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Molecular and Immunological Characterization of Hepatitis B Virus in Patient With Chronic Infection

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Degree of Master in Biology

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Dedication

To God, by whose guiding light this path was made possible, and through his boundless grace this achievement was attained.

To the soul of my father, may he rest in peace, whose enduring presence remains a source of inspiration and a strength from which I draw determination throughout my journey.

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Summary

Hepatitis B virus (HBV) is a major health problem that affects the liver, especially when the infection becomes chronic. To evaluate the immunological and molecular characteristics of chronic hepatitis B virus (HBV) infection through the assessment of serum IL-6 and IL-10 levels and the molecular detection and sequencing of the HBV precore/BCP region.

The present study examined the genetic variation of the Chronic active HBV Patients in Diwaniya province and the role of cytokines in disease progression. Both IL-6 and IL-10 levels were measured in patients and the control group.

Blood samples were collected from 60 HBV-infected patients attending Al-Diwaniyah General Hospital and the Central Blood Bank, Al-Diwaniyah, Iraq, and from 24 healthy individuals who served as controls during the period from August 2025 to December 2025. Exclusion criteria included HIV, HCV, other viral infections, autoimmune liver diseases, diabetes mellitus, and chronic renal failure. Serum IL-6 and IL-10 levels were determined using ELISA kits.

Molecularly. Viral DNA was extracted from EDTA plasma samples using a commercial DNA extraction kit. Then, nested PCR amplification was carried out using a commercial master PCR mix. Subsequently, Sanger sequencing was performed to confirm the genetic identity and genotype of hepatitis B virus (HBV) from the amplified precore and basal core promoter (BCP) regions. Samples that were hemolyzed, degraded, or insufficient in volume were excluded from the final analysis. The final analyzed samples included 24 samples for immunological analysis and 24 samples for molecular analysis.



The results indicated that IL-6 levels were significantly higher in HBV patients compared to the control group, with an interquartile range (IQR: 49.31–78.37) for the patient group and (IQR: 7.34–13.55) for the control group. IL-10 levels were also elevated in HBV patients (IQR: 25.25–47.16) and in healthy controls (IQR: 2.22–6.25), indicating an enhanced regulatory immune response in HBV infection, which may reflect immune-modulatory mechanisms during viral persistence.

Nested PCR targeting the precore and basal core promoter (BCP) region of the HBV genome produced a 316-bp amplicon. Sequencing analysis confirmed successful amplification of the target precore/BCP region and provided reliable nucleotide data for mutation detection, genotype classification, and phylogenetic analysis. The sequencing results revealed a high degree of similarity among the HBV isolates, indicating limited genetic variation within this region. Furthermore, sequence analysis enabled the identification of mutations in the precore and BCP regions that may influence viral replication and disease progression. Phylogenetic analysis showed that the studied isolates clustered predominantly within HBV genotype D, which is the most prevalent genotype in the Middle East, confirming its predominance among the investigated Iraqi HBV samples..

The study also showed that HBV persistence results from its ability to evade the host immune response, which explains the prolonged infection in some patients. This is achieved through multiple mechanisms, including the formation of covalently closed circular DNA (cccDNA) within hepatocytes, allowing the virus to establish long-term intracellular persistence. Additionally, the elevated IL-10 levels observed in this study may contribute to suppressing virus-specific immune responses and creating an immunotolerant environment. Meanwhile, increased IL-6 levels reflect ongoing inflammatory activity that is inadequate for effective viral clearance. The simultaneous elevation of IL-6 and IL-10



indicates a cytokine imbalance, with opposing pro-inflammatory and anti-inflammatory responses coexisting. This dysregulated immune state may hinder coordinated antiviral activity, thereby promoting viral persistence and ongoing replication within liver cells, ultimately leading to chronic HBV infection



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List Of Abbreviations

NO	Abbreviation	Full Term
1	ALP	Alkaline Phosphatase
2	ALT	Alanine Aminotransferase
3	AST	Aspartate Aminotransferase
4	BCP	Basal Core Promoter
5	cccDNA	Covalently Closed Circular DNA
6	CTLA-4	Cytotoxic T-Lymphocyte Antigen-4
7	CTLs	Cytotoxic T Lymphocytes
8	DNA	Deoxyribonucleic Acid
9	ELISA	Enzyme-Linked Immunosorbent Assay
10	GGT	Gamma-Glutamyl Transferase
11	HBeAg	Hepatitis B e Antigen
12	HBcAg	Hepatitis B Core Antigen
13	HBsAg	Hepatitis B Surface Antigen
14	HBV	Hepatitis B Virus
15	HCV	Hepatitis C Virus
16	HIV	Human Immunodeficiency Virus
17	IFN	Interferon
18	IL-6	Interleukin-6
19	IL-10	Interleukin-10
20	IQR	Interquartile Range
21	NGS	Next-Generation Sequencing
22	NK cells	Natural Killer Cells
23	NTCP	Sodium Taurocholate Cotransporting Polypeptide
24	PCR	Polymerase Chain Reaction
25	PD-1	Programmed Death-1

26	pgRNA	Pregenomic RNA
27	PRRs	Pattern Recognition Receptors
28	rcDNA	Relaxed Circular DNA
29	RNA	Ribonucleic Acid
30	TLRs	Toll-Like Receptors
31	TNF	Tumor Necrosis Factor

Chapter One

Introduction

1. Introduction

Chronic HBV infection remains a major public health problem due to its progression to liver cirrhosis and hepatocellular carcinoma. Limited local data are available regarding the immunological response, particularly IL-6 and IL-10 levels, as well as the molecular characteristics, mutations, and genotype distribution of HBV in Iraqi patients. Therefore, this study was conducted to investigate selected immunological and molecular parameters associated with chronic HBV infecti (WHO, 2024).

Hepatitis B virus (HBV) is one of the most clinically important hepatitis viruses such that it has the ability to develop chronic infection. Quite a significant percentage of the infected people are unable to clear the virus and develop chronic hepatitis, which is one of the most significant determinants of cirrhosis and hepatocellular carcinoma. HBV infection is on the rise all over the world, especially in areas of intermediate and high endemicity, such as the Middle East, despite an effective vaccine (Loomba & Liang, 2021). HBV is a DNA virus with a partially double stranded genome and an unusual replication cycle which creates covalently closed circular DNA (cccDNA) in the nucleus of the infected hepatocytes. This mini chromosome of virus acts as a stable transcriptional template and it allows the virus to keep replicating continuously and it becomes hard to eliminate with the current treatment methods. Therefore, cccDNA maintenance is one of the greatest challenges in controlling chronic HBV infection (Rehermann & Bertoletti, 2020).

Hepatitis B virus (HBV) has a significant genetic diversity, and viral genotypes have been attributed to the variation in disease progression, clinical outcomes, and antiviral therapy responses. Molecular analyses have demonstrated that sequence differences in particular genomic loci could determine the ability of the virus to replicate and the pathogenicity. Thus, HBV molecular characterization and genotyping play a crucial role in the comprehension of the regional epidemiology and disease behavior (Kramvis, 2022). HBV genotype D has been reported as the most common genotype in the Middle East and is commonly linked with chronic infection. In Iraq, a study has also indicated that the prevalence of genotype D in HBV-infected patients is high, and molecular studies serve as essential tools to explain the local distribution of HBV genotypes and their possible clinical consequences

The host immune response greatly determines the prognosis of HBV infection and not necessarily the viral factors. The innate and adaptive immune systems take part in the control of infection wherein cytokines, especially interleukins, are the central part in immune regulation and inflammation. The complexity of the immune response during infection has been highlighted by the implication of several interleukins in the infection, including pro-inflammatory cytokines, IL-10, IL-2, and IL-12, and regulatory cytokines, IL-10 and transforming growth factor-beta (TGF-0) (Khanam *et al.*, 2021). The pro-inflammatory interleukins help in amplifying the immune reaction and containing the virus but extravagant or sustained inflammatory reactions might cause immune mediated liver damages. Regulatory cytokines, conversely, restrict overreacting immune responses, but can also facilitate viral persistence through inhibition of antiviral immunity. Two of these cytokines are the IL-6

and IL-10 which play contrary roles of immunology. The IL-6 is inflammatory and represents the evidence of immune activation, whereas IL-10 is essential in the regulation of immunity and tolerance.

The combination of those two cytokines can give information about the ratio between the inflammatory and the regulatory immune responses to chronic HBV infection (Xia *et al.*, 2017; Dimitriadis *et al.*, 2023). The interactions between the viral molecular features and the host immune responses are also important in discussing the mechanisms involved in the persistence and progression of the HBV disease. Combining immunological research of the chosen cytokines with the determination of HBV by molecular methods and sequencing would give an overall picture of host-virus interactions and improve the interpretation of the processes of the disease in patients with HBV (Zheng *et al.*, 2023).

1.1 Aim of the Study

Under the necessity of medical significance of the HBV virus as one of the most harmful causes of chronic hepatitis, this paper was aimed at researching immunological and molecular peculiarities of the virus in chosen clinical samples. This was done by the following objectives.

1. Detection of HBV DNA in the plasma of patient by the nested polymerase chain reaction (PCR) system
2. Study of precore/BCA-DNA region character by using sequencing and determine the genotype.
3. Determine the immune response in patients with HBV by determining the level of serum interleukin-6 (IL-6) and interleukin-10 (IL-10) through enzyme-linked immunosorbent assay(ELISA).

Chapter Two

Literature Review

2. Literature Review

2.1. General Overview of Hepatitis

2.1.1. Viral Causes of Hepatitis

Viral infections are regarded as the primary cause of hepatitis in the world and a major health issue. There are a number of viruses that are particular to the liver such as hepatitis A, B, C, D and E. Though they all have an impact on the same organ, these viruses vary in terms of genetic make-up, transmission, and clinical results (Quirino et al., 2024).

Other such viruses like hepatitis A (HAV) and hepatitis E (HEV) are normally spread by contaminated food or water. Such infections usually vary as acute and do not cause any serious consequences in the long term (Revill et al., 2022). Conversely, hepatitis B (HBV), hepatitis C (HCV) and hepatitis D (HDV) are primarily spread by blood or body fluid. Such kinds are more alarming as they may remain in the body and cause a long-term infection (Schweitzer et al., 2022).

HBV and HCV long-term infection are closely associated with liver progressive damage. This could cause fibrosis, cirrhosis, and even hepatocellular carcinoma with time (Gan et al., 2025). Moreover, HDV infection, that only affects persons who are already infected with HBV is known to be more severe and lead to cirrhosis more rapidly. This emphasizes how viral hepatitis is a significant cause of liver-related disease and mortality on a global scale (Revill et al., 2022; Quirino et al., 2024)

2.1.2. Metabolic and Genetic Etiology of Hepatitis.

Less prevalent, yet important and not to be ignored, are metabolic and genetic disorders as the causes of hepatitis. Such circumstances are typical as a result of inherited issues in the metabolic pathways that cause the buildup of substances that are harmful within the liver cells. In the long-term, this accumulation might lead to progressive hepatic damage

(EASL, 2022). Although these disorders are rarer as compared to viral hepatitis, they must be taken into consideration, particularly in individuals with unexplainable liver disease or with a family history of such conditions (Zoller *et al.*, 2022). Hereditary hemochromatosis is one of the most famous ones. This is a condition that is marked by the excessive absorption of iron that slowly builds up in both the liver and other organs. The increase in iron may cause liver inflammation, fibrosis, and cirrhosis or even hepatocellular carcinoma in the end unless it is prevented (Brissot *et al.*, 2018). Laboratory tests and genetic analysis can be used to diagnose early in order to achieve early diagnosis. The disease can be treated and prevented with severe liver damage, in particular, with the help of therapeutic phlebotomy (EASL, 2022). One more notable condition is Wilson disease that is caused by mutations in the ATP7B gene. These mutations disrupt the normal excretion of copper and the result is the accumulation of copper in the liver. It may lead to a variety of liver issues, including a mild rise in liver enzymes and to more serious ones, like acute hepatitis or liver failure (Roberts and Schilsky, 2021). Considering that Wilson disease is commonly manifested among younger patients, it is necessary to diagnose it early and use chelation therapy to avoid the development of the disease and its complications with other organs (AASLD, 2022).

2.2. Physiological Functions of The Liver and The Etiology of Hepatic inflammation

Liver is at the center of ensuring that the body is running well since it is a part of most of the critical processes. It assists in controlling metabolism through the breaking of carbohydrates, lipids and proteins as well as the elimination of toxic substances in the body. Moreover, the liver synthesizes some essential proteins and contains vitamins and minerals required to perform normal physiological processes (Trefts *et al.*, 2021).

Besides the metabolic one, the liver is also closely associated with the immune system. It serves as a defense against pathogens and antigens that get into the body by the digestive system. This role contributes to why it is significant not only during metabolism but also the immune balance (Jenne & Kubes, 2018).

Since liver is involved in most of the activities that are essential, any damage to it may implicate various body systems. Liver inflammatory states may impair regular liver functioning and cause such symptoms as jaundice, weakness, metabolic disproportion, and clotting issues (StatPearls, 2024). Hepatitis is another of the most common liver diseases that is also known as the inflammation of the liver. Nevertheless, the onset of this condition may be caused not by one particular reason but by a variety of reasons (Schweitzer et al., 2022).

Hepatitis can be caused by a variety of causes, such as viral infection, autoimmune diseases, drug or toxin exposure, and hereditary metabolic complications. Viral hepatitis is however the most common cause but non-viral causes should also be considered in the diagnosis. The different causes are significant to understand when classifying the disease properly and selecting the most relevant management strategy, not mentioning that it helps to minimize the risk of long-term complications (Khanam et al., 2021; Gan et al., 2025).

2.3. Epidemiology of Hepatitis B Virus (HBV)

The infection of the hepatitis B virus (HBV) is a significant health problem in the whole world and one of the leading causes of chronic liver disease. It is estimated that over 250 million individuals across the globe reside with chronic HBV infection that exposes them to severe side effects like cirrhosis and hepatocellular carcinoma (Schweitzer *et al.*, 2022). Though an effective vaccine exists, the infection is still spreading

in most corners of the world. This is primarily due to discrepancies in vaccination rates, inaccessible healthcare facilities, and delayed disease diagnosis in certain territories (Polaris Observatory Collaborators, 2022). The prevalence of chronic HBV infection varies among countries in the Middle East. Iraq is considered a country of intermediate endemicity, with an estimated HBsAg prevalence of approximately 2.94%. Similar prevalence rates have been reported in neighboring countries, including Iran (3.4%) and Turkey (3.0%), whereas a lower prevalence has been observed in Saudi Arabia (1.7%). These findings indicate that HBV infection remains a significant public health concern in the region despite the implementation of vaccination programs and preventive measures (Schweitzer et al., 2015; WHO, 2022).

The other consideration is the age of infection. Acquiring the virus at infancy or early childhood increases the probability of the emergence of chronic infection significantly. However, a majority of adults can get rid of the virus following an acute infection without any long-term consequences (Schweitzer *et al.*, 2022). Due to this fact, nations with prevalence of early transmission have higher chronic HBV infection and complications associated with it. Knowledge of such patterns can be useful in planning the prevention measures, enhancing the screening programs, and decreasing the total disease burden (Polaris Observatory Collaborators, 2022; Gan *et al.*, 2025).

2.4. Transmission of Hepatitis B Virus (HBV)

Hepatitis B virus (HBV) is primarily acquired by being exposed to infected blood or body fluids. The virus may spread in a number of ways and they include mother to child, at an early age, sexually, and through infected needles or blood products. Nevertheless, the relevance of every route varies in all regions and it will be determined by the conditions in the region, the demographics present, and the state of their health

information (Razavi-Shearer *et al.*, 2023). In places where the prevalence of HBV is high, the commonest mode of transmission is that of mother to child. This is notably observed in places like East Asia and some parts of the sub-Saharan Africa. Newborn babies of infected women are highly exposed to the risk of being contracted at birth. Without the use of preventive measures, the majority of such infants can become chronically infected since their immune system is not fully developed at that point (Nelson *et al.*, 2022). That is why this path of transmission is significant in supporting such a great number of chronic HBV cases in these areas. Conversely, in lower prevalence or moderate, e.g., most Middle Eastern and Western countries, the infection is acquired more frequently later in life. In such environments, they are believed to be passed on during sexual interactions, use of shared needles or coming into contact with infected medical/cosmetic devices (Hajarizadeh *et al.*, 2023). Moreover, healthcare workers can also be exposed to risk, particularly within the setting where infection control measures are not adhered to (Westernmann *et al.*, 2022). It is significant to understand the ways in which HBV is spread among various groups of people in order to create efficient preventive measures. They are vaccination programs, screening of pregnant women, safe blood transfusion, and raising awareness in the general way. The implementation of preventive actions on the basis of the most widespread methods of spreading the infection will substantially minimise the rate of new infections and assist in dealing with the long-term consequences of the disease (Nelson *et al.*, 2022; Razavi-Shearer *et al.*, 2023).

Table 2.1: Comparison of hepatotropic viruses based on genome type, transmission routes, chronicity.

Virus	Genome	Transmission	Chronicity
HAV	RNA (Picornaviridae)	Fecal–oral (food, water, close contact)	No
HBV	DNA (Hepadnaviridae)	Blood and body fluids (perinatal, sexual, injections)	Yes
HCV	RNA (Flaviviridae)	Bloodborne (transfusion, unsafe injections, IDU)	Yes
HDV	RNA (Defective virus, requires HBV)	Same as HBV (co-infection or superinfection)	Yes
HEV	RNA (Hepeviridae)	Fecal–oral (contaminated water)	Rare

Schweitzer *et al.*, 2022; Revill *et al.*, 2022.

2.5. Structure of Hepatitis B Virus (HBV)

Hepatitis B virus (HBV) is a small DNA virus and is a member of the Hepadnaviridae family with rather simple yet distinct structure. The entire viral particle is the so-called Dane particle, which is made up of an external envelope and an internal core. The surface antigen of hepatitis B is found in the outer surface (HBsAg), and the core antigen (HBcAg) on the inside. The viral genome is located in the form of partially double-stranded circular DNA with the viral polymerase enzyme in this core (Seeger & Mason, 2015). The latter structural arrangement is strictly connected to the manner the virus replicates and survives in the host. The HBV is a highly organized genome which is approximately 3.2 kilobase.

