Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
College of Medicine
Chemistry and Biochemistry Department

Hepcidin Levels and Sodium Taurcholate Cotransporting Polypeptide (NTCP) Gene Polymorphism in Patients with Chronic Hepatitis B in Babylon Province

A Thesis
Submitted to the Council of the College of Medicine / University of Babylon as a partial fulfillment for the requirements of the Degree of Master in Science /Clinical Biochemistry

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2023 A. D. **1444 A. H.**

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صدق الله العلي العظيم

سورة يوسف: (الآية ٧٦)

Supervisor Certification

We certify that this thesis entitled " **Hepcidin Levels and Sodium Taurcholate Cotransporting Polypeptide (NTCP) Gene Polymorphism in Patients with Chronic Hepatitis B in Babylon Province**" was carried out under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

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In review of the available recommendation, I forward this thesis for debate by the examining committee.

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Dedication

This work is dedicated to:

To…… my lovely father and mother

To…… my dear wife

To…… my lovely sons

To…… all members of my family

To…… all friends and lovers

To…… every knowledge student

To my great supervisor (Dr. Zinah Abbass Ali)

To…… my great country

Amjed

Acknowledgment

I would like to express my extreme thanks to **Allah, Most gracious and most merciful.**

I would like to introduce my deepest thanks to my supervisor's **Asst. Prof. Dr. Zinah Abbass Ali** and **Asst. Prof. Dr. Nihad Abdallah Selman** for their supervision and continuous encouragement throughout the work.

I would like to express my thanks to the Head of Chemistry and Biochemistry Department **Prof. Dr.Abdulsamie Hassan Alta'ee and all academic staff** of Chemistry and Biochemistry Department, College of Medicine, University of Babylon.

I would like to thank the **Dean** of the College of Medicine, University of Babylon.

I would like to thank my dear wife **Dr. Hiba Resheed Behayaa** for her great assisted.

I would like to thank the working staff in **Gastro Intestinal Tract and Hepatic Center (GIT&H Center) in Merjan Medical City in Babylon province** for their assistance in the collection of samples. Special thanks to all people participating in this study as patients and control.

Also, I'd want to express my thanks to everyone who has assisted me.

Amjed

Summary

Hepatitis can be defined as inflammation of the liver organ cells, such as hepatocyte infection or dysfunction. Hepatitis is classified as acute if it resolve within a half-year period or chronic if it persists for more than 6 months.

Hepcidin, a 25-amino acid peptide in humans is encoded by the Hepcidin antimicrobial peptide (*HAMP)* gene. Hepcidin is a central regulator of iron metabolism. Hepcidin is mostly produced by hepatocytes in response to iron-load in cells. Whenever this load increases, hepcidin expression goes up in hepatocytes, which results in increased serum hepcidin levels.

Sodium taurocholate co-transporting polypeptide (NTCP), is a protein in human encoded by SLC10A1(Solute carrier family 10 member 1) gene. In the human genome, NTCP gene is located on band q 24.1 of chromosome 14 and consist of 5 exons. Genetic polymorphisms in SLC10A1 have been found to affect NTCP protein function, several SNPs have been identified in NTCP and some of these SNPs are found in specific ethnic backgrounds.

This study was designed to investigate hepcidin, ferritin and iron level and to find the possible correlation between these parameters with age and gender in patients with chronic hepatitis B, and to evaluate the role of single nucleotide polymorphism SNP (rs2296651 G/A) of NTCP gene and its associated with HBV infectivity and disease progression in Babylon Province.

To achieve these aims, the present study included 100 subjects. The first group included 50 patients with chronic hepatitis B. The second group included 50 apparently healthy individuals which was the control group.

Hepcidin, ferritin and NTCP concentration were determined by enzyme linked immunosorbent assay (ELISA) method.

DNA was extracted from blood and genotyped for the SNP (rs2296651 G/A) by probe-based real-time quantitative polymerase chain reaction technique. Results were evaluated by various statistical analysis.

NTCP was increased significantly ($p<0.05$) in chronic hepatitis B patient. Hepcidin and ferritin concentration were increased significantly ($p<0.05$) in chronic hepatitis B individuals (compared to the control group) this result attributed to increased iron levels which stimulate hepcidin production, Hepcidin is able to respond to variations in body iron demand and its concentration varies accordingly.

Also, liver function tests were disturbed significantly in patients compared to control group.

On the other hand all genotype SNP (rs2296651 G/A) with chronic hepatitis B (studies in this search) didn't show any significant association ($p>0.05$) with the disease.

The present study concluded that chronic hepatitis B patients in Babylon Province have higher serum level of hepcidin, ferritin and iron comparing to normal subjects and this elevation may be represent the degree of hepatocyte damage in these patients.

Finally, polymorphism of NTCP gene (rs2296651 G/A) was not associate with chronic hepatitis B virus, we detected only wild type GG genotype in all our studied participants, and none of them presented the mutant GA or AA genotypes.

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List of Abbreviations

Abbreviation	Meaning
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CHB	Chronic hepatitis B
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immune sorbent assay
GSH	Reduced glutathione
HAMP	Hepcidin antimicrobial peptide
HBcAg	hepatitis B core antigen
HBeAg	Hepatitis B envelope antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus.
HCC	hepatocellular carcinoma
HRP	Horseradish peroxidase
M	Mean
NTCP	Sodium taurocholate co-transporting polypeptide
PT	Prothrombin time
r	Correlation-coefficient
RT-qPCR	Real-time quantitative polymerase chain reaction
SD	Standard deviation
SLC10A1	Solute carrier family 10 member 1
SNP	Single nucleotide polymorphism
TSB	Total serum bilirubin

Chapter One

Introduction and Literature Review

1. Introduction and literature review

1.1 Hepatitis disease

Hepatitis can be defined as inflammation of the liver organ cells, such as hepatocyte infection or dysfunction. Certain patients show no signs of hepatitis, whereas other patients develop yellow-colored skin, which is called jaundice, reduced appetite, vomiting, nausea, diarrhea, and headache **[1]**.

Hepatitis is classified as acute if it recurs within a half-year period or chronic if it persists for more than 6 months. Self-recurrent occurs in acute hepatitis or the development of chronic hepatitis, or it leads to acute liver failure in a very few cases. Chronic hepatitis can also advance to scar fibrous tissue liver (cirrhosis), liver failure and hepatic malignancies such as hepatocarcinoma **[2]**.

Hepatitis, is one of the most prevalent forms of disease in the world. It can be caused by alcohol consumption, drugs, toxins, or an auto-immune disease, but it is caused by hepatitis viruses in most cases. This is so-called viral hepatitis. Hepatitis is most commonly caused by the virus hepatovirus A, B, C, D, and E. Other viruses can also cause liver inflammation, including cytomegalovirus, *Epstein–Barr* virus, and yellow fever virus **[3]**.

Hepatitis A and E are mainly spread by contaminated food and water. Hepatitis B is mainly sexually transmitted, but may also be passed from mother to baby during pregnancy or childbirth and spread through infected blood. Hepatitis C is commonly spread through infected blood such as may occur during needle sharing by intravenous drug users. Hepatitis D can only infect people already infected with hepatitis B. The five hepatitis viruses can cause acute illness, but hepatitis B and C can cause cirrhosis and liver cancer, which occur after decades of chronic infection, result the largest number of deaths **[4]**.

Chronic hepatitis is not considered just a single condition but is a clinical and pathological disorder, which results from several causes and is distinguished by various degrees of hepatocellular necrosis. The necrosed and inflammed parts of the liver can be identified as focal areas for parenchymic necrosis and dropout, big lobular areas of confluent necrosis, anteroseptal or periportal part necrosis with or without a connection. Chronic hepatitis remains an unimproved disease for at least 6 months without recovery **[5]**.

1.2 Sign and Symptom

Hepatitis has a broad spectrum of presentations that range from a complete lack of symptoms to severe liver failure. The acute form of hepatitis, generally caused by viral infection, is characterized by constitutional symptoms that are typically self-limiting. Chronic hepatitis presents similarly, but can manifest signs and symptoms specific to liver dysfunction with long-standing inflammation and damage to the Organ **[6]**.

Chronic hepatitis is often asymptomatic early in its course and is detected only by liver laboratory studies for screening purposes or to evaluate non-specific symptoms. As the inflammation progresses, patients can develop constitutional symptoms similar to acute hepatitis, including fatigue, nausea, vomiting, poor appetite, and joint pain. Jaundice can occur as well, but much later in the disease process and is typically a sign of advanced disease **[7]**.

Chronic hepatitis interferes with hormonal functions of the liver which can result in acne, hirsutism (abnormal hair growth), and amenorrhea (lack of menstrual period) in women. Extensive damage and scarring of the liver over time defines cirrhosis, a condition in which the liver's ability to function is permanently impeded. This results in

jaundice, weight loss, coagulopathy, ascites (abdominal fluid collection), and peripheral edema (leg swelling). Cirrhosis can lead to other life-threatening complications such as hepatic encephalopathy, esophageal varices, hepatorenal syndrome, and liver cancer **[8]**.

1.3 Causes of Hepatitis

Hepatitis causes can already be categorized further into corresponding important categories:

Infectious, metabolic, ischemic, immune system dysfunction, hereditary, and others. Viruses, bacteria, and parasites are examples of infectious agents. Metabolic causes incorporate drugs, poisons (most importantly, alcohol), and non-alcoholic fatty liver disease. Hepatitis is caused by the immune system and inherited factors include genetic predispositions affecting particular populations in general **[3]**.

1.3.1 Infectious Hepatitis Caused by Viruses

At least five major viruses are known to cause hepatitis. Hepatitis that isn't caused by hepatitis A or B was known as non-A, non-B hepatitis in the past. Practically all viral etiologies of non-A, non-B infections can now be detected, which has led to the discovery of the hepatitis viruses, C, E, and G. Certain viruses, such as *Epstein–Barr* virus as well as cytomegalovirus, can still cause liver inflammation, but they really are not the main cause of hepatitis **[9]**.

Hepatitis B (HBV) mostly progress to chronic hepatitis and are significant worldwide general medical issues. Concerning sickness trouble, Organization of the World Health assessed that in excess of 350 million and 170 million individuals are ongoing transporters of HBV. HBV has a place in a group of firmly related DNA infections called the

hepadnaviruses that lead to the reasons for chronic hepatitis and hepatocellular carcinoma **[1]**.

The nucleocapsid that coats the viral DNA yields hepatitis B core antigen (HBcAg).When peptides determined by HBcAg are transmitted to the outside of liver hepatocytes, they trigger an immune reaction that is necessary to destroy infected cells. Hepatitis B envelope antigen (HBeAg), a circulating peptide derived from the core genome before being modified and shared by liver cells, serves as a marker of active viral replication **[10]**.

HBeAg might also serve as a tolerogen, as its presence in the blood has been linked to a reduced immune response, primarily due to its close resemblance to HBcAg, the putative immune response's target. With a few exceptions, HBeAg is only found in people who already have HBV DNA in their blood **[11]**.

Antibodies against an HBV surface antigen that are produced in response to envelope antigen exposure and provide protective immunity. Antibodies to HBsAg remain observable in patients who have recovered from acute hepatitis B, even those who have received the HBV vaccination, but they may become undetectable in people who have completely recovered from the disease **[4]**.

An antibody to the HBV core antigen is found in almost all individuals that have been exposed to HBV at some stage. This antibody, unlike an antibody to the HBV surface antigen, does not protect the patient from HBV infection and could not be used to distinguish between acute and chronic infections. Patients with chronic HBV infection, as well as those that have recovered from HBV infection, have antibodies to HBcAg. As some patients with chronic hepatitis B test positive for immunoglobulin M (IgM) antibody during infection flare-ups, it is obviously not a fully accurate predictor of acute disease **[12]**.

1.3.2 Bacterial and Parasitic Hepatitis

Liver bacterial infection usually creates liver pyogenic abscesses or acute or chronic liver hepatitis (granulomatous inflammation). Liver abscesses of pyogenic bacteria are generally come from in enteric microbes. Parasites are another infectious particle that can invade the liver and stimulate the immune response, cause acute hepatitis in the serum and lead to increased immunoglobulin E (IgE) **[13]**.

Also, chronic hepatitis can be caused by chronic parasitic infections). Most parasites that can infect liver include *Entamoeba histolytica*, which also causes liver abscesses with hepatitis. Dog tapeworms are multicellular parasite worms that infiltrate the liver and form typical hepatic hydatid cysts called *Echinocococo granulosus*. Liver flukes such as *Fasciola hepatica* and *Sinensis Clonorchis* will live in the bile, causing hepatitis and hepatic fibrosis in advanced cases **[14]**.

1.3.3 Metabolic Hepatitis

Alcohol, non-alcoholic hepatitis, fatty liver disease, toxic and drug induced hepatitis are included in this category:

1.3.3.1. Alcoholic Hepatitis

Regular alcohol consumption, in any event, for only a few days, can lead to fatty liver disease (also named steatosis), an issue where hepatocytes have macrovesicular beads of fatty substances (triglycerides) Albeit alcoholic fatty liver is associated with abstinence, steatosis inclines individuals who keep on drinking to hepatic fibrosis and cirrhosis **[15]**.

Increased bowel permeability and changes in the composition of intestinal microbiota are also associated with chronic alcohol consumption, as well as with ethanol causing hepatocyte damage and

changes to lipid metabolism. Bacterial translocation from the intestines to the liver contributes to alcohol-related liver disease **[16]**.

1.3.3.2. Non-Alcoholic Fatty Liver Disease

Non-alcoholic steatohepatitis (NASH) can be defined as a developing type of nonalcoholic fatty liver disease (NAFLD) that can lead to liver cirrhosis and its related complications. A specific diagnosis of NASH, normally not done without approved treatments, can only be done with a liver biopsy **[17]**.

The factors accelerating NAFLD-NASH development are obsessive, older, non-African Americans, females, hypertensives, higher Alanine amino transferase (ALT) and cholesterol levels, higher AST/ALT ratios, low platelet counts and ultrasound steatosis ratings **[18]**.

NAFLD and NASH pathogenesis were traditionally viewed as a "two-hit" process, with the first causing hepatic triglyceride aggregation and the second causing hepatocyte dying and scarring **[19]**.

1.3.3.3. Drug-Induced Hepatitis

The liver is the chief organ for the metabolism and excretion of numerous medications. Despite the fact that a few medications lead to hepatotoxicity if the source formulation attacks explicit organelles, for example, mitochondria or nuclei, the most harmful medications require metabolism of toxic metabolites **[6]**.

The liver provides three stages of drug metabolism. In phase I, cytochromic compounds P-450 are used to treat medications. This activity will produce toxic synthetic electrophilic and free radicals. In phase II, reduced glutathione (GSH), sulfate, or glucuronide are used to produce water-dissolving compounds in the parent medicine or

metabolites. As a result, the substances can be removed from the body through bile or urine **[19]**.

The removal route is primarily defined by hepatocyte canalic and sinusoidal membrane excretory transporters (phase III). The variations between populations in phases I, II, and III drug metabolism can represent environmental causes, along with concomitant drugs or alcohol, or genetic polymorphisms, which may affect liver exposures to toxic metabolizations and may decide the sensitivity of idiosyncratic drugs that stimulate liver damage **[20]**.

The dangerous metabolites that result from drug metabolism can actually influence liver cells' biochemistry, resulting in cell injury or triggering an immune-mediated liver attack. These metabolites can bind covalently to lipid, protein, and DNA and lead to cellular defects by stimulating biochemical effects such as oxidation, GSH reduction, lipid peroxidation (LPO) and redox changes **[15]**.

As a result, these changes can affect the work of mitochondria, endoplasm reticulum, microtubules, cytoskeletal, and nucleus, leading to a major direct insult. Put another way, the events may lead to activating or inhibiting signaling kinases, transcriptional factors and gene expressions which can make hepatocytes or cholangiocytes sensitive to the toxicity of the innate immune system, like cytokines or chemokines, which are triggered by primary liver damage**[21]**.

1.3.4. Ischemic Hepatitis

Ischemical Hepatitis (IH) is a disease due to inadequate blood volume and/or oxygen content in the liver to support the hepatocytes. IH, hypoxic hepatitis and shocking liver are also the same hepatic necrosis surrounding the central veins **[22]**.

Congestive hepatopathy describes a spectrum of clinical and biological manifestations (e.g., mild increase in plasma aminotransferase level) because of passive and frequently prolonged hepatic obstruction in the setting of right-sided heart failure (e.g., dilated cardiomyopathy, mitral stenosis, and constrictive pericarditis). The morphological pattern is centrilobular congestion. Congestive hepatopathy may precede the onset of IH **[23]**.

1.3.5. Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is a condition which causes chronic liver to be differentiated by drug-cause and alcohol-cause hepatitis and chronic idiopathic hepatitis. AIH exists in all ethnic groups worldwide and has a prevalence of women among children and adults of all ages. A lack of tolerance to the liver-antigens of the patient's is seen as based on pathological processes likely to be caused by genetically sensitive people, such as pathogens and xenobiotics **[24]**.

