnancy. As the foetus develops, the liver divides into two sections, the right and the left lobes and ultimately the right lobe will be six times bigger than the left. The right and the left lobes are further divided into a total of eight segments in accordance with subdivisions of the hepatic portal veins. The segments each have their own hepatic artery branch and biliary tree. Each segment is made up of multiple smaller units known as lobules comprised of a central vein, radiating sinusoids separated from each other by single liver cell (hepatocytes) plates and peripheral portal racks. The functional unit of the liver is the hepatic acinus, which is anatomically almost the reverse of the hepatic lobule. The acinus is divided into 3 zones on the basis of the distance from the supplying vessels.

Liver contains hepatocytes or liver cells, a porous lining tissue of macrophages or Kupffer cells derived from blood monocytes, stellate cells found in the space of Disse and endothelial cells lining the hepatic sinusoids. Approximately 15 % of the liver is composed of cells other than hepatocytes (Sleisenger and Fordtran, 1993).

The liver has high blood flow and low vascular resistance. About 1050 ml of blood flow from the portal vein into the liver sinusoids each minute and an
additional 3000 ml flow into the sinusoids from the hepatic artery, the total averaging about 1350 ml per minute. This amounts to 27% of the resting cardiac output. The liver is a large, chemically reactant pool of cells that have a high rate of metabolism, sharing substrates and energy from one metabolic system to another, processing and synthesizing multiple substances that are transported to other areas of the body, and performing a myriad of other metabolic functions.

In carbohydrate metabolism, liver performs 1) storage of large amounts of glycogen (Radziuk et al., 1993) 2) conversion of galactose and fructose to glucose, 3) gluconeogenesis (Pilkis and Granner, 1992) and 4) formation of many clinical compounds from intermediate products of carbohydrate metabolism (Felber and Golay, 1995).

 Certain aspects of fat metabolism occur mainly in the liver. Liver is involved in the 1) oxidation of fatty acids to supply energy for other body functions, 2) synthesis of large quantities of cholestereol, phospholipids and most lipoproteins and 3) synthesis of fats from proteins and carbohydrates. The body cannot dispense without the services of the liver in protein metabolism for more than a few days without death ensuing. The most important functions of the liver in protein metabolism are 1) deamination of amino acids, 2) formation of urea for removal of ammonia from the body fluids, 3) formation of plasma proteins and 4) inter
conversions of the various amino acids and synthesis of other compounds from other amino acids (Guyton and Hall, 2000).

Essentially all the plasma proteins with the exception of part of the γ-globulins are formed by the hepatic cells. This accounts for about 90% of all the plasma proteins. The liver can form plasma proteins at a maximum rate of 15 – 50 gm/day. Therefore, after loss of as much as one half of the plasma proteins from the body, they can be replenished in one or two weeks. It is particularly interesting that plasma protein depletion causes rapid mitosis of the hepatic cells and growth of the liver to a larger size. These effects are coupled with rapid output of plasma proteins until the plasma concentrations returns to normal.

Liver can synthesize all non essential amino acids. It has a particular propensity for storing vitamins and has been long known as an excellent source of certain vitamins in treating patients. The vitamins stored in greatest quantity in the liver is vit A, but large quantities of vit D and vit B12 are normally stored as well. Liver stores iron in the form of ferritin. When the iron in the circulating body fluid reaches a low level, the ferritin releases the iron. The apoferritin-ferritin system acts as a blood iron buffer as well as an iron storage medium. Coagulation factors like fibrinogen, prothrombin, accelerator globulin, factor VII and several other important factors are synthesized in liver.

The liver removes or excretes drugs, hormones and other substances. The active chemical medium of the liver is well known for its ability to detoxify or excrete into the bile, many drugs including sulphonamides, penicillin, ampicillin and erythromycin. Several of the hormones secreted by the endocrine glands, are either chemically altered or excreted by the liver. Liver damage can often lead to excess accumulation of one or more of these hormones in the body fluids and therefore can
cause over activity of the hormonal system. Bilirubin, the major end product of hemoglobin degradation is excreted in the bile by liver (Guyton and Hall, 2000).

1.2. Liver diseases

Liver is so complex and is susceptible to a wide variety of adverse effects caused by excess of alcohol or drugs, infections such as viral hepatitis, cancer and other metabolic disorders. But the liver is also resilient. It has the remarkable ability to regenerate itself following injury or inflammation and it has nutrient reserves, which can be tapped when it is damaged.

Classification of liver diseases based on etiology

<table>
<thead>
<tr>
<th>Viral</th>
<th>Toxic or drug induced</th>
<th>Autoimmune</th>
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</thead>
<tbody>
<tr>
<td>Hepatitis A, B, C, D, E.</td>
<td>Alcohol</td>
<td>Autoimmune chronic active hepatitis</td>
</tr>
<tr>
<td>Epstein Barr virus</td>
<td>Drugs</td>
<td>Primary biliary cirrhosis</td>
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<tr>
<td>Cytomegalovirus</td>
<td>Poisons</td>
<td>Vascular</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>Biliary tract obstruction</td>
<td>Bud-chiari syndrome</td>
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<tr>
<td>Exotic viruses</td>
<td>Tumors</td>
<td>Portal vein thrombosis</td>
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<tr>
<td><strong>Metabolic</strong></td>
<td>Strictures</td>
<td>Neoplastic</td>
</tr>
<tr>
<td>Haemochromatosis</td>
<td>Gall stones</td>
<td>Primary malignant</td>
</tr>
<tr>
<td>Wilson's disease</td>
<td>Sclerosing cholangitis</td>
<td>Benign</td>
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<tr>
<td>The hereditary hyper bilirubinaemia</td>
<td>Primary or secondary biliary atresia</td>
<td>Secondary</td>
</tr>
<tr>
<td>A 1 anti trypsin deficiency</td>
<td>Miscellaneous</td>
<td>Hemorrhagic</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Polysystic liver diseases</td>
<td>Ascariasis</td>
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<tr>
<td>Hepatic porphyria</td>
<td>Congenital hepatic fibrosis</td>
<td>Toxocariasis</td>
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<tr>
<td><strong>Bacterial/spirochaetal</strong></td>
<td>Amyloid</td>
<td>Clonorchis</td>
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<tr>
<td>Leptospirosis</td>
<td>Protozoan</td>
<td>Schistosomiasis</td>
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<tr>
<td>Tuberculosis</td>
<td>Kala – azar (visceral leishmaniasis)</td>
<td>Cryptogenic</td>
</tr>
<tr>
<td>Pyogenic liver abscess</td>
<td>Amoebiasis, Malaria</td>
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When a liver is sieged from viral hepatitis, its liver cells are damaged or destroyed. This type of injury can be initially tolerated and resisted due to the liver’s ability to regenerate and compensate for the damage. This phase of liver disease is called compensated liver disease because the liver is able to continue all
its functions. When the liver begins to lose the battle, and when it is not able to regenerate liver tissue and its filtering and storing abilities are damaged by scar tissue, it reaches the end stage of liver disease called decompensated liver disease as the liver cannot compensate for the ongoing damage.

**Liver disease progression pathway**

![Liver disease progression pathway diagram](image)

Liver inflammation refers to the presence of special cells called inflammatory cells in the liver. Chronic inflammation is inflammation that persists over a long period of time. It leads to changes in liver structure, slowed blood circulation, and the death of liver cells (necrosis). Chronic inflammation eventually causes scar tissue formation, a condition known as fibrosis. Fibrosis is the harmful outcome of chronic inflammation. When fibrosis become widespread and progresses to the point that the internal structure of the liver has become abnormal, fibrosis progresses...
to cirrhosis. Cirrhosis is the result of long term liver damage caused by chronic inflammation and liver cell death. The most common causes include viral hepatitis, excessive intake of alcohol, inherited diseases and hemochromatosis. Cirrhosis is accompanied by a reduction in blood supply to the liver. The loss of healthy liver tissue and reduced blood supply can lead to abnormalities in liver function. Clinical manifestations of liver disease include jaundice, portal hypertension, abnormal hepatorenal function, altered drug metabolism, nutritional and metabolic abnormalities, disordered homeostasis and the release of enzymes into various body fluids (Carl et al., 2006).