It has four similar overlapping segments that encode the major viral proteins such as surface proteins, core proteins, polymerase proteins and X proteins. Since these regions were found to overlap, a mutation in a single part of the genome can impact on more than one function. Although small, the virus can effectively manage various steps of its life cycle, including host cell entry, replication, and new viral particle assemblies (Tong and Revill, 2016). The large quantities of subviral particles containing HBsAg only are one of the typical characteristics of HBV infection. These are non-infectious particles that are found in the bloodstream, in great quantities. They are said to assist the virus to evade the immune system because they are decoys, which are bound to antibodies, making them less effective against the virus itself (Durantel and Zoulim, 2016).

The knowledge of the structure of HBV is valuable since it can correspond to the opinion of how this virus acts within the body and interacts with the immune system. It also offers a foundation on which diagnostic instruments and treatment plans are developed. Table 2.2 summarizes the main structural components of HBV and their functions whereas the overall structure of the virus is demonstrated in Figure 2.1.

Table 2.2: Structural components of hepatitis B virus (HBV) and their biological functions

Component	Description	Function
Envelope (lipid bilayer)	Outer lipid layer derived from the host cell membrane; embedded with surface antigens (HBsAg)	Protects the virion and mediates attachment and entry into hepatocytes via the NTCP receptor
HBsAg (surface antigens)	Three forms: Large (L), Middle (M), and Small (S) proteins	Essential for receptor binding and viral entry; diagnostic marker of active infection
Nucleocapsid (core particle)	Icosahedral protein shell composed of HBcAg	Encapsidates the viral genome and polymerase; protects rcDNA during replication
HBcAg (core antigen)	Structural protein forming the nucleocapsid	Marker of viral replication and important target of immune recognition
HBeAg (e antigen)	Soluble protein derived from the precore region of the HBV genome	Secreted into serum; marker of viral replication and infectivity
Viral polymerase	Multifunctional enzyme with reverse transcriptase, DNA polymerase, and RNase H activities	Converts pgRNA into rcDNA; essential for viral replication
Genome (rcDNA)	Partially double-stranded relaxed circular DNA (~3.2 kb)	Template for conversion to cccDNA in the hepatocyte nucleus
cccDNA (covalently closed circular DNA)	Stable episomal form of viral DNA located in the nucleus	Serves as the transcriptional template for all viral RNAs and maintains viral persistence

Seeger & Mason, 2015; Tong & Revill, 2016.

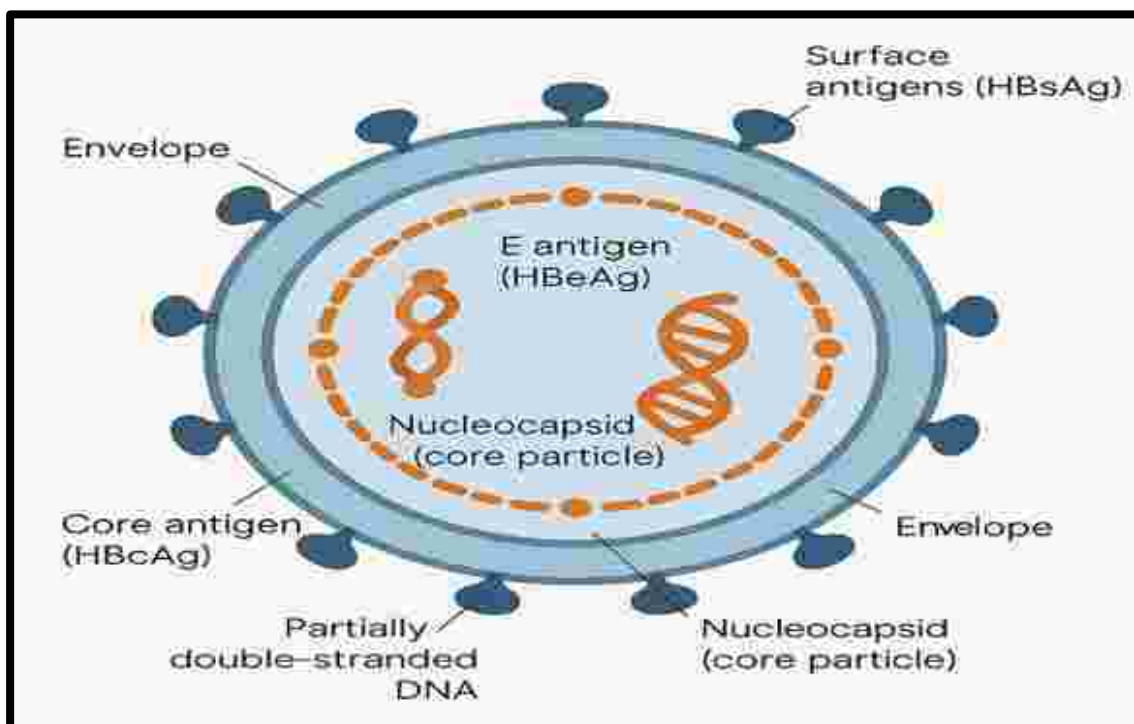


Figure 2.1: Structural organization of the hepatitis B virus (HBV) particle. Seeger & Mason, 2015.

2.6. Hepatitis B Virus (HBV) Infection Pathogenesis

Host immune response primarily in relation to hepatitis B virus (HBV) infection development is not due to direct virus-induced destruction, but indirectly through the immune response. HBV is deemed to be a non-cytopathic virus and this implies that the liver cell damage is not directly caused by the virus, but rather by immune-mediated mechanisms against infected hepatocytes. This is why the degree of the disease is mostly conditioned by the interplay between viruses replication and host immune system (Bertoletti & Ferrari, 2016). Once the virus gains entry into the hepatocytes, it initiates its replication cycle by establishing covalently closed circular DNA (cccDNA) in the nucleus which serves as a stable template of viral transcription. Viral antigens like hepatitis B surface antigen (HBsAg), core antigen (HBcAg) and e antigen (HBeAg) are expressed and cause the activation of host immune response. This response may be successful and lead to viral clearance in

certain locations, but in other locations, an insufficient or unregulated response permits the virus to persist and is one of the causes of continued liver damage (Maini and Burton, 2019). Continuous immune-mediated inflammation contributes significantly to the development of liver disease due to HBV infection. Repeated episodes of hepatocyte damage and regeneration may trigger fibrogenesis by hepatic stellate cell activation. This can eventually cause fibrosis, cirrhosis and high chances of hepatocellular carcinoma. The current interaction between viral activity and host immune mechanisms is exposed to these changes throughout the infection period (Ringelhan *et al.*, 2018). Figure 2.2 shows the key processes involved in the pathogenesis of HBV that are viral replication, antigen expression, liver injury induced by the immune system, and progression of the disease.

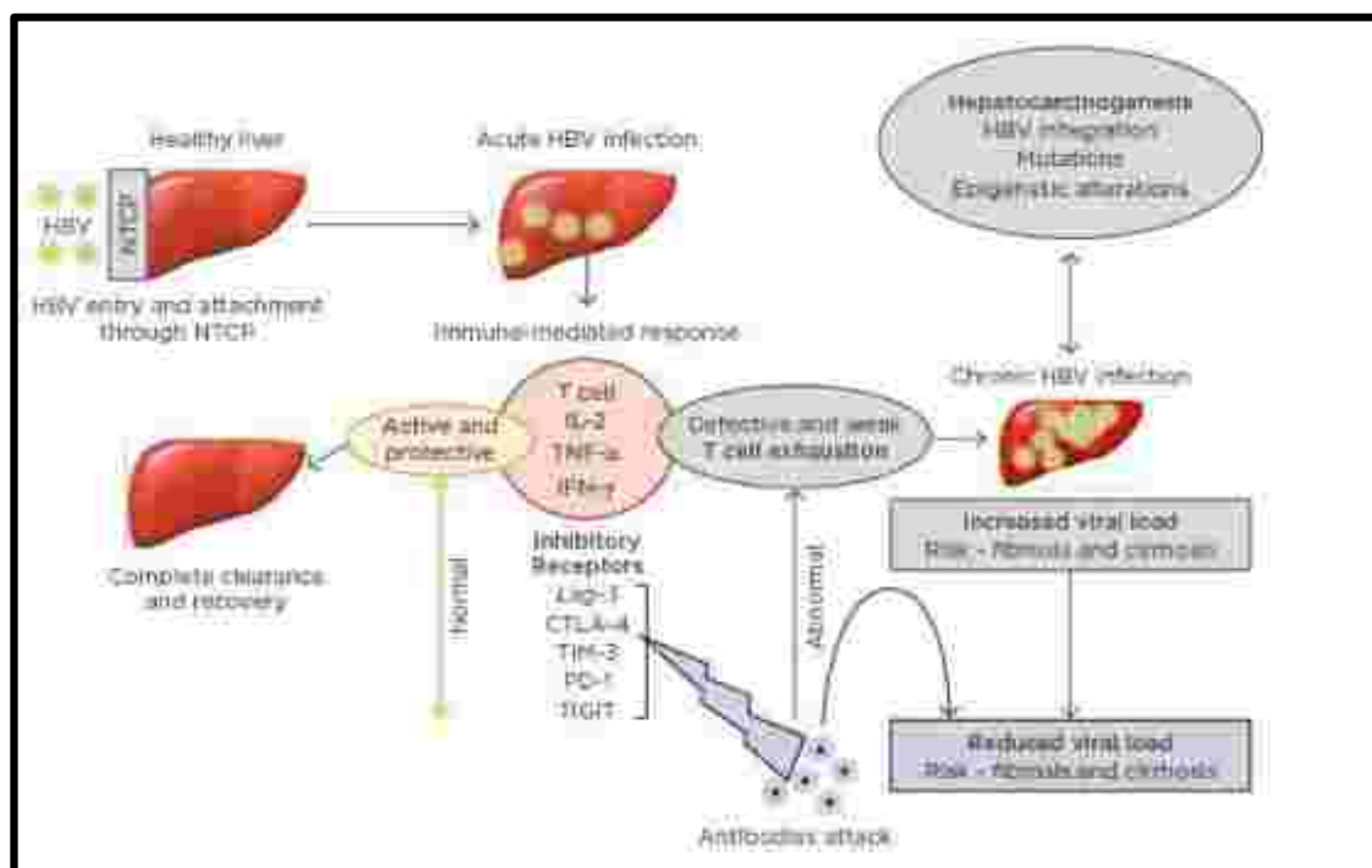


Figure 2.2: Pathogenesis of hepatitis B virus (HBV) infection.

Bertoletti & Ferrari, 2016; Ringelhan *et al.*, 2018.

2.6.1. Acute Hepatitis B Virus Infection

Acute hepatitis B virus (HBV) infection refers to the early stage that occurs after exposure to the virus. This phase is marked by active viral replication along with a strong immune response from the host. In most healthy adults, the immune system responds effectively through both innate and adaptive mechanisms, which play an important role in clearing the virus. Immune responses, in particular, cytotoxic lymphocyte attack on infected liver cells, rather than the virus itself, is responsible for liver damage in this stage (Bertoletti & Ferrari, 2016).

This stage involves the recognition of the viral antigens on the surface of the infected hepatocytes by the HBV-specific CD8⁺ cytotoxic T cells. The cells operate to remove the infected hepatocytes in various ways, such as direct cell destruction and secretion of antiviral cytokines that assist in the process of suppressing viral reproduction. This is an immune reaction often coupled with the short-term elevation of liver enzymes and inflammation, which would eventually recover when the virus is eliminated (Maini and Burton, 2019). The eventual result of acute HBV infection determines much on the age of the affected individual and the development of the immune system. The infection is normally resolved in adults and long-term protective immunity is developed as indicated by the presence of hepatitis B surface antibodies (anti-HBs). Nevertheless, the immune response is weak in most cases when infection takes place during infancy or early childhood, which predisposes the disease to chronic infection (Wieland & Chisari, 2019).

2.6.2. Chronic Hepatitis B Virus Infection

The chronic infection of hepatitis B virus (HBV) is caused when the immune system fails to get rid of the virus during the acute period, and the virus is left to persist in the hepatocytes. The following conditions are

linked with the chronic presence of viral antigens, a persistence of cccDNA, and immune tolerance or impaired immune activity. Unlike acute infection, in chronic HBV infection, there is a weaker/impaired T-cell response that permits the virus to remain in replication and results in persistent but rather mild levels of liver inflammation (Bertoletti & Ferrari, 2016). Under persistent infection, viral antigens influence the activity of the HBV-specific T cells (CD8+ and CD4+) in the long run. These cells eventually become ineffective which is called T-cell exhaustion. This lower immune response inhibits the capacity of the body in both clearing the virus and in addition leads to persistence of inflammation within the liver. Consequently, the cycles of liver cell damage and recovery can be repeated, which, in turn, over time, can cause fibrosis and liver architecture (Maini and Burton, 2019).

Prolonged inflammation is one of the determinants of the occurrence of complications of chronic HBV infection. Such complications are liver cirrhosis and hepatocellular carcinoma. Activation of hepatic stellate cells and deposition of extracellular matrix elements which mediate fibrosis occur during the process. Moreover, persistent immune dysregulation and cyclical cell turnover are added as risk factors promoting the malignant transformation. These processes prove the importance of immune-related mechanisms in the development of the disease in chronic HBV infection (Ringelhan *et al.*, 2018).

2.6.3. Viral Entry Through NTCP Receptor.

The Hepatitis B virus (HBV) infects a hepatocyte cell and the selectivity is attributed to the existence of the sodium taurocholate cotransporting polypeptide (NTCP) receptor on the hepatocyte surface. This receptor is possessed on the basolateral wall of hepatocytes and serves as the prime site of getting attacked by the virus. HBV binds to NTCP, via the receptor with the pre-S1 region of hepatitis B surface

antigen (HBsAg). This is necessary to provoke the viral entry and is the first stage of infection (Yan *et al.*, 2012). After the virus attaches to NTCP, it is endocytosed into the cell using receptors. Once in, the uncoating of the viral particle occurs and transfer of the nucleocapsid to the nucleus takes place. The viral DNA is released and transformed into covalently closed circular DNA (cccDNA) there, a template of subsequent viral replication, that is in the form of a relaxed circular DNA (rcDNA). HBV infection is confined mostly to the liver tissue since NTCP is predominantly exhibited in the hepatocytes. Viruses have been demonstrated to be prevented from entering the cell by blocking or inhibiting NTCP expression and establishing it as an essential component in early infection and its possible use as a therapeutic target (Hagenbuch and Meier, 2020).

The process of acting between circulating HBV particles and NTCP is ongoing during chronic infection and hepatocytes can again be infected repeatedly which is why the amount of cccDNA in the cells is maintained. Such a continuous process leads to the continuation of the virus. This interaction has been targeted in recent treatments, including Myrcludex-B (bulevirtide), which has the potential to decrease viral entry and cut the infection cycle short. This shows that NTCP is of clinical significance in HBV infection (Blank *et al.*, 2022). Figure 2.3 shows the mechanism by which viruses enter the cell by binding to NTCP and then going down the pathway.

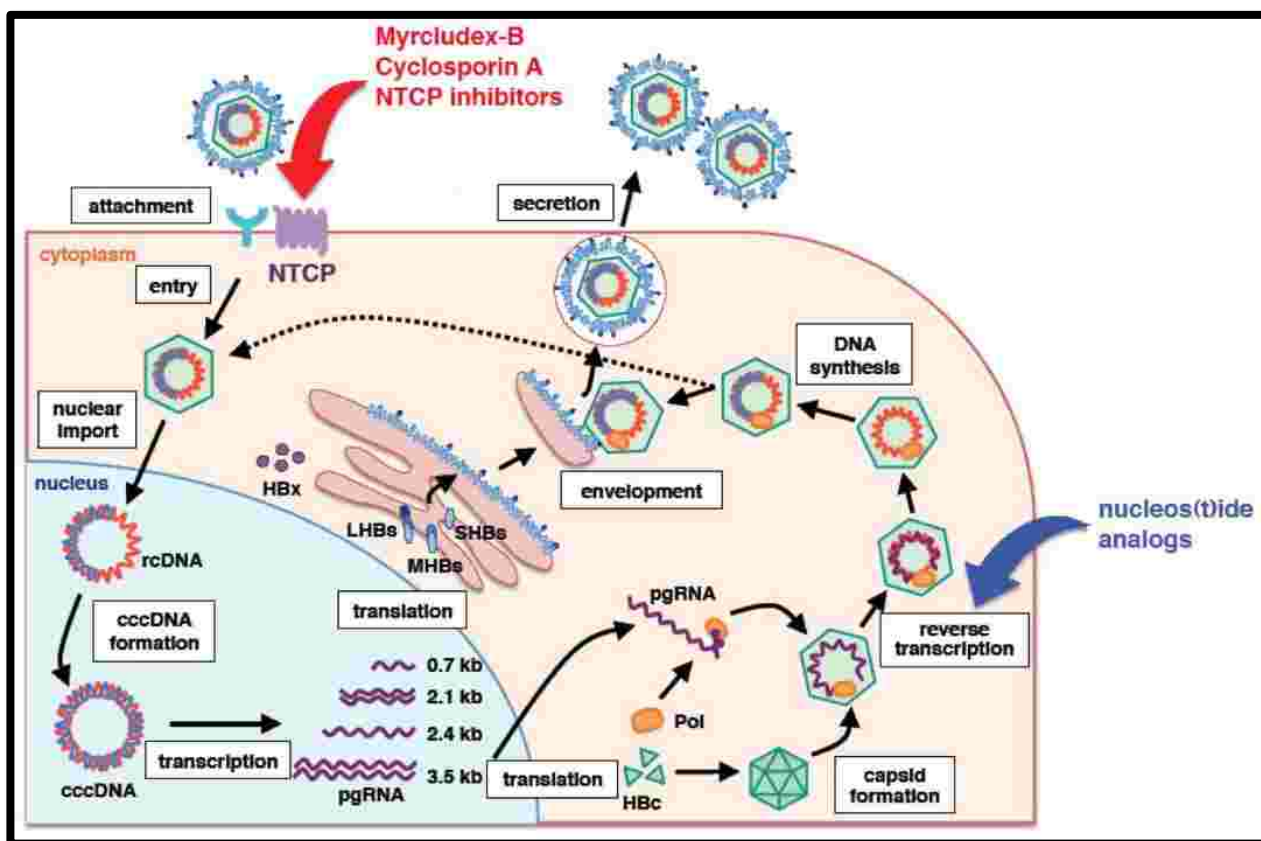


Figure 2.3: Hepatitis B virus (HBV) entry into hepatocytes via NTCP receptor binding.

