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HEMATOLOGY AND IMMUNOLOGY INDICATORS AND DETERMINATION SOME MUTATION'S FOR *HEPATITIS B VIRUS*

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY DEPARTMENT

BY
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Thesis Approval

The study, titled "**Hematology and Immunology Indicators and Determination of Some Mutations in Hepatitis B Viruses**" and prepared by Samer Kareem Hanoon, HANOON was accepted as **a Master's Thesis** in the Department of Biology of Çankırı Karatekin University, Institute of Science and Technology, by the following jury on January 1,2022. The Ayes have it.

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The thesis on "**Hematology and Immunology Indicators and Determination of Some Mutations in Hepatitis B Viruses**", which I prepared according to the Çankırı Karatekin University Graduate Education and Examination Regulations, is my own and original study; I have acted in accordance with scientific ethical principles and rules in all stages of my study, including preparation, data collection, analysis and presentation of information, that I have not taken the innovations and results of the thesis from anywhere else, that I have duly cited the works I have used in the thesis, that I have submitted the thesis from Çankırı Karatekin University, Institute of Science and Technology. I declare that I did not submit it to another scientific committee for academic purposes and to obtain a title, and that this study was screened by the "Scientific Plagiarism Detection Program" used by Çankırı Karatekin University and that it "does not contain plagiarism". I declare that I consent to all moral and legal consequences that may arise in the event of a situation contrary to this statement I made regarding my work. I request that necessary action be taken in accordance with the relevant articles of Çankırı Karatekin University Postgraduate Education and Examination Regulations (01/01/2022).

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ÖZET

Yüksek Lisans Tezi

HEPATİT B VİRÜSLERİNDE HEMATOLOJİ VE İMMÜNOLOJİ GÖSTERGELERİ VE BAZI MUTASYONLARIN BELİRLENMESİ

Samer Kareem Hanoon HANOON

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Bu tez çalışması, hematoloji ve immünolojinin göstergelerini doğrulamak amacıyla Mart-Temmuz 2021 tarihleri arasında Thi Qar vilayetindeki *Hepatit B virüsü*ndeki bazı mutasyonları belirlemek amacıyla yapılmıştır. Bu çalışma, Nasiriyah kentindeki ana kan bankasına bağışta bulunan görünüşü sağlıklı kan bağışçılarının ve Thi Qar vilayetindeki Al-Hussein Eğitim Hastanesine kabul edilen hastaların dahil olduğu bir anketi kapsamaktadır. Bu çalışma, *Hepatit B virüsü* ile enfekte olmuş 120 örneği içermektedir. Çalışmada ELISA, PCR ve CBC testleri kullanılmıştır. Analiz sonuçları değerlendirilerek çeşitli önerilerde bulunulmuştur.

2022, 71 sayfa
ANAHTAR KELİMELER: *Hepatit B virüsü*, enfeksiyon, gen, PCR, ELISA

ABSTRACT

Master of Science Thesis

HEMATOLOGY AND IMMUNOLOGY INDICATORS AND DETERMINATION OF SOME MUTATIONS FOR HEPATITIS B VIRUS

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This thesis was conducted to verify the indicators of hematology and immunology and to identify some mutations in the hepatitis B virus in the Thi Qar governorate for the period from March 2021 to July 2021. This study included a survey of apparently healthy people from blood donors in the main blood bank in Nasiriyah city, as well as of patients admitted to Al-Hussein teaching hospital in Thi Qar governorate. This study included 120 samples infected with the Hepatitis B virus. The study was carried out using the following tests: ELISA, PCR, and CBC. Various suggestions were made by evaluating the results of the analysis.

2022, 71 pages

Keywords: Hepatitis B virus, infection, gene, PCR, ELISA

PREFACE AND ACKNOWLEDGEMENTS

I would like to my heartfelt thanks my thesis advisor, Assist. Prof. Dr. Melda DÖLARSLAN, for your patience, guidance and understanding. I also extend my heartfelt thanks to Dr. Imran Mezher, the secondary supervisor. Finally, I would like to extend my sincere thanks to my family for their patience and endurance.

SAMER KAREEM HANOON
Cankiri, January 2022

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LIST OF SYMBOLS

IQ.No	Isolation number
Cal_x^2	The calculated chi-square value
Tab_x^2	Tabular chi-square value
Df	Degree of freedom
P. value	Moral value
α	Morale level
OD	Value of the negative control
NC	Absorbance value
C.O.	Cut-off value
LSD	Less statistical differences

LIST OF ABBREVIATIONS

AuAg	Australia antigen
CBC	Complete blood count
cccDNA	covalently closed circular DNA
CDC	Centers for disease control and prevention
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immune sorbent assay
EOS	Eosinophil
ETV	Entecavir
HAV	*Hepatitis A virus*
HbcAg	Hepatitis B virus core antigen
HBe	Hepatitis B e protein
HbsAg	Hepatitis B virus surface antigen
HBV	*Hepatitis B virus*
HBx	Hepatitis B x protein
HCC	*Hepatocellular carcinoma*
HCV	*Hepatitis C virus*
HDV	*Hepatitis D virus*
HEV	*Hepatitis E virus*
HBIG	*Hepatitis B virus* immune globulins
HGV	*Hepatitis G virus*
HRP	*Horseradish peroxidase*
NCBI-BLAST	National center for biotechnology information
NEUT	Neutrophil
PCR	Polymerase chain reaction
Peg IFN	Pegylated interferon
Pol gene	Polymerase gene
RNA	Ribonucleic acid
S gene	Large S protein gene
WHO	The world health organization

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1. INTRODUCTION

Hepatitis is one of the oldest diseases to have been recognized. Hepatitis is not a disease only, but rather a term that means inflammation of the liver cells. Because the liver plays a key role in cellular metabolism, it can be affected by any virus, so hepatitis usually occurs due to a viral infection caused by infectious viruses. However, there are other probable causes of hepatitis, such as auto immune hepatitis and hepatitis caused by medications, pharmaceuticals, toxins, or alcohol (Nielsen *et al.* 2017).

Viral hepatitis is the world's second most common cause of cirrhosis, behind alcoholic hepatitis. Hepatitis causes about 1,000,000 deaths each year, the majority of which are caused by liver scarring or illness (Judith 2020). Hbv has become a serious public health concern that affects hundreds of millions of individuals throughout the globe. In the case of the Hepatitis B, C, and D viruses, viral hepatitis causes major morbidity and mortality in the human population, both as a consequence of acute infection and chronic sequelae, which include chronic active hepatitis and cirrhosis. Chronic infection leads to liver cancer that is incurable (Seeger and Mason 2000).

Hepatitis is classified as acute if it clears up in six months or chronic if it lasts longer than that. Immediate hepatitis can go away on its own, turn into chronic hepatitis, or (in rare cases) cause acute liver failure (Denniston *et al.* 2014). Cirrhosis, liver failure, and liver disease are all possible outcomes of chronic hepatitis. Depending on the type of hepatitis you have, you have several treatment choices. Some types of hepatitis can be avoided with vaccines and lifestyle changes (Vos *et al.* 2016). Hepatocellular carcinoma is one of the ten most prevalent malignancies globally, and it is intimately linked to the hepatitis B and, in some parts of the world, the hepatitis C viruses (Alexopoulou *et al.* 2003). Adult viral hepatitis disease is usually self-limiting, with just 5% of those infected becoming chronic, and 20 to 30% of those who become chronically infected developing cirrhosis or liver disease. Infection in infants and children, on the other hand, commonly leads to persistent infection. Most cases of viral hepatitis, unlike Hepatitis B, result in chronic infection (Rutherford *et al.* 2016).

The Hepatitis B virus (HBV) causes an infectious inflammatory illness of the liver that affects hominoidea. The frequency of HBV infection varies greatly from nation to country

(Shepard *et al.* 2006). HBV infection characterized a form of disease describes by the existence of detectable HbsAg in the serum or blood (Ali *et al.* 2019). Depending on WHO, 887,000 deaths in 2015 attributed to *Hepatitis B virus* complications (WHO 2019). *Hepatitis B virus* (HBV) is an Orthohepadnavirus with features such as retroviruses. The human *Hepatitis B virus* is a small enveloped DNA virus which causes two patterns of hepatitis acute and chronic (Ginzberg *et al.* 2018).