1.2.1. Cirrhosis

The term cirrhosis was first used by Rene Laennec (1781-1826), to describe the abnormal liver colour of individuals with alcohol induced liver disease. The word cirrhosis comes from the Greek word Kirrhos, the name for yellowish colour. Normal functioning of the liver depends on its proper organization. Cirrhosis is a diffuse process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules (Blaker et al., 2001). It is the final pathologic and clinical expression of a wide variety of chronic liver diseases (Pan et al., 2004). The major complications reported to be associated with cirrhosis are jaundice (Gubernick et al., 2000), infections, portal hypertension (Bilbao et al., 2002), variceal and gastrointestinal bleeding (Odelowo et al., 2002). Liver failure in cirrhosis is assessed by features such as ascites (Aalami et al., 2000), encephalopathy (Butterworth, 2000), low serum albumin and prothrombin deficiency not corrected by vitamin K (Bustamante et al., 1999).

Cirrhosis can be irreversible and life threatening. It is a public health concern because of its association with mortality and morbidity. The only available and definitive treatment is liver transplantation. Cirrhosis is however reversible in
most cases. Classically cirrhosis has been classified as 1) micro nodular, 2) macro nodular and 3) mixed, based on the histology and gross appearance of the liver.

The earliest abnormalities found to develop in cirrhosis are 1) fall in platelet count (Giannini et al., 2003) 2) increase in prothrombin time, 3) decrease in the albumin to globulin ratio to less than one (Luo et al., 2002) and 4) increase in the Aspartate amino transferase/Alanine amino transferase (AsT/AlT) activity ratio to greater than one (Luo et al., 2002; Giannini et al., 2003).

Women are more susceptible to liver damage than men. They are likely to develop at an earlier stage. When it is severe, it leads to more complications (Wong and Blendis, 2001). Liver function is usually impaired in patients with cirrhosis and because cirrhotic livers are less able to regenerate, it is important to stimulate both the regeneration and function of the remnant cirrhotic liver after hepatectomy (Burroughs et al., 2004).

1.2.2. Alcoholic liver cirrhosis

Alcohol abuse is a leading cause of morbidity and mortality throughout the world. Alcohol affects many organ systems of the body but perhaps most notably affected are the central nervous system and the liver. Almost all ingested alcohol is metabolized in the liver and excessive alcohol use can lead to acute and chronic liver disease. The three alcohol abuse conditions are fatty liver, hepatitis and cirrhosis (Howard, 1998). Ethanol is the most common causes of cirrhosis in the US. Genetics may play a role in the development of alcoholic liver disease. Risk factors for developing alcoholic liver disease include 1) duration and magnitude of alcohol ingestion. The risk dosage is 80 gm of alcohol per day. Daily drinking appears to be riskier than intermittent drinking (Lelbach, 1975). 2) Gender: In women there is a greater likelihood of progression of cirrhosis because of reduced activities of alcohol dehydrogenase in gastric mucosa leading to
increased blood levels of alcohol (Svikis and Reid-Quinones, 2003). 3) Hepatitis B or C infection or both may increase the severity in patients who drink heavily (Mendenhall et al., 1991). 4) Genetic factors: Patterns of alcohol drinking behaviour is inherited (Whitefield, 1999; Whitefield et al., 2004). 5) Nutritional status: Protein calorie malnutrition is extremely common in alcoholics. There is evidence for an immunological component in alcoholic liver disease (French, 2002; Ishii et al., 1993) and there is modification of liver proteins by ethanol metabolites involved in the pathogenesis (Teare et al., 1993). Alcohol is metabolised to acetaldehyde by alcohol dehydrogenase (Tanaka et al., 1996) and then to acetate by acetaldehyde dehydrogenase. Genetic pleomorphism of the enzyme systems that metabolize alcohol, leading to different rates of alcohol elimination, also contributes to the individual’s susceptibility to alcohol damage. Alcoholics with decreased acetaldehyde dehydrogenase activity develop alcoholic liver disease at a lower cumulative intake than others (Wong and Blendis, 2001).

Acetaldehyde formed from alcohol is toxic to different tissues, especially liver. Alcohol induced cytochrome P2E1 that leads to lipid peroxidation and low glutathione level (Nicholas et al., 2006). Raised serum lipid peroxide concentrations can be found during acute inflammatory liver disease. Acute changes in liver function, reflected by high bilirubin concentrations seems to be more important for intravascular liberation of lipid peroxides than existence of specific etiologic factors or of severe long lasting global liver damage (Southorn and Powis, 1988). Consumption of alcohol increases the gut permeability to endotoxins, which induces the Kupffer cells to release tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), leading to liver inflammation (Abbas et al., 1994; Male et al., 1996).

Alcohol abuse can lead to the accumulation of fat within the hepatocytes, the predominant cell type in the liver. A similar condition can also be seen in some
obese people who are not alcohol abusers. Fatty liver is reversible if the patients stop drinking alcohol. However, fatty liver can lead to steatohepatitis (Howard, 1998). Fatty liver is the most frequent hepatic abnormality found in alcoholics. It is a toxic manifestation of ethanol ingestion appearing within 3–7 days of excess alcohol intake. Metabolic changes associated with ethanol ingestion result in increased triglyceride synthesis, decreased lipid oxidation and impaired secretion of the liver. This results in the accumulation of triglycerides in the hepatocytes mainly in the terminal hepatic venular zone. Fatty liver may occur alone or be part of the picture of alcoholic hepatitis or cirrhosis (Reynard et al., 2002).

In some clinically important conditions such as hepatitis fibrosis, cirrhosis and fatty liver, the collagen content has been reported to be abnormal (Brandao et al., 2006). Collagen deposition is a complex process that depends on synthesis in hepatic stellate cells and degradation by collagenases. Excessive deposition of collagen could occur during an imbalance in its metabolism. Alcohol intoxication activate hepatic stellate cells and Kupffer cells to secrete reactive oxygen species, that induce the production of Transforming Growth Factor-β (TGF-β) all of which induce the fibrogenic process. TGF-β and IL-6 upregulate the expression of type-1 collagen genes (Purohit and Brenner 2006). Reactive oxygen species (ROS) can inactivate enzymes containing sulphydryl groups, especially collagenases and proteases responsible for collagen degradation, which results in accumulation of collagen in liver (McCullough, 2006).

1.2.3. Non alcoholic liver cirrhosis

Nonalcoholic liver cirrhosis is mainly due to nonalcoholic fatty liver disease (NAFLD). The pathophysiology of NAFLD is complex and available data suggests that environmental factors such as exercise and toxins are likely to be important in its causation (Cotrim et al., 1999). Nonalcoholic fatty liver disease is an increasingly recognized form of chronic liver disease. It encompasses a spectrum of
conditions associated with lipid deposition in hepatocytes. It ranges from steatosis (simple fatty liver) to nonalcoholic steatohepatitis and advanced to fibrosis and cirrhosis. Studies suggest that although simple fatty liver is a benign condition, nonalcoholic steatohepatitis may progress to fibrosis and lead to end-stage liver disease. It is strongly associated with obesity and insulin resistance and is currently considered by many as a hepatic component of the metabolic syndrome (Bugianesi et al., 2005).