Yan *et al.*, 2012; Blank *et al.*, 2022

2.6.4. Viral Immune Evasion Mechanisms of Hepatitis B Virus

Hepatitis B virus (HBV) can survive within hepatocytes since it has a number of strategies that aid it to evade the host immune system. Such processes diminish the immune detection and minimize the efficacy of antiviral actions, which causes the virus to stay in the liver. HBV is more likely to remain inside the cell and have a low level of activity in comparison to viruses, which damage the infected cells directly, particularly in the early phases of infection, which allows this virus to suppress immune activation (Wieland and Chisari, 2019).

The existence of covalently closed circular DNA (cccDNA) in the nucleus of the hepatocytes that are infected is one of the key reasons that help the viruses to stay in the body of the organism. This type of viral DNA is stable and may spend a considerable period in the cell. It is a persistent source of the production of viral RNA and is not readily recognized and eliminated by the immune system, which enables the virus to persistently replicate despite the presence of immune pressure (Allweiss & Dandri, 2017). The other significant mechanism is the generation of subviral quantities of particles which are primarily comprised of hepatitis B surface antigen (HBsAg). These particles find their way into the blood system and have the ability of attaching themselves to the circulating antibodies. Consequently, they inhibit the functionality of the immune system by being decoys. It is also capable of influencing B-cell and T-cell responses due to such high exposure to antigens and the development of immune tolerance, especially during chronic infection (Durantel & Zoulim, 2016). HBV may also disrupt innate immune signaling of infected cells. Protein viruses such as HBx have been revealed to influence host signal pathways and inhibit interferon synthesis. This impairs the initial immune response and puts off the viral clearance, which sustains the infection (Liu *et al.*, 2020). All in all, these processes can help HBV to persist in host cells and lead to the emergence of chronic infection. Their contribution to the development of liver disease in HBV is also significant. It is relevant to understand these processes to explain how the virus stays and what potential targets can be identified to find a cure (Bertoletti and Ferrari, 2016).

2.7. HBV Replication Cycle

Hepatitis B virus (HBV) is said to have the unique replication cycle due to its features of DNA and RNA viruses, and having a reverse

transcription step. After infection in hepatocytes, the virus develops a stable viral DNA, called covalently closed circular DNA (cccDNA) in the nucleus. This form will stay in the cell and serve as a continuous source of viral genes expression. Consequently, it contributes to the long-term infection and sustenance of viruses (Seeger and Mason, 2015; Revill *et al.*, 2020).

2.7.1. Viral Attachment and Entry Through NTCP

Infection with HBV begins with the fact that the pre-S1 part of hepatitis B surface antigen (HBsAg) attaches itself to the sodium taurocholate cotransporting polypeptide (NTCP) receptor on the basolateral hepatocyte membrane. This contact between the virus and the receptor results in endocytosis and enables the virus to access the cytoplasm (Yan *et al.*, 2012).

2.7.2 Transport of rcDNA and Formation of cccDNA

The cccDNA is formed as a result of the transportation of the rcDNA. Once in the cell, the viral nucleocapsid proceeds to the nucleus, and there releases the partially double-stranded relaxed circular DNA (rcDNA). The cellular repair mechanisms then convert this DNA into covalently closed circular DNA (cccDNA). This structure is maintained within the cell and is a crucial model of viral action, which is crucial in the cause of chronic infection (Allweiss & Dandri, 2017).

2.7.3 Viral protein Transcription-Translation.

The cccDNA serves as a template of the synthesis of pregenomic RNA (pgRNA) and other associated RNA molecules. These RNAs are delivered to the cytoplasm, where they help to make various viral parts,

such as the core protein, surface antigens, viral polymerase, and the regulatory HBx protein (Seeger & Mason, 2015).

2.7.4. Reverse Transcription and Genome Synthesis.

Within newly-formed nucleocapsids, a reverse-transcription is carried out to change the pregenomic RNA (pgRNA) into a DNA form. In this process, the viral polymerase synthesises a new negative strand of DNA and partially makes the positive strand, to create the relaxed circular DNA (rcDNA). This form of DNA synthesis that is dependent on RNA is a characteristic feature of HBV when compared to normal DNA viruses (Durantel & Zoulim, 2016).

2.7.5 Assembly, Secretion, and Replenishment of cccDNA

Once the synthesis of genomes is accomplished, two options of the nucleocapsids are possible. Others go back to the nucleus and they can assist in the maintenance and amplification of intracellular pool of cccDNA. The others obtain an outer envelope that carries hepatitis B surface antigen (HBsAg) inside the endoplasmic reticulum and Golgi apparatus, and then discharges them into the blood in the form of fully developed viral particles. Besides these infectious particles, non-infectious but large quantities of sub vials are also generated. These particles primarily comprise of HBsAg and their release is high. They are capable of positively interfering with the immune system by attaching to antibodies and inhibiting the effectiveness (Revill *et al.*, 2020). HBV has a full replication cycle, which includes viral entry via NTCP, cccDNA, transcription of pgRNA, reverse transcription, and viral particles release, as shown in Figure 2.4.

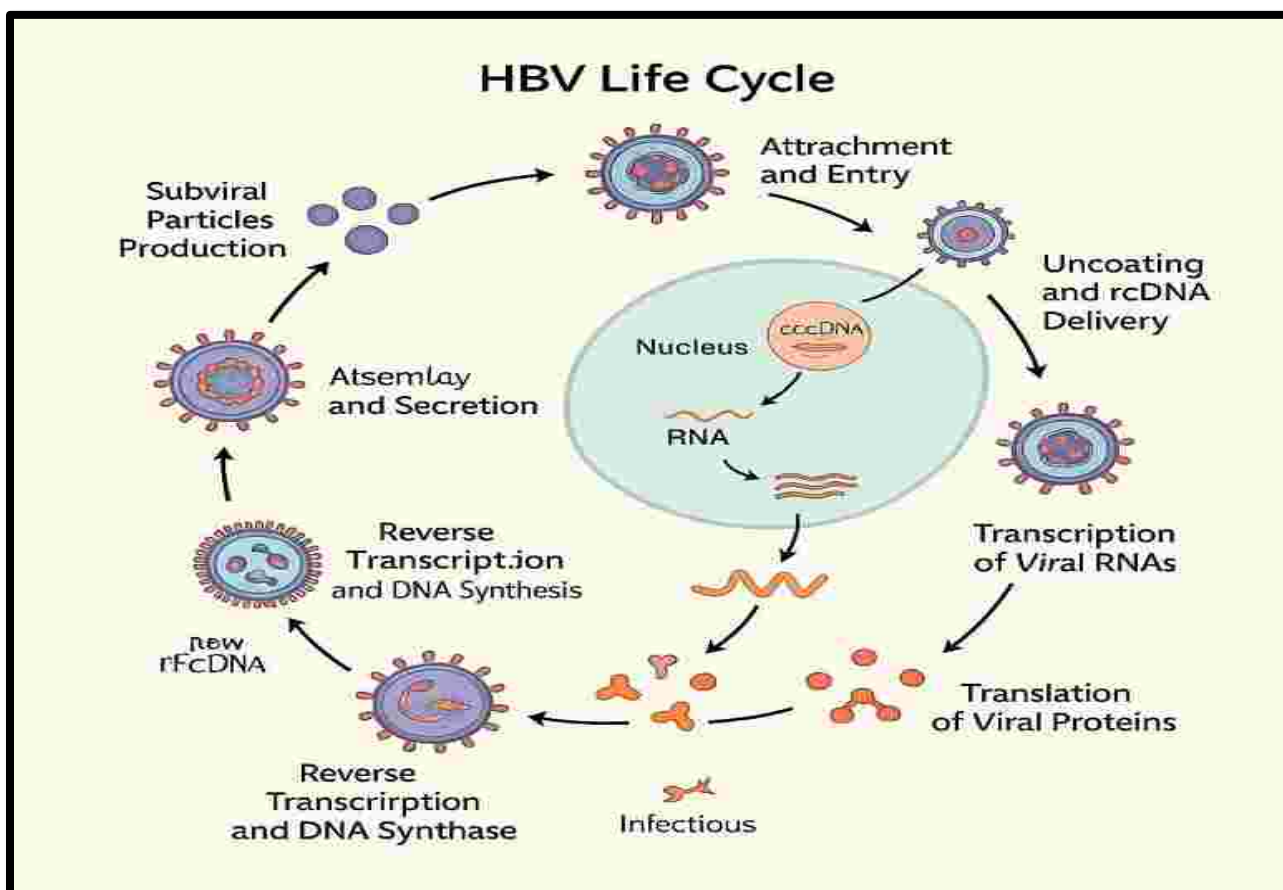


Figure 2.4: Replication cycle of hepatitis B virus (HBV).

Seeger & Mason, 2015; Revill *et al.*, 2020.

2.8. HBV Genotypes

Hepatitis B virus (HBV) shows a high level of genetic variation, which contributes to its wide distribution around the world and differences in disease patterns among infected individuals. Based on differences in the viral genome of more than 7.5%, HBV is divided into at least nine recognized genotypes (A–I), while a tenth genotype (J) has been reported in a limited number of studies. Each genotype has specific characteristics that may affect viral behavior, including replication patterns, interaction with the immune system, transmission, and long-term disease outcomes (Kramvis, 2014; Sunbul, 2014; Schaefer, 2021). The distribution of these genotypes is not random and tends to follow

geographic patterns. Genotypes A and D are commonly found in Europe, the Middle East, and parts of Africa. In contrast, genotypes B and C are more common in East and Southeast Asia. Genotype E is mainly reported in West Africa, while genotypes F and H are seen mostly in Central and South America, particularly among indigenous populations. The less common genotypes, such as I and J, have been identified mainly in some Asian regions (Toyé *et al.*, 2023; Schaefer, 2021).

These differences between genotypes are also important from a clinical point of view. For example, genotypes C and F are linked to delayed HBeAg seroconversion and a higher risk of hepatocellular carcinoma. On the other hand, genotype A is generally associated with a better response to interferon therapy. Genotype D, which is more common in certain regions, has been associated with more severe liver disease, a higher chance of persistent infection, and a lower response to interferon treatment (Elizalde *et al.*, 2021; Hussain *et al.*, 2022). In Iraq, available studies show that genotype D is the most common type, representing the majority of HBV cases. Other mixed genotype patterns, such as B, C, and D combinations, may be detected but are relatively rare. The predominance of genotype D is important because it has been linked to more persistent infection, weaker treatment response, and a higher risk of developing cirrhosis. This makes it relevant to focus on related immunological markers, including cytokines such as IL-6 and IL-10, which are examined in this study (Hussain *et al.*, 2022; Amini *et al.*, 2019). The main clinical and epidemiological differences between HBV genotypes are summarized in Table 2.2

Table 2.3: Clinical and Epidemiological Characteristics of HBV Genotypes

Genotype	Geographic Distribution	Transmission & Prevalence	Clinical Characteristics
A	North America, Western Europe, sub-Saharan Africa, India	Sexual transmission, young adults; also vertical in Africa	Chronicity risk if acquired perinatally; associated with HCC at older age
B	East Asia (China, Japan, Taiwan, Vietnam)	Predominantly vertical/perinatal	Milder disease, slower progression, but HCC may develop at a younger age
C	East & Southeast Asia	Perinatal & horizontal	More severe disease, higher risk of cirrhosis and HCC; delayed HBeAg seroconversion
D	Mediterranean, Middle East, India, Central Asia	Endemic; common in IV drug users	More aggressive, associated with fulminant hepatitis and cirrhosis
E	West & Central Africa	Horizontal and maternal	High prevalence; mainly acute, but can become chronic
F	South & Central America (Indigenous populations)	Horizontal	Severe outcomes, higher HCC risk
G	USA, France, Mexico	Often in co-infection with A or in HIV+ patients	Rare alone; low replication efficiency
H	Mexico, Central America	Endemic in Latin America	Associated with mild to moderate liver disease
I	Vietnam, Laos, China	Rare; recombinant genotype	Associated with severe disease and HCC
J	Japan (Ryukyu Islands)	Very rare; possible zoonotic origin	Severe cases reported

As shown in Table 2-3, genotype D—which predominates in Iraq—is associated with more persistent disease and reduced response to interferon-based therapy, underscoring its clinical relevance to the present study Schaefer, 2021

2.9. Diagnosis

Determination of the hepatitis b virus (HBV) infection is based on a combination of various methods that include serology, molecular, and histological tests. All these methods have certain information that contributes to the nature of the infection and aids in the clinical decision-making. The diagnostic tools may include simple rapid screening tests or more complex molecular tests that can determine the degree of the viral replication (WHO, 2024). In order to have the right diagnosis, one should not only identify viral antigens and nucleic acids, but also examine the state of the liver. That is why assessment of biochemical indicators, especially liver enzymes, is regarded as the important element of the diagnosis process. These indicators assist in the assessment of the level of liver inflammation and are significant in the process of making decisions as far as treatment is concerned. Additional information regarding such indicators is presented in the next section.

2.9.1. Rapid HBsAg Screening Tests

The most commonly employed initial screening tool used is the rapid diagnostic tests (RDT), particularly when the programs are large-scale or when resources are limited. Such tests rely on the lateral-flow immunoassay methods and are easy to administer, it takes just a small amount of blood or serum. The results are generally ready in 10-20 minutes and hence can be used in fast screening. Nevertheless, such tests

tend to be less sensitive and specific than more sophisticated procedures including enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR). This is why positive results achieved in the rapid tests are likely to require confirmation using laboratory-based methods (Osiowy *et al.*, 2019).

2.9.2. Serological Diagnosis

Serological testing has been regarded as the primary method of screening infection of HBV and differentiating between various phases of the disease. The hepatitis B surface antigen (HBsAg) presence is an indicator of an active infection. The anti-HBs antibodies detection on the other hand indicates immunity, either through vaccination or an already healed infection. Moreover, anti-HBc IgM is usually connected with acute infection whereas anti-HBc IgG demonstrates the previous exposure to the virus. The amount of viral replication and the extent of infectivity is also determined using markers like HBeAg and anti-HBe (CDC, 2023).

2.9.3. Molecular Testing

Molecular testing is regarded as one of the more precise techniques of assessing HBV infection, especially in the determination of the amount of viral load. Quantitative HBV DNA PCR is popular as a reference test in the detection of viremia. Such tests are significant in the treatment of whether treatment is required, the reaction to antiviral treatment, and the presence of potential viral rebound. Moreover, the genotyping techniques, such as sequencing and probe-based assays can give additional data about the virus. The techniques can be used to predict the disease outcome and to understand the epidemiological trends of HBV in various areas (WHO, 2024).

2.9.4. Emerging Biomarkers

Recent studies have identified several emerging biomarkers that may improve the monitoring and management of chronic HBV infection. These biomarkers provide additional information about viral replication, cccDNA activity, disease progression, and treatment response. The most important emerging biomarkers and their clinical significance are summarized in Table 2.4 (Pawlotsky, 2024; Agarwal, 2024).

Table 2.4. Emerging biomarkers used in chronic HBV infection and their relationship with cccDNA

Biomarker	What it Measures	Reflects	Sample Type	Relationship with cccDNA
HBcrAg	Core-related viral proteins	Intrahepatic viral activity	Serum	Indirect surrogate marker
HBV RNA	Pregenomic RNA	Active viral replication	Serum/Plasma	Reflects transcriptionally active cccDNA
qHBsAg	Quantitative HBsAg	Viral transcriptional activity	Serum	Correlates with cccDNA activity
miRNA-122	Liver-specific microRNA	Hepatocyte injury and inflammation	Serum/Plasma	Indirect association

2.9.5. Fibrosis and Histological Examination.

Even though these techniques are not direct HBV infection diagnostic techniques they are utilized in assessing liver condition. The extent of fibrosis and the intensity of necroinflammation are usually assessed by liver biopsy and non-invasive methods, including transient elastography (FibroScan). They are also useful in the process of making treatment decisions. Together with the serological and molecular tests, they present a more detailed clinical picture of the situation with a patient (WHO,

2024).

2.10. Liver Enzymes as Indicators of HBV-Related Liver Injury

The liver enzyme is a good predictor of liver injury caused by HBV-related liver injury. The liver enzyme test plays a significant role in assessing the damage of the liver in a hepatitis B virus (HBV) patient. The most commonly used enzymes include alanine aminotransferase (ALT) and the aspartate aminotransferase (AST). These are enzymes that are discharged into the blood when the liver cells are destroyed (Terrault, 2022). During acute HBV infection levels of ALT and AST are typically and dramatically elevated due to immune-mediated damage of infected hepatocytes. Clinical symptoms that accompany this increase include jaundice. The degree of ALT increase is typically regarded as the indirect measure of the intensity of the immune response (Yuen, 2023). The levels of ALT can be different in case of chronic infection of HBV and this is with regard to the phases of the disease. In the immune-active phase, patients regularly exhibit constant or intermittent high levels of ALT whereas in the immune-tolerant phase, normal levels of ALT could be witnessed despite high levels of viral replication. This emphasizes the role of combining the results of ALT with those of the HBV DNA (Terrault, 2022). Continuous increase in ALT is linked to an increased threat of fibrosis, cirrhosis, and hepatocellular carcinoma (Yuen, 2023). In some cases, especially in severe fibrosis or cholestasis, other liver enzymes can also rise e.g. alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). Nevertheless, ALT and AST are the most effective predictors of hepatocellular injury. This is why liver enzyme testing can be regarded as quite a helpful and affordable method, yet must be combined with serological and molecular results to conduct the right clinical examination (Terrault, 2022; Yuen, 2023).

