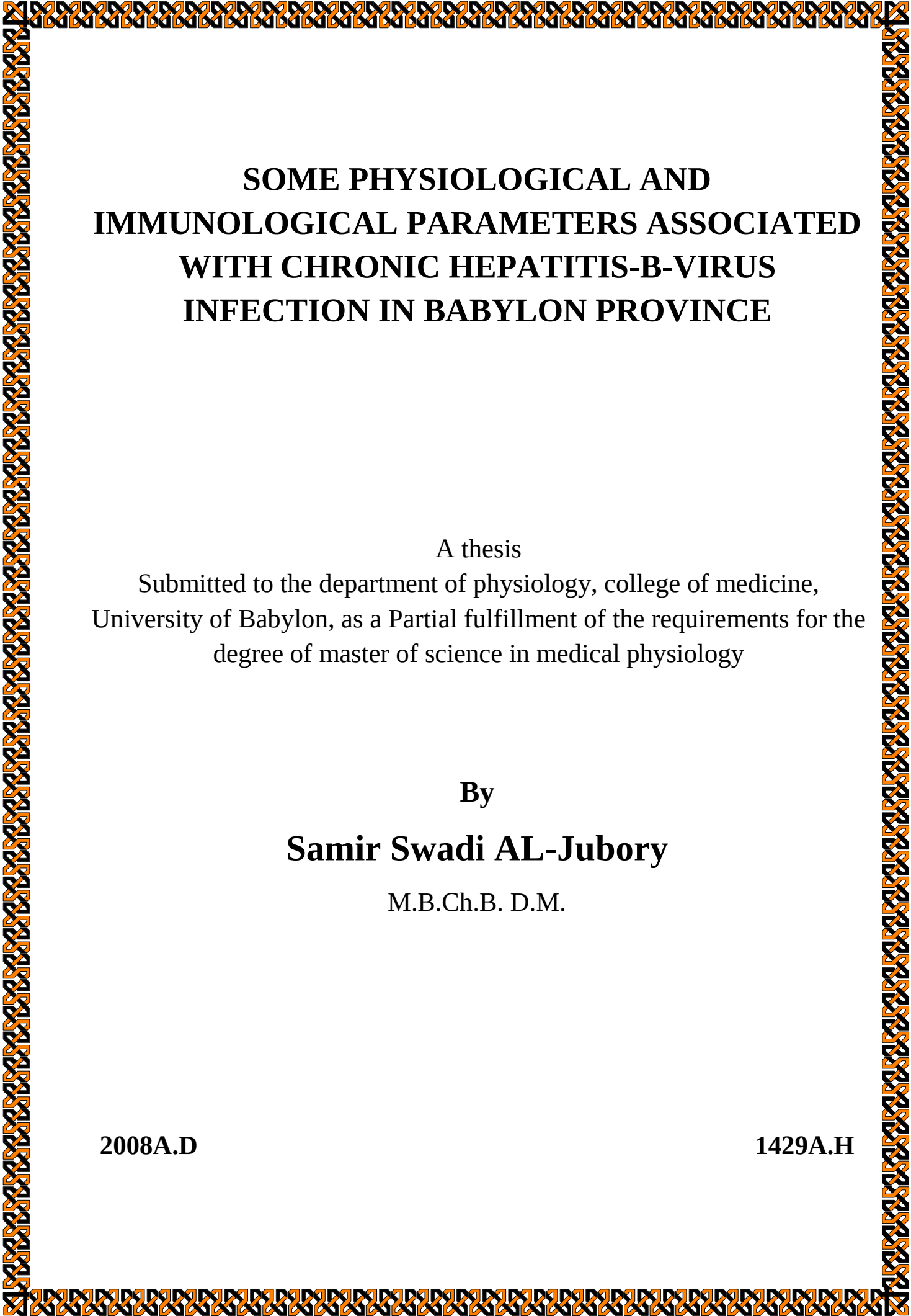




SOME PHYSIOLOGICAL AND IMMUNOLOGICAL PARAMETERS ASSOCIATED WITH CHRONIC HEPATITIS-B-VIRUS INFECTION IN BABYLON PROVINCE

A thesis

Submitted to the department of physiology, college of medicine,
University of Babylon, as a Partial fulfillment of the requirements for the
degree of master of science in medical physiology

By

Samir Swadi AL-Jubory

M.B.Ch.B. D.M.

2008A.D

1429A.H

بعض المتغيرات الفسلجية والمناعية المترامنة مع التهاب
الكبد الفيروسي نوع "ب" المزمن
في محافظة بابل

رسالة مقدمة إلى
مجلس كلية الطب جامعة بابل وهي جزء من متطلبات نيل شهادة
الماجستير في علم وظائف الأعضاء

من قبل
سمير سوادي حمود الجبوري
بكالوريوس طب وجراحة عامة
دبلوم عالي بالطب الباطني

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَأَنذَرْتُ الْوَيْلَ مِنَ الْقَارِعَةِ إِنَّكُمْ لَأُنذِرُكُمْ يَوْمَ الْبَاسِ

رَأَيْتُمْ أَصْحَابَ الْوَيْلِ يَوْمَ الْبَاسِ إِنَّهُمْ لَكَاظِمُونَ

((صدق الله العلي العظيم))

(الإسراء: 85)

1.1. Background:

Hepatitis B virus (HBV) infection is a significant public health problem that may lead to acute and chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC) (Atkinson, *et al.*, 2000). Hepatitis B is a viral disease with a high incidence and prevalence worldwide and it can cause acute and chronic liver disease (Tsuyoshi and Nagayama, 2004). Approximately (8%) of the world's population has been infected with HBV, and about (350 million, 5–6%) are persistent carriers of HBV (Inlin, *et al.*, 2005). The role of host genetic factors and their interactions with environmental factors lead to chronic HBV infection and its complications are not well understood. A better understanding of these factors and interactions will lead to more effective diagnostic and therapeutic options (Zheng, *et al.*, 2008). The clinical presentation ranges from subclinical to symptomatic and, in rare instances, fulminant hepatitis (Parveen and Michael, 2006). Perinatal or childhood infection is associated with few or no symptoms, but it has a high risk of becoming chronic. There are limited number of medications that can be used to effectively treat chronic hepatitis B; a safe and effective vaccine is available to prevent hepatitis B infection (Alexander and Kourtis, 2007).

1.2. Liver anatomy:

The liver is one of the heaviest organs in the body weighting about (1.2-1.5kg) and it has traditionally been divided into the left and right lobes, by the falciform ligament, fissure of

the ligamentum teres and fissure of the ligamentum venosum; the right and left hemi livers are further divided into a total of eight segments, in accordance with subdivisions of the hepatic and portal veins; each segments has their own hepatic artery branches and biliary tree (Nicholas, *et al.*, 2006). 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 (hepatocyte) plates and peripheral portal tracts and it lies in the right upper quadrant (hypochondrium) of the abdominal cavity, beneath the protective cover of the lower ribs(Clancy and Mcvicar, 2002).

1.3. Liver structure:

The liver is mainly composed of tiny cylindrical lobules with a hexagonal cross section, the lobules are made up of sheets of hepatocytes that radiate from the centre like spokes of a wheel, separating the sheets are channels (bile canaliculi) that collect bile produced by the hepatocytes, and also channels (blood sinusoids) that carry blood flowing through the lobule (Porth, 2002). The liver lobules receive their blood supply from branches of the hepatic artery and branches of the portal vein, and blood enters a liver lobule at its outer aspect, flows inwards through the sinusoids and leaves via the central vein in the middle of the lobule (Nicholas, *et al.*, 2006). The central veins of the lobules unite to form the hepatic vein, and hepatic vein carries the blood away from the liver towards the heart and the bile flows in the

bile canaliculi outwards into the bile ducts, which in turn drain into progressively larger ducts; eventually these form the common bile duct, and the common bile duct carries the bile to empty into the duodenum (Parveen and Michael, 2006).

1.4. Major hepatic functions:

1.4.1. Metabolism of carbohydrates, protein and fats:

The liver has many essential functions and is vital to life. The liver is important in metabolism of carbohydrates, protein and fats and has a central role in maintaining the correct level of glucose in the blood. It is also important in the metabolism of protein and in the safe disposal of ammonia (Parveen and Michael, 2006). There are two important enzymes involved in protein metabolism produced in the liver: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Brooker, 1998). The liver performs a wide variety of functions following a meal, more than half the glucose absorbed is taken up by liver and stored as glycogen or converted to lactate and released into the systemic circulation; in contrast, during fasting, the liver releases glucose derived either from the breakdown of glycogen or from gluconeogenesis using amino acids released from extra hepatic tissues such as muscle. Synthesis of urea, endogenous protein and hepatic amino acid release are suppressed during fasting (Mark, *et al.*, 1998). In both the fed and fasting state, the liver plays a central role in lipid metabolism, producing very low-density lipoproteins and further metabolizing

low and high-density lipoproteins, and a considerable part of fat metabolism takes place in the liver e.g. the liver is involved in the storage of fat and its release, when required, to provide energy (Brooker, 1998).

1.4.2. Metabolism of bilirubin:

The liver plays a central role in the metabolism of bilirubin and bile salts, drugs and alcohol. It is involved in breaking down of the old red blood cells and bilirubin is a by-product of this process. There are two types of bilirubin (fat-soluble and water-soluble) (Alexander and Kourtis, 2007). Fat-soluble bilirubin is toxic to cells; therefore, the body needs to get rid of it, and is carried in the plasma bound to a protein; while in the hepatocytes, the unconjugated bilirubin converted into a less toxic water-soluble form known as conjugated bilirubin. This forms a component of bile and is excreted via the bile channels in the liver lobules into the duodenum via the common bile duct (Marshall, 2000). These small bile channels merge and form the hepatic duct; some bile flows to the gall bladder for storage and release into the gut when required for digestion while the rest flows through the common bile duct into the gut (Huether, 2000). In the gut, the conjugated bilirubin converted into urobilinogen, and some urobilinogen reabsorbed into the circulation to be excreted in the urine, but most excreted in the stool as stercobilin (Marshall, 2000). The hepatocytes secrete about 600-1200 ml of bile every day and the production of bile is essential for the

digestion and absorption of dietary fats and fat-soluble vitamins from the gut (Porth, 2002).

1.4.3. Storage and metabolism of vitamins and minerals:

The liver stores some vitamins e.g. vitamin B12, D, E and K (Huether, 2000). Some vitamin, such as A and D are stored by the liver in large amounts, while others, such as vitamin k and folate, are stored in smaller concentrations and disappear rapidly if dietary intake is deficient. It is also used to metabolize vitamin D and vitamin k (essential for the hepatic synthesis of coagulation factors II, VII, IX and X) (Mark, *et al.*, 1998). The liver stores some minerals such as copper and iron (in ferritin and haemosiderin states) (Nicholas, *et al.*, 2006).

1.4.4. Synthesis of plasma proteins:

The liver synthesizes plasma proteins, including albumin, and other important proteins such as (vitamin K-dependent blood coagulation proteins, gamma carboxylated glutamic acid, α_1 -antitrypsin, ceruloplasmin, serum albumin, alpha fetoprotein, iron storage and binding proteins)(Porth, 2002).

1.4.5. Detoxification function:

The liver also alters (detoxifies) potentially toxic chemicals such as alcohol and so facilitates their excretion from the body, and in a case of alcohol, however, the end-product of detoxification is also toxic (Huether, 2000). Excessive alcohol intakes over a prolonged period causes these products to damage the hepatocytes (Mark, *et al.*, 1998).

1.5. Regulation of liver function:

The liver's ability to store and modulate availability of systemic nutrients is regulated by numerous local factors and by the requirements of peripheral organs for energy sources (Porth, 2002). Hepatic metabolic function is subjected to hormonal modulation by endocrine organs such as pancreas, adrenal and thyroid glands, as well as to neural regulation (Arthur and John, 2006). The liver regulates nutrients flux during periods of nutrients absorption (meals), when absorbed nutrients are either metabolized or modified for storage in the liver and fatty tissues or are made available to the remaining organs as sources of energy metabolism, and during non absorptive nutritional periods (fasting), when metabolic requirements need to be maintained from stored fuel sources or synthesized (Mark, *et al.*, 1998).

1.6. Hepatitis in general:

1.6.1. Pathology of hepatitis:

Hepatitis is a general term that means inflammation of the liver, and microscopically, there is spotty parenchymal cell degeneration, with necrosis of hepatocytes, a diffuse lobular inflammatory reaction, and disruption of liver cells cords (Carol, 2007). These parenchymal changes are accompanied by reticuloendothelial (Kupffer) cells hyperplasia, periportal infiltration by mononuclear cells, and cell degeneration; localized areas of necrosis are frequently observed, and in the later course of the disease, there is an accumulation of macrophages near

degenerating hepatocytes (Geo, *et al.*, 2007). The histological characteristics of chronic hepatitis B include visible inflammation in the portal triad; the infiltrating cells are predominantly lymphocytes, with few plasmocytes and macrophages (Ishak, 2000). The aggregation of the inflammatory cells usually results in the expansion of the portal triad and the destruction of the interface, which is referred to as interface hepatitis or piecemeal necrosis, and is the characteristic change seen in active chronic hepatitis B with the progression of hepatitis, hepatocellular degeneration and necrosis within the lobules worsens, necrosis and interface hepatitis may result in an excessive deposition of collagen in the liver and fibro separation formation, and cirrhosis occurs once chronic hepatitis B patients develop pseudo lobules (Vinay, *et al.*, 2005).

1.6.2. The causes of hepatitis:

There are many causes of hepatitis ranging from; drug reactions, chemical toxins, sepsis or shock; however, the most common cause of hepatitis in the world is a viral infection (Carol, 2007). This is a common cause of jaundice and must be considered in any one presenting with high transaminase, and these viral causes are classified as follows:

Common: (hepatitis A, hepatitis B with or without hepatitis D, hepatitis C, hepatitis E, hepatitis F, hepatitis G, and hepatitis Sen. V).

Less common: (cytomegalovirus and Epstein Bar virus).

Rare: (herpes simplex and yellow fever) (Nicholas, *et al.*, 2006).

All These viruses cause illnesses with similar clinical and pathological features and they are frequently an icteric or even asymptomatic. They differ in their tendency to cause acute or chronic infection; and to be development to a carrier state (Geo, *et al.*, 2007). Because hepatitis constitutes a generalized pattern of inflammatory injury in the liver and because of the central role of the liver in metabolism, just about all viruses are capable of involving the liver (Eugene, 2006). However, there are at least eight distinct viruses, which specifically seem to infect and damage hepatocytes; two of these originate from the same viral family, the different forms of viral hepatitis have similar clinical signs and symptoms, but differ in their means of spread, infectivity and potential outcome (Carol, 2007). (Eugene, 2006) found about 70% of all patients with hepatitis A, 50% of patients with hepatitis B, and 80% of patients with hepatitis C recover without overt clinical symptoms and these patients do not become jaundiced, nor do they complain of any acute illness, and for types A and B hepatitis lasting immunity results in the investigational studies suggest that immunity does not occur following exposure to C type hepatitis infection. (Wright, 2006) found that approximately 0.05 - 0.1% of all cases of type A hepatitis and 1-3% of adult patients with acute, icteric type B hepatitis develops a fulminant hepatitis, which is associated with

hepatic failure, signs and symptoms of hepatic encephalopathy and death. Serological hepatitis markers in fulminant hepatitis are similar to those in acute hepatitis, and a bilirubin levels may increase and a prolongation of prothrombin time commonly occur, while chronicity is unknown in humans infected with type A or E hepatitis, it occurs in 5-10% of all cases of type B hepatitis and in the majority of cases of type C hepatitis (Eugene, 2006).

1.7. Hepatitis A virus (HAV):

Type -A- hepatitis is a member of the *picornavirus* family and it is simple and non-enveloped. The virus is approximately 27 nm in size, consisting of a single stranded ribonucleic acid (RNA) molecule of approximately 8100 nucleotides surrounded by a nucleocapsid consisting of 32 capsomeres, and each capsid consists of four polypeptides (Maguire, *et al.*, 1995), although HAV is endemic globally, there are wide variations in prevalence throughout the world, in the USA, 35-45% of the adult populations have anti-HAV markers and type -A- hepatitis accounts for 20% of all viral hepatitis in the US (Hutin, *et al.*, 1999). Type -A- hepatitis spreads primarily via the 'oral-fecal' route, and the virus is relatively hardy and is commonly associated with poor sanitary conditions and overcrowding (Massoudi, *et al.*, 1999). The disease is common among institutionalized persons (prisons, military groups, mentally retarded), children in day care centers, male homosexuals and

drug addicts. The prevalence of this disease is not increased among health care workers or dialysis patients (Eugene, 2006). The sero prevalence of HAV increases with increasing age, ranging from 11% in children less than 5 years to 74% in those more than 50 years (Koff, 1995).

1.8. Hepatitis D virus (HDV):

HDV is a *hepatotropic*, circular, single-stranded RNA viroid that codes hepatitis delta antigen (HDV-Ag) as the sole HDV protein and HDV is defective and dependent on coinfection with HBV to provide hepatitis B surface antigen (HBsAg) for virion assembly (Tsuyoshi and Nagayama, 2004). An incomplete RNA virus requires the HBV outer coat, and it is only found in patients with hepatitis B. Also it is largely an infection of intravenous drug users and their sexual partners but also in female sex workers, and sporadically in other groups such as medical staff. Suspect of HDV with hepatitis B particularly if the acute hepatitis is severe, if chronic hepatitis B carriers get a further attack of acute hepatitis or if the liver disease in chronic HBV is rapidly progressive (Bianco, *et al.*, 2003). If HBV co infected with HDV, there is a high rate of fulminant hepatitis and progression of chronic hepatitis to cirrhosis and the diagnosis is confirmed by a positive anti-HDV antibody or HDV-RNA test (Vandamme, *et al.*, 2003). Response to anti-viral therapy is poor, and because of the obligate presence of HBsAg, HDV can be either a self-limited acute process or a chronic process and the

usual incubation period for HDV is 30-50 days, and the primary means of spread is parenteral (Lau, *et al.*, 1999).

1.9. Hepatitis C virus (HCV):

HCV is an RNA virus of the *flaviviridae* family. There are six major genotypes and more than 90 subtypes of HCV that vary in their worldwide distribution, in addition, HCV has a high rate of spontaneous mutation; producing discrete “quasi species” that vary from one individual to another (Aoyagi, *et al.*, 1999). HCV is a major cause of hepatitis and cirrhosis worldwide. The most efficient mode of transmission of HCV is associated with percutaneous exposures to blood, but such exposures account for less than half of reported cases, and the sexual, household, and perinatal transmission also seems to occur, but the risks associated with these types of exposures are still unknown (Nicholas, *et al.*, 2006). Maternal-neonatal transmission of hepatitis C is also probably inefficient, and most babies will have passive transfer of maternal hepatitis C antibodies and most studies do show a lack of viremia as detected by polymerase chain reaction at 12-24 month follow-up (Lau, *et al.*, 1999).

1.10. Hepatitis E virus (HEV):

HEV is an enterically transmitted RNA virus that causes sporadic and epidemic acute hepatitis in developing countries. It does not cause chronic hepatitis, in the US, HEV infection has been seen rarely as a cause of hepatitis, predominantly among those who have traveled to endemic areas, although at least one

case has occurred without history of travel to endemic areas (Erker, *et al.*, 2001). (Aoyagi, *et al.*, 1999) revealed that in the US, approximately 1.4% of all blood donors show evidence of previous infection with hepatitis E, but it is a major cause of hepatitis in India, central and Southeast Asia, Africa and Mexico cities. Hepatitis E is associated with fecal-oral transmission in unsanitary conditions associated with contaminated water; it is endemic in Southeast Asia, India, Russia, the Mid-East, Africa, and Central America and the infections tend to be acute, self-limiting process, except among pregnant women in the third trimester, and mortality rates among infected pregnant women are between 10 and 20% (Eugene, 2006).

1.11. Hepatitis G virus (HGV):

The designation of HGV has been applied to a *flavivirus* that is percutaneously transmitted and associated with chronic viremia lasting at least 10 years. It is an agent that rarely, if ever, causes frank hepatitis (Lawrence, *et al.*, 2005).

1.12. TT virus (TTV):

The researchers described a deoxyribonucleic acid (DNA) virus designated (TTV) has been identified in up to 7.5% of blood donors and found to be transmitted readily by blood transfusion, but an association between this virus and liver disease has not been established (Lawrence, *et al.*, 2005).

