# Biochemical and Socio-epidemiological Analysis of Different Types of Hepatitis

By

**Asma Sadia**

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# DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

**Asma Sadia**

# CERTIFICATE

This thesis, submitted by **Ms. Asma Sadia** to the Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, is accepted in its present form as satisfying the thesis requirement for the Degree of Master of Philosophy in Biochemistry/Molecular Biology.

Supervisor:

Dr. Samina Shakeel
Assistant Professor

External Examinar:

Dr. Atika Umer
Scientific Officer
National Institute of Health, (NIH)
Islamabad

Chairperson:

Dr. Bushra Mirza
Professor

Dated: Oct 07, 2013

*He it is Who sends Blessings on you,*
*as do His Angels,*
*that He may bring you out from the depths of*
*Darkness into the Light:*
*(Al Ahzab – 43)*

*.....To my Parents, with Love*

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| µl | Microlitre |
| AIH | Autoimmune Hepatitis |
| ALD | Alcoholic Liver Disease |
| ALP | Alkaline Phosphatase |
| ALT | Alanine Aminotransferase |
| AMPK | Adenosine Monophosphate-activated Protein Kinase |
| ANA | Antibodies to Nuclear Antigen |
| ANOVA | Analysis of Variance |
| AST | Aspartate Transaminase |
| BMI | Body Mass Index |
| CI | Confidence Interval |
| CRP | C-reactive Protein |
| CTL | Cytotoxic T-lymphocytes |
| CVD | Cardiovascular Disease |
| DEA | Diethanolamine |
| DILI | Drug Induced Liver Injury |
| DMSO | Dimethyl Sulfoxide |
| DNA | Deoxyribonucleic Acid |
| ELISA | Enzyme Linked Immunosorbent Assay |
| FasL | Fas Ligand |
| FFA | Free Fatty Acid |
| GGT | Gamma-glutamyltransferase |
| GPT | Glutamic-pyruvate Transaminase |

| | |
|---|---|
| HBcAg | Hepatitis B Core Antigen |
| HBeAg | Hepatitis B e Antigen |
| HBsAg | Hepatitis B Surface Antigen |
| HBV | Hepatitis B Virus |
| HCC | Hepatocellular Carcinoma |
| HCl | Hydrochloric Acid |
| HCV | Hepatitis C Virus |
| HLA | Human Leukocyte Antigen |
| IFN□ | Interferon Gamma |
| IL-1 β | Interleukin-1 β |
| IR | Insulin Resistance |
| L/B/K ALP | Liver/Bone/Kidney ALP |
| LC 1 | Liver Cytosol type 1 |
| LFT | Liver Function Test |
| LKM | Liver/Kidney Microsomes |
| MCV | Mean Corpuscular Volume |
| mg/dl | Milligram Per Decilitre |
| MHC | Major Histocompatibility Complex |
| min | Minute |
| ml | Millilitre |
| mmol/L | Millimol Per Litre |
| NADH | Nicotinamide Adenine Dinucleotide |
| NAFLD | Non-alcoholic Fatty Liver Disease |
| NASH | Non-alcoholic Steatohepatitis |
| nm | Nanometer |

| | |
|---|---|
| NO | Nitric Oxide |
| NSAIDs | Non-steroidal Anti-inflammatory Drug |
| OR | Odd Ratio |
| ORF | Open Reading Frame |
| PAPR | Pathogen Associated Pattern Recognition |
| PCR | Polymerase Chain Reaction |
| PIMS | Pakistan Institute of Medical Sciences |
| pNPP | p-Nitrophenylphosphate |
| p-Value | Probability Value |
| rpm | Revolutions Per Minute |
| SE | Standard Error |
| SLA | Soluble Liver Antigen |
| SMA | Smooth Muscle Antigen |
| SPSS | Statistical Package for the Social Sciences |
| SREBP | Sterol Regulatory Element Binding Protein |
| TMB | 3, 3', 5, 5'- Tetramethylbenzidine |
| TNF | Tumor Necrosis Factor |
| U/L | Units Per Litre |
| US | United States |

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# ABSTRACT

Hepatitis is a major disease burden associated with high rates of morbidity and mortality especially in developing countries.

This study was conducted to determine the frequency of hepatitis B and C viral infections among abnormal liver test patients. Association of viral infections with liver function test parameters and socio-epidemiological factors was also determined.

We obtained socio-epidemiological data of 1000 individuals, suspected of liver dysfunction. Serum levels of alanine aminotransferase, bilirubin and alkaline phosphatase were determined using chemistry analyzer. Presence of hepatitis B surface antigens and anti-hepatitis C virus antibodies was detected by an enzyme immunoassay in individuals having raised levels of alanine aminotransferase and bilirubin.

Out of 1000 subjects tested for liver function markers, only 132 (13.2%) had abnormal liver function test with higher prevalence in males (7.7 % versus 5.5 %), 20-40 years age group (20-40 years; 6.3 % , 41-60 years; 5.1 % , > 60 years; 1.8 %), socially deprived individuals (79.5 %) and subjects using medicines (41.7 %). A statistically significant association was observed between hepatitis B surface antigen and socio-epidemiological factors; female gender, self/family history of hepatitis and low socioeconomic status, anti-hepatitis C virus antibodies and socio-epidemiological factors; female gender, surgical history, body piercing, history of dental treatment and low socioeconomic status. Odd ratios, 95 % confidence interval and probability value were used to determine statistical significance. Alanine aminotransferase and bilirubin levels were significantly higher in subjects with viral hepatitis than without viral hepatitis.

Higher level of endemicity of hepatitis B and C viral infections and their association with elevated levels of alanine aminotransferase and bilirubin highlights the importance of diagnostic tests for viral hepatitis especially in individuals with marked elevations in the level of alanine aminotransferase and bilirubin. To reduce viral infections due to social inequity and nosocomial factors, some regulatory policies should be considered.

# *Introduction*

# 1. INTRODUCTION

Liver is the largest and most important organ in our body. It is found in the right upper part of abdomen, just beneath the diaphragm. A layer of connective tissues called Glisson's capsule covers the whole organ. It is halved into a larger right lobe and a smaller left lobe via falciform ligament (Sutherland and Harris, 2002; Abdel-Misih and Bloomston, 2010).

Liver plays a central role in perfoming body's functions. Some of important functions performed by the liver include defense, detoxification, synthesis, storage and recycling (Pimstone *et al.*, 1994; Michalopoulos, 2007).

Liver screens all blood coming from small and large intestines, spleen and pancreas via portal vein. Liver serves as a biochemical defense against toxic chemicals in blood. It also reprocesses some components of absorbed food. Some nutrients are converted into transmembrane and secretory proteins such as albumin, coagulation factors and plasma carrier proteins (Akhavan and Lingappa, 2007). Lipids are transported to other tissue in the form of lipoprotein (Cohn *et al.*, 2008). Carbohydrates are stored in the liver as glycogen (Tudhope *et al.*, 2012). Bile synthesized by liver is important for emulsification and disposal of lipids (Meier and Stieger, 2000). It also helps in absorption and transport of other nutrients and serve as signaling and inflammatory agent (Chiang, 2009). As a regulator of blood glucose and ammonia, it protects brain from encephalopathy (Lobley *et al.*, 1995; Tudhope *et al.*, 2012). Owing to physiological importance of liver, diagnosis and management of liver damage is very important. To know about the causes and risk factors, play a crucial role in this respect.

## 1.1 Important Markers of Liver Dysfunction

### 1.1.1 Alanine Aminotransferase

Alanine transaminase (ALT) also known as glutamic-pyruvate transaminase (GPT) is a member of transaminase family of enzymes. It is a pyridoxal enzyme that catalyzes reversible transamination between alanine and 2-oxoglutarate to form pyruvate and glutamate. By catalyzing the conversion of these major intermediate metabolites, ALT has an important role in gluconeogenesis and amino acid metabolism (Yang *et al.*, 2002). In muscle and certain other tissues which degrade amino acids for fuel, amino

groups are collected from glutamate by transamination. ALT catalyzes the transfer of amino group from glutamate to pyruvate to form alanine, which is a major amino acid in blood during fasting. Alanine is taken up by the liver to form glucose from pyruvate in a reversal of the ALT reaction, called alanine- glucose cycle. This cycle is also important during exercise when skeletal muscles operate anaerobically, producing ammonia groups from protein breakdown and pyruvate from glycolysis (Felig, 1973). Moreover, alanine transamination is also involved in formation of glutamate, an important neurotransmitter in the brain.

ALT is found in many tissues including liver, muscle, heart, kidney, pancreas, spleen lung and brain(Clark *et al.*, 2003). However, liver is the main resident of this clinically important enzyme. Thus, the most significant aspect of ALT is that it is used clinically as a surrogate marker for liver function. Serum ALT activity is significantly elevated during liver damage caused by drug toxicity, infection, alcohol, and steatosis (Sherman, 1991).

### 1.1.2 Bilirubin

Bilirubin is produced in the reticuloendothelial system by the breakdown of Hemoglobin. After formation, it is released into the blood in unconjugated form. In the liver, it is conjugated with glucuronic acid to form bilirubin mono and diglucuronides. This reaction is catalyzed by the enzyme UDP-glucuronyltransferase. Conjugated forms are now water soluble and are excreted in bile. The value of normal serum total bilirubin varies from 2-21 μmol/L. The level of unconjugated bilirubin is about 12 μmol/L and conjugated bilirubin 8 μmol/L. jaundice occurs when presence of bilirubin is evident in sclera, skin and mucous membranes (Titcomb, 2003; Gowda *et al.*, 2009).

### 1.1.3 Alkaline Phosphatase

Alkaline phosphatases (ALPs) are a group of membrane-bound glycoproteins that hydrolyze a wide range of monophosphate esters at alkaline pH (Weiss *et al.*, 1988). It is found that human serum contains at least five isoforms of ALP (Wieme and Demeulenaere, 1970). However, some studies consider these as three distinct forms viz placental, intestinal, and liver/bone/kidney (L/B/K ALP). A placental-like ALP is found in small amounts in testis and thymus also. It is considered that all of the human ALP genes have arisen from a single ancestral gene. Placental and intestinal

ALPs are more closely related, showing about 87% identity at the amino acid level. L/B/K ALP is evolutionarily more distinct from the other ALPs. It shows 52 and 57% amino acid identity to placental and intestinal ALP, respectively (Weiss *et al.*, 1988).

Different forms of human ALPs show a specific pattern of tissue distribution. Placental and intestinal phosphatases are found predominantly in placenta and small intestine, respectively. While, L/B/K ALP is found to be present in various tissues, including liver, bone and kidney (Weiss *et al.*, 1988). ALP originates mainly from two sources that are liver and bone (approximately 80% being of liver or bone origin). In healthy individuals, most of the rest comes from the intestine (Titcomb, 2003). In the liver, the enzyme is located in the bile canaliculi (McKenna, 2008) whereas, it is found in mucosal epithelia of small intestine and proximal convoluted tubule of kidney (Gowda *et al.*, 2009). It is involved in lipid transportation in the intestine and bone calcification in bones. The serum ALP activity is mainly from the liver with 50% contribution from bone (Gowda *et al.*, 2009). The elevation in enzyme level may be physiological or pathological. Tissues undergoing metabolic stimulation exhibit high levels. Elevations are also observed in the third trimester of pregnancy, due to an efflux of placental ALP. Adolescents may show increases as a result of bone ALP into the blood, owing to growth activities. Individuals with blood groups B and O show increased intestinal ALP after a fatty meal. In addition, ALP values usually increase between ages 40 and 65, particularly in women (Limdi and Hyde, 2003; Titcomb, 2003).

### 1.1.4 Other Markers

**Aspartate aminotransferase (AST)** being a member of transaminase family of enzymes catalyzes transamination reaction. AST has two genetically distinct isoforms, the mitochondrial and cytoplasmic form. ALT activity is found in liver, heart, kidney and skeletal muscles. However, highest concentration is present in heart compared with other tissues (Panteghini *et al.*, 1983).

**Gamma-glutamyltransferase (GGT)** is a microsomal enzyme found in hepatocytes, biliary epithelial cells, renal tubules, pancreas and intestine. It is also present in cell membrane where it is involved in glutathione metabolism and transport of peptides into the cell across the cell membrane. Serum GGT activity is contributed

predominantly by hepatobiliary system although it is found in high concentration in renal tissues (Carey, 2000).

**Albumin** synthesis is an important function of the liver by reprocessing absorbed food components. Approximately 10 g of albumin is synthesized and secreted per day. Any changes in albumin levels indicate about synthetic activity of liver (Limdi and Hyde, 2003).

## 1.2 Etiological Factors of Liver Dysfunction

### 1.2.1 Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is considered to be a leading cause of liver disease in western countries with a prevalence of upto 30%. Among children, prevalence of NAFLD is 3% (Jou *et al.*, 2008; Malhi and Gores, 2008; Dowman *et al.*, 2010).

Its risk is increased is people with obesity and insulin resistance. This may lead to the formation of lipid vacuoles in hepatocytes if uncontrolled. This paves the way for development of steatohepatitis and fibrosis in the presence of factors such as free fatty acids (FFAs), inflammatory cytokines, oxidative stress and mitochondrial dysfunction. Once fibrosis is developed, a person becomes prone to cirrhosis and hepatocellular carcinoma (Jou *et al.*, 2008; Malhi and Gores, 2008).