2.11. Immune Response in HBV

The immune system has the key role of deciding whether to clear or to develop a chronic infection of hepatitis B virus (HBV). The virus per se does not do much direct hepatocytic damage. Most of the liver damage, on the other hand, takes place due to the host immune response. That is why, HBV infection has an outcome defined by the equilibrium between the immune response to the virus and immune tolerance (Xia, 2020).

2.11.1. Innate Immune Response

The first line of defense is the innate immune system and is activated once the virus has been introduced into the body. Pattern-recognition receptors (PRRs) detect different viral components, including HBV DNA, RNA intermediates, and viral proteins. These are Toll-like receptors (TLR3, TLR7, and TLR9), cytosolic sensors (RIG-I and MDA-5). The intracellular signaling pathways that are evoked by the activation of such receptors include interferon-regulatory factors (IRF3 and IRF7) and nuclear factor-KB (NF-KB). This leads to the production of type I interferons (IFN- α and IFN- 2) and interferon-stimulated genes (ISGs) to curb early viral replication (Li *et al.*, 2022; Frontiers in Immunology, 2022).

Nonetheless, HBV can suppress these early immune responses. Viral proteins, especially HBx and viral polymerase can inhibit Toll-like receptor signaling, lower interferon production, and antiviral genes expression. This enables the virus to remain in cells (Li *et al.*, 2022). The natural killer (NK) cells also act as a part of the innate immune response by destroying the infected cells and production of interferon-gamma

(IFN- γ). Their role is usually minimized in chronic HBV infection despite this role. These are reduced cytotoxic activities, disappearance of activating receptors, and production of amplified inhibitory signals, all of which contribute to the maintenance of the virus (Khanam, 2021).

2.11.2. Adaptive Immune Response

Adaptive immune system contributes significantly to the long term outcome of HBV infection. The responsibility of CD8⁺ cytotoxic T lymphocytes (CTLs) is to eliminate infected hepatocytes using perforin and granzyme as well as the release of antiviral cytokines like interferon-gamma (IFN- γ) and tumor necrosis factor- α (TNF- α). This process is supported by CD4⁺ helper T cells which preserve the activity of CTLs and stimulate the B-cell to produce antibodies against hepatitis B surface antigen (HBsAg), which helps to get rid of the virus (Xia, 2020).

With chronic HBV infection, immune efficiency is lost gradually, and this is as a result of uninterrupted contact with viral antigens. The functions of CTLs decline with time, and they become less active and express more inhibitory molecules (programmed death-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4). They also generate a lesser amount of cytokines, as a result of which the virus can remain (Li *et al.*, 2022). Also, chronic infection is linked to alterations of B-cell activity, such as the rise of exhausted-like B-cells and a decrease in memory B-cell production. This can help to cause delayed or even absent seroconversion of HBsAg (Shen, 2022). These immune-related processes are significant to understand in order to make sense of cytokine signaling changes. A change in the inflammatory cytokines like interleukin-6 (IL-6) and that of regulatory cytokines like interleukin-10 (IL-10) can indicate the degree of either immune activation or immune suppression in chronic HBV infection.

Cytokines play a significant role in regulating immune responses during HBV infection. IL-6 is a pro-inflammatory cytokine produced by hepatocytes, Kupffer cells, and activated T cells in response to viral infection. Elevated IL-6 levels are associated with increased hepatic inflammation, fibrosis, and a higher risk of hepatocellular carcinoma. In contrast, IL-10 is an anti-inflammatory cytokine secreted by regulatory T cells, B cells, and Kupffer cells. It suppresses antiviral immune responses and may contribute to viral persistence and chronic infection. Therefore, IL-6 and IL-10 represent two opposing aspects of the immune response in chronic HBV infection, reflecting inflammation and immune regulation, respectively (Shen, 2022; Hoshino, 2023; Zheng, 2021; Yin, 2022).

The clinical relevance of IL-6 and IL-10 in HBV infection is related to their ability to reflect disease activity and immune status. Elevated IL-6 levels are associated with liver inflammation, fibrosis progression, and disease severity. In contrast, increased IL-10 levels indicate immune suppression, viral persistence, and chronic infection. Together, these cytokines provide complementary information regarding HBV progression, making them useful biomarkers for disease evaluation and prognosis (Hoshino, 2023; Shen, 2022; Zheng, 2021; Yin, 2022).

2.12. Sequencing Technologies in HBV Molecular Analysis

Sequencing is an important molecular tool for studying HBV genetic variability, identifying viral genotypes, and detecting mutations associated with viral replication, disease progression, and treatment response. Among the available methods, Sanger sequencing remains widely used because of its high accuracy in analyzing specific genomic regions, such as the precore (PC), basal core promoter (BCP), and S genes. These regions are frequently examined to identify clinically significant mutations and determine genotype distribution. Although next-generation sequencing (NGS) provides more comprehensive genomic information and can detect minor viral variants,

Sanger sequencing remains a reliable and cost-effective approach for targeted HBV molecular analysis (Garcia-Garcia et al., 2021).

The precore/core region of the hepatitis B virus (HBV) genome plays an important role in viral replication and antigen production. Transcription from cccDNA is regulated by the Basal Core Promoter (BCP), which controls the synthesis of precore and core mRNAs. The precore region encodes HBeAg, a secreted protein commonly associated with active viral replication and high infectivity, whereas the core gene encodes HBcAg, the structural protein that forms the viral nucleocapsid and contributes to the assembly of infectious Dane particles.

Mutations in the precore and BCP regions are clinically significant because they can reduce or abolish HBeAg expression without completely suppressing viral replication. Consequently, some patients may present with HBeAg-negative chronic hepatitis B despite having detectable HBV DNA levels. Clinically, HBeAg positivity is generally associated with active viral replication, while HBeAg-negative patients may represent either an inactive carrier state or active chronic infection resulting from precore/BCP mutations.

Due to their important role in viral replication and antigen expression, the precore and BCP regions are common targets for molecular analysis. Sequencing of these regions enables the detection of clinically relevant mutations, supports genotype identification, and improves understanding of HBV pathogenesis. These mutations are particularly common in HBV genotype D, the predominant genotype in Iraq and many Middle Eastern countries.

Chapter Three

Materials & Method

3. Materials and Methods

3.1 Materials

3.1.1 Laboratory Equipment and Instruments

The table below lists the laboratory equipment and instruments used in this study (3-1).

Table (3-1): Lists the laboratory equipment used in this study

NO.	Laboratory Equipment and Instruments	Manufacturer/Origin
1	Biological safety cabinet class I	Thermo Fisher Scientific, USA
2	ELISA system- ELISA system	Bio Tek (USA)
3	Eppendorf centrifuge	Hettich (Germany)
4	Freezers (−20 °C and −80 °C)	Panasonic Biomedical, Japan
5	Gel electrophoresis apparatus	LKB (Sweden)
6	Hot plate with magnetic stirrer	Heidolph (Germany)
7	Microcentrifuge	Heraeus (Germany)
8	Micropipettes	Capp (Denmark)
9	Microwave oven	Elekta (Japan)
10	Nano Drop Spectrophotometer	Thermo fisher(USA)
11	PCR thermocycler system	Techne (UK)
12	pH-meter	WTW (Germany)
13	UV Transilluminator	UVP (USA)
14	Vortex mixer	IKA, Germany

3.1.2 Technical Instruments and Disposable Materials

Technical Instruments and disposable materials used in this study were listed in table (3-2).

Table (3-2): Technical Instruments and Disposable Materials Used in This Study

No.	Item	Manufacture (Origin)
1	Biohazard waste bags and sharps containers	Local suppliers
2	Cryovials (1.8 mL) for serum storage	Nunc (Denmark)
3	Disposable lab coats and masks	Local suppliers
4.	K2 EDTA-tubes	Afco (Jordan)
5	Medical gloves	Broche (PRC)
6	Microtiter plates	Biofil (korea)
7	Parafilm	Bemis (USA)
8	PCR tubes 1.5 ml(Eppendorf)	Bio basic (Canada)
9	PCR tubes 200µl (Eppendorf)	Bio basic (Canada)
10	Tips	Dolphin (Syria)

3.1.3 Biological and Chemical Material

The biological and chemical materials used in this study were listed in table (3-3).

Table (3-3): Biological and Chemical Materials Used in This Study

No.	Biological and Chemical Materials	Manufacturer (Origin)
1	Agarose	Bio basic (Canada)
2	DNA safe stain (alternative to Ethidium bromide)	Intron (Korea)
3	EDTA solution (0.5 M, pH 8.0)	Bio Basic (Canada)
4	Ethanol "70%"	Sigma-Aldrich
5	Ethanol "99%"	Sigma-Aldrich
6	Loading dye (6X)	Thermo Fisher Scientific (USA)
7	Nuclease Free Water	Bioneer (Korea)
8	Phosphate Buffer Solution (PBS)	Promega (USA)
9	Sodium chloride (NaCl)	Merck (Germany)
10	TBE buffer (10X)	Bio Basic (Canada)
11	Tris-HCl buffer	Bio Basic (Canada)

3.1.4 Molecular and Immunological Materials and kits

The commercial kits used in the present study were illustrated in table (3-4).

Table (3-4): Commercial kits which used in this study

NO.	Type of Kit	Company (Origin)
1	DNA extraction Kit	Geneaid (Taiwan)
2	ELISA kit for IL-10	Elabscience (china)
3	ELISA kit for IL-6	Elabscience (china)
4	Green master mix	Bioneer (Korea)
5	PCR product purification kit	QIAGEN (Germany)
6	HBS Ag Kit (rapid)	Elabscience (china)
8	HBS Ag Kit (ELIZA)	Elabscience (china)

3.1.5 Molecular Materials

The molecular-related materials and kits used in this study, including reagents required for DNA extraction, nested PCR amplification, agarose gel electrophoresis, and PCR product purification, are summarized in Table (3-5).

Table (3-5): Molecular Materials and Kits Used in This Study

NO	Item	Company	Origin
1.	2X PCR Taq MasterMix	Bioneer.	Korea
2	DNA ladder (100–3000 bp, 12 fragments)	Bioneer	Korea
3	Nuclease free water	Bioneer	Korea
4	Primer pairs	IDT	Canada
5.	TBE buffer (10X, pH 8.3)	Bio basic	Canada
6	DNA stain (SYBR Safe)	Thermo Fisher Scientific	USA
7	6X loading dye	Thermo Fisher Scientific	USA
8	Positive control DNA for HBV PCR	Previously confirmed HBV-positive sample Local laboratory	Iraq

3.1.6 Primers used for Nested PCR amplification

Table (3-6): Primers used for Nested PCR amplification of HBV Precore/BCP region

Target Region	PCR Round	Forward Primer (5'–3')	Reverse Primer (5'–3')	Expected Product Size
Precore / Basal Core Promoter (BCP)	First Round (Outer)	BCP1-F: TCGCATGGAGACCACCGTGA	BCP1-R: ATAGCTTGCCTGAGTGC	467 bp
Precore / Basal Core Promoter (BCP)	Second Round (Inner)	BCP2-F: CATAAGAGGACTCTTGGACT	BCP2-R: GGAAAGAAGTCAGAAGGC	316 bp

3.2 Study Design

This control –case study was conducted between August and December 2025 and included patients with chronic hepatitis B virus (HBV) infection and healthy controls. Chronic HBV infection was defined as the persistence of hepatitis B surface antigen (HBsAg) positivity for more than six months. A total of 60 HBV-infected patients and 24 healthy controls were recruited from Al-Diwaniyah General Hospital and the Main Blood Bank in Al-Diwaniyah, Iraq. Inclusion criteria for patients included positive HBsAg and clinical or laboratory confirmation of chronic HBV infection. Exclusion criteria included co-infection with HCV, HIV, or other viral hepatitis viruses, as well as autoimmune liver disease, diabetes mellitus, and chronic renal failure. Healthy controls consisted of voluntary blood donors who tested negative for HBsAg, anti-HCV, and HIV.

3.3 Sample Collection

Venous blood samples (5 mL) were collected aseptically by trained personnel and distributed according to the required analyses. Blood samples collected in EDTA tubes were used for molecular investigations, whereas serum samples obtained after centrifugation at 3000 rpm for 10 minutes were used for immunological assays. For cytokine analysis, serum samples from 24 HBV-infected patients and 24 healthy controls were included in the measurement of IL-6 and IL-10 levels. Serum samples were inspected for hemolysis, transferred into sterile cryovials, and stored at -80°C until analysis to preserve cytokine stability. EDTA plasma samples were processed immediately or stored at -20°C before DNA extraction. To maintain sample quality, repeated freeze–thaw cycles were avoided, and samples showing visible hemolysis or insufficient volume were excluded from the study.

3.3 Ethical Approval

The study protocol was reviewed and approved by the Ethical Committee of Al-Diwaniyah Health Directorate under approval number (374). The research was conducted as part of a Master's thesis in the Department of Biology, College of Science, University of Al-Qadisiyah. All clinical samples were collected from Al-Diwaniyah General Hospital and the Main Blood Bank in Al-Diwaniyah, Iraq, between August and December 2025, after receiving official permissions from the relevant health authorities. Written informed consent was obtained from all participants before sample collection, following an explanation of the study objectives, procedures, and potential risks.

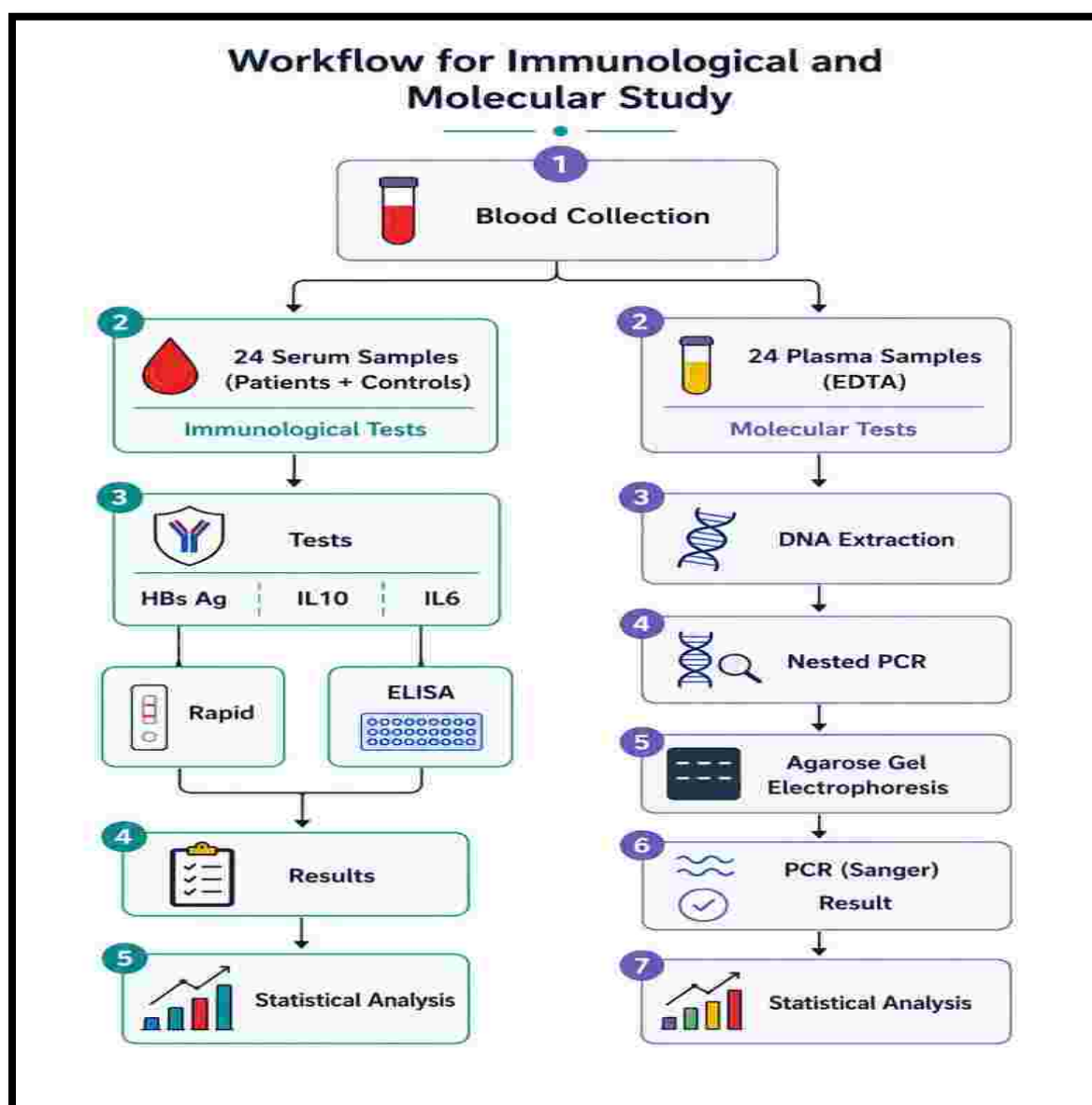


Figure (3-1): Study Workflow Diagram

Figure (3-1): Workflow of the study showing sample collection, DNA extraction, nested PCR, gel electrophoresis, sequencing, bioinformatics analysis, immunological analysis by ELISA (IL-6 and IL-10), and statistical analysis.

3.4 Laboratory Methods

This part explains the laboratory processes undertaken in the serological, immunological, and molecular study of the hepatitis B virus (HBV) infection. Every experimental procedure was carried out by

standardized laboratory procedures in order to render accuracy, reproducibility, and reliability of results. The laboratory program involved serological screening, quantitative cytokine assay by ELISA, molecular detection of HBV DNA by nested PCR and confirmatory genetic analysis using Sanger sequencing.

3.4.1 Serological Methods

Qualitative detection of hepatitis B surface antigen (HBsAg) was done by rapid immunochromatographic testing using the instructions of the manufacturer. Enzyme-linked immunosorbent assay (ELISA) was used to confirm reactive samples in accordance with the usual laboratory procedures. All the assays were performed under regulated laboratory conditions and the results were interpreted based on suggested cut-off values of the manufacturer.