1.13. Hepatitis B virus (HBV):

1.13.1. Over view:

HBV is a member of the family *hepadnaviridae*, viruses with a double-stranded circular DNA genome that replicates through an RNA intermediate. HBV infects only humans and higher apes, but other similar hepadnaviruses are endemic in rodent and bird species (Seeger and Mason, 2000). The virus is detectable in serum in high levels and on electron microscopy is a 50 nm double-shelled particle with an outer envelope (HBsAg) and an inner nucleocapsid (HBcAg) protein; within the nucleocapsid is a partially double stranded, circular molecule of HBV DNA. The HBV genome is 3.2 kb in length (Lau, *et al.*, 2003). There are four partially overlapping open reading frames encoding the envelope (pre-S/S), core (precore/core), polymerase, and X proteins, replicates through an intermediate reverse transcription step (Maini, *et al.*, 2000). The virus gains entry into hepatocytes, the primary site of infection, through yet undefined pathways and receptors (Wright, 2006). After entry, HBV DNA is transported to the nucleus and converted to covalently closed circular DNA (cccDNA), which serves as the stable template for transcription of both messenger RNA (for translation of viral proteins) and pre-genomic RNA (for reverse transcription into genomic DNA) (Strader, *et al.*, 2004). HBV cccDNA is present in the range of 5-50 copies per hepatocyte and is the stable form of viral DNA that is the most resistant to antiviral therapy and host

immunological response (Lok, *et al.*, 2001). Most persons exposed to HBV have a transient infection, which may or may not be accompanied by symptoms and jaundice (Seeger and Mason, 2000). In a proportion, however, chronic infection ensues because of failure of the host immune response to eliminate virus (Lok, *et al.*, 2001). Viral clearance mediated by both cytopathic and non-cytopathic mechanisms, the existence of both pathways being supported by extensive evidence in cell culture and animal models (Lok and McMahon, 2001). Cytopathic clearance based on direct killing of virus-harboring hepatocytes by virus-specific T- cells, followed by compensatory proliferation of hepatocytes and this proliferation may also result in gradual loss of cccDNA (Doo and Liang, 2001). Uninfected hepatocytes are protected from reinfection through induction of neutralizing antibodies and innate immune mechanisms (Lemire, *et al.*, 2005). The early decrease in virus correlates with the appearance of antiviral cytokines such as interferon and tumor necrosis factor (TNF), which have direct inhibitory effects on HBV replication, thus both cytopathic and non-cytopathic mechanisms are important in clearance of HBV during transient infection, in addition to the humeral response, cell-mediated immunity is crucial for the control of HBV infection (Chisari and Ferrari, 1995). The continued presence of T cell responses suggest that HBV persists at low levels despite absence of detectable HBV DNA in serum and presence of antibodies to HBsAg (anti-HBs), thus

reactivation of hepatitis B in previously recovered people during immunosuppressive therapy has amply documented (Guidotti, *et al.*, 2002). Thus, recovery from HBV may not indicate virus eradication so much as firm immunological control, an acute evolves into chronic infection if T cell responses to type B hepatitis virus are not induced or are not capable of controlling the virus because of T cell responses to HBV antigens disappear gradually with the onset of chronic infection, probably because of exhaustion and/or tolerance induction, and most patients with chronic hepatitis B have no or few HBV specific T cells in the circulation, an increase in the HBV specific T cell response can occur during acute exacerbations of chronic hepatitis B or during seroconversion from HBeAg to anti-HBe (Rehermann, *et al.*, 1996). (Lok, *et al.*, 2001) found that the HBV in the US, cause about 20%- 30% of acute viral hepatitis and 15% of chronic viral hepatitis, and a multiple sex partners and injection drug use are the major risk factors for disease acquisition in this country (Lok and McMahon, 2001). Type B hepatitis is spread mainly by the parenteral route. It is commonly associated with the transfusion of blood, blood products, needle stick accidents and contaminated needles and syringes, also the infectious viral particles can be found in serum, saliva, semen, vaginal secretions and breast milk (Eugene, 2006). HBV is not infectious by the oral, nasal or respiratory route; it is not capable of crossing intact skin or mucous membrane and the prevalence of type B hepatitis is

increased among multiply transfused individuals, drug addicts, medical personnel (particularly emergency room and laboratory workers) and dialysis patients (Carol, 2007). The other study shows approximately 95% of adults recover from acute hepatitis B and have subsequent life-long immunity, and the remainder have chronic infection, also the immunosuppressed persons with acute hepatitis B are more likely to become chronically infected, because of an insufficient immune response against HBV spontaneous clearance of HBs-Ag occurs in up to 1% of chronically infected patients per year and a clearance rates are lowest in those who acquired HBV at a very young age (Mahoney, 1999). Patients with chronic hepatitis B who have normal liver enzymes values, a HBV DNA level less than 10^5 copies/ml and normal findings on liver biopsy are “inactive carriers” such patients have a good prognosis and their overall survival rate is similar to that of non infected persons with abnormal liver enzyme values. Active viral replication and abnormal liver biopsy finding have chronic hepatitis B and are at higher risk for developing cirrhosis and HCC than are inactive carriers (Clancy and Mcvicar, 2002). In endemic areas, most individuals are infected by vertical transmission, or in the early childhood. HBV may cause an acute viral hepatitis however; the acute infection is often asymptomatic particularly when acquired at birth, and many individuals with chronic hepatitis B are also asymptomatic (Pol, *et al.*, 1998). The risk of progression to

chronic liver disease depends on the source of infection; horizontal transmission, the risk of chronic infection in this state is 10% and vertical transmission, the risk of chronic infection in HBs-Ag positive mother, is 90%, vertical transmission (from mother to child in the perinatal period) is the most common cause of infection world- wide and carries the highest risk (Harpaz, *et al.*, 1996). If a person is infected when the immune response is “immature,” there is little or no response to the HBV, and the concentrations of HBV DNA in serum are very high, the hepatocytes contain abundant viral particles (surface antigen and core antigen), but little or no ongoing hepatocyte death is seen on liver biopsy because of the defective immune response, over some years, the degree of immune recognition usually increases; at this stage, the concentration of viral DNA tends to fall and liver biopsy shows increasing inflammation in the liver (Ryder and Beckingham, 2001). Both the adaptive and the innate immune responses play critical roles in viral clearance. Activation of innate immunity occurs early in HBV infection, and animal studies have highlighted the important role of interferon gamma (IFN- δ) and TNF- α in controlling the bulk of viral replication without necessarily inducing the perforin or Fas-dependent apoptotic pathway of cell killing (Guidotti and Chisari, 2000). The marked reduction in viral replication occurs typically before the peak infiltration of T cells and the onset of liver injury; thus, activation of innate immunity during the early stages of

infection appears to be essential for the control of HBV infection and accounts for the bulk of the antiviral activity of the host's immune response (Ganem and Schneider, 2001). (Hoofnagle, *et al*, 2006) illustrated that the prevalence of HBV infection is higher among black populations than among white populations. Also the exacerbations of chronic HBV infection are observed more often in men than in women, although the reason for this difference is not clear. The higher frequency of exacerbations in men may account, in part, for the higher incidence of type B hepatitis-related cirrhosis and HCC among men. (Alexander and Kourtis, 2007) mentioned that the age at infection primarily determines the rate of progression from acute infection to chronic infection, which is approximately 90% in the perinatal period, 20-50% in children aged 1-5 years, and less than 5% in adults. The incubation period for HBV infection ranges from 6 weeks to 6 months, and the clinical manifestations depend on the age at infection, level of HBV replication, and host's immune status and the age of adult patients at the time of initial presentation with HBeAg positive chronic hepatitis B ranges between 24 and 36 years (mean 31 years) in several reports and men usually outnumber women, the male to female ratio ranging from 1.5 to 4.9 (Yuen, *et al.*, 2001). In a previous study, the researchers found approximately 1-3% of healthy adults, 5-10% of immunocompromised adults, and 90% of neonates exposed to HBV develop chronic infection (Scaglioni, *et al.*, 1997).

1.13.2. Virus-host interaction:

Infection with HBV of a specific subtype, e.g., HBsAg (*adw*), appears to confer immunity to other HBsAg subtypes, probably because of their common, group *a* specificity and the immunopathogenetic mechanisms that result in viral persistence and hepatocellular injury in type B hepatitis remain to be elucidated; as the virus is not cytopathic, it is believed that hepatocellular injury during acute disease represents a host immune attack against HBV-infected hepatocytes (Decker, 1998). Host responses both immunologic and genetic have been proposed to account for the frequency of HBV chronicity in the infected as infant and in a study done on newborns showed that about 95% of newborns infected at birth become chronic carrier of virus, often for life, and this risk decreases steadily with time, so that the risk of infected adults becoming carriers decreases to 10% (Kurt, *et al.*, 2006).

1.14. Hepatic injuries:

1.14.1. Acute hepatic injury:

Acute hepatic injury can be recognized by the presence of jaundice or non-specific symptoms of acute illness accompanied by elevation of AST and/or ALT activities. Estimated 80% of individuals with acute viral hepatitis are never diagnosed clinically, although some of them may be detected by elevated aminotransferases in the face of non-specific or absent clinical symptoms, AST and ALT activities are seldom greater than 10

times the upper reference limit in liver diseases other than acute hepatic injury (Whitehead, *et al.*, 1999). The acute type B hepatitis is usually a benign disease showing spontaneous resolution and recovery in more than 90 % of cases, it may evolve into chronic hepatitis, while in 0.1-1% of the patients with acute hepatitis may have a fulminant course (Decker, 1998).

1.14.2. Chronic hepatic injury:

Chronic hepatic injury is a relatively common disorder with minimal symptoms, yet with long term risk of significant morbidity and mortality, it is defined pathologically by ongoing hepatic necrosis and inflammation in the liver, often accompanied by fibrosis, it may progress to cirrhosis (15-20% in the case of chronic HCV) and predisposes to HCC (Chen, *et al.*, 2007). Most commonly, it is due to chronic viral infection, and in the US alone, there are an estimated 2.1-2.7 million people chronically infected with HCV. There are also approximately 1-1.25 million chronic carriers of HBV, while prevalence rates for HCV infection generally are between 0.5 and 5% in other parts of the world, prevalence rates for HBV vary markedly, and in many areas HBV is an endemic infection (Alter, *et al.*, 2005). ALT often fluctuates between normal and abnormal values particularly in chronic HCV; 60% of patients with multiple ALT measurements have at least occasional normal ALT values (Hoofnagle, 1997). The majority of patients with normal ALT have histological evidence of chronic hepatitis on biopsy, but in

general, milder inflammation, less fibrosis, and lower rates of progression to cirrhosis than do HCV patients with elevated ALT (Mathurin, *et al.*, 1998). ALT should therefore be measured periodically even if initially normal, with both chronic HBV and HCV, and the clearance of viral markers is the most reliable method for detecting resolution of infection and in case of untreated hepatitis B, a small percentage of patients spontaneously clear viral antigens; in long-term studies, loss of HBeAg occurs in 1/3 to 1/2 of patients, and in those that lose hepatitis B e antigen (HBe-Ag), 5-10% will subsequently clear HBs-Ag over 10 years of follow-up and HBe-Ag should be rechecked periodically if initially positive, so if HBe-Ag is negative and anti-HBe is positive, this may indicate either the beginning of viral clearance from the body, or integration of HBV DNA into host DNA or loss of ability to form replicating virus (Brown, *et al.*, 1995). HBs-Ag should be measured periodically to look for viral clearance, as HBs-Ag will remain positive in those with integration of HBV DNA, and the possible risk factors for developing liver disease include older age, male gender, presence of HBe-Ag, HBV genotype, mutations in the precore and core promoter regions of the viral genome, and coinfection with type D hepatitis infection (Lai, *et al.*, 2003). Although most persons will not develop any illness related to this chronic infection, some will develop chronic liver disease, which can lead to cirrhosis or HCC, or both (Boni, *et al.*, 2003). Chronic

infection begins when the immune response that normally clears the infection fails to take place or is too weak to be effective; thus, infections are almost always chronic following exposure of children younger than 1 year or of immunocompromised individuals and chronic infections also occur in about 5% of healthy adults (Lok, *et al.*, 1991). The patients whose infection as adults often have the most severe and progressive liver disease and these patients may also experience recurrent bouts of acute liver disease, because their immune system attempts to produce the response that normally leads to virus clearance and the immune system-mediated liver disease may be less active in these carriers because infection occurred during birth or in the early years of life, producing a degree of tolerance to the viral antigens (Fonseca, 2007).

1.15. Stages of chronic HBV infection:

Chronic hepatitis B is classified into 2 major forms: HBeAg-positive disease (wild-type HBV) and HBeAg negative disease (pre-core/core promoter HBV variant), and both forms can lead to liver cirrhosis, hepatic decompensation and liver cancer (Fonseca, 2007). It is currently thought that there are 4 phases of chronic hepatitis B; these include:

1. Immune tolerance phase.
2. Immune clearance phase.
3. Immune control phase.
4. Immune escape phase. (Sam, *et al.*, 2008).

1.15.1. Immune tolerant phase:

A phase in childhood and adolescence, in which there are very high levels of virus, but no accompanying active liver disease (Sam, *et al.*, 2008). The immune tolerant phase most commonly occurs after perinatal transmission and is characterized by the presence of HBeAg, high levels of serum HBV DNA but normal ALT levels, no evidence of active liver diseases and minimal or no inflammation on liver biopsy (Fonseca, 2007).

1.15.2. Chronic hepatitis B phase or immune clearance phase:

The chronic hepatitis B phase is seen after horizontal transmission of HBV during early childhood or adulthood (Mcmahon, *et al.*, 2001). In this phase (HBeAg-positive, high levels of serum HBV-DNA, elevated ALT, and active liver disease) (Fonseca, 2007).

1.15.3. Inactive hepatitis B carrier phase:

In this state the (HBs-Ag-positive in serum without HBe-Ag, HBV-DNA levels less than 10^5 copies/mL, and normal ALT levels) (Fonseca, 2007). Three outcomes have been observed once a patient enters the inactive carrier phase of HBV infection, and most carriers (as many as 70 to 80%) remain in the inactive carrier phase indefinitely; usually accompanied by elevations in ALT levels, during which liver inflammation reactivates (Mcmahon, *et al.*, 2001). Regardless of what course HBV infection takes in a patient, all affected persons, even those who

remain in the inactive carrier state, are at risk of developing HCC, and a carriers in the inactive phase could have a flare of liver disease at anytime during their lifetimes (and thus could benefit from antiviral therapy) or could develop HCC, all persons chronically infected with HBV should be followed routinely with aminotransferase levels every 6 to 12 months (Lok and McMahon, 2004).

1.15.4. The immune response and the carrier state:

Following HBV infection, the first marker to appear is HBsAg, which is evident 2 to 8 weeks before the appearance of the jaundice and biochemical evidence of liver damage (Bortolotti, *et al.*, 1998). Next to appear are the markers of the virion, such as virus-specific DNA polymerase activity, the viral DNA and the soluble antigen, HBeAg; although HBcAg is present, it is not detectable in the serum due to the early appearance of anti-HBc, and anti-HBc is found 2 - 4 weeks after the appearance of the surface antigen, at around the same time as the development of the signs and symptoms (Lok and McMahon, 2004). In acute infections, clearance of the virus is marked by the disappearance of HBeAg and the appearance of anti-HBe, later during convalescence, HBsAg also disappears with the appearance of anti-HBs, and cell mediated immunity (CMI) to HBsAg appears near the end of the acute phase of hepatitis and appears to be mainly responsible for the disappearance of

HBsAg, in contrast, anti-HBs does not appear until months after the termination of the clinical illness, in chronic HBsAg carriers specific CMI is generally decreased and circulating anti-HBs is not demonstrable; however, on electron microscopic examination of the patients' sera reveal HBsAg - anti-HBs immune complexes, and the increase in anti-HBs is clearly correlated with immunity (Mcmahon, *et al.*, 2005).

1.16. Epidemiology of chronic HBV:

Age at the time of initial HBV infection is the major determinant of chronicity (Beasley and Hwang, 1991). (David and Daniel, 2003) found that as many as 90% of infected neonates become carriers, the carrier rate falls with increasing age at the time of infection, so that only 3% to 5% of newly infected adults fail to clear HBsAg, and the important risk factor for chronicity is the presence of intrinsic or iatrogenic immunosuppression, also a gender is a well-established but poorly understood, determinant of chronicity; women are more likely than men to clear HBsAg. Hepatitis B spread predominantly parenterally, through intimate personal contact, and perinatally, so individuals at risk are intravenous drug users, children of mothers with HBV infection, homosexuality, patients on hemodialysis, medical staff and those exposed to blood or blood products (Sam, *et al.*, 2008). Approximately 5% of the world's populations are carriers of HBV; it is endemic in many

areas of the world, such as Asia, and Africa as well as in certain populations in Australia, New Zealand, South America, the Middle East and the Arctic (Lee, 1997). An estimated 1.25 million people in the US are positive for hepatitis B surface antigen. fifteen percent to forty percent of these carriers may develop hepatitis B-related sequelae in there life times (Lok and McMahon, 2004). (Kursad, *et al.*, 2005) found in their study in Turkey, that the mean ages of chronic HBV and cirrhotic HBV patients were 31 ± 9 years in carriers, chronic hepatitis and cirrhotic patients; however, chronic HBV infection was 2.2 times, 2.9 times and 2.75 times more frequent in males than in females in carriers, chronic hepatitis and cirrhotic patients, respectively. Other studies revealed that the people who have lower educational and socioeconomical level and less hygienic living conditions are more susceptible to chronic HBV than the other, although chronic HBV infection was three times more frequent in males than in females (Mistik and Balik, 2001). A study in Japan showed the prevalence of HBV is higher in lower socioeconomic state and the men predominate more than the women (Dennis, *et al.*, 2005). Intrafamilial transmission rate of HBV infection has been reported as 25% in Eastern and Southeastern Anatolia (Degertekin, 2001). HBV vaccination in the childhood period, hygienic living conditions and improved socioeconomical and educational status are the cornerstones in the prevention of liver morbidity and mortality in Eastern Turkey (Kursad, *et al.*, 2005).

1.17. Clinical features and natural history of chronic HBV:

The natural history of hepatitis B is variable, complex and still not completely well defined. An epidemiological study was done on the HBV infection, revealed that only one-third of adults experience symptoms of acute hepatitis, while the majority (65%) has subclinical disease, about 5% of adults (2% of women and 7% of men) develop chronic hepatitis B, and rates of chronicity are higher in newborn babies (about 90%), children (about 30%) and in immune deficient individuals (Maguire, *et al.*, 1995). Some of the variation in outcome of HBV infection may relate to the genetic heterogeneity of the virus, eight genotypes of HBV have been described, genotype A being most common in the United States and Northern Europe, B and C in Asia, and D in Mediterranean countries and the Middle East (Chu, *et al.*, 2003). Chronic infection with HBV genotype C appears to have a poorer prognosis and greater likelihood of cirrhosis and HCC than genotype B, the transition from acute to chronic infection appears to represent a failure of immune clearance of virus-infected cells and is marked by persistence of high levels of HBV DNA and HBeAg in serum (Lok and McMahon, 2001). The accompanying acute hepatitis is typically mild and subclinical with only modest serum ALT elevations and no jaundice (Lok, 2002).

1.18. Serology of chronic HBV:

HBV Contains several antigens to which infected persons can make immune responses: these antigens and their antibodies

are important in identifying HBV infection, and in acute infection, the hepatitis B surface antigen (HBsAg) is a reliable marker of HBV infection, and a negative test of HBsAg makes HBV infection very unlikely but not impossible (Nicholas, *et al.*, 2006). HBsAg appears in blood late in the incubation period and before the prodromal phase of acute type B hepatitis; it may be present for only a few days, and disappearing even before jaundice has developed, but usually lasts for 3-4 weeks and can persist for up to 5 months (Porth, 2002). Chronic HBV infection is marked by the presence of HBsAg and anti HBC (IgG) in the blood (Van and Graaff, 2002).