It has been analyzed that obesity and insulin resistance commonly results in hepatic steatosis but progression to steatohepatitis and cirrhosis occurs in relatively less number of people. It shows that genetic predisposition is an important player in this regard. Risk of NASH and cirrhosis is increased due to polymorphism in genes associated with lipid metabolism, IR, oxidative stress, cytokines and fibrosis (Rodríguez-Suárez *et al.*, 2010; Rossi *et al.*, 2012; Souza *et al.*, 2012).

### 1.2.2 Drug Induced Liver Injury

The liver is the main organ involved in metabolizing and detoxifying drugs and other toxins. As a result, liver is more prone to toxic damage than any other organ (Hartleb *et al.*, 2002; Bleibel *et al.*, 2007).

Drugs reach the liver through portal circulation after being absorbed from intestine. In hepatic cells, these drugs undergo complex metabolic reactions to be converted into

hydrophilic substances. Now these substances are soluble in blood stream and easily eliminated afterwards (Lee, 2003; Bleibel *et al.*, 2007).

Drug induced liver injury (DILI) is responsible for 0.1 – 3.0% of hospital admissions. It is the major cause of death from acute liver failure in United States (Hartleb *et al.*, 2002; Lazerow *et al.*, 2004; Lee and Senior, 2005). One half of drugs reported in Physician desk reference can cause liver damage at any level (Lewis, 2002). Leading cause of DILI is acetaminophen followed by antibiotics, NSAIDs, amiodarone and anti-TB medicines (Rashid *et al.*, 2004; Bleibel *et al.*, 2007). Acetaminophen alone is responsible for 50 % of cases of acute liver failure in US (Lee and Senior, 2005).

Different drugs damage different liver cells by different mechanisms. Liver cell types affected by drug include hepatocytes, bile duct cells, sinusoidal epithelial cells, stellate cells and kupffer cells (Cullen, 2005; Ramachandran and Kakar, 2009).

Drugs and their metabolites result in either biochemical changes in cell or mediate immune response (Mackay, 1999; Kaplowitz, 2002).

Biochemical changes occur due to reactions resulting in deficiency of reduced glutathione and increase in cellular oxidative stress. These reactions result in damaging effect on proteins, DNA and other cellular molecules. It also results in imbalance between apoptotic and survival signals (Kaplowitz, 2002; Park *et al.*, 2005; Grattagliano *et al.*, 2009).

Immune mediated DILI is triggered by pro-inflammatory cytokines such as TNF, Fas L, NO, IFN-□ ultimately leading to apoptosis (Mackay, 1999; Lee, 2003; Ju, 2005; Bleibel *et al.*, 2007). A part of drug or its metabolite binds to MHC type II and presented on surface of liver cells. This promotes the formation of antigen recognizing helper and cytotoxic T-cells. Binding of drug/drug metabolites with MHC type I and its presentation on liver cell surface activate humoral and cellular immune response (Kaplowitz, 2002; Bleibel *et al.*, 2007).

The complication of DILI may vary from mild elevation of LFT to severe liver damage which is characterized by high level of transaminases and bilirubin, jaundice and decreased synthetic activity of liver (Hartleb *et al.*, 2002; Lee, 2003; Rashid *et al.*, 2004). Diagnosis of DILI is not very easy. It requires to exclude other types of liver damages such as by viral hepatitis, autoimmune liver disease and NASH (Gunawan

and Kaplowitz, 2004; Lee and Senior, 2005). LFT elevation proves helpful if it occurs within 5 - 90 days of drug use. And after drug use is withdrawn, LFT becomes normal within 8 days (Gunawan and Kaplowitz, 2004; Bleibel *et al.*, 2007). Thus exclusion of other possible causes and taking thorough history of patient is important.

Based on biochemical pattern of LFT abnormality, DILI can be divided into 3 different groups. Predominant elevation in transaminase level indicates hepatocellular damage, whereas bile duct damage is favoured by rise in ALP. When none of the markers show abnormality but symptoms are there, the type of liver cell injury is considered as mixed pattern (Bussieres and Habra, 1995; Rashid *et al.*, 2004).

### 1.2.3 Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is periportal hepatitis characterized by presence of elevated immunoglobulin and autoantibodies (Malik and Saeed, 2010). Prevalence of AIH is low however it varies in different regions of world (Boberg, 2002; McFarlane, 2002). It affects women more than men (Boberg, 2002). AIH is classified into 2 main groups (Albert and Inman, 1999; Manns and Vogel, 2006; Malik and Saeed, 2010).

In type 1 AIH, antibodies to nuclear antigen(ANA), smooth muscle antigen (SMA) and soluble liver antigen (SLA) are found (Albert and Inman, 1999; Malik and Saeed, 2010).

Type 2 AIH is characterized by presence of antibodies to liver/kidney microsomes (LKM) and liver cytosol type 1 (LC 1) (Albert and Inman, 1999; Malik and Saeed, 2010).

Factors responsible for liver cell damage and autoimmunity are not well known (Manns and Vogel, 2006). However, it is proposed that some intrinsic factors or extrinsic infectious agents trigger autoimmunity (Ermann and Fathman, 2001; Regner and Lambert, 2001). AIH usually results in fibrosis leading to cirrhosis and chronic liver failure in 15% of patients (Czaja and Carpenter, 2004; Kogan *et al.*, 2002). Risk of hepatocellular carcinoma in AIH patients is very low (Park *et al.*, 2000). Individuals having AIH show genetic predisposition to the disease. HLA polymorphism is found to show association with this disease (Klein and Sato, 2000).

Patients with AIH show marked elevation of LFT. Moreover, this disease tends to accumulate in families due to genetic predisposition. Therefore taking family/self-

history along with liver function test can provide a clue to AIH though it does not run in families in mandalian fashion (Ben-Ari *et al.*, 1993; Czaja and Freese, 2002a; Hennes *et al.*, 2008). However for better diagnosis, exclusion of other causes of chronic hepatitis is required such as viral hepatitis, NASH, ALD, hereditary liver diseases and other hepatic/extrahepatic causes (Czaja and Freese, 2002b; Manns *et al.*, 2010). Histological assessment and evidence of serological markers is also essential to confirm diagnosis (Czaja and Freese, 2002b).

### 1.2.4 Alcoholic Liver Disease

Alcohol is the leading cause of liver disease worldwide. Alcoholic liver disease (ALD) range in severity from simple steatosis to development of cirrhosis (Lefkowitch, 2005; Mendez-Sanchez *et al.*, 2005). Upto 90% of individuals who consume about 60g of alcohol per day are likely to develop steatosis (Crabb, 1999 ). However, this state is usually self limiting (O'Shea *et al.*, 2010). Steatosis may develop into fibrosis and cirrhosis in 5 to 15% of patients (Sorensen *et al.*, 1984 ; O'Shea *et al.*, 2010). In 2003, ALD was found to be the cause of 44% of all deaths resulting from liver disease in American population (Yoon and Yi, 2006).

Above data suggests that there are some additional factors, which play a role in progression of liver injury due to alcohol abuse (O'Shea *et al.*, 2010). These include dose (Savolainen *et al.*, 1993), duration (Lu *et al.*, 2004), type (Becker *et al.*, 2002 ), and time of alcohol consumption (Lu *et al.*, 2004). Other factors are gender (Sato *et al.*, 2001), ethnicity (Lu *et al.*, 2004), improper diet (Mendenhall *et al.*, 1995 ) and other comorbidities (Befrits *et al.*, 1995; Monto *et al.*, 2004). Women are more prone to liver injury than men using same type and dose of alcohol (Sato *et al.*, 2001). Possible explanation to this disparity lies in the difference of gastric alcohol dehydrogenases, obesity, and alcohol absorption (Frezza *et al.*, 1990).

However, these factors are still insufficient to explain differential progression of liver disease in alcoholics. Involvement of genetic factors offers most likely explanation to this (Day and Bassendine, 1992; Jarvelainen *et al.*, 2001). Polymorphs of genes involved in alcohol metabolism such as alcoholic dehydrogenases are important contributors (Jarvelainen *et al.*, 2001). Other genes involved are those associated with fibrosis such as COL1A1 and COL1A2. HLA polymorphism is also found to play a role in this regard (Day and Bassendine, 1992). It is found that expression of CD 14 is

increased on monocytes and macrophages in alcohol abusers. On CD 14 receptors, endotoxins together with LPS binding protein bind preferentially. This triggers release of proinflammatory cytokines such as TNF-α and IL-1 β. Such inflammatory pathways may lead to liver injury (Jarvelainen *et al.*, 2001).

Alcohol consumption leading to liver injury results in abnormal liver function test (Lee *et al.*, 2001). Levels of serum ALT and AST are found elevated. However, these are not sensitive markers of alcohol abuse (Conigrave *et al.*, 2003). Elevation in GGT and MCV together may increase reliability of diagnosis (Hannuksela *et al.*, 2007; Hartmann *et al.*, 2007). GGT is also a marker of oxidative stress caused by alcohol metabolism (Conigrave *et al.*, 2003). Taking patient history is also very important. CAGE questionnaire is commonly used screening tool in this regard (Aertgeerts *et al.*, 2001; Levitsky and Mailliard, 2004).

## 1.3 Other Risk Factors

### 1.3.1 Socioeconomic and Demographic Factors

Socioeconomic factors that influence the health of individuals or communities include early childhood conditions, education, employment, food access, health services and many others.

Age, gender, ethnicity and geographic location can be considered as demographic determinants of health.

Different studies show that many chronic and infectious diseases tend to accumulate in a population or a specific group of people rather occurring randomly. Genetic predisposition only minimally explains such disparities. Instead, social and demographic determinants offer the best explanation in this regard (Sharpe *et al.*, 2010).

Through above parameters, low socioeconomic status can be considered as a risk factor of liver cirrhosis (Jepsen *et al.*, 2009).

Chronic liver disease and cirrhosis is an important cause of death with differential mortality rates based on social and demographic determinants (Singh and Hoyert, 2000).

It is also suggested that large exposure to infections and inflammation during early ages increases the chances of morbidity and mortality at later life time. Biological pathways involved are not explored fully yet. However, it is found that frequent exposure to infections contribute to morbidity and mortality via immune function down regulation and persistent inflammation (Dowd *et al.*, 2009). Markers of inflammation such as C-reactive protein have been found to vary by socioeconomic status in US population (Alley *et al.*, 2006).

### 1.3.2 Smoking

Cigarette smoking is considered to be a risk factor of liver cell damage (Mendez-Sanchez *et al.*, 2004) while some studies have shown that there is no link between the two (Bedogni *et al.*, 2003; Chavez-Tapia *et al.*, 2006). Some others have not considered smoking as a risk factor of liver damage (Chen *et al.*, 2006; Zelber-Sagi *et al.*, 2006).

Cigarette contains upto 4,000 harmful chemicals (Hamabe *et al.*, 2011). Clinical research shows that cigarette smoke affects enzymatic and inflammatory pathways thus altering liver function (Marrero *et al.*, 2005). In a 10 years follow up study conducted in Japan, smoking was found to be an independent risk factor of NAFLD. However, quitting smoking was also found to increase the risk of NAFLD progression probably due to increase in BMI (Hamabe *et al.*, 2011).

Cigarette smoking has also some link with other chronic hepatic diseases such as hepatitis B and C, primary biliary cirrhosis and alcoholic liver disease (Bataller, 2006; El-Zayadi, 2006; Zein, 2010).

It contributes to chronic hepatic damage through various mechanisms.

$H_2O_2$ and nicotine released by smoking decrease adiponectin expression (Iwashima *et al.*, 2005; Kawamoto *et al.*, 2010).

Smoking also aggravates the formation of reactive oxygen species by activated NADPH oxidase. This results in oxidative stress and lipid peroxidation resulting in hepatocytic damage (Agarwal, 2005; Avti *et al.*, 2006; Muriel, 2009). Oxidative stress also induces necrosis in fatty liver (Sanyal *et al.*, 2001).

In a one year follow up study on rats exposed to second hand cigarette smoke, it was found that cigarette smoke cease AMPK activity leading to enhanced SREBP activity.

SREBP is involved in fatty acid synthesis in liver while AMPK regulates expression of SREBP. This reversal of protein activities led to NAFLD in those rats (Martins-Green, 2009; Yuan *et al.*, 2009).

Due to elevated levels of carbon monoxide and inability of red blood cells to transport oxygen, hypoxia occurs. This triggers enhanced erythropoiesis and iron absorption from intestine. Surplus iron is stored in liver resulting in liver cell damage (El-Zayadi *et al.*, 2002).

Chemicals contained in cigarette also affect lipid metabolism and release of inflammatory cytokines (Zeidel *et al.*, 2002; Arnson *et al.*, 2010; Azzalini *et al.*, 2010).

Second hand smoking also results in elevated triglyceride level which may lead to NAFLD, cirrhosis and finally liver failure (Martins-Green, 2009).

In a study conducted by Suzuki et al, it was found that cigarette smoking in NAFLD patients results in raised ALT (Suzuki *et al.*, 2005). Smoking is associated with raised ALT in people who are positive for anti-HCV antibodies (Gordon *et al.*, 2005). However, no such association is found in healthy population. In a study by Chavez-Tapia NC et al, it was concluded that smoking is not linked to NAFLD or raised liver enzymes in general population (Chavez-Tapia *et al.*, 2006).

### 1.3.3 Obesity, Diabetes and Cardiovascular disease

There exists an association between obesity, diabetes and liver pathology. Liver plays a significant role in the regulation of carbohydrate metabolism. Due to increased hepatocellular glycogen synthesis and accumulation, hepatomegaly occurs leading to biochemical disturbances. Liver can also be affected by accumulation of fats, a condition known as non-alcoholic fatty liver disease. Increased transport of FAs to the liver, increased hepatic fat synthesis as well as decreased oxidation of fats from liver lead to fat accumulation. Associated with obesity, diabetes and, NAFLD is the most common cause of chronic liver disease. It ranges in severity from mild steatosis to fibrosis and cirrhosis (Levinthal and Tavill, 1999; Ni *et al.*, 2012).