3.4.2 Immunological Analysis

Quantitative sandwich ELISA kits were used to determine the serum levels of IL-6 and IL-10 as per the standard laboratory procedures. The experiment was performed in the following way: A total of 48 serum samples were analyzed for immunological assessment, including 24 patients with confirmed hepatitis B virus (HBV) infection and 24 apparently healthy individuals serving as controls. Serum cytokine concentrations were measured using enzyme-linked immunosorbent assay (ELISA) and expressed in picograms per milliliter (pg/mL) according to the generated standard curves. Before statistical comparison, the distribution of IL-6 and IL-10 concentrations was evaluated using the Shapiro–Wilk normality test.

Step 1: Preparation

Reagents and serum samples were left in equilibrium to room temperature. Serial dilution was done in the recommended concentration range to

prepare calibration standards.

Step 2: Sample and Standard Addition.

The serum samples and standards, 100 μL of each, were placed on the antibody-coated microplate wells. Incubation of the plate was done at 37 $^{\circ}\text{C}$ in order to facilitate cytokine-immobilized capture antibody binding.

Step 3: Washing

After incubation the wells were washed 3-5 times with wash buffer in order to remove unbound material.

Step 4: Antibody Addition: Detection.

Each well was added to biotinylated IL-6 or IL-10 detection antibody and incubated at 37 $^{\circ}\text{C}$.

Step 5: Enzyme Conjugate Reaction.

Following the washing, the Horseradish peroxidase (HRP)-conjugated reagent was added and incubated to enable the amplification of the signal of the enzyme.

Step 6: Substrate Development

The substrate solution of Tetramethyl benzidine (TMB) was put on and the plate was incubated in the dark until development of a color occurred.

Step 7: Reaction Termination

The solution was stopped by adding stop solution to carry out the enzymatic reaction to get a measurable color change.

Step 8: Measurement and analysis.

A microplate reader was used to measure the absorbance at 450 nm. Quantification of cytokine concentrations was performed using a four-parameter logistic (4-PL) logistic nonlinear regression model to ascertain that the correct quantification was performed throughout the entire

dynamic range of the assay. The concentrations were given in picograms per milliliter (pg/mL). Each sample was analyzed twice to achieve reliability and reproducibility of the analysis.

3.4.3 Molecular Analysis

3.4.3.1 DNA Extraction

step, the column was centrifuged at high speed to remove residual wash buffer. The bound DNA was then eluted by adding the elution buffer directly onto the center of the silica membrane and centrifuging according to the manufacturer's protocol. The purified DNA was collected in sterile microcentrifuge tubes and stored at -20°C until further molecular analysis, including nested PCR amplification of the HBV precore/basal core promoter (BCP) region. Nested PCR Amplification Viral DNA was extracted from plasma samples using a commercial spin-column DNA extraction kit (Geneaid, Taiwan) according to the manufacturer's instructions. Briefly, 200 μL of plasma was transferred into a sterile microcentrifuge tube, followed by the addition of the appropriate lysis buffer and Proteinase K provided in the kit. The mixture was vortexed thoroughly and incubated to ensure complete lysis of viral particles and release of nucleic acids. After lysis, ethanol was added to the lysate and mixed well. The entire mixture was then transferred to a silica membrane spin column and centrifuged, allowing viral DNA to bind selectively to the membrane under optimized salt conditions. The flow-through was discarded, and the column was washed sequentially using the washing buffers supplied with the kit to remove proteins, cellular debris, and other contaminants.

3.4.3.2 Nested PCR Amplification

HBV DNA was amplified by nested polymerase chain reaction (nested PCR) targeting the precore/basal core promoter (BCP) region of the HBV

genome. The amplification process consisted of two successive PCR rounds to enhance the sensitivity and specificity of viral DNA detection. The first-round PCR was performed using outer primers to amplify a 467 bp fragment, whereas the second-round (nested) PCR utilized inner primers to amplify a 316 bp fragment within the first-round product. The composition of the reaction mixtures used in both PCR rounds is presented in Tables (3.7) and (3.8), respectively.

First-Round PCR

The first-round PCR was performed in a final reaction volume of 25 μL using outer primers specific for the precore/BCP region. The composition of the reaction mixture is shown in Table (3.7).

Table (3.7). Components of the First-Round PCR Reaction Mixture

Component	Volume (μL)
2 $\times$ PCR Master Mix	12.5
Forward Outer Primer (10 pmol/ μL)	1.0
Reverse Outer Primer (10 pmol/ μL)	1.0
DNA Template	5.0
Nuclease-Free Water	5.5
Total Volume	25.0

The thermal cycling conditions used for the first-round PCR are presented in Table (3.8)

Table (3.8). Thermal Cycling Conditions for the first -Round (Nested) PCR

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	30 sec	35
Annealing	55	30 sec	35
Extension	72	45 sec	35
Final Extension	72	7 min	1
Hold	4	∞	—

Second-Round (Nested) PCR

The first-round PCR product was used as a template for the second-round (nested) PCR using inner primers targeting an internal fragment of the HBV precore/BCP region. The composition of the nested PCR reaction mixture is presented in Table (3.9), whereas the corresponding thermal cycling conditions are shown in Table (3.10).

Table (3.9). Components of the Second-Round (Nested) PCR Reaction Mixture

Component	Volume (μL)
2× PCR Master Mix	12.5
Forward Inner Primer (10 pmol/μL)	1.0
Reverse Inner Primer (10 pmol/μL)	1.0
First-Round PCR Product	2.0
Nuclease-Free Water	8.5
Total Volume	25.0

Table (3.10). Thermal Cycling Conditions for the Second-Round (Nested) PCR

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	30 sec	35
Annealing	52	30 sec	35
Extension	72	45 sec	35
Final Extension	72	7 min	1
Hold	4	∞	—

3.4.3.3 Agarose Gel Electrophoresis.

The amplified PCR products were analyzed by agarose gel electrophoresis to confirm the presence and expected size of the amplified DNA fragments. A 1.5% agarose gel was prepared by dissolving 1.5 g of agarose powder in 100 mL of 1× TBE buffer. The mixture was heated in a microwave oven until the agarose was completely dissolved and a clear solution was obtained.

After cooling to approximately 50–60°C, 5 µL of nucleic acid staining dye (RedSafe DNA stain) was added and mixed thoroughly. The agarose solution was then poured into a gel casting tray fitted with an appropriate comb to form sample wells and allowed to solidify at room temperature for approximately 20–30 minutes.

Following solidification, the gel was placed in an electrophoresis tank containing sufficient 1× TBE running buffer to completely cover the gel surface. PCR products were mixed with 6× loading dye at a ratio of 5:1.

Subsequently, 5 μ L of a 100–3000 bp DNA ladder and 10 μ L of each PCR product were carefully loaded into separate wells. Electrophoresis was performed at 90–100 V for approximately 45–60 minutes. After completion of electrophoresis, DNA bands were visualized using a UV transilluminator and photographed using a gel documentation system. The sizes of the amplified fragments were determined by comparison with the DNA ladder. Expected fragment sizes were 467 bp for the first-round PCR and 316 bp for the second-round (nested) PCR.

3.4.3.3 PCR Product Purification

PCR products that were amplified specifically were purified before being sequenced. The purification was done through a commercial PCR purification kit, following the standard laboratory protocols. With the help of amplified products, the mixture containing them was added to the binding buffer and then transferred to a silica-based purification column to be able to selectively bind DNA fragments. To eliminate the remaining primers, nucleotides, enzymes, and product of the reaction, the column was washed. Elution buffer was then used to purify DNA and it was stored at -20 °C awaiting sequencing. This was necessary to have quality sequencing output to remove contaminants that have the potential to disrupt subsequent genetic analysis.

3.4.3.4 Sanger Sequencing

Confirmatory genetic analysis and HBV genotyping were done by Sanger sequencing of purified PCR products. Amplified samples with distinct and specific bands (n=12) were then picked to undergo sequencing. Forward and reverse primers against the precore/ basal core promoter (BCP) region were sequenced. Sequencing was conducted on the premise of the chain-termination technique in which the fluorescently labelled dideoxynucleotides (ddNTPs) were used, and nucleotide

sequences were determined by capillary electrophoresis. The sequence chromatograms were obtained and checked to ensure that the quality of the sequences was clear and good. To get the consent sequences of the individual samples, forward and reverse reads were matched to come up with the consensus sequences of the sample before subjecting the samples to bioinformatic analysis.

3.4.4 Bioinformatics Analysis

The acquired nucleotide sequences were analyzed using an extensive bioinformatic approach to define the genotype classifications and genetic variation in the precore/basal core promoter (BCP) of the hepatitis B virus (HBV). Raw sequencing chromatograms were also closely examined to guarantee the clarity of peaked signals, levels of signal, and general quality of sequences. The forward and reverse reads were edited and assembled to obtain high-quality consensus sequences per sample. The ambiguous bases were manually revisited and corrected where needed in order to achieve accuracy and reliability of sequences. The alignment tool provided in MEGA 12.1 software was used to compare the constructed sequences with some reference HBV strains stored in the GenBank database of various geographic locations. Multiple sequence alignment was used to compare the sequences generated with the reference strains of HBV. The sequence similarity analysis was done to facilitate the identification of a genotype and determination of the nucleotide variation among isolates. The evolutionary relationship of the isolates in the study and reference strains was assessed using phylogenetic analysis by the Neighbor-Joining method as implemented in MEGA 12.1 software. The genetic distances were calculated by Kimura two-parameter model. Bootstrap resampling (1,000 replicates) was used to measure the strength of the phylogenetic tree topology to identify the statistical strength of the branching patterns. The phylogenetic tree resulting was plotted and ready

to be shown without distorting the postulated evolutionary pattern.

3.4.5 Statistical Analysis

The apparatus of statistical analysis of the immunological data was the Statistical Package of the Social Sciences (SPSS) software (version 26.0, IBM Corp., Armonk, NY, USA). First, data were evaluated with regard to normality of distribution. The variables followed no normal distribution and, therefore, non-parametric tests were used. The results were in the form of median and interquartile range (IQR). The Mann Whitney U test was used to perform comparisons between study groups. The p-value less than 0.05 was taken as statistically significant.

Besides the immunological data analysis, molecular sequence data were also subjected towards bioinformatic and phylogenetic analysis. The MEGA 12.1 software was used to offer multiple sequence alignment in order to determine nucleotide variation as well as sequence similarity between the obtained HBV isolates and reference strains retrieved in the GenBank database. The Neighbor-Joining method was used to build phylogenetic trees.

Bootstrap resampling of 1,000 replicates was used to assess the strength of the phylogeny tree topology. Intersection values were computed to determine the statistical stability of the patterns of branching, and any value above 50% were estimated as evidence of statistically supported nodes. The Kimura two-parameter model was estimated over the MEGA 12.1 software to calculate genetic distances. A p-value of less than 0.05 was regarded as statistically significant (Daniel & Cross, 2018).

Chapter four

Results

4 Results

4.1. Immunological Results: Serum IL-6 and IL-10 Levels

The results indicated that cytokine levels were not normally distributed among the study groups. Therefore, non-parametric statistical methods were applied. Data were presented as median values with interquartile ranges (IQR), and comparisons between HBV-infected patients and healthy controls were performed using the Mann–Whitney U test. The analysis revealed statistically significant differences in serum cytokine levels between HBV-infected patients and healthy controls ($p < 0.001$).

4.1.1. IL-6 Serum levels

Serum IL-6 concentrations were markedly elevated in patients with HBV infection compared with healthy controls. The median IL-6 concentration in HBV-infected patients was **61.96 pg/mL (IQR: 49.31–78.37)**, whereas healthy controls demonstrated a substantially lower median concentration of **10.40 pg/mL (IQR: 7.34–13.55)**, as presented in Table 4.1.

The interquartile range in the HBV group indicates consistently elevated IL-6 levels with limited overlap relative to the control group. In contrast, IL-6 concentrations among healthy individuals remained within a narrow and lower range, suggesting baseline physiological cytokine expression.

Statistical comparison using the Mann–Whitney U test revealed a highly significant difference between the two groups ($p < 0.001$). This finding indicates a pronounced pro-inflammatory response associated with HBV infection, reflected by elevated circulating IL-6 levels. The distribution of IL-6 concentrations between the two study groups is illustrated in Figure 4.1 and Table 4.1, where a clear separation between HBV patients and controls can be observed.

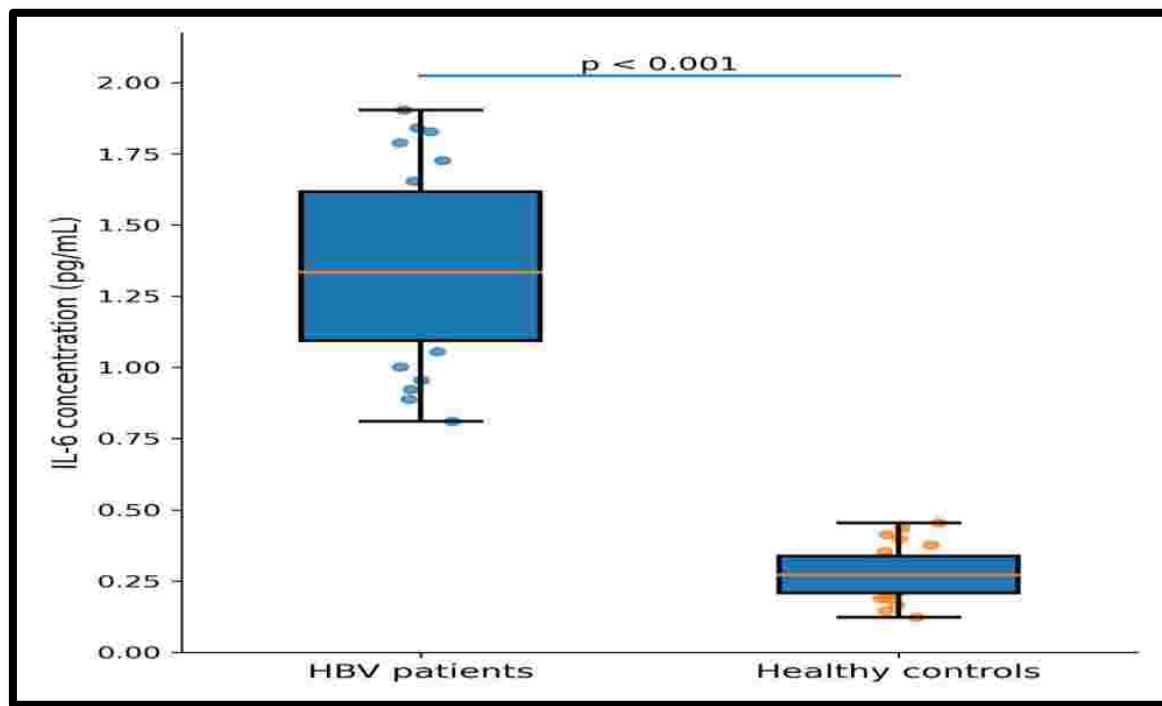


Figure (4.1): Distribution of serum IL-6 concentrations (pg/mL) in HBV patients and healthy controls. Box plots represent median, interquartile range, and minimum–maximum values. Mann–Whitney U test; $p < 0.001$.

Table 4.1: Serum IL-6 Concentrations (pg/mL) in HBV Patients and Healthy Controls

Group	n	Median (pg/mL)	IQR
HBV patients	24	61.96	49.31–78.37
Healthy controls	24	10.40	7.34–13.55

In addition to IL-6, the anti-inflammatory cytokine IL-10 was also evaluated in HBV patients and healthy controls.

4.1.2. IL-10 Serum levels

The serum IL-10 levels were also significantly increased in the HBV-infected patients than in the healthy controls. As shown in Table 4.2, the median IL-10 concentration among patients with HBV was 35.19 pg/mL (IQR: 25.25-47.16) whereas the median IL-10 concentration among healthy controls was much smaller, 4.37 pg/mL (IQR: 2.22-6.25). The interquartile of IL-10 of the HBV group implies that there is a stable response of anti-inflammatory cytokine in the infected people. On the other hand, the IL-10 levels of the control group were within a small physiological range as they represent normal immune regulation in non-infected conditions. The statistical analysis with Magnitude Whitney U test showed that the difference between HBV patients and healthy controls is highly significant ($p < 0.001$). This significant increase of IL-10 may indicate an increase in regulatory immune response which goes hand in hand with HBV infection and may indicate immune regulation mechanisms during viral persistence. The pattern of IL-10 distribution among the two groups is graphically depicted in Figure 4.2

and this confirms further the significant divide between the HBV-infected patients and controls.