While AIH is a chronic condition that can lead to cirrhosis, liver transplantation and/or death and also liver cancer such as hepatocellular carcinoma (HCC) , it can also begin with an acute attack of hepatitis (that is, with malaise, nausea, abdominal pain, jaundice and elevation of transaminase levels). AIH can also be fulminant hepatic failure, so it must be considered when differentiating the diagnosis of acute liver failure **[25]**.

1.3.6. Genetic Hepatitis

The most common genetic disorders that cause hepatitis are alpha-1 antitrypsin deficiency, iron hemochromatosis, and Wilson disease. In alpha-1-antitrypsin (AAT), deficiency results from co-dominant mutations in the AAT gene, leading to excessive deposition of the mutant AAT protein inside hepatocytes, resulting in liver disease **[3]**.

Wilson's disease as well as hemochromatosis are also autosomal recessive digestive disorders that are characterized by irregular mineral processing. Hemochromatosis is a disease in which excess iron builds up in different parts of the body, such as the liver, causing cirrhosis. Wilson's disease causes cirrhosis and dementia by accumulating too much copper within the liver and also in the brain **[18]**.

1.4 Risk Factors

Due to the HBV viruses are transmitted by exposure to infectious blood, the risk factors includes blood transfusion or organ transplantation without HBV screening, working in a healthcare setting, dialysis, acupuncture, tattooing, extended overseas travel, multiple-use of medication vials, infusion bags, and improperly sterilized surgical equipment. Intravenous drug use (IDU) is a major risk factor for viral hepatitis in many parts of the world **[26]**.

Vertical transmission is the mother to child transmission, it is a major route of infection in endemic countries. It may occur both during gestation and at delivery. Personal care items such as razors, toothbrushes, and manicuring or pedicuring equipment can be contaminated with blood, sharing such items can potentially lead to exposure to HBV **[26]**.

Hepatitis can be transmitted between family members within households, possibly by contact of non-intact skin or mucous membrane with secretions containing the virus **[27]**.

The risk for the development of chronic hepatitis B following acute HBV infection is age-dependent. Where only fewer than 5 % of adults undergo chronic infection, while approximately 90 % of infants become chronically infected **[28]**.

1.5 Epidemiology of HBV

Viral hepatitis is responsible for an estimated 1.4 million deaths per year. HBV are responsible for about 90% of these mortality, whilst the remaining 10% of mortality are caused by other hepatitis viruses **[29]**.

Al-badry & Al-tamemi in 2019 **[30]**, showed the pattern spread of hepatitis in the urban more than the rural, this may be due to the people of urban more referred to the hospitals and thus exposure to the risk of infection due to contact with contaminated tools.The prevalence of HBV infection varies, ranging from 0.5% in some developed countries to up to 8% in some East Asian countries. A previous study in Turkey, a neighboring country of Iraq, showed that the prevalence of HBV varied markedly from 1% to 14.3%, according to the geographical region of the study **[31]**.

While the prevalence of HBV infection was below 1% in some regions in Iran, studies from Saudi Arabia showed that approximately 3% of the study populations were infected with the virus. Chronic hepatitis B virus (HBV) infection represents a major global health problem, around 2.57 billion people are estimated to be infected with HBV, and about 750000 deaths annually. among them, more than 250 million are chronically infected with an increased risk of developing HBV-related

liver diseases, including liver cirrhosis (LC) and hepatocellular carcinoma (HCC) **[32]**.

Chronic infection with the hepatitis B virus (HBV) is a major cause of chronic liver-disease and remains endemic in many countries despite the worldwide implementation of vaccination **[33]**.

The previous study on the prevalence of hepatitis B among blood donors in Baghdad showed 6.2% positive for HBV **[34]**.

Viral hepatitis infection obtained in Basra- Iraq by that indicated hepatitis prevalence was 0.12% **[35]**.

A similar study showed the prevalence was 0.7% in Babylon Province **[36]**, another study in Najaf Province showed the prevalence was 0.66% **[37]**.

The prevalence rate in Karbala Province, Iraq , of HBV was 2.8% in 2008, this prevalence rate rose to 3.9% in 2016 **[38]**.

The World Health Organization declared an ambitious plan to eliminate viral hepatitis by 2030, calling for a 90% reduction in new infections and a 65% reduction in mortality. However, it has been previously estimated that only 10% of HBV-infected patients are aware of their infection **[39]**.

1.6 Hepatitis B Virus

1.6.1 HBV Biology and Structure

Hepatitis B virus is the prototype member of the Hepadnaviridae family, which are the smallest known deoxyribonucleic acid (DNA)-containing, enveloped animal viruses. The identification of cellular receptor, as well as the development of innovative infection models and molecular techniques, have opened up new possibilities for study into particular stages of the HBV lifecycle as well as the organization and activity of the covalently closed circular DNA (cccDNA), (the viral minichromosome that serves as the template for HBV transcription in the nucleus of the infected hepatocytes, enabling maintenance of chronic HBV infection **[40]**.

Electron microscopy revealed three distinct viral structures in the serum of HBV-infected patients: Dane particles, circular particles, and filamentous particles, fig. 1-1. A common HBsAg on the surface of all three particles. Circular (20 nm) and filamentous (22 nm) particles are noninfectious because they are composed of HBsAg and host-derived lipids, but lack the HBV genome **[41]**.

On the other hand, Dane particle (42 nm sphere) is a fully infectious HBV virion. A small circular, partially double-stranded DNA molecule and viral DNA polymerase are found in the core of the Dane particle, which is surrounded by nucleocapsid. Nucleocapsid is built by assembled hepatitis B core antigen (HBcAg), and it is covered with a lipid envelope containing HBsAg. Thus, a nucleocapsid composed of hepatitis B core protein (HBc), viral polymerase (Pol), and viral genome DNA **[42]**.

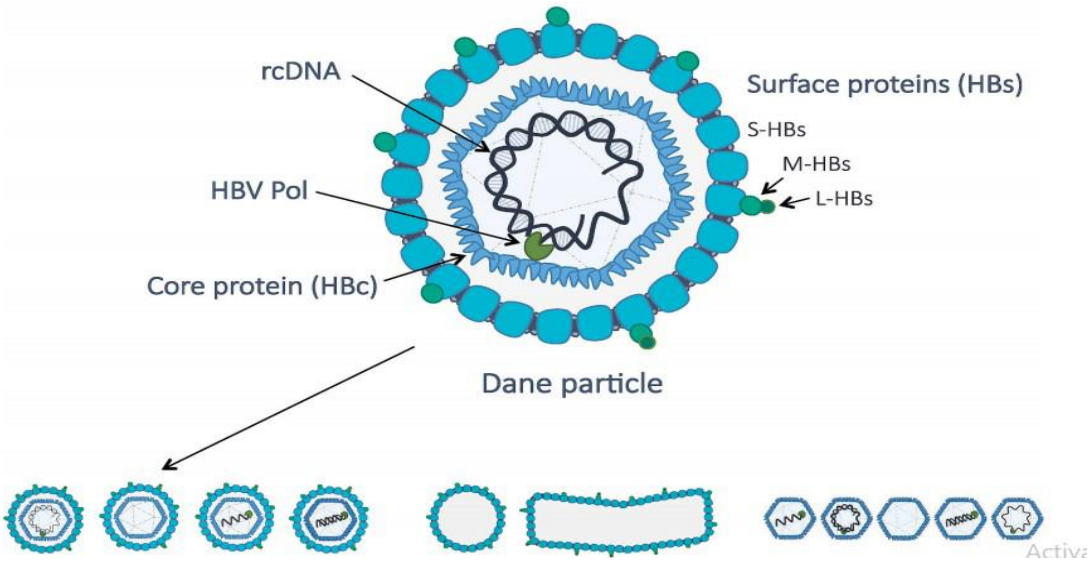

Figure 1-1. Schematic representation of HBV particles. Infectious HBV virion (Dane particle) (upper) and non-infectious HBV particles, including enveloped capsids containing immature DNA/RNA, subviral particles (circular and filament), and naked nucleocapsids (lower) [42].

HBV's genome is unique since it's not completely double-stranded. The viral DNA polymerase is attached to one end of the full-length strand.The full-length strand of the genome is 3020–3320 nucleotides long, while the short-length strand is 1700–2800 nucleotides long **[43]**.

C, X, P, and S are the four recognized genes encoded by the genome. Gene C codes for the core protein (HBcAg) and its start codon is preceded by an upstream in–frame AUG start codon, from which produces the pre-core protein. Proteolytic processing of the pre-core protein produces hepatitis B envelope antigen (HBeAg) **[44]**.

Gene P is responsible for encoding DNA polymerase. The surface antigen is coded for by Gene S (HBsAg). The HBsAg gene is one long open reading frame with three in frame "start" (ATG) Codons that divide it into three sections: pre-S1, pre-S2, and S. Polypeptides of three sizes

are formed as a result of the multiple start codons, they are: large, middle, and small [Pre-S1+Pre-S2+S, Pre -S2 + S, or S] **[45]**.

Although the function of the protein encoded by gene X is not fully known, it is linked to the development of liver cancer, where it stimulates cell growth-promoting genes while inactivating growth-regulating molecules. The lipid envelope's cholesterol-rich composition is necessary for viral infectivity. The nucleocapsid induces an orderly and condensed arrangement of the three different surface glycoproteins: L (large), M (middle), and S (small) on the envelope membrane during budding from the endoplasmic Reticulum (ER) **[46]**.

The persistence of the S HBsAg in the serum across a period of more than 6 months is usually trusted as a chronic infection and most commercial assays were designed to recognize S HBsAg. Other non-infectious particles, such as enveloped particles without a viral genome, those containing viral Ribonucleic Acid (RNA), and envelope-less particles (naked nucleocapsids), are currently known to be formed by infection **[47]**.

1.6.2 Replication of Hepatitis B Virus

The virus enters the cell after binding to a cell surface receptor. The sodium taurocholate cotransporting polypeptide (NTCP), which is a liver specific bile acid transporter, has been identified as a cell receptor necessary for HBV entry **[48]**.

HBV loses its coating and becomes uncoated and being endocytosed in. Since the virus multiplies by RNA made by a host enzyme, the viral genomic DNA must be transported to the cell nucleus by host proteins called charperones **[27]**.

The viral genome that is partially double-stranded, switched to covalently closed circular double stranded DNA in the nucleus

(cccDNA). All viral transcripts, including a 3.5-kb pregenome RNA, use the cccDNA as a template. The freshly synthesized HBcAg encapsidates the pregenome RNA **[49]**.

The viral polymerase creates a negative-strand DNA copy within the cores by reverse transcription. The polymerase begins the process of synthesising the positive DNA strand, but it does not complete it. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes and potentially exiting the cell. Cores may also be reimported into the nucleus and begins a new round of replication in the same cell figure 2 **[50]**.

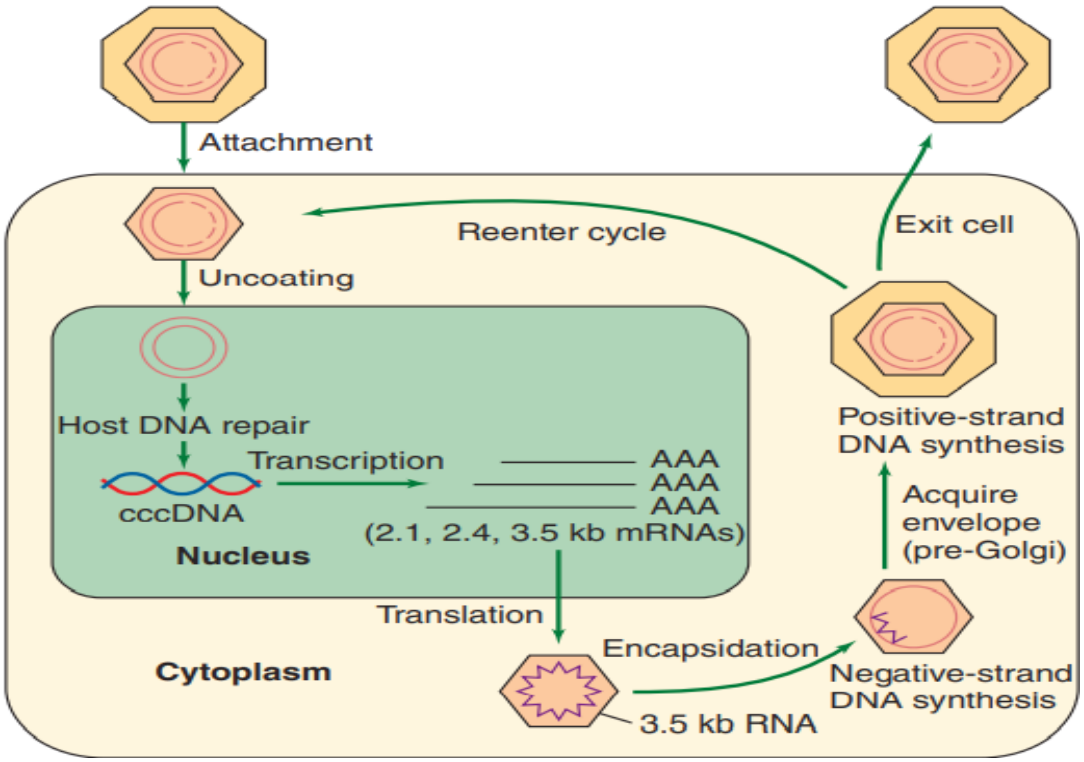

Figure 1-2: Replication cycle of Hepatitis B virus (HBV) [50].

1.6.3 Chronic Hepatitis B Virus

The incubation period for HBV infection ranges from one to four months, before acute hepatitis progresses. At least 70% of patients will develop subclinical or anicteric hepatitis, with just less than 30% developing icteric hepatitis. The most obvious clinical symptoms of hepatitis are nausea, fever, jaundice, vomiting, right upper quadrant discomfort, hepatomegaly, changes in stool color, dark urine and other unspecific constitutional symptoms **[51]**.

HBV DNA can usually be identified one to two weeks after exposure, and patients usually have no symptoms and only slightly elevated serum alanine aminotransferase (ALT) levels during this period. HBsAg and HBeAg are the first serological HBV markers that can be detected. Fulminant hepatic failure is extremely rare. The causes and risk factors for fulminant HBV are not quite understood **[52]**.

This may be linked with coinfections with other viruses or substance use. Massive immunemediated lysis of infected hepatocytes is thought to be the cause of fulminant HBV. This is why many fulminant HBV patients have no indication of HBV replication at the presentation **[53]**.

In adults, the risk of developing fulminant HBV is less than 1% . Chronic HBV infections are described as the presence of HBsAg in the serum of an infected person for at least six months or the presence of HBsAg in a patient who is negative for immunoglobulin (IgM) antibodies to HBcAg **[54]**.

Around 90% of babies infected perinatally with HBV and 30% of children horizontally infected with HBV develop chronic infections, on the other hand, only 5-10% of adult infections convert chronic. After 10-30 years, the majority of chronically infected individuals develop HBeAg-positive chronic hepatitis B.

As a result, a physical examination would be normal in the vast majority of cases. Jaundice, peripheral edema, ascites, and encephalopathy, can be present in patients with decompensated cirrhosis **[55]**.

Many patients' serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are mild to moderately elevated. There are numerous typical patterns of chronic hepatitis B (CHB) acquired in adult or later childhood: first, infection with a wild type HBV variant, with high HBV DNA, HBeAg positivity, high ALT, active liver disease and there is the classic necro inflammatory state **[56]**.

Second, infection with a procure mutant virus, which has recently become much more widespread than wild-type virus **[57]**.

Despite significant HBV DNA replication and elevated ALT, HBeAg is negative after infection with a precore mutant virus. Third, there is a low or non-replicative period, in which serum ALT is normal, anti-HBe antibodies are common, HBeAg is negative, and HBV DNA is low or undetectable, this situation is described by the partial immune control of HBV infection **[58]**.

1.7 Diagnosis of HBV

There are several methods used to diagnose HBV mainly serological tests that used to detect HBV serological markers; HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, and anti-HBc-immunoglobulin M (IgM) are HBV serological markers. HBV infections are indicated by the presence of HBsAg. Anti-HBs is a protective antibody that indicates immunity to HBV. It is found in patients who have had their hepatitis B infections resolved and in people who have been inoculated with the hepatitis B vaccine. Patients with acute hepatitis B and CHB reactivation are more likely to have anti-HBc-IgM positivity **[41]**.

An immunoglobulin G (IgG) antibody is the most common anti-HBc antibody, it is positive in most cases as long as people are infected with HBV, regardless of whether the virus is eliminated or not. Quantitation of HBsAg in the blood can be used to predict disease progression, antiviral effectiveness, and prognosis **[59]**.