Africa, Southeast Asia, China, Indonesia, the Philippines, the Middle East, and parts of South America have high endemicity (8–15%); Japan, eastern and southern Europe, and parts of South America and Central Asia have intermediate endemicity (2–7%); and North America, northern Europe, and Australia have low endemicity (2%) (Kao *et al.* 2002). The prevalence of HBV in Iraq is estimated to be approximately 1%. Globally, it is believed that 400 million individuals are infected with the virus. This adds to the already overburdened health systems in poor nations like Iraq (Hussein 2015). HBV infection may be spread by surgical and dental procedures, and certain sexual acts. HBV transmission via sexual activity has been documented for over three decades. Homosexual guys have higher incidence of HBV infection, which has been well documented (Akselrod *et al.* 2014).

Immunization against hepatitis A, B, and D is possible. Chronic hepatitis is also treated with medications. Except for those with disorders that restrict their effectiveness, antiviral medicines are advised for all people with chronic hepatitis C. Interferon Alfa is the most common treatment for chronic HBV infection. Medication to suppress the immune system is also used to treat autoimmune hepatitis (Voss *et al.* 2016). In both acute and chronic liver failure, a liver transplant is an option. Hepatitis A infected around 114 million individuals in 2015, chronic hepatitis B infected approximately 343 million people, and chronic hepatitis C infected approximately 142 million people (Friedman *et al.* 2015). Blood transfusions were a key risk for spreading the viral hepatitis virus in the 1970s and 1980s. The risk of contracting hepatitis C as a result of an insertion has declined from about 10% in the 1970s to about 1 in 2 million now (Harder 2008).

When blood or mucous membranes come into touch with contaminated blood or bodily fluids such as sperm or vaginal secretions, the hepatitis B, C, and D viruses are transferred (Bauer 2013). Saliva and breast milk have also been reported to contain viral particles. However, unless these fluids are injected into open wounds or cuts, kissing, sharing

utensils, and nursing do not result in transmission (Friedman *et al.* 2015). HBV transfer from patient to patient has also been thoroughly established, and is frequently linked to contaminated equipment (Kondili *et al.* 2006).

1.1. Aim of study:

1- Finding a relationship between some immunological indicators and Infection with the *hepatitis B virus* in Thi Qar Governorate.

2- A comparison between the percentage of age, gender, educational level and place of residence of the person and their relationship to infection with the *hepatitis B virus* in the research area.

3- Implementation of the DNA sequencing method to determine the genetic relationship and analysis of genetic variation (alternative mutations) in the large S protein gene and the protein polymerase gene. For local Iraqi *Hepatitis B virus* by PCR technique.

4- Comparison between our obtained local isolates and global *Hepatitis B virus* isolates associated with NCBI-Blast.

2. LITERATURE REVIEWS

2.1. History :

The term hepatitis (Inflammation of the liver) was first described by Hippocrates (460-375 BC). In 1918 Martin's description of a non-fatal form of infectious hepatitis among military troops, while in 1943 Beeson's account of cases of jaundice resulting from whole blood transfusion, and at the same year the 1943 Cameron's report of an epidemic hepatitis among troops during World War II (Ray and Ryan 2004).

The causes of hepatitis are varied and include viruses, bacteria, and parasite, as well as drugs, toxins and tumors. Virology was still a new science in the 1960s, devoted mostly to basic research. While scientists have succeeded in growing viruses from a variety of important infections in cell cultures, these approaches remained inadequate for diagnosing the majority of viral disorders. In some circumstances, utilizing an electron microscope to look at the viral particles or using biological approaches is useful. These virus detection methods allowed for the creation of vaccines, but they were too time-consuming, complicated, or unsuitable for individual clinical diagnosis (Gerlich 2013).

In 1963, Nobel Laureate Baruch Samuel Blumberg, an American physician and medical scientist (1925–2011), announced that he and a colleague had discovered a new antigen called Australia antigen (AuAg) in the blood serum of Australian aborigines, which was later determined (in 1967) to be part of a virus that causes Hepatitis B virus. This finding enabled the creation of a blood screening test for Hepatitis B virus transmission, as well as the development of a safe vaccination to prevent Hepatitis B virus infections(Niu and Hann 2017). AuAg antigen is now known as the Hepatitis B virus surface antigen (HBsAg). In the 1970s by using electron microscopy specified the whole viral particle (Halegoua and Hann 2014).

The first commercially ready Hepatitis B virus approved by a food and a drug administration, in 1980 and the genome of HBV was sequenced once for vaccine (Candotti and Laperche 2018). In Iraq the first seroepidemiological study of Infection with the hepatitis B virus from 1989 to 2002, has shown that positivity rate was 1.5 of the prevalence of HBsAg (Hamied *et al.* 2010).

2.2.Viral hepatitis :

2.2.1.Viruses :

In Greek, the term virus means "toxin" or "poison," implying that the disease is small and may only multiply within the cells of another creature. Viruses may infect all living things, including animals, plants, bacteria, and fungus. Despite the fact that there are many different types of viruses, only approximately 5,000 have been detected. As a result, a virus might be thought of as a microscopic parasite that is lesser comparing to bacterium. It may not replicate without the assist of active cell. The virus infects living cells and takes use of their chemical mechanisms to keep itself alive and replicate (Koonin *et al.* 2006).

2.2.2.Viral hepatitis :

Viral hepatitis is a systemic illness that predominantly affects the liver as the primary site of viral replication. It is characterized clinically by fever, jaundice, and the most prevalent type of parenteral transmitted viral hepatitis, and is a leading cause of acute and chronic liver infection (Salisbury *et al.* 2006). The five hepatitis viruses are hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV) (HEV). The hepatitis G virus (HGV) was discovered in 1995. (Linnen *et al.* 1996). Viral hepatitis is often asymptomatic and anicteric, however it may cause a severe sickness with jaundice and, in rare cases, abrupt liver failure (Fattovich *et al.* 2003).

Hepatitis may show up in a number of ways, from no symptoms to severe liver failure. Acute hepatitis is characterized by self-limiting constitutional symptoms and is typically caused by a virus (Rutherford *et al.* 2016). Viral hepatitis has become a serious public health concern that affects hundreds of millions of individuals throughout the globe. In the case of the Hepatitis B, C, and D viruses, viral hepatitis causes major morbidity and mortality in the human population, both as a consequence of acute infection and chronic sequelae, which include chronic active hepatitis and cirrhosis (Zuckerman 1996; Longo *et al.* 2013).

2.3. The hepatitis B virus:

HBV is the smallest human DNA virus, with a genome that is extremely tiny (Fig. 2.1). It is a member of the Hepadnaviridae family, which comprises a group of highly species-specific DNA viruses. The hepatitis B virus is composed of double-stranded DNA viruses that multiply in an unusual manner known as reverse transcription. In many places in the world, the hepatitis B virus is endemic or hyper-endemic. A variety of viral variations have been identified. The Hepatitis B virus was considered as the cause of "serum hepatitis," the most prevalent form of viral hepatitis transmitted by parents (Wang and Seeger 1993).

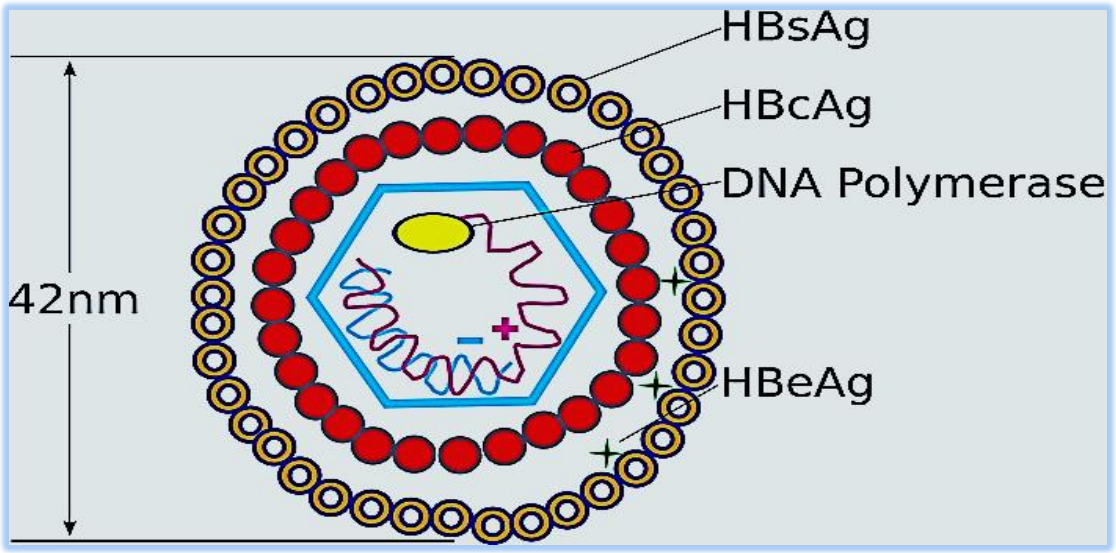

Figure 2.1. The hepatitis B virus's virion structure (HBV) (Keeffe *et al.* 2008).