Liver cell damage also has some association with cardiovascular disease. People with metabolic syndrome are at increased risk of CVD and liver injury via NAFLD (Dekker *et al.*, 2005).

Certain stimuli such as reactive oxygen species, stimulate the production of cytokines such as TNF-α and IL-6 in hepatic tissues particularly fat rich tissues (Kern *et al.*, 2001). These cytokines amplify inflammatory pathway by inducing CRP production. Elevated serum CRP is an independent risk factor of CVD. So, CVD patients with abnormal LFT may also have an underlying NAFLD or it can be the other way round (Schindhelm *et al.*, 2007).

## 1.4 Viral Hepatitis

Viral hepatitis is a major global public health problem and predominant cause of liver cells' insult. It is caused by atleast six distinct viruses viz hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis delta virus, hepatitis E virus and hepatitis G virus. Except the target organ affected by them, they only share a few common features.

### 1.4.1 Hepatitis B Virus

Hepatitis B virus was identified in 1967 as serum hepatitis by Krugman and colleagues. Later on, identification of hepatitis B surface antigen opened up the way to serologic diagnosis of HBV infection (Ganem and Prince, 2004).

#### 1.4.1.1 Genomic and Proteomic Organisation

Hepatitis B virus (HBV) is the prototype member of the *Hepadnaviridae* family of viruses. Members of this family have a strong preference for infecting liver cells, but small amounts of hepadnaviral DNA can be found in other cell types such as kidney, pancreas, and mononuclear cells. However, these sites are not prone to develop extrahepatic disease (Marion, 1988).

HBV virions are double-shelled particles, with a diameter ranging from 40 to 42 nm. An outer lipoprotein envelope that contains three related envelope glycoproteins called surface antigens is present surrounding the nucleocapsid (core). The core contains the viral genome which is a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase that synthesizes viral DNA in infected cells (Ganem, 1991).

In addition to virions, HBV-infected cells also produce subviral lipoprotein particles that may be spherical or filamentous with 20 nm diameter. These HBsAg particles contain envelope glycoproteins and host-derived lipids (Ganem, 1991).

The HBV genome consists of four long open reading frames (Fig 1.1) (Khawaja and Khawaja, 2009).

The preS–S also called presurface surface region of the genome encodes three viral surface antigens due to differential initiation of translation owing to the presence of three in-frame initiation codons. The most abundant protein is the 24-kD S protein that is commonly known as HBsAg. Other proteins are M protein or pre S 1 and L protein or pre S 2. L protein is thought to play key roles in the binding of the virus to host-cell receptors, assembly of the virion and its release from the cell (Robinson and Lutwick, 1976; Bruss and Ganem, 1991).

The preC–C also known as precore–core region encodes hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg) by same differential initiation of translation at two in-frame start codons (Ganem, 1991).

The P coding region encodes the viral polymerase, which is involved in DNA synthesis and RNA encapsidation (Ganem and Prince, 2004).

The X open reading frame encodes the viral X protein, HBx, which alters host-cell signal transduction and affect host and viral gene expression (Ganem and Prince, 2004).

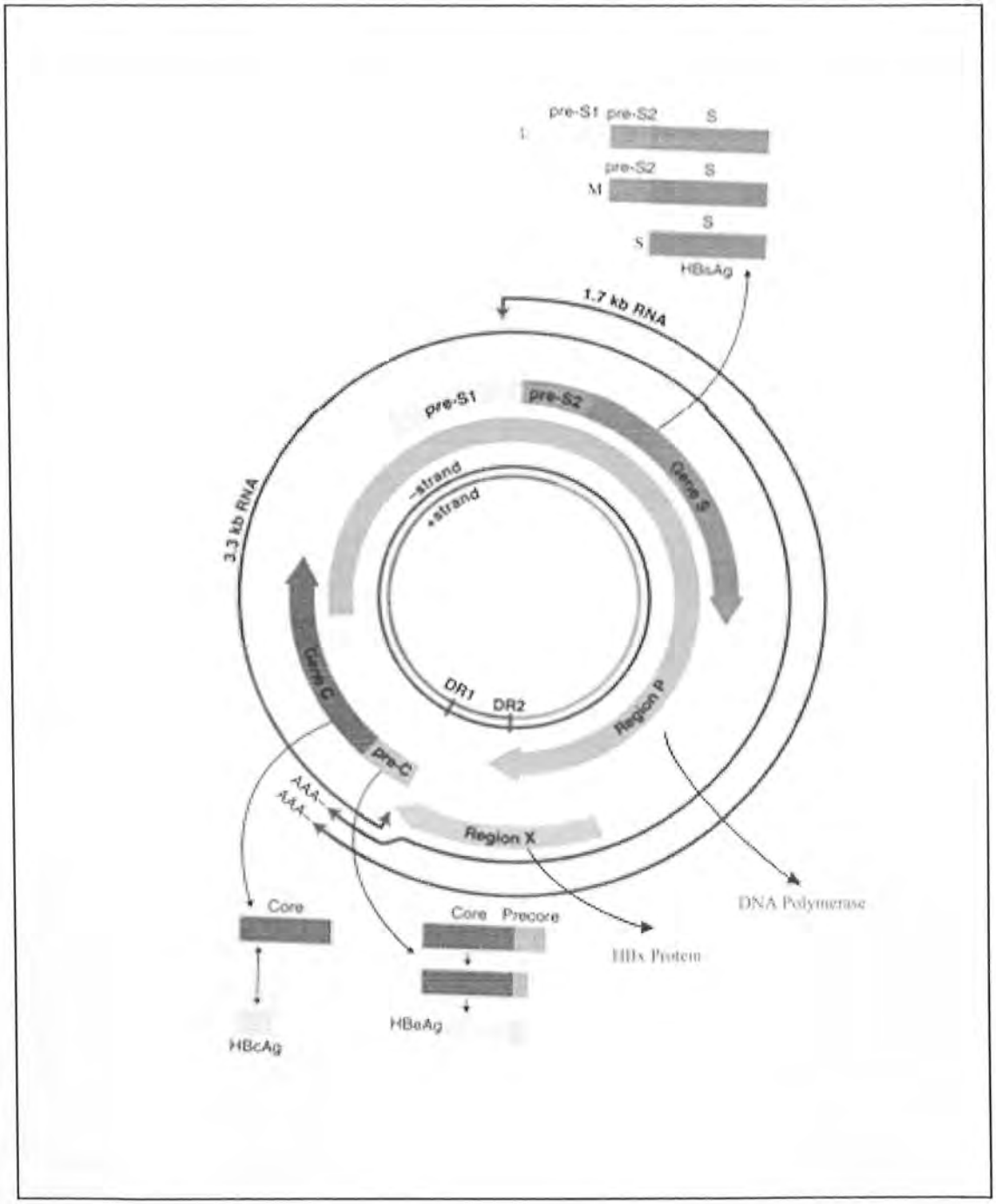



**Fig 1.1 Organisation of HBV genome and its proteomic expression**

### 1.4.1.2 Prevalence and Risk factors

Almost two billion people worldwide are infected with HBV. More than 400 million develop chronic infection and 600,000 are died each year by HBV related chronic liver diseases (Shepard *et al.*, 2006). However, prevalence varies distinctly based on different geographic locations. Developing countries with primitive or limited medical

facilities have higher prevalence rates than developed countries (www.who.int/csr/disease/hepatitis/whocdscsrlyo20022/en/index3.html). In United States, incidence of HBV infection in 2010 was estimated to be < 10,000 individuals per year (www.cdc.gov/hepatitis/statistics/2010surveillance/Commentary.htm). In Northeast China, HBV infection rate in 2011 was found to be 4.38 % (Zhang *et al.*, 2011). A study conducted at southwest of Iran showed a prevalence of 1.2% (Khosravani *et al.*, 2012). A review analysis of eleven different studies in Pakistan showed 2.46- 8.06 % prevalence of HBV among general population (Ali *et al.*, 2011).

Risk factors of HBV include age, gender, ethnicity, contact with infected blood, infected sexual partner, body piercing and use of surgical instruments or syringes. However, pattern of prevalence of these factors varies according to geographical distributions (Ali *et al.*, 2011; Zhang *et al.*, 2011; Khosravani *et al.*, 2012).

### 1.4.1.3 Pathogenesis of HBV Mediated Liver Injury

It is found that hepatitis B virus is not directly cytopathic for infected hepatocytes. Hepatic pathogenesis is due primarily to the response of the body's immune system to the presence of HBV. In general, there are two types of host's immune response to viral infection, viz. innate immune response and adaptive immune response. Recent evidence shows that the innate immune response does not play a significant role in HBV clearance or in liver injury. Whereas, the adaptive immune response mediated by cytotoxic T-lymphocytes is found linked to viral clearance and liver injury. It suggests that the pathogenesis of HBV is closely linked to the cytotoxic T-lymphocyte mediated immune response. CTL cells mediate viral clearance by releasing serine protease granzymes such as granzyme A and granzyme B. Secretion of these granzymes upon interaction with infected cells leads to the apoptosis of infected cells. In response to this, HBV replication may upregulate the expression of apoptosis inhibitors such as serine protease inhibitor Kazal or SPIK. These inhibitors help in the resistance of the cells to CTL-mediated immune killing. The inability of the immune system to clear HBV-infected cells can result in chronic hepatitis B, HBV cirrhosis and hepatocellular carcinoma (Baumert *et al.*, 2007; Chang and Lewin, 2007; Lu, 2011). One likely explanation to developing cirrhosis or HCC even in the absence of CTL mediated immune response may be the inability of the immune system to remove virus-infected cells from the body. Failure to remove virus-infected cells

usually results in slowly accumulating cellular genetic changes that ultimately lead to the development of HCC (Lu, 2011).

#### 1.4.1.4 Diagnosis

Diagnosis of HBV infection is made by assessing biochemical markers of liver function (www.cdc.gov/std/treatment/2006/hepatitis-b.htm). Diagnosis is confirmed by the presence of serological markers such as HBsAg, anti-HBs, HBeAg, anti-HBe, HBcAg and anti-HBc. Specific diagnostic tests involve amplification of viral DNA by PCR which also provides information about number of viral particles in infected cells (You *et al.*, 2008).

### 1.4.2 Hepatitis C Virus

Hepatitis C virus (HCV) was identified and cloned in 1989 as a virus responsible for most transfusion associated non-A non-B hepatitis. Frequency of HCV infections is increasing globally. It is one of the main causes of cirrhosis, hepatocellular carcinoma and end stage liver disease leading to liver transplantation (Chen and Morgan, 2006).

#### 1.4.2.1 Genomic and Proteomic Organisation

HCV contains 9.6 kb long single stranded, positive, sense RNA molecule with one long open reading frame (ORF). This ORF codes for a large protein of 3000 amino acids which undergoes co- and post-translational cleavage to yield individual viral proteins. A highly conserved 5' untranslated region of 340 nucleotides and 3' untranslated region of variable length also exists. The N-terminal one fourth of genome encodes the core and structural proteins while non-structural proteins are encoded by rest of the genome (Fig 1.2) (Krekulova *et al.*, 2006).

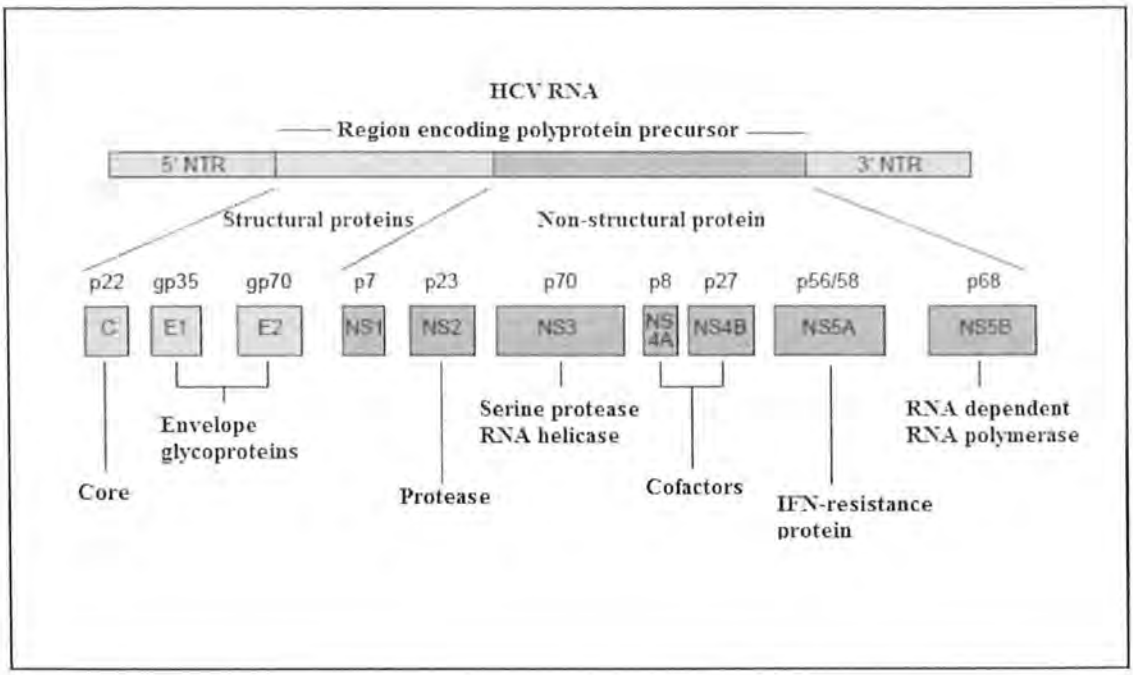



**Fig 1.2 HCV genome structure and proteins encoded by HCV genome**

### 1.4.2.2 Prevalence and Risk Factors

HCV belongs to family of viruses *Flaviridae* having atleast six major genotypes and various subtypes. HCV genotypes 1, 2 and 3 infections are common worldwide but their relative prevalence varies in different geographical areas (Idrees and Riazuddin, 2008). In United States as well as Europe, subtypes 1a and 1b are the most common genotypes (McOmish *et al.*, 1994; Zein *et al.*, 1996). Subtype 1b is also a predominant genotype in Japan. HCV genotype 4 is found most prevalent in North Africa and Middle East (Abdulkarim *et al.*, 1998). Genotype 1 is predominant in Brazil with 1a and 1b most common subtypes (Pereira *et al.*, 2013). In Pakistan, HCV genotype 3 followed by 1 is the most predominant (Idrees and Riazuddin, 2008).