The real-time quantitative polymerase chain reaction (RT-PCR) used to detect HBV DNA, genotype, and mutation. HBV-DNA quantification is primarily used to assess the extent of viral replication in chronic HBV infections, it's also used to select antiviral therapy indications and assess efficacy. Because of its high sensitivity and accuracy, the real-time quantitative PCR approach is recommended **[60].**

Several biochemical testes which in majority used to monitoring hepatitis diseases mainly including serum ALT, AST, and serum bilirubin.Serum ALT and AST levels are the most widely used markers for show degree of hepatic cell injury. Hepatic cell damage, intrahepatic and extrahepatic biliary tract obstruction and hemolysis are the most common causes of elevated serum bilirubin. Patients with hepatic failure may experience a gradual rise in serum bilirubin levels, with an increase of more or equally 1 time upper limit of normal (ULN) per day, and a divergence phenomenon (i.e. bilirubin elevation and ALT and AST decrease) may occur **[61]**.

1.8 Prevention

1.8.1 Vaccination

The center for disease control and prevention (CDC) recommends the routine vaccination of all children under the age of 19 with the hepatitis B vaccine. They also recommend it for those who desire it or are at high risk **[62]**.

1.8.2 Behavioral Changes

As hepatitis B are transmitted through blood and multiple bodily fluids, prevention is aimed at screening blood prior to transfusion, abstaining from the use of injection drugs, safe needle and sharps practices in healthcare settings, and safe sex practices **[63]**.

1.9 Treatment

The treatment of hepatitis varies according to the type, whether it is acute or chronic, and the severity of the disease**.**

Chronic hepatitis B management aims to control viral replication, which is correlated with progression of disease **[64]**.

1.10 Iron Regulatory Parameters

One of the important trace essential minerals, iron, controls the differentiation and development of living cells, as well as Iron may interact and carry oxygen to various areas of the body via transfer of electrons across cells, which has an influence on genome synthesis, as well as contribute in a variety of metabolic process that are necessary to life **[65]**.

The liver is a major storage organ for iron. Approximately one-third of the body's total iron is deposited in hepatocytes, sinusoidal mesenchymal cells, and reticuloendothelial cells **[66]**.

liver plays an important role in iron metabolism. It is the major production site of the iron regulatory peptides: ferritin, transferrin, and hepcidin. Liver derangements, therefore, have a direct effect on iron regulation **[67]**.

Abnormal serum iron markers have been observed in chronic HBV infection, but earlier findings seemed to suggest that altered serum iron levels were associated with chronic HBV infection. Chronic HBV infection may be or may not be accompanied by liver injury, whereas altered iron metabolism could be a reflection of pathological changes in the organs, such as the liver, involved in iron metabolism **[68]**.

1.10.1 Hepcidin

Hepcidin, a 25-amino acid peptide in humans is encoded by the *HAMP* gene, human hepcidin, also known as liver-expressed antimicrobial peptide-1, or LEAP-1, was first discovered and characterized as a highly disulfide-bonded peptide with antimicrobial activity **[69]**.

Hepcidin is an acute phase reactant protein that is produced and secreted predominantly by hepatocytes **[70]**.

Hepatocytes express 15-1500 times more hepcidin than other cells in the body, thus making them the primary source of hepcidin **[71]**.

Hepcidin is the primary hormone responsible for regulating iron homeostasis. It does this by bonding to ferroportin (FPN1), which leads to its ubiquitination **[72]**.

Hepcidin activity relies on interactions with ferroportin. Hepcidin regulates ferroportin expression. Hepcidin binds to ferroportin, causing it to be internalized and destroyed in endolysosomes. When iron stores are large, increased hepcidin expression lowers intestinal iron absorption and macrophage iron release. Iron deficiency reduces hepcidin production. **[73]**. figure 1-3.

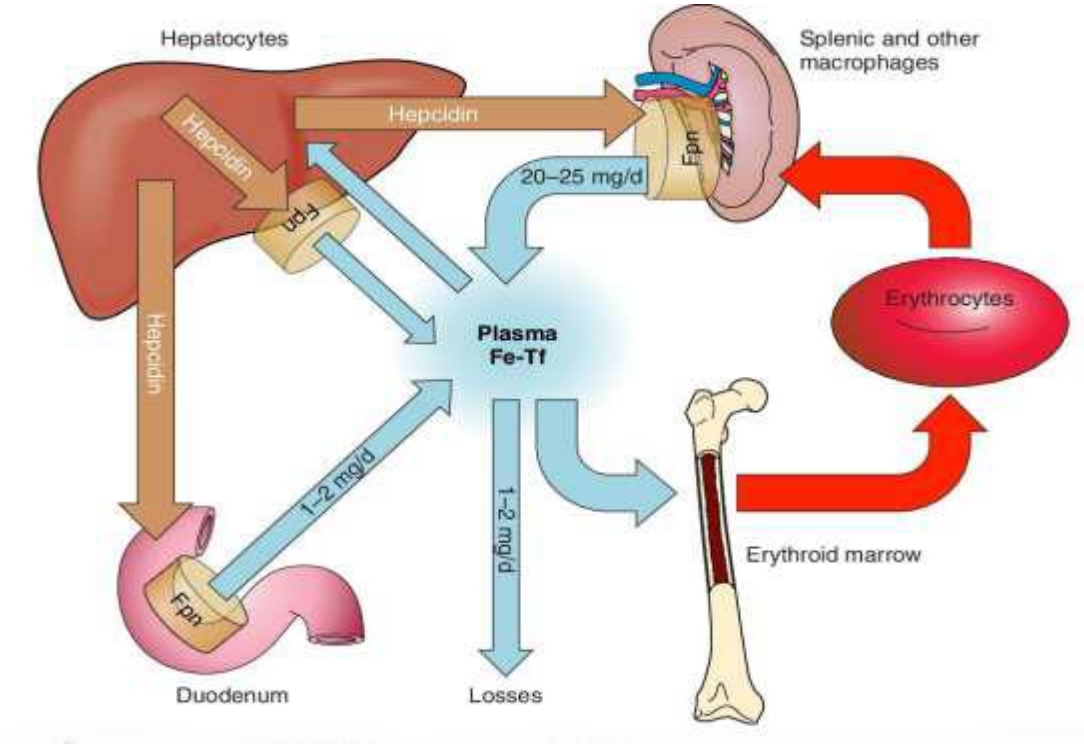

Fig. 1-3: The regulation of major iron flux by hepcidin and ferroportin [73].

Iron found in transferrin is represented by the color blue, whereas iron found in erythrocytes is shown by the color red. Through activating the endocytosis and proteolysis of the iron exporter ferroportin, hepcidin is able to regulate the amount of iron that enters the plasma (brown).

Hepcidin expression is up-regulated by excess iron or inflammation, whereas increased erythropoiesis and reduced iron stores all down-regulate hepcidin expression **[74]**.

In this way hepcidin protects us from iron-overload. This role of hepcidin is important since there is no known excretory pathway for body iron. Keeping iron in check through hepcidin is vital for our cells, because high levels of iron saturate the capacity of the proteins to keep iron in bound form **[73]**.

1.10.2 Hepcidin and Chronic HBV

Hepcidin is a central regulator of iron metabolism. As hepcidin is produced mainly by hepatocytes, pathologic changes in the liver may affect hepcidin production. Abnormal hepcidin expression has been reported following liver injury, including liver cirrhosis, alcoholic liver disease, and chronic hepatitis B **[75]**.

Hepcidin expression in the setting of chronic viral hepatitis has been extensively studied, but remains controversial. **[76]**.

In chronic HBV infection levels of hepcidin change; they rise in early stages of the disease, only to be reduced in the cirrhotic stage of the disease, reflecting the inability of hepatocytes to control hepcidin levels caused by destruction of liver architecture during fibrosis **[77]**.

Differences in pathophysiological mechanisms induced by HBV explain why hepcidin levels have a specific mode of fluctuation in these infections; they include differences in the level of oxidative stress, level of viral load and presence of inflammation **[73]**.

1.10.3 Ferritin

Ferritin is highly symmetrical and persistent iron-containing protein that was crystallized, named, and discovered in 1937. Because it contains a huge cavity that really can store a lot of iron, it was named the major iron storage protein **[78]**.

Ferritin's capacity to bind iron ions and promote mineralization among those ions by coupling its ferroxidase action with the chemical characteristics of the cavity environment is one of its most essential features, The mineral core, which may contain reach to 4000 Fe atoms in mineral form, is protected and kept in solution by the protein covering**[79]**.

Ferritin would be almost abundant, and it has a wide range of functions. It's mostly present in the cytoplasm, but it's also find in the nucleus, mammalian mitochondria, plant plastids, insect endoplasmic reticulum (ER), and circulation plasma. Ferritins have such a ferroxidase action that uses the same chemicals as the harmful Fenton reaction, thus they operate as antioxidants and control the availability of ferrous iron within cells. Over the last few decades, periodic reviews on ferritin have appeared, each focusing on a different aspect of this molecule **[80]**.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO\bullet$$

(Fenton's Reaction) [80].

1.10.4 Ferritin and Chronic HBV

Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body **[81]**.

Several studies have shown that serum ferritin can be increased in chronic liver diseases due to hepatitis C or B and alcohol. It has been shown that increased ferritin has important prognostic significance in chronic liver disease **[82]**.

In a large group of patients with chronic viral Hepatitis , shown that more than one third had elevations in serum ferritin levels, Thus, many patients with chronic hepatitis have abnormal results of serum ferritin status tests that are probably clinically significant. The most plausible reason individuals with chronic hepatitis have elevated serum ferritin levels in the absence of increased hepatic iron stores is that the serum levels reflect increased release of iron from damaged liver cells, because the increase in serum ferritin correlated strongly with the degree of hepatic injury (as measured by the serum AST activity) **[83]**.

The determination of serum ferritin levels is a simple test and is both reproducible and inexpensive, forming part of the routine investigations of patients being assessed for liver diseases to determine iron homeostasis **[84]**.

1.11 Sodium Taurocholate Co-transporting Polypeptide (NTCP)

Sodium taurocholate co-transporting polypeptide (NTCP) also known as sodium/bile acid cotransporter) or liver bile acid transporter (LBAT), is a protein in human encoded by SLC10A1(Solute carrier family 10 member 1) gene **[85]**, with highly expressed on the sinusoidal membranes, plays a crucial role in bile duct enterohepatic circulation and regulating functions of the hepatocytes **[86]**.

In the human genome, NTCP gene is located on band q 24.1 of chromosome 14 and consist of 5 exons, human NTCP encodes a 349-amino acid (approximately 45 kDa) membrane glycoprotein that

functions as an electrogenic sodium-solute cotransporter to mediate the uptake of all the major glycine/taurine-conjugated bile acids. As a cotransporter, NTCP binds two sodium ions and one (conjugated) bile salt molecule, thereby providing a hepatic influx of bile salts. NTCP is molecule that influences the outcome of HBV infection. HBV enters into the hepatocytes via a recently identified entry receptor, sodium taurocholate co-transporting polypeptide (NTCP), which specifically interacts with the pre-S1 region of HBV **[87]**.

Due to the high NTCP expression in the sinusoidal membrane of hepatocytes and its extreme high affinity to conjugated bile acids, NTCP efficiently extracts them from the portal blood and thereby maintains enterohepatic circulation of bile acids and keeps plasma concentrations at minimum **[88]**.

The functional receptor mediating HBV entry into hepatocytes remained unknown for more than 20 years since the discovery of the virus, until NTCP was identified as being critical for preS1 binding and HBV infection . NTCP is located on the basolateral membrane domain (bloodside) of hepatocytes and is broadly expressed in humans, rats, and monkeys, among other species **[89]**. NTCP is expressed exclusively in the liver although the crystal structure of hNTCP has not been solved, a series of modeling, mutagenesis, and biochemical analyses suggest that this protein has a putative nine transmembrane domains with a topology predicted to consist of an extracellular N-terminus and an intracellular C-terminus figure 1-4. Which region(s) of NTCP is essential for viral attachment and the subsequent triggering of internalization remains incompletely understood **[90]**.

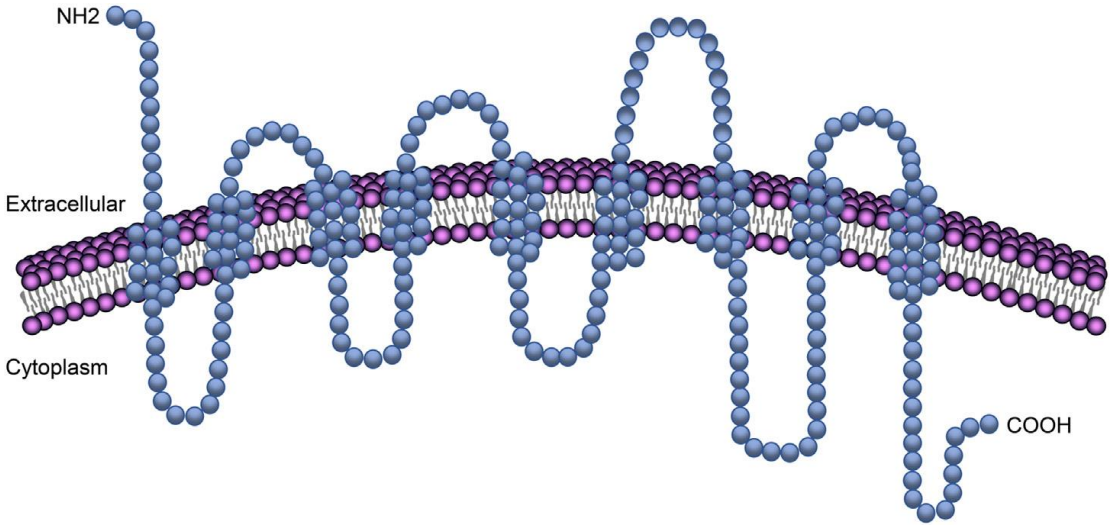

Figure 1-4: NTCP transmembrane domains in the plasma membrane. The transmembrane protein NTCP has a putative nine transmembrane domains with atopology predicted to consist of an extracellular N-terminus and an intracellular C-terminus. [90]

Current evidence suggests that hNTCP residues 157 to 165 are important for preS1 binding and, thus, HBV infection . By analyzing the susceptibility of NTCP variants to HBV attachment, showed that a single amino acid at position 158 of NTCP is critical for HBV binding and subsequent infection. Similarly, demonstrated that the sequence of amino acid 158 was a determining factor for the attachment of the HBV envelope protein to the host cell **[91]**.

In addition, it was found that the molecular determinants for the transporter function of NTCP overlapped with those for its ability to support HBV entry. HBV infection into host hepatocytes involves multiple steps. In the initiation of infection, HBV reversibly attaches to heparin sulfate proteoglycans on the cell surface via a highly conformational determinant region 1) of the HBsAg glycoprotein. HBV subsequently interacts with its specific receptor with high affinity, thereby triggering viral internalization **[92]**.

After endocytosis-mediated internalization, the virus fuses with the cellular membrane compartment. However, the precise mechanisms are

not yet fully understood. NTCP mediates the specific binding of HBV to the host cell surface with high affinity by interacting with the preS1 region of the large surface protein (LHB) of HBV. NTCP confers HBV susceptibility to human hepatic cell lines such as HepG2, Huh7, or undifferentiated HepaRG cells, which are originally non susceptible to infection **[93]**.

This easily manipulated model of cell infection has been used to decipher the early steps of HBV entry. However, the expression of NTCP alone is not sufficient for efficient HBV internalization into hepatocytes, and additional host factors are likely to be required for susceptibility to HBV infection, potentially through the formation of a complex and a multistep entry process **[94]**.

For example, host epidermal growth factor receptor (EGFR) was reported to interact with NTCP and mediate HBV internalization, a finding that potentially accounts for the low rate of infection in the HepG2 cell line, in which EGFR expression is undetectable. Kinesin family member 4 (KIF4) regulates the levels of surface NTCP via the anterograde transport of NTCP to the cell surface, potentially also mediating HBV entry **[95]**.

1.11. 1 NTCP Gene Polymorphism and Chronic HBV Virus

Genetic polymorphisms in SLC10A1 have been found to affect NTCP protein function, several SNPs have been identified in NTCP and some of these SNPs are found in specific ethnic backgrounds. Previous studies demonstrated that specific single nucleotide polymorphisms (SNPs) alter the physiological function of NTCP including bile salt homoeostasis, HBV entry and clinical outcomes of HBV infection in diverse ethnic groups and countries **[96]**.

Recent data in Asian cohorts have shown that genetic variations in the NTCP genes might be associated with HBV infection and disease progression **[97]**. The relationship between NTCP polymorphisms and HBV infection, liver cirrhosis, or HCC is controversial, with some studies concluding that NTCP polymorphisms have been associated with resistance to HBV infection, while others have demonstrated that it promotes HBV infection. Another study demonstrated that NTCP mutation (S267F) could result in a reduction of bile acid uptake and inhibition of HBV cell entry and viral replication. Accordingly, it remains to be explored whether NTCP polymorphisms influence the susceptibility to HBV infection and the occurrence of liver cirrhosis or HCC **[98]**.