2.3.1. Classification :

The family *Hepadnaviridae* has been divided into two genera: the *orthohepadnaviruses* (mammals) were the human HBV is belongs. Second genus is the *avihepadnaviruses* (avian) which includes the *Heron Hepatitis B virus*, *Ross goose Hepatitis B virus*. All the viruses in this family show a strong preference for infecting hepatocytes and narrow host-range specificity .

Characteristics of Orthohepadnavirus Viruses in this genus infect animals, with each viral species having a specific host range. Humans and apes are the only known natural hosts of members of the Hepatitis B virus species (chimpanzees, gorillas, orangutans, and

gibbons). Hepatitis B virus is a member of the Orthoheapdnavirus genus with a circular DNA genome that is partly double-stranded and has a core antigen (HbcAg) surrounding by a cortex carrying surface antigen (HbsAg) (Abdulghani *et al.* 2016).

2.3.2. Genome :

Hepatitis B virus genetic material consists of a single circular molecule that is not segmented. The genome is a partly double-stranded DNA molecule , At the 5′ end of the full length minus strand, it forms a covalently closed circle with the viral DNA polymerase. The genetic material of the virus contains four open-read genetic fragments that contain genetic information for seven viral proteins. The full length genome is 3020-3320 nucleotides long (for the full length strand) or 1700-2800 nucleotides long (for the short length strand) , the amount of guanine G and cytosine C 48% , and the size of the genomes are 3.2 kbp. The core gene and the polymerase gene are the most important genes in virus replication, while the envelope gene is a key gene in the evolution of the nuclear capsule (Kramvis 2014).

2.3.3. The hepatitis B virus's virion structure (HBV):

The virion of *Hepatitis B virus* has a complex structure and includes an isometric nuclear envelope The core , with a diameter of 27 nm, surrounded by an outer layer called the nuclear capsid approximately 4 nm thick. The virus particle is surrounded by sensitive determinants composed of lipoproteins. The'surface antigen,' or HbsAg, is a protein found in the virion layer. On one side of the viral particle, the surface antigen is occasionally extended as a tubular tail. It is usually generated in large quantities and is seen in the blood of afflicted people as filamentous and spherical particles (Lavanchy 2004). The Hepatitis B virus also includes an inner protein envelope, known as the core particle or "HbcAg," that contains viral DNA and replication enzymes (called "DNA polymerase"). The antigenic determinant HBeAg (Hepatitis B virus antigen) is closely connected to the nucleocapsid of HBV. It is also found in the blood serum as a soluble protein (Liang 2009). Electron microscopy sees three kinds of virus particles in infectious serum: Dyne-ferion particle with a diameter of 42 nm, spherical structures with a diameter of 20 nm, and filamentous particles with a diameter of 22 nm (Fig.2.2) (Keeffe *et al.* 2008).

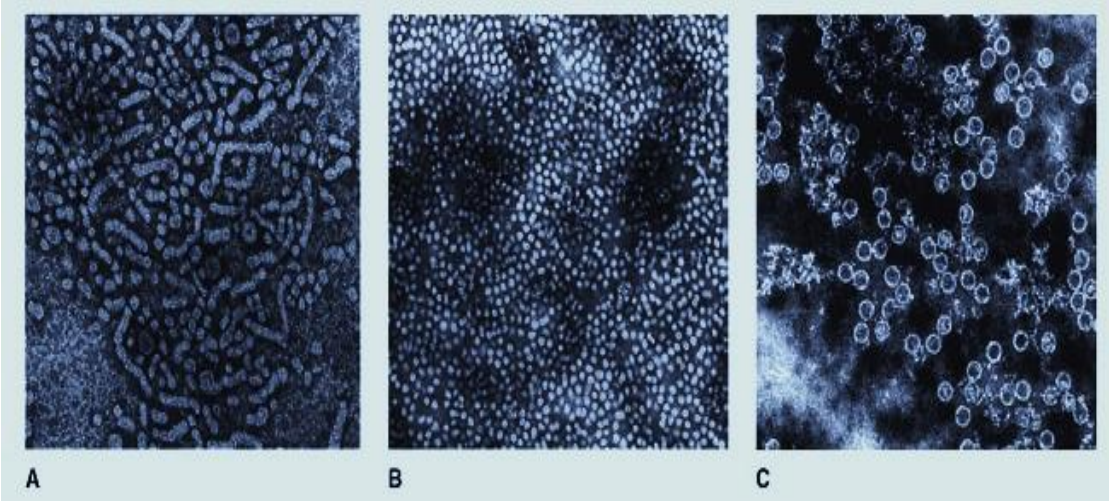

Fig. 2.2 : Unfractionated human plasma positive for the Hepatitis B virus surface antigen (HbsAg). (Keeffe *et al.* 2008).

2.3.4. Proteins produced by viruses:

Hepadnaviruses have a small genome, about 3.2 kbp in length, and encode a small number of proteins. The core and polymerase proteins are required for viral DNA replication, whereas the envelope proteins, which are divided into three subspecies based on the hepadnavirus, are required for nucleocapsid envelopment. And HBe protein , X protein (İnan and Tabak 2015).

(a) Surface proteins:

Hepatitis B virus surface proteins are divided to three proteins : small proteins (SHBs), medium proteins (MHBs) and large proteins (LHBs) , all of them are created from the same gene but have different degrees of immune response. Used as alternatives for three start codes for protein synthesis (Mast *et al.* 2006).

(SHBs) antigen, which accounts for 85 percent of Hepatitis B virus surface antigen (HBsAg), is highly immunogenic and elicits an immune response against HBV in the host. The host's immune response to the virus is hypothesized to be diluted by excess surface protein circulating in sub viral particles. (LHBs) is essential for viral infection and morphogenesis, in contrast to MHB. It accounts for 10–30% of the HBsAg in virions and filaments. Although SHB may also be required for viral entrance into hepatocytes, LHB plays a function in this process.

(b) Core protein :

Capsid consists of 180 copies of one of the size of (22KDa), Immunoactive Core molecules induce to production of a large number of antibodies during natural Infection with *Hepatitis B virus* as well as during the prevention (Kann 2002).

(c) Polymerase protein:

It is a basal protein rich in histidine and a molecular weight of about (93 KDa), and has an effective role in doubling the liver viruses family. The polymerase protein's terminal domain serves as a protein primer for reverse transcription of pregenomic viral RNA; a non-functional spacer region; the reverse transcription-active polymerase domain; and the RNase H domain, which is responsible for RNA template degradation during reverse transcription (Kann 2002).

(d) HBx protein:

Consist protein X of 154 amino acid, acts protein X out the body as a cloning activator (Benhenda *et al.* 2009).

(e) HBe protein:

HBe antigen (HBeAg) is found as a soluble protein, it usually correlates with viremia . The gene of HBeAg is conterminal with the core gene; it is a non-structural viral protein not necessary for HBV infectivity. Because of its resemblance to the viral core protein, it competitively inhibits the immune response directed against the viral nucleocapsid (Block *et al.* 2007).

2.3.5. Virus multiplication :

HBV enters the cell by docking with receptors on the cell envelope. The non-enveloped virus enters the cell. Passive diffusion or microtubules are used to deliver the virus core to the cell nucleus. The virus's genetic material is generated in the nucleus (Umetsu *et al.* 2018). Some HBV DNA fragments bind to hepatocyte DNA and act as a template for the production of mRNA. Cellular RNA polymerase II transcribes several genomic and sub-genomic RNAs using cccDNA as a transcriptional template. The polyadenylated pregenomic RNA is packed into nucleocapsids preferentially, with a length equal to the whole genome plus a 120-nucleotide terminal redundancy. The co-packaged polymerase then reverse-transcribes it into new rc-DNA genomes. The polymerase commences

negative-strand DNA synthesis by reverse transcription once the pregenomic RNA-polymerase complex is encapsidated (Wei *et al.* 2020).

With up to 20 double bases produced from the fifth end of DNA (5), the negative strand acts as a template for the positive strand of viral DNA. It acts as a catalyst for the production of the positive strand, indicating that the DNA molecule is double-stranded. The DNA of the virus is cloned and transmitted to the cytoplasm, where it is translated into the envelope, pulp, and surrounds, protein X, and the DNA polymerase enzyme. As the viral polymerase enzyme is coupled to the loop assembly from the fifth end of the DNA (5), the virus core is transferred to the cell nucleus and its transit to the inner endoplasmic begins reticulum or the Golgi apparatus and acquires the protein coat there before it is released from the cells (Cui *et al.* 2015).