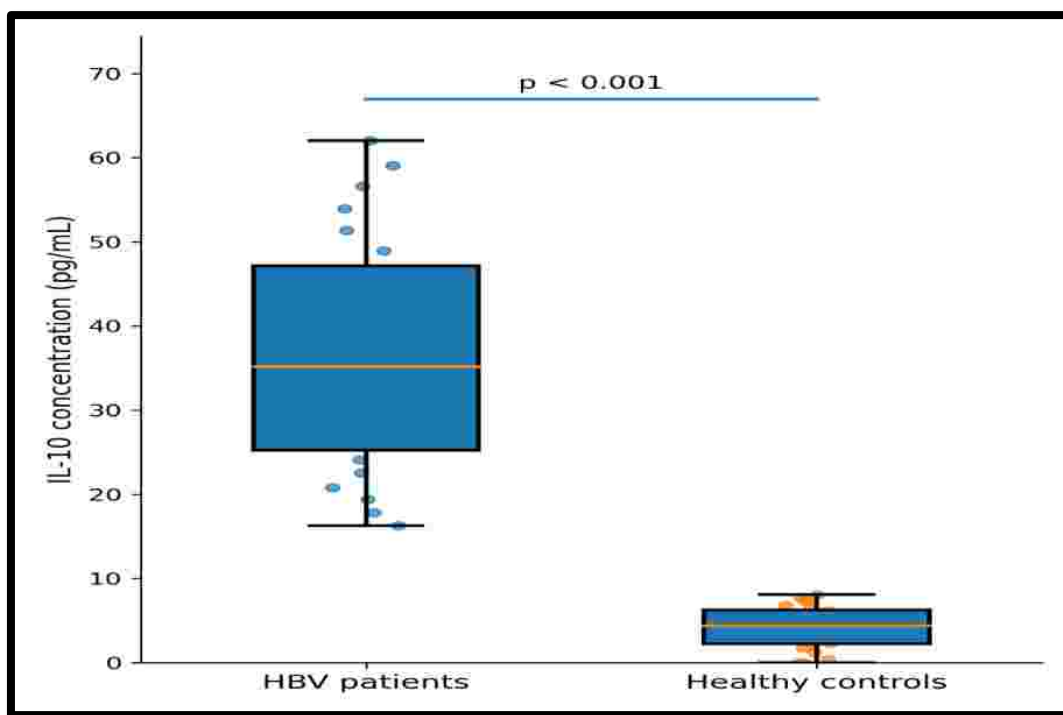


Figure (4.2): Distribution of serum IL-10 concentrations (pg/mL) in HBV patients and healthy controls. Box plots show the median, interquartile range, and minimum–maximum values, along with individual data points. Mann–Whitney U test; $p < 0.001$. table 4.2

Table 4.2: Serum IL-10 Concentrations (pg/mL) in HBV Patients and Healthy Controls

Group	N	Median (pg/mL)	IQR (pg/mL)
HBV patients	24	35.19	25.25–47.16
Healthy controls	24	4.37	2.22–6.25

A comparative summary of IL-6 and IL-10 concentrations between the study groups is presented in Table 4.3.

Table 4.3: Comparison of Serum IL-6 and IL-10 Concentrations Between Study Groups

Cytokine	HBV Median (IQR) pg/mL	Control Median (IQR) pg/mL	P- Value
IL-6	61.96 (49.31–78.37)	10.40 (7.34–13.55)	<0.001
IL-10	35.19 (25.25–47.16)	4.37 (2.22–6.25)	<0.001

4.2. Molecular Detection of HBV DNA by Nested PCR

For molecular investigation, 30 EDTA plasma samples obtained from HBV-infected patients were subjected to viral DNA extraction. Following DNA quantification and quality assessment, six samples were excluded due to insufficient DNA yield. Consequently, 24 samples were included in the final nested PCR analysis. Nested PCR targeting the precore and basal core promoter (BCP) region of the HBV genome was performed in two sequential rounds. The first-round PCR generated an amplicon of 467 bp, while the second-round (nested) PCR produced a specific fragment of 316 bp. Successful amplification of the expected nested PCR product (316 bp) was observed in the analyzed samples. Clear and distinct DNA bands corresponding to the expected molecular size were visualized on agarose gel electrophoresis, confirming the presence of HBV DNA in the tested samples. The amplification results and representative gel electrophoresis patterns are shown in Figure 4.3. Out of the 24 analyzed samples, a subset showing clear amplification bands was selected for subsequent sequencing analysis.

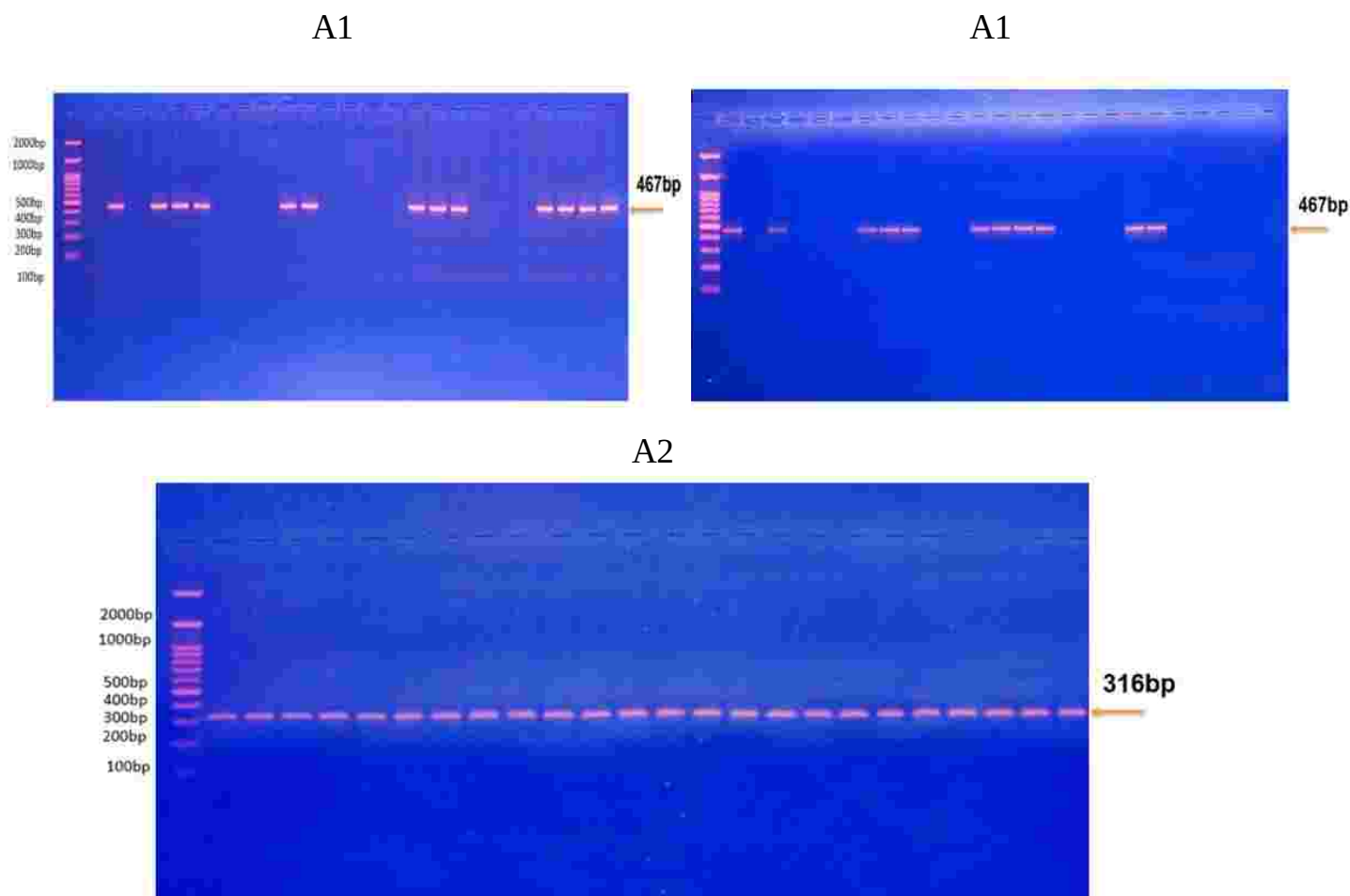


Figure (4.3): Agarose gel electrophoresis of nested PCR products targeting the HBV precore/BCP region. Lane M: DNA ladder (100-3000 bp). Lane P: positive control. Lane N: negative control. A1,: represent the first stage of the amplified band at 467. A2: The second stage, Lanes 1–12 represent representative HBV-positive samples showing the expected amplification band at 316 bp.

4.3. HBV DNA Sequencing Results (Precore/BCP Region)

The obtained sequences (Seq1–Seq12) were partial HBV genomic sequences with lengths of 322–323 bp. Minor differences from the expected PCR product size were likely due to primer nucleotides and sequence trimming during processing.

All sequences were obtained from human blood samples collected in Iraq in 2025. Chromatogram analysis showed clear peaks and low background noise, indicating good-quality sequencing reads. These results confirmed successful amplification and sequencing of the targeted precore/BCP region. The sequence characteristics are summarized in Table 4.4, and phylogenetic analysis was performed to evaluate genetic relatedness among the isolates.

Table 4.4. Summary of HBV precore/BCP sequences generated in this study

Sequence ID	Sample code (from note)	Length (bp)	Country	Collection year	Sequencing method
Seq1	ZDQSB1	322	Iraq	2025	Sanger
Seq2	ZDQSB2	322	Iraq	2025	Sanger
Seq3	ZDQSB3	323	Iraq	2025	Sanger
Seq4	ZDQSB4	323	Iraq	2025	Sanger
Seq5	ZDQSB5	323	Iraq	2025	Sanger
Seq6	ZDQSB6	322	Iraq	2025	Sanger
Seq7	ZDQSB7	323	Iraq	2025	Sanger
Seq8	ZDQSB8	323	Iraq	2025	Sanger
Seq9	ZDQSB9	323	Iraq	2025	Sanger
Seq10	ZDQSB10	323	Iraq	2025	Sanger
Seq11	ZDQSB11	323	Iraq	2025	Sanger
Seq12	ZDQSB12	322	Iraq	2025	Sanger

4.3.1 Molecular and Phylogenetic Analysis of HBV Precore/BCP Sequences

To further investigate the genetic characteristics and evolutionary relationships of the sequenced HBV isolates, detailed molecular and phylogenetic analyses were performed based on the obtained precore/basal core promoter (BCP) nucleotide sequences.

4.3.1.1 Sequence Characteristics

A total of twelve HBV nucleotide sequences (Seq1–Seq12) targeting the precore/BCP region were successfully generated in this study. All sequences were derived from human EDTA plasma samples collected in Iraq in 2025 and were obtained using Sanger dideoxy sequencing technology. The amplified fragments ranged from 322 to 323 base pairs in length, consistent with the expected nested PCR amplicon size. Minor variation in sequence length was attributed to trimming procedures during chromatogram editing and consensus sequence assembly.

All isolates were taxonomically classified as *Hepatitis B virus* (taxon:10407), genus *Orthohepadnavirus*, family *Hepadnaviridae*. Metadata associated with each sequence confirmed uniform host origin (*Homo sapiens*), isolation source (blood), geographic origin (Iraq), and collection year (2025), ensuring reliability and consistency for downstream comparative analysis.

4.3.1.2 Nucleotide Composition and Sequence Variability

Analysis of nucleotide composition revealed slight variation in base distribution among the twelve isolates. For example, Seq1 contained 72 adenine (A), 70 cytosine (C), 79 guanine (G), and 101 thymine (T) residues, whereas Seq2 exhibited 72 A, 74 C, 80 G, and 96 T. Comparable minor differences in nucleotide

frequencies were observed across the remaining sequences..

Multiple sequence alignment demonstrated a high degree of nucleotide conservation across most positions, with conserved motifs maintained among the majority of isolates. However, scattered single nucleotide substitutions were identified at specific positions in several sequences. These variations were limited to point mutations and did not involve insertions, deletions, or major structural rearrangements. No large deletions, frameshift mutations, or extensive sequence divergence were detected within the analyzed region. The observed variability therefore reflects natural intra-genotypic polymorphism within circulating HBV strains rather than significant genomic instability.

4.3.1.3 Phylogenetic Analysis

Phylogenetic analysis was conducted to investigate the evolutionary relationships of the HBV precore/BCP sequences obtained in this study in comparison with selected reference strains retrieved from the GenBank database. The analysis aimed to determine the genetic relatedness, clustering behavior, and evolutionary proximity of Iraqi isolates within a broader regional and international context. The sequence showed that all HBV isolates belonged to the D genotype. As illustrated in Figure 4.4, the Iraqi isolates (PX955235–PX955258) were distributed into several closely related subclusters rather than forming a single homogeneous lineage. This clustering pattern indicates the circulation of genetically related yet distinguishable HBV variants within the studied population. Several internal nodes displayed high bootstrap support values, reaching up to 100, indicating strong statistical confidence in the inferred evolutionary relationships. Other nodes showed moderate to lower support values, reflecting natural genetic diversification within the precore/BCP region. The phylogenetic tree showed that the Iraqi HBV isolates clustered into several closely related subgroups rather than

forming a single homogeneous lineage. Several internal nodes displayed high bootstrap support values, reaching up to 100, indicating strong statistical confidence in the inferred evolutionary relationships. The clustering pattern suggests genetic relatedness among circulating HBV strains in the studied population. Finally, phylogenetic analysis revealed that the HBV isolates identified in this study clustered closely with previously reported HBV strains from Iraq, Iran, China, and South Korea. This clustering indicates a high level of genetic similarity and suggests a common evolutionary origin. Furthermore, the high bootstrap values observed in most branches provided strong statistical support for the inferred phylogenetic relationships.

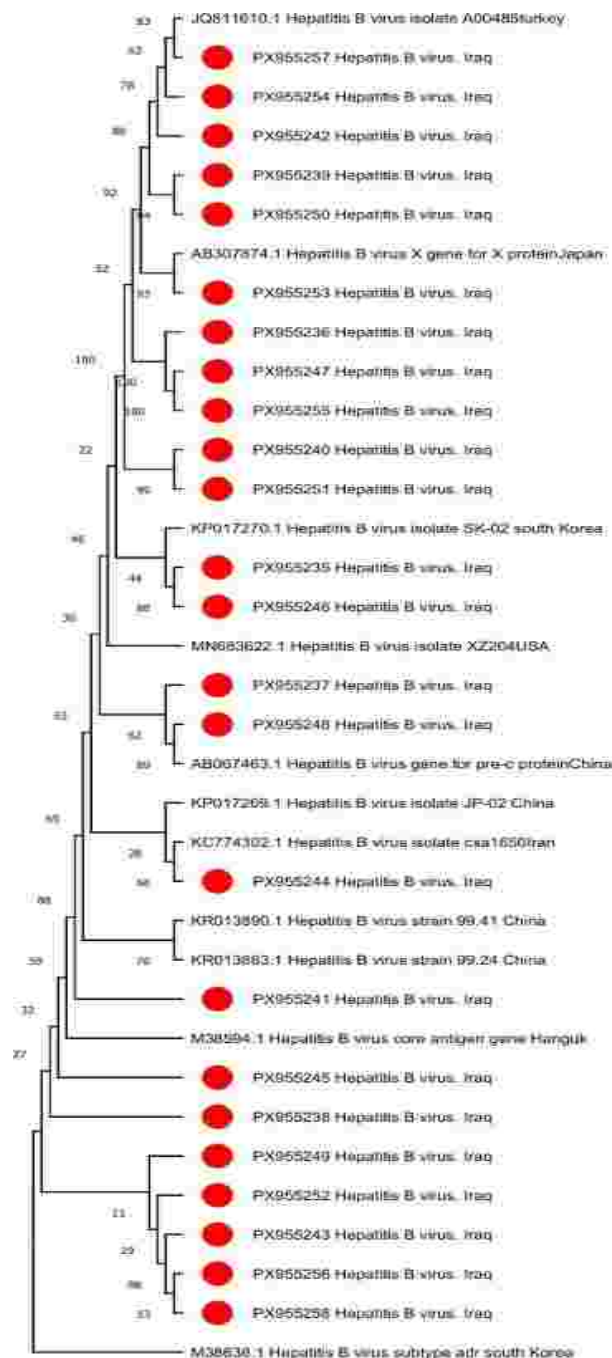


Figure (4.4): Phylogenetic Analysis of HBV PC/BCA gene

Chapter Five

Discussion

5 Discussion

5.4 Immunological Significance of Elevated IL-6 Levels in HBV Infection

The results of this research showed that IL-6 serum levels were increased in chronic hepatitis B patients in comparison to healthy controls. This increase was evident as indicated in Chapter 4 and could be an indication of a persistent inflammatory response to the chronic nature of the infection. This finding is consistent with a number of other past studies (Kareem and Mohammed, 2025), which also reported high levels of IL-6 in the HBV-infected patients. These observations indicate that IL-6 is likely to have a significant role in the inflammatory responses that take place in the liver in the face of persistent infection. The elevated IL-6 levels are commonly linked to the amplified inflammatory signaling, which, eventually, can lead to the damage of liver tissue (Kim *et al.*, 2020; Zhang *et al.*, 2022). Also, recent reports indicate that the IL-6 could potentially mediate the necroinflammation formation, potentially by recruiting immune cells to the infected liver tissue and promoting the inflammatory response (Wang *et al.*, 2023). IL-6 is one of the pro-inflammatory cytokines that are important in controlling immune responses, particularly in cases of viral infections. It is synthesized by a number of cell-types, such as macrophages, hepatocytes and T lymphocytes and is generally produced in response to infection or inflammatory stimuli. Part of its main roles is to stimulate intracellular signaling cascades and facilitate the acute-phase response, hence facilitating host defense. In chronic hepatitis B infection, an active immune system is maintained over a long period of time due to the persistent presence of the virus. Such continuous stimulation may cause a

sustained cytokine production including IL-6 that may maintain the liver in a chronic inflammatory state. The recent Iraq and world research has reported that IL-6 correlates with liver damage and could be a disease progression in HBV patients (Abbas, 2024; Gao *et al.*, 2022). It may also be possible that the imbalance of the immune response is disrupted by the dysregulation of IL-6 production, and the body may not be able to properly regulate the viral replication (Dimitriadis *et al.*, 2023; Li *et al.*, 2022). The disease severity in chronic hepatitis B is closely associated with elevated levels of IL-6. Increases in this cytokine in most instances indicate increased liver inflammation and are associated with immune-mediated tissue injury. This relationship is supported by a number of clinical studies. Indicatively, a study conducted by Chen *et al.* (2022) found that IL-6 was significantly higher in active hepatitis B patients, and the latter was related to the increase in the inflammatory activity of liver tissue. Likewise, Shen *et al.* (2022) recommended that IL-6 high levels may be a sign of lack of cytokine balance and may be the reason behind the ongoing inflammation in some individuals. Furthermore, there is also some recent evidence that IL-6 can also lead to liver fibrosis, where the persistent inflammatory signaling slowly leads to structural damage of liver tissue (Zheng *et al.*, 2023).