Nonalcoholic fatty liver disease is the most common cause of mild alteration of liver enzyme levels in the western world, and, according to the National Health and Nutritional Survey, point-prevalence is about 23% among American adults (Harrison et al., 2002). The biochemical picture includes mildly raised aminotransferase levels, and γ-glutamyl transferase (GGT) levels can be elevated up to 3 times the upper reference value in nearly half of patients in the absence of ethanol consumption (Brunt, 2004). The AST/ALT ratio greater than 1, which is observed in 61% of patients with advanced fibrosis and 24% of patients with no or initial fibrosis, is highly suggestive of advanced liver disease (Angulo et al., 1999). Suspicion of nonalcoholic fatty liver disease is increased by the presence of conditions linked to the metabolic syndrome and insulin resistance (increased body mass index, diabetes, hyperlipemia, hypertension), although the disease may occur in patients without these associated factors (Brunt, 2004).

1.2.4. Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the 5th most common tumor worldwide. The epidemiology of HCC exhibits two main patterns, one in North America (El-Serag, 1999) and Western Europe and another in non-Western countries, such as sub-Saharan Africa, central and Southeast Asia, and the Amazon basin, suggesting that both host and environmental factors are involved in its etiology. There is a two
to three fold higher incidence of carcinoma in females and is usually more common between the 3rd and 5th decades of life (Bosch, et al., 1999; Kumar et al., 2003). Hepatocellular carcinoma causes 662,000 deaths worldwide per year. Liver cells (hepatocytes) make up 80% of the liver tissue, thus the majority of primary cancers arises from liver cells and is called hepatocellular carcinoma. Hepatocellular carcinoma, like any other cancer, develops when there is a mutation to the cellular machinery that causes the cell to replicate at a higher rate and/or results in the cell, avoiding apoptosis. (Dennis, 2007).

Once cirrhosis is developed, the rate of development of HCC is about 1.5 – 5% per year in both hepatitis B virus (HBV) and hepatitis C virus (HCV) (Fattovich et al., 1995; Fattovich et al., 1997). The risk of HCC doubles in the case of those infected with both viruses (Fattovich, 1998). The mechanism of increased risk in HBV is thought to be related to the integration of HBV DNA into the host genome, which may block the activity of p53 (Feitelson et al., 1993; Ueda et al., 1995). The role of hepatitis B virus (HBV) infection in causing liver cancer is well established (Fattovich 1997; Fattovich 1995). Hepatitis C infection also associated with the development of liver cancer (El-Serag, 1999).

Cirrhosis caused by chronic alcohol consumption is the most common association of liver cancer in the developed world. When an individual with alcoholic cirrhosis has stopped drinking for ten years, develop liver cancer. It is unusual for an actively drinking alcoholic to develop liver cancer, because when drinking is stopped, the liver cells try to heal by the regeneration process. It is during this active regeneration that a cancer producing mutation can occur, which explains the occurrence of liver cancer after drinking has been stopped. Food infected with Aspergillus flavus (especially peanuts and corns stored during prolonged wet seasons) which produces aflatoxin, poses another risk factor for HCC. It is thought to cause liver cancer by producing changes (mutations) in the p53
gene. These mutations work by interfering with the gene’s important tumor suppressing functions (Puisieux et al., 1991).

Female hormones (estrogens) and protein building drugs are associated with the development of hepatic adenoma that can later become malignant. Liver cancer will develop in up to 30 % of patients with hereditary hemochromatosis. Individuals with more types of cirrhosis of the liver are at an increased risk of liver cancer than are other causes.

It is a primary malignancy of the hepatocytes that generally leads to death within 6 months of the onset. HCC frequently arises in the setting of cirrhosis, appearing 20–50 years after the initial insult to the liver (Burrough et al., 2004). The usual outcome is poor, because only 10–20 % of hepatocellular carcinoma can be removed completely by surgery. If the cancer cannot be completely removed, the disease is usually deadly within 3 to 6 months.

Symptoms of liver cancer include abdominal pain, unexplained weight loss or unexplained fever, ascites, jaundice, muscle wasting without any causative factors. It is often detected by ultrasound screening, and so can be discovered by health-care facilities much earlier than in developing regions such as Sub-Saharan Africa. Tumor markers of hepatocellular carcinoma include, α fetoprotein, des-γ carboxyprothrombin, α-L fucosydase and isoenzymes of γ glutamyl transferase.

Liver function tests are useful in detecting, diagnosing, evaluating severity, monitoring therapy and assessing the prognosis of the liver disease and liver function. The array of tests useful for these purposes include measurement in plasma of total bilirubin, albumin concentrations and the activity of enzymes such as the aminotransferases (AST and ALT), ALP, lactate dehydrogenase and GGT. By using a combination of these tests, it is possible to categorize broad types of liver disease, which can then be more accurately diagnosed through disease specific tests.
1.3. Bilirubin

Bilirubin consists of an open chain of four pyrrole-like rings (tetrapyrrole). In heme, by contrast, these four rings are connected into a larger ring, called a porphyrin ring. Bilirubin is created by the activity of biliverdin reductase on biliverdin. Bilirubin, when oxidized, reverts to become biliverdin once again. This cycle, in addition to the demonstration of the potent antioxidant activity of bilirubin, has led to the hypothesis that bilirubin's main physiologic role is as a cellular antioxidant (Baranano et al., 2002).

Bilirubin is the product of hemoglobin catabolism within the reticuloendothelial system mainly by spleen, which is then transported to liver. Uptake of bilirubin across the hepatocyte sinusoidal membrane occurs by carrier mediated mechanism, which is shared by other organic anions. The uptake process is rapid, has a great capacity and is not rate limiting for hepatic bilirubin transport. Several carrier proteins that mediate bilirubin uptake into the hepatocytes have been identified. Once inside the hepatocytes, bilirubin appears to interact with membrane lipids such that it may transport from membrane to membrane (plasma membrane to endoplasmic reticular membrane). In endoplasmic reticulum, the COOH groups of one or both of its propionic acid side chains are esterified with glucuronic acid by the enzyme bilirubin UDP glucoronlyl transferase to form bilirubin mono or di glucuronides, which are more water soluble. The hepatocyte microtubular system, hepatic bile salt excretion, and membrane carrier proteins, all appear to facilitate the excretion of bilirubin glucuronides (conjugated bilirubin) into bile (Berk and Noyer, 1994).

In plasma, four bilirubin fractions are identified. 1) Unconjugated bilirubin (α-bilirubin), which is usually bound with albumin, 2) bilirubin mono glucuronate (β-bilirubin), 3) bilirubin di glucuronide (γ bilirubin) and 4) conjugated bilirubin bound to albumin (δ-bilirubin or biliprotein) (Carl et al., 2006).
Unconjugated bilirubin may increase because of augmented bilirubin production or decreased hepatic uptake or conjugation or both. In adults, the most common conditions associated with unconjugated hyperbilirubinemia are hemolysis and Gilbert’s syndrome (Fevery and Blanckaert, 1986). Other, less frequent causes of unconjugated hyperbilirubinemia include reabsorption of large hematomas and ineffective erythropoiesis (Pratt and Kaplan, 2000).

In healthy people, conjugated bilirubin is virtually absent from serum mainly because of the rapid process of bile secretion (Green and Flamm, 2002). Bilirubin levels increase when the liver has lost at least half of its excretory capacity. Therefore, the presence of increased conjugated bilirubin is usually a sign of liver disease. Conjugated hyperbilirubinemia (usually <34 μmol/L) and concomitant, markedly elevated aminotransferase levels may suggest acute viral hepatitis or toxic or ischemic liver injury. Furthermore, this biochemical picture can be the presenting feature of autoimmune hepatitis (Krawitt, 1996; Alvarez et al., 1999; Kessler et al., 2004).