2.3.6. Pathogenicity :

Hepatitis B virus is the leading cause of chronic liver disease worldwide, as each period of HBV induces specific viral kinetics and host immune responses that lead to cirrhosis and liver damage. However, immune system-mediated cytotoxicity plays a role in prevalent role within causing liver damage (Abdulghani *et al.* 2018).

Liver injury still caused by the weaken immune reaction, not certain, but that is consider, to be the elementary mediator of hepatocellular carcinoma (HCC) and cirrhosis. There appears to be an obvious role in cirrhosis, but the association with carcinogenesis is more fragile It is also considered the cause of hepatocellular carcinoma, and it was found in the study of molecular biology that the cause of infection is due to the presence of gene HBx . And *Hepatitis B virus* are also the known cause of infection Membranous glomerulo nephritis or Membrano proliferative glomernous nephritis or nephropathy , and these diseases are associated with Infection with *Hepatitis B virus* and lead to kidney injury and affect it. It was also noted that *Hepatitis B virus* plays an important role in the development of Atherosclerosis, and it was found that there is a relationship between patients with atherosclerosis and those with chronic *Hepatitis B virus* (Nelson *et al.* 2016).

2.3.6.1. Acute infection with HBV :

The period incubation of Infection with the *hepatitis B virus* is 40-150 days (average, approximately 12 weeks). The clinical disease associated for acute Infection with the *hepatitis B virus* may range from moderate disease to severe. 5- 10% of infected infants and 95% of adult patients after acute hepatitis resolves in the end develop antibodies against *Hepatitis B virus* surface antigen (HBsAg) and fully improve. Chronic infection develops in around 5% of adult patients, 90% of infected babies, and 30-50 percent of children aged 1 to 5 years. Some infected individuals, particularly young infants and neonates, have a positive blood test for envelope antigen (HBeAg) and high serum levels of Hepatitis B viral DNA but have limited histologic evidence of liver damage (Sorrell *et al.* 2009).

2.3.6.2. Chronic infection with HBV :

Hepatitis B virus carriers often are asymptomatic without considerable liver injury. Individuals that seroconvert of a positive-HBsAg condition to a negative-HBsAg condition may be enter the "inactive carrier state" (formerly known as the "healthy carrier state"). Such persons have minimally abnormal liver biopsy results and have normal liver chemistry test results, or asymptomatic have normal. *Hepatitis B virus* replication should be minimal or nonexistent of blood test evidence, the range of 0 to 2000 IU/mL for a serum *Hepatitis B virus* DNA level. (Lok and McMahon 2009). Some but not all of those individuals may enter the "immune-active phase" of disease after years, the liver experiences active fibrosis and inflammation, in state DNA of *Hepatitis B virus* may stay high. Usually the phase of immune-active ends with the loss of HBsAg and the development of antibodies to HBsAg (Sorrell *et al.* 2009).

Inactive carriers stay infectious to others through sexual transmission or parenteral, they may ultimately clear the virus and develop anti-HBs. However, some inactive carriers improve chronic hepatitis, as specified through HBV DNA levels liver, chemistry results, and liver biopsy results. Although the risk is minimal, inefficient carriers remain at risk for hepatocellular carcinoma (HCC). At this stage, for the patients in an inactive carrier condition no active anti-viral therapies are available. May enter other seroconvert patients the "reactivation phase" from disease. Those persons have serum *Hepatitis B virus* DNA levels higher than 2000 IU/mL and show directory of active liver inflammation; and stay

HBeAg-negative. Those patients have HBeAg-negative with chronic hepatitis. The disease may develop manifestation extra hepatic (e.g. glomerulonephritis, cryoglobulinemia, and polyarteritis nodosa) (Keeffe *et al.* 2008).

2.3.7. Transmission :

Hepatitis B is most often passed from mother to child before birth (perinatal transmission) or by horizontal transmission (exposure to contaminated blood) in highly endemic regions, particularly during the first five years of life from an infected kid to an uninfected child (WHO 2021).

Infected things that pierce the mucous membranes or skin, such as body-penetrating equipment, tattoo equipment, acupuncture equipment, toothbrushes, and razor blades, can also spread the Hepatitis B virus. Infected blood and body fluids, such as menstrual, vaginal, and seminal fluids, may be detected using saliva, tears, and bile. Unvaccinated persons who have several sexual partners (gay or heterosexual), dentistry or invasive medical procedures with poor infection control, and transfusion operations of contaminated blood or blood components are more prone to spread the Hepatitis B virus sexually (Mast *et al.* 2006).

Transmission may occur in places like schools, child care centers, and institutions for developmentally disabled persons. They might get infected by organ donation or dialysis, for example. The Hepatitis B virus may survive outside the body for up to 7 days. The virus may still cause sickness if it enters the body of someone who has not been vaccinated during this time period. The virus may be diagnosed 30 to 60 days after infection and can progress to chronic Hepatitis B, particularly if it is transmitted during infancy (Trépo *et al.* 2014).

2.3.7.1 Blood and blood derivatives:

HBV is a blood-borne virus that is found all throughout the world, particularly in developing countries. And the majority of those countries have yet to completely adopt the criteria for contemporary, sterile blood transfusion systems. Blood transfusions remain a concern in Arab countries due to a lack of coordinated infrastructure and competent personnel (Khan and Attaullah 2011).

Blood is still mostly donated by the patient's relatives and friends. Donors are more likely to come forward in an emergency, and queries concerning high-risk behaviors are rarely raised. Having the means and organizations to set up a mechanism for recruiting altruistic volunteers would be excellent. According to current statistics, all Arab countries require HBV marker screening in all blood banks; nevertheless, in some countries, patients may be charged a fee for such tests. The reliability of such tests is a big worry because some of these countries, particularly poor African Arab governments, employ the Rapid test kit, which has a low sensitivity (Laperche *et al.* 2013).

As a result, even if an HBV screening test is performed, the risk of infection still exists. In Arabian countries, blood transfusion and hospital-related practices are potential HBV risk factors. As a result, solutions to these problems must be devised, as well as a well-organized blood screening and transfusion system. International and local standards must be met, and these requirements must be revised and monitored on a regular basis (Bjoerkvoll *et al.* 2010).

2.3.8. Symptoms :

The Hepatitis B virus has an incubation period of 30 to 180 days. When people are first infected, they usually have no symptoms. Acute hepatitis can cause acute liver failure, which can result in death. Adults and children over the age of five are more prone than younger children to experiencing symptoms. Symptoms of acute Hepatitis B virus infection can appear 2 to 5 months after infection in certain people (WHO 2021).

Some of the symptoms include dark yellow urine, diarrhea, lethargy, fever, gray or clay-colored stools, joint pain, lack of appetite, nausea and vomiting, stomach discomfort, and jaundice (yellowish eyes and skin). If you have the chronic Hepatitis B virus, you may not experience any symptoms until complications arise. It's possible that this will happen years after you've been afflicted. As a result, even if you don't have any symptoms, you should get tested for the Hepatitis B virus. Even if you don't have symptoms, you might be tested for a disease through screening. If you are at high risk, your doctor may recommend screening (Liu *et al.* 2018).

2.3.9. Treatment of infection with the *hepatitis B virus* :

The major goal of treatment is to preserve lives by lowering liver cancer mortality and reducing or reversing liver disease and infection development (Terrault *et al.* 2018). Acute hepatitis C normally does not require treatment. Antiviral drugs help you fight the virus and are used to treat chronic hepatitis B. It may also lower the risk of future liver problems. Medication that inhibits the virus's capacity to replicate and multiply is commonly used to treat chronic hepatitis B.

Treatment can last usually from one year with pegylated interferon to indefinitely with oral antiretroviral. Drugs to entirely remove the infection are being researched. The chronic hepatitis B virus should be treated by individuals who are familiar with these conditions, the medications used to treat them, and the side effects of these medications (Terrault *et al.* 2018).

2.3.10. *Hepatitis B virus* vaccines and immune globulins (HBIG) :

The mainstay of HBV prevention efforts is the hepatitis B vaccine. In many countries, the HBV immunization program has reduced perinatal and horizontal HBV transmission. In addition, knowing the frequency of hepatitis B infection by location and age is critical for evaluating vaccination programs and national disease prevention and control initiatives. HBV vaccination has been found to dramatically reduce the number of HBV infections among health-care personnel. Furthermore, population shifts and increased vaccination may result in novel viral epidemiological patterns, which may have an impact on endemicity levels in specific regions (Madani 2007).