1.19. Investigation of chronic HBV:

1.19.1. Serum aminotransferase:

AST, also sometimes termed SGOT and ALT, also sometimes termed SGPT are widely distributed in cells throughout the body (Zheng, *et al.*, 2008). AST is found primarily in heart, liver, skeletal muscle, and kidney, while ALT is found in liver and kidney, with lesser amounts in heart and skeletal muscle (Carol, 2007). Generally, serum ALT and AST levels reflect the extent of hepatocellular damage and are most frequently used to assess liver function. AST and ALT activity in liver are about 7,000 and 3,000 times serum activities, respectively and ALT is exclusively cytoplasmic; both mitochondrial and cytoplasmic forms of AST are found in all cells (Zhuang, 2007). In adults, AST and ALT activities are significantly higher in males than in

females, and reference intervals vary with age until about age 15, AST activity is slightly higher than that of ALT, with the pattern reversing by age 15 in males but persisting till age 20 in females. In adults, AST activity tends to be lower than that of ALT until approximately age 60, when they become roughly equal and upper reference limits vary little between the ages of 25 and 60 years, so the age-adjusted reference limits need to be used for this population (Zheng, *et al.*, 2008). Separate reference limits are needed for children and older adults; these may require national efforts to obtain enough samples from healthy individuals to accurately determine reference limits, liver disease is the most important cause of increased ALT activity and a common cause of increased AST activity (Nalpas, *et al.*, 1994). (Olut, *et al.*, 2007) found that in chronic HBV infection and during the one year period of the study that 89% of the patients yielded continuously normal ALT levels, while 11% showed at least one ALT value above the normal levels (ALT more than 1.2 time of normal value). Other study showed that, the serum ALT levels were constantly normal in 57.4% of patients HBV-DNA positive and above the normal in 42.6% of the patients (Myers, *et al.*, 2003). In conclusion, although ALT levels may be normal, it should always be taken into consideration that more than half of inactive HBsAg carriers exhibited low level viral replication, thus HBV-DNA and liver enzyme levels should be monitored routinely in order not to miss the acute manifestations (Olut, *et*

al., 2007). A prospective study shows that the ALT and AST levels in chronic HBV infection were greater than 60 IU/L for the testing hospital before or at enrollment (Yu, *et al.*, 2005). AST or ALT values less or equal 30IU/L excluded significant activity with 96% certainty. So the aminotransferases are accurate noninvasive markers of hepatitis B virus-related activity and fibrosis (Myers, *et al.*, 2003). (Lai, *et al.*, 2007) revealed in their study, were the patients divided into 3 groups; persistently normal ALT (PNALT); ALT 1-1.5 time the upper limit of normal (ULN), and ALT more than 1.5 time ULN and the results of this study; about 18% of patients with PNALT had stage 2 fibrosis and 34% had grade 2 or 3 inflammation, and overall 37% of patients with PNALT had significant fibrosis or inflammation; subgroup analysis showed the majority with fibrosis belonged to the high normal ALT group and that only a minority who were young and immune tolerant had significant findings on biopsy; there is significant fibrosis and inflammation in 37% of patients with PNALT and a liver biopsy should be considered in patients older than 40 with high normal ALT. Other study showed that the serum ALT and AST levels are usually elevated in patients with chronic hepatitis, although a small number of patients with histological chronic hepatitis have transiently normal aminotransferase levels (David and Daniel, 2003). Even mild elevation of aminotransferase levels (5-10 IU/L) higher than the upper limit of normal should lead the physician to consider the

presence of chronic hepatitis, and elevation of more than 400 IU/L are common in the case of untreated severe chronic viral hepatitis (Lawrence, *et al.*, 2005). In a study was done in Turkey the researchers found that serum mean ALT values were elevated three to six times and mean AST values were approximately three times elevated in chronic hepatitis B (Kursad, *et al.*, 2005). Another study was done on HCV revealed that the 15-50% of individuals with chronic hepatitis C infection had normal ALT and it was consistently higher than AST with all causes of chronic hepatic injury except alcohol; AST is normal in a significant number of cases, ALT may be normal in patients with cirrhosis, while AST remains elevated in a minority of individuals with only one elevated ALT had liver disease (Inglesby, *et al.*, 1999). Patients with slightly elevated ALT (1-2 times the upper reference limit) are more likely to have transient elevation not due to disease; however, about 30% of those with chronic HCV infection have peak ALT less than 2 times the upper reference limit. In patients with chronic HBV infection without elevated ALT “chronic carriers”, about 10% will develop increased ALT on follow-up (Dufour, 1998). Other studies were done on patients with acute hepatitis who found that serum AST and ALT values were greater than 500 IU/L, with ALT being greater than AST (Flink, *et al.*, 2006). The prevalence of elevated ALT was 28.5% in HBV, and elevation was more common in men compared to women (Chen, *et al.*, 2007). (Veldhuijzen, *et*

al., 2008) revealed that a single ALT determination can successfully predict viral load in chronic HBV patients and can be used in primary care to select patients for referral to specialist care. Some times patients with high serum HBV DNA but have a normal serum ALT activity, this occurs in cases known as (immunotolerant hepatitis B) (Wang, *et al.*, 2008).

1.19.2. Serum alkaline phosphatase (AP):

AP is an enzyme involved in metabolite transport across cell membranes and is found in a decreasing order of abundance, in placenta, ileal mucosa, kidney, bone, and liver. Alkaline phosphatase share a common protein structure, coded by the same gene (Weiss, *et al.*, 1998). Elevation of AP in the setting of liver disease results from increased synthesis and release of the enzyme into serum rather than from impaired biliary secretion and there are two primary categories of abnormal liver chemistry studies are hepatitis and cholestatic liver disease, and the cholestatic liver diseases primarily cause elevation of serum alkaline phosphatase values (Mark, *et al.*, 1998). (Whitehead, *et al.*, 1999) showed in a study done on patients with HBV, that the AP was over 3 times the upper reference limit in less than 10% of cases and about 90% of those patients had normal value. Another study was done for hepatitis revealed that the elevation of AP above normal limit was in less than 8% of cases (Doumas, *et al.*, 2004).

1.19.3. Total serum bilirubin (TSB):

Daily production of unconjugated bilirubin is 250 to 350 mg, mainly from senescent erythrocytes clearance at normal values is 5 mg/kg/day, or about 400 mg/day in adults; the rate does not increase significantly with hemolysis (Chowdhury, *et al.*, 1995). The half-life of unconjugated bilirubin is less than 5 minutes, and delta bilirubin (also sometimes termed biliprotein) is produced by reaction of conjugated bilirubin with albumin and it has a half-life of about 17-20 days, accounting for prolonged jaundice in patients recovering from hepatitis or obstruction and total bilirubin rarely exceeds (4-5 mg/dl), even during prolonged fasting; unless other factors increasing bilirubin are also present and increases in conjugated bilirubin are highly specific for disease of the liver parenchyma or bile ducts (David and Daniel, 2003). Increased conjugated bilirubin may also occur with impaired energy-dependent bilirubin excretion in sepsis, total parenteral nutrition, and following surgery; the serum bilirubin level is associated with the extent of hepatocellular necrosis, elevated serum bilirubin levels should be differentiated from cholestatic liver disease that can occur both within and outside the liver (Zhuang, 2007). Serum bilirubin rises markedly and keeps rising in patients with liver failure, and the total serum bilirubin is often greater than 10 times ULN, and the daily increment is greater than 1time ULN, and sometimes, the bilirubin diverges from the aminotransferase levels (Mark, *et al.*,

1998). Serum bilirubin level is normal (0.0-1.0 mg/dl) in chronic viral hepatitis, the frequency of jaundice in patients with acute viral hepatitis differs both by age and etiologic agent, and jaundice is rare in children than in adults and only 1% of children with acute hepatitis had peak bilirubin value over 171 mol/L (10 mg/dL), and only 27% of adults had, while in another study found that the jaundice develops in 70% of cases of acute hepatitis A (Hoofnagle, 1997). (Gitlin, 1997) in a study was done for acute hepatitis B and C viral infection revealed that jaundice develops in 33-50% of acute HBV and 20-33% of cases of acute hepatitis C. There is a direct correlation between age and peak serum bilirubin in children; an increase of 10 years in age was associated with an average increase of 85 mol/L (5 mg/dl) in bilirubin. In adults, there is no relationship between age and peak bilirubin, in addition to that the distribution of direct bilirubin as a percentage of total bilirubin is similar in acute hepatic injury and obstructive jaundice (Flink, *et al.*, 2006).

1.19.4. The immunity of patients with chronic HBV:

Viruses can kill immune cells indirectly and focus more specifically on those that are directed against their antigens; for example HBV can establish persistent infection (chronic hepatitis B infection) (William, 2003). Although this is more common in maternal-fetal transmission, in which the immature immune system becomes tolerant of the viral antigens, some adults are also incapable of completely clearing an HBV infection (Flink, *et*

al., 2006). The immunological state of the patients assessed by the following parameters:

1.19.5. Total serum immunoglobulin G (IgG):

Each IgG molecule consists of two L chains and two H chains linked by disulfide bonds (molecular formula H_2L_2); it is an important host defense against encapsulated bacteria (Geo, *et al.*, 2007). IgG is the predominant antibody in secondary responses and constitutes an important defense against bacteria and viruses, it is the only antibody to pass the placenta and is therefore the most abundant relationship between liver damage and immune regulation in HBV infection, and this study showed; in HBsAg carriers, spontaneous IgG production was elevated only in those with chronic active hepatitis whilst those with less severe inflammation had values comparable to normal, because of concanavalin A (Con A) induced suppressor cell regulation of IgG producing cells was impaired in those with chronic active hepatitis, and those with chronic persistent hepatitis, and there was a correlation in both groups of patients with the severity of portal tract inflammation. In contrast those with minimal liver damage had values in the normal range (Kayhan, *et al.*, 1986). In a study done to find the relationship between HBsAg, HBeAg and IgG it was found that the patients who were serologically negative to these markers show frequent elevation of IgG (Segovia, *et al.*, 1996). Another study revealed that the patients presenting with HBV and an elevation in IgG production due to

impaired Con A induced suppressor cell regulation of IgG production and there was no association between spontaneous IgG production or Con A induced suppressor cell function and the titles of HBsAg, e Ag status, serum IgG, immune complexes, and liver function tests (Alexander, *et al.*, 1983). Sequential study of three patients prior to the detection of HBsAg in serum and subsequently during and after acute hepatitis B showed that such abnormalities were transient and closely related to the onset of liver damage (Kayhan, *et al.*, 1986). (Voiculescu, *et al.*, 1993) revealed that in chronic HBV the immunoglobulin (IgG and IgM) will increase. Another study illustrated that if IgG concentrations were higher than 2000 mg%, indicate chronic aggressive hepatitis or cirrhosis, and exclude all other groups of HBV, thus persistent high levels of IgG following acute hepatitis indicate the development into a chronic hepatitis (Kleinschmidt and Schlicht, 1997).

1.19.6. Total serum immunoglobulin M (IgM):

IgM is the main immunoglobulin produced early in the primary immune response, IgM is present on the surface of virtually all uncommitted B cells it is composed of five H₂L₂ units (each similar to one IgG unit) and one molecule of J (joining) chain, it's the most efficient in defense against bacterial and viruses (Geo, *et al.*, 2007). A study was done on patients with chronic HBV in the United Kingdom showed that there was a

increased spontaneous IgM production due to defect in Con A induced suppressor cell function have been identified in association with chronic active hepatitis and a proportion of those with chronic persistence hepatitis, in contrast, patients with minimal liver damage have completely normal profiles (Kayhan, *et al.*, 1986). (Schwarz, *et al.*, 1988) illustrated in a study on HBV, that the IgM found rather frequently in acute hepatitis and chronic aggressive hepatitis and occasionally also in chronic persistent hepatitis, and IgM-test may be used as an easily measurable additional criteria for diagnosis and course of chronic liver diseases. Another study found that the elevation of IgG and IgA was frequently observed in healthy subjects and patients with acute viral hepatitis, liver cancer and miscellaneous liver disorders, while IgG and IgM were elevated in cirrhosis of the liver (Zuberi, *et al.*, 1997).

1.19.7. Tuberculin skin test (TST):

The tuberculin test is performed by intracutaneous injection of 5 tuberculin unit (TU); (usually 0.1ml) of purified protein derivative (PPD) and skin reaction is either positive, negative, false-positive and false-negative. The false-negative tests are due to immunosuppression (Carol, 2007). A positive skin test indicates active cell-mediated immunity, whereas a negative skin test indicates a weak cell-mediated immunity (Geo, *et al.*, 2007). (Mcglynn, *et al* 1985) revealed that in hepatitis B carriers there

are a statistically significant association between a negative tuberculin skin test and HBV replication, and this finding suggests that BCG vaccination might reduce the prevalence of infectious carriers, thereby ultimately reducing the incidence of HBV. In a previous study for the same team and the researchers found that the HBV carriers who reacted to a PPD skin test were more likely to be negative for the hepatitis B e antigen (HBeAg) than carriers who did not react to PPD, because it was not known whether the PPD reactivity was due to natural infection or vaccination with Bacilli Calmette Guerin (BCG). The researchers conducted a further study in 1985 in Alaskan Native hepatitis B carriers, a group not vaccinated with BCG, and the inverse association of HBeAg and PPD reactivity was confirmed across all age groups and was similar in magnitude to that observed in the refugee population, so if the host response to BCG is similar, BCG vaccination may be of therapeutic value in chronic hepatitis B infection (Mcglynn, *et al.*, 1987). In a study on patients with HBsAg-positive, it was shown that a person with tuberculin test negative is about 6.2 times more likely to be HBsAg-positive than those who were tuberculin test positive. Further analyzed a group whose status was known for HBV serologic markers and PPD negativity, and found the overall, a significantly greater proportion (76.2%) of HBsAg positives were PPD negative in comparison to those with anti-HBs positive (67.2%) or who had no HBV markers (43.2%) (Larke, *et al.*, 1991).

1.19.8. Total and differential leucocytes count:

The absolute amount of blood lymphocytes decreased with the increase of the age of the patients, but in chronic HBV this was accompanied by the decrease of the morphological activity of hepatitis, while in (HBV+HCV) it was accompanied by its increase, and the amount of lymphocytes rise with the increase of liver fibrosis in chronic HBV. In children with chronic hepatitis C and B + C the amount of blood lymphocytes was found to be unrelated to the morphological activity of hepatitis (Filimonov, *et al.*, 2004). (Vassilopoulos, *et al.*, 2008) revealed that the lymphocytes and monocytes will increase in patients with chronic HBV infection. Another study was found that the peripheral blood monocytes in chronic HBV infection proliferate and increase in number (Vingerhoets, *et al.*, 2000). (Craig, *et al.*, 2002) illustrated that the lymphocytes will increase while the neutrophils will decrease and no changes in total leucocytes count. (Vassilopoulos, *et al.*, 2008) revealed that the lymphocytes and monocytes will increase, while the neutrophils will decrease in patients with chronic HBV infection.

1.19.9. Erythrocytes sedimentation rate (ESR):

The ESR is a nonspecific screening test that indirectly measures how much inflammation is in the body. It is a simple and inexpensive laboratory test that is frequently used in clinical medicine (Saadeh, 1998). (Par, *et al.*, 1995) found that the ESR elevated in chronic HBV. Another study was done to find the

relationship between ESR and all types of hepatitis and was found that the high ESR were more frequent in HBA than in other types of hepatitis (Kassas, 1997).

1.19.10. Fasting blood sugar (FBS):

The study was done for HBV showed that diabetes mellitus was also significantly more frequent in chronic HBV infected patients (Kassas, 1997). (Paraschiv, *et al.*, 2002) found that the frequency of diabetes and impaired glucose tolerance in chronic liver diseases was 30% in patients with HCV infection and 17% with HBV infection who showed an altered response to a standard oral glucose tolerance test. (Kwon, 2003) found that the impaired glucose tolerance and overt diabetes mellitus (DM) frequently occurs in patients with chronic liver disease, and among the cirrhotic patients 57.1% had HBV related disease and 10% had HCV related disease, and a DM was observed in 22.4% of the cirrhotic patients; and in the diabetic group, the frequency of HCV infection was significantly greater, and only 20.6% of the diabetic patients had normal range blood glucose levels even though most of them received medical therapy. Also diabetic patients, the cases with well controlled blood glucose, showed higher survival rate than poorly controlled cases. (Arao, *et al.*, 2003) revealed that the prevalence of DM was higher in HCV-infected patients (20.9%) than in HBV-infected subjects (11.9%) and in the case-control study of the diabetic patients, 10.5% of patients were infected with type C hepatitis compared with 1.1%

with type B hepatitis. (Kwon, *et al.*, 2005) showed that the prevalence of DM was 19.7% in the HBV, and the DM was detected before the diagnosis of cirrhosis or simultaneously in 79% of HBV. (Papatheodoridis, *et al.*, 2006) found that the diabetes was present in (13%) of chronic hepatitis patients, without any difference between those with chronic hepatitis B (14%) or chronic hepatitis C (13%), and the presence of diabetes was independently associated with more severe fibrosis or presence of cirrhosis. In a previous study the researchers illustrated that the prevalence of diabetes mellitus was significantly higher in patients with HBV than in those without HBV (58.9% vs. 33.3%), and the prevalence of diabetes mellitus in patients with HBV was significantly higher than in those without HBV among Asians (65.0% vs. 27.5%) but not in Pacific Islanders (43.8% vs. 37.1%, $P=0.60$), and in patients who were tested for both HBV and HCV, the prevalence of diabetes mellitus was 29.4% in uninfected subjects, 44.4% in patients with HBV monoinfection, 47.2% in patients with HCV monoinfection and 85.0% in patients with HBV and HCV coinfection, so HBV infection is strongly associated with diabetes mellitus among Asians, Americans, but not in Pacific Islanders, whereas HCV infection was associated with diabetes mellitus in both ethnic groups. Asians have a higher prevalence of both diabetes mellitus and chronic hepatitis B infection compared to Caucasians (Ling, *et al.*, 2007). (Imazeki, *et al.*, 2008) found that the prevalence of

DM in the chronic HBV, and chronic HCV groups was 6.3%, and 13.6%, respectively. Other studies revealed that the HBV was detected in 11% of the diabetic patients and in 3% of the controls. These data suggest that the prevalence of occult HBV infection is higher in diabetics compared with healthy controls and this may contribute to the increased prevalence of primary hepatocellular carcinoma in diabetics (Demir, *et al.*, 2008).

1.20. The aim of this study:

Chronic HBV is associated with many anatomical, epidemiological, physiological, biochemical and immunological changes. Owing to the fact that insufficient informations concerning the effect of these factors on Iraqi patients are available, this study was carried out to provide insight to this question and to know some physiological changes in Iraqi patients. Such information is of no doubt necessary as a background for any programs devised in the future for studying chronic HBV, and treating this disease. Also this study will aid in gaining a better understanding of the pathogenesis of the chronic HBV, and this ultimately leads to advances in the design of drugs of choice to prevent and treat this disorder. To fulfill these objectives, the present study has dealt with samples of subjects in Babylon province to determine:

1. The epidemiology of chronic HBV.
2. AST

3. ALT.
4. AP.
5. TSB.
6. FBS.
7. The immunity of the patients which consist of the following:
 - 5.1. Total serum IgG.
 - 5.2. Total serum IgM.
 - 5.3. TST
 - 5.4. Total and differential leucocytes count
8. ESR.

الخلاصة:

أعدت هذه الدراسة لتوضيح بعض المتغيرات الفسلجية والمناعية عند المرضى المصابين بالتهاب الكبد الفيروسي نوع "ب" المزمن في محافظة بابل. لقد شملت هذه الدراسة سبعين مريضاً مصاباً بالتهاب الكبد الفيروسي نوع "ب" المزمن والتي استمرت من تشرين الثاني 2007 ولغاية أيار 2008 المكونين من (57 من الذكور) و(13 من الإناث) وكان متوسط أعمارهم (31,5 سنة) وتم مقارنة نتائجهم بالأصحاء في مجموعة السيطرة والذي كان عددهم ثلاثون شخصاً مكونين من (21 من الذكور) و(9 من الإناث) وكان متوسط أعمارهم (30,5 سنة). وقد تبين من هذه الدراسة إن الأشخاص الذين يتراوح أعمارهم بين (25-35 سنة) أكثر عرضة لظهور المرض. وكان الذكور أكثر إصابة من الإناث بنسبة 4 إلى 1 والمتزوجون أكثر من الغير متزوجين. وتبين إن هذا المرض أكثر انتشاراً عند الأشخاص ذوي الحالة المعيشية والمستوى العلمي المتدنيين وإن الإصابة أكثر بالمدن من القرى والمدخنين أكثر من الغير مدخنين. وأظهرت هذه الدراسة إن الإصابة أكثر عند الأشخاص الذين لا يتعاطون الخمر والغير مدمنين على المخدرات وعند الأشخاص الذين لديهم تاريخ عائلي للمرض.

لقد أجري للمرضى والأصحاء في مجموعة السيطرة تحليلات مختبرية في مستشفى مرجان التعليمي ومختبر الدراسات العليا لفرع الفسلجة في كلية الطب- جامعة بابل وكانت النتائج كالآتي:

إن متوسط قيمة إنزيم ناقل أمين الاسبارتات (AST) للمرضى (45,33 وحدة/لتر) وهو أعلى من مجموعة السيطرة (19,57 وحدة/لتر) وكذلك متوسط قيمة إنزيم ناقل أمين الالانين (ALT) للمرضى (49,33 وحدة/لتر) وهو أعلى قيمة من مجموعة السيطرة (16,47 وحدة/لتر). لا يوجد اختلاف مهم بين المرضى والأصحاء بالنسبة إلى الفسفاتاز القلوية (AP) وبليروبين المصل الكلي (TSB). إن متوسط قيمة سكر الدم للمرضى (8,25 ملي مول/لتر) أكثر من متوسط قيمته عند مجموعة السيطرة (4,24 ملي مول/لتر), وكذلك متوسط قيمة الغلوبولين المناعي- G (IgG)

للمرضى (18373,86 ملغم\تر) وهو أعلى من مجموعة السيطرة (7893,33 ملغم\تر) بالإضافة إلى ذلك إن متوسط قيمة الغلوبولين المناعي- M (IgM) للمرضى (3742,43 ملغم\تر) وهو أكثر من قيمته عند الأصحاء (1045 ملغم\تر). أيضا هذه الدراسة بينت انه لا يوجد اختلاف في العدد الكلي لكريات الدم البيض وعدد الخلايا القعدة والخلايا الحمضة ولكن عدد الخلايا العدلة للمرضى (3324,04 خلية\ملم³) وهي اقل من عددها عند مجموعة السيطرة (4179,23 خلية\ملم³) أما الخلايا اللمفاوية للمرضى (3263,3 خلية\ملم³) هي أكثر من متوسط عددها عند مجموعة السيطرة (2104,5 خلية\ملم³) وكذلك متوسط عدد الخلايا الوحيدة للمرضى (701,7 خلية\ملم³) أكثر من متوسط عددها عند الأصحاء (366,07 خلية\ملم³). كذلك بينت هذه الدراسة إن هنالك اختلاف مهم بين متوسط قيمة سرعة ترسيب الكريات الحمراء (ESR) عند المرضى (26,29 ملم\ساعة) وقيمته عند مجموعة السيطرة (8,87 ملم\ساعة). إن نسبة سلبية فحص الجلد للتوبركولين (TST) أعلى عند المرضى (84,3%) من مجموعة السيطرة (30%).