A purely cholestatic picture, with conjugated hyperbilirubinemia, an increase in ALP levels and a negligible increase in aminotransferase levels, may be present in cholestatic drug reactions (Lee, 2003; Velayudham and Farrell 2003). Sometimes, the same biochemical picture may be present in the late presentation of previously unrecognized autoimmune cholestatic diseases (primary biliary cirrhosis, primary sclerosing cholangitis). In these patients, the presence of other signs of chronic liver disease may facilitate diagnosis (Ponsioen and Tytgat, 1998; Morrison and Kowdley, 2000; Heathcote, 2000). Biliary obstruction can cause various degrees of conjugated hyperbilirubinemia. The severity of alteration depends upon the degree and duration of obstruction and the functional reserve of the liver. Biliary obstruction may have an abrupt onset and be preceded by typical symptoms.
(right upper quadrant pain, nausea) or may be silent and progressive. With the presence or absence of concomitant aminotransferase alteration, a liver ultrasound is essential to identify and locate the obstacle to bile flow. Once the causal condition of conjugated hyperbilirubinemia has resolved, whatever be the cause, bilirubin serum levels decrease in a bimodal fashion. There is a first, rapid decrease and then a later, slower decrease caused by the binding of bilirubin to albumin and the formation of a complex (δ-bilirubin) that has the same half-life as serum albumin (Berk and Noyer, 1994; Fevery and Blankaert, 1986; Van Hootegem et al., 1985).

Gilbert's syndrome is a genetic disorder of bilirubin metabolism, which can result in mild jaundice, found in about 5% of the population. Moderate rise in bilirubin may be caused by drugs (especially anti-psychotic, some sex hormones, and a wide range of other drugs), hepatitis (levels may be moderate or high), biliary stricture (benign or malignant). Very high levels of bilirubin may be caused by neonatal hyperbilirubinemia, where the newborn's liver is not able to properly conjugate the bilirubin. Hyperbilirubinemia is also reported in large bile duct obstruction, e.g., stone in common bile duct, tumour obstructing common bile duct etc, severe liver failure with cirrhosis, severe hepatitis, Crigler-Najjar syndrome, Dubin-Johnson syndrome and Choledocholithiasis (chronic or acute).

1.4. Albumin

Albumin has a single polypeptide chain of 580 amino acids, with 17 intra chain SS bonds aligned in a multiple loop structure. It has a molecular weight of 69 Kd and it contains 17 histidine residues (Vasudevan and Sreekumari, 2003). Albumin is one of only a few plasma proteins with no carbohydrate side chain. It is a very stable protein with a high net negative charge at physiological pH and partly as a result is very high solubility in water. There is one free SH group at position 34 that reacts completely with thiol compounds such as cysteine at physiological pH (Peters, 1996).
Albumin is primarily synthesized by the hepatic parenchymal cells except in early foetal life, when it is synthesized largely by the yolk sac. The synthetic rate is controlled primarily by colloidal osmotic pressure and secondarily by protein intake (Peters, 1996; Rothschild et al., 1972). Liver produces about 12 grams of albumin per day and its half life is about 20 days (Vasudevan and Sreekumari, 2003). Catabolism occurs primarily by pinocytosis by all tissues. By the process of lysosomal catabolism proteins are converted to free amino acids for the synthesis of cellular proteins. The rate of pinocytosis is proportional to the local tissue metabolic rate.

The primary function of albumin is generally considered to be the maintenance of colloid osmotic pressure in both the vascular and extra vascular compartments. The presence of many charged surface groups and many specific binding sites, both ionic and hydrophobic gives albumin the ability to bind and transport a large number of compounds. This includes free fatty acids, phospholipids, cholesterol, metallic ions, amino acids, drugs, hormones and bilirubin. It functions as an amino acid source for pheripheral tissues and act as a buffer (Vasudevan and Sreekumari, 2003). The binding of albumin to endothelial membrane associated glycoproteins increases capillary permeability to small proteins that are important for metabolism in the extra vascular space. Albumin inhibits leukotriene and actin
production, thus reducing the inflammatory response of platelets and neutrophils. Albumin also possesses anti oxidant activity (Carl et al., 2006).

Inflammatory disorders both acute and chronic are the most common causes of decreased plasma albumin levels. The most common cause of decreased plasma albumin levels are 1) hemodilution, 2) loss into the extra vascular space caused by increased vascular permeability, 3) increased consumption by cells locally and 4) decreased synthesis as a result of direct inhibition by cytokines (Ryffel et al., 1994) 5) decreased release by hypokalaemia (Rothschild et al., 1972). 6) small amounts are also lost into the gastrointestinal tract and the glomerular filtrate (Peters, 1996).

Plasma albumin measurements are useful in assessing the chronicity and severity of liver diseases. Low serum albumin, often found with severe chronic liver disease is probably due to its reduced synthesis. The plasma albumin level is an indication of the synthetic capacity of liver.

1.5. Liver enzymes

There are four liver enzymes that are commonly used in the diagnosis of liver diseases. They are aspartate aminotransferase (AsT; EC:2.6.1.1), alanine aminotransferase (AlT; EC 2.6.1.2), alkaline phosphatase (ALP; EC 3.1.3.1) and γ-glutamyltransferase (GGT; EC 2.3.2.2). AlT and GGT are present in several tissues, but plasma activities primarily reflect liver injury. AsT is found in liver, muscle and to a limited extent in red blood cells. Bone and liver are good sources of ALP in normal individuals, though it is seen in a number of other tissues. Based on tissue distribution, AlT and GGT would seem to be the most specific markers for liver injury (Carl et al., 2006).
1.5.1. Aminotransferases

Injury to liver, whether acute or chronic, eventually results in an increase in serum concentrations of aminotransferases. AST and ALT are enzymes that catalyze the transfer of α-amino groups from aspartate and alanine to the α-keto group of ketoglutaric acid to generate oxaloacetic and pyruvic acids respectively, which are important contributors to the citric acid cycle. Both enzymes require pyridoxal-5'-phosphate (vitamin B6) in order to carry out this reaction, although the effect of pyridoxal-5'-phosphate deficiency is greater on ALT activity than on that of AST (Dufour et al., 2000; Vanderlinde, 1986). This has clinical relevance in patients with alcoholic liver disease, in whom pyridoxal-5'-phosphate deficiency may decrease ALT serum activity and contribute to the increase in the AST/ALT ratio that is observed in these patients (Cohen and Kaplan, 1979; Diehl et al, 1984). Both aminotransferases are highly concentrated in the liver. AST is also diffusely represented in the heart, skeletal muscle, kidneys, brain and red blood cells, and ALT has low concentrations in skeletal muscle and kidney (Panteghini, 1990). An increase in ALT serum levels is, therefore, more specific for liver damage. In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20 % of total activity) and mitochondrial (80 % of total activity) (Rej, 1989). Zone 3 of the hepatic acinus has a higher concentration of AST, and damage to this zone, whether ischemic or toxic, may result in greater alteration to AST levels. Aminotransferase clearance is carried out within the liver by sinusoidal cells (Kamimoto et al, 1985). The half-life in the circulation is about 47 hours for ALT, about 17 hours for total AST and, on average, 87 hours for mitochondrial AST (Dufour et al., 2000).

Patients with a marked increase in aminotransferase levels more than 10 times the upper reference limit, typically have acute hepatic injury. However, data from a series of patients with acute hepatic injury due to viral hepatitis suggest that the most sensitive and specific aminotransferase threshold level to identify acute
injury lies within the moderate range of increase of 5–10 times the upper reference limit, at 200 IU/L for AST and 300 IU/L for ALT (Rozen et al., 1970). Thus, the academic attribution of cause and “severity” of acute damage on the basis of the magnitude of enzyme elevation may sometimes be misleading, since there can be grey areas in which a range of causes overlap. Moreover, the degree of elevation varies during the course of injury and depends on the time of enzyme levels tested. Despite these ambiguities, the magnitude and rate of change of aminotransferase alteration may provide initial insight into a differential diagnosis.