The HBV vaccination has been included in the WHO-recommended Expanded Programme on Immunization in all Arab nations. And it went into effect in 2005 (Al-Bataineh 2005). The majority of these countries claim that the long-term goal of the HBV vaccination program is to prevent virus transmission in all age groups (newborns, children, adolescents, and at-risk adults), despite the fact that chronic HBV carriers make this difficult. The short- and medium-term goals are to reduce health-care expenses while preventing acute and persistent symptomatic infections. Another long-term goal is to prevent HBV consequences, including cirrhosis and HCC, which are associated with high morbidity and death (André 2000).

Plasma-derived HBsAg was used in the first Hepatitis B vaccinations. By the late 1980s, recombinant Hepatitis B vaccinations comprising yeast-derived HBsAg refined using biochemical and biophysical separation techniques had largely supplanted plasma-derived vaccines in the US (Schillie *et al.* 2013). In infants delivered to HBsAg-positive mothers and in some other postexposure prophylactic scenarios, HBIG is commonly used as an adjuvant to the Hepatitis B vaccine. HBIG can help supplement protection until a vaccine response is achieved (Schillie *et al.* 2013).

2.3.11. Epidemiology :

The World Health Organization (WHO) has confirmed that there are more than 248 million patients infected with *Hepatitis B virus* , and *Hepatitis B virus* causes about 600,000 deaths annually, and is the fifteenth cause of death around the world (WHO 2017). There are also millions around the world who are chronically infected with hepatitis and have the ability to infect other individuals, and a large part of them turn the virus into a carcinogenic virus, which leads to cirrhosis or liver cancer. In Europe alone, it is estimated that there are one million people infected with the virus annually, and about 90,000 of them become carriers of the disease, as well as 22,000 of them progress to the disease and develop cirrhosis or liver cancer (Raimondo *et al.* 2010). Studies conducted around the world have shown that *Hepatitis B virus* accounts for 20-25% of acute viral hepatitis cases accompanied by jaundice, and that most infections occur in adolescents and adults aged 15-45, and *Hepatitis B virus* causes less chronic infection. from 5% for adults (Guha *et al.* 2004).

The virus is found in the blood and in other body fluids such as semen, vaginal secretions and saliva. Although the virus is available in other body fluids, its concentration is 1-1000 of its concentration in the blood. Infection with the *hepatitis B virus* occurs worldwide, with 45% of the world's population living in areas of high prevalence of chronic infection, 43% of the world's population living in areas of medium prevalence, 12% of the world's population living in areas of low prevalence, and about 2% of the world's population tested positive for HBsAg (Wang *et al.* 2004).

Hepatitis B virus is a blood-borne viruses, spread in Iraq and all over the world (Hasan and Mustaf 2017). In 2015, about 1.34 million people died globally due to complications of viral hepatitis (Salam *et al.* 2020). According to WHO, Iraq is considered a low

endemic country for *Hepatitis B virus* through the study in Maysan (Kadhem *et al.* 2019) this is done through the implementation of the disease control program. And out of the total population of (37,140,000) Iraqis, 3674 cases of *Hepatitis B virus* have been reported (WHO 2017).

There are ten genotypes of the Hepatitis B virus, which are found in different parts of the globe. Genotypes A, B, and C, for example, are common on the Asian continent, whereas viral mutations are strongly linked to genotype C. In Africa and Europe, genotype A is also quite frequent; however, West Africa is recognized for having a specific genotype, E. Furthermore, in the Middle East, genotype D was shown to be the most common genotype. HBV genotypes are recognized by an 8% difference in nucleotide sequence, whereas subtypes within each genotype are defined by a 4% difference. In comparison to other genotypes, genotype H, which is present in Central America, has a reduced pathogenicity (Al-Sadeq *et al.* 2019).

2.3.12. Genotypic variation :

In terms of the diversity of HBV, two opposing forces can be discerned. The error-prone polymerase causes a high rate of nucleotide substitutions, while the genome's severe compactness inhibits a great deal of genetic variation. There have been various efforts to investigate HBV's evolutionary history in terms of its genesis, the period when it separated from other hepadnaviruses, and the time when HBV genotypes diverged (Shuhart *et al.* 2006).

2.3.13. HBV serotypes :

Following the identification of "AuAg," HBV's primary envelope protein, it became clear that sera from patients who had seroconverted to anti-HBs did not react the same manner to HBsAg from different chronic carriers, indicating that viral diversity was to blame. The primary immunogenic portion of HBsAg, the "a" determinant, which spans residues 124–147 amino acids, is found in practically all HBV isolates and so is not useful for categorization. As a result, subtypes are used to classify items. The d/y and r/w determinants are the two most important subtype epitopes. Both of these determinants are made up of two mutually exclusive epitopes that are dependent on HBsAg amino acids 122 and 160, respectively (Kidd *et al.* 2002).

2.3.14. HBV genotype and distribution:

Many genotypes and subtypes have been discovered thus far. At least ten HBV genotypes (A-J) and various subgenotypes have been identified based on viral sequence homogeneity (Kurbanov *et al.* 2010). The regional distributions of HBV genotypes and subgenotypes are well described, with the exception of the recently found genotypes I and J. In Asia, for example, genotypes A, B, and C are widespread, and viral mutations are typically linked with genotype C. (Sunbul 2014). In Europe and Africa, genotype A is also quite common, although West Africa is renowned for a specific genotype, E. Genotype D, on the other hand, was shown to be the most common genotype in the Middle East. In South and Central America, genotype F has been discovered (Sozzi *et al.* 2018). Genotype G has been discovered in France and the United States. Central America is home to genotype H. In Vietnam and Laos, genotype I was discovered, whereas genotype J was discovered in Japan (Kurbanov *et al.* 2010).

In 1979, the first full HBV genome sequence was released (Galibert *et al.* 1979). By the end of the 1980s, Okamoto had classified HBV strains based on genomic sequence rather than surface protein antigenicity. He studied 18 full-length genomes and grouped them into four genotypes, A to D (Okamoto *et al.* 1988). Since then, further four genotypes, E to H, have been found. In Vietnam and Laos, genotype I was discovered (Sozzi *et al.* 2018).

HBV genotypes are characterized by an 8 percent variation in nucleotide sequence, whereas subtypes within each genotype have a 4 percent divergence (fig. 2.3) (Al-Sadeq *et al.* 2019). HBV genotyping is thought to be significant because different HBV genotypes have different disease severity and pathogenicity responses to interferon therapy. HBV genotypes C and D, for example, are more typically related with liver cirrhosis and progression to liver cancer than other genotypes (Sunbul 2014). In comparison to other genotypes, genotype H, which is present in Central America, has a reduced pathogenicity. According to Sozzi *et al.*, genotype H's reduced pathogenicity may be linked to its low viral replication rate and altered expression of envelope proteins when compared to genotype D. (Sozzi *et al.* 2018). The serotype and genotype have a definite relationship. Genotype A was found to correlate to adw2 (Europe) and ayw1 (Africa), whereas genotype B was found to belong to adw2 (Asia) (Schaefer 2005).

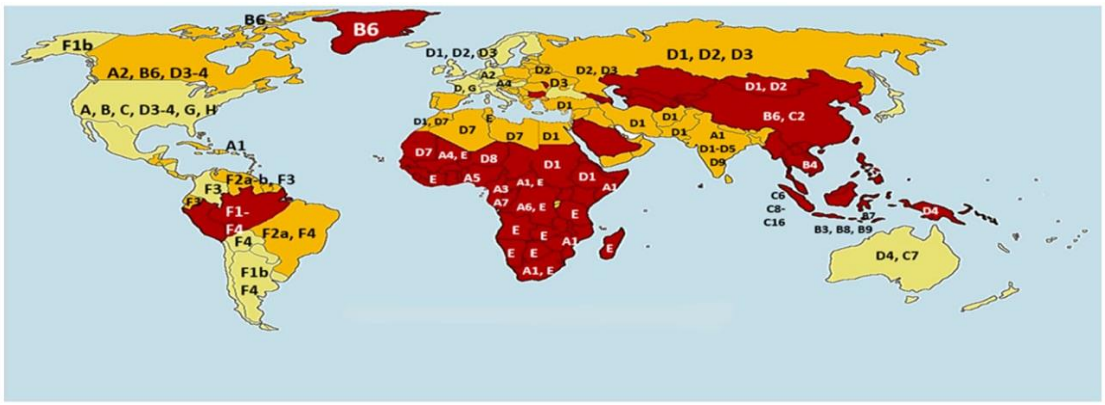

Figure 2.3. HBV genotypes are distributed geographically all over the world.(Al-Sadeq *et al.* 2019).