Overall prevalence of HCV infection in Brazil was found to be 1.38% from 2005-2009 (Pereira *et al.*, 2013). In Taiwan, prevalence of HCV among intravenous drug users was estimated to be between 59.5% and 89.6% (Yen *et al.*, 2012). In USA, anti-HCV prevalence determined by National Health and Nutrition Examination Survey was found to be 1.6% (Daniels *et al.*, 2009).

Different studies have shown different risk factors to be positively associated with HCV infection. These include drug use related factors, sexual behavior related factors

and blood route related factors such as surgery, dental treatment, blood transfusion and body piercing (Gates *et al.*, 2004; Yen *et al.*, 2012; Pereira *et al.*, 2013).

### 1.4.2.3 Pathogenesis by HCV

Like HBV, hepatitis C virus is also non-cytopathic itself. Liver damage in hepatitis C virus infection is usually associated with immune cell response to the virus. Viral proteins interact with immunity related pathways thus disrupting pathogen associated pattern recognition. Cytotoxic T-lymphocytes not only kill infected cells through granzyme pathway but also release inflammatory cytokines. These cytokines attract other lymphocytes to the site of infection resulting in non-specific killing of healthy liver cells (Spengler and Nattermanin, 2007). On the other hand, due to changes in pathogen associated pattern recognition (PAPR), viral particles keep on replicating unchecked. Consequent changes in sequence of hypervariable region 1 (epitope) of an envelope protein during infection also results in variants that escape from immune system. All this ultimately results in fibrogenesis and cirrhosis (Bukh *et al.*, 1995).

### 1.4.2.4 Diagnosis

HCV infection is diagnosed by detection of anti-HCV antibodies through different immunoassays. To determine viral load, amplification techniques such as PCR is used commonly. Liver function test is important to assess liver damage by hepatitis C virus infection (Ghany *et al.*, 2009).

# STUDY OBJECTIVES

- ✓ To determine the level of liver function markers in individuals suspected of liver dysfunction
- ✓ To investigate the prevalence of HBsAg and anti-HCV antibody among patients with abnormal LFT by enzyme linked immunosorbent assay
- ✓ To find out an association between socio epidemiological factors and viral hepatitis seropositivity
- ✓ Comparative analysis of the level of liver function markers in different study groups
- ✓ To determine the frequency of different risk and etiological factors of liver tests abnormality

# *Materials and Methods*

# 2. MATERIALS and METHODS

## 2.1 Blood Sampling

### 2.1.1 Socio-epidemiological Data

Our study subjects included individuals visiting PIMS hospital who were advised LFT by their consultant. Socio-epidemiological data and history was obtained from 1000 individuals by a proper questionnaire filling. It included age, gender, status, smoking, DM, cardiac disease, alcohol use, history of blood transfusion, injection, surgery, travel abroad, dental therapy, body piercing, medication and family history about under study disease.

This study was approved by PIMS hospital ethical committee.

### 2.1.2 Blood Collection

5 ml blood was taken from each individual in disposable sterile (BD) syringes using aseptic vein puncture method. It was then transferred into yellow top gel tubes (BD) and allowed to coagulate. After an hour, it was centrifuged at 4000 rpm for 10 minutes to separate serum from it. Same procedure was followed for healthy control individuals.

Informed consent was obtained from all individuals under observation.

## 2.2 Determination of LFT

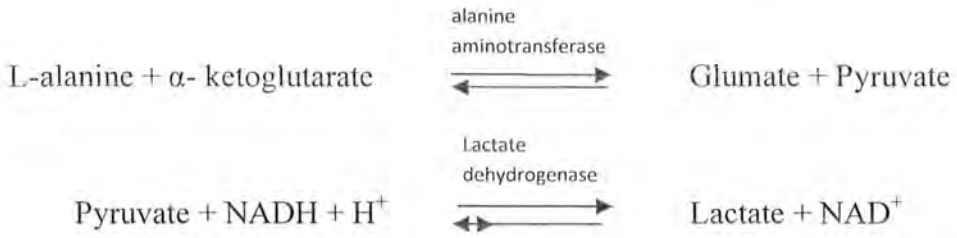
Although appropriate estimation of liver function requires the determination of transaminases, bilirubin, alkaline phosphatase, gammaglutamyl transferase, albumin and prothrombin time but we mainly focused on ALT and bilirubin. Level of ALP was also determined.

### 2.2.1 Quantitative Determination of ALT

Alanine aminotransferase level was determined by spinreact kit method using Selectra e chemistry analyzer.

**Principle**

By catalyzing the reversible transfer of an amino group from alanine to α-ketoglutarate, ALT is responsible for the formation of glutamate and pyruvate. In the presence of lactate dehyrogenase and NADH, this pyruvate is reduced to lactate.




Photometrically determined rate of decrease in NADH concentration is proportional to active concentration of ALT present in sample.

**Procedure**

Working reagent was prepared by mixing 4 volume R 1 buffer (Tris pH 7.8 100 mmol/L, Lactate dehydrogenase 1200 U/L, L-alanine 500 mmol/L) and 1 volume R 2 substrate (NADH 0.18 mmol/L, α-ketoglutarate 15 mmol/L). 1 ml of this working reagent and 100 µl of serum sample were mixed and incubated for one minute. Initial absorbance (A) of the sample was read at 340 nm followed by absorbance reading at one minute interval thereafter for three minutes. Difference between absorbances and the average absorbance difference per minute (ΔA/min) was calculated.

$$\Delta A/min \times factor\ (1750) = ALT\ activity\ (U/L)$$

### 2.2.2 Total Bilirubin

For the determination of total bilirubin level, spinreact kit method was used.

**Principle**

Diazotized sulfanilic acid converts bilirubin to colored azobilirubin which is measured photometrically. The intensity of the color formed is directly proportional to the total bilirubin concentration in serum sample.

**Procedure**

After calibrating the instrument to zero, 1.5 ml R1 (Sulfanilic acid 30 mmol/L, HCl 50 mmol/L, DMSO 7 mol/L), 50 µl R2 (Sodium nitrite 29 mmol/L) and 100 µl sample were mixed and incubated for five minutes at room temperature. For blank, 1.5 ml R1 and 100 µl sample were mixed and incubated for five minutes. Absorbance (A) was read at 555 nm and T.bilirubin was determined as follows;

$$(A)Sample - (A)Blank \times factor\ (19.1) = T.bilirubin\ (mg/dl)$$

### 2.2.3 Alkaline Phosphatase

Spinreact kit method was used for the quantitative determination of alkaline phosphatase.

**Principle**

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{alkaline phosphatase}} \text{p-Nitrophenol} + \text{Phosphate}$$

The rate of p-Nitrophenol formation, determined photometrically, is proportional to active concentration of ALP present in the serum sample.

**Assay Procedure**

Working reagent was prepared by mixing 4 volumes of R1 buffer (DEA 1 mmol/L, Magnesium chloride 0.5 mmol/L) and 1 volume R2 substrate (p-NPP 10 mmol/L). 20 µl sample and 1.2 ml were mixed well and incubated for one minute. Initial absorbance was read at 405 nm followed by three more readings at one minute interval each. Calculations were done as follows

Average absorbance difference per min (ΔA/min) × 3300 = U/L ALP in the sample

## 2.3 Serum Storage

For those samples which showed elevated levels of ALT and T.bilirubin, serum was transferred from gel tubes to eppendorf tubes and stored at -20°C for further analysis.

## 2.4 ELISA Test for the Detection of HBsAg

### 2.4.1 Principle

Bioelisa HBsAg 3.0 is a direct and sandwich type immunoenzymatic assay in which anti HBs antibodies coated on microplate wells serve as the capture antibodies and anti-HBs antibodies conjugated with an enzyme act as conjugate antibodies. If the sample to be analysed contains HBsAg, it binds to the antibody on plate. Now, anti-HBs antibody marked with an enzyme can bind to the previously formed antigen-antibody complex. The addition of enzyme substrate containing a chromogen results in color development if the sample is positive for HBsAg. This color changes to another color after blocking the reaction with stop solution. The intensity of the color is proportional to the amount of HBsAg in the test sample.

### 2.4.2 Procedure

**Previous operations**

Of all the reagents mentioned above, a few needed to be diluted before use.

10 X washing solution was diluted to 1 X using distilled water. The volume of working conjugate solution was selected based on number of samples operated in one run. For the dilution of 51 X concentrate conjugate to 1 X, conjugate diluent was used. Volume was adjusted as required.

**Assay procedure**

Before starting the assay, wells of the microplate were labeled properly, at least three wells for negative control, one well for positive control, one well for substrate blank and others for the samples. Blank well was left empty while 100 µl of each positive control, negative control and samples was added to the assigned wells. Microplate was covered with an adhesive seal and incubated for 1 hour at 37°C. 350 µl of washing solution was added in each well and removed after 15 seconds. This process of washing was repeated three more times and after the last washing, plate was blotted on adsorbent material to remove any excess liquid. After that, 100 µl of diluted conjugate was added into each well except blank, plate was covered with an adhesive seal and incubated for half an hour at 37°C. Meanwhile, substrate-chromogen solution was prepared in 1:20 ratio. Adhesive seals were removed after completion of incubation and plate was washed the same way. Then, 100 µl of substrate-TMB solution was added to each well including the blank and again incubated for 30 minutes at room temperature. Reaction was then stopped by adding 100 µl of 1N $H_2SO_4$ in each well. Absorbance was taken with spectrophotometer at 450 nm using a 630 nm reference filter.

Cut-off value was calculated by using formula as given in manufacturer's instructions.

**Cut-off** = mean absorbance of the negative control + 0.040

Whole assay was repeated twice for all samples and thrice if two readings were not concordant.

## 2.5 Detection of Anti-HCV Antibodies

### 2.5.1 Principle

Serum sample is added to the wells of a microplate coated with recombinant antigens representing HCV epitopes. Antibodies in the serum form stable complexes with HCV antigens on the well. Anti-human antibodies conjugated with an enzyme can now bind to antigen-antibody complexes formed. Addition of enzyme substrate and chromogen results in the development of blue color if the sample is positive.

### 2.5.2 Procedure

**Pre-assay procedure**

Concentrated reagents provided in the kit were changed to working dilutions as follows.

Based on number of samples in one run, 1 X wash solution of a particular volume was made from 10 X washing solution using distilled water and 51 X conjugate was diluted to 1 X with the conjugate diluent.

**Assay procedure**

After labeling the wells, 200 µl of each negative control, low positive control and high positive control was added to 2, 3 and 2 wells respectively. First well was left empty for substrate blank. Then 200 µl of sample diluents was being pipetted to the rest of the wells followed by the addition of 10 µl of each sample. All wells were covered with an adhesive seal, mixed gently and incubated for 60 minutes at 37°C. After incubation, content of the wells was washed by adding 350 µl of washing solution in each well for atleast 15 sec. The process of washing was repeated five more times and microplate was blotted after the last washing to remove any unbound material. Then 100 µl of diluted conjugate was added into each well except substrate blank and covered microplate was kept for 30 minutes incubation at 37°C. Washing was done again after incubation. 100 µl of freshly prepared substrate-TMB solution was added to all wells including blank and allowed to incubate for 30 minutes at room temperature. 100 µl of stop solution was added into each well to stop the reaction and absorbance was taken with microplate reader at 450 nm using 630 nm reference filter.

Following formula was used for calculating cut-off value

**Cut-off** = mean absorbance of the low positive control × 0.9

Assay was repeated twice or thrice in different runs if results did not fulfill the criteria as given in manufacturer's instructions.

## 2.6 Statistical Analysis

Based on sociodemographic data and experimental findings, patients were categorized into different groups and analysed statistically by using SPSS 16.0 software. The odd ratios and confidence intervals of 95 % for odd ratios also determined. Some parameters were recorded as mean ± SE. A level of $p < 0.05$ was used to show statistical significance.

# *Results*

# 3. RESULTS

Liver function test involving determination of ALT, bilirubin and ALP levels was done for 1000 individual who were suspected of liver function abnormality.

## 3.1 Prevalence of Abnormal LFT

### 3.1.1 Gender-wise Prevalence

Only 132 individuals showed abnormal LFT with a prevalence of 13.2%. Level of ALT and bilirubin was found raised in 77 males and 55 females. Overall prevalence of male subjects was 7.7% and females were 5.5% (Fig. 3.1).