Very high aminotransferase levels of more than 75 times the upper reference limit indicate ischemic or toxic liver injury in more than 90% of cases of acute hepatic injury, whereas they are less commonly observed with acute viral hepatitis (Dufour et al., 2000). In ischemic or toxic liver injury, AST levels usually peak before those of ALT because of the enzyme’s peculiar intralobular distribution (Dufour and Teot, 1988; Singer et al., 1995; Seeto et al., 2000). Zone 3 of the acinus is more vulnerable to both hypoxic (hepatocytes are exposed to an already oxygen-poor milieu) and toxic (hepatocytes are richer in microsomal enzymes) damage. Furthermore, in ischemic injury aminotransferase levels tend to decrease rapidly after peaking. In about 80% of patients with ischemic injury, the serum bilirubin level is lower than 34 \( \mu \text{mol/L} \). It is important to stress that a decrease in aminotransferase levels alone after a marked increase does not have prognostic meaning, since both resolution and massive hepatic necrosis may draw a similar biochemical picture. In this setting, patients with high bilirubin serum levels and prolonged prothrombin time should be closely monitored for the risk of hepatic failure. In cases of acute viral hepatitis, aminotransferase levels usually peak before jaundice appears and have a more gradual decrease thereafter, and there is a greater increase in serum bilirubin levels (Clermont and Chalmers, 1967). Jaundice occurs in about 70% of cases of acute hepatitis A infection, 33 – 50% of cases of acute
hepatitis B infection and 20 – 33 % of cases of acute hepatitis C infection (Dufour et al., 2000).

1.5.2. **Alkaline phosphatase**

Alkaline phosphatases are zinc metalloenzymes that release inorganic phosphate from several organic orthophosphates. They are present in nearly all tissues. Their natural substrates may include pyrophosphate, phosphoserine and phosphoethanolamine. Intestinal ALP acts as a calcium dependent ATPase. In liver, alkaline phosphatase can be found histochemically in the microvilli of the bile canaliculus and on the sinusoidal surface of hepatocytes. Alkaline phosphatase exists in tissue specific isoforms, some of which are true isoenzymes in that they are the products of separate genes. Biliary, liver, bone, placental, renal and intestinal isozymes are identified (Rosalki, 1975; Nemesanszky, 1986). The alkaline phosphatases from liver, bone and kidney are thought to be coded for the same gene, while the ALP from intestine and placenta has different genes. Alkaline phosphatase activity in normal serum is mainly due to the isoforms from bone and liver with near equal proportions. Intestinal ALP may also contribute up to 20 % of total activity. Placental enzyme may appear in mid-pregnancy.

ALP activity varies with age and sex and with several other factors. There are two peaks, one in neonatal period and the other in adolescence. Levels also tend to rise in older subjects with increased liver ALP in elderly men and bone ALP in elderly women (Kuwana et al., 1988). Cellulose acetate electrophoresis identifies two liver fractions, a major fraction of \( \alpha-1 \) globulin mobility accompanied by a fraction of \( \alpha-2 \) globulin mobility, together with bone isoenzymes. Intestinal isoenzyme is easily identified by its \( \beta \) globulin mobility. Other methods for the detection of ALP isoenzymes are by using monoclonal antibodies and wheat germ lectin precipitation (Rosalki and Foo, 1984).
The serum ALP activity rises in many liver diseases, the highest level occurring in intrahepatic or extrahepatic obstruction to the flow of bile. In acute viral hepatitis ALP is usually either normal or moderately raised but up to 40% of patients have levels two and half times the upper reference limit. The serum alkaline phosphatase is increased by drugs that cause cholestasis liver disease. Elevation was also reportedly caused by cimetidine (Payne et al., 1982), frusemide (Math, 1982), phenobarbitone (Balazs et al., 1978) and phenitoin (Moss, 1975). Low ALP has been found in Wilson’s disease presenting with hemolytic anemia and evidence of severe liver dysfunction (Shaver et al., 1986).

Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissue, such as the placenta, kidneys or intestines or from leukocytes (Fishman, 1990). The third trimester of pregnancy (placenta origin) and adolescence (bone origin) are associated with an isolated increase in serum ALP levels (Dufour et al., 2000). Hepatic ALP is present on the surface of bile duct epithelia. Cholestasis enhances the synthesis and release of ALP, and accumulating bile salts increase its release from the cell surface (Schlaeger et al., 1982; Moss, 1997). ALP half-life in the circulation is about 1 week (Dufour et al., 2000). These characteristics explain why ALP levels usually rise late in bile duct obstruction and decrease slowly after resolution.

In some patients (e.g., pregnant women, adolescents) the reason for increased ALP levels may be straightforward, but in other patients it is necessary to identify the origin of the enzyme. This task can be accomplished in 2 ways: assessment of GGT levels or dosage of ALP isoenzymes. From a practical point of view, measurement of GGT is preferred since it relies on automated analysis rather than on more sophisticated and expensive techniques. The degree and rate of enzyme alteration may provide minor and nonspecific clues to diagnosis, but the presence of
symptoms and the patient's history, with particular emphasis on comorbid conditions, may provide fundamental clues. Liver ultrasound may reveal the presence of bile duct dilation, demonstrate signs of chronic liver disease or even liver cirrhosis, and identify hepatic masses.

Drug-induced liver injury may present with a cholestatic pattern (preferential increase in ALP or ALT/ALP ratio < 2), although the degree of ALP alteration is variable and may be accompanied by hyperbilirubinemia (Velayudham and Farrell, 2003). Commonly used drugs such as antihypertensives (e.g., angiotensin converting enzyme inhibitors) or hormones (e.g., estrogen) may cause cholestasis and can be overlooked. It has been found that ALP acts as an acute phase reactant in Hodgkin's disease, congestive heart failure and in infectious and inflammatory diseases (Brensilver and Kaplan, 1975; Parker et al., 1989) Normal alkaline phosphatase is also observed in patients with primary biliary cirrhosis (Sherlock and Scheuer, 1973) and in primary sclerosing cholangitis (Cooper and Brand, 1988).

Increased serum intestinal ALP activity is found in cirrhosis and may result from diminished hepatic uptake, perhaps due to disruption of receptors for intestinal alkaline phosphatase of the liver cell surface, or due to diminished hepatic excretion of catabolism (David and Vincent, 1998). Tumors may secrete ALP into plasma. Some tumors may produce specific isoenzymes- for example Regan, Nagao and Kasahara isoenzymes may be found in patients with carcinoma of the bile duct. The Kasahara isoenzyme (a fetal intestinal like phosphatase) has been found in the serum of about 30% of patients with primary liver cell carcinoma (Higashino et al., 1975).

1.5.3. Gamma glutamyl transferase

Gamma glutamyltransferase (GGT) or gamma glutamyltranspeptidase (GGTP) is a membrane bound glycoprotein that catalyses the transfer of $\gamma$ glutamyl
groups from γ-glutamyl peptides particularly glutathione to other peptides to amino acids and to water. It is found mainly in the membranes of cells with a high rate of secretory or absorptive activity. Large amounts are present in the kidneys, pancreas, liver, intestine and prostate and is also found in many other tissues. The γ-GT activity in bile is approximately 100 times greater than in normal serum (Rosalki, 1975). Several isoforms of GGT have been described but there is no clear evidence of tissue specificity. The heterogeneity is related to the number of sialic acid residues, to the degree of glycosylation and to binding to lipoproteins (Rosalki, 1984). The half life of GGT has been reported as 1-4 days. It is proposed that the enzyme is removed by receptor-mediated endocytosis by liver macrophages.