2.3.15. Mutations :

A mutation is any alteration in genetic information in biology. DNA sequences, chromosomes contained in DNA, and, in the case of some viruses in RNA sequences, vital genetic information is encoded. Nucleotides also known as nitrogen bases, are the components of DNA, which is a double chain. Viruses, a jumping gene, mutagenic chemicals, or radiation exposure can all cause mutations, as can errors in DNA replication or meiosis in the formation of gametocytes. It can be caused by cellular processes such as hyper mutation or by the organism itself (Al-Nahi *et al.* 2018).

Mutations are both the raw material for genetic variety and the driving force behind organism evolution in general. Mutations can have detrimental, helpful, or neutral effects (neither harmful nor beneficial to the organism in which they live). Changes that result from mutations may have no effect, may modify genetic outcomes or offspring, or may prevent the gene from working properly or completely. The great majority of mutations are unnoticeable and have little effect on the environment. However, mutations that alter proteins produced by genes are frequently deleterious (Buti *et al.* 2005).

2.3.16. Viral load of HBV :

HBV infection is a long-standing medical problem that affects people all around the globe. Serologic approaches are routinely used to diagnose it. However, owing to the necessity for sensitivity of serologic indicators as severity or prognostic markers, deciding

whether or not to treat a patient remains a challenge. The use of polymerase chain reaction (PCR) methods to determine viral load is a useful tool in decision-making (Niederhauser 2011).

For HbsAg negative Hepatitis B virus chronic, the blood HBV DNA level is more than 2000 IU/mL in the vast majority of patients. Hepatitis B viral DNA levels in HBsAg-positive individuals are usually greater than in HBsAg-negative patients. Hepatitis B viral DNA levels are typically no more than 2 104 IU/mL. (Keeffe *et al.* 2008 ; Lok and McMahon 2009).

2.3.17. Laboratory diagnosis of HBV infection :

The most frequent laboratory test for HBV infection is the detection of the Hepatitis B virus surface antigen (HBsAg). WHO recommends that all blood donors be tested for HBV to guarantee blood safety and avoid unintentional transmission to individuals who receive blood products. Acute Hepatitis B virus infection is indicated by the presence of HBsAg and immunoglobulin M (IgM) antibodies to the primary antigen, HBsAg. During the early stages of infection, persons with hepatitis B virus e antigen (HBsAg) are also seropositive. HBsAg is a common indicator of strong viral replication. Because HBsAg is present, it means that the infected person's body fluids and blood are extremely infectious (World Health Organization, 2019).

2.3.18. Complete blood count (CBC) :

The complete blood count (CBC) is a series of tests used to determine the number of red blood cells, white blood cells, and platelets in the blood. Additionally, it regulates the quantity of blood in the body. The complete blood count (CBC) is a test that may be used to assess your overall health and to identify a variety of diseases and ailments, including infections, anemia, and leukemia (Keohane *et al.* 2016). The CBC looks at three different sorts of cells: Red blood cells are the cells that make up blood. The bone marrow releases red blood cells into the circulation as they mature. They contain hemoglobin. It's a protein that helps the body transport oxygen.

White blood cells are cells found in the blood, lymphatic system, and tissues that help the body's natural defense (immune) system function properly. They aid in the fight against infection and are involved in inflammation and allergic responses. WBCs are classified

into five categories, each with a distinct function. There are many types of white blood cells. They include neutrophils, lymphocytes, basophils, eosinophils, and monocytes. The quantity of white blood cells (WBCs) in the blood is rather stable. However, these levels may momentarily rise or fall depending on what is going on in the body. For example, a bacterial infection may drive the bone marrow to produce more neutrophils in order to combat the infection. The amount of eosinophils in the body may grow as a consequence of allergies. Lymphocytic production may rise as a result of a viral infection.

Platelets are tiny cell fragments that circulate in the circulation and are necessary for blood clotting. Platelets stick to the injury site and cluster together to create a temporary plug, which helps to stop bleeding (Keohane *et al.* 2016).

2.3.19. HBV Molecular diagnosis :

The correct identification of hepatitis B virus infection is crucial for effective therapy. Hepatitis B virus infection is divided into phases, each of which is defined by the presence of biomarkers such as human antibodies to viral antigens or viral DNA. As a result, the disclosure approaches are divided into serological tests with varying specificities and sensitivities, as well as molecular assays for detecting Hepatitis B viral DNA using various types of PCR. These tests may help detect the development and progression of Hepatitis B virus infection. Indeed, combining molecular and serological approaches will significantly improve the accuracy of HBV infection detection (Al-Sadeq *et al.* 2019).

Covalently closed circular DNA of Hepatitis B virus (cccDNA) level observation in hepatocytes is a more exact technique to evaluate the number of infected hepatocytes for HBV diagnosis using PCR, but it necessitates invasive procedures and so is not part of standard diagnostics. Quantitation of HBV DNA in the serum, on the other hand, provides a less intrusive alternative to cccDNA detection. According to the Taormina Group's suggestions, extremely sensitive PCR using primers specific to highly preserved sequences (genotype independent) of diverse HBV genomic areas is required to reveal such low quantities of HBV DNA. It has been shown that the sensitivity of Hepatitis B viral DNA detection by PCR varies across different genetic areas of the virus genome (Datta *et al.* 2014).

3. MATERIALS AND METHODS :

3.1. Materials :

3.1.1. Laboratory apparatuses :

Table 3.1. explains the laboratory apparatuses which are used in this study, also it explains the manufacture company for each one:

NO	Instrument / equipment	Company / Country
1	ELISA System: - Micro well system washer - Micro well system reader - Automatic printer	BioTek/ USA
2	pH Meter	Sartorius/ Germany
3	Microwave	Bajaj/ India
4	Balance	Sartorius/ Germany
5	Stopwatch	Termaks/ Germany
6	centrifuge	Axiom/ Germany
7	High speed Cold Centrifuge	Eppendorf/ Germany
8	Incubator	Memmert/ Germany
9	Nano drop	Thermo Scientific/ UK
10	Vortex	CYAN/ Belgium
11	Micropipettes different size	Eppendorf / Germany
12	Eppendorf tubes	Biobasic/ Canada
13	Thermo cycler PCR	Bio-Rad/ USA
14	Gel electrophoresis apparatus	Bio-Rad/ USA
15	Water bath	Kottermann/ Germany
16	Exispin vortex centrifuge	Bioneer/ Korea
17	Refrigerator	Concord/ lebanon
18	Deepfreeze -20 Arctiko	Meiling/ China
19	UV Trans illuminator	Wisd/ Germany
20	Camera	Nikon/ Japan
21	Complete blood count device (CBC)	Mindray/ China

3.1.2. HbsAg ELISA test kit :

Fortress HbsAg is an in vitro diagnostic kit that detects HBV surface antigen (HbsAg) in human serum or plasma. The purpose of screening blood donors is to monitor those at a greater than average risk of getting hepatitis, such as patients, technicians, or nursing employees in renal dialysis units or clinical labs. As a diagnostic tool in the diagnosis of liver disease **(fortress. BXE0741A. UK):**

Table 3.2. ELISA Kit Contents :

No	Kit Contents	Volume
1	Microwell Plate 96 Test's	1 plates (12x8 well strips per plate)
2	Negative control	1 x 1 ml
3	Positive control	1 x 1 ml
4	HRP-Conjugate reagent	1 x 7 ml
5	Stock wash buffer	1 x 30 ml (Dilute 1 to 20 with distilled water before use. Once diluted stable for two weeks at 2-8C˚)
6	Chromogen solution A	1 x 7ml (Ready to use and once open ,stable for one month at 2-8C˚)
7	Chromogen solution B	1 x 7ml (Ready to use and once open ,stable for one month at 2-8C˚)
8	Stop solution	1 x 7 ml
9	Plastic sealable bag	1 units
10	Plate cover	1 sheets
11	Package inserts	1 copy

3.1.3. PCR materials :

The PCR primers for detection *Hepatitis B virus* Large S protein gene (S gene) and Polymerase protein gene (pol gene) were designed in this study by NCBI-Genbank and Primer3 plus design. The primers were provided by (Macrogen. Company, Korea) as following table:

Table 3.3. The PCR primers **:**

Primers	Sequence (5'-3')		Amplicon	Genbank design code
HBV-Polymerase protein gene primers	F	CAGCATGGGAGGTTGGTCTT	571bp	MK138694.1
	R	TAACACGAGCAGGGGTCCTA		
HBV- Large S protein gene primers	F	CAAGGCATGGGGACGAATCT	692 bp	KX354997.1
	R	GGACAGGAGGTTGGTGAGTG		