Acknowledgements

I pray to Allah for his help to give me power to conduct this work.

It is pleasure to express my deep appreciation to Medical College deanery of Babylon university for their assistance to apply me as postgraduate student.

I would like to express my sincere indebtedness and gratitude to my supervisors; Professor Dr. Monem Al-Shook (Chairman of Internal Medicine Department in Medical College of Babylon University) and assist. Prof. Dr. Hassan G. AL-Awady (Dean of Veterinary Medicine & Surgery College of Babylon University) for their valuable guidance during the progress of the present work.

I am greatly indebted to Dr. Mohammed Mhna (Consultant of Gastroenterology and Hepatology in Marjan Teaching Hospital) for his kind help and encouragement throughout the period of my work.

I would like to thank the Chairman of Medical Physiology Department "Dr. Abdul-Kareem AL-bermany" and other members of the department for their assistance to apply me as postgraduate student and their scientific support.

I am greatly thankful to the staff of the Gastroenterology and Hepatology center, and laboratories in Marjan Teaching Hospital for their assistance and technical support.

Samir

Summary

This study was done to illustrate some physiological and immunological parameters in chronic hepatitis B viral (HBV) infection in Babylon district. Seventy patients with chronic hepatitis B viral infection were enrolled in this study which lasted from November (2007) to May (2008), and consists of (57 males) and (13 females) with a mean age of (31.5 ± 7.8) years). Those patients were matched with thirty apparently healthy subjects (as controls), consisted of (21 males) and (9 females) with a mean age of (30.5 ± 6.7) years). The study found out that the patients with the age group between (25-35 years) were more affected than others. Males were more affected than females at a ratio of (4:1) and the married were more frequent than singles (3:1). Also this study revealed that the chronic HBV was more incidence in low economic status peoples and with low educational level. Chronic HBV was more frequent in urban areas than rural areas at a ratio of (2:1), also the prevalence of it was more in smokers than in non smokers (2:1). In addition to that, it was more in non alcoholic than alcoholic patients and no history of drugs addiction was registered. The prevalence of chronic HBV was more common in subjects with a positive family history for HBV (46:70, 65.7%) than subjects with negative family history (24:70, 34.3%). The investigations for those patients and controlled subjects were done in laboratory of Marjan Teaching Hospital, AL-Hilla Teaching Hospital and

advanced physiology laboratory in Medical College of Babylon University. The results of this study were as in the following: The mean value of aspartate aminotransferase (AST) for patients (45.33 ± 8.25 IU/L) was higher than of controls (19.57 ± 5.40 IU/L), also alanine aminotransferase (ALT) for patients (49.33 ± 8.07 IU/L) was higher than of controls (16.47 ± 4.51 IU/L). There were no significant differences between patients and controls for serum alkaline phosphatase (AP), and total serum bilirubin (TSB). The fasting blood sugar (FBS) for patients (8.25 ± 1.95 mmol/L) was more than of controls (4.24 ± 0.61 mmol/L), also the total serum immunoglobulin-G (IgG) for patients (18373.86 ± 5629.85 mg/L) was higher than of controls (7893.33 ± 2589.09 mg/L), the total serum immunoglobulin-M (IgM) for patients (3742.43 ± 1444.33 mg/L) was more than in controls (1045.00 ± 611.06 mg/L). There were no significant differences in the total numbers of leucocytes, eosinophils, basophils, while the mean numbers of neutrophils for patients (3324.04 ± 844.86 cells/mm³) was less than for controls (4179.23 ± 856.47 cells/mm³). The mean numbers of lymphocytes for patients (3263.30 ± 698.00 cells/mm³) was more than controls (2104.50 ± 473.46 cells/mm³) and the mean numbers of monocytes for patients (701.70 ± 192.77 cells/mm³) was higher than controls (366.07 ± 97.76 cells/mm³). This study revealed a significant difference in the mean values of erythrocyte sedimentation rate (ESR) for patients (26.29 ± 5.51 mm/hr) and controls (8.87 ± 3.95

mm/hr). The present study illustrated that the incidence of negativity of tuberculin skin test (TST) was higher in patients (84.3%) than in controls (30%).

List of Contents

Subjects	Pages
Chapter one: Introduction and review of literatures.	1
1.1. Background	1
1.2. Liver anatomy	1
1.3. Liver structures	2
1.4. Major hepatic functions	3
1.4.1. Metabolism of carbohydrates, protein and fats	3
1.4.2. Metabolism of bilirubin	4
1.4.3. Storage and metabolism of vitamins and minerals	5
1.4.4. Synthesis of plasma proteins	5
1.4.5. Detoxification function	5
1.5. Regulation of liver function	6
1.6. Hepatitis in general	6
1.6.1. Pathology of hepatitis	6
1.6.2. The causes of hepatitis	7
1.7. Hepatitis A virus (HAV)	9

1.8. Hepatitis D virus (HDV)	10
1.9. Hepatitis C virus (HCV)	11
1.10. Hepatitis E virus (HEV)	11
1.11. Hepatitis G virus (HGV)	12
1.12. TT virus (TTV)	12
1.13. Hepatitis B virus (HBV)	13
1.13.1. Over view	13
1.13.2. Virus-host interaction	19
1.14. Hepatic injuries	19
1.14.1. Acute hepatic injury	19
1.14.2. Chronic hepatic injury	20
1.15. Stages of chronic HBV infection	22
1.15.1. Immunetolerant phase	23
1.15.2. Chronic hepatitis B phase or immune clearance phase	23
1.15.3. Inactive hepatitis B carrier phase	23
1.15.4. The immune response and the carrier state	24
1.16. Epidemiology of chronic HBV	25
1.17. Clinical features and natural history of chronic HBV	27

1.18. Serology of chronic HBV	27
1.19. Investigation of chronic HBV	28
1.19.1. Serum aminotransferase	28
1.19.2. Serum alkaline phosphatase (AP)	32
1.19.3. Total serum bilirubin (TSB)	33
1.19.4. The immunity of patients with chronic HBV	34
1.19.5. Total serum immunoglobulin G (IgG)	35
1.19.6. Total serum immunoglobulin M (IgM)	36
1.19.7. Tuberculin skin test (TST)	37
1.19.8. Total and differential leucocytes count	39
1.19.9. Erythrocytes sedimentation rate (ESR)	39
1.19.10. Fasting blood sugar (FBS)	40
1.20. The aim of this study	42
Chapter two: Materials and methods.	44
A. Materials	44
1. Subjects of the study	44
1.1. Patients	44
1.2. Controls	44
2. Instruments	45

3. Chemicals	47
B. Methods	48
1. Blood collection	48
2. Hematological studies	49
2.1. Total leucocytes count	49
2.2. Differential leukocytes count (DLC)	49
2.3. Measurement of ESR	50
3. Biochemical's analysis	50
3.1. Determination of AST	50
3.2. Determination of ALT:	51
3.3. Determination of AP	51
3.4. Determination of TSB	51
3.5. Determination of FBS	51
4. Immunological studies	52
4. 1 Measurement of total serum IgG and IgM	52
4.2. Tuberculin skin test	52
5. Serological study	53
5.1. Determination of HBsAg	53
5.2. Determination of HCV	53

C. Statistical analysis	53
Chapter Three: The results.	55
3.1. The age distribution	55
3.2. The gender influence	55
3.3. The marital status	55
3.4. The economic status	55
3.5. Residency	56
3.6. Educational levels	56
3.7. History of smoking	56
3.8. Alcohol intake	56
3.9. Drugs addiction	56
3.10. Family history of HBV	58
3.11. AST	59
3.12. ALT	59
3.13. AP	60
3.14. TSB	61
3.15. FBS	61
3.16. Total serum IgG	62
3.17. Total serum IgM	63

3.18. Total leucocytes count	63
3.19. Leucocytes differential count	64
1. Neutrophils count	64
2. Lymphocytes count	64
3. Monocytes count	65
4.. Eosinophils count	65
5. Basophils count	65
3.24. ESR	67
3.25. Evaluation of tuberculin test	67
Chapter Four: Discussion.	69
4.1. The age distribution	69
4.2.The gender influence	69
4.3. The economic status	70
4.4. Residency	71
4.5. Educational levels	71
4.6. History of smoking	72
4.7. Alcohol intake and Drugs addiction	72
4.8. Family history of HBV	72

4.9. AST and ALT	73
4.10. AP	75
4.11. TSB	76
4.12. FBS	76
4.13. Total serum IgG	79
4.14. Total serum IgM	80
4.15. Total leucocytes and differential count	81
4.16. ESR	83
4.17. Tuberculin skin test	84
4.20. Conclusion	86
4.21. Recommendation	87
Chapter five: References.	88

List of Tables

Tables	Pages
Table (2-1): Tools used in this study and their sources	45
Table (2-2): Instruments used throughout the study and their sources.	46
Table (2-3): The chemical and biological materials used throughout the study and their sources.	47
Table (3-1): Distribution of patients and control group by (mean age, gender, residency, marital status, economical status, educational level, history of smoking, and alcohol intake).	57
Table (3-2): Distribution of patients and control group by (family history for HBV).	58
Table (3-3): Mean values of AST (IU/L) for patients and control group.	59
Table (3-4): Mean values of ALT (IU/L) for patients and control group.	60
Table (3-5): Mean values of AP (IU/L) for patients and control group.	60
Table (3-6): Mean values of TSB ($\mu\text{mol/L}$) for patients and control group.	61
Table (3-7): Mean values of FBS (mmol/L) for patients and control group.	62

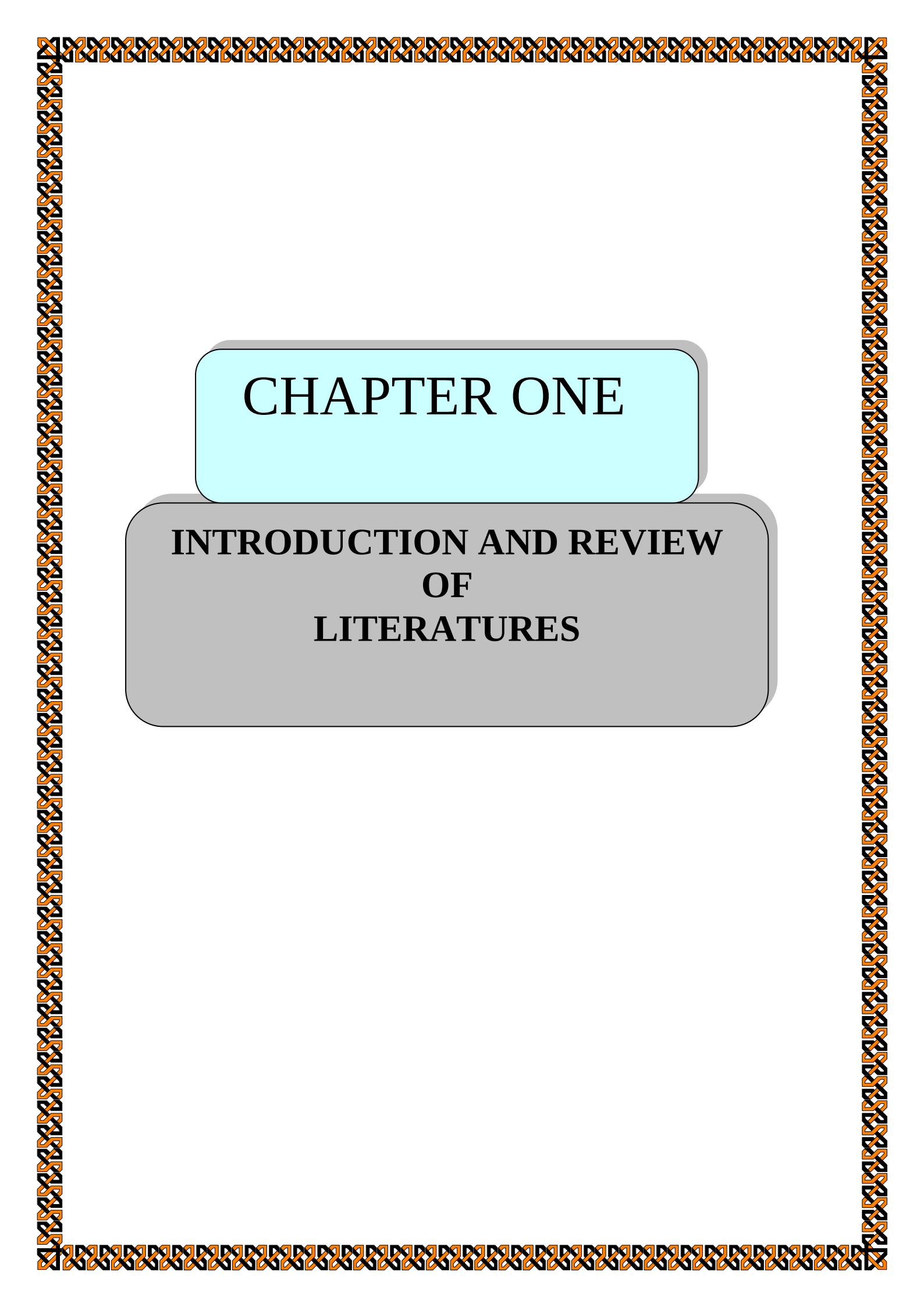
Table (3-8): Mean values of IgG (mg/L) for patients and control group.	62
Table (3-9): Mean values of IgM (mg/L) for patients and control.	63
Table (3-10): Mean numbers of total leucocytes (cells/mm ³) for patients and control group.	64
Table (3-11): Mean numbers of leucocytes differential count (cells/mm ³) for patients and control group.	66
Table (3-12): Mean values of ESR (mm/hr) for patients and control group.	67
Table (3-13): Distribution of patients and control group by (response to tuberculin test (PPD)).	68

List of Abbreviations

ALT	Alanine aminotransferase
ALTPN	Alanine aminotransferase persistently normal
AP	Alkaline Phosphatase
AST	Aspartate aminotransferase
BCG	Bacilli Calmette-Guérin
cccDNA	Covalently closed circular DNA
CMI	Cell mediated immunity
Con-A	Concanavalin-A
dl	Deciliter
DLC	Differential leukocytes count
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ESR	Erythrocytes sedimentation rate
FBS	Fasting blood sugar
HAV	Hepatitis A virus
HBe-Ag	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen

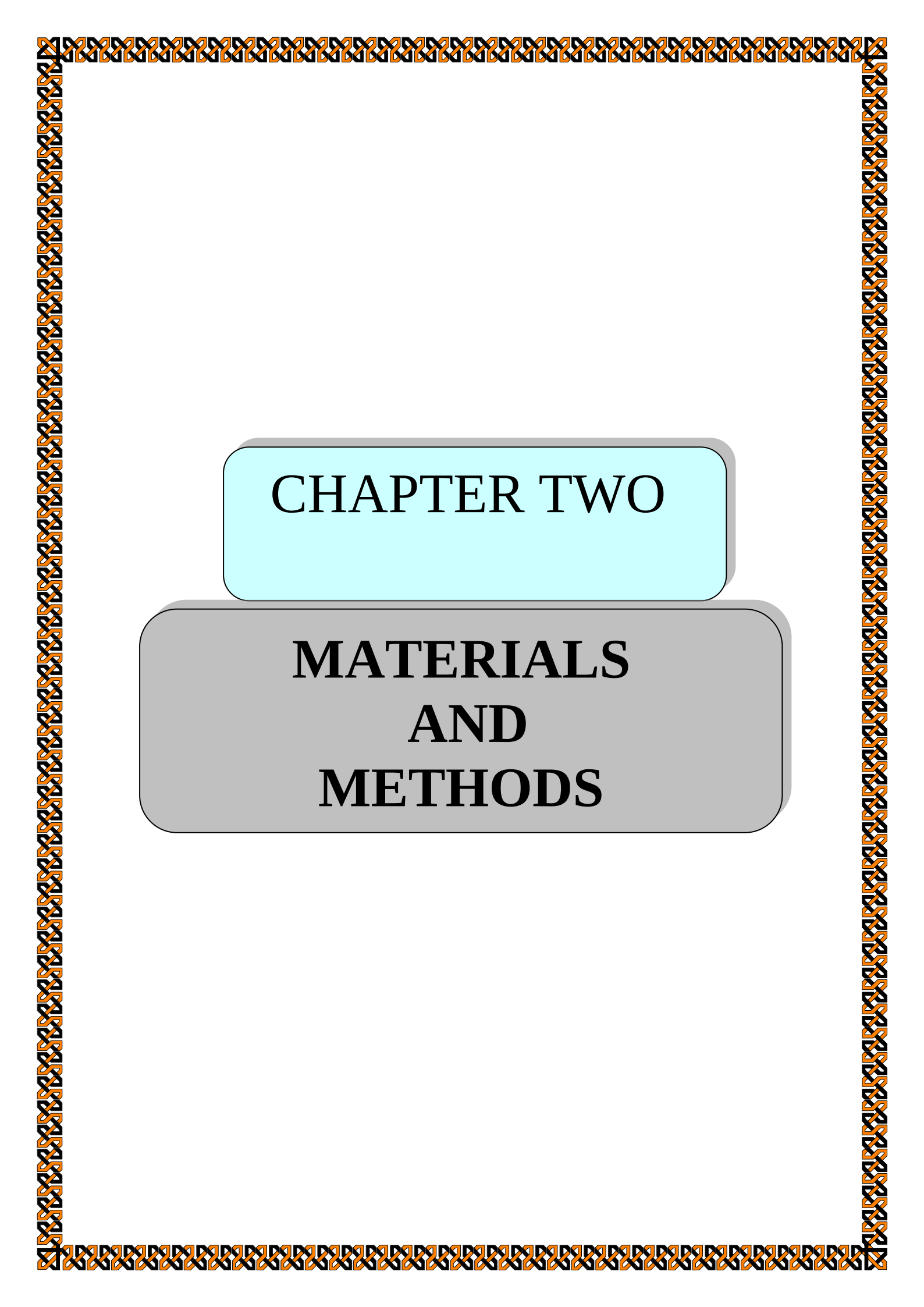
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HDV-Ag	Hepatitis delta antigen
HEV	Hepatitis E virus
HGV	Hepatitis G virus
IFN- δ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU	International unit
Kg	Kilogram
L	Liter
mg	Milligram
mm ³	Cubic millimeter
μ mol	Micromole
mmol	Millimole
PPD	Purified protein derivative
RNA	Ribonucleic acid

S. D.	Standard deviation
TLC	Total leukocytes count
TNF	Tumor necrosis factor
TSB	Total serum bilirubin
TST	Tuberculin skin test
TTV	TT virus
TU	Tuberculin unit
UK	United Kingdom
ULN	Upper limit of normal
US	United States