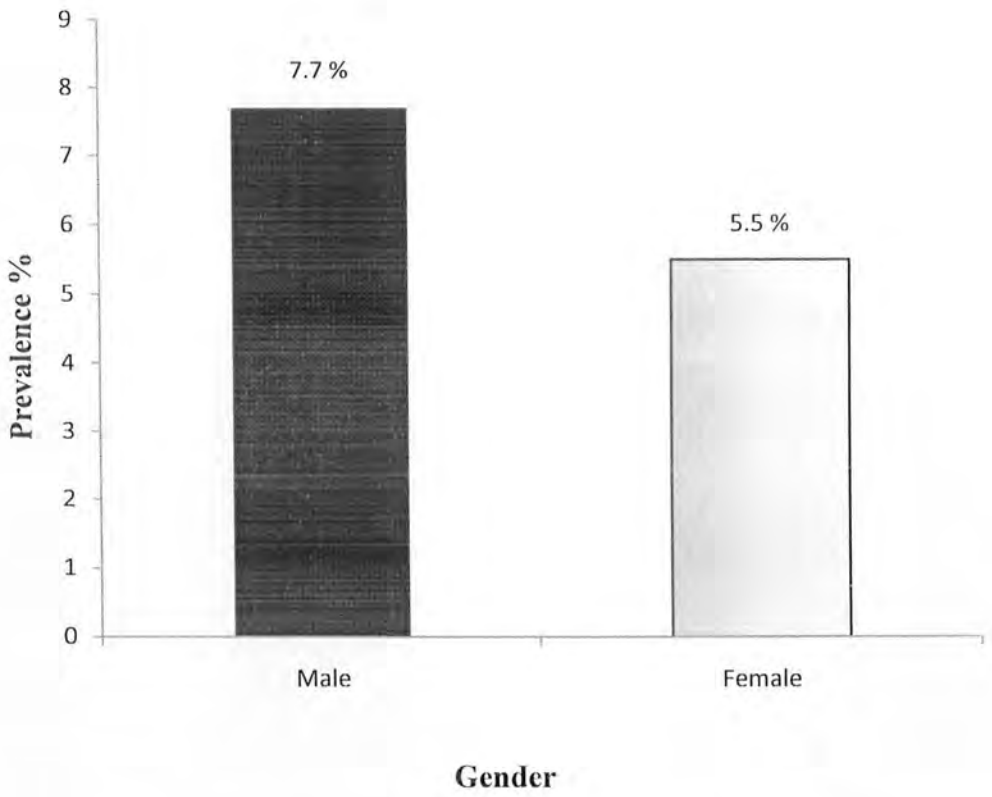



**Figure 3.1: Gender-wise prevalence of abnormal LFT**

### 3.1.2 Age-wise Prevalence

To determine age-wise prevalence, study subjects were classified into three age groups i.e 20-40 years, 41-60 years and >60 years. Prevalence of abnormal LFT was found to be 6.3%, 5.1% and 1.8% respectively which showed a decreasing trend with increasing age (Fig. 3.2).

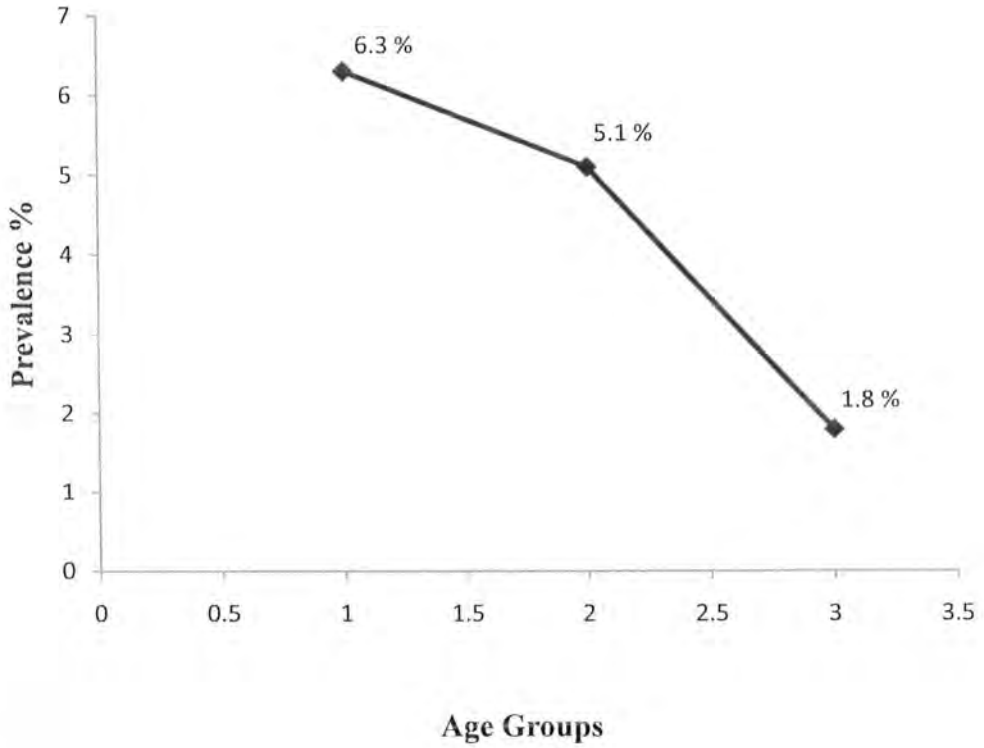



**Fig 3.2: Prevalence of abnormal LFT among participants by age groups**

Group 1: 20-40 years
Group 2: 41-60 years
Group 3: > 60 years

## 3.2 Risk Factors Associated with LFT Elevation

Patient history taken was used to determine common risk factors associated with LFT elevation in our sampling population (Table 3.1, Fig. 3.3).

**Table 3.1 Frequency of risk factors associated with abnormal LFT**

| Risk Factors | Subjects with abnormal LFT (n) | Subjects with abnormal LFT (%) |
|---|---|---|
| Socioeconomic status | | |
| Middle | 27 | 20.4 |
| Low | 105 | 79.5 |
| Obesity | | |
| Normal | 109 | 82.6 |
| Obese | 23 | 17.4 |
| Blood transfusion | | |
| No | 112 | 84.8 |
| Yes | 20 | 5.21 |
| Smoking | | |
| No | 123 | 93.2 |
| Yes | 09 | 6.8 |
| Alcohol use | | |
| No | 132 | 100 |
| Yes | 00 | 0 |
| Diabetes mellitus | | |
| No | 124 | 93.9 |
| Yes | 08 | 6.1 |
| Risk of cardiac disease | | |
| No | 129 | 97.7 |
| Yes | 03 | 2.27 |
| Family history | | |
| No | 125 | 94.7 |
| Yes | 07 | 5.3 |
| Previous medication | | |
| No | 77 | 58.3 |
| Yes | 55 | 41.7 |

Most frequent risk factor encountered in our study population was low socioeconomic status. Out of 132 people having elevated liver function, 105(79.5 %) were found socially deprived. Criteria of social deprivation were based on individual's ease of

access to health care centers for diagnosis and treatment. Individuals having no breadwinner in the family or having income less than 6000 and living from hand to mouth were considered socially deprived.

Another common risk factor observed was use of medicines with 55 people (41.7 %) having elevated levels of ALT and bilirubin.

Co-incidence of obesity and abnormal LFT was observed in 23 (17.4 %) study subjects.

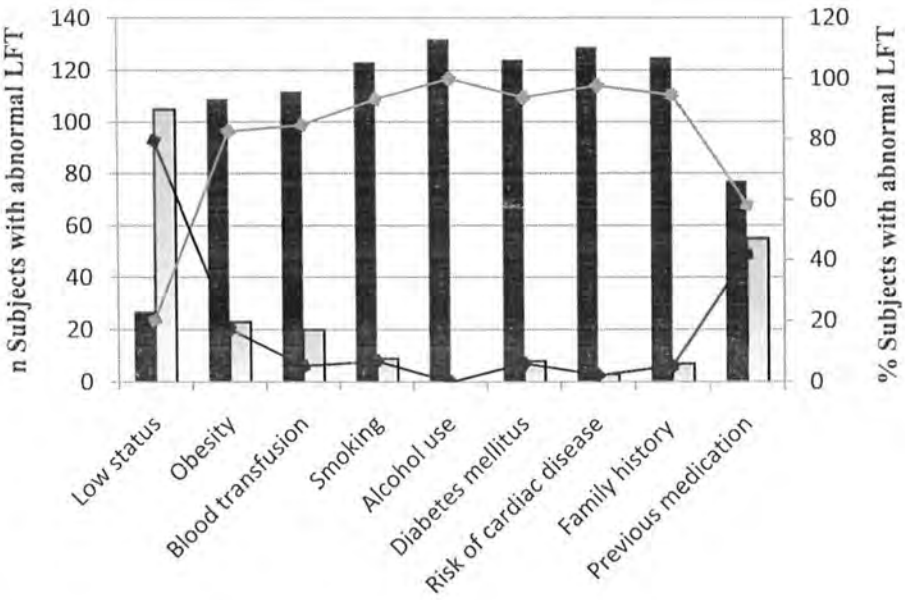



**Fig 3.3 Frequency of risk factors associated with abnormal LFT**

## 3.3 Prevalence of HBV Infection

As our study objective was to determine the prevalence of HBV and HCV infections among patients with abnormal LFT so, ELISA was done with only 132 subjects who had elevated levels of ALT and Bilirubin. Out of 132 subjects, HBsAg seropositivity was found among 34 (25.76%) individuals. 22 (16.7%) females were HBsAg seropositive whereas 12 (9.1%) males were found infected with HBV which showed a lower infection rate than females. Prevalence of HBsAg by age groups did not show a significant difference. Eighteen subjects (13.6%) who were less than 40 years had HBV infection whereas prevalence in > 40 years age group was 16 individuals (12.1%).

## 3.4 Socio-epidemiological Analysis of HBV Infection

Patient history taken was used to determine association between possible risk factors and HBsAg seropositivity (Table 3.2). Odd ratios at 95% CI were determined. p-value < .05 was used to show statistical significance. p-value was calculated using Pearson chi-square, Likelihood ratio chi-square and Fisher's exact test.

Factors which were found to be significantly associated with HBV infection were female gender (OR= 3.6; p < .05), self/family history of hepatitis (OR= 4.3; p < .05) and low socioeconomic status (OR= 5.5; p < .05). On the other hand, no significant association was observed between HBV seropositivity and age factor, multiple blood transfusions, history of dental therapies and history of travel abroad. Association between HBV seropositivity and history of surgery (OR=2.6; p .050) and body piercing (OR= 2.1; p .059) represented a borderline case.

An association between HBV infection and reuse of syringes was considered inconclusive as p-value and odd ratio of this association were not found correlated. It was probably due to small sample size or it may also be due to the fact that usually Pakistanis are reluctant to expose the truth so there may be an error in data collection.

**Table 3.2 Association between socio-epidemiological factors and HBV seropositivity**

| Risk factor | N= 132 | | Odd ratio (CI 95%) | p-value | | |
|---|---|---|---|---|---|---|
| | $HBV^+$ subjects n (%) | $HBV^-$ subjects n (%) | | Pearson Chi square | Likeli-hood ratio | Fisher's exact test |
| Gender | | | | | | |
| Male | 12 (9.1) | 65 (49.2) | | | | |
| Female | 22 (16.7) | 33 (25.0) | 3.6 (1.6-8.2) | .002 | .002 | .002 |
| Age | | | | | | |
| < 40 | 18 (13.6) | 45 (34.1) | | | | |
| >40 | 16 (12.1) | 53 (40.2) | 0.8 (0.3-1.6) | .480 | .480 | .306 |
| Self/family history of hepatitis | | | | | | |
| No | 11 (8.3) | 66 (50.0) | | | | |
| Yes | 23 (17.4) | 32 (24.2) | 4.3 (1.9-9.9) | .000 | .000 | .000 |
| Multiple blood transfusions | | | | | | |
| No | 31 (23.5) | 81 (61.4) | | | | |
| Yes | 3 (2.3) | 17 (12.9) | 0.5 (0.1-1.7) | .232 | .210 | .181 |
| Surgical history | | | | | | |
| No | 25 (18.9) | 86 (65.2) | | | | |
| Yes | 9 (6.8) | 12 (9.1) | 2.6 (1.0-6.8) | .051 | .061 | .050 |
| Body piercing | | | | | | |
| No | 19 (14.4) | 71 (53.8) | | | | |
| Yes | 15 (11.4) | 27 (20.4) | 2.1 (0.9-4.7) | .074 | .079 | .059 |
| Dental therapy | | | | | | |
| No | 24 (18.2) | 80 (60.6) | | | | |
| Yes | 10 (7.6) | 18 (13.6) | 1.8 (0.8-4.5) | .175 | .186 | .133 |
| Reuse of syringes* | | | | | | |
| No | 27 (20.4) | 52 (39.4) | | | | |
| Yes | 7 (5.3) | 46 (34.8) | 0.3 (0.1-0.7) | .007 | .005 | .005 |
| History of travel abroad | | | | | | |
| No | 32 (24.2) | 86 (65.2) | | | | |
| Yes | 2 (1.5) | 12 (9.1) | 0.4 (0.1-2.1) | .299 | .273 | .245 |
| Socioeconomic status | | | | | | |
| Middle | 2 (1.5) | 25 (18.9) | | | | |
| Low | 32 (24.2) | 73 (55.3) | 5.5 (1.2-24) | .014 | .007 | .010 |

*Inconclusive association

Association is significant at 0.05 level

Association is significant at >1 OR

## 3.5 Prevalence of HCV Infection

Enzyme linked immunosorbent assay was done to determine the prevalence of HCV infection in patients with abnormal LFT. 132 serum samples were analyzed for the presence of anti-HCV antibodies.