In liver, GGT is present in hepatocytes and biliary epithelial cells, renal tubules, and the pancreas and intestine. The mechanisms of alteration are similar to those described for alkaline phosphatase. It is a microsomal enzyme, and its activity can be induced by several drugs, such as anticonvulsants and oral contraceptives (Rosalki, 1971). Elevated GGT levels can be observed in a variety of nonhepatic diseases, including chronic obstructive pulmonary disease and renal failure, and may be present for weeks after acute myocardial infarction. Increased serum levels observed in alcoholic liver disease can be the result of enzyme induction and decreased clearance. In these patients, GGT serum levels can be markedly altered (>10 times the upper reference value), whereas ALP levels may be normal or only slightly altered (GGT/ALP ratio > 2.5). The whole spectrum of liver diseases, regardless of cause, may be responsible for altered GGT serum levels. In particular, GGT levels may be 2–3 times greater than the upper reference value in more than 50% of the patients with nonalcoholic fatty liver disease and above the upper reference value in about 30% of patients with chronic hepatitis C infection (McCullough et al., 2002; Giannini, et al., 2001). Furthermore, an increase in GGT levels in patients with chronic liver disease is associated with bile duct damage and fibrosis (Giannini et al., 2001). Thus, because of its lack of specificity but high sensitivity for liver
disease, GGT can be useful for identifying causes of altered ALP levels, or elevated levels, together with other biochemical abnormalities (AST/ALT ratio > 2) may support the diagnosis of alcoholic liver disease (Cohen and Kaplan, 1979).

1.6. Acute phase proteins

Acute phase proteins (APP) or acute phase reactants (APR) is a generic name given to a group of approximately 30 different biochemically and functionally unrelated proteins. These proteins are secreted by hepatocytes (Ruminy et al., 2001) and their levels in the serum are either increased (positive acute phase reactants) or reduced (negative acute phase reactants) approximately 90 minutes after the onset of a systemic inflammatory reaction. Acute phase proteins are synthesized predominantly in the liver with all hepatocytes possessing the capacity to produce the entire spectrum of these proteins in response to tissue injury as a result of neoplasia, trauma and infection (Heinrich et al., 1990). Following stimulation of single hepatocyte within individual lobule, one observes a stimulation of further hepatocytes and this process continues until almost all hepatocytes produce these proteins and release them into the circulation. The various APP differ markedly in the rise or decline of their plasma levels and also in their final concentrations. APP generates a characteristic serum protein profile. Levels of elevated expression can differ widely from species to species and some proteins that can function as APP in one species may not be an acute phase protein in another species (Baumann, 1988).

Acute phase proteins regulate immune responses, function as mediators and inhibitors of inflammation, act as transport proteins for products generated during the inflammatory process, and play an active role in tissue repair and tissue remodelling. Acute phase response is a protective physiological reaction of the organism to disturbances of its homeostasis due to inflammation caused by tissue injury, infection or neoplastic growth (Heinrich et al., 1990; Fey and Gauldie, 1990; Baumann and Gauldie, 1994). Van Molle et al (1999) have suggested that at least
some APP might constitute an inducible system of factors protecting against cell
death by apoptosis. They have observed that α-1 antitrypsin and α-1 acid
glycoprotein activate the major executioners of apoptosis, caspase 3 and caspase 7.

Some of the APP behaves like cytokines, C-reactive protein, for example
activates macrophages, some influence the chemotactic behaviour of cells, some
possess antiproteolytic activity and presumably block the migration of cells onto the
lumen of blood vessels thus helping to prevent the establishment of generalized
systemic inflammation. A failure to control these processes or an uncontrolled APR
eventually has severe pathologic consequences.

The elevated serum concentrations of certain acute phase proteins are of
diagnostic relevance and also of prognostic value. Their measurement, for example,
allows inflammatory processes to be distinguished from functional disturbances
with similar or identical clinical pictures. Under normal circumstances an APR is
not observed with functional disturbances that are not the result of an inflammatory
process, thereby allowing the differentiation between failure of function and organic
disease (Dofferhoff et al., 1992).

Some acute phase reactions are observed in chronic disorders such as
rheumatoid arthritis and chronic infections, while malignant diseases are almost
invariably associated with an APR and therefore the determination of acute phase
protein cannot be used for differential diagnosis in these instances. There are many
diseases in which the rise in the synthesis of acute phase proteins parallels the
degree and progression of the inflammatory processes (Ramadori et al., 1999).

Acute phase response is a systemic reaction of the organism to non specific
systemic stimuli that is accompanied by increased production of a cytokine cascade,
which includes tumor necrosis factor-α (TNF-α), IL-1, IL-6, IL-11, leukemia
inhibitory factor and oncostatin M (Heinrich et al., 1990; Fey and Gauldie, 1990; Baumann and Gauldie, 1994). Characteristic of AP response after a local injury includes the release of cytokines, which in turn induce a systemic reaction manifested by fever elevated secretion of glucocorticoids and changes in the concentration of acute phase proteins. These APP are either upregulated (positive APP) or down regulated (negative APP) during the acute phase response. Protease inhibitors, blood coagulation factors, transport proteins and compliment components are examples for positive APP, which are commonly upregulated 2–10 fold on both the mRNA and protein levels. Typical negative APP include albumin and transferrin. The spectrum of acute phase proteins produced in hepatoma cells however varies quantitatively and qualitatively between the different cytokines studied (Semenza and Wang, 1992, 1993).

The two cytokine mediators IL-1 and IL-6 have been used to classify APP into two subgroups. Type 1 APP are those that require the synergistic action of IL-6 and IL-1 for maximum synthesis e.g., CRP, serum amyloid A and α-1 acid glycoprotein. Type 2 APP are those that require IL-6 only for maximum induction e.g., fibrinogen chains, haptoglobin, α-2 macroglobulin. Expression of genes encoding type 2 APP is suppressed rather than being enhanced frequently by IL-1 (Ramadori et al 1999; Fey et al, 1994). Additive synergistic, co operative and antagonistic effects between cytokines and other mediator substances influencing the expression of APP do occur and have been observed in almost all combinations. Many cytokines also show differential effects, inducing the synthesis of one or two APP but not others (Benigni et al., 1996).

Several animal models were used for the analysis of the acute phase response including rats (Baumann et al., 1983) mice (Beaudet et al., 1982; Baumann et al., 1984) and rabbits (MacIntyre et al., 1983), each of which has a slightly different subset of proteins that responds to the appropriate stimulation in vivo. In vivo
studies are complicated by the multiplicity of cell types in the body and pose some questions whether the stimulus acts directly or indirectly upon the hepatocytes. Primary hepatocyte cultures have shown that stimulating agent such as turpentine and bacteria do not operate directly on hepatocytes. Rupp and Fuller (1979) have shown that one acute phase protein fibrinogen was elevated when supernatants derived from isolated peripheral monocytes were added to primary rat hepatocytes in vitro. This demonstration clarified the role of cellular intermediates in the acute phase response although the primary hepatocytes culture system is not a totally pure population of parenchymal cells. Sipe et al., (1982) have reported the induction of serum amyloid A after the injection of mice with purified fractions of mouse IL-1 and endogenous pyrogens. Woloski and Fuller (1985) have shown that a separate factor, which they have called hepatocytes stimulating factor elevated fibrinogen in primary rat hepatocytes cultures.

The altered hepatic transcription of the APP genes represents an adaptive response to minimize damage during the acute phase response. Activated macrophages invade damaged tissues and release a number of factors into the bloodstream including interleukin-1β. This 17.4 Kd lymphokine reproduces most acute phase changes when administered to rats. Some of these in vivo responses are also reproduced by the administration of recombinant IL-1β to hepatoma cells grown in vitro (Karin et al., 1985).