5.5 Immunoregulatory role of IL-10 in Chronic HBV Infection

Besides the observed IL-6 increase, the current study also indicated a higher level of serum IL-10 in patients with chronic HBV infection relative to healthy controls, which is also consistent with the findings of (Rubayii and Meesher, 2025) and AbdulAl-Abass (2025), who found that IL-10 levels are also significantly higher in patients with hepatitis B, which This observation can point to the occurrence of immunoregulatory

processes during chronic infection. The anti-inflammatory cytokine IL-10 is well known as an important part of keeping immune balance by preventing excessive inflammation. It is secreted by a number of immune cells such as regulatory T cells, macrophages and dendritic cells and primarily acts by suppressing the release of pro-inflammatory cytokines and lowering antigen-presenting functions (Ouyang *et al.*, 2011). Within the framework of chronic hepatitis B, an increase in the level of IL-10 is a common finding that is commonly regarded as a component of an immune response that takes place during chronic infection. Recent works have postulated that elevated IL-10 levels correlate with the alterations in the immune response patterns and the emergence of immune tolerance in patients with HBV (Shen *et al.*, 2022; Yin *et al.*, 2022). The high rate of IL-10 in this research could be an indication of an effort by the immune system to keep down over inflammation and minimise liver tissue damage. In chronic HBV infection, there is a possible compensatory mechanism of augmented IL-10 production that can curtail immune-mediated tissue damage. Nevertheless, there can be a negative side to this effect. The continuous increase of the IL-10 can decrease the effectiveness of antiviral immunity and possibly lead to the development of viral infection. This is primarily by inhibiting HBV-specific T-cell activity and inhibiting production of pro-inflammatory antiviral cytokines. Past research has defined chronic HBV infection as a disease with a regulatory immune setting, with anti-inflammatory signaling, such as IL-10, contributing to incomplete viral elimination (Bertoletti & Ferrari, 2016; Wieland and Chisari, 2019). Still more recent studies also highlight that regulatory immune cells and inhibitory cytokines contribute to immune tolerance in the context of HBV infection, inhibit cytotoxic T-cells, and impair antiviral immunity (Zheng *et al.*, 2023; Lin *et al.*, 2023). Moreover, recent evidence indicates that HBV-specific CD8⁺ T-cell

responses could be directly inhibited by IL-10 secreted by regulatory B cells, which could also contribute to viral persistence in chronic infection (Yu *et al.*, 2024).

Meanwhile, long-term increase in IL-10 can lead to the weakening of the immune system to eliminate the virus. Though IL-10 is beneficial in preventing the liver against a lot of inflammation, the chronic overexpression of this cytokine may override key antiviral responses. Some studies have demonstrated that the functional exhaustion of HBV-specific CD8⁺ T cells is linked to high IL-10 levels and reduces their capacity to destroy infected hepatocytes (Rehermann & Thimme, 2019). Further, the suppression of the generation of important antiviral cytokines like interferon- γ and tumor necrosis factor- α by IL-10 has been associated with immunosuppression, which is vital in controlling the virus (Zhang *et al.*, 2021). Recent evidence also indicates that sustained IL-10 expression can help create an immunotolerant environment in the liver, which enables the virus to persist and the chronicity of the infection (Chen *et al.*, 2023; Dimitriadis *et al.*, 2023). In general, these data are not only consistent with the current findings of the study but also indicate that high IL-10 levels can be viewed as a regulatory mechanism that can be used to contain liver damage along with promoting long-term viral persistence.

5.6 Cytokine Imbalance and Immune Dysregulation in Chronic HBV Infection

The findings of the current research revealed that the levels of IL-6 and IL-10 were high in chronic HBV infected patients as compared to healthy controls. The concomitant rise of these two cytokines could signify a cytokine imbalance in chronic infection. This trend indicates that there is coexistence of both the pro-inflammatory and anti-inflammatory reactions. This is typical of chronic viral infections and

represents the multivariate manner in which immune activation and regulation interplay. The same results were observed earlier, as both inflammatory and regulatory cytokines were found to increase in HBV patients as a part of the immune response to the continued viral replication (Bertoletti and Kennedy, 2015; Wieland and Chisari, 2019). This dual response can be the dynamic equilibrium between immune activation and control. Cytokines such as IL-6 have been identified to mediate inflammatory signaling and aid in the recruitment of immune cells to infected liver tissue. These reactions are necessary to restrict the viral replication, however, when prolonged too much, they may cause liver inflammation and tissue damage as well. Anti-inflammatory cytokines like IL-10, on the other hand, aid in curbing excessive immune response and safeguarding the liver against immune-mediated damage. Thus, a balance between these two types of cytokines is crucial in stabilizing immunity in chronic infection (Bertoletti and Ferrari, 2016; Rehmann and Thimme, 2019). Nevertheless, this balance may be perturbed, which may influence disease progression in chronic HBV infection. This chronic high IL-6 might cause inflammatory state in the liver and result in necroinflammation, fibrosis, and hepatocellular injury. Concurrently, increased IL-10 can inhibit efficient antiviral immunity, which can enable the virus to survive. A number of studies indicate that the imbalance of cytokines is a major factor in HBV pathogenesis and may affect the course of the disease, including the development of more severe forms of it, such as cirrhosis and hepatocellular carcinoma (Wieland *et al.*, 2019; Zheng *et al.*, 2023). In light of these results, it appears that the concomitant increase in IL-6 and IL-10 in this case, is representative of a complex immune environment that not only restrains excessive inflammation but also may lead to persistent viral infection and liver injury.

Results of this study are mostly in line with the previous studies, which have indicated that chronic HBV infection is associated with alterations in cytokine responses. The result of this change is often manifested in the form of a concomitant increase in pro-inflammatory and anti-inflammatory cytokines. The trend denotes that the immune system is reacting to persistent viral infection by stimulating and suppressing immune activities. Other previous studies concur that incessant virus replication is triggered through the production of inflammatory cytokines and regulatory processes. The changes in cytokine profiles are probably due to the complicated relationship between viral persistence and the host immune responses in chronic infection. The fact that chronic HBV infection is linked to cytokine network changes has also been noted by recent research, which may lead to immune dysregulation and disease progression (Zheng *et al.*, 2023; Dimitriadis *et al.*, 2023).

The high levels of IL-6 and IL-10 in this study can be of significant clinical significance in the understanding of immune responses in chronically infected patients of HBV. An elevation in IL-6 levels is usually regarded as an increased inflammatory activity and has been identified to be linked with liver necroinflammation, severity, and conversion to fibrosis. The IL-6 in most clinical studies has been considered as a sign of active liver inflammation. As an example, Kim *et al.* (2020) and Wang *et al.* (2023) found that the more IL-6 was expressed, the more active the inflammatory processes and the disease involved the patient. Thus, the IL-6 levels monitoring can be an important source of information concerning the inflammatory condition of patients and can help to assess the progression of the disease with time. On the other hand, high IL-10 concentrations depict a significant regulatory feature of the immune response in chronic HBV infection. IL-10 helps

limit excessive inflammation in the liver by decreasing the production of pro-inflammatory cytokines and controlling immune cell activity. This effect is normally positive, in that it safeguards liver tissue against damage. Nevertheless, the long-lasting presence of IL-10 can also suppress antiviral immunity and enable the virus to stay. Some reports have revealed that a rise in the levels of IL-10 can reduce the T-cell response to the virus and general antiviral response (Bertoletti & Ferrari, 2016; Rehmann and Thimme, 2019). Furthermore, other more recent studies indicate that sustained IL-10 signaling could impose an immunosuppressive environment in the liver that would favor the persistence of infection and disease development (Dimitriadis *et al.*, 2023). Together, the evaluation of both IL-6 and IL-10 could give a better picture of the immune status of the patients with chronic HBV infection. IL-6 mainly indicates the degree of inflammatory response, and IL-10 indicates the degree of immune regulation. The analysis of these two markers simultaneously can contribute to understanding the balance between immune activation and control in case of chronic infection. This combined evaluation may prove value in tracking disease progress, and may have some therapeutic ramifications in the future as well.

5.7 Molecular and Phylogenetic Analysis of HBV Precore/BCP Sequences

5.7.1 Sequence characteristics

The outcomes of the present research proved the successful amplification and sequencing of the precore BCP (HBV). Polymerase chain reaction (PCR) was used to obtain twelve positive successive samples, which were subjected to further analysis. The amplified fragments had a size from 322 to 323 base pairs, which is similar to the

size of this region. The sequencing data were well-defined with clear peaks and low background noise. This shows that the sequencing was of good quality to continue further analysis and this agrees with the results of Kramvis (2014) and Tong and Revill (2016) which confirms the validity of molecular methods utilized in this study. The pre-nuclear and core binding regions of HBV are usually studied to get insight into the viral mutations, genetic diversity and viral dispersal.

5.7.2 Nucleotide Variability Among HBV Isolates

The sequence analysis revealed that there were minimal differences in this region and the HBV isolates were very similar. There were some small changes in the nucleotide sequences, which were mostly simple replacements with no significant insertions and deletions. Such variation is typical of HBV since the virus is mutated in the process of replication. The replication of HBV is characterized by reverse transcription which is not very accurate and may cause mutations with time. Also, the immune system is capable of shaping the development of new variants (Seeger & Mason, 2015; Kramvis, 2014). The pre-core and BCP mutations have the potential to influence the viral behavior. As an illustration, they can modify the synthesis of HBeAg, which is involved in the immune response. There are mutations that have been reported to reduce or abolish the HBeAg production, such as G1896A and A1762T/G1764A, and these mutations are frequently associated with chronic infection and immune escape (Tong and Revill, 2016; Reuter *et al.*, 2023).

5.7.3 Phylogenetic Relationships of HBV Isolates

The relationship between the HBV isolates in this study and the reference sequences in global databases was also analyzed using phylogenetics. The findings indicated that the isolates were clustering in a

few closely related clusters instead of a one cluster. This would imply that there are related yet a little different viral strains within the population under study. This trend indicates the natural variation of HBV and shows that the virus is still evolving. The progression of new variants is also due to the HBV replication process which involves frequent mutations (Seeger & Mason, 2015; Tong and Revill, 2016). Phylogenetic analysis is an essential instrument to understand virus diversity, distinguish various genotypes, and trace the virus spreading. The differences between the viral strains can contribute to the severity of the disease, immunity, and clinical outcomes (Kramvis, 2014; Schaefer, 2021).

The results overall suggest that there exists a persistent variation among HBV isolates in this population. This contributes to the knowledge of the evolution and spread of the virus and the significance of molecular analysis in the investigation of the HBV infection. According to this research, longitudinal studies of patients might be useful in the future to understand the dynamics of immune responses and changes in viral genome in chronic infection. Such studies may enhance our knowledge on the interplay between the virus and the immune system and may result in the creation of more effective diagnostic methods and treatments of the HBV infection.

Conclusion and Recommendations

6.1 Conclusion

- 1.The immunological results of the research showed that, in patients with chronic HBV infection, the serum concentration of cytokine IL-6 and IL-10 was significantly greater, compared to healthy controls.
- 2.Raised IL-6 levels indicate an increase in inflammatory processes associated with chronic infection and can lead to inflammation and progression of liver diseases.
- 3.The increase in IL-10 suggests the involvement of regulatory immune response that may assist in the control of the excessive inflammation and potentially aid in viral persistence.
- 4.The concomitant rise of IL-6 and IL-10 in this research indicates that there is an imbalance of cytokines in chronic HBV infection. This trend means that there is a complicated interplay between pro-inflammatory and anti-inflammatory immune reactions, which are essential in the immunopathogenesis of chronic hepatitis B.
- 5.Follow-up sequencing of the selected samples revealed that there was a high degree of nucleotide similarity among the isolates obtained, and there were only minor genetic variations in the region under analysis.
- 6.Phylogenetic analysis revealed that the HBV isolates obtained in the present study belonged to a few closely related clusters, which indicates the presence of genetically similar, but distinct HBV variants in the studied population and underscores the dynamic nature of viral evolution in a chronic infection.

6.2 Recommendations

1. In future studies, the study of the cytokine responses must be integrated with the analysis of genetic variability of the virus to develop a more detailed picture of how hosts and viruses interact. This can aid in the formation of better plans to diagnose, monitor and manage infection of the hepatitis B virus.
2. To make the future research more inclusive, a bigger sample of patients should be implemented to offer a more thorough assessment of immunological reaction and virus genetic diversity relating to chronic HBV infection.
3. It is proposed that additional research needs to be conducted in order to quantify more cytokines and immune biomarkers in order to have a more holistic view of the immune processes that are argued in the pathogenesis of chronic hepatitis B.
4. It is proposed to subject the different sections of the HBV genome to molecular analysis which will provide in-depth details about viral mutations, genetic variation and their potential association with the pathogenesis of the disease.
5. Additional information on the HBV genetic diversity and evolution of virus that can be obtained through use of other advanced molecular techniques, such as next-generation sequencing.
6. It has been proposed that longitudinal investigations to ascertain modifications in cytokines and viral genetic changes overtime would be more suitable to comprehend the dynamic nature of the interaction between the host immune responses and the viral persistence in chronic HBV infection.

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Appendices

Appendices

Appendix (A): Genomic Sequence of Hepatitis B Virus Isolate ZDQSB12 (Accession No. PX955258.1)

GenBank

UNVERIFIED: Hepatitis B virus isolate ZDQSB12 genomic sequence

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Appendices

Appendix (B): IL-10 ELISA Standards (Concentration vs OD 450 nm)

Standard	Concentration (pg/mL)	OD (450 nm)
Std-0	0	0.089
Std-1	1.56	0.131
Std-2	3.13	0.177
Std-3	6.25	0.308
Std-4	12.5	0.451
Std-5	25	0.872
Std-6	50	1.544
Std-7	100	2.236

Appendix (B): OD Values of IL-10 in HBV Patients

Sample	OD (450 nm)
HBV-1	0.602
HBV-2	0.655
HBV-3	0.707
HBV-4	0.753
HBV-5	0.809
HBV-6	0.857
HBV-7	0.905
HBV-8	0.951
HBV-9	1.002
HBV-10	1.056

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HBV-11	1.104
HBV-12	1.156
HBV-13	1.201
HBV-14	1.255
HBV-15	1.302
HBV-16	1.353
HBV-17	1.404
HBV-18	1.455
HBV-19	1.506
HBV-20	1.557
HBV-21	1.608
HBV-22	1.659
HBV-23	1.703
HBV-24	1.755

Appendix (C): OD Values of IL-10 in Healthy Controls

Sample	OD (450 nm)
H-1	0.099
H-2	0.106
H-3	0.111
H-4	0.126
H-5	0.138
H-6	0.144
H-7	0.151
H-8	0.166

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H-9	0.179
H-10	0.182
H-11	0.193
H-12	0.201
H-13	0.216
H-14	0.228
H-15	0.233
H-16	0.248
H-17	0.251
H-18	0.264
H-19	0.272
H-20	0.283
H-21	0.298
H-22	0.307
H-23	0.311
H-24	0.326

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Appendix (D): IL-6 ELISA Standards

Standard	Concentration (pg/mL)	OD (450 nm)
Std-0	0	0.051
Std-1	3.12	0.129
Std-2	6.25	0.197
Std-3	12.5	0.344
Std-4	25	0.623
Std-5	50	1.057
Std-6	75	1.526
Std-7	100	1.988

Appendix (E): OD Values of IL-6 in HBV Patients

Sample	OD (450 nm)
HBV-1	0.811
HBV-2	0.888
HBV-3	0.923
HBV-4	1.001
HBV-5	1.055
HBV-6	1.129
HBV-7	1.183
HBV-8	1.258
HBV-9	1.326
HBV-10	1.384
HBV-11	1.455
HBV-12	1.523
HBV-13	1.582

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HBV-14	1.654
HBV-15	1.726
HBV-16	1.788
HBV-17	1.841
HBV-18	1.903
HBV-19	0.955
HBV-20	1.107
HBV-21	1.269
HBV-22	1.341
HBV-23	1.603
HBV-24	1.827

Appendix (F): OD Values of IL-6 in Healthy Controls

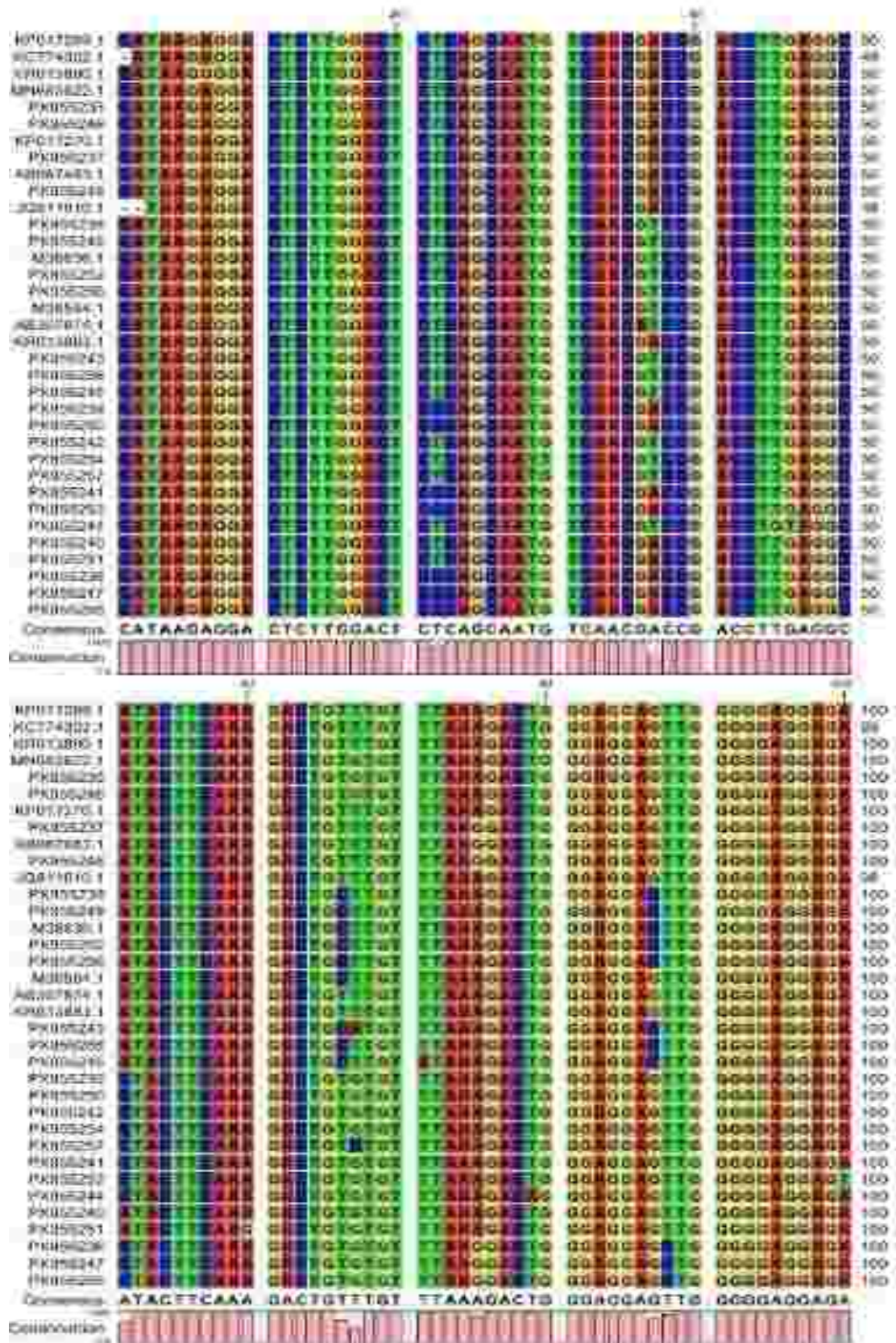
Sample	OD (450 nm)
H-1	0.122
H-2	0.144
H-3	0.166
H-4	0.188
H-5	0.201
H-6	0.223
H-7	0.245
H-8	0.267
H-9	0.289
H-10	0.301
H-11	0.189