Aims of the Study

1- To evaluate the role of NTCP protein and gene polymorphism and its associated with HBV infectivity and disease progression in Babylon Province.

2- Investigate hepcidin, ferritin and iron level in patients with CHB in Babylon province.

3- To find the possibility of correlation between iron regulatory parameters with age and gender in CHB patients.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Materials and Patients

2.1.1 Chemicals and kits

Chemicals and kits that used in this study were listed in Table 2-1:

Table 2-1: Chemical and kits used in the study

No.	Chemicals	Company
1.	AddPrep Genomic DNA Extraction Kit / Cat. No.: 10023	Addbio/Korea
2.	ALT (GPT) kit	Biolabo SAS (France)
3.	AST(GOT) kit	Biolabo SAS (France)
4.	GoTaq® Probe qPCR/ Cat. No.: A6101	Promega/USA
5.	Human Hepcidin ELISA kit	BT Laboratory (china)
6.	Human Ferritin ELISA kit	BT Laboratory (china)
7.	Human NTCP ELISA kit	BT Laboratory (china)
8.	HBV Rabid test	EUGENE (china)
9.	Prothrombin time kit	Spectrum (china)
10.	Primers and probes	Macrogen (korea)
11.	Serum Iron Kit	Biolabo SAS (France)
12.	Serum albumin kit	Biolabo SAS (France)
13.	Total serum bilirubin kit	Biolabo SAS (France)

2.1.2 Instruments and Material

The instruments and equipment used in this study are shown in Table 2-2 :

Table 2-2: Instruments and equipment used in the study

No.	Instruments and Materials	Origin
1.	Autoclave	Haramaya / Japan
2.	Centrifuge EBA 20	Hettich /Germany
3.	Distillator	GFL/Germany
4.	Disposable syringes (5 mL)	Medical jet /Syria
5.	Deep Freeze	GFL / Germany
6.	Dry microtubes incubator	ae /UK
7.	EDTA tube	AFCO / Jordan
8.	ELISA system	Bio-tech instruments /USA
9.	Eppindorf tube (0.5 ml)	China
10.	Gel tube	Al-Rawan/ Iraq
11.	Microfuge IB Centrifuge	Beckman Coulter (Germany)
12.	Micropipettes	Slamed (Germany)
13.	Multiple micropipettes	Watson Nexty (Japan)
14.	Magnetic Stirrer with Hot plate	Grant / England
15.	Nanodrop	Nanodrop 2000 (USA)
16.	Stratagene Real-Time Thermal Cycler	Stratagene / USA
17.	Spectrophotometer	Cecil 7200

2.1.3 Place of Study

This study was carried out on patients attended to Gastro Intestinal Tract (GIT) and Hepatic center in Merjan Medical City, Hilla City, Babylon Province, Iraq, during the duration from August 2022 to November 2022. The diagnosis of patients was based on the clinical and laboratory examinations.

The practical side of the study was performed at the laboratory of Chemistry and Biochemistry Department/ College of Medicine in University of Babylon.

2.1.4 Study Design

This is a case control study.

2.1.5 Study Individuals

The present study included 100 subjects. The first group include 50 patients with chronic hepatitis B virus. The second group include 50 apparently healthy individuals which was the control group. The age of both group ranged between (20-60) years.

2.1.6 Research and Sampling Ethics

Depends on the following:

a- Approval of scientific committee in Chemistry and Biochemistry Department , in College of Medicine at University of Babylon.

b- Approval of scientific committee of Merjan Medical City in Hilla City, Babylon Province.

c- The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptance.

2.1.7 Patients Criteria

The sample which have collected from adult both sex male and female who have chronic hepatitis B virus.

2.1.8 Exclusion Criteria

The patients that excluded were:

- Patients with renal failure.
- Patients with autoimmune diseases like rheumatoid rrthritis.
- Obesity.
- Patients with cirrhosis and hepatocellular carcinoma.

2.1.9 Questionnaire

The socio-demographic characteristics that composed of age, gender, residence, height , weight and family history. The questionnaire shown in appendix .

2.1.10 Calculation of BMI

Body mass index is calculated by dividing weight(Kg) by height square (m^2),(BMI=weight Kg/(height)^2m^2 **[18]**.

- BMI < 18kg/m^2 is underweight.
- BMI 19-24.9 kg/ m^2 is normal.
- BMI 25 - 29.9kg/ m^2 is overweight.
- BMI 30 - 34.9 kg/ m^2 is class I obesity
- BMI 35 - 39.9 kg/ m^2 is class II obesity.
- BMI 40 kg/m_2 and above is Class III obesity.

2.1.11 Sample collection

Five milliliters of venous blood was obtained by fife ml disposable syringe, and for this two milliliters of the blood was drained into EDTA tube and mixed gently, the blood in EDTA (ethylene diamine tetra acetic acid) tube stored at -20 ˚C and used for DNA extraction while the rest 3ml of blood was drained into gel plain tube for serum preparation. Blood in plain tube was allowed to clot at 37C° for 10-15 minutes and then centrifuged at 14000 rpm for approximately 10-15 minutes then the serum are divided into four parts in labeled eppindorf tubes and given a serial number together with the patients names then stored at -20˚C until analysis.

2.2 Methods (Biochemical part)

2.2.1 Alanine Aminotransferase (ALT)

2.2.1.1 Principle of the Assay:

2-oxoglutarate + L-Alanin $\xleftrightarrow{\text{ALT}}$ **L-glutamate + pyruvate**

Pyruvate +NADH + H^+ $\xrightarrow{\text{LDH}}$ **L- Lactate +NAD^+**

The decrease in absorbance proportional to ALT activity in the specimen, is measured at 340 nm **[99]**.

2.2.1.2 Procedure:

The procedures were performed according to the manufacture's manual.

The normal range **[99]**:

For men 10-40 (IU/L)

For women 7-35 (IU/L)

2.2.2 Aspartate Aminotransferase (AST)

2.2.2.1 Principle of the Assay:

2-oxoglutarate + L-Aspartate $\xleftrightarrow{\text{AST}}$ **L-Glutamate + Oxaloacetate**

Oxaloacetate +NADH + H^+ $\xrightarrow{\text{MDH}}$ **L- Malate + NAD^+**

The decrease in absorbance proportional to AST activity in the specimen, is measured at 340 nm **[99]**.

2.2.2.2 Procedure:

The procedures were performed a cording to the manufacture's manual.

The normal range **[99]**:

For Adult 13-31 (IU/L)

2.2.3. Total Serum Bilirubin (TSB)

2.2.3.1 Principle of the Assay:

To enable the assay of TB, it is necessary to break the link between unconjugated bilirubin and albumin. This step is done by adding dimethyl sulfoxide (DMSO).

The absorbance of azobilirubin produced is proportional to the concentration of bilirubin and is measured at 550 nm (530-580) **[100]**.

2.2.3.2 Procedure:

The procedures were performed a cording to the manufacture's manual.

The normal range (0.3-1.2 mg/dl) or (5-21 µmol/l) **[100]**.

2.2.4 Prothrombin Time (PT)

2.2.4.1 Principle of the Assay:

Tissue thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When SP-NORMOPLASTIN reagent is added to normal antigoagulated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specific period of time. The time required for clot formation would be prolonged if there is a deficiency of factors / factor activity in the extrinsic pathway of the coagulation mechanism **[101]**.

2.2.4.2 Procedure:

The procedures were performed a cording to the manufacture's manual.

The normal range (10-14 seconds) **[101]**.

2.2.5 Serum Albumin

2.2.5.1 Principle of the Assay:

In buffer solution at PH 4.2 bromocresol green binds albumin to form a colored compound which absorbance, measured at 630 nm (620-640) is proportional to the albumin concentration in the specimen **[102]**.

2.2.5.2 Procedure:

The procedures were performed a cording to the manufacture's manual.

The normal range (3.4-4.8 g/dl) or (512-722 µmol/l) **[102]**.

2.2.6 Serum Iron

2.2.6.1 Principle of the Assay:

After dissociation of iron- transferrin bound in acid medium, ascorbic acid reduces Fe^{3+} iron into Fe^{2+} iron. Fe^{2+} iron then form a colored complex with 3-(2-Pyridyl) -5, 6- difuryl-1, -2,-4-triazine-disulfonate (Ferene). The absorbance thus measured at 600nm (580-620) is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent to prevent the copper interference **[103]**.

2.2.6.2 Procedure:

The procedures were performed a cording to the manufacture's manual.

The normal range **[103]**:

For men	65-175(µg/dl)	11.6-31.3 (µmol/l)
For women	50-170 (µg/dl)	9.0- 30.4 (µmol/l)

2.2.7 Determination of Human Hepcidin (HEPC) Concentration

Hepcidin concentration is measured by enzyme linked immunosorbent assay kit [Bioassay Technology Laboratory].

2.2.7.1 Assay Principle

This ELISA kit uses sandwich–ELISA as the method for the accurate quantitative detection of human HEPC. The plate has been pre-coated with human HEPC antibody. HEPC present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human HEPC Antibody is added and binds to HEPC in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated HEPC antibody. After incubation unbound Streptavidin-HRP is washed away during a

washing step. Substrate solution is then added and color develops in proportion to the amount of human HEPC. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm **[104]**.

2.2.7.2 Reagents Preparation

Wash buffer

A volume of 20 ml of concentrated wash buffer was diluted into 480 ml of distilled water to yield 500 ml of washing buffer.

Standard:

Reconstituted the 120 µl of the standard (4800pg/ml) with 120 µl of standard diluent to generate a 2400pg/ml standard stock solution.

The lid was tightened and the standard was let to stand for 15 minutes and turned it upside down for several times. Reconstitution was produced a stock solution of 2400pg/ml. Then serial dilutions were made as needed. The recommended concentrations were as follows:2400, 1200, 600, 300, 150 and 0 pg/ml.

2.2.7.3 Assay procedure

1- All reagents were brought to room temperature before use.

2-Fifty microliter of the each standard was added to the standard well.

3- A volume of 40 µl of sample and then 10 µl of hepcidin antibody were add to testing sample wells.

4- A volume of 50 µl of streptavidin -HRP reagent was dispensed into each well covered with a sealer and incubated for 60 minutes at 37 C˚.

5- The wash process was repeated for five times by filling each well with wash buffer (approximately 300 µl).

6- The liquid was removed at each step was essential to good performance then remaining wash solution was removed by aspirating and the plate was invert and blot it against clean paper towels.

7- A 50 µl volume of substrate solution A and 50 µl volume of substrate solution B were add to each well.

8-The microplate wells covered with a new sealer then incubated for 10 minutes at 37 C˚.

9-The reaction was stopped by adding 50 µl of stop solution to each well. The color was changed from blue to yellow color.

10-The absorbance was read the optical density at 450 nm using microtiter plate reader within 10 minutes. The standard curve is depicted in figure 2-1.

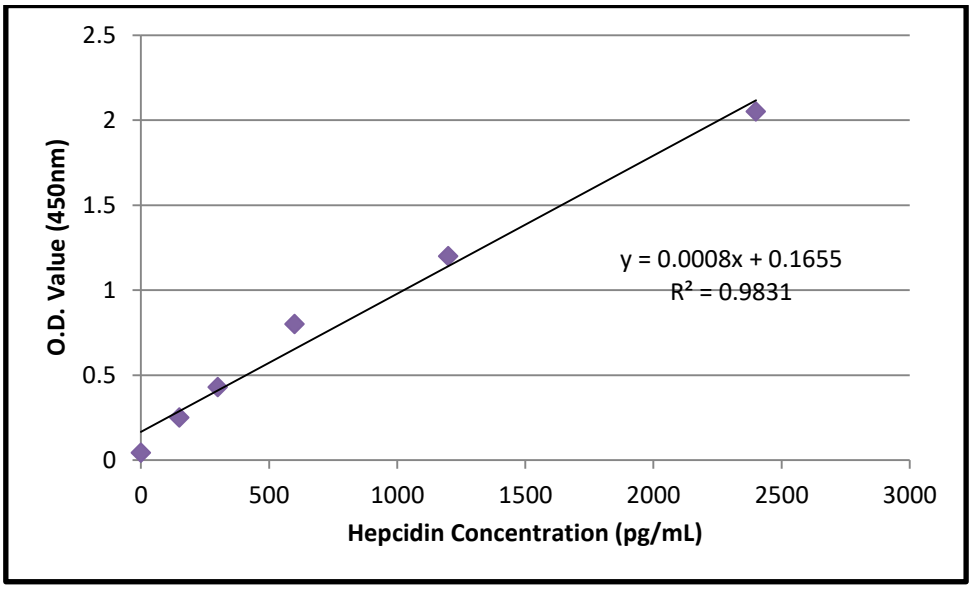

Figure 2-1 : **Standard Curve for Hepcidin Concentration by ELISA**

2.2.8 Determination of Serum Human Ferritin (FE) Concentration

Ferritin concentration is measured by enzyme linked immunosorbent assay kit (ELISA). [Bioassay Technology Laboratory].

2.2.8.1 Assay Principle

This ELISA kit uses sandwich–ELISA as the method for the accurate quantitative detection of human Ferritin. The plate has been pre-coated with human FE antibody. FE present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human FE Antibody is added and binds to FE in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated FE antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human FE. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm **[105]**.

2.2.8.2 Reagents Preparation:

Wash buffer

A volume of 20 ml of concentrated wash buffer was diluted into 480ml of distilled water to yield 500 ml of washing buffer.

Standard: Reconstituted the 120 µl of the standard (480ng/ml) with 120 µl of standard diluent to generate a 240ng/ml standard stock solution.

The lid was tightened and the standard was let to stand for 15 minutes and turned it upside down for several times. Then serial dilutions were made as needed. The recommended concentrations were as follows:240, 120, 60, 30, 15 and 0 ng/ml.

2.2.8.3 Assay procedure

1- All reagents were brought to room temperature before use.

2- Fifty microliter of the each standard was added to the standard well.

3- A volume of 40 µl of sample and then 10 µl of ferritin antibody were add to testing sample wells.

4- A volume of 50 µl of streptavidin -HRP reagent was dispensed into each well .Covered with a sealer and incubated for 60 minutes at 37 C˚.

5- The wash process was repeated for five times by filling each well with wash buffer (approximately 350 µl).

6- The liquid was removed at each step was essential to good performance then remaining wash solution was removed by aspirating and the plate was invert and blot it against clean paper towels.

7- A 50 µl volume of substrate solution A and 50 µl volume of substrate solution B were add to each well.

8- The microplate wells covered with a new sealer then incubated for 10 minutes at 37 C˚.

9- The reaction was stopped by adding 50 µl of stop solution to each well. The color was changed from blue to yellow color .

10- The absorbance was read the optical density at 450 nm using microtiter plate reader within 10 minutes. The standard curve is depicted in figure 2-2.

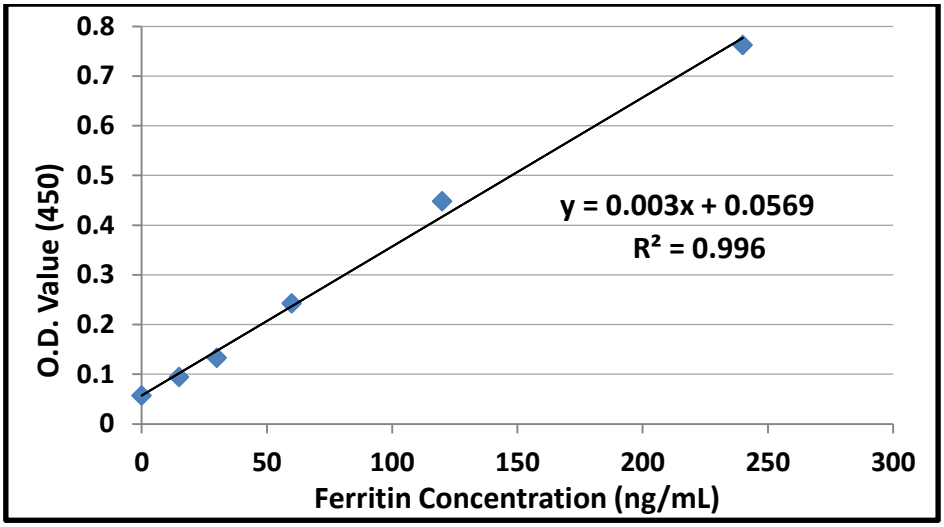

Figure 2-2: Standard Curve for Ferritin Concentration by ELISA

2.2.9 Determination of Serum Human Sodium Taurocholate Cotransporting Polypeptide Concentration

Human Na+ Taurocholate Cotransporting Polypeptide concentration is measured by enzyme linked immunosorbent assay kit (ELISA). [Bioassay Technology Laboratory].