Prevalence of HCV infection was 24.24% representing 32 subjects. 10 (7.6%) males and 22 (16.7%) females were HCV positive. Like HBV infection, prevalence was higher in females than males. Age-wise observation showed a prevalence of 12.1% (n=16) and 19.7% (n=26) among age groups < 40 and >40 years respectively.

## 3.6 HCV Infection and Socio-epidemiological Factors

To determine any significant association between different socio-epidemiological factors and anti-HCV Ab seropositivity, data collected by interviewing patients was statistically evaluated. Odd ratios at 95% CI were calculated. P-value less than .05 was considered statistically significant. It was calculated by pearson chi-square, likelihood ratio chi-square and fisher's exact test (Table 3.3).

Statistically significant association was found between HCV seropositivity and female gender (OR 4.5; $p < .05$), surgical history (OR 2.9; $p < .05$), body piercing (OR 3.6; $p < .05$), history of dental therapies (OR 2.6; $p < .05$) and low socioeconomic status (OR 5.0; $p < .05$).

Factors that did not show significant association with HCV infection were age, multiple blood transfusions, reuse of syringes and history of travel abroad.

Association between HCV infection and self/family history of hepatitis was a borderline case (OR 0.4; p-value .056).

**Table 3.3 Distribution of HCV serological marker among different risk groups**

| Risk factor | N= 132 | | Odd ratio (CI 95%) | p-value | | |
|---|---|---|---|---|---|---|
| | $HCV^+$ subjects n (%) | $HCV^-$ subjects n (%) | | Pearson Chi square | Likeli-hood ratio | Fisher's exact test |
| Gender | | | | | | |
| Male | 10 (7.6) | 67 (50.8) | | | | |
| Female | 22 (16.7) | 33 (25) | 4.5 (1.9-10) | .000 | .000 | .000 |
| Age | | | | | | |
| < 40 | 16 (12.1) | 47 (35.6) | | | | |
| >40 | 26 (19.7) | 43 (32.6) | 1.8 (0.8-3.8) | .130 | .185 | .092 |
| Self/family history of hepatitis | | | | | | |
| No | 23 (17.4) | 54 (40.9) | | | | |
| Yes | 9 (6.8) | 46 (34.8) | 0.4 (0.2-1.1) | .074 | .070 | .056 |
| Multiple blood transfusions | | | | | | |
| No | 25 (18.9) | 87 (65.9) | | | | |
| Yes | 7 (5.3) | 13 (9.8) | 1.9 (0.7-5.2) | .223 | .239 | .173 |
| Surgical history | | | | | | |
| No | 23 (17.4) | 88 (66.7) | | | | |
| Yes | 9 (6.8) | 12 (9.1) | 2.9 (1.1-7.6) | .030 | .039 | .033 |
| Body piercing | | | | | | |
| No | 12 (9.1) | 78 (59.1) | | | | |
| Yes | 15 (11.4) | 27 (20.4) | 3.6 (1.5-8.7) | .003 | .004 | .004 |
| Dental therapy | | | | | | |
| No | 21 (15.9) | 83 (62.9) | | | | |
| Yes | 11 (8.3) | 17 (12.9) | 2.6 (1.0-6.2) | .036 | .044 | .036 |
| Reuse of syringes | | | | | | |
| No | 22 (16.7) | 57 (43.2) | | | | |
| Yes | 10 (7.6) | 43 (32.6) | 0.6 (0.2-1.4) | .238 | .233 | .165 |
| History of travel abroad | | | | | | |
| No | 28 (21.2) | 90 (68.2) | | | | |
| Yes | 4 (3.0) | 10 (7.6) | 1.3 (0.4-4.4) | .689 | .694 | .454 |
| Socioeconomic status | | | | | | |
| Middle | 2 (1.5) | 25 (18.9) | | | | |
| Low | 30 (22.7) | 75 (56.8) | 5 (1.1-22.4) | .022 | .012 | .015 |

Association is significant at 0.05 level

Association is significant at >1 OR

## 3.7 Prevalence of HBV and HCV Co-infection

Regarding the prevalence of HBV and HCV dual infection, high prevalence of 20.45% (n= 27) was observed in subjects showing elevated levels of ALT and bilirubin. Gender wise prevalence observation showed that 9 males (6.8%) and 18 females (13.6%) were victims of dual infection. In age group of < 40 years, 13 (9.8%) individuals were found co-infected while 14 (10.6%) subjects who were > 40 years had dual infection.

## 3.8 Socio-epidemiological Analysis of HBV and HCV Co-infection

Subjects with HBV and HCV dual infection were found by noting the presence of both HBsAg and anti-HCV antibodies. An association between different factors and co-infection was determined by calculating odd ratios at 95% CI. P-value was calculated by pearson chi-square test, likelihood ratio test and fisher's exact test and was considered significant if less than .05 (Table 3.4).

There was a significant association between HBV and HCV dual infection and female gender (OR 3.7; $p < .05$), surgical history (OR 3.0; $p < .05$), body piercing (OR 3.6; $p < .05$) and reuse of syringes (OR 2.7; $p < .05$).

No significant association was observed between co-infectivity and age factor, self/family history of hepatitis, multiple blood transfusions, history of dental therapies, history of travel abroad and socioeconomic status.

**Table 3.4 Association between different risk factors and HBV, HCV co-infection**

| Risk factor | N= 132 | | Odd ratio CI(95%) | p-value | | |
|---|---|---|---|---|---|---|
| | Co-infection n (%) | No infection n (%) | | Pearson Chi square | Likeli-hood ratio | Fisher's exact test |
| Gender | | | | | | |
| Male | 9 (6.8) | 68 (51.5) | | | | |
| Female | 18 (13.6) | 37 (28.0) | 3.7 (1.5-9.0) | .003 | .003 | .003 |
| Age | | | | | | |
| < 40 | 13 (9.8) | 50 (37.9) | | | | |
| >40 | 14 (10.6) | 55 (41.7) | 1.0 (0.4-2.3) | .961 | .961 | .566 |
| Self/family history of hepatitis | | | | | | |
| No | 17 (12.9) | 60 (45.4) | | | | |
| Yes | 10 (7.6) | 45 (34.1) | 0.8 (0.3-1.9) | .584 | .583 | .374 |
| Multiple blood transfusions | | | | | | |
| No | 21 (15.9) | 91 (68.9) | | | | |
| Yes | 6 (4.5) | 14 (10.6) | 1.8 (0.6-5.4) | .251 | .269 | .195 |
| Surgical history | | | | | | |
| No | 19 (14.4) | 92 (69.7) | | | | |
| Yes | 8 (6.1) | 13 (9.8) | 3.0 (1.1-8.2) | .029 | .040 | .034 |
| Body piercing | | | | | | |
| No | 12 (9.1) | 78 (59.1) | | | | |
| Yes | 15 (11.4) | 27 (20.4) | 3.6 (1.5-8.7) | .003 | .004 | .004 |
| Dental therapy | | | | | | |
| No | 19 (14.4) | 85 (64.4) | | | | |
| Yes | 8 (6.1) | 20 (15.2) | 1.8 (0.7-4.7) | .230 | .244 | .174 |
| Reuse of syringes | | | | | | |
| No | 11 (8.3) | 68 (51.5) | | | | |
| Yes | 16 (12.1) | 37 (28.0) | 2.7 (1.1-6.4) | .023 | .024 | .021 |
| History of travel abroad | | | | | | |
| No | 23 (17.4) | 95 (72.0) | | | | |
| Yes | 4 (3.0) | 10 (7.6) | 1.6 (0.5-5.7) | .426 | .443 | .313 |
| Socioeconomic status | | | | | | |
| Middle | 5 (3.8) | 22 (16.7) | | | | |
| Low | 22 (16.7) | 83 (62.9) | 1.2 (0.4-3.4) | .780 | .778 | .508 |

Association is significant at 0.05 level

Association is significant at >1 OR

## 3.9 Comparative Analysis of LFT Markers in Different Groups

Levels of LFT markers (ALT, Bilirubin and ALP) determined by chemistry analyzer were then comparatively analyzed among different groups by status of disease, age and gender.

### 3.9.1 Analysis of LFT Markers in Groups by Disease Status

After determining the level of ALT, Bilirubin and ALP and doing ELISA, 132 study subjects were classified into four groups i.e abnormal LFT (group 1 i.e those having elevated LFT but no viral infection), HBV positive subjects with abnormal LFT (group 2), HCV positive subjects with abnormal LFT (group 3) and subjects having co-infection with abnormal LFT (group 4). Control group included 30 healthy individuals.

### 3.9.1.1 Analysis of ALT Levels

Mean level of ALT (U/L) in groups under study i.e abnormal LFT, HBV+, HCV+, co-infection and control was 107.8 ± 14.1, 218.1 ± 69.8, 206.4 ± 52.8, 222.6 ± 36.8 and 31.9 ± 1.3 respectively.

One way analysis of variance (ANOVA) was done to compare any significant difference in the mean value of ALT in disease groups with control. Tukey test was done for multiple comparisons and mean difference was considered significant at .05 level. There was a significant difference in mean value of ALT between HBV+, HCV+, co-infection and control with a p-value of .024, .036 and .028 respectively. There was no significant difference between abnormal LFT and control (p-value .697) (Fig. 3.4).

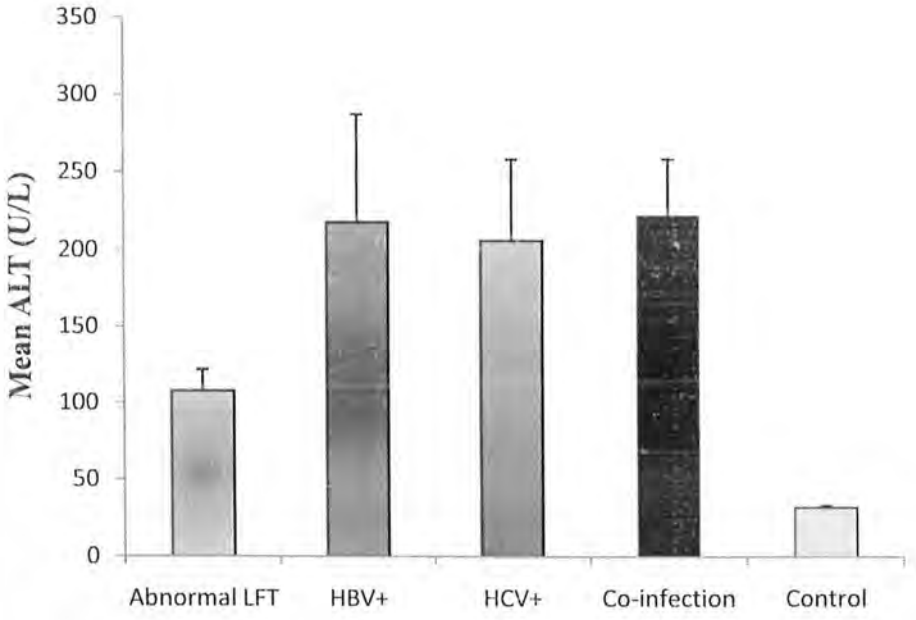



**Fig 3.3: A Comparison of the level of ALT in disease groups and control**

### 3.9.1.2 Analysis of Bilirubin Levels

One way ANOVA was done to determine significant elevation in the level of bilirubin in abnormal LFT subjects (3.8 ± 0.8), HBV+ (5.0 ± 1.4), HCV+ (4.6 ± 0.6) and co-infected subjects (4.8 ± 1.2) with control (0.6 ± 0.1). Bilirubin level was taken in mg/dl. Tukey test was done for multiple comparisons and $p < .05$ was considered significant. There was no significant difference between mean bilirubin levels of subjects having only their LFT elevated and control. Mean differences for other three groups were significant (p .011; .034; .031 respectively) (Fig 3.5).

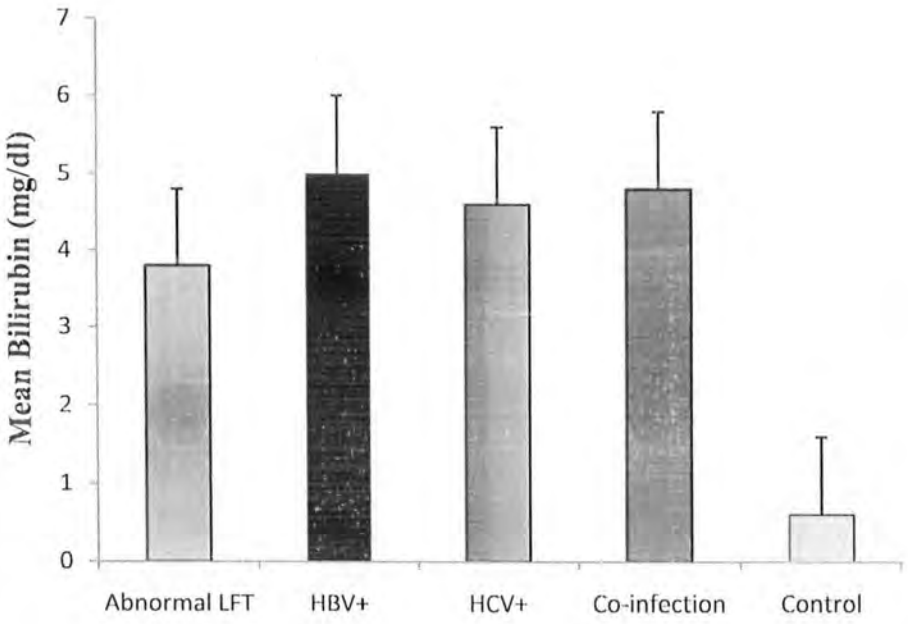



**Fig 3.5: Variation in the level of Bilirubin studied in disease groups and controls**

### 3.9.1.3 Analysis of ALP Levels

Mean value of ALP in groups under study was; group 1 (315.5 ± 36.7), group 2 (256.4 ± 38.7), group 3 (153.6 ± 9.8), group 4 (201.3 ± 29.2). Mean ALP value of controls was 182.0 ± 10.9.