Appendices

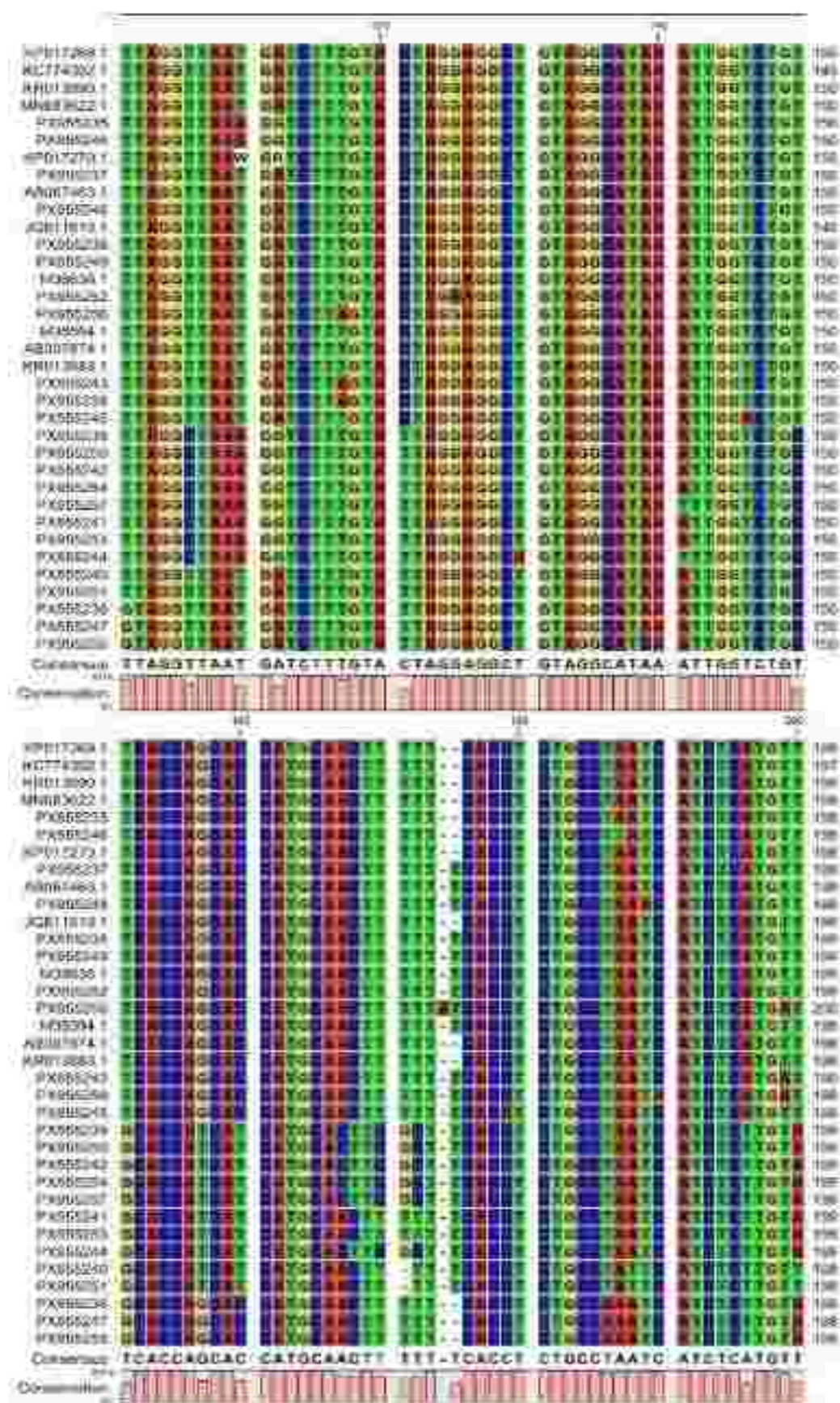
H-12	0.211
H-13	0.233
H-14	0.255
H-15	0.275
H-16	0.297
H-17	0.319
H-18	0.332
H-19	0.354
H-20	0.376
H-21	0.398
H-22	0.412
H-23	0.437
H-24	0.454

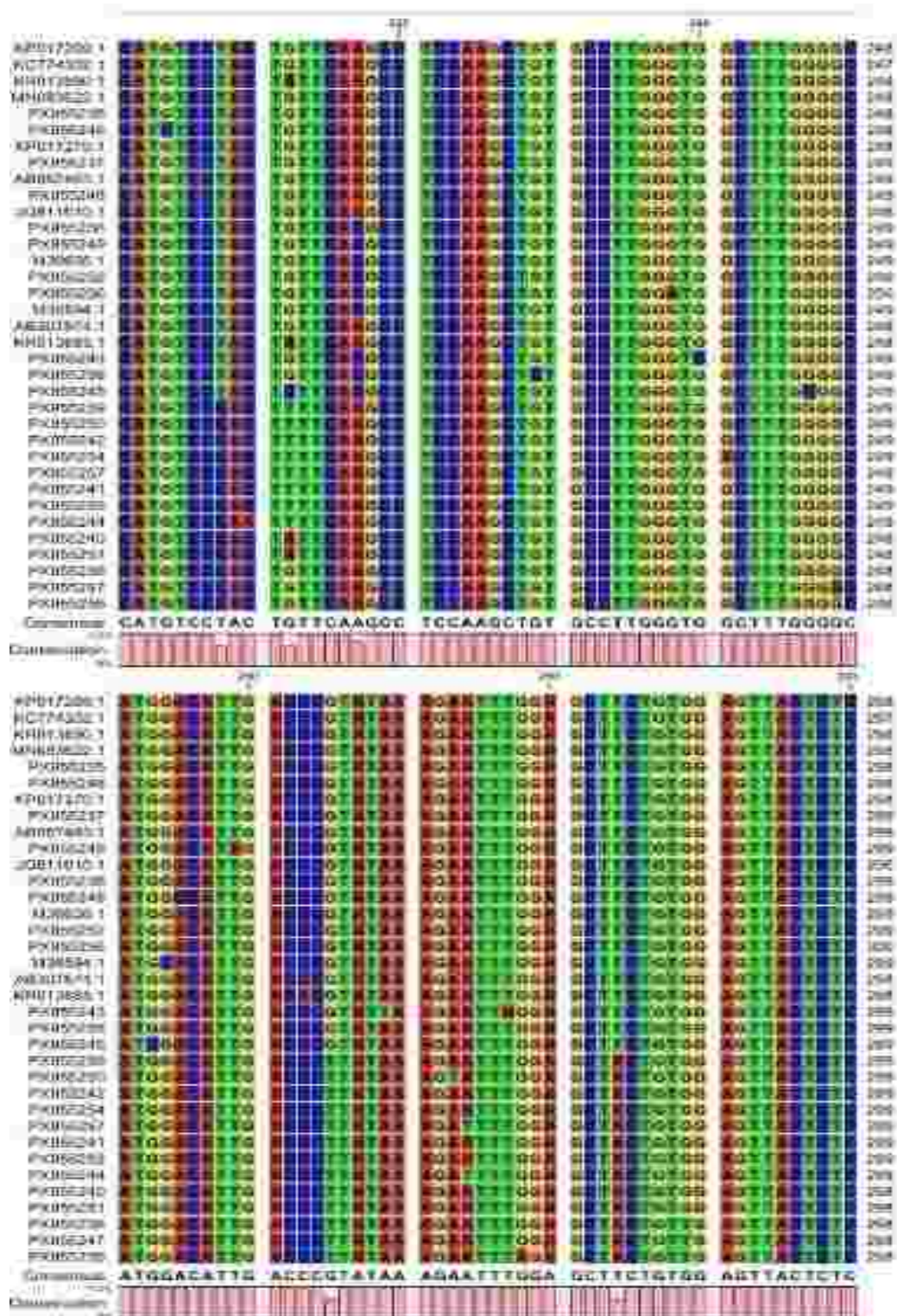
Appendices

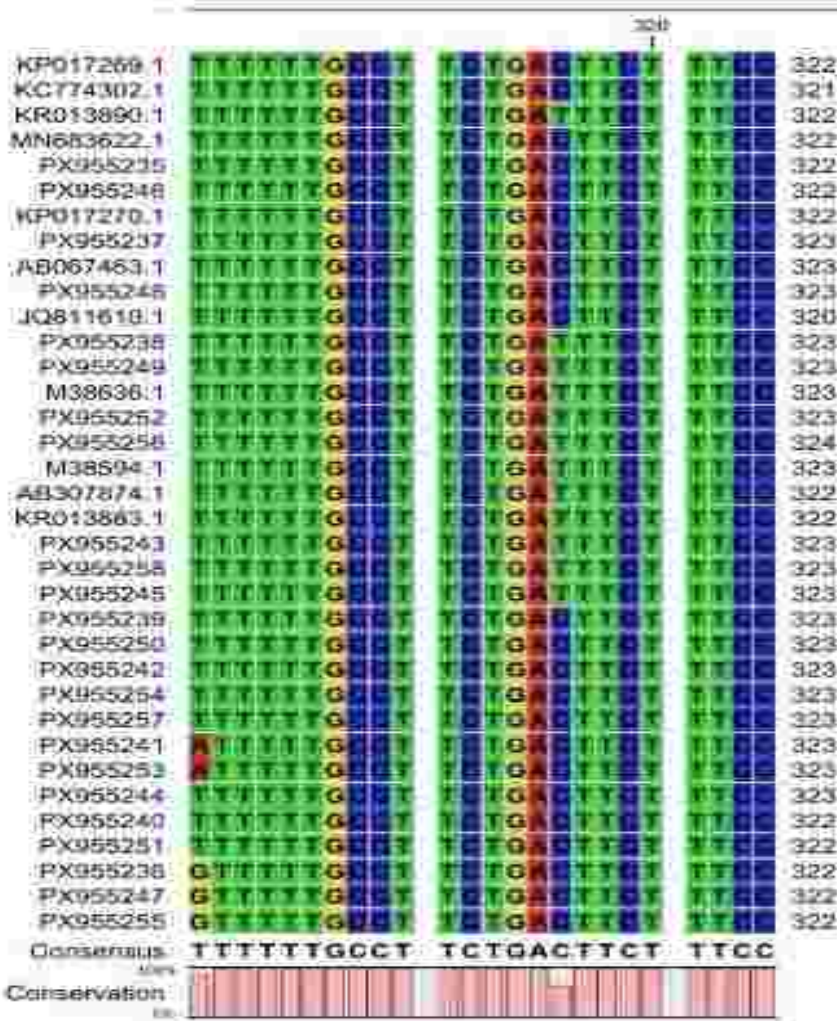
Appendix (G): Comparative Genomic Analysis of Hepatitis Virus via Multiple Sequence Alignment and Conservation Profiling



Appendices







الخلاصة

يُعد فيروس التهاب الكبد (HBV) مشكلة صحية رئيسية تؤثر على الكبد، خاصةً عندما تتحول العدوى إلى حالة مزمنة. ويُعتبر الكبد عضوًا حيويًا، وأي خلل في وظائفه قد يؤدي إلى مضاعفات خطيرة مثل التليف أو سرطان الكبد. ومن السمات الرئيسية لفيروس HBV قدرته على البقاء لفترات طويلة داخل خلايا الكبد بسبب وجود الحمض النووي الدائري المغلق تساهميًا (cccDNA)، مما يجعل القضاء التام على الفيروس أمرًا بالغ الصعوبة.

هدفت الدراسة الحالية إلى فحص التباين الجيني لفيروس التهاب الكبد B في محافظة الديوانية، ودراسة دور السيتوكينات في تطور المرض. تم قياس مستويات كل من الإنترلوكين-6 (IL-6) والإنترلوكين-10 (IL-10) لدى المرضى ومقارنتها مع مجموعة السيطرة.

تم جمع عينات الدم من 60 مريضًا مشخصين بالإصابة بفيروس HBV اعتمادًا على إيجابية مستضد سطح الفيروس (HBsAg)، بالإضافة إلى 24 عينة من أشخاص أصحاء كمجموعة سيطرة. امتدت فترة جمع العينات من أغسطس 2025 إلى ديسمبر 2025 في مستشفى الديوانية التعليمي العام ومصرف الدم الرئيسي. تضمنت معايير الاستبعاد المرضى المصابين بفيروس نقص المناعة البشرية (HIV)، أو فيروس التهاب الكبد (HCV) C، أو أي عدوى فيروسية أخرى، إضافةً إلى مرضى أمراض الكبد المناعية الذاتية، وداء السكري، أو الفشل الكلوي المزمن. تم قياس مستويات IL-6 و IL-10 باستخدام تقنية الإليزا (ELISA).

على المستوى الجزيئي، تم استخلاص الحمض النووي الفيروسي من مصل الدم باستخدام عدة استخلاص تجارية (Geneaid، تايوان). بعد ذلك، أُجري تفاعل البوليميراز المتسلسل المتداخل (Nested PCR) باستخدام خليط تفاعل جاهز. تلا ذلك إجراء تسلسل سانجر (Sanger sequencing) لتأكيد الهوية الجينية والنمط الجيني لفيروس HBV من المناطق المستهدفة (precore و basal core promoter (BCP)). كما تناولت الدراسة آلية انتقال الفيروس ودخوله إلى خلايا الكبد عبر مستقبل NTCP، مع تقديم نظرة عامة على دورة تكاثره، بالإضافة إلى تسليط الضوء على قدرته على التهرب من الجهاز المناعي، مما يفسر استمرارية العدوى لفترات طويلة لدى بعض المرضى.

أظهرت النتائج أن مستويات IL-6 كانت أعلى بشكل معنوي لدى مرضى HBV مقارنةً بمجموعة السيطرة، حيث بلغ المدى الربيعي (IQR: 49.31–78.37) في مجموعة المرضى مقابل (IQR: 7.34–13.55) في مجموعة السيطرة. كما لوحظ ارتفاع مستويات IL-10 لدى المرضى (IQR: 25.25–47.16) مقارنةً بالأصحاء (IQR: 2.22–6.25)، مما يشير إلى استجابة مناعية تنظيمية متزايدة في عدوى HBV، وقد يعكس ذلك آليات تعديل المناعة أثناء استمرارية الفيروس.

أنتج تفاعل Nested PCR الموجه لمنطقتي precore و BCP قطعة مضخمة بطول 316 زوج قاعدي. وأكدت نتائج التسلسل نجاح عملية التضخيم وتوفير بيانات نوكلويدية دقيقة لتصنيف الأنماط الجينية والتحليل التطوري. كما أظهرت النتائج تشابهًا عاليًا بين عزلات الفيروس، مما يدل على محدودية التباين الجيني في هذه المنطقة. كذلك بينت أن الطفرات في منطقتي precore و BCP يمكن أن تؤثر على سلوك الفيروس، مثل التأثير على إنتاج مستضد HBeAg المرتبط بالاستجابة المناعية.

أوضحت الدراسة أيضًا أن استمرارية فيروس HBV تعود إلى قدرته على التهرب من الاستجابة المناعية للمضيف، مما يفسر استمرار العدوى لفترات طويلة لدى بعض المرضى. ويتم ذلك عبر عدة آليات، من بينها تكوين cccDNA داخل الخلايا الكبدية، مما يسمح للفيروس بالبقاء داخل الخلية لفترات طويلة. بالإضافة إلى ذلك، قد تساهم المستويات المرتفعة من IL-10 في تثبيط الاستجابات المناعية النوعية للفيروس وخلق بيئة مناعية متسامحة. في المقابل، تعكس زيادة IL-6 نشاطًا التهابيًا مستمرًا غير كافٍ للقضاء على الفيروس. إن الارتفاع المتزامن لكل من IL-6 و IL-10 يشير إلى اختلال في توازن السيتوكينات، حيث تتواجد استجابات التهابية ومضادة للالتهاب في آن واحد. وقد يعيق هذا الخلل المناعي التنسيق الفعال للاستجابة المضادة للفيروس، مما يعزز من استمرارية تكاثره داخل خلايا الكبد، ويؤدي في النهاية إلى الإصابة المزمنة بفيروس HBV.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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كلية العلوم
قسم علوم الحياة

التوصيف الجزيئي والمناعي لفايروس التهاب الكبد B المزمّن

رسالة

مقدمه الى مجلس كلية العلوم جامعة القادسية

وهي جزء من متطلبات نيل درجه الماجستير في علوم الحياة- علم الأحياء المجهرية

من قبل

زهراء عبد الامير عطيه

بكالوريوس علوم الحياة 2014 - كلية العلوم - جامعة القادسية

بإشراف

أ.م.د ضحى مهدي العابدي