Table 3.4. The kit used in this study with their companies and countries of origin :

No	Kit	Company/ Country
1	G-spin™ Total DNA Extraction Kit : -CL Buffer (Lysis buffer) -BL Buffer (Binding buffer) -Proteinase K 11 mg 1 vial -WA Buffer (W1 buffer) -WB Buffer (W2 buffer) -CE Buffer (Elution buffer) -Spin column -Collection tube 2ml	iNtRON/ Korea
2	GoTaq™ Green PCR Master Mix : -Tap DNA polymerase dNTPs (dATP, dCTP, dGTP, dTTP) Tris-HCl pH 9.0, KCl, $MgCl_2$ Stabilizer and Tracking dye	Promega/ USA

Table 3.5. All the chemicals materials that used in this with their company and country of origin :

No.	Chemical	Company and Origin
1	Absolute Ethanol	BDH/ England
2	TBE buffer	BioBasic/ Canada
3	Agarose	BioBasic/ Canada
4	Ethidium Bromide	BioBasic/ Canada
5	Free nuclease water	Biolabs/ USA
6	DN A marker ladder (100bp)	Intron/ Korea

3.2. Methods :

3.2.1. Design of the study:

All the samples used in the research are HBV-positive samples tested using an ELISA test that looks for HBsAg. The positive sample shown by the ELISA assay was confirmed using PCR technology to search for viral DNA in the blood, in addition to genotyping to detect HBV genotype in the study area.

3.2.2. Specimen collection sites:

Blood donors at Nasiriyah's main blood bank and inpatients at Al-Hussein teaching hospital were used to take a sample. The samples were tested for the first time at the virus unit of Al-Hussein teaching hospital's main laboratory and the virus unit of the major blood bank. The infected samples were then sent to Thi Qar's central public health laboratory's virus unit for confirmation tests. The hepatitis B virus surface antigen (HbsAg) was detected in human serum or plasma using an ELISA assay. The samples were kept frozen until they were used in the virus laboratory's PCR technology.

3.2.3. Study groups:

The present study was conducted in the Thi-Qar governorate in the Republic of Iraq for the period from March 2021 to the end of July 2021. This study included a survey of apparently healthy people from blood donors in the main blood bank in Nasiriyah city, as

well as of patients admitted to Al-Hussein teaching hospital in Thi-Qar governorate. This study included 120 samples infected with the hepatitis B virus from males and females, and the average age ranged between (19-73 years). A special forma was prepared for this study to collect the information directly from the patient and indirectly by the statistical and information units in the places where the samples were collected, and this information included age, gender, blood type, and residence (Appendix 1). A complete blood picture was also tested for all patients.

3.2.4. Samples collection:

A samples of venous blood was drawn using disposable plastic syringes (5cc) after sterilizing the area of blood drawing with alcohol ethanol 70%. were placed (2cc) of the blood in test tube containing an anticoagulant for CBC test. And put (3cc) of the blood left in disposable gel tube and then left the gel tube at room temperature (20-25 °C) for 30 minutes to clot the blood, then separate the blood in a centrifuge at 3000 rpm for 5 minutes to obtain the blood serum, then Serum was taken and transferred to new test tubes by automatic pipette, one sample was divided into aliquots (500 µl) and stored at -20°C until assay. Each portion of serum was used once to avoid sample degradation due to repeated freeze-thaw cycles. All sera and reagents were allowed to stand at room temperature before being used in all tests.

3.3. Complete blood count examination:

After we collected the blood sample and placed amount (2 cc) in a test tube containing an anticoagulant substance (K3 commercially prepared), the test tubes were transferred to the hematology unit, where the complete blood count was examined using a blood count device.

3.4. HBsAg ELISA test :

1. Assay principle :

The test is based on the "sandwich" principle and is an enzyme-immunoassay. The solid-phase antibody has been coated in microtiter wells with monoclonal anti-HBs (antibody to HbsAg). In this well, the test sample is incubated; if HbsAg is present in the sample, it binds to the solid-phase antibody. Following that, a guinea-pig anti-HBs is introduced that has been tagged with the enzyme horseradish peroxidase (HRP). With a positive reaction, the tagged antibody binds to any previously created solid-phase antibody HbsAg complex. When the enzyme substrate is incubated in the test-well, it becomes blue, but when the reaction is halted with sulphuric acid, it turns yellow. If the sample lacks HbsAg, the labeled antibody is unable to attach accurately, leaving just a weak background color.

2. Method of assay:

Step 1: The reagents prepare:

To attain a temperature of 18–30°C, we left the reagent and samples in a room atmosphere for at least 15-30 minutes. Then we checked the concentration of the washing solution for the presence of salt crystals. We reduce the samples were washed 1 to 20 times with distilled or deionized water, only using clean containers**.**

Step 2 - Numbering the **Wells:**

We placed the appropriate strips and a suitable number of wells in the strip holder, including three negative controls (e.g. B1, C1, and D1), two positive controls (e.g. E1 and F1), and one blank (e.g. A1).

Step 3 - Adding a Model and HRP Comparator:

We filled each well with 50 liters of positive control, negative control, and sample. Then, with the exception of the blank, we added 50L HRP-Conjugate to each well and gently mixed it in.

Step 4 - Incubation:

We covered the plate with a plate lid and incubated it for 60 minutes at 37 degrees Celsius.

Step 5 - Washing:

At the conclusion of the incubation, we removed and disposed the plate cover. Then, using a diluted washing solution, we washed each well five times. We soaked the microwells for 30-60 seconds each time. We turned the board onto a clean blotting paper or towel and pressed it to remove any residue after the last wash cycle.

Stage 6 - Coloring:

In this step, we put 50 liters of Chromogen A and 50 liters of Chromogen B to each well, including the blank, and gently mixed them together. After that, we incubated the plate at 37 °C for 15 minutes without exposing it to light. In positive control and HbsAg-positive sample wells, the enzymatic interaction between Chromogen solutions and HRP-Conjugate creates a blue hue.

Step 7 - Stop the reaction:

We poured 50 l stop solution to each well and carefully mixed it with a multichannel pipette or by hand. Positive control and HbsAg positive sample wells produce an intense yellow color.

Step 8 - Absorbance Measurement:

After calibrating the plate reader to the blank well, we measure the absorbance at 450 nm. After that, we computed the final value and assessed the outcomes.

3. Calculation of Cut-off value

Cut-off value (C.O.) =*Nc × 2.1

***Nc =** The mean absorbance value for three negative controls.

Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05. If higher than 0.05 see the quality control range.

3.5. Molecular detection :

3.5.1. Genomic DNA extraction:

The following processes were used to extract genomic DNA from patient blood samples using the G-spinTM Total DNA Extraction Kit according to the manufacturer's instructions:

1. 200 l of frozen serum was transferred to a sterile 1.5 ml micro centrifuge tube, which was then combined by vortex with 20 l of proteinase K. After that, it was incubated for 15 minutes at 60°C.

2. Next, 200 l of GSB cell lysis buffer was added to each tube and thoroughly mixed by vortexing, after which all tubes were incubated at 70°C for 15 minutes, inverting every 3 minutes.

3. 200 liters of 100% ethanol were added to the lysate and aggressively stirred.

4. The DNA filter column was put in a 2 ml collection tube, and the whole mixture (including any precipitate) was transferred to the column. Then it was centrifuged for 5 minutes at 10000rpm. The flow Through 2 ml collection tube was discarded, and the column was put in a fresh 2 ml collection tube.

5. 400 l W1 buffer was added to the DNA filter column, and it was centrifuged for 30 seconds at 10000 rpm. The column was put back in the 2 ml collecting tube after the flow through was discarded.

6. Each column received 600 l of Wash Buffer (ethanol). Then it was centrifuged for 30 seconds at 10000rpm. The column was put back in the 2 ml collecting tube after the flow through was discarded.

7. To dry the column matrix, all of the tubes were centrifuged for 3 minutes at 10000 rpm.

8. Transfer the dried DNA filter column to a clean 1.5 ml micro centrifuge tube, and add 50 l of warmed elution buffer to the middle of the column matrix.

9. To ensure that the elution buffer was absorbed by the matrix, the tubes were let to stand for at least 5 minutes. The purified DNA was then centrifuged for 30 seconds at 10000 rpm to elute it.