There are many diseases in which the rise in the synthesis of acute phase proteins parallels the degree and progression of the inflammatory processes. The coordinated expression of many acute phase proteins as a direct consequence of the activities of several cytokines can be explained, at least in part, by the fact that the regulatory sequences of the genes encoding these acute phase proteins contain so-called cytokine response elements. These elements are recognized specifically by
transcription factors that mediate the activity of these genes in a cell- and/or tissue-specific manner (Richards et al., 1991).

Interleukin-1 and also interferon-γ reduce some of the effects of IL-6. Some of the effects of IL-2 and IL-6 are antagonized by TGF-β. The combined action of two or even more cytokines may produce effects that no factor on its own would be able to achieve. In cultured HepG2 hepatoma cells IL-1, IL-6, TNF-α and TGF-β induce the synthesis of antichymotrypsin and at the same time repress the synthesis of albumin and α-fetoprotein (AFP). The synthesis of fibrinogen is induced by IL-6 and this effect is, in turn, suppressed by IL-1α, TNF-α or TGF-1β. The increased synthesis of Haptoglobin mediated by IL-6 is suppressed by TNF-α. Insulin inhibits the synthesis of some negative acute phase proteins (prealbumin, Transferrin, and fibrinogen, in HepG2 hepatoma cells (Ikawa and Shozen, 1990).

Different patterns of cytokines are involved in systemic and localized tissue damage, which is supported by observations with knock-out mice for IL-1 and IL-6. Inflammatory acute phase response after tissue damage or infection is severely compromised in IL-6 knock-out mice, but only moderately affected after challenge with bacterial lipopolysaccharides (Kopf et al, 1994). Fattori et al (1994) show that in the absence of IL-6, the induction of acute phase proteins is dramatically reduced in response to turpentine injections but that parameters are altered to the same extent both in wild-type and IL-6 deficient mice following injection of bacterial lipopolysaccharides. These mice, however, produce three times more TNF-α than wild-type controls. Bopst et al (1998) observed a normal acute phase reaction to both turpentine and bacterial lipopolysaccharides in TNF-β knock-out mice. They have reported a striking absence of elevated major acute phase proteins, serum amyloid P and serum amyloid A, in mice deficient in TNF-β and IL-6. Fantuzzi et al (1996) demonstrated that IL-1β knock-out mice, on the other hand, show a normal response to bacterial lipopolysaccharides.
1.6.1. C-reactive protein

The major site of synthesis of C-reactive protein is the hepatocyte (Hurlimann et al., 1966, Kushner and Feldmann, 1978). CRP is secreted from the liver cells and can be observed in the serum as an annular disc consisting of five identical mature polypeptide subunits (Osmond et al., 1977). The genomic sequence of human CRP indicates that the precursor subunit is composed of 206 amino acids of mature peptide as well as an 18 amino acid signal sequence assay (Lei et al., 1985).

C-reactive protein is named for its capacity to precipitate the somatic C polysaccharide of Streptococcus pneumoniae, was the first acute phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage (Pepys et al., 2003). CRP has been shown to bind specifically to phosphorylcholine in a calcium-dependent manner (Volanakis and Kaplan, 1971). Another ligand with higher affinity than phosphorylcholine, chromatin, has been shown to bind to CRP (Robey et al., 1984). The binding of these ligands to CRP has been shown to initiate the classical complement pathway by the subsequent binding of complement component Clq (Kaplan and Volanakis, 1974, Robey et al., 1984). In addition, CRP has been found to play an important role in the platelet-dependent killing of immature schistosomes (Bout et al., 1986). CRP also has been shown to bind to a specific class of T-lymphocytes (James et al., 1983) large granular lymphocytes. CRP, the major acute phase reactants in humans, derives mainly from hepatocytes in response to IL - 6 and is then secreted into the systemic circulation (Ross, 1999). CRP down regulates endothelial nitric oxide synthase resulting in decreased release of NO and this facilitates endothelial cell apoptosis and inhibition of angiogenesis (Libby, 2002).

Of the markers of inflammation, CRP has been shown in multiple prospective studies to predict the incidence of recent myocardial infarction, stroke,
Peripheral vascular disease and sudden cardiac death (Ridker, 2001, Ridker et al. 2003). Recent studies have shown that elevated CRP is strongly associated with various characteristics of the metabolic syndrome (Yudkin et al., 1999; Festa et al., 2000; Ridker et al. 2003).

The levels of CRP increased in parallel with the progression of chronic liver diseases. Levels of α-2 macroglobulin is not changed with chronic hepatitis or liver cirrhosis, while those in patients with hepatocellular carcinoma are significantly higher than in controls or liver cirrhosis. Serum hepatocyte growth factor (HGF) showed a positive correlation with CRP and a negative correlation with albumin. The serum levels of acute phase proteins such as albumin and α-2 macroglobulin are more closely associated with the degree of hepatic dysfunction than serum HGF levels (Shiota et al., 1995). Highly enhanced fucosylation of serum glycoproteins was found in HCC compared with liver cirrhosis and that the combination of measurements of fucosylated alpha feto protein or transferrin was useful for the diagnosis of HCC (Naitoh et al., 1999). Serum CRP is not a good marker for HCC, however very high values of CRP in patients with cirrhosis may suggest the presence of a diffused type HCC (Lin et al., 2000).

C-reactive protein is the prototype acute phase protein in humans (Mortensen, 2001) and its synthesis is stimulated by cytokines, especially IL-1β and IL-6. Transcription factors involved in IL-6 mediated CRP synthesis included signal transducer and activator of transcription 3 and members of the C/EBP family, especially C/EBP α, β and δ (Ghosh and Karin, 2002; Moscat et al., 2003).

CRP synthesis increases during infections, allergic complications of infections, inflammatory disease, necrosis, trauma and malignancy (Pepys and Hirschfield, 2003). The elevation of serum CRP is known in various liver diseases (Atono et al., 1989; Lee et al., 1989; Murakami et al., 1989). The CRP
concentration is a useful nonspecific biochemical marker of inflammation, measurement of which contributes importantly to a) screening for organic disease, b) monitoring of the response to treatment of inflammation and infection and c) detection of inter current infection in immuno compromised individuals, and in the few specific diseases characterized by the modest or absent acute phase responses (Pepys et al., 2003).

### 1.6.2. Transferrin

The iron binding protein of the plasma is transferrin, which is very similar to lactoferrin found in granulocytes and milk. It is a monomeric glycoprotein with 79.6 Kd and made up of 679 amino acids organized in 2 homologous domains, each containing an iron binding site (van Eden and Young, 1995). It is synthesized almost exclusively in the liver, with lesser amounts in the choroids plexus of the brain. Plasma levels are regulated primarily by the availability of iron. In iron deficiency, plasma transferrin level rise and on successful treatment with iron return to normal. It has a half life of approximately 8 -10 days.

Transferrin is involved in iron metabolism. Apotransferrin binds with iron absorbed from the intestine or released from catabolism of hemoglobin to form transferrin. The iron is then transported to storage sites such as the liver and the endothelial system and to sites of synthesis of iron containing compounds especially the erythropoietic tissue. These cells have surface receptors for transferrin (Lash and Saleem, 1995). After binding, transferrin iron complex is internalized into
caltherin coated endosome that lowers the internal pH, resulting in the release of iron from transferrin. Iron is transported across the vesicle membrane for utilization or storage within the cell and the transferrin receptor complex recycles back to the cell surface, where apotransferrin is released at the higher pH of blood (Aisen et al., 2001).

High levels of transferrin are seen in pregnancy and during estrogen administration. Transferrin is a negative acute phase protein, and low levels are observed in inflammation, malignancy, liver disease, malnutrition, protein losing enteropathies (Carl et al., 2006).