2.2.9.1 Assay Principle

This ELISA kit uses sandwich–ELISA as the method for the accurate quantitative detection of human NTCP. The plate has been pre-coated with human NTCP antibody. NTCP present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human NTCP Antibody is added and binds to NTCP in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated NTCP antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human NTCP. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm **[106]**.

2.2.9.2 Reagents Preparation:

Wash buffer

A volume of 20 ml of concentrated wash buffer was diluted into 480ml of distilled water to yield 500 ml of washing buffer.

Standard: Reconstituted the 120 µl of the standard (2400 ng/L) with 120 µl of standard diluent to generate a 1200 ng/ml standard stock solution. The lid was tightened and the standard was let to stand for 15 minutes and turned it upside down for several times. Then serial dilutions were made as needed. The recommended concentrations were as follows:600, 300, 150, 75 and 0 ng/ml.

2.2.9.3 Assay procedure

1- All reagents were brought to room temperature before use .

2-Fifty microliter of the each standard was added to the standard well.

3- A volume of 40 µl of sample and then 10 µl of NTCP antibody were add to testing sample wells.

4- A volume of 50 µl of streptavidin -HRP reagent was dispensed into each well covered with a sealer and incubated for 60 minutes at 37 C˚.

5- The wash process was repeated for five times by filling each well with wash buffer (approximately 350 µl).

6- The liquid was removed at each step was essential to good performance then remaining wash solution was removed by aspirating and the plate was invert and blot it against clean paper towels.

7- A 50 µl volume of substrate solution A and 50 µl volume of substrate solution B were add to each well.

8-The microplate wells covered with a new sealer then incubated for 10 minutes at 37 C˚ .

9- The reaction was stopped by adding 50 µl of stop solution to each well. The color was changed from blue to yellow color .

10- The absorbance was read the optical density at 450 nm using microtiter plate reader within 10 minutes. The standard curve is depicted in figure 2-3.

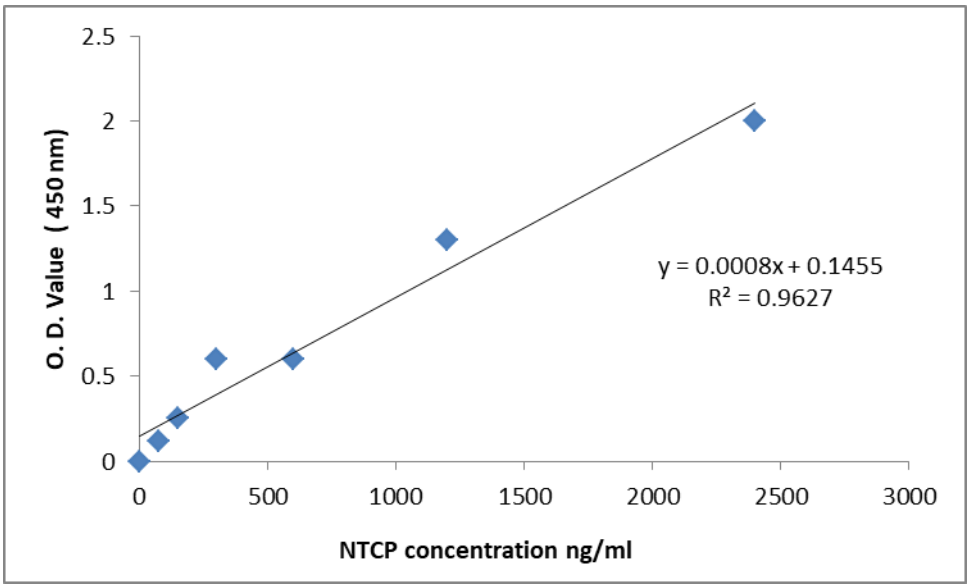

Figure 2-3: Standard curve for NTCP concentration by ELISA

2.3. Serological Test to HBV

2.3.1 Principle

HBsAg and HBeAg:

The HBsAg and HBeAg tests are qualitative, two-site sandwich immunoassays for the detection of HbsAg or HbeAg in serum. The membrane is pre-coated with anti HBsAg or anti HBeAg antibodies on the test line region of the strip. During testing, the serum specimen reacts with the particle coated with anti-HBsAg or anti-HBeAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg or anti-HbeAg antibodies on the membrane and generated a colored line. The presence of this colored line

in the test line region indicates a positive result, while its absence indicates a negative result **[107]**.

HBsAb:

Hepatits B surface antibody (HBsAb) is also known as anti-hepatitis B surface Antigen (anti-HBs). This test is a qualitative, lateral flow immunoassay for detection of HBsAb in serum. The membrane is pre-coated with HBsAg on the test line region of the strip. during testing ,the serum specimen reacts with the particle coated with HBsAg. the mixture migrates upward on the membrane chromatographically by capillary action to react with HBsAg on the membrane and generated a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result.

HBeAb and HBcAb:

Hepatits B envelope antibody (HBeAb) is also known as anti-hepatitis B envelope Antigen (anti-HBe). Hepatits B core antibody (HBcAb) is also known as anti-hepatitis B core Antigen (anti-HBc). These tests are immunoassays based on the principle of competitive binding.

For **HBeAb**, the membrane is pre-coated with anti-HBe antibody on the test line region of the strip. During testing, if anti-HBe antibody present in the specimen, it will combine with the HBeAg in the sample pad and particle coated anti-HBe antibody and compete for limited amount of HBeAg in the sample pad with anti-HBe antibody on the membrane. No line will form in the test region. And a visible colored line will form in the test region if there is no anti-HBe antibody in the specimen because all the antibody coated particles combined with the

antigen in the sample pad will be captured by the antibody on the test region.

For **HBcAb**, the membrane is pre-coated with anti-HBc antibody on the test line region of the strip. During testing, anti-HBc antibody, if present in the specimen, will compete with the anti-HBc antibody coated on the test region for limited amount of particle coated HBcAg, and no line will form in the test line region, indicating a positive result. a visible colored line will form in the test line region if there is no anti-HBc antibody in the specimen because all the HBcAg coated particles will be captured by the anti-HBc antibody coated in the test line region.

To serve as a procedural control, a colored line will always appear in the control line region. If the control line does not appear, the test result is not valid.

2.3.2 Procedure

- The test device and specimen was allowed to equilibrate to room temperature (15-30 °C) before testing.
- The pouch was brought to room temperature before opening it. The test device was removed from the sealed pouch and used as soon as possible. The best results were obtained when the assay was performed within one hour.
- The test device was placed on a clean and level surface. By micropipette was transferred 75 µl of serum to each specimen well (S) of the test device respectively, with avoid trapping air bubbles in the specimen well (S), then started the timer.
- Waited for the red line(S) to appear. The result was read at 15 minutes.

2.3.3 Interpretation of results

HBsAg, HBsAb, HBeAg:

Positive:

Two red lines appeared. one line was in the control line region (C) and another line was in the test line region (T).

The intensity of the color in the test line region (T) was vary depending on the concentration of HBsAg, HBsAb, and HBeAg present in the specimen. Therefore, any shade of red in the test line region (T) should be considered positive.

Negative:

One red line appears in the control line region (C). No apparent red or pink line appears in the test line region (T).

HBeAb , HBcAb

Positive:

One red line appears in the control line region (C). No apparent red or pink line appears in the test line region (T) or the line intensity in the test region)T(is obviously weaker than in control region (C).

Negative:

Two red lines appear, and the line intensity in the test region(T) should be equivalent to or stronger than control region (C).

2.4 Methods (Genetic part)

2.4.1 DNA Extraction

DNA extraction from whole Blood by (AddPrep Genomic DNA Extraction Kit)

2.4.1.1 Principle (Silica Based Technology):

Silica matrices have special properties for DNA binding. They have positive charge and consequently they have large affinity for the negative charge of the DNA.

High salt conditions and pH are performed to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column, by using sodium cations that bind strongly to the negatively charged oxygen of the DNA phosphate.

Contaminants were removed with multiple washing steps by using a wash solution (containing ethanol) and the purified genomic DNA was eluted by low ionic strength (pH ≥7) through TE buffer. These methods was faster and simpler procedure than other methods like organic extraction method **[108]**.

2.4.1.2 DNA Extraction Procedure

The protocol of DNA extraction from blood involved several steps:

1. Two hundred µl of blood was carry over to a 1.5 ml micro centrifuge tube, then 200 µl of lysis was added.
2. Twenty µl of proteinase K (20mg/ml) was added and briefly mixed. Then it was incubated for 15 minutes at 56℃.
3. Two hundred µl of binding solution was added to the samples then mixed by shaking vigorously.
4. The sample was incubated in a 56℃ water bath for 10minutes, during incubation the sample was inverted every 3 minutes.

5. Two hundred µl of absolute ethanol was added to the test and vortex for 15 second.
6. The lysate was transfer into the upper reservoir of the spin with 2ml collection tube.
7. The sample were centrifuged for 1 minutes at full speed (13,000 rpm).
8. The 2ml collection tubes containing the flow-through were discared, after that the spin column was placed in a new 2ml collection tube.
9. The spin column was washed with 500 µl washing 1 solution and centrifuged for 1 minutes at (13,000 rpm) then the flow-through was discarded.
10. The spin column was putted back in the 2ml collection tube and washed with 500 µl of washing 2 solution then centrifuged for 1 minute at (13,000 rpm) and discarded the flow-through.
11. The spin column was placed back in the 2ml collection tube and centrifuged for additional 3 min to dry the column.
12. The spin column was placed to a new 1.5 ml microcentrifuge tube.
13. One hundred µl of Elution solution was added to the spin column and the spin column stayed standing at least 1 min.
14. Centrifuged for 1 minutes at (13,000 rpm) in order to elute the purified DNA.
15. The DNA fragment was stored at 4°Cor -20°C.

2.4.2 Determination of Concentration and Purity of DNA

Agarose gel electrophoresis and spectrophotometric methods were used to measure the concentration and purity of extracted DNA.

2.4.2.1 Agarose Gel Electrophoresis Method

The quality of the isolated DNA was assessed by running 8 μL of each DNA sample on 1% agarose gel stained with RedSafe nucleic acid staining solution, then DNA sample was visualized by U.V. transilluminator according to Harisha method, figure 2-4 **[109]**.

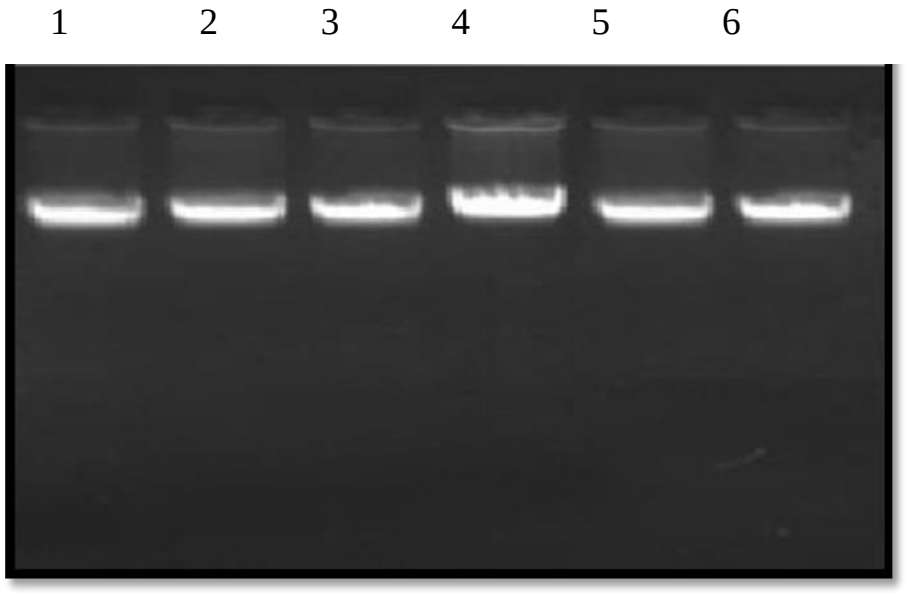

Figure 2-4: lanes 1-6 represents agarose gel electrophoresis for extracted DNA

2.4.2.2 Spectrophotometric Method

The purity and concentration of DNA were measured by nanodrop instrument (absorbance method) **[110]**.

Absorbance readings were accomplished at 260 nm and at 280 nm. At 260 nm, the DNA strongly absorbs light while at 280 nm, the protein absorbs light most strongly. DNA purity can be estimated by A260/A280

ratio. The A260/A280 ratio between 1.7 and 2.0 is generally accepted and it represent a high quality DNA sample **[110,111]**.

2.4.2.3 Procedure:

- One microliter of distilled water was applied on the highly sensitive micro detector of nanodrop as blank.
- The micro detector was cleaned up from blank.
- Then 1 µL of sample was applied on the micro detector of nanodrop.
- The concentration and A260/A280 ratio of DNA were recorded from the instrument **[112]**.

2.4.3 Detection of Single Nucleotide Polymorphism

Detection of Human SNP rs2296651 G/A in the NTCP gene by Probe-based Real-Time quantitative polymerase chain reaction (RT-qPCR) technique, figure 2-5.

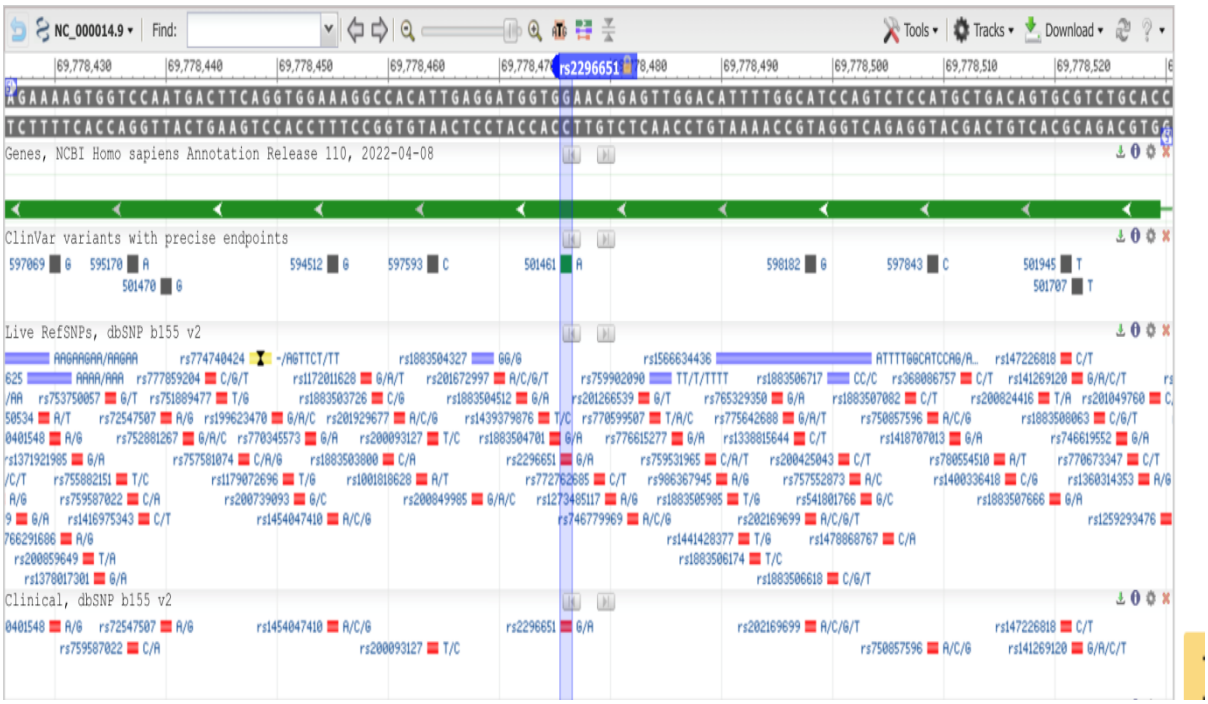

Figure 2-5: Location of SNP rs2296651 G/A in the gene *SLC10A1* of *Homo sapiens.* The adjacent SNPs are also highlighted as red boxes. (Source: https://www.ncbi.nlm.nih.gov/snp/rs2296651)

2.4.5 Primers of Amplification:

The primer that have been used in Probe RT-qPCR reaction for amplified the SNPs (rs2296651 G>A) in the NTCP gene have been designated by UGene lab, as shown in Table 2- 3.

Table 2-3 :The forward and reverse primer for the SNP (rs2296651 G/A) by RT-qPCR assay with the product size.