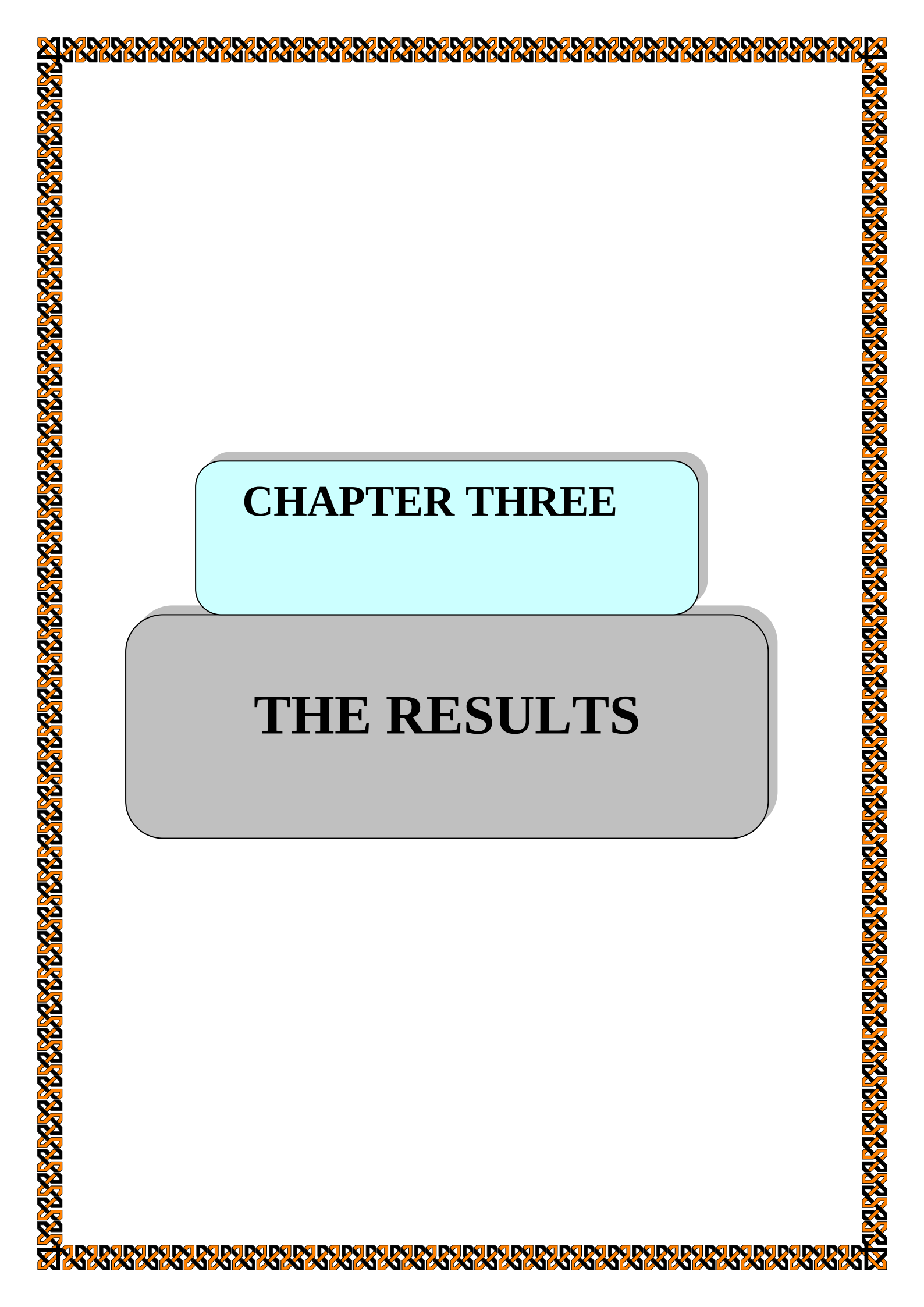
CHAPTER ONE

INTRODUCTION AND REVIEW OF LITERATURES



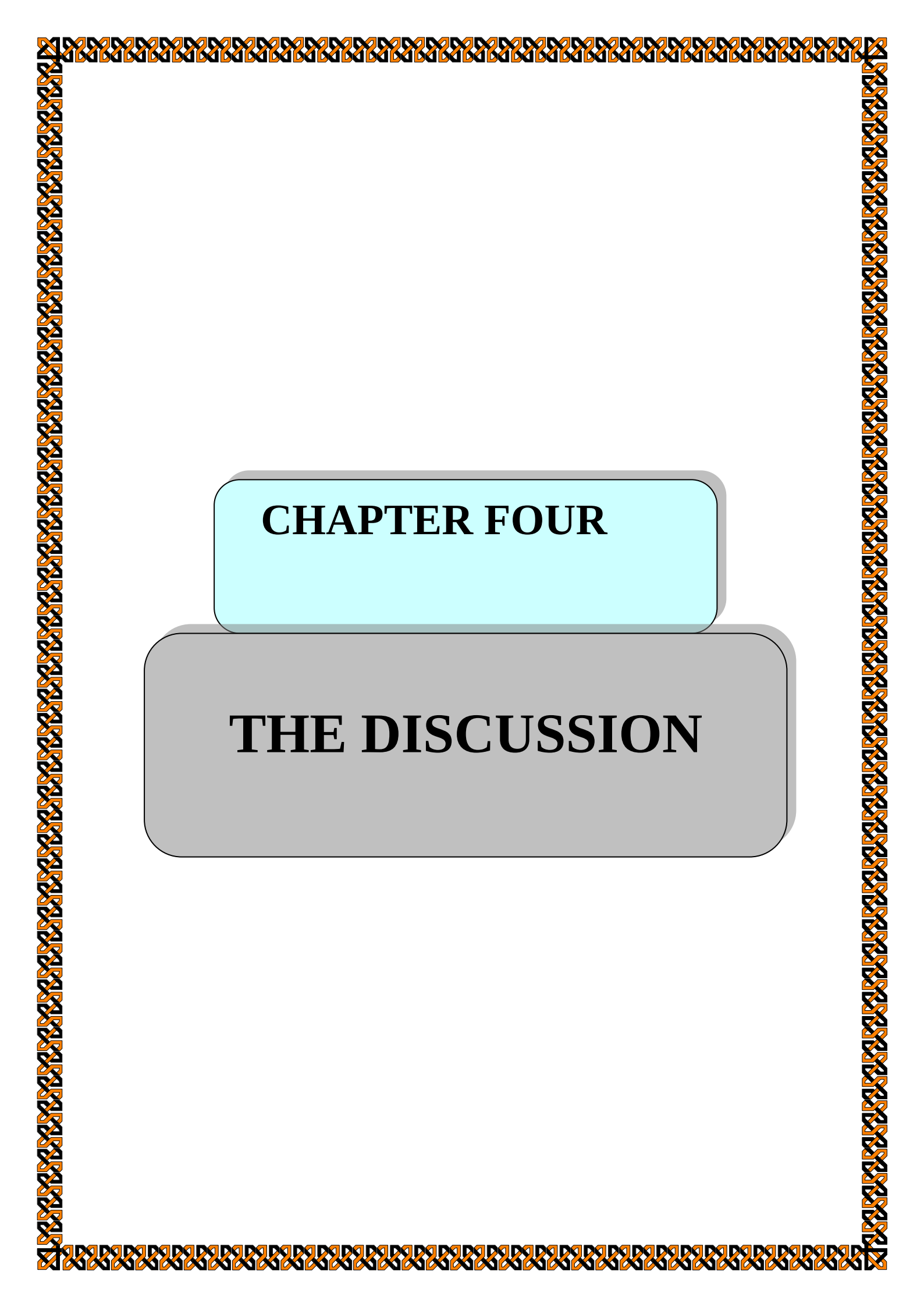
CHAPTER TWO

MATERIALS AND METHODS



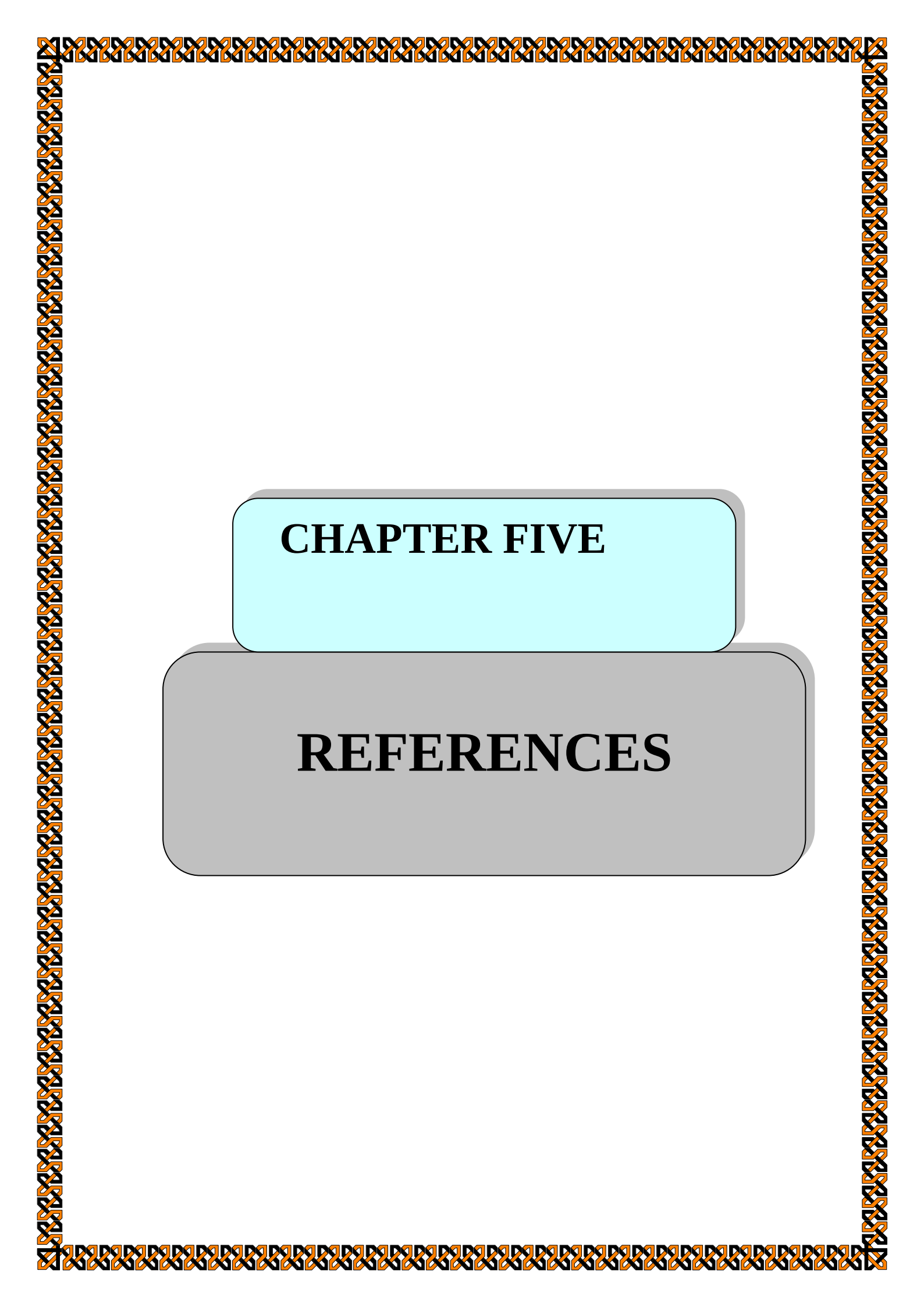
CHAPTER THREE

THE RESULTS



CHAPTER FOUR

THE DISCUSSION



CHAPTER FIVE

REFERENCES

We, certify that this thesis titled "*Some Physiological and Immunological Parameters Associated with Chronic Hepatitis -B- Virus Infection in Babylon Province*", was carried out under our supervision at the Babylon University as partial requirement for the degree of "*Master of Science in Medical Physiology*".

Prof. Dr.

Monem AL-Shook
C.A.B.M.S./Medicine
College of Medicine
Babylon University
Supervisor

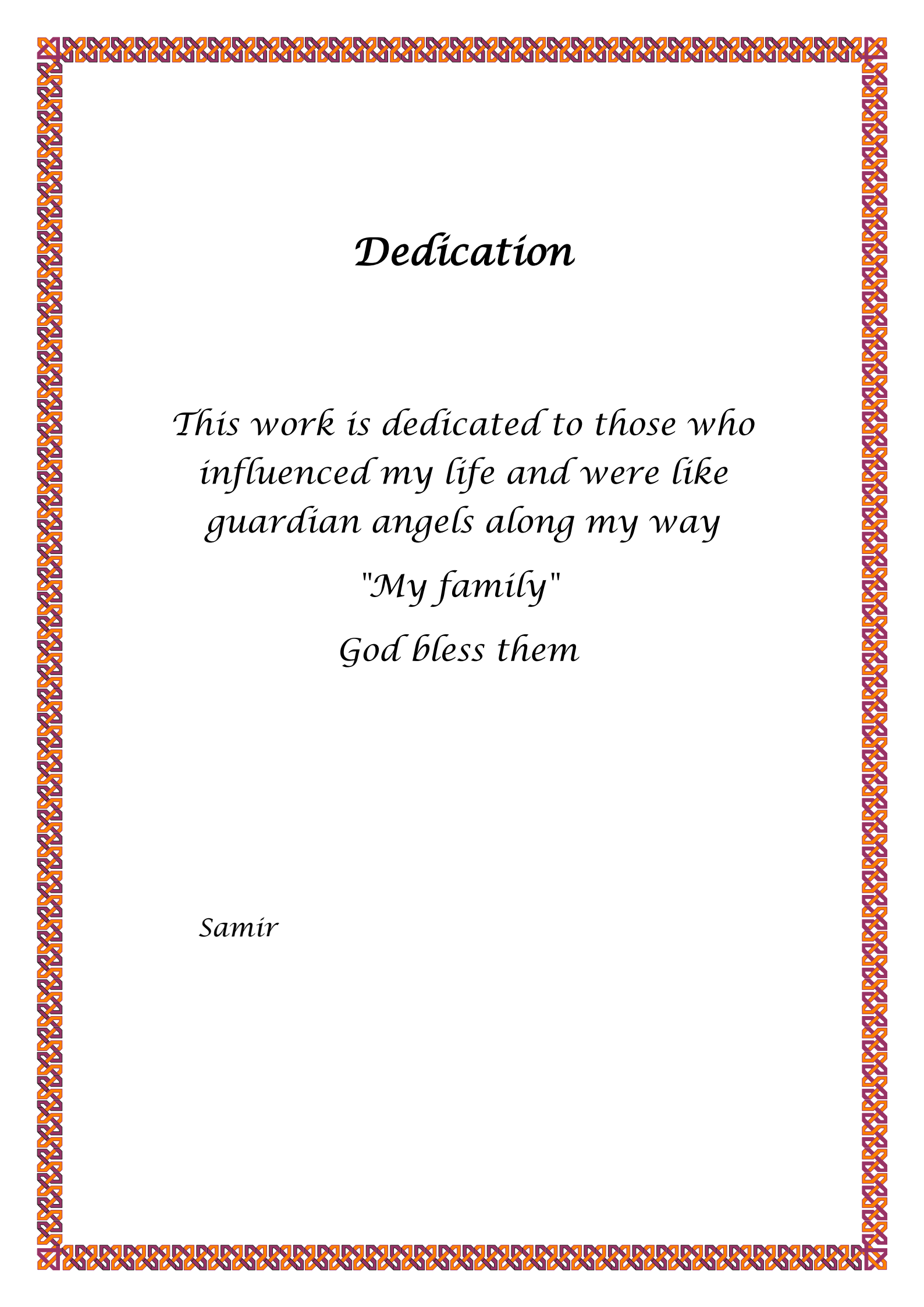
Assist. Prof. Dr.

Hassan G. AL-Awady
PH.D./Medical Physiology
College of Veterinary Medicine & Surgery
Babylon University
Supervisor

In view of the available recommendations. I put forward this thesis for debate by the examining committee.

Assist. Prof. Dr. Abdul-Kareem AL-bermany

Chairman of Medical Physiology & Physics Department
College of Medicine
Babylon University



Dedication

*This work is dedicated to those who
influenced my life and were like
guardian angels along my way*

"My family"

God bless them

Samir

We, the "*Examination Committee*", certify that we have read this thesis entitled "*Some Physiological and Immunological Parameters Associated with Chronic Hepatitis -B- Virus Infection in Babylon Province*", and have examined the student "*Samir Swadi AL-Jubory*" in it's contents, and that in our opinion it's adequate with "*Excellent Estimation*" as a thesis for the degree of "*Master of Science in Medical Physiology*".

Prof. Dr. Karim Hamid Rashid
PH.D./ Animal Physiology
Chairman

Assist. Prof. Dr.
Naseer J. AL-Mukhatar
PH.D./ Medical Physiology
Member

Assist. Prof. Dr.
Ahmed AL-Mayalli
F.I.C.M.S.
Member

Prof. Dr.
Monem AL-Shook
C.A.B.M.S.
Member - Supervisor

Assist. Prof. Dr.
Hassan G. AL-Awady
PH.D./Medical Physiology
Member - Supervisor

Approve for the College Committee on Graduate Studies

Assist. Prof. Dr. Ali K. AL-Shaaeli
Dean
College of Medicine
Babylon University

4.1. The age distribution:

The patients enrolled in this study were divided into three age groups: first group with age less than 25 years old with a mean of (19 ± 5.2) , second age group between 25-35 years old with a mean of (30.7 ± 4.14) and third age group more than 35 years old with a mean of (45.9 ± 5.3) . 9 patients were in group 1, 41 were in group 2 and 20 were in group 3. That means the first presentation of patients with chronic HBV was in age group between 25-35 years with a mean of 31. This study agrees with other study results that revealed that the time of initial presentation with chronic hepatitis B ranges was between 24-36 years a mean of 31 years (Yuen, *et al.*, 2001). In addition, the results of present study were similar to those done in Turkey by (Kursad, *et al.*, 2005) who found that the mean age of chronic HBV patients were 31 ± 9 years. The age of infection is mainly at birth time by the vertical transmission (from mother to baby), but the time of presentation between 25 to 35 years old is because the patients are asymptomatic and discovered accidentally by routine investigation during blood donation, married or employment and all of them at this age group.

4.2. The gender influence:

In this study the males to females ratio was about 4:1. Meaning that the males are more affected with chronic HBV than females. The study under discussion agrees with other study that

has revealed the males to females ratio ranging from (4.9:1.5) (Yuen, *et al.*, 2001). It is also similar to other study that illustrated that the chronic HBV infection was 2.9 times more frequent in males than in females (Kursad, *et al.*, 2005). (Mistik and Balik, 2001) found the chronic HBV infection was three times more frequent in males than in females. Also (Dennis, *et al.*, 2005) and (Alexander, *et al.*, 2007) found that men predominate women in all populations of HBsAg carriers. The gender is well established but poorly understood, determinant of chronicity; however women are more likely than men to clear HBsAg (David and Daniel, 2003).

4.3. The economic status:

This study revealed that, the chronic HBV is more frequent in low economic status than the other groups enrolled in this study. The ratio of good, medium, and low economic status was about (1:1:3) respectively. The present study agrees with other study done by (Mistik, *et al.*, 2001) who found a lower socioeconomical level and less hygienic living conditions are more susceptible to chronic HBV than the others. Also it is similar to results of other study was done in Japan by (Dennis, *et al.*, 2005) who showed that the prevalence of HBV is higher in lower socioeconomic state. Chronic HBV infection begins when the immune response that normally clears the infection fails to take place or is too weak to be effective; thus, infections are

more common in low immunity subjects as a result of poverty (Hoofnagle, *et al.*, 1997).

4.4. Residency:

The incidence of chronic HBV is more frequent in urban areas than in the rural areas with a ratio of (2:1) respectively, and by statistical analysis there are significant ($P < 0.05$). This finding is may be, due to the health education in the urban is better than in rural areas, which leads to early discovery of this disease.

4.5. Educational levels:

This study found that, the subjects with low educational level are more affected than those with higher educational level at ratio of (6:1) respectively. The present study agrees with other study that revealed the people who have lower educational level were more affected than the higher educational level (Mistik, *et al.*, 2001). The results of this study under discussion agree with the results obtained by other study was done in Turkey by (Kursad, *et al.*, 2005), who they found that the incidence of HBV infection is more common in low educational level than the other, and improvement of socioeconomical and educational status are the cornerstones in the prevention of liver morbidity and mortality in Eastern Turkey. The mode of transmission is mainly by (homosexual and drugs abuse), and both of them are behaviors of low educational level and that explain, why the HBV is more common in this community groups .

4.6. History of smoking:

The prevalence of chronic HBV in this study is more in smokers than in non smokers patients with a ratio of (about 2:1). This study is in agreement with other study who found that the incidence of chronic HBV is more in smokers than nonsmokers, because smoking alter the immune system (Dennis, *et al.*, 2005).

4.7. Alcohol intake and drugs addiction:

The prevalence of chronic HBV in this study is significantly ($P < 0.05$) more in non alcoholic than the alcoholic patients with a ratio of (about 6:1). Also there are no history of drugs addiction to any patients enrolled in this study, and this finding disagrees with (Alexander, *et al.*, 2007), and (Beasley, *et al.*, 1983) who illustrated that the prevalence of type B hepatitis increased among drinkers and drug addicts. (Harpaz, *et al.*, 1996) mentioned that, the risk of progression to chronic liver disease depends on the source of infection and in drugs addiction the risk of chronic infection is 10%. No explanation to this deference, but may be due to the patients denied of alcohol intake and drugs addiction.