The amino acid backbone of transferrin contains two side chains at Asn 413 and Asn 611, which may bear bi or tri antinnary oligosaccharide side chains with terminal sialic acid groups. In a healthy patient the majority of the blood transferrin molecules carry 4 or 5 sialic acid groups. However, alcoholic patients possess transferrin with either no or less number of sialic acid groups. Such sialic acid group deficient transferrin level is found to be increased in alcoholic liver cirrhotic patients (Stibler, 1991). Serum acute phase proteins exist in different glycoforms. Abnormally glycosylated transferrin and other glycoproteins are found to be increased in different types of diseases including liver diseases. Besides transferrin, other clinically relevant proteins exist in differently glycosylated isoforms including glycosylated markers for cancers and other diseases.

Insulin is able to inhibit the synthesis of prealbumin, transferrin and fibrinogen (Thompson et al., 1991). The micro heterogeneity analysis of human serum transferrin is useful for distinguishing patients with hepatocellular carcinoma from those with liver cirrhosis and normal controls (Suzuki et al., 1996). Both albumin and transferrin gene expression has been shown to be regulated by various
agents such as cell density, human growth hormone and temperature (Sporn and Roberts, 1983).

**1.6.3. Ferritin**

Ferritin is a ubiquitous iron storage protein, the shell of which consists of a mixture of 24 heavy (H-21,000 Da) and light (L-19,000 Da) subunits (Theil, 1987). The subunits are roughly cylindrical in shape and form nearly a spherical shell that encloses a central core containing up to 4500 atoms of iron in the form of ferric hydroxyphosphate. *in vitro*, ferritin behaves as an iron storage protein. Human ferritins are made up of two types of subunits (H and L subunits) in varying proportions. The gene for the L subunit is located on chromosome 19 and the gene for the H subunit is found in chromosome 11 (Worwood, 1990). Apoferritin will bind and oxidise Fe$^{2+}$ and deposit Fe$^{3+}$ within the protein. The release of iron may be effected by reducing agents.

Studies in animal cell culture show that apoferritin is synthesized in response to iron administration and this control is largely exercised at the level of translation (Worwood, 1990). The 5' end of untranslated ferritin mRNA contains 28 base sequence that forms a stem loop structure. This has been termed an iron response element. A cytoplasmic protein that binds to this sequence and prevents translation has been also been identified. In the presence of iron, this protein is unable to bind to the mRNA, and polysomes forms to proceed translation.

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Detailed investigation of L-subunit mRNA expression revealed that translational control mechanisms regulate L-sub unit synthesis in response to IL-1β in human hepatoma cells. Northern blot and RNase protection analyses show that L-ferritin mRNA levels in HepG2 cells are unaffected by IL-1β treatment. The response of ferritin synthesis to IL-1β is accompanied by a redistribution of L-ferritin mRNA towards the polyribosomes consistent with an increase in translational efficiency. This occurs within two hours of cytokine administration and persists for at least 14 hours. Rat liver and spleen ferritin synthesis is elevated 3-4 fold 6 hours after the onset of an experimentally induced inflammatory response (Konijn and Hershko, 1977; Campbell et al., 1989). Konijn et al., (1981) suggested that increased ferritin synthesis occurs as the result of translational mechanisms since cytoplasmic extracts taken from rat liver reproduced this induction in the absence of nuclei in vitro. L-subunit mRNA was shown to be recruited from mRNAs to polyribosomes in rat liver and spleen cells 12 h after a turpentine induced inflammation (Campbell et al., 1989). The mRNAs for both H and L-ferritins are translationally activated within the first 2 hours of administering iron to human and rat hepatoma cells (Rogers and Munro, 1987), human erythroleukemia cells (K562) (Rouault et al., 1988) and mouse fibroblast cell lines (Walden and Tahch, 1986). In intact animals, a similar induction by iron results in a 10–20 fold increase in liver ferritin synthesis (White and Munro, 1988).

1.6.4. Ceruloplasmin

Ceruloplasmin is a major plasma protein containing most of the circulating copper (Ryden and Bjork, 1976; Linder and Hazegh-Azam, 1996). Ceruloplasmin is an α2-glycoprotein mainly synthesized in the liver parenchymal cells with small amounts synthesized by macrophages and lymphocytes and secreted into the serum as the major copper transporting protein (Sas Kortsak and Bearn, 1965). This glycosylated serum protein has a molecular weight of 132 Kd and contains 6-8 copper molecules. Copper appears to be essential for the normal folding of the
polypeptide chain and possibly for normal CHO side chain attachment. The peptide chain is formed first and copper is added from an intra cellular ATPase. Apo Cp is synthesized even in the absence of copper or ATPase. In this condition most of it is degraded intracellularly but moderate amounts are released into the circulation, where Apo Cp has a very short half life.

Numerous functions have been attributed to ceruloplasmin, including a crucial role in iron metabolism through its peroxidase activity (Young et al., 1997; Mukhopadhya et al., 1998). It exhibits oxidase activity and oxidizes among other substrates, Fe$^{2+}$ to Fe$^{3+}$ that can then be stored by ferritin and transported into cells by transferrin (Ozaki and Johnson, 1969; Harris et al., 1999). It is involved in the antioxidant functions in the prevention of the formation of free radicals in serum (Gutteridge, 1983; Miyajima et al., 1996; Richardson et al., 1999). It is also involved in a number of metabolic processes related to copper metabolism (Harris, 1993), biogenic amines and nitric oxide metabolism (Bianchini et al., 1999). A lack of ceruloplasmin leads to iron accumulation in the liver and finally to liver damage (Harris et al., 1999; 1995).

Ceruloplasmin is a member of the acute phase protein family, and, consequently, its serum level is increased during inflammation as well as in various malignancies (Gitlin, 1988; Ramadori et al., 1988). Ceruloplasmin is absent in aceruloplasminemia (Loreal et al., 2002) and may be slightly decreased in other diseases such as fulminant hepatitis (Walshe et al., 1962) and genetic hemochromatosis (Laine et al., 1992). Serum ceruloplasmin is found to be decreased in decompensated cirrhosis with hepatic failure (Cauza et al., 1997).

Dominique et al., (2001) reported an increase in the ceruloplasmin level to extremely high levels during the development of HCC in ATIII-Tag transgenic mice and investigated the factors responsible for this increased synthesis.
Ceruloplasmin is found in the globulin fraction in the mammalian plasma. It shows significant size and charge heterogeneity because of differences in glycosylation, the number of copper atoms present, peptide chain variations, secondary to alternative DNA splicing and polymerization. In addition, it is very susceptible to proteolysis both *in vivo* and *in vitro*, by many proteases including trypsin, plasmin, leucocyte elastase and a plasma metalloproteinase. There are two important molecular isoforms, one predominating in bile (125 Kd) and the other in plasma (132 Kd). The biliary form is important for copper excretion, which is absent in Wilson’s disease.

Serum ceruloplasmin is an important diagnostic marker in Wilson’s disease, in which the plasma level is usually reduced. Low ceruloplasmin is also seen in neonates, Menke’s disease, Kwashiorkar and marasmus, protein losing enteropathy, nephrotic syndrome, severe hepatic insufficiency, copper deficiency and in hereditary hypoceruloplasminaemia (David and Vincent, 1994).
AIM OF THE STUDY

1) To determine the amount of bilirubin, albumin, serum enzymes (Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase) and acute phase proteins (C-reactive protein, transferrin, ferritin, ceruloplasmin) in the serum of liver disease patients like nonalcoholic liver cirrhosis (NALC), alcoholic liver cirrhosis (ALC), hepatocellular carcinoma (HCC).

2) To compare the above serum parameters between control and all the diseased categories (NALC, ALC and HCC categories) of this study.

3) To compare the above serum parameters among the diseased categories (NALC, ALC and HCC categories).