Target gene	Primer name	Sequence (5'-3')	Ta (°C)	Product size	Reference	Accession number
SLC10A1	F	GGTCCAATGACTTCAGGTGGAAAG	56	99 bp	UGene lab	HQ447437.1
	R	CAGGTGCAGACGCACTGTC				
	Allele G	FAM-5'-CATTGAGGATGGTGGAACAG-3'-BHQ1	60			
	Allele A	HEX-5'-CATTGAGGATGGTGAAACAG-3'-BHQ1	60			

2.4.6 Preparation of Primers

According to instruction of the primer synthesiser company, the primers (originally lyophilized), were dissolved in the free ddH_2O to obtain a final concentration of 100 µM/µl which served as a stock solution that stored at -20 °C. A concentration of 10 µM/µl was prepared from the stock primers to be used as a work primer.

2.4.7 Real-Time PCR assay

All the suspected samples were used in the implementation of the conventional Real-Time PCR. One pair of specific primers was used in the amplification process of the conserved region of gene *SLC10A1* of *Homo sapiens*.

The SNP rs2296651 G/A was genotyped using primers and probes specific for this SNP. Reactions were conducted in 0.2 µl wells, in a total 20 ml volume using 2 ng of genomic DNA and a GoTaq® Probe qPCR

Master Mix (Promega, USA). The wells were then positioned in a thermal cycler (Stratagene, USA) and heated for 10 min at 95℃, followed by 40 cycles of 95 C˚ for 15 s and 60 C˚ for 1 min. The genotyping success rate was better than 95%, with a calculated error rate based on PCR duplicates of less than 1%.

The reaction components for PCR amplification of NTCP genes that give the best results were described in Table 2-4.

Table 2-4: Preparation of PCR solutions

Components	Concentration	Volume (20 µl)
ddH_2O	-	4 µl
GoTaq® Probe qPCR Master Mix	1X	10 µl
Forward primer	10 µM/µl	1 µl
Reverse primer	10 µM/µl	1 µl
Probe 1	10 µM/µl	1 µl
Probe 2	10 µM/µl	1 µl
DNA	40 ng	2 µl

2.4.8 Optimization of Real-Time qPCR Conditions

The PCR thermocycler program that gave the best results of amplification of NTCP gene rs2296651 G > A are shown in Table 2-5.

Table 2-5: RT-qPCR conditions

Phase	Tm (°C)	Time	Cycles
Initial denaturation	95°C	**10 min**	**1X**
Denaturation	95°C	**15 sec.**	**40X**
Annealing	60°C	**1 min**	
Final Extension	72°C	**30 sec**	

2.5 Statistical Analysis

In the current study a t-test was performed to determine whether group variance was significant or not, the difference were considered significant when the P value less than 0.05.

Data were expressed as mean ± standard deviation in expression of results. Statistical analyses were performed with SPSS (version 20).

Chapter Three
Results and Discussion

3. Results and discussion

3.1 General characteristic of the study group

3.1.1. Age

In the present study, the age of subjects is stratified into 2 groups to elucidate the effect of age on study parameters; the results show no significant differences ($p > 0.05$) in age between control group and Chronic hepatitis B patients group; as demonstrated in Table 3-1.

This age matching helps to eliminate differences in parameters' results that may originate due to the significant variation in age.

Table 3-1: Means Age ± SD of patients and control groups.

Subjects	Number	Age (Years) Means ±SD	Range (Years)	P-value
Chronic hepatitis B group	50	38.72 ± 10.79	20-60	**P > 0.05**
Control group	50	35.52 ± 11.92	20-60	

SD: standard deviation; **non-significant at p > 0.05**

The frequency distribution of patients with Chronic hepatitis B according to age was as following: 34 (68 %) cases from 20-40 years and 16 (32%) cases between 41-60 years.

Age distribution of our study showed that most people were in the age range of 20-40 years, and this is relabel with another reviews like (Kolou M *et al*.,2017) **[113]**, who found that more than half (58.26%) of hepatitis B patients were represented by young adults (20-39 years).

Our finding is agreed with another study in Thi-Qar province, showed that high rate of hepatitis infection occur with age 21-40 years old (48.5%), while in the age group between 61-70 was (8.5 %) **[114]**.

Mode of transmission for HBV may be aid the increase of high rate infection in age group less than 30 to 40 years old. Share use of razor

blade, Botox injection, tattoo, and sexually activity are the major cause of high incidence of HBV infection **[115]**.

3.1.2. Gender

The gender of chronic hepatitis B patients and control group in the present study was selected to be equal for both males and females to avoid sex genome interference with the activity of liver enzymes interested in this study.

Table 3-2 showed that the percentage of males was 50 % and percentage of females was 50% for each patients and control, this finding was similar with the study conducted by Maya R W, 2021 **[26]**, while Ayano G *et al.*,2018 **[116]** and Ezzikouri S *et al.*,2017 **[117]** demonstrated that there was a significant increase in the risk of HBV infections in men compared to women.

Table 3-2: Distribution of Study Groups According to gender.

Gender	CHB		control	
	Count	%	Count	%
Male	25	(50%)	25	(50%)
Female	25	(50%)	25	(50%)

CHB= Chronic Hepatitis B

3.1.3 Body Mass Index (BMI)

In this study, patients with a normal body mass index were selected. Results in Table 3-3, shows non-significant differences in BMI between patients with chronic hepatitis B virus and control group.

Yu MW, *et al* in 2008 found that an increased body-mass index (BMI) has been demonstrated to worsen the disease outcome of chronic HBV.

Obesity (BMI ≥ 30 kg/m^2) was associated with increased risk of both hepatocellular carcinoma (HCC) and liver-related mortality **[118]**.

Table 3-3: Means BMI ± SD of patients and control groups.

Subjects	Number	BMI Means ±SD	P-value
Chronic hepatitis B group	50	23.08 ± 1.69	**P > 0.05**
Control group	50	22.66 ± 1.95	

BMI: Body Mass Index ;SD: standard deviation; **non-significant at p > 0.05**

3.1.4 Serological Results

Hepatitis patients in the current study were selected and for detection of their serological response to HBV, a serological tests were performed , Table 3-4.

Table 3-4: Serological panel for diagnosis of CHB patients.

NO.(%)of patients	HBsAg	HBsAb	HBeAg	HBeAb	HBcAb	Most likely interpretation
44 (88%)	+	-	-	+	+	**CHB virus carrier state**
6 (12%)	+	-	-	-	+	**Chronic state**

HBsAg: Hepatits B surface antigene; HBsAb: Hepatits B surface antibody; HBeAg: Hepatits B envelope antigene; HBeAb: Hepatits B envelope antibody; HBcAb: Hepatits B core antibody

For HBV patients, parameters including HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb were tested in immunochromatography principle using a rapid combo test device. The results showed that 44 (88%) of HBV patients had HBeAg negative, anti-HBe positive chronic hepatitis B, 6 (12 %) had HBeAg-negative chronic hepatitis infection.

The current study showed all patients were HBsAg-positive, HBcAb positive. HBsAg is the first serological marker to appear indicating active infection. Chronic infection is characterized by the persistence of this marker for more than 6 months **[119]**.

The HBcAg is intracellular and for this reason, is not detected in the serum of infected individuals. Anti-HBc appears shortly after HBsAg in acute infection and persists after the acute phase indicating a previous exposure. The IgM anti-HBc antibody is the first detected during acute infection, approximately 1 month after the onset of HBsAg, and disappears after 6 months of infection. Anti-HBc IgG remains detectable in patients cured of hepatitis B and among chronic cases of HBV infection **[120]**.

HBeAg marker indicates viral replication and risk of transmission of infection, seroconversion of HBeAg to anti-HBe is associated with remission of liver disease. But many of anti-HBe reactive subjects continue to have active viral replication and hepatic disease caused by mutations in the pre-core and core region in the HBV genome, which reduces the production of HBeAg **[119]**.

Thus CHB can be broadly divided into two major forms namely, HBeAg positive and HBeAg negative **[121]**.

The current study showed there was 88% of patients have HBeAg negative and anti-HBeAb positive, meaning that these patients either have a reduction of HBV replication activity, or that the spontaneous HBeAg loss is complicated by the emergence of a mutant form of HBV and the patients have active viral replication and liver damage.

Also the current study showed there was 2% of HBV patients have HBeAg positive, meaning these patients either have acute infection or active replication of chronic HBV infection. HBeAg-negative CHB is also referred to as anti-HBe-positive and precore mutant hepatitis, it

emerges during the course of a typical HBV infection with the wild-type virus and is selected during the immune clearance phase (HBeAg seroconversion) **[122]**.

HBeAg-negative CHB can develop either soon after HBeAg seroconversion or decades later. HBeAg negative CHB usually has an aggressive course, with rapid progression to cirrhosis and frequent development of HCC.

The annual rate of progression to cirrhosis is 8–10% in HBeAg-negative patients compared with 2–5% in HBeAg-positive patients. Long-term prognosis is poorer among HBeAg negative individuals compared with their HBeAg-positive counterparts **[123]**. Therefore, appropriate use of effective therapy is an important issue in the management of this group of patients.

Complete eradication of HBV is difficult because it tends to integrate into the host genome. Patients with HBeAg-negative CHB generally require a longer duration of treatment than those with HBeAg-positive CHB.

The recommended ALT levels for treatment initiation in these patients are different, as the ALT levels often fluctuate and may even be normal. As about 50% of HBeAg negative patients have an active liver disease with HBV DNA levels <105 copies/ml **[124]**.

All patients of the current study were HBsAb-negative. Anti-HBs is a neutralizing antibody, and its presence indicates immunity to HBV infection and vice versa in the absence of it. The simultaneous presence of anti-HBs and HBsAg has been documented in HBsAg-positive patients, probably due to the incapability of antibodies to neutralize the circulating virions. In this situation, these people are classified as carriers of HBV infection **[125]**.

3.2 Biochemical result

3.2.1 Liver Function Tests

The results of the present study showed significant differences between chronic hepatitis B patients and controls ($p < 0.05$) regarding the value of ALT, AST, TSB, PT and Albumin, Table 3-5.

Table 3- 5: Liver Function Tests in the Studied Groups.

Parameters	Mean ± SD		P-value
	Chronic HBV group N=50	Control group N=50	
AST (IU/L)	58.74±25.96	19.96±3.22	< 0.05
ALT (IU/L)	65.05±23.52	21.22±2.46	< 0.05
TSB (µmol/L)	24.19±7.17	12.64±2.39	< 0.05
Albumin (g/dl)	3.54±0.34	4.32±0.33	< 0.05
PT (second)	12.02±0.54	11.42±0.53	< 0.05

AST:Aspartate aminotransferase; ALT:Alanine aminotransferase; TSB:Total serum bilirubin; PT:Prothrombin time; N:number ; SD:standard deviation; significant at $p < 0.05$

According to liver functional tests, there was a significant association between hepatitis and each of ALT, AST, TSB, PT and albumin. Because of the liver tissue contains two different types of cells, hepatocytes and bile duct cells (cholangiocytes), enzymes as ALT and AST are found inside hepatocytes and are released into the circulation when there is hepatocellular inflammation or injury. On the other hand, inflammation of cholangiocytes can produce elevations in serum bilirubin, which may be released during intrahepatic (or extrahepatic) bile duct obstruction or inflammation **[48]**.

Our finding is concomitant greatly with Al-Haidary B *et al*., 2008 **[126]**, who showed that all liver function tests raise in all hepatitis patients at a higher rate than that in the healthy control group. While Abulude O A *et al*. 2017 **[127]**, revealed that, among HBV patients, only 75% had elevated levels of AST.

The results of current study were in line with previous studies which showed significantly elevated serum concentration of ALT, AST, and TSB in hepatitis patient compared to healthy control **[128]**.

An increase in serum AST and ALT concentrations constitute a reliable marker of necroinflammatory activity. Generally, ALT, AST, and TSB considered recognized markers for liver diseases **[129]**.

This contrasts with the findings of Saleh D A, 2015 **[130]**, who found no significant differences in the levels of ALT, AST, and total bilirubin between hepatitis B patients and control group.

ALT is a liver enzyme, it's sometimes known as serum glutamic pyruvic transaminase (SGPT), present mainly in the cytosol of the liver and in low concentration elsewhere. When liver cells are damaged, ALT leaks out into the bloodstream and elevated the level of ALT in the blood **[113]**.

On the other hand, AST is another liver enzyme also known as serum glutamic oxaloacetic transaminase (SGOT), in declining order of concentration AST, it can be found in the liver, heart, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes, and erythrocytes. High levels of AST can occur due to viral hepatitis, acute liver cell damage, in addition to several other cases such as myocardial infarction **[132]**.

In the liver, ALT is localized only in the cellular cytoplasm, whereas AST is in the cytosol and mitochondria, therefore, an increase in ALT levels is more specific for liver damage.

Bilirubin is the product of haemoglobin breakdown during normal process of breaking down red blood cells, then excreted into bile which is fluid in the liver that helps digest food **[131]**.

If the liver is healthy, it will remove most of the bilirubin from the body but when the liver is damaged, bilirubin can leak out of liver and into the blood and when too much bilirubin gets into the bloodstream, it can cause jaundice **[133]**.

Results in Table 3-5 shows decrease level of albumin concentration in chronic hepatitis B patients compared with control group. This finding was coinciding with Maulidia V N R *et al*.,2020 **[134]**, were reporting that serum albumin levels will decrease if there is impaired function of albumin synthesis in hepatocytes, and chronic liver cell lesions.

Albumin synthesis is an important function of the liver. Approximately 10 g is synthesised and secreted daily. With progressive liver disease serum albumin levels fall, reflecting decreased synthesis. Albumin levels are dependant on a number of other factors such as the nutritional status, catabolism, hormonal factors, and urinary and gastrointestinal losses. These should be taken into account when interpreting low albumin levels. Having said that, albumin concentration does correlate with the prognosis in chronic liver disease **[133]**.

The results of the study revealed that prothrombin time is prolonged in chronic hepatitis B patients compared to control subjects, Table 3-5. The synthesis of coagulation factors (except factor VIII) is an important function of the liver.

The prothrombin time measures the rate of conversion of prothrombin to thrombin (requiring factors II, V, VII, and X) and thus reflects a vital synthetic function of the liver. Vitamin K is required for the gamma carboxylation of the above named factors. Prothrombin time

may therefore be prolonged in vitamin K deficiency, warfarin therapy, liver disease, and consumptive coagulopathy **[129]**.

3.2.1.1. Association of Liver Function Tests with Age and Gender Groups of Chronic Hepatitis B Patients

Abnormal liver tests occur commonly in elderly people and are associated with a modest increase in all-cause mortality. Fleming K M *et al.*,2011 **[135]**, revealed a strong association of age with liver disease.

The result of the present study revealed non-significant association of liver function tests with different age groups, except total serum bilirubin values decline significantly with increasing age, as in Table 3-6.

This study concomitant with Schmucker D L, 1998 **[136]**, they observed that numerous tests have failed to identify significant age-associated deficits in hepatic functions.

The study of Tietz N W *et al.*, 2018 **[102]**, concluded that many liver functions were well maintained in the elderly subjects based on parameters such as hepatic enzyme profiles, and serum albumin.

Bilirubin values decline with increasing age, perhaps reflecting reduced muscle mass and hemoglobin concentration **[136]**, this explain the result of the present study. But our results contradicted the study of Cieslak K P *et al.*,2016 **[137]**, shown that liver function deteriorates with age.

Table 3- 6: Association of Liver Function Tests in Chronic Hepatitis B patients with age and gender groups.

Association of Liver Function Tests in CHB patients with age groups			
Parameters	**Age 20-40 (years) N=34 (Mean ± SD)**	**Age 41-60 (years) N=16 (Mean ± SD)**	**P value**
ALT (IU/L)	61.39 ± 24.84	72.81±18.83	> 0.05
AST (IU/L)	56.82 ± 26.67	62.83±24.69	> 0.05
TSB (µmol/L)	28.81 ± 4.4	22.01±7.2	< 0.05
Albumin (g/dl)	3.58 ± 0.35	3.46±0.32	> 0.05
PT (second)	12.02 ± 0.58	12.01 ± 0.46	> 0.05
Association of Liver Function Tests in CHB patients with gender			
Parameters	**Male (N=25) (Mean ± SD)**	**Female (N=25) (Mean ± SD)**	**P value**
ALT (IU/L)	68.8 ± 24.04	61.3 ± 22.85	> 0.05
AST (IU/L)	67.08 ± 22.87	50.4 ± 26.6	< 0.05
TSB (µmol/L)	24.38 ± 7.13	24 ± 7.35	> 0.05
Albumin (g/dl)	3.59 ± 0.33	3.54 ± 0.36	> 0.05
PT (second)	11.95 ± 0.53	12.09 ± 0.54	> 0.05

AST:Aspartate aminotransferase; ALT:Alanine aminotransferase; TSB:Total serum bilirubin; PT:Prothrombin time; N:number; SD:standard deviation; p< 0.05: significant.

This study demonstrated a significant gender difference in AST concentration. While, there was non-significant gender difference in other liver parameters. Our study is in accordance with Adiga U S, 2016 **[138]**, who suggest a low AST levels in women.