4.8. Family history of HBV:

A careful family history for HBV from patients and control subjects was taken, it was found that the prevalence of chronic HBV more common in subjects with positive family history for HBV (46:70, 65.7%) than in the subjects with negative family

history (24:70, 34.3%), in comparison with control group only (4:30, 13%) with positive family history, while the majority with negative family history for HBV (26:30, 87%). This study correlates with the results of (Degertekin, 2001) who reported that intrafamilial transmission rate of HBV infection has been reported as 65% in Eastern and Southeastern Anatolia. (Vinay, *et al.*, 2005) revealed that, in endemic regions such as Africa and Asia, spread from an infected mother to a neonate during birth (vertical transmission) is common, and these neonatal infection often leads to the carrier state for life and this may explain why more than one member in the same family have a chronic HBV.

4.9. AST and ALT:

The mean values of AST for patients enrolled in this study is (45.33 ± 8.25 U/L), while in control group it is (19.57 ± 5.40 U/L), and the mean values of ALT for patients is (49.33 ± 8.07 U/L), while for control group it is (16.47 ± 4.51 U/L). The results of present study indicated that, the aminotransferase enzymes will mildly elevate in chronic HBV infection and this finding is consisted with other study done by (Olut, *et al.*, 2007), who found that in chronic HBV infection and during the one year period of study 89% of the patients yielded continuously normal ALT levels, while 11% showed at least one ALT value above the normal levels (ALT more than 1.2 time of normal value). (Myers, *et al.*, 2003) revealed that, the serum ALT levels are

constantly normal in 57.4% of patients were HBV-DNA positive and above the normal in 42.6% of the patients. This study is in agreement with other a prospective study done by (Yu, *et al.*, 2005); who showed that the ALT and AST levels in chronic HBV infection was about 60 IU/L for the testing hospital before or at enrollment. (Lai, *et al.*, 2007) revealed in a study done on the chronic HBV, the incidence of ALT values increase with increment hepatic fibrosis, and a liver biopsy should be considered in patients with high normal ALT. Another study showed that the serum ALT and AST levels are usually elevated in patients with chronic hepatitis, although a small number of patients with histological chronic hepatitis have transiently normal aminotransferase levels (David and Daniel, 2003). Also the results of the present study are similar to the study was done in Turkey and the researchers founds that serum mean ALT values were three to six times higher and the mean AST values were approximately three times higher in chronic hepatitis B (Kursad, *et al.*, 2005). (Bonacini, *et al.*, 1997) who are reported that the ALT is consistently higher than AST with chronic hepatic injury and these results are the same as in present study results. A minority of individuals with only one elevated ALT were found to have liver disease, while in patients with chronic HBV infection without elevated ALT “chronic carriers”, approximately 10% developed increased ALT (Kundrotas and

Clement, 1993). (Veldhuijzen, *et al.*, 2008) revealed that a single ALT determination can successfully predict viral load in chronic HBV patients and can be used in primary care to select patients for referral to specialist care. Sometimes patients with high serum HBV DNA can have a normal serum ALT activity. This occurs in cases known as immunotolerant HBV (Wang, *et al.*, 2008). Aminotransferases are normally intracellular enzymes (mainly hepatic cells), and low levels found in the plasma represent the release of cellular contents during normal cells turnover, so elevation of plasma aminotransferases level indicates damage to cells rich in these enzymes, such as viral hepatitis (Pamela, *et al.*, 2005).

4.10. AP:

The present study revealed that no significant differences in AP between patients and control group, and the mean values of AP for patients was (51.34±12.03 U/L), and for control group (50.43±9.99 U/L). In other words the elevation of AP in chronic HBV is unusual. This finding is in agreement with (Whitehead, *et al.*, 1999); that showed elevation of AP above reference limit in less than 10% of examined cases and about 90% of those patients are of normal values. Another study done for uncomplicated hepatitis revealed that the elevation of AP above normal limit is present in less than 8% of examined cases (Doumas, *et al.*, 2004). The mild elevation of serum AP in chronic HBV infection

showed no significant statistical difference (Mark, *et al.*, 1998) and (Vinay, *et al.*, 2005).

4.11. TSB:

This study revealed that there were no significant statistical differences in mean values of TSB for patients ($11.71 \pm 3.89 \mu\text{mol/L}$), and control group ($10.43 \pm 3.99 \mu\text{mol/L}$), and this finding is consistent with (David and Daniel, 2003), who mentioned that, the serum bilirubin level is normal in chronic viral hepatitis. This finding indicate that the TSB didn't increase in chronic HBV infection which is different from an acute hepatitis B infection (when the jaundice develops in 33-50% of cases) as illustrated by (Gitlin, 1997). The bilirubin is metabolized in the liver and secreted via biliary ducts, so any defect in hepatocytes lead to increase in TSB, but in chronic HBV infection it is mildly elevated, as mentioned by (Vinay, *et al.*, 2005), that is why no statistical difference between patients and controls was noticed.

4.12. FBS:

This study revealed that there is a significant difference between the mean values of FBS for patients ($8.25 \pm 1.95 \text{ mmol/L}$), and control group, ($4.24 \pm 0.61 \text{ mmol/L}$), which is in agreement with that mentioned by (Kassas, 1998) in which diabetes mellitus was significantly more frequent in chronic HBV cases. The present study was similar in it is finding to another study done by (Kwon, 2003) who found that the impaired glucose

tolerance and overt DM frequently occurs in patients with chronic liver disease, and among the cirrhotic patients 57.1% had HBV related disease, and a DM was observed in 22.4% of the cirrhotic patients; and only 20.6% of the diabetic patients had normal range of blood glucose levels even though most of them received medical therapy. Also diabetic patients with well controlled blood glucose showed higher survival rate than poorly controlled cases. (Paraschiv, *et al.*, 2002) found that the frequency of diabetes and impaired glucose tolerance in chronic liver diseases was 30% in patients with HBV infection who showed an altered responds to a standard oral glucose tolerance test which was investigated in order to evaluate their pancreatic-endocrine features. The results of this study are in agreement with those obtained by (Ling, *et al.*, 2007) who found that the prevalence of diabetes mellitus was significantly higher in patients with HBV than in those without HBV (58.9% vs. 33.3%), and the prevalence of diabetes mellitus in patients with HBV was significantly higher than in those without HBV among Asians (65.0% vs. 27.5%) but not in Pacific Islanders (43.8% vs. 37.1%), and in patients who were tested for HBV, the prevalence of diabetes mellitus was 44.4% in patients with HBV, and 85.0% in patients with HBV and HCV coinfection, so HBV infection is strongly associated with diabetes mellitus. The present study is also in agreement with the following studies: (Papatheodoridis, *et al.*, 2006) found that the

diabetes was present in (13%) of chronic hepatitis patients, without any difference between those with chronic hepatitis B (14%) or chronic hepatitis C (13%), and the presence of diabetes was independently associated with more severe fibrosis or presence of cirrhosis. (Imazeki, *et al.*, 2008) found that the prevalence of DM in the chronic HBV, and chronic HCV groups was 6.3%, and 13.6%, respectively. (Kwon, *et al.*, 2005) showed that the prevalence of DM was 19.7% in the HBV, and the DM would be detected before the diagnosis of cirrhosis or simultaneously in 79% of HBV. (Demir, *et al.*, 2008) found that the HBV was detected in 11% of the diabetic patients and in 3% of the controls, these data suggest that the prevalence of occult HBV infection is higher in diabetics compared with healthy controls and this may contribute to the increased prevalence of primary hepatocellular carcinoma in diabetic patients. The explanation of these results as mentioned by (Kwon, 2003) is that hyperinsulinaemia and peripheral insulin resistance contribute to the development of DM in these patients. Patients with HBV infection showed signs of enhanced insulin resistance but overt diabetes develops in those who, in addition to insulin insensitivity, have a relative defect of insulin secretion which is the main mechanism leading to both glucose intolerance and overt diabetes (Paraschiv, *et al.*, 2002).

4.13. Total serum IgG:

This study it was found that the mean values of total serum IgG for patients is (18373.86 ± 5629.85 mg/L), while in control group it is (7893.33 ± 2589.09 mg/L). that means, the titer of IgG increased in chronic HBV infection and the results of the present study are in agreement with those obtained by (Alexander, *et al.*, 1983) who found that, elevation in IgG production occurs in patients with chronic HBV infection and explained that due to impaired concanavalin A (Con A) induced suppressor it cell regulation of IgG production. In addition to that, results of this study are consistent with the results of other studies was illustrated that, if IgG concentration was higher than 2000 mg/l it would indicate chronic aggressive hepatitis or cirrhosis has occurred, and exclude all other groups of HBV, thus persistent high levels of IgG following acute hepatitis indicate the development into a chronic hepatitis (Kleinschmidt and Schlicht, 1997). (Segovia, *et al.*, 1996) mentioned in their study that there is a relationship between HBsAg, HBeAg and IgG; and found that those patients who were serologically negative to these markers showed frequent elevation of IgG. (Par, *et al.*, 1995) illustrated that the IgG elevated in chronic HBV. (Voiculescu, *et al.*, 1993) revealed that in chronic HBV the immunoglobulin (IgG and IgM) would increase. Sequential study of three patients prior

to the detection of HBsAg in serum and subsequently during and after acute hepatitis B showed that such abnormalities were transient and closely related to the onset of liver damage (Kayhan, *et al.*, 1986). Explanation of these results as mentioned by the researchers is that spontaneous IgG production was elevated in patients with chronic active hepatitis while those with less severe inflammation had values comparable to normal, because of Con A-induced suppressor cell regulation of IgG producing cells was impaired in those with chronic active hepatitis, and those with chronic persistent hepatitis, and there was a correlation in both groups of patients with the severity of portal tract inflammation, in contrast to those with minimal liver damage who had values in the normal range (Kayhan, *et al.*, 1986).

4.14. Total serum IgM:

The present study revealed that the mean values of total serum IgM for patients is (3742.43±1444.33 mg/L), while in control group it is (1045.00±611.06 mg/L), i.e. the total serum IgM increased in patients with chronic HBV infection and the current study compatible with another study done for patients with chronic HBV in the United Kingdom, and found that, the increase spontaneous IgM production in association with chronic active hepatitis and a proportion of those with chronic persistence hepatitis. In contrast, patients with minimal liver damage have

completely normal profiles (Kayhan, *et al.*, 1986). In addition to that, the present study is in agreement with (Schwarz, *et al.*, 1988) who illustrated in a study done for HBV, that the IgM was found rather frequently in acute hepatitis and chronic aggressive hepatitis and occasionally also in chronic persistent hepatitis, and IgM-test may be used as an easily measurable additional criteria for diagnosis and course of chronic liver diseases. (Zuberi, *et al.*, 1997) found that the elevation of IgG and IgA were frequently observed in healthy subjects and patients with acute viral hepatitis, liver cancer and miscellaneous liver disorders, whereas IgG and IgM were elevated in cirrhosis of the liver. Elevation of IgM in 35% chronic aggressive hepatitis is believed to be an atavistic primary IgM response, occurring in the case of elevated humoral immune reactions and disorders of lymphatic tissues (Schwarz and Scheurlen, 1995). The finding occurs due to defective in Con A-induced suppressor cell regulation of IgM producing cells was impaired in those with chronic hepatitis (Kayhan, *et al.*, 1986, Schwarz, *et al.*, 1988 and Zuberi, *et al.*, 1997).

4.15. Total leucocytes and differential count:

This study revealed the following:

- No significant difference in the mean numbers of total leucocytes for patients (7462.86 ± 1607.70 cells/mm³), and controls (6836.67 ± 1422.84 cells/mm³).

- The mean numbers of neutrophils in patients was (3324.04 ± 844.86 cells/mm³) which is less than in controls (4179.23 ± 856.47 cells/mm³).
- There are increase in the mean numbers of lymphocytes for patients (3263.30 ± 698.00 cells/mm³) compared to controls (2104.50 ± 473.46 cells/mm³).
- This study revealed a significant difference in the mean numbers of monocytes for patients (701.70 ± 192.77 cells/mm³) and controls (366.07 ± 97.76 cells/mm³).
- There is no significant differences in the mean numbers of eosinophils for patients (110.41 ± 68.14 cells/mm³) and control group (119.07 ± 64.32 cells/mm³).
- Also there is no significant differences in the mean numbers of basophils for patients (63.40 ± 46.84 cells/mm³), and control group (67.80 ± 47.83 cells/mm³).

The results of this study are in agreement with other studies which found that, the number of lymphocytes rise while the number of neutrophils decrease with the increase of liver fibrosis in chronic HBV (Filimonov, *et al.*, 2004). (Vingerhoets, *et al.*, 2000) found that the peripheral blood monocytes in chronic HBV infection proliferate and increase in number. Another study was done for patients with chronic HBV and showed that the lymphocytes will increase while the neutrophils will decrease and no changes in total leucocytes count (Craig, *et al.*, 2002).

(Vassilopoulos, *et al.*, 2008) revealed that the lymphocytes and monocytes will increase, while the neutrophils will decrease in patients with chronic HBV. However mild increment in total leucocytes count showed no significant difference between patients and control group in statistical analysis, the lymphocytes will increase in viral infection due to activation of lymphocytes proliferation, while monocytosis occur as a result of chronic infection and decrement in neutrophils number due to increment in lymphocytes number upon computation of neutrophils, while the eosinophils and basophils have no value in viral infection.

4.16. ESR:

This study revealed that there is a significant difference in the mean values of ESR for patients (26.29 ± 5.51 mm/hr) and control group (8.87 ± 3.95 mm/hr). Meaning that the ESR elevated in patients with chronic HBV infection, and this finding is similar to that by (Par, *et al.*, 1995) who found that the ESR elevated in chronic HBV. Also the current study is consistent with another study done to find the relationship between ESR and all types of hepatitis and the researchers found that the high ESR were more frequent in HBV than in other types of hepatitis (Kassas, 1997). Explanation of this finding is that, the ESR is a nonspecific screening test that indirectly measures how much inflammation is in the body (Saadeh, 1998). The amount of fibrinogen and

immunoglobulin (specially IgG) in the blood directly correlates with the ESR, so in patients with chronic HBV, the ESR will increase due to increment in total serum IgG (Veldhuijzen, *et al.*, 2008).

4.17. Tuberculin skin test:

The present study illustrated that the incidence of negative tuberculin test is higher in patients with chronic HBV (84.3%) than in the subjects of control group (30%). The finding of the present study is similar to a study done by (Larke, *et al.*, 1991) who showed that, a person with tuberculin test negative is about (6.2 times) more likely to be HBsAg-positive than those who were tuberculin test positive, and further analyzed a group whose status was known for HBV serologic markers and PPD negativity, and found that a significantly greater proportion (76.2%) of HBsAg positives were PPD negative in comparison to those with anti-HBs positive (67.2%) or who had no HBV markers (43.2%). Also the results of this study are in agreement with a previous study in 1982-1984 for Southeast Asian refugees in Philadelphia. The researcher found that HBV carriers who reacted to a PPD skin test were more likely to be negative for the HBeAg than carriers who did not react to PPD. The researchers conducted a further study in 1985 in Alaskan native hepatitis B carriers, a group not vaccinated with BCG, and the inverse

association of HBeAg and PPD reactivity was confirmed across all age groups and was similar in magnitude to that observed in the refugee population, so if the host response to BCG vaccine is similar, BCG vaccination may be of therapeutic value in chronic hepatitis B infection (Mcglynn, *et al.*, 1987). (Mcglynn, *et al.*, 1985) revealed that in hepatitis B carriers there are a statistically significant association between a negative tuberculin skin test and HBV replication, and this finding suggests that BCG vaccination might reduce the prevalence of infectious carriers, thereby ultimately reducing the incidence of hepatitis B infection. (Geo, *et al.*, 2007) explained that, the positive skin test indicates active cell-mediated immunity, whereas a negative test indicates a weak cell-mediated immunity. False-negative reaction may be produced by certain viral infection such as chronic hepatitis B infection either due to defects in mononuclear cells or defect in cytokines (IL-12, IFN- γ , IL-2 and TNF) that produced in the liver (Vinay *et al.*, 2005). In case of hepatitis the liver function was altered, and this lead to subnormal production of cytokines and in turn it farther leads to abnormal TST.

Conclusions and Recommendations:**A. Conclusions:**

1. The age, gender, residency, economical state, educational level, history of (smoking, alcohol intake and drugs addiction); and family history for HBV infection play an important role in the development of chronic HBV infection.
2. Chronic HBV infection induces abnormal serum aminotransferases (ALT and AST) and no significant effect on AP and TSB.
3. The prevalence of chronic HBV infection increases among diabetic patients and chronic HBV infection may induce diabetes mellitus.
4. Chronic HBV infection altered the immune system of the patients.
5. The ESR will increase in chronic HBV infection.
6. In chronic HBV infection, the TST is necessary for checking the immune system of the patients.

B. Recommendations:

1. Chronic HBV should be considered to any patient presented with high serum transaminase (ALT and AST) while normal TSB and AP, not be excluded chronic HBV infection.
2. Blood glucose should be checked in any patient presented with chronic HBV infection and HBsAg should be done to any diabetic patient.
3. Because of high incidence of vertical transmission of chronic HBV infection, HBV vaccine should be given to any pregnant women mainly in the third trimester.
4. For early detection of chronic HBV complications; the total serum (IgG and IgM), leucocytes differential count, ESR and TST should be added to routine investigations of those patients.
5. A farther study on the "role of TST in the diagnosis of chronic HBV infection" is recommend.

A. Materials:**1. Subjects of the study:****1.1. Patients:**

Seventy patients were included in this study which lasted from November (2007) to May (2008), and consisted of (57 males) and (13 females). The mean age of those patients was (31.5 ± 7.8 years). Those patients were attended to the gastroenterology center in the Marjan teaching hospital and diagnosed by specialist doctors as chronic hepatitis B infection.

The epidemiological distribution of those patients was as the following: The residency (49 urban and 21 rural area), marital status (51 married and 19 single), economic status (14 good, 16 medium and 40 low level), educational level (9 high education levels and 61 low educational levels), history of smoking (44 with positive history and 26 with negative history), alcohol intake (60 not alcoholic while 10 alcoholic) and all patients included in this study have no history of drugs addiction.

1.2. Controls:

Thirty apparently healthy subjects (clinically assessed by specialist doctors) were included as controls in this study, which consist of (21 males) and (9 females). The mean age of those subjects was (30.5 ± 6.7 years). Those subjects were selected randomly from the population.

The epidemiological distribution of those subjects was as the following: The residency (23 urban and 7 rural area), marital

status (19 married and 11 single), economic status (4 good, 9 medium and 17 low level), educational level (4 high education, 26 low education), history of smoking (21 with positive history and 9 with negative history), alcohol intake (26 not alcoholic while 4 alcoholic) and all subjects enrolled in this study have no history of drugs addiction.

Excluded criteria for the patients and controls:

1. Renal failure.
2. Pregnancy.
3. Asthma.
4. Immunosuppressant drugs intake.
5. Any chronic illness.
6. No other causes of hepatitis (autoimmune, HCV... etc.).

2. Instruments:

Table (2-1): The tools used in this study and their sources

No.	Tools	Company	Country
1.	EDTA tubes	Afma-dispo	Jordan
2.	Micro-pipette	Oxford,	USA
3.	Westergren tubes	Witeg	Germany
4.	Plain tubes	Afma-dispo	Jordan
5.	Disposable syringes	Witeg	Malaysia
6.	Microscope slides	Sail brand	China
7.	Microscope cover slides	Ataco	China

**Table (2-2): The main instruments used throughout the study
and their sources**

No.	Instruments	Company	Country
1.	Hemocytometer	Witeg	Germany
2.	Light microscope	Olympus	Japan
3.	Water bath	Memmert	Germany
4.	Centrifuge	Hermle	Japan
5.	Spectrophotometer	Cecil	England
6.	Refrigerator	Concord	Lebanon
7.	Incubator	Memmert	Germany
8.	Water distillator	G.F.L.	Germany
9.	Manual leucocytes counter	Hermle	Japan

3. Chemicals:**Table (2-3): The chemical and biological materials used throughout the study and their sources**

No.	Chemical materials	Company	Country
1.	AST- kit	Randox	U. K.
2.	ALT- kit	Randox	U. K..
3.	AP -kit	Biomerieux	France.
4.	Bilirubin- kit	Human	Germany
5.	Glucose -kit	Human	Germany
6.	Turk's solution	Crescent	Saudi Arabia
7.	Leishman stain solution	Crescent	Saudi Arabia
8.	Trisodium citrate solution	Crescent	Saudi Arabia
9.	Human IgG plate	Birmingham	U. K.
10.	Human IgM plate	Birmingham	U. K.
11.	PPD	Amertek	USA
12.	HBsAg-kit	Biokit	Spain
13.	HCV-kit	Biokit	Spain

B. Methods :**1. Blood collection:**

The collection of blood was done in gastroenterology center in the Marjan teaching hospital at (9-12:am). The brachial vein on the front of the elbow was employed. The arm should be warm to improve blood circulation and distended the vein. A tourniquet was applied directly on the skin around the arm (usually from the left arm), approximately (6-8cm) above the site of collection. The skin over the vein was sterilized with a small pad of cotton wool soaked with 70% ethyl alcohol, and the site was dried with clean gauze to prevent hemolysis. Needles used were 21 gauge. Two groups of labeled tubes were used; the first tubes contain disodium ethylene diamine tetra acetic acid (EDTA) as anticoagulants to prevent clotting of blood to be used for hematological studies. The second group tubes were without anti-coagulant as plain tubes, for blood to be used for preparing sera for subsequent biochemical tests. The blood was allowed to clot for 15 minutes, the clot shrinks and screw can be obtained by centrifugation and precautions were taken to avoid hemolysis. The serum samples were liquated in sterile test tubes using micropipette with sterile disposable tips. Each sample was labeled and given a serial number together with the patient name, the serum samples were frozen at (-20°C) for biochemical analysis (Lewis, *et al.*, 2006).