One way ANOVA followed by post hoc Tukey test showed a significant difference in mean value (U/L) of group 1 (315.5 ± 36.7) and control (p =.010) but no significant mean difference was observed for group 2 (256.4 ± 38.7), 3 (153.6 ± 9.8) and 4 (201.3 ± 29.2) (Fig. 3.6).

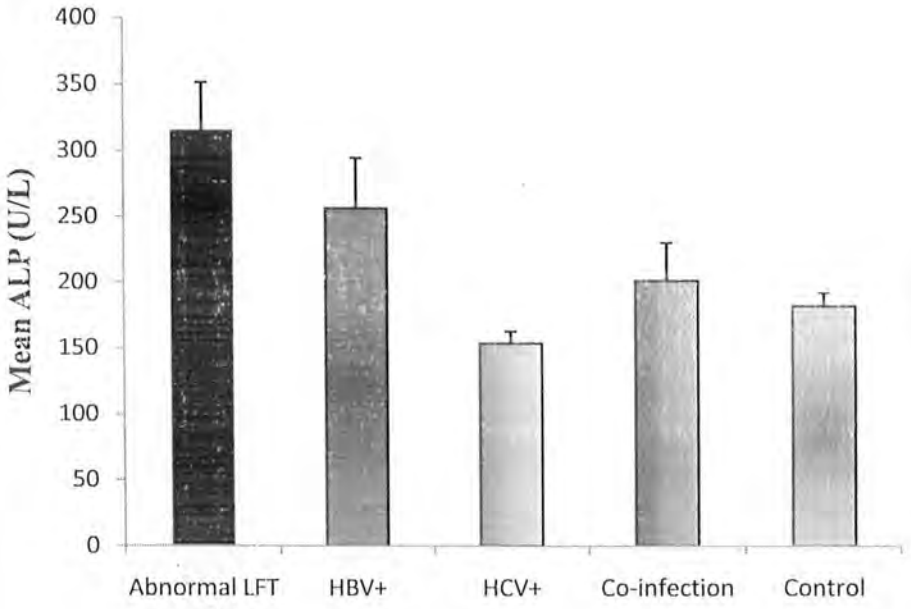



**Fig 3.6: A comparison of the ALP levels in patients and control**

### 3.9.2 Analysis of LFT Markers by Age Groups

To study any difference in the level of ALT, bilirubin and ALP by age, 132 participants were categorized into 3 groups which included 20-40 years (group 1), 41-60 years (group 2) and > 60 years (group 3). There were 30 healthy controls, 10 from each age group.

#### 3.9.2.1 Comparative Analysis of ALT Levels

Difference in the level of ALT (measured in U/L) between different age groups was determined by one way ANOVA. No significant difference in the mean value of ALT among three groups (group 1; 166.3 ± 23.6, group 2; 195.2 ± 45.1; group 3; 209.9 ± 83.3) was found although an increase in ALT level was observed by increasing age. Mean values of ALT in controls of group 1, 2 and 3 were 31.3 ± 2.0, 28.5 ± 2.5 and 35.8 ± 1.3 respectively (Fig 3.7).

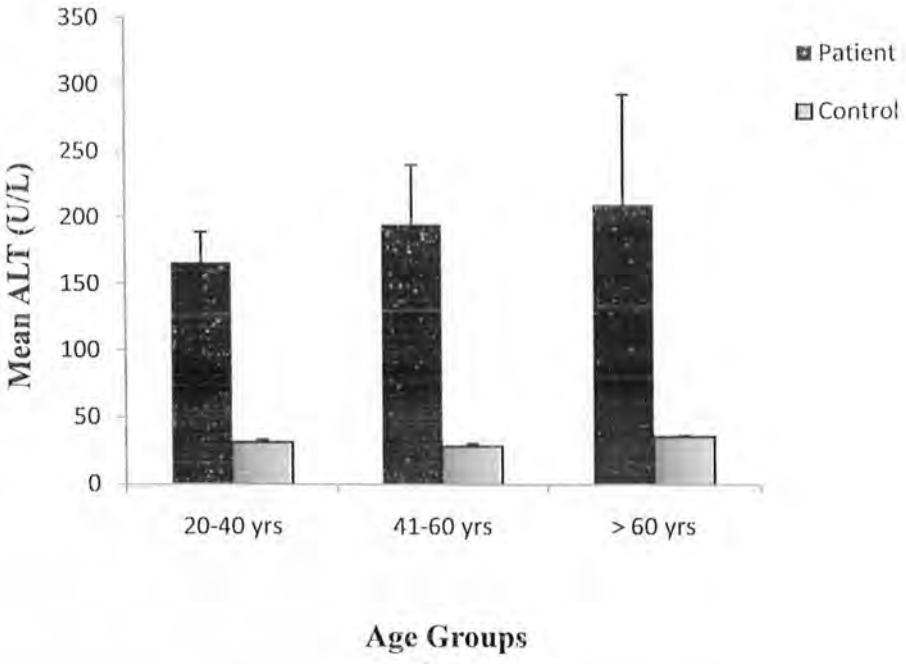



**Fig 3.7: A comparison of ALT levels in different age groups**

### 3.9.2.2 Comparative Analysis of Bilirubin Levels

Mean value of bilirubin in different age groups and their respective control groups was; group 1 (4.0 ± 0.74; 0.6 ± 0.14), group 2 (3.9 ± 0.41; 0.7 ± 0.09), group 3 (8.0 ± 2.4; 0.6 ± 0.10) (Fig. 3.8).

By one way ANOVA and multiple comparisons Tukey test, it was found that mean value of bilirubin measured in mg/dl in group 3 (8.0 ± 2.4) was significantly higher when compared to group 1 (4.0 ± 0.74; p = .053) and group 2 (3.9 ± 0.41; p = .058). However, mean difference of bilirubin levels was insignificant between group 1 and group 2. Each age group had elevated levels of bilirubin when compared to control.

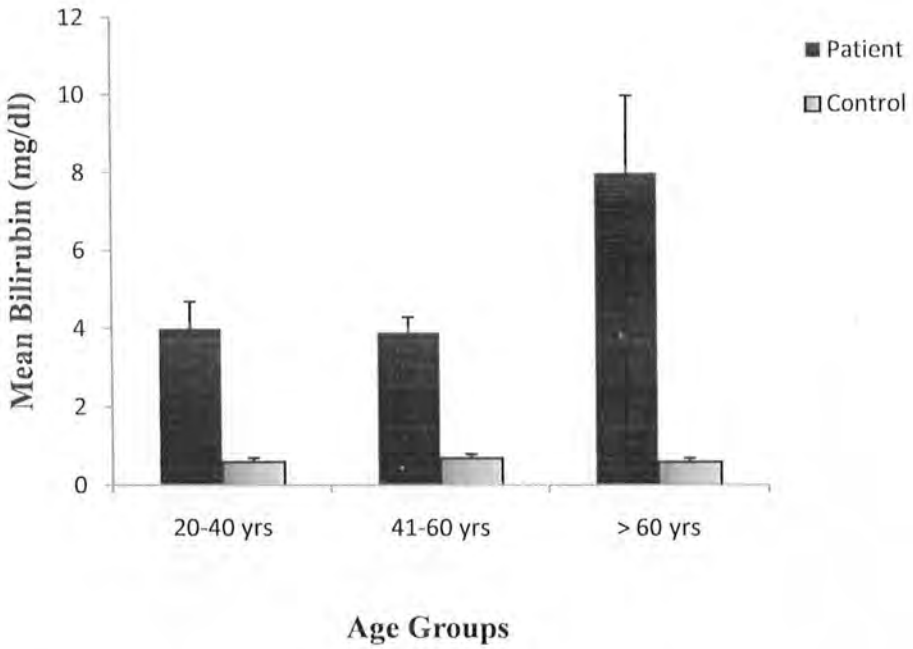



**Fig 3.8: A comparison of Bilirubin levels in different age groups**

### 3.9.2.3 Analysis of ALP Levels

Mean value of ALP in under study age groups and respective controls was; group 1 (233.3 ± 25.6; 165.4 ± 19.4), group 2 (237.7 ± 24.9; 186.2 ± 13.7), group 3 (279.0 ± 55.7; 194.3 ± 22.9). Mean value of ALP in all age groups was found within reference limit (98-279 U/L) (Fig. 3.9).

There was no significant difference ($p > 0.05$) in mean value of ALP among all the age groups.

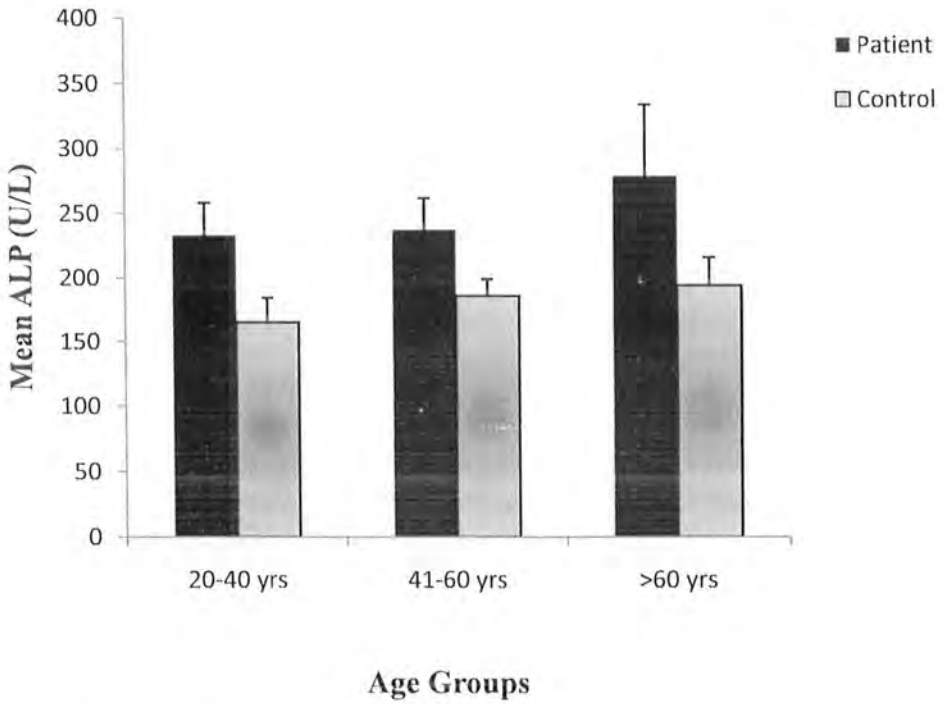



**Fig 3.9: A comparison of ALP levels in different age groups**

### 3.9.3 Gender-wise Comparison of LFT Markers

Gender-wise comparison of understudy LFT markers was done by one way ANOVA. There were 77 males and 55 females and control group included 30 healthy individuals 15 males and 15 females.

#### 3.9.3.1 Comparison of ALT Levels

Mean level of ALT in females was 190.4 ± 42.5 whereas in males 178.4 ± 26.6. In female controls, level was 29.9 ± 2.0 and in male controls, its value was 33.9 ± 1.5 (Fig. 3.10).

Reference value of ALT was considered as 2-40 U/L.

No significant difference ($p > 0.05$) was observed between males and females.

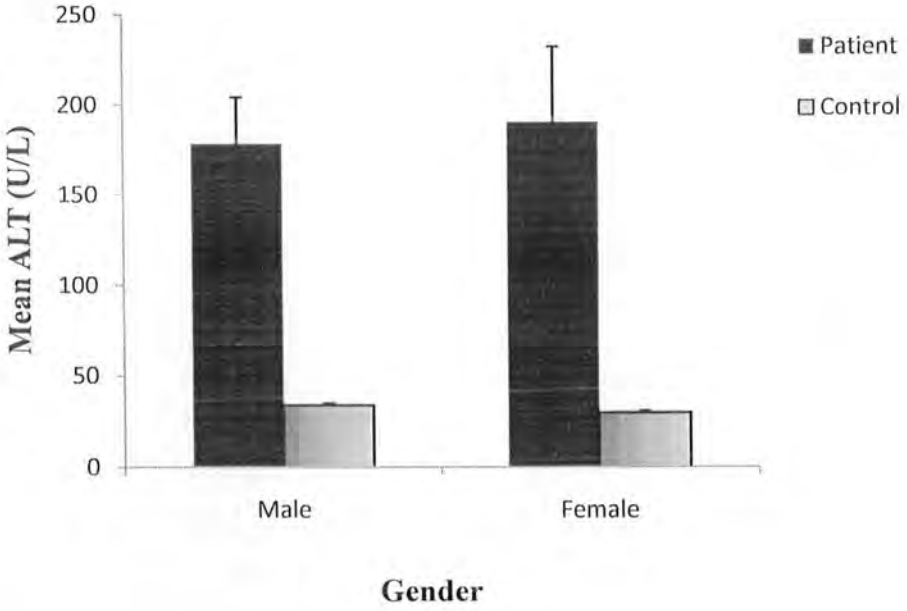



**Fig 3.10: Difference in the level of ALT by gender**

### 3.9.3.2 Comparison of Bilirubin Levels

Mean value of bilirubin determined by chemistry analyzer was 4.2 ± 0.8 in males and 4.8 ± 0.8 in females. In control group, its value was 0.7 ± 0.1 in males and 0.6 ± 0.1 in females (Fig 3.11).