Cotler S *et al.*, 2017 **[139]**, they found decreased ALT in women. Discrepancies were noted in ALT in different genders with same living conditions. Comparatively high ALT was reported in females **[140]**.

This variation in liver enzymes is attributed to the hormonal status and difference in the muscle mass.

Rosenthal P *et al.*,2018 **[141]**, reported a declined bilirubin levels in females. This could be attributed to increased rate of conjugation and excretion of bilirubin in females due to the influence of gonadal hormones.

Contradictory reports were given by Guattery J M *et al.*,2017 **[142]**, who stated heightened bilirubin levels in biliary cirrhotic women.

Our findings on serum albumin and PT are supported by Adiga U S, 2016 **[138]**, they reported low albumin levels and prolong PT in women could be due to increased degradation, this mean the rate of protein synthesis is lower in females compared to males.

3.2.2. NTCP, Iron, Ferritin and Hepcidin Concentration in Chronic Hepatitis B Patients and Control Groups

NTCP concentration was increased significantly ($p<0.05$) in chronic hepatitis B patients compared to the control group, Table 3-7.

Both enterohepatic circulation of bile acids and hepatocyte function. Although NTCP had been known as a bile acid transporter for a long time **[86]**, a completely different function as a cell surface receptor for both HBV and HDV infection was recently uncovered **[87]**.

Interestingly, the functional determinants as HBV receptor and bile acid transporter are at least in part shared on the same NTCP protein **[143]**.

Iron is crucial to humans' biological functions and cellular biochemical processes. Both iron deficiency and iron overload pose significant and potentially fatal health risks, and the homeostasis of iron

is tightly regulated. When this regulation is disrupted leads to cellular toxicity, tissue injury, and organ fibrosis. Such harmful effects are mediated through the deposition of iron in parenchymal cells of a number of vital organs, including the heart, pancreas, and liver **[144]**.

The liver plays a fundamental role in recycling iron as the organ synthesizes both transferrin (the main transporting protein) and ferritin (the major storage protein). Hepatic injury and dysfunction can disturb iron homeostasis. Excessive iron deposition in the liver leads to further injuries by triggering hepatocellular necrosis, inflammation, fibrosis, and even carcinoma **[145]**.

In this study, serum iron concentration were detected in both groups, serum iron elevated significantly in chronic HBV patients compared with healthy control, as in Table 3-7.

Blumberg B S *et al.* 1981 **[146]**, was the first to describe an association between changes in iron levels and hepatitis B infection in 1981, these authors found that serum iron were higher in patients who developed chronic HBV than in those who eliminated the virus.

Also, this finding was observed by Maoa W *et al.,* 2015**[68]** and Wang J *et al.,* 2016**[77]**, they suggested that elevated serum iron levels were associated with chronic HBV infection. Chronic HBV infection may be accompanied by liver injury, whereas altered iron metabolism could be a reflection of pathological changes in the liver.

While, Wei Y *et al.*, 2018 **[147]** revealed that physiological consequences resulting from the high proliferation rate of the tumor cells decreased the availability of serum iron in patients with HBV-related HCC.

Table 3-7: Mean difference of NTCP, Hepcidin, Ferritin and Iron in studied groups.

Parameters	Mean ± SD		P-value
	CHB patients group (n=50)	Control group (n=50)	
NTCP (ng/ml)	2520 ± 410	2425 ± 320	< 0.05
Iron (µmol/L)	24.94 ± 3.82	17.93 ±3.07	< 0.05
Ferritin (ng/ml)	345.64 ± 29.06	310.1 ± 31.5	< 0.05
Hepcidin (pg/ml)	165.56 ± 31.45	95.56 ± 32.47	< 0.05

SD: standard deviation; **p< 0.05: significant**

Ferritin is an acute phase protein, and its levels are increased in response to iron overload and systemic inflammation. Ferritin is the primary tissue iron storage protein in the liver, where most extra body iron is stored, and its expression in this organ is induced in primary or secondary iron overload disorders, resulting in increased hepatic and circulating ferritin levels **[84]**.

The serum ferritin concentration is a widely available and easily measured biochemical parameter. Elevated serum ferritin concentration may reflect systemic inflammation as well as elevated iron stores. In many patients with chronic liver disease, serum ferritin could be elevated as an acute phase reaction during the necro-inflammatory process of CHB. The serum ferritin concentration is also frequently increased during infection and malignant conditions **[148]**.

In the present work, serum ferritin levels in CHB group were significantly higher than controls group (P< 0.05) Table 3-7. Our study agrees with Gao Y H *et al.,* 2018 **[149]** and Abdalwahed I B *et* al., 2018 **[81]**, they reported elevated serum ferritin concentration in patients with HBV compared to healthy control. Furthermore, Some studies have

indicated that serum ferritin levels could be used to assess the degree of hepatocyte damage in CHB.

Datz C *et al*., 2017 **[150]** Abdalwahed I B *et* al., 2018 **[81]**, found that in HBV-infected patients, serum iron and serum ferritin were higher compared with control group. They conclude that the main cause of iron metabolism disorder in chronic HBV patients is liver injury. The major factors that affect hepcidin levels are iron stores, inflammation, hypoxia and erythropoietic activity. In addition, viral or bacterial infection has been reported to stimulate hepcidin synthesis **[151]**.

Overall, our investigations demonstrated an increase in hepcidin level in chronic HBV patients relative to control group Table 3-7.

These results are in accordance with Wang J *et al.*, 2016 **[77]**,who reported that serum hepcidin was elevated in CHB patients. While another study shown that serum hepcidin is decreased in CHB relative to healthy controls **[152]**.

Increased tissue and systemic iron levels also stimulate hepcidin production. As an iron regulatory peptide hormone, hepcidin is able to respond to variations in body iron demand and its concentration varies accordingly **[153]**.

3.2.2.1 Association of Hepcidin, Ferritin and Iron level with Age and Gender groups of Chronic Hepatitis B Patients

The synthesis of hepcidin is regulated by certain physiologic and pathologic processes. Hepcidin concentrations are decreased in situations that require increased concentrations of circulating iron. A decreased hepcidin concentration will result in the release of stored iron and in an increase in the dietary iron absorption.

On the other hand, infection and inflammation cause an increase in hepcidin synthesis resulting in decreased availability of circulating iron, which is considered to represent a defense mechanism of the human body against extracellularly proliferating pathogens.

Still, Hepcidin concentration is associated with age and gender **[145]**. This explain the result of the present study revealed significant association of hepcidin with different age and genders groups, Table 3-8.

Table 3- 8: Association of Hepcidin, Ferritin and Iron in Chronic Hepatitis B patients with age and gender groups.

Association of Hepcidin, Ferritin and Iron in CHB patients with age groups			
Parameters	**20-40 (years), N=34 (Mean ± SD)**	**41-60 (years), N=16 (Mean ± SD)**	**P value**
Hepcidin (pg/ml)	154.61 ± 32.4	188.81±8.42	< 0.05
Ferritin (ng/ml)	333.5 ± 26.98	371.4±10.67	< 0.05
Iron (µmol/L)	23.14 ± 3.03	28.61±2.62	< 0.05
Association of Hepcidin, Ferritin and Iron in CHB patients with gender			
Parameters	**Men (N=25) (Mean ± SD)**	**Women (N=25) (Mean ± SD)**	**P value**
Hepcidin (pg/ml)	188.8±9.7	142.32±28.26	< 0.05
Ferritin (ng/ml)	363.84±16.09	327.44±27.89	< 0.05
Iron (µmol/L)	26.93±3.6	22.86±2.97	< 0.05

SD: standard deviation; **p< 0.05: significant**

This study concomitant greatly with Ferrucci L *et al.*,2016 **[154]**, they observed that serum hepcidin were virtually dependent on age and sex, but our results contradicted other study by Ganz T *et al.,* 2008 **[155]**, reported nonsignificant associated of hepcidin with age and gender.

Results of serum iron and ferritin concentrations in patients group have demonstrated that levels are significantly higher in the 41-60 years old group than in 20-40 years old group and significantly higher in male than female, as in Table 3-8.

This finding is confirm by Aiguo Ma *et al.*, 2016. **[156]**, who revealed that the median of serum ferritin concentration in 65–70 years old was nearly two-fold higher than that in 45–50 years old and suggest an increasing prevalence of iron overload with age.

Also, Cankurtaran M et al., 2012 **[157]**, find that the level of serum ferritin higher in males than in females and increase of iron storage with ageing.

3.2.3. Correlations of Hepcidin with Iron, Ferritin and Age in patients with chronic hepatitis B virus

The current study observed significant (p value < 0.05) positive correlation for hepcidin with ferritin and iron, and non-significant correlation (p value > 0.05) for Hepcidin with age in (CHB) patients, as in figure 3- 1, 3-2 and 3- 3 respectively.

Our data showed that both serum iron and ferritin levels were higher in CHB patients than in controls, and serum hepcidin was positively correlated with ferritin in all patients (r = 0.735, p < 0.05). These data indicate that hepcidin synthesis in CHB patients may be responding to elevated iron, in addition to infectious stimuli. This is in agreement with the findings of Wang J *et al.*, 2016 **[77]** and El-Lehleha A M *et al.*, 2017 **[158]**, reported positive correlations between serum hepcidin and ferritin in CHB patients; But Nagashima M *et al.*,2006 **[76]**, reported negatively correlated between them.

Correlations between iron and hepcidin are often observed with chronic viral hepatitis. In the present study, there was a significant positive correlation between serum hepcidin and serum iron (r = 0.476, p <0.05). Similarly, El Wakil R *et al.*,2012 **[159]**, found a positive correlation between serum hepcidin and serum iron.

However, it is in disagreement with the findings of Olmez S *et al.*, 2016 **[84]**, who found no significant correlation between serum iron and serum hepcidin. Other studies, using hepcidin, found either no association with iron parameters in CHB patients or a negative correlation **[160,161]**.

Aoki CA *et al.*, 2005 **[162]**, found a nonsignificant correlation between serum hepcidin and age of CHB patients and this is reliable with our finding.

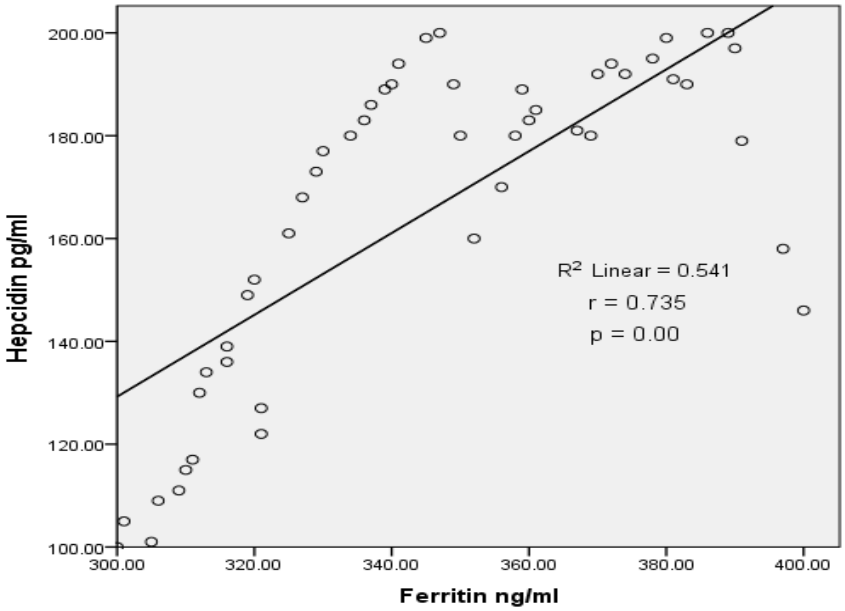

Figure 3- 1: Correlation between Hepcidin and Ferritin in CHB Patients

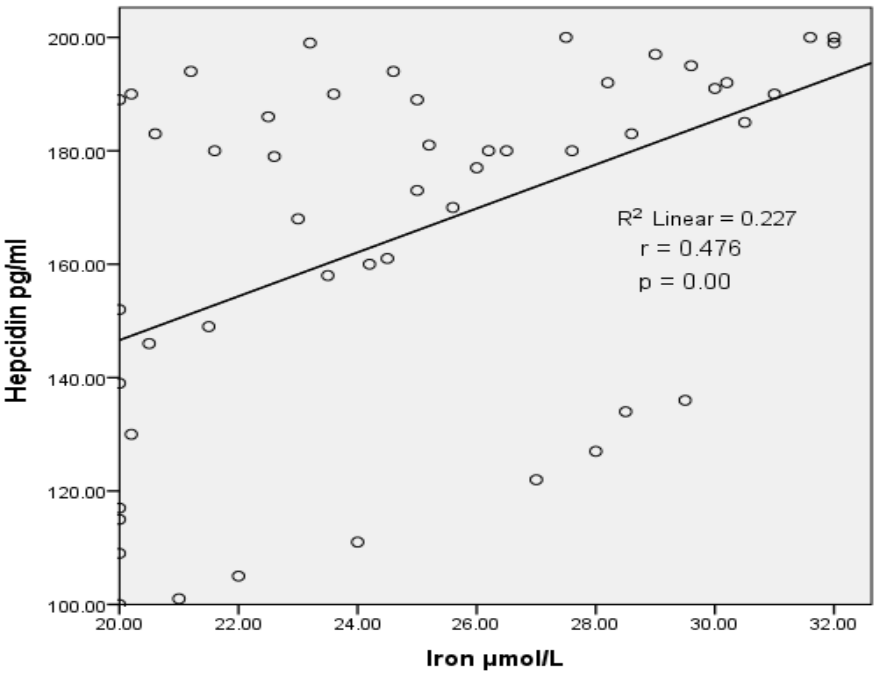

Figure 3- 2: Correlation between Hepcidin and Iron in CHB Patients

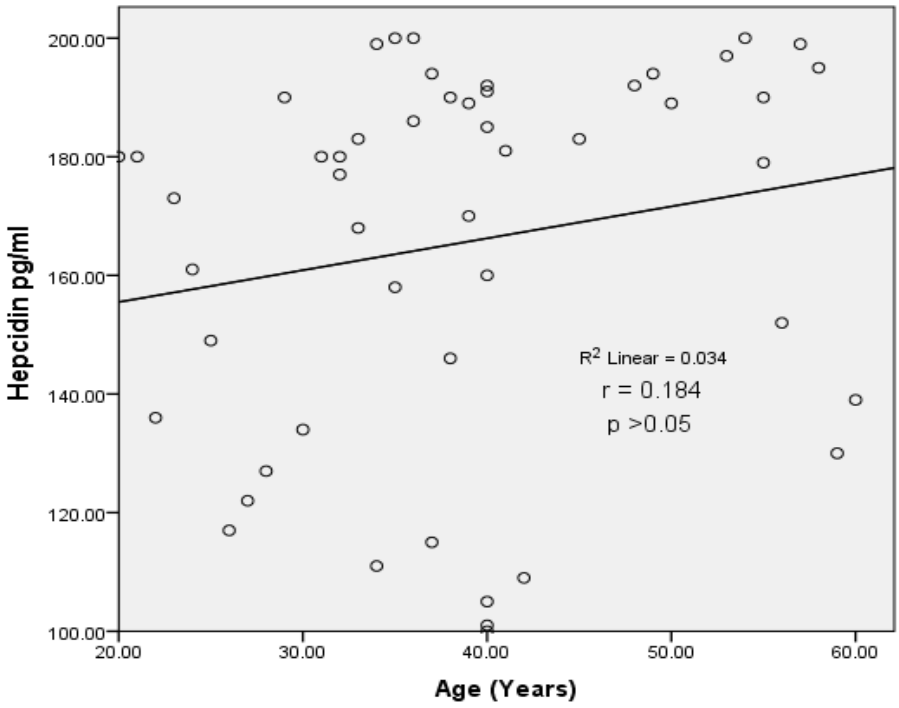

Figure 3- 3: Correlation between Hepcidin and Age in CHB Patients

3.3 Genetic Results

3.3.1. Measurement of Concentration and Purity of Deoxyribonucleic Acid

A ratio of absorbance at 260 nm and 280 nm was measured for the estimation of DNA concentration and purity. Data was demonstrated in Table 3-9 and figures 3-4, 3-5 respectively.

Table 3-9: DNA concentration and purity.