The investigations for those patients and control group were done in the laboratory of Marjan Teaching Hospital and post graduate physiology laboratory of medical college of Babylon university.

2. Hematological studies:

2.1. Total leucocytes count:

Blood was drawn in a clean and dry WBC pipette up to the mark 0.5 and the outside of the pipette was wiped off with a gauze. Then diluting fluid (Turk's solution) was drawn up to mark 11 (dilution 1:20), the contents were mixed for three minutes; 1-2 drops were discarded then counting chamber (Neubaur chamber) was filled. It was left for three minutes to let the cells for setting down and then the chamber was examined under 40X objective lens of the microscope to count WBCs in the four corners secondary squares (Lewis, *et al.*, 2006).

2.2. Differential leucocytes count (DLC):

Blood film was made immediately by careful mixing of the blood, an appropriate drop was delivered by a glass capillary and placed in the center line of a clean microscope slide about one cm from one end. Then without delay, placed a spreader in front of the drop at an angle of about 45° to the slide and moved it back to make contact with the drop. The drop was spread out quickly along the line of contact. With a steady movement of the hand, the drop of blood was spread along the slide. The spreader should not be lifted off until the last trace of blood has been spread out.

After air drying, the blood film was stained by Leishman's stain; the slide was flooded with the Leishman's stain. After two minutes, double the volume of buffered water was added for 5-7 minutes. Then washed in a stream of buffered water until it had acquired a pinkish tinge (up to two minutes), the back of the slide has been wiped and set it upright to dry. Preparation were examined under oil immersion lens (100X). Counted 100 leukocytes were taken and calculated the percentage of each type of cells (Lewis, *et al.*, 2006).

2.3. Measurement of ESR:

A Westergren tube: length- 300mm (open at both ends), diameter 2.5mm were used. One part of anticoagulant (3.8% trisodium citrate solution) was added to 4 parts of blood (0.5 ml of anticoagulant is added to 2 ml of blood). The mixture was drawn into a Westergren tube up to the zero mark and the tube was set upright in a stand with a spring clip on the top and rubber at the bottom. The level of the top of the red cell column was read at the end of 1 hour (Lewis, *et al.*, 2006).

3. Biochemical's analysis:

3.1. Determination of AST:

Colorimetric method was used to measure the amount of oxaloacetic acid produced under fix conditions. A reddish brown by hydrazone formed when 2,4 dinitrophenyl hydrazine in alkaline solution was added and measured at 530-550 nm by the

spectrophotometer according to procedure recommended by the company (Randox, UK) (Reitman and Frankel, 1957).

3.2. Determination of ALT:

Colorimetric method was used for the determination of GPT reaction with 2,4 dinitrophenyl hydrazine in alkaline solution, a brown colored hydrazone was formed and measured by using spectrophotometer at 490-560 nm as procedure recommended by the company (Random, UK) (Reitman and Frankel, 1957).

3.3. Determination of AP:

The method used was that in which phenyl phosphate is hydrolyzed with the liberation of phenol and formation of phosphate. The amount of phenol which formed was estimated colorimetrically by using spectrophotometer at 510 nm according to the procedure recommended by the company (Biomerieux, France) (Kind and King, 1954).

3.4. Determination of TSB:

Working solution was prepared by mixing reagent 1(RI) with reagent 3 (R3); mix well and incubate exactly for 5 minutes at 37°C and read at wave length 555 nm by using spectrophotometer according to the procedure recommended by the company (Human, Germany) (Walter and Gerarde, 1970).

3.5. Determination of FBS:

The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide

reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a red-violet quinoneimine dye as indicator. The absorbance of standards and samples are measured against reagent blank at 546nm according to the procedure recommended by the company (Human ,Germany) (Dingeon, 1975).

4. Immunological studies:

4. 1 Measurement of total serum IgG and IgM:

The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate monospecific antibody. Antigen-antibody complex are formed which, under the right conditions, will form a precipitin ring. The ring size will increase until equilibrium is reached between the formation and breakdown of these complexes, this point being termed ‘completion’. At this stage, a linear relationship exists between the square of the ring diameter and the antigen concentration. By measuring the ring diameter produced by a number of samples of known concentration of the antigen in an unknown sample may then be determined by measuring the ring diameter produced by that sample and reading off the calibration curve according to the procedure recommended by the company (Birmingham ,Germany) (Fahey and Mckelvey, 1965).

4.2. Tuberculin skin test:

The tuberculin test is performed by intracutaneous injection of 5 tuberculin unit (TU); (0.1ml) of PPD using a gage number 27

needle. The reaction is read at 48-72 hours, and a positive test is indurations of 10mm or more in diameter; erythema is not considered in determining a positive test (Carol, 2007 and Geo, *et al.*, 2007).

5. Serological study:

5.1. Determination of HBsAg:

Put 100 µl of sample dilutant in a well, then add 50 µl of serum and incubate for 30 minutes at 37°C, then wash 5 times and add 100 µl of conjugate and incubate for 90 minutes at 37°C then wash 5 times and add 100 µl of substrate incubate for 30 minutes at 37°C and add 100 µl of stopping solution and read at 450nm according to (Biokit, Spain). (Lennette, *et al.*, 1974).

5.2. Determination of HCV:

Put 100 µl of sample dilutant in a well, then add 10 µl of serum and mix then wash 5 times and add 100 µl of conjugate and incubate for 30 minutes at 37°C then wash 5 times and add 100 µl of substrate incubate for 30 minutes at 37°C and add 100 µl of stopping solution and read at 450nm according to (Biokit, Spain). (Lennette, *et al.*, 1974).

C. Statistical analysis:

All values were expressed as means $\pm$ SD. The data were analyzed by using of SPSS program and taking $p < 0.05$ as the

lowest limit of significance. Student's t-test was used to examine the differences between different groups. Both t-test, chi square and ANOVA test were applied to determine the differences between one group and another, and between all groups and within group (Wayne, 1999)..

- Alexander, I. K. and A. P. Kourtis. (2007). Hepatitis B. Last Updated. Am J Gastroenterol. 201(3).297-298.
- Alexander, J. G., A. N. Eddleston and R. Williams. (1983). Contrasting relations between suppressor cell function and suppressor cell number in liver disease. Lancet. 1. 1291.
- Alter, M. J., D. Kruszon and O. V. Nainan. (2005). The prevalence of hepatitis C virus infection in the United States, 1998 through 2004. N Engl J Med. 341. 556-562.
- Aoyagi, K., C. Ohue and K. Iida. (1999). Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol. 37. 1802-1808.
- Arao, M., K. Murase, A. Kusakabe, K. Yoshioka, Y. Fukuzawa, T. Ishikawa, T. Tagaya, K. Yamanouchi, H. Ichimiya and S. Kakumu. (2003). Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. J Gastroenterol. 38(4). 355-60.
- Arthur, C. G. and E. H. John. (2006). Textbook of medical physiology. 11th edition. W. B. Saunders company. USA. 881-882.
- Atkinson, W., C. Wolfe, S. Humiston and R. Nelson. (2000). Epidemiology and Prevention of Vaccine-

Preventable Diseases. Sixth Atlanta, GA: Public Health Foundation, Centers for Disease Control and Prevention.

Beasley, R. P., L. Y. Hwang and G. C. Lee. (1983) .Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. *Lancet*. 2.1099-1102.

Bianco, E., T. Stroffolini and E. Spada. (2003). Case fatality rate of acute viral hepatitis in Italy. An update. *Dig. Liver. Dis*. 35.404-8.

Bonacini, M., G. Hadi, S. Govindarajan and K. L. Lindsay. (1997). Utility of a discriminate score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C infection. *Am J Gastroneterol*. 92. P: 1302-1304.

Boni, C., A. Penna, A. Bertoletti, V. Lamonaca, I. Rapti and G. Missale. (2003). Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. *J Hepatol*. 39.595-605.

Bortolotti, F., P. Jara, C. Crivellaro, L. Hierro, P. Cadrobbi and. (1998). Outcome of chronic hepatitis B in Caucasian children during a 20-year observation period. *J Hepatol*. 29.184-190.

- Brooker, C. (1998). Nursing applications in clinical practice. Human structure and function. 2nd edition. Mosby London. 816.
- Brown, S. D., A. J. Barbara, T. Lambert and D. V. Wilson. (1995). Spontaneous loss of HBeAg and development of anti-HBe during long-term follow-up of blood donors found to be HBsAg positive. Br J Biomed Sci. 52.106-109.
- Carol, M. P. (2007). Essentials of pathophysiology. 2nd edition. Lippincott Williams and Wilkins. USA. 638-640.
- Chen, C. H., M. H. Huang , J. C. Yang, C. K. Nienand, C. C. Yang. (2007). Prevalence and etiology of elevated serum alanine aminotransferase level in an adult population in Taiwan. Gastroenterol Hepatol. 22(9).1482-9.
- Chisari, F.V. and C. Ferrari. (1995) Hepatitis B virus immunopathogenesis. Annu Rev Immunol. 13.29-60.
- Chowdhury, J. R., A. W. Wolkoff, N. R. Chowdhury and I. M. Arias. (1995). Hereditary jaundice and disorders of bilirubin metabolism. McGraw-Hill. USA. 2161-2208.
- Chu, C. J., E. B. Keefte, S. H. Han, R. P. Perrillo, A. D. Min and P. C. Soldevila. (2003). Hepatitis B virus genotypes in the United States. Gastroenterology. 125.444-451.

- Clancy, J. and A.J. Mcvicar. (2002). Physiology and anatomy. A homeostatic approach. 2nd edition. Arnold. London. 761-764.
- Craig, J. I., A. P. Haynes, D. B. McClelland, and C. A. Ludlam. (2002). Blood disorders in viral diseases. *Annu Rev Med.* 53.15-33.
- David, C. D. and D. F. Daniel. (2003). Scientific American medicine. WebMD inc. USA. 824.
- Decker, R. H. (1998). Diagnosis of acute and chronic hepatitis B. In Zuckerman A J, Thomas HC, editors. *Viral Hepatitis*. Churchill Livingstone. 201-215.
- Degertekin, H. (2001). Chronic hepatitis B and D in eastern and southeastern Anatolia. *Symposium of Hepato-gastroenterology in Eastern and South Eastern Anatolia, Turkey. Viral Hepatitis.* 1. 8.
- Demir, M., E. Serin, S. Gokturk, N. A. Ozturk, S. Kulaksizoglu, and U. Ylmaz. (2008). The prevalence of occult hepatitis B virus infection in type 2 diabetes mellitus patients. *Eur J Gastroenterol Hepatol.* 20(7). 668-73.
- Dennis, L. K., B. Eugene, S. F. Anthony, L. H. Stephen, L. Dnal and J. L. Jameson. (2005). *Harrison's principles of internal medicine.* 16th edition. McGraw-Hill. USA. 1830.
- Dingeon, B. (1975). Determination of serum glucose. *Ann. Biol. Clin.* (33).3.

- Doo, E., T. J. Liang. (2001). Molecular anatomy and pathophysiologic implications of drug resistance in hepatitis B virus infection. *Gastroenterology*. 120.1000-1008.
- Doumas, B. T., T. W. Wu and Jendrzecz. (2004). Delta bilirubin: absorption spectra, molar absorptivity, and reactivity in the diazo reaction. *Clin Chem*. 33.769-774.
- Dufour, D. R. (1998). Effects of habitual exercise on routine laboratory tests. *Clin Chem*. 44.136.
- Erker, J. C., S. M. Dessai, G. G. Schlauder, G. J. Dawson and I. K. Mushawar. (2001). A hepatitis E variant from the United States: molecular characterization and transmission. *J Gen Virol*. 80.681-690.
- Eugene, G. M. (2006). Viral hepatitis an overview. *Robert Wood Johnson Medical School Journal*. 3(3).1-13.
- Fahey, J. L. and E. M. Mckelvey. (1965). Quantitive determination of serum immunoglobulins in antibody-agar plates. *J. Immunol*. (94).84-90.
- Filimonov, P. N., N. I. Gavrilova, G. Ivanov, and V. A. Shkrupi. (2004). Interrelation between the activity of hepatitis, liver fibrosis and immune status in children with chronic hepatitis B. *Mikrobiol Epidemiol Immunobiol*. 2. 50-56.

- Flink, H., M. Vanzonneveld, B. E. Hansen. (2006). Treatment of chronic hepatitis B. *Gastroenterol.* 101(2).297-303.
- Fonseca, J. C. (2007). Natural history of chronic hepatitis B. *Rev. Soc. Bras. Med.* 40(6).672-7.
- Ganem, D. and R. Schneider. (2001). Hepadnaviridae: the viruses and their replication. *Fields Virology.* Lippincott-Raven. USA. 2923–2970.
- Geo, B., J. S. Butel, K. C. Carroll and S. A. Morse. (2007). *Medical microbiology.* 24th edition. Mc Graw Hill .U.S.A. 398-399.
- Gitlin, N. (1997). Hepatitis B: diagnosis, prevention and treatment. *Clin Chem.* 43. 1500-15006.
- Guidotti, L. G. and F. V. Chisari. (2000). Non cytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol*; 19. P: 65-91.
- Guidotti, L. G., K. Ando, and R. D. Schreiber. (2002). Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a non cytolytic mechanism in transgenic mice. *Proc. Natl. Acad. Sci. U S A.* 91.3764-3768.
- Harpaz, R., L. Von Seidlein, and F. M. Averhoff. (1996). Transmission of hepatitis B virus to multiple patients from a surgeon without evidence of inadequate infection control. *N. Engl. J. Med.* 334. 549–554.
- Hoofnagle, J. H. (1997). Hepatitis C: the clinical spectrum of disease. *Hepatology.* 26. P: 15-20.

- Hoofnagle, J. H., G. M. Dusheiko, L. B. Seef, E. A. Jones, J. G. Waggoner and Z. B. Bales. (2006). Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann Intern Med.* 104.744-748.
- Huether, S. E. (2000). Structure and function of the digestive system. *Understanding Pathophysiology*. 2nd edition.. Mosby. USA. 917-941.
- Hutin, Y. J., V. Pool and E. H. Cramer. (1999). A multistate, food borne outbreak of hepatitis A. *N Engl J Med.* 340.595-602 .
- Imazeki, F., O. Yokosuka, K. Fukai, T. Kanda, H. Kojima, and H. Saisho. (2008). Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients. *Liver Int.* 28(3).355-62.
- Inglesby, T. V., R. Rai and J. Astemborski. (1999). A prospective community-based evaluation of liver enzymes in individuals with hepatitis C after drug use. *Hepatology.* 29.590-596.
- Ishak, K. G. (2000). Pathologic features of chronic hepatitis. A review and update. *Am J Clin Pathol.* 113.40-45.

- Inlin, H., L. Zhihua and G. Fan. (2005). Epidemiology and Prevention of Hepatitis B Virus Infection. *Int J Med Sci.*2(1).50–57.
- Kassas, A. L. (1997). Clinical, laboratory, and serological findings of adult Hungarian hospitalized acute hepatitis patients, and possible source of the infection. *Acta Microbiol Immunol Hung.* 44(4).327-342.
- Kassas, A. L. (1998). Follow up of clinical, laboratory, and serological findings of adult Hungarian hospitalized acute hepatitis patients and characteristics of recovery. *Acta Microbiol Immunol Hung.* 45. (2):209-219.
- Kayhan, T., A. Nouri, G. J. Alexander and B. Portmann. (1986). In vitro study of IgG production and concanavalin A induced suppressor cell function in acute and chronic hepatitis B virus infection. *Clin. Exp. Immunol.* 64.50-58.
- Kind, P. R. N. and E. J. King. (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J. Clin. Path.* (7).322-326.
- Kleinschmidt, J., and I. Schlicht. (1997). Quantitative serum immunoglobulin determination: differential

- diagnostic significance for liver disease. *Med. Klin.* 72(12).505-512.
- Koff, R. S. (1995). Seroepidemiology of hepatitis A in the United States. *J Infect Dis.* 171.119-123.
- Kundrotas, L. W. and Clement. (1993). Serum alanine aminotransferase (ALT) elevation in asymptomatic US Air Force basic trainee blood donors. *Dig Dis Sci.* 38. 2145-2150.
- Kursad, M., B. Hamza, U. Ismail and T. Ilyas. (2005). Chronic hepatitis delta virus infection in Van region of eastern Turkey. *The Turkish Journal of Gastroenterology.* 16(1).17-20.
- Kurt, K., J. Ende and R. M. Hoffman. (2006). General internal medicine, MKSAP. 14th edition. ISBN. USA. P: 5.
- Kwon, S. Y. (2003). Prevalence and clinical significance of diabetes mellitus in patients with liver cirrhosis. *Taehan Kan Hakhoe Chi.* 9(3).205-211.
- Kwon, S. Y., S. S. Kim, O. S. Kwon and K. A. Kwon. (2005). Prognostic significance of glycaemic control in patients with HBV and HCV-related cirrhosis and diabetes mellitus. *Diabet Med.* 22(11).1530-1535.
- Lai, C. L., V. Ratziu, M. F. Yuen, and T. Poynard. (2003). Viral hepatitis B. *Lancet.* 362.2089–2094.

- Lai, M., B. J. Hyatt, I. Nasser, M. Curry and N. H. Afdhal. (2007). The clinical significance of persistently normal ALT in chronic hepatitis B infection. *J Hepatol.* 47(6).260-267.
- Larke, R. P., D. D. Harley, and D. A. Enarson. (1991). Relationship between tuberculin reactivity and hepatitis B virus infection in the Northwest Territories. *Arctic Med Res. Suppl.* 371-373.
- Lau, D. T., E. Doo, Y. Park et al: (1999). Lamivudine for chronic delta hepatitis. *Hepatology*; 30. P: 546-9.
- Lau, G. K., H. H. Yiu , D. Y. Fong and L. S. Lai. (2003). Early is superior to deferred preemptive lamivudine therapy for hepatitis B patients undergoing chemotherapy. *Gastroenterology.* 125.1742-1749.
- Lawrence, M. T., J. M. Stephen and A. P. Maxine. (2005). Current medical diagnosis and treatment. 44th edition. McGraw Hill. USA. 623, 630-635.
- Lee, W. M. (1997).Hepatitis B virus infection. *N Engl J Med*; 337. 1733-1745.
- Lemire, M. F., D. S. Miller, W.K. Foster, C. J. Burrell and A. R. Jilbert. (2005). Covalently closed circular DNA is the predominant form of duck hepatitis B virus DNA that persists following transient infection. *J Virol.* 79.12242-12252.

- Lennette, E. H., E. H. Spaulding and J. P. Truant. (1974). Manual of clinical microbiology. 2nd edition. Amer. Soc. Washington. 268.
- Lewis, S. M., B. J. Bgin and A. Bates. (2006). Daci and Lewis practical hematology. 10th ed. Churchill Livingston. USA. 1-5.
- Ling, M., S. Tropp, A. Danoff, and E. J. Bini. (2007). Association between chronic hepatitis B virus infection and diabetes among Asian Americans and Pacific Islanders. Dig Liver Dis. 39(6).549-556.
- Lok, A. S. (2002). Chronic hepatitis B. N Engl J Med. 346.1682-1683.
- Lok, A. S. and B. J. McMahon. (2001). Chronic hepatitis B. Hepatology. 34.124-1225.
- Lok, A. S. and B. J. McMahon. (2004). Chronic hepatitis B: update of recommendations. A. A. S. L. D. Practice Guideline. Hepatology; 39. 857–861.
- Lok, A. S., C. L. Lai, H. T. Chung, J. Y. Lau, E. K. Leung, and L. S. Wong. (1991). Morbidity and mortality from chronic hepatitis B virus infection in family members of patients with malignant and nonmalignant hepatitis B virus-related chronic liver diseases. Hepatology. 13. 834-837.