Significance of the difference of bilirubin level between male and female gender was determined by one way ANOVA. It was not found significant ($p > 0.05$).

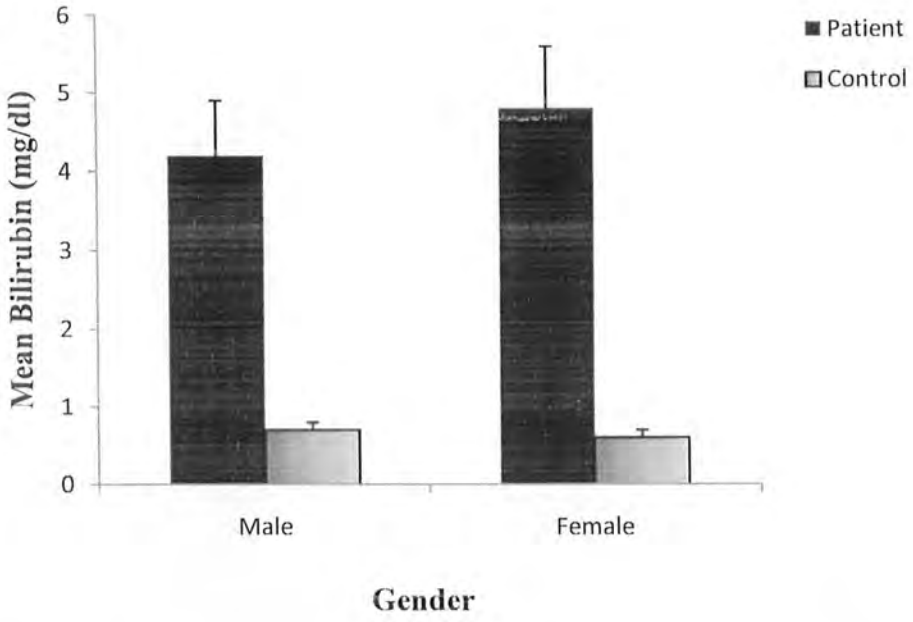



**Fig 3.11: Difference in the level of Bilirubin by gender**

### 3.9.3.3 Comparison of ALP Levels

Level of ALP in males (225.4 ± 17.7) and females (263.4 ± 33.0) was within reference limit as controls (males; 196.7 ± 15.0, females; 167.3 ± 15.3) (Fig 3.12). Difference was not significant ($p > 0.05$).

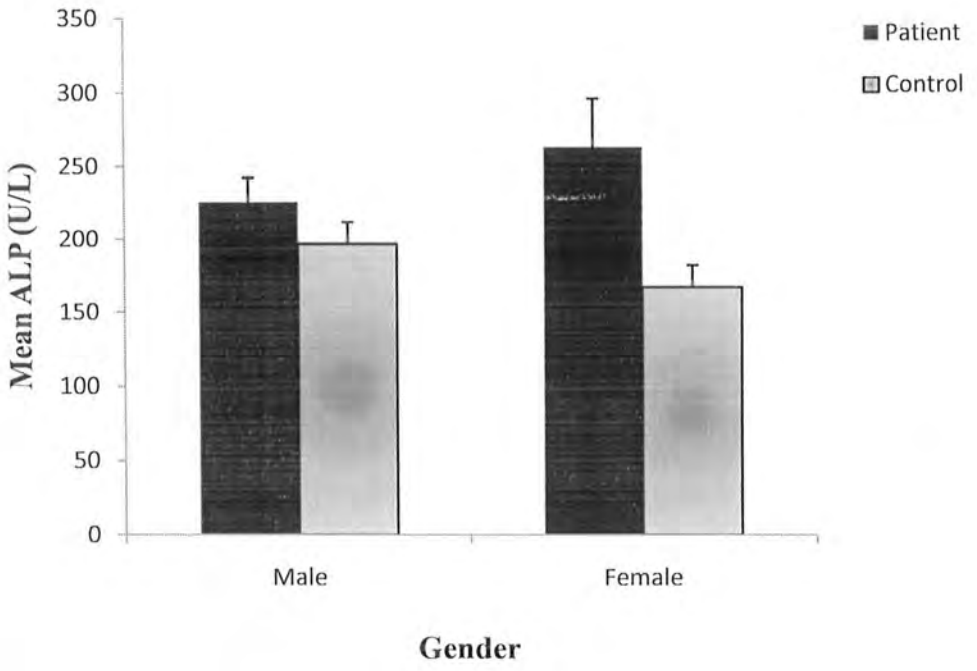



**Fig 3.12: Difference in the level of ALP by gender**

## 3.10 Etiological Factors of Liver Test Abnormality

From the follow up study of subject population, different etiological factors were evaluated for their contribution to liver disease.

Major factor responsible for elevation of the levels of ALT and bilirubin was viral hepatitis. Of all the individuals having abnormal LFT, 24.24 % had HCV infection, 25.76 % had HBV infection and 20.45 % were found infected with both HBV and HCV. Ultrasonography reports showed 3.79 % individuals to be having non-alcoholic fatty liver disease. Only one of the study subjects (0.76 %) had Wilson's disease and in 25 % individuals, cause of elevated liver test remained unidentified (Fig 3.13).

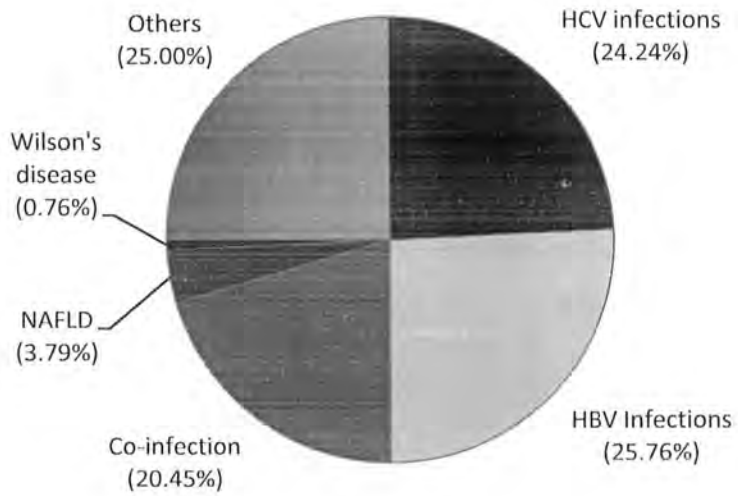



**Fig 3.13 Etiological factors of LFT elevations in sampling population**

# *Discussion*

# 4. DISCUSSION

Wide variety of serum markers is used to assess the liver function. Most common include alanine aminotransferase, bilirubin, gamma glutamyltransferase, alkaline phosphatase and albumin. But, accurate diagnosis requires patient history and other tests like ultrasonography, liver biopsy if required and specific tests of liver diseases to exclude all possible causes.

We reported here the prevalence of abnormal LFT, its possible risk factors and etiologies, prevalence of HBV and HCV infection among abnormal LFT and their association with possible socio-epidemiological factors and some LFT parameters.

Low socioeconomic status was observed as the most common risk factor (79.5 %) for elevation in the level of bilirubin and ALT. This is because Pakistan is a developing country where literacy rate and level of public awareness is low. Use of medicines was found as the second important risk factor (41.7%) of LFT elevation. Due to unawareness, use of over-the-counter drugs is common in our population and instead of preventive and precautionary measures, use of medicines as a curative is highly practiced. 17.4 % of study subjects having abnormal LFT were obese also. Other risk factors studied were history of blood transfusion, smoking, diabetes mellitus and family history of liver dysfunction whose frequency of occurrence was low. All the study subjects claimed that they did not use alcohol. Such finding may be due to selective study population or because it's a taboo in our society so people usually hide it.

There are a few prospective studies on abnormal LFT and viral hepatitis. Levels of liver function markers were determined by chemistry analyzer. Prevalence of abnormal LFT was found to be 13.2 % which is in the range of 7.4 – 16 % ; 7.4 % in US adults (Fraser *et al.*, 2007), 11.4 % in adult population of Taiwan (Chen *et al.*, 2007), 14.77 % in Jilin, China (Zhang *et al.*, 2011) and 16 % in a study at Gorgan in Iran (Mansourian *et al.*, 2011). Its incidence was higher in males and the results were concomitant with a study by Zhang et al (Zhang *et al.*, 2011).

Most common etiological factor of elevated liver tests, determined by follow up study was viral hepatitis. Incidence of HBsAg with abnormal LFT was 25.76 % and anti-HCV antibodies was 24.24 %. A population based study conducted at Taiwan showed

that 28.5 % individuals with abnormal LFT were HBV positive and 13.2 % were HCV positive. However, NAFLD was found as the major cause (33.6 %) (Chen *et al.*, 2007). In a population based survey at Mediterranean town, Pendino et al found that in 18.6 % of study subjects, cause of abnormal liver test is HCV infection while only 1 % individuals with abnormal LFT had HBV infection. Alcohol use and NAFLD were leading causes with a prevalence of 45.6 % and 24 % respectively (Pendino *et al.*, 2005 ). This disparity may be due to difference in sampling population and sample size.

Viral hepatitis is a major public health problem targeting billions of people globally. In a survey conducted by Pakistan Medical Research Council, 16 million people in Pakistan are estimated to be exposed to HBV and HCV representing 7 – 10 % of total population. Studies on selective population have shown many independent risk factors to be associated with HBsAg and anti-HCV antibody seropositivity.

In this study we found that nosocomial acquisition of these viral infections is common in our study population. It may be due to the fact that clinics run by quack doctors and paramedicals is a common practice in Pakistan. Although, in big hospitals auto disposable syringes are used but due to poverty, people cannot afford to visit these hospitals. Some public sector hospitals also use non-sterile instruments. Other studies in Pakistan also show the same nosocomial factors like surgical instruments, therapeutic injections, reuse of syringes and dental extraction to have an association with hepatitis viruses infections (Bosan *et al.*, 2010; Qureshi *et al.*, 2010).

Injecting drug use is a reported factor in many studies but none of our study subjects agreed if they used any injecting or other abused drugs (Bosan *et al.*, 2010; Qureshi *et al.*, 2010).

Low socioeconomic status is another important factor associated with HBV and HCV infections which is also highlighted by Alam et al who determined association of patients' epidemiological characteristics with hepatitis B status (Alam *et al.*, 2007).

We also found that anti-HCV antibody and HBV, HCV dual infection has significant association with body piercing. One likely explanation to this may be our selective sampling population which included Afghan immigrants with high rate of tattooing.

Females are found more prone to hepatitis viral infections probably because of their frequent visits to hospital than males resulting in high exposure to potential risk

factors but due to low socioeconomic status they do not go for the treatment of such infectious diseases. Frequency of infection remained unaffected by increasing age which is contrary to the results of a nationwide survey about hepatitis B, C virus infections (Qureshi *et al.*, 2010).

We also did comparative analysis of the levels of ALT, bilirubin and ALP among different groups. A significant association was observed in the level ALT and bilirubin and hepatitis virus infection. However, no significant elevation was observed in patients having abnormal LFT but no viral infection. It shows that frequency of viral hepatitis is higher in patients having more than three times elevation in the level of ALT and bilirubin compared to control. And these patients should be tested for viral hepatitis as reported by Arnold et al in their study (Arnold *et al.*, 2011). No significant association between viral hepatitis and level of ALP provides clue that ALP is not very helpful marker in this regard. When we compared the level of these markers among different age groups and genders, we did not observe any significant difference.

In 25 % of individuals, factors responsible for liver test elevation remained unknown. It may be a shortcoming of our study that the population was not studied in detail. It also provides clue to the fact that the level of LFT markers may sometimes get elevated in normal healthy individuals.

# CONCLUSION

Our results indicate high level of endemicity of HBV and HCV infections in individuals with abnormal LFT, which figures out the importance of diagnostic tests for hepatitis viral infections especially for asymptomatic individuals having marked elevations in the level of ALT and bilirubin. We found that ALP is not a good marker of viral hepatitis so role of other markers should be elucidated to improve diagnostic procedure.

We also identified use of contaminated instruments as an important contributor to viral hepatitis that highlights the need of regulatory policies to prevent hospital-acquired infections.

Higher prevalence in socially deprived population suggests that social inequity should be reduced so that every individual can have better access to diagnostic and therapeutic options.

Our findings also point out to the need of nationwide public awareness programmes about potential risk factors and vaccination.

# FUTURE PERSPECTIVES

Use of advanced and more sensitive quantitative detection techniques and assays such as PCR, RT-PCR, COBAS AMPLICOR HCV monitor 2.0, VERSANT HCV RNA 3.0, and other qualitative detection techniques like COBAS AMPLICOR HCV 2.0, VERSANT HCV RNA and HCV RNA transcription- mediated amplification.

Comparative analysis of different diagnostic techniques based on their sensitivity, specificity and cost effectiveness.

Identification of novel viral variants by sensitive techniques like restriction fragment length polymorphism (RFLP) and assays based on 5' nuclear activity of Taq DNA polymerase.

Need to conduct nationwide prospective and retrospective studies to validate the results of current study and to elucidate potential common factors associated with the transmission of viral hepatitis.

Making and implementation of regulatory policies to improve living standards and public health.

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