DNA concentration and purity	Mean ± SD
DNA concentration ng/µL	30.08 ± 7.25
DNA purity (260/280)	1.84 ± 0.07

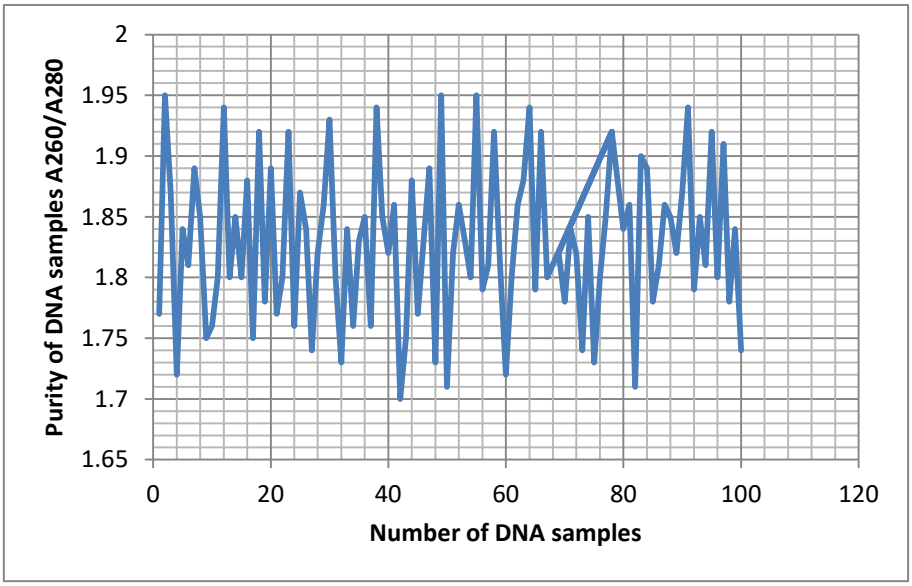

Figure 3-4: Purity of Extracted DNA Samples

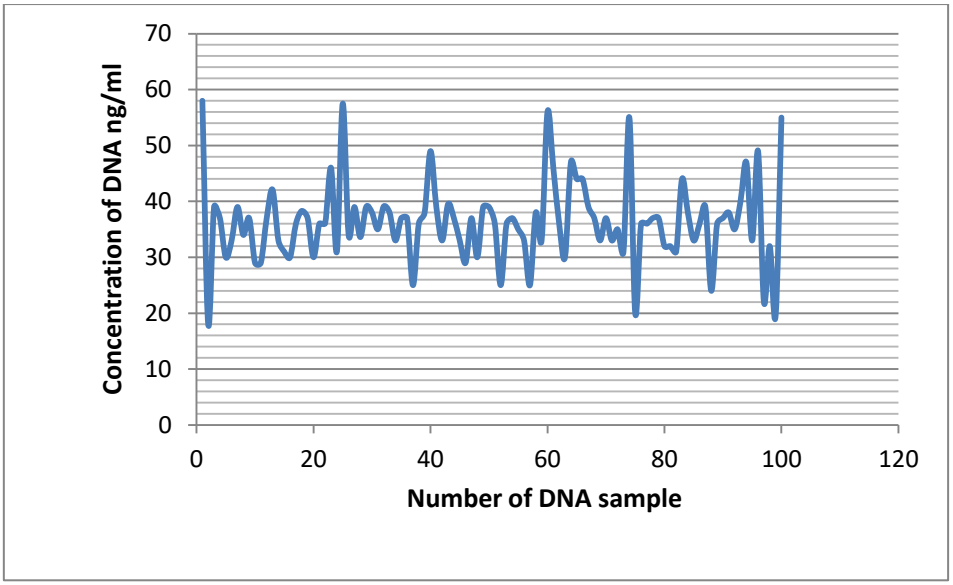

Figure 3-5: Concentration of Extracted DNA Samples

3.3.2. NTCP rs2296651 Variants among the Studied Groups

In current study the gene polymorphism of NTCP was studied in Iraqi patients with chronic hepatitis B virus and healthy control groups. The genotyping were detected by Probe-based Real-Time quantitative polymerase chain reaction (RT-qPCR) technique used to confirm the results.

Thus, genetic variations of NTCP could potentially have effects on both HBV entry and bile acid uptake. To date, several SNPs have been identified in NTCP and some of these SNPs are found in specific ethnic backgrounds. Among them, the S267F substitution in exon 4 of NTCP gene affects both HBV entry and bile acid uptake and has been identified only in East Asian people **[163]**.

Single nucleotide polymorphisms (SNPs) in the SLC10A1 gene, coding for sodium taurocholate co-transporting polypeptide (NTCP) functional receptor of HBV, has been associated with the natural history of HBV infection in some populations gene **[164]**.

Several genetic association studies have been carried out, which explored the relationship between NTCP polymorphisms and HBV-related clinical outcomes. Most of these studies focused on rs2296651, as non-synonymous G/A single nucleotide transition substitution, missense variant in the fourth exon of SLC10A1 gene, located on Chr.14 **[165]**, with a minor allele frequency ranging from 3.1% to 9.2% among different Asian populations, which could impairs the function of NTCP protein as both bile acid transporter and cellular receptor for HBV infection. Although the effect of this specific SNP was demonstrated in *in vitro* cell culture experiments, the clinical implication of this polymorphism remained to be evaluated **[166]**.

Based on the genome Aggregation Database (genom AD), the minor allele frequency of rs2269951 SNP is A = 0.005 in the global population, A = 0.095 in East Asian, A = 0.002 in African, A = 0.002 in American, and A = 0.00005 in European populations (SNPs with a minor allele frequency of 0.05 (5%) or greater are considered a common variant in this population) **[98]**.

Surprisingly, we detected only wild type GG genotype in all our studied participants, and none of them presented the mutant GA or AA genotypes, with no difference between groups, figure 3-6 and Table 3-10.

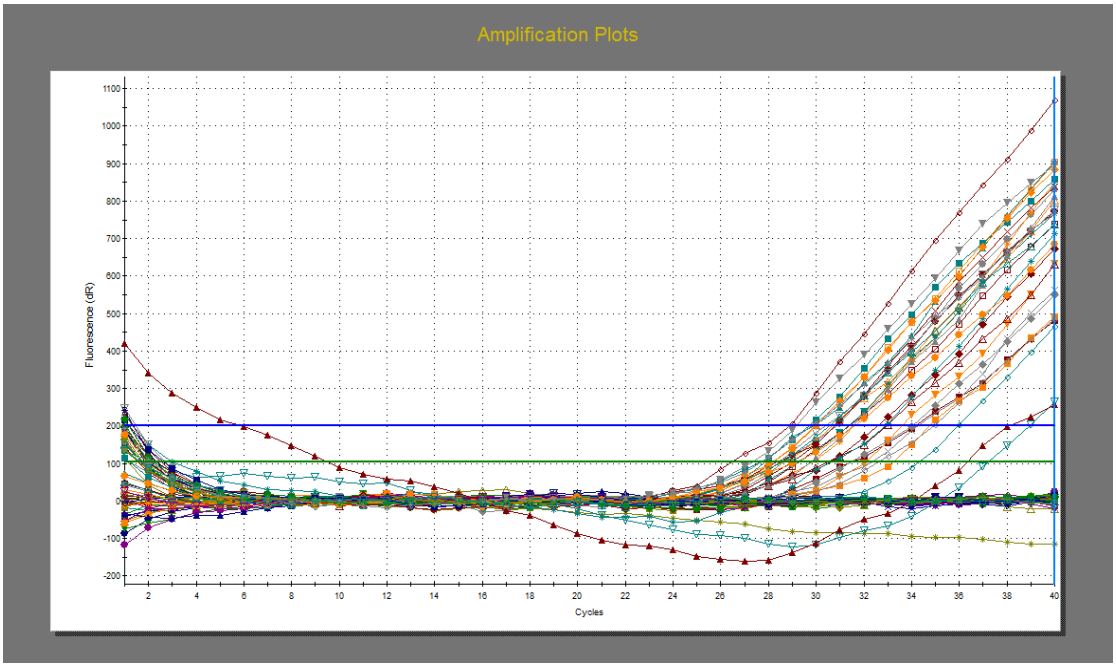

Figure 3- 6:Amplification Plots for Detection of SNP rs2296651 G/A

Each colored line is represented a sample. The horizontal blue line represents the threshold point of FAM emission. On the other hand, the horizontal green line represents the threshold point of HEX emission. An NTCP reactions were used as a control to eliminate the possibility of contaminated reactions.

Table 3-10: The wild genotype (G/G) (rs2296651 G/A) for Patients and Control

.

Genotype	rs2296651 G/A			
	Case (n=50)	Control(n=50)	OR(95% CI)	P- value
GG Wild type	50 (100%)	50 (100%)	Reference	1.000
GA Mutant heterozygous	0	0	1(0.019-51.38)	
AA Mutant homozygous	0	0	1(0.019-51.38)	

Our results were similar to those of other populations such as Moroccan **[167]**, Spanish Caucasian **[168]**, Polish Caucasian **[169]**, European American, and African American **[170]** populations, where the rs2296651 mutant genotypes were not detected in any of their participants.

In contrast to our results, several previous studies in the Chinese Han population showed that rs2296651 mutant variants were inversely correlated with HBV susceptibility, where GA and AA genotypes had lower frequencies in patients with CHB compared with healthy controls and were demonstrated to be protective, reducing the risk of liver cirrhosis, hepatocellular carcinoma, and liver failure in CHB patients **[96,170]**.

Some studies conducted in other Asian populations, such as Taiwanese **[171]**, Vietnamese **[172]**, Korean **[173]**, populations, reported that the SLC10A1 A allele consistently decreased HBV infection risk compared with the G allele.

The possible protective role of rs2296651 polymorphism was also demonstrated in a meta-analysis that extracted eight studies, including 14,591 chronically HBV-infected patients and 12,396 healthy controls from different populations (Chinese, Taiwanese, and Moroccan).

It concluded that the mutant variant was inversely associated with the risk of HBV infection (OR = 0.593, p = 0.028), where the A allele and GA genotype frequencies were lower in the CHB group compared with HCs group **[97]**.

However, there were conflicting data in Asian populations that the rs2296651 mutant variant may be associated with increased susceptibility to HBV infection **[174]** or may not be a risk factor for HBV infection at all **[165]**.

These rather contradictory results suggest that the rs2296651 variant varies among different ethnic populations and may be specific to Asian populations, and that it might display its advantage in conferring resistance to HBV infection.

Our findings may suggest that there is no relation between these SNPs of the NTCP gene and the susceptibility or chronicity of HBV infection in Babylon province.

3-4 Conclusion

1. Chronic hepatitis B virus patients in Babylon province have higher serum level of hepcidin, ferritin and iron comparing to normal subjects and this elevation may be represent the degree of hepatocyte damage in CHB. Concluded that increased iron levels stimulate hepcidin production as an iron regulatory peptide hormone.

2. Hepcidin, ferritin and iron levels are significantly elevated in age ranged between (20-40) and in male compared to female, this might be due to increase iron storage in this age and in male than female of chronic hepatitis B virus patients.

3. NTCP level was significantly elevated in chronic hepatitis B virus while, the NTCP gene SNP (rs2296651 G/A) do not associate with chronic hepatitis B in Babylon province.

3.5 Recommendations:

1. Further study need of specific link between hepcidin levels and different type of hepatitis.
2. Analysis of more SNPs of NTCP gene and observing the relation with progression of chronic hepatitis B virus in Babylon province and in other provinces in Iraq to give a complete picture about genotype distribution in Iraqi population.
3. Conduct whole NTCP gene sequencing to detect the possible SNPs related to CHB infection.

3.6 Limitation of study:

There were certain limitations to our research.

The following were the limitations:

1. It was based on data from a single center.
2. The main limitation of Genetic result is the sample size. Clearly 100 participants are not enough to generalize the results over our population or ethnic group.
3. rs2296651 was one of the most studied NTCP SNPs in patients with HBV infection. It would be better to conduct whole NTCP gene sequencing to detect the possible SNPs related to HBV infection and disease.

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مستويات الهيبسيدين و صوديوم تاركوليت كوترانسبورتنك بولي ببتيد (NTCP) متعدد الاشكال الجيني لدى مرضى التهاب الكبد المزمن نوع ب في محافظة بابل

رسالة

مقدمة إلى مجلس كلية الطب في جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في العلوم / الكيمياء الحياتية السريرية

من قبل
امجد حميد سلمان عيسى الفاضلي

بكالوريوس علوم كيمياء/ كلية العلوم / جامعة بابل
(٢٠٠١-٢٠٠٢)

اشراف

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الخلاصة

يعرف التهاب الكبد على أنه التهاب في خلايا الكبد، مثل عدوى خلايا الكبد أو الخلل الوظيفي في خلايا الكبد. يصنف التهاب الكبد على أنه حاد إذا تكرر خلال ستة اشهر أو مزمن إذا استمر لأكثر من ٦ أشهر.

الهيبسيدين هو ببتيد يتكون من٢٥ حامضًا أمينيًا، في البشر يتم ترميزه بواسطة جين الهيبسيدين الببتيد المضاد للميكروبات(HAMP).

الهيبسيدين هو منظم مركزي لعملية التمثيل الغذائي للحديد. ان خلايا الكبد تنتج في الغالب مادة الهيبسيدين استجابةً لزياده الحديد في الخلايا. كلما زاد الحديد في الخلايا، يرتفع مقدار الهيبسيدين في خلايا الكبد، مما يؤدي إلى زيادة مستويات الهيبسيدين في الدم.

صوديوم تاركوليت ببتيد مشترك في النقل (NTCP)، هو بروتين في الإنسان مشفر بواسطة الجين SLC10A1 (عائلة الناقل المذاب ١٠ الفرد ١). في الجينوم البشري، يقع جين NTCP في النطاق 24.1 q من الكروموسوم ١٤ ويتكون من ٥ مواقع مشفرة. تم العثور على تعدد الأشكال الجينية في الجين SLC10A1 لتؤثر على وظيفة بروتين NTCP، وقد تم تحديد العديد من الطفرات الوراثية في جين ال NTCP وتم العثور على بعض هذه الطفرات الوراثية في خلفيات عرقية محددة.

صُممت هذه الدراسة للتحقيق في مستوى صوديوم تاركوليت ببتيد مساعد النقل و الهيبسيدين والفريتين والحديد ولإيجاد الارتباط المحتمل بين هذه المعلمات مع العمر والجنس في مرضى التهاب الكبد المزمن ب، ولتقييم دور الطفرة الوراثية (rs2296651 G / A) في جين ال NTCP وما يرتبط بها مع العدوى بفيروس التهاب الكبد المزمن ب وتطور المرض في محافظة بابل.

ولتحقيق اهداف هذه الدراسة تم اجراء اختبار على (١٠٠) شخص قسموا على مجموعتين، ضمت المجموعة الأولى ٥٠ مريضاً مصاباً بالتهاب الكبد المزمن ب، أما المجموعة الثانية فضمت ٥٠ من الأفراد الأصحاء ظاهرياً وهم مجموعة السيطرة.

تم تحديد تركيز الهيبسيدين والفيريتين وال NTCP بروتين بواسطة تقنية الاليزا (ELISA). تم استخراج الحمض النووي من الدم والتنميط الجيني للطفرة الوراثية (rs2296651 G / A) بواسطة تقنية تفاعل البلمرة المتسلسل الكمي المستند إلى البروب (RT-qPCR) وتم تقييم النتائج من خلال تحاليل إحصائية متنوعة.

لوحظ ان تركيز صوديوم تاركوليت ببتيد مساعد النقل يزداد بشكل واضح لدى مرضى التهاب الكبد المزمن ب (مقارنة مع مجموعه السيطرة) كما ان تركيز الهيبسدين والفيريتين يزداد بشكل واضح ($p < 0.05$) لدى مرضى التهاب الكبد المزمن ب (مقارنة مع مجموعه السيطرة)، وتعزى هذه النتيجة إلى زيادة مستويات الحديد التي تحفز إنتاج الهيبسيدين (كهرمون ينظم مستوى الحديد)، الهيبسيدين قادر على الاستجابة تبعا لمستوى الحديد في الجسم وسوف يختلف تركيزه وفقًا لذلك.

كذلك لوحظ ان اختبارات وظائف الكبد اظهرت اضطرابات واضحة لدى مجموعة المرضى مقارنة مع مجموعة السيطرة.

من ناحية أخرى، ان الأنماط الجينية للطفرة الوراثية (rs2296651 G / A) لدى مرضى التهاب الكبد المزمن ب (التي تم دراستها في البحث) لم تبين أي ارتباط مهم بالمرض ($p > 0.05$).

خلصت الدراسة الحالية إلى أن مرضى التهاب الكبد المزمن ب في محافظة بابل لديهم مستوى مصل أعلى من الهيبسيدين والفيريتين والحديد مقارنة بالأشخاص الطبيعيين وقد يمثل هذا الارتفاع درجة تلف خلايا الكبد لدى هؤلاء المرضى.

أخيرًا، لا يرتبط تعدد الأشكال الجينية (rs2296651 G / A) لجين ال NTCP بفيروس التهاب الكبد المزمن ب، اكتشفنا فقط النمط الوراثي من النوع GG (السائد) في جميع المشاركين المدروسين، ولم يقدم أي منهم الأنماط الجينية GA أو AA التي تمثل الطفرة الوراثية.