- Lok, A. S., E. J. Heathcote, and J. H. Hoofnagle. (2001). Management of hepatitis B. *Gastroenterology*. 120:1828-1853.
- Maguire, H. C., S. Handford and K. R. Perry. (1995). A collaborative case-control study of sporadic hepatitis A in England. *CDR Review*. 5:33-40.
- Mahoney, F. J. (1999). Update on diagnosis, management, and prevention of hepatitis B virus infection. *Clin Microbiol Rev*. 12(2):351–356.
- Maini, M. K., C. Boni, C. K. Lee and J. R. Larrubia. (2000). The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med*. 191:1269-1280.
- Mark, F., M. H. Sleisenger and B. F. Scharchmidt. (1998). Sleisenger and Fortran's Gastrointestinal and liver disease. 6th edition. Vol. 2. W. B. Saunders Company. USA. 1062.
- Marshall, W. J. (2000). Clinical chemistry. 4th edition. Mosby. Edinburgh. 812.
- Massoudi, M. S., B. P. Bell, V. Paredes, and C. N. Shapiro. (1999). An outbreak of hepatitis A associated with an infected food handler. *Pub Health Rep*. 114:157-64.
- Mathurin, P., J. Moussali and J. F. Cadranel. (1998). Slow progression rate of fibrosis in hepatitis C patients with persistently normal alanine aminotransferase activity. *Hepatology*. 27:568-572.

- Mcglynn, K. A., E. D. Lustbader and W. T. London. (1985). Immune responses to hepatitis B virus and tuberculosis infections in Southeast Asian refugees. *Am J epidemiol.* 122(6).1032-1036.
- Mcglynn, K. A., E. D. Lustbader, W. T. London, W. L. Heyward, and B. J. McMahon. (1987). Hepatitis B virus replication and tuberculin reactivity: studies in Alaska. *Am J epidemiol.* 126(1).38-43.
- McMahon, B. J., D. L. Bruden, K. M. Petersen and L. R. Bulkow. (2005). Antibody levels and protection after hepatitis B vaccination: results of a 15-year follow-up. *Ann Intern Med.* 142.333-341.
- McMahon, B. J., P. Holck, L. Bulkow and M. M. Snowball. (2001). Serologic and clinical outcomes of 1536 Alaska natives chronically infected with hepatitis B virus. *Ann Intern Med.* 135.759–768.
- Mistik, R. and I. Balik. (2001). Epidemiological analysis of viral hepatitis in Turkey. *Viral Hepatitis.* 9. 29.
- Myers, R. P., M. H. Tainturier, V. Ratziu and A. Piton. (2003). Prediction of liver histological lesions with biochemical markers in patients with chronic hepatitis B. *J Hepatol.* 39(2).222-30.
- Nalpas, B., A. Vassault and A. L. Guillou. (1994). Serum activity of mitochondrial aspartate aminotransferase:

- a sensitive marker of alcoholism with or without alcoholic hepatitis. *Hepatology*. 14.893-896.
- Nicholas, A. B., N. R. College, R. Walker and J.A. Hunter. (2006). *Davidson's principles and practice of medicine*. 20th edition. Printed in Churchill Livingstone Elsevier. India. 939,962-966.
- Olut, A. I., H. Ozunlu, H. Bozdag and N. Ozkalay. (2007). The follow-up of serum aminotransferase levels and investigation of hepatitis B virus load in inactive HBsAg carriers. *Mikrobiyol Bul*. 41(3).429-433.
- Pamela, C. C., A. H. Richard and R. F. Denise. (2005). *Lippincott's illustrated reviews biochemistry*. 3rd edition. Lippincott Williams and Wilkins. Philadelphia. 248-249.
- Papatheodoridis, G. V., N. Chrysanthos and S. Savvas. (2006). Diabetes mellitus in chronic hepatitis B and C: prevalence and potential association with the extent of liver fibrosis. *J Viral Hepat*. 13(5).303-310.
- Par, A., G. Bajtai, K. Barna, A. Gogl and A. Patakfalvi. (1995). Clinical and immunological findings in hepatitis B antigen-positive and hepatitis B antigen-negative chronic active hepatitis. *Acta Med Acan Sci Hung*. 32(1).15-25.

- Paraschiv, C., M. Graur, G. Butnariu, B. Mihai, and D. Constantinescu. (2002). The pathological mechanisms of glycoregulation disturbances in chronic hepatitis B and C. *Rev Med Chir Soc Med Nat Iasi*. 107(2).294-297.
- Parveen, K. and C. Michael. (2006). Kumar and Clark clinical medicine. 6th edition. Elsevier. Spain. 364-371.
- Pol, S., I. Couillin and M. L. Michel. (1998). Immuno-therapy of hepatitis B by anti HBV vaccine. *Acta Gastroenterol Belg*. 61(2).228-233.
- Porth, C. M. (2002). Pathophysiology. Concepts of altered health states. 6th edition. Lippincott Williams & Wilkins. Philadelphia. 643-644.
- Rehermann, B., C. Ferrari, C. Pasquinelli and F. V. Chisari. (1996). The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat. Med*. 2.1104-1108.
- Reitman, S. and S. Frankel. (1957). *In vitro* determination of glutamic-pyruvic transaminase (GPT) in serum. *J. Clinic. Path.* (28).56.
- Ryder, S. D. and I. J. Beckingham. (2001). ABC of diseases of liver, pancreas, and biliary system: Chronic viral hepatitis. *BMJ*. 322.219-221.

- Saadeh, C. (1998). The erythrocyte sedimentation rate: old and new clinical applications. *South Med J.* 3:220-225.
- Sam, G., D. Crawford, G. Mccaughan, J. Olynyk and S. Strasser. (2008). The relationship between the hepatitis B virus and the immune system changes over the life of a person. *Ashm.* 23.7.
- Scaglioni, P. P., M. Melegari and J. R. Wands. (1997). Biologic properties of hepatitis B viral genomes with mutations in the precore promoter and precore open reading frame. *Virology.* 233.374-381.
- Schwarz, J. A., and P. G. Scheurlen. (1995). Low molecular weight IgM in human sera. *Z Immunitatsforsch Exp Klin Immunol.* 149(1).51-60.
- Schwarz, J. A., U. Kaboth, H. Reikowski, H. Jost, D. Muting, and H. Ortmanns. (1988). Monomeric IgM in acute and chronic liver diseases. *Klin wochensh.* 53(11).535-538.
- Seeger, C. and W. Mason. (2000). Hepatitis B virus biology. *Microbiol Mol Biol Rev.* 64.51-68.
- Segovia, E., N. Rattoni, and L. Landa. (1996). Serum markers of B virus in chronic active hepatitis. *Rev. Gastroenterol. Mex.* 46(1).23-26.

- Strader, D. B., T. Wright, D. L. Thomas and L. B. Seeff. (2004).
Diagnosis, management, and treatment of hepatitis C.
Hepatology. 39.1147-1171.
- Tsuyoshi, Y. and K. Nagayama. (2004). Hepatitis Delta Virus
Levels May Predict Disease Severity in HBV. The
Journal of Infectious Diseases. 1(189).1151-1157.
- Van, D. and K. M. Graaff. (2002). Human anatomy. 6th edition. :
McGraw-Hill. Boston. 802-804.
- Vandamme, P., J. Banatvala and O. Fay. (2003). Hepatitis A
vaccination. Lancet. 362.1065-1071.
- Vassilopoulos, D., I. Rapti, M. Nikolaou and S. J. Hadziyannis .
(2008). Cellular immune responses in hepatitis B
virus e antigen negative chronic hepatitis B. J Viral
Hepat.14(8).352-357.
- Veldhuijzen, I. K., M. C. Mostert, H. G. Niesters and R. A.
deman. (2008). Chronic hepatitis B virus infection:
usefulness of the combination of HBeAg and ALT
determination to predict a high HBV-DNA level and
therefore the necessity of referral to a specialist for
possible antiviral treatment. Ned Tijdschr Geneeskd.
152(25).1426-1430.
- Vinay, K., K. A. Abul and F. Nelson. (2005). Robbins and
Cotran pathologic basis of disease. 7th edition.
Elsevier Saunders. China. 891-894.

- Vingerhoets, J., P. Michielsen, G. Vanham and E. Bosmans. (2000). HBV-specific lymphoproliferative and cytokine responses in patients with chronic hepatitis B. *J Hepatol.* 33(1).169.
- Voiculescu, C., M. Balasoiu and A. Turculeanu. (1993). Comparative study on some systemic humoral and cellular immune markers in only HIV or HIV and hepatitis B infected children. *Rom J Virol.* 44(12).97-111.
- Wachs, M. E., W. J. Amend, N. L. Ascher, P. N. Bretan, J. Emond and J. R. Lake. (1995). The risk of transmission of hepatitis B from HBsAg (-), HBcAb (+), HBIgM (-) organ donors. *Transplantation*; 59. 230-234.
- Walter, J. and P. A. Gerarde. (1970). Determination of total serum bilirubin. *Ann. Biol. Clin.* (11). 25.
- Wang, C. C., L. Y. Lim, H. Deubner and K. Tapia. (2008). Factors predictive of significant hepatic fibrosis in adults with chronic hepatitis B and normal serum ALT. *J Clin Gastroenterol.* 42(7).820-6.
- Wayne, W. D. (1999). *Biostatistics a foundation for analysis in the health sciences.* 7th edition. John Wiley and sons, INC. USA. 186-188.

- Whitehead, M. W., N. D. Hawkes, I. Hainsworth and J. G. Kingham. (1999). A prospective study of the causes of notably raised aspartate aminotransferase of liver origin. *Gut*. 45.129-133.
- William, E. P. (2003). *Fundamental immunology*. 5th edition. Lippincott Williams &Wilkins. USA. 61-62, 1217.
- Wright, T.L.(2006). Introduction to chronic hepatitis B infection .*AmJ Gastroenterol*. 101. 1-6.
- Yu, M. W.,S. H. Yeh, P. J. Chen, Y. F. Liaw and C. L. Lin. (2005). Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst*. 97.265–272.
- Yuen, M. F., C. K. Hui, C. C. Cheng and C. L. Lai. (2001). Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection. the effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. *Hepatology*. 34.139-145.
- Zheng, Z. L., P. Guan, S. Sun, and C. A. Winkler. (2008). A population-based study to investigate host genetic factors associated with hepatitis B infection and pathogenesis in the Chinese population. *BMC Infect Dis*. 8.1.
- Zhuang, H. (2007). Guideline on prevention and treatment of chronic hepatitis. *Chinese Medical Journal*. 120. (24).2159-2173.

- Zuberi, S. J., M. Siddiqui, and T. Z. Lodi. (1997).
Immunoglobulin in liver disease. Jpak Med Assoc.
27(9).391-2.

3.1. The age distribution:

The patients were divided into three age groups: first age group < 25 years of age with mean age (19 ± 5.2) years), second age group was between 25-35 years of age with a mean of (30.7 ± 4.14) and the third age group > 35 years old with a mean of (45.9 ± 5.3) and found 9 patients were in (group-1), 41 were in (group-2) and 20 were in (group-3) i.e. the age group between 25-35 years old had more incidence than other groups and the result was statistically significant ($P < 0.05$).

3.2. The gender influence:

In this study the males were more affected with chronic HBV than females, the ratio of males to females about (4:1) respectively. Statistical analysis give significant differences ($P < 0.05$).

3.3. The marital status:

In those patients enrolled in this study a marries are more frequent than a singles, the ratio about (3:1) respectively. There are statistically significant ($P < 0.05$).

3.4. The economic status:

This study revealed that the chronic HBV was more incident in low economic status group than other groups, and the ratio of good, medium, and low economic status were

(1:1:3) respectively. Also there were significant differences between these groups ($P < 0.05$).

3.5. Residency:

The incidence of chronic HBV was significantly ($P < 0.05$) more frequent in urban areas than in the rural areas and the ratio was (2:1) respectively.

3. 6. Educational levels:

This study revealed that the patients with low education were significantly ($P < 0.05$) more affected with chronic HBV than other, and the ratio of high educational level to low educational level was (1:6) respectively.

3.7. History of smoking:

The prevalence of chronic HBV in this study was significantly ($P < 0.05$) more in smokers than in non smokers patients with a ratio of (2:1).

3.8. Alcohol intake:

Also the prevalence of chronic HBV in this study was significantly ($P < 0.05$) more in nonalcoholic than in alcoholic patients with a ratio of (6:1).

3.9. Drugs addiction:

No history of drugs addiction to any patients enrolled in this study was registered. The results from (1-8) illustrated in the (table 3-1).

Table (3-1): Distribution of patients by (mean age, gender, residency, and marital status, economical status, educational level, history of smoking, and alcohol intake).

		Cases
Mean age $\pm$ SD		31.5 $\pm$ 7.8
Gender	Male	57
	Female	13
Residency	Rural	21
	Urban	49
Marital status	Married	51
	Single	19
Economical status	Good	14
	Medium	16
	Low	40
Educational level	High	9
	Low	61
History of smoking	Smoker	44
	Non smoker	26
Alcohol intake	Drinker	10
	Non drinker	60

Significant differences within groups for all the above ($P < 0.05$).

3.10. Family history of HBV:

After taken a careful family history for HBV from patients and control subjects it was found that the prevalence of chronic HBV more common in subjects with positive family history for HBV (65.7%) than in the subjects with negative family history (34.3%), in comparison with control group only (4:30, 13%) with positive family history, while the majority (26:30, 87%) with negative family history for HBV. There are a significant difference between patients and control group ($P < 0.05$). (Table 3-2).

Table (3-2): Distribution of patients and control group by (family history for HBV).

Family history for HBV		+ve	-ve	Total
Groups	patients	46	24	70
	Control	4	26	30
Total		50	50	100

Significant differences between patients and control group ($P < 0.05$).

3.11. AST:

The mean values of AST for patients enrolled in this study was (45.33±8.25 IU/L), whereas in control group it was (19.57±5.40 IU/L). There were a significant ($P < 0.05$) difference between patients and control group. (Table 3-3).

Table (3-3): Mean values of AST (IU/L) for patients and control group.

AST		Mean ± S.D.	No.
Groups	patients	45.33 ± 8.25	70
	Control	19.57 ± 5.40	30
Total			100

Significant differences between patients and control group ($P < 0.05$).

3.12. ALT:

The mean values of ALT for patients was (49.33±8.07 IU/L), compared to (16.47±4.51 IU/L) in control group. Significant differences ($P < 0.05$) between patients and control group were found. (Table 3-4).

Table (3-4): Mean values of ALT (IU/L) for patients and control group.

ALT		Mean $\pm$ S. D.	No.
Groups	patients	49.33 $\pm$ 8.07	70
	Control	16.47 $\pm$ 4.51	30
Total			100

Significant differences between patients and control group ($P < 0.05$).

3.13. AP:

There were insignificant differences ($P > 0.05$) between patients and control group, and the mean values of AP for patients was (51.34 $\pm$ 12.03 IU/L), and for control group it was (50.43 $\pm$ 9.99 IU/L). (Table 3-5).

Table (3-5): Mean values of AP (IU/L) for patients and control group.

AP		Mean $\pm$ S. D.	No.
Groups	patients	51.34 $\pm$ 12.03	70
	Control	50.43 $\pm$ 9.99	30
Total			100

No significant differences between patients and control group ($P > 0.05$).

3.14. TSB:

This study revealed that there was in significant ($P > 0.05$) statistical differences in the mean values of TSB for patients ($11.71 \pm 3.89 \mu\text{mol/L}$), and control group ($10.43 \pm 3.99 \mu\text{mol/L}$). (Table 3-6).

Table (3-6): Mean values of TSB ($\mu\text{mol/L}$) for patients and control group

TSB		Mean $\pm$ S. D.	No.
Groups	patients	11.71 ± 3.89	70
	Control	10.43 ± 3.99	30
Total			100

No significant differences between patients and control group ($P > 0.05$).

3.15. FBS:

This study revealed that there was a significant difference ($P < 0.05$) between the mean values of FBS for patients ($8.25 \pm 1.95 \text{ mmol/L}$), and control group, ($4.24 \pm 0.61 \text{ mmol/L}$). (Table 3-7).

Table (3-7): Mean values of FBS (mmol/L) for patients and control group.

FBS		Mean $\pm$ S. D	No.
Groups	patients	8.25 $\pm$ 1.95	70
	Control	4.24 $\pm$ 0.61	30
Total			100

Significant differences between patients and control group ($P < 0.05$).

3.16. Total serum IgG:

The mean values of total serum IgG for patients was (18373.86 $\pm$ 5629.85 mg/L), while in control group it was (7893.33 $\pm$ 2589.09 mg/L). There was a significant difference between patients and control group ($P < 0.05$). (Table 3-8).

Table (3-8): Mean values of IgG (mg/L) for patients and control group.

IgG		Mean $\pm$ S. D.	No.
Groups	patients	18373.86 $\pm$ 5629.85	70
	Control	7893.33 $\pm$ 2589.09	30
Total			100

Significant differences between patients and control group ($P < 0.05$).

3.17. Total serum IgM:

The mean values of total serum IgM for patients was (3742.43±1444.33 mg/L), while in control group it was (1045.00±611.06 mg/L), and this was statistically significant difference between patients and control subjects enrolled in this study ($P < 0.05$) as demonstrated in (table 3-9).

Table (3-9): Mean values of IgM (mg/L) for patients and control group.

IgM		Mean ± S. D.	No.
Groups	patients	3742.43 ± 1444.33	70
	Control	1045.00 ± 611.06	30
Total			100

Significant differences between patients and control group ($P < 0.05$).

3.18. Total leucocytes count:

This study revealed that there was no significant difference ($P > 0.05$) in the mean numbers of leucocytes for

patients (7462.86 ± 1607.70 cells/mm³) and control group (6836.67 ± 1422.84 cells/mm³). (Table (3-10)).

Table (3-10): Mean numbers of total leucocytes (cells/mm³) for patients and control group.

Leucocytes		Mean $\pm$ S. D.	No.
Groups	patients	7462.86 $\pm$ 1607.70	70
	Control	6836.67 $\pm$ 1422.84	30
Total			100

No significant differences between patients and control group ($P > 0.05$).

3.19. Leucocytes differential count:

1. Neutrophils count:

The mean numbers of neutrophils for patients (3324.04 ± 844.86 cells/mm³) was lower than in control group (4179.23 ± 856.47 cells/mm³) and the statistical analysis revealed a significant difference between patients and control group ($P < 0.05$). (Table 3-11).

2. Lymphocytes count:

There was an increase in the mean numbers of lymphocytes for patients (3263.30 ± 698.00 cells/mm³) compared to control group (2104.50 ± 473.46 cells/mm³).

There was a significant difference between patients and control group ($P < 0.05$). (Table 3-11).

3. Monocytes count:

There was a significant difference ($P < 0.05$) between the mean numbers of monocytes for patients (701.70 ± 192.77 cells/mm³) and control group (366.07 ± 97.76 cells/mm³). (Table 3-11).

4. Eosinophils count:

There were insignificant differences ($P > 0.05$) in the mean numbers of eosinophils for patients (110.41 ± 68.14 cells/mm³) and control group (119.07 ± 64.32 cells/mm³). (Table 3-11).

5. Basophils count:

There were insignificant differences ($P > 0.05$) in the mean numbers of basophils for patients (63.40 ± 46.84 cells/mm³), and control group (67.80 ± 47.83 cells/mm³). (Table 3-11).

Table (3-11): Mean numbers of leucocytes differential count (cells/mm³) for patients and control group.

Types of leucocytes	Groups	Mean $\pm$ S. D.
Neutrophils	Patients	3324.04 $\pm$ 844.86**
	Control	4179.23 $\pm$ 856.47**
Lymphocytes	Patients	3263.30 $\pm$ 698.00**
	Control	2104.50 $\pm$ 473.46**
Monocytes	Patients	701.70 $\pm$ 192.77**
	Control	366.07 $\pm$ 97.76**
Eosinophils	Patients	110.41 $\pm$ 68.14*
	Control	119.07 $\pm$ 64.32*
Basophils	Patients	63.40 $\pm$ 46.84*
	Control	67.80 $\pm$ 47.83*

**Significant differences between patients and control group (P < 0.05).

* No significant difference between patients and control group ($P > 0.05$).

3.20. ESR:

This study revealed that there was a significant difference ($P < 0.05$) in the mean values of ESR for patients (26.29 ± 5.51 mm/hr) and control group (8.87 ± 3.95 mm/hr). (Table (3-12)).

Table (3-12): Mean values of ESR (mm/hr) for patients and control group

ESR		Mean $\pm$ S. D.	No.
Groups	patients	26.29 $\pm$ 5.51	70
	Control	8.87 $\pm$ 3.95	30
Total			100

Significant differences between patients and control group ($P < 0.05$).

3.21. Evaluation of tuberculin skin test:

The incidence of negative tuberculin test was higher in patients with chronic HBV (84.3%) than in the subjects control group (30%). There was a significant difference

between patients and control group ($P < 0.05$). As illustrated in (table 3-13).

Table (3-13): Distribution of patients and control group by
(response to tuberculin skin test (PPD)).

PPD		+ve	-ve	Total
Groups	patients	11	59	70
	Control	21	9	30
Total		32	68	100

Significant differences between patients and control group ($P < 0.05$).