3.5.2. Genomic DNA estimation:

1. 1. The isolated genomic DNA was checked and measured the purity of DNA using a Nanodrop spectrophotometer (THERMO, USA) by measuring the absorbance in at (260 /280 nm) as follows:
2. 2. Select the relevant program after starting the Nanodrop software (Nucleic acid, DNA).
3. 3. The measuring pedestals were cleaned multiple times with a dry wipe. Then, to blank the system, gently pipette 2l of free nuclease water over the surface of the bottom measurement pedestals.
4. 4. The sample arm was lowered, the Nanodrop was activated by clicking OK, the pedestals were cleaned, and 1l of DNA was introduced to the measurements.

3.5.3. PCR:

The PCR technique was performed for detection *Hepatitis B virus* based on pol and S gene from patient serum samples. This method was carried out according to (Brody and Kern 2004) following steps:

3.5.3.1. master mix preparation:

It was prepared by using (**GoTaq™ Green PCR Master Mix**) and this master mix done according to company instructions as following tables :

Table 3.6. HBV Pol gene PCR master mix contains :

No	PCR Master mix	Volume
1	DNA template 5-50ng	5µL
2	Pol gene Forward primer (10pmol)	2µL
3	Pol gene Reverse primer (10pmol)	2µL
4	Green PCR Master mix	12.5µL
5	Nuclease Free water	3.5 µL
	Total volume	**25µL**

After that, these PCR master mix components from the table above were centrifuged at 3000rpm for 3 minutes in an Exispin vortex centrifuge. The samples were then put in a PCR thermocycler.

Table 3.7. HBV S gene PCR master mix contains :

No	PCR Master mix	Volume
1	DNA template 5-50ng	5µL
2	S gene Forward primer (10pmol)	2µL
3	S gene Reverse primer (10pmol)	2µL
4	Green PCR Master mix	12.5µL
5	Nuclease Free water	3.5 µL
	Total volume	**25µL**

The components of the PCR master mix, as listed in the table above, were then transferred to an Exispin vortex centrifuge and spun at 3000rpm for 3 minutes. Then into the PCR Thermocycler it goes. The following were the PCR thermocycler settings for both genes using a standard PCR thermocycler system: (Table 3.8).

Table 3.8. The PCR thermocycler conditions for both genes by using conventional PCR thermocycler system.

PCR step	Temperature	Time	Repeat
Initial Denaturation	95ºC	5min.	1
Denaturation	95 ºC	30sec.	35 cycle
Annealing	60 ºC	30sec	
Extension	72 ºC	1min.	
Final extension	72 ºC	5min.	1
Hold	4 ºC	Forever	-

The following procedures were used to examine the PCR results using agarose gel electrophoresis:

1. A 1.5 percent Agarose gel was made by dissolving 1X TBE in a water bath at 100°C for 15 minutes, then cooling to 50°C.

2. The ethidium bromide dye was then added to the agarose gel solution in a volume of 3 liters.

3. After placing the comb in the right position, the agarose gel solution was poured into the tray and allowed to solidify for 15 minutes at room temperature before carefully removing the comb from the tray.

4. The gel tray was placed in the electrophoresis chamber and 1X TBE buffer was added.

5. In each comb well, 10 l of PCR product was added, with 3 l of (100bp Ladder) in the first well. Then, for 1.5 hours, electric current was applied at 80 volts and 80 Hz.

6. UV Transilluminator was used to view the PCR products.

4. RESULTS AND DISCUSSIONS

4.1. The group of study:

This study was conducted to verify the indicators of hematology and immunology and to identify some mutations of the hepatitis B virus in apparently healthy patients from blood donors at the main blood bank and inpatients at Al-Hussein teaching hospital in Thi Qar governorate. This study included 120 samples infected with the hepatitis B virus. The study group consisted of 84 (70%) males, while females numbered 36 (30%). The age range was 20–69 years. There were 64 (53.33%) from urban areas and 56 (46.67%) from rural areas. As for the level of learning, it was 54 (45%) of those with a high level of learning and 66 (55%) of those with a low level of learning(Table 4.1). The study has been implemented by using the following tests: ELISA, PCR, and CBC.

Table 4.1. Frequency distribution of the study group according to social and demographic changes, and percentages of the data. For gender, habitation and level of education:

Variables	No. included	Percentage %
Gender:		
-Female	36	30 %
-Male	84	70 %
Total:	**120**	**100.0 %**
Habitation:		
-Urban	64	53.33 %
-Rural	56	46.67 %
Total:	**120**	**100.0 %**
Level of education:		
- High level of learning	54	45%
- Low level of learning	66	55 %
Total:	**120**	**100.0 %**

4.2. Demographic and social distribution of HBV patients:

4.2.1. The distribution of HBV patients according to gender:

This study concluded that the number of males infected with *Hepatitis B virus* in the study area is greater than the number of females, as the number of males was (84) while the number of females was (36) (Fig. 4.1). And by 70% males and 30% females (Table 4.1).

This is consistent with the study conducted by (Abass *et al.* 2008) as well (Hussein 2015). This study differs from what was recorded in New York, where females reached the highest percentage of males, and in Turkey, where it was found that males had a slightly higher percentage than females (Kacar *et al.* 2003). This study also differs with (Ibtahal 2012), which says that the detection rate of HIV DNA is greater in women than in men. The reason may be that males have a higher proportion of women in this study is that males are mostly blood donors, and the difference may be because men are more productive and more susceptible to infection, in addition to male responsibilities more, and this can happen through the common use of razors and razors in Barber shops. Perhaps the reason is that males travel more than women.

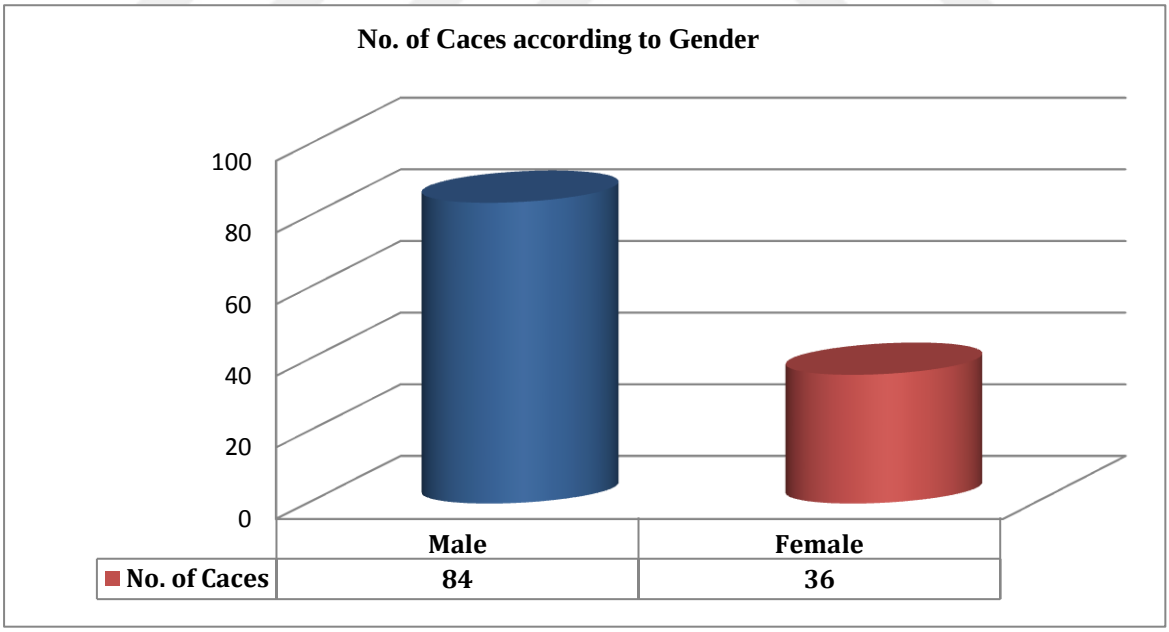

Figure 4.1. Show No. of cases according to gender.

4.2.2. Distribution of HBV patients according to age:

Distribution of *Hepatitis B virus* patients by age. In the current study, it was found that the age ranged between 20-69 years. It was also distributed over four age groups: (20-34 years) by 22%, (35-49 years) by 27%, (50-60 years) by 42% and (more than 60 years) by 9%. Where it was found that most patients were within the category (50-60 years) by 42% and the lowest percentage of 9% was found in the category (more than 60 years)(Fig.4.2). The reason is that the age group (50-60 years) is more susceptible to infection is due to the failure to take the vaccine because the vaccination program was not implemented in Iraq except in 1989 (Chessab and Abdul 2020), while those born in this age group before 1980. Perhaps the difference in sensitivity of the tests used is another reason as well. As for the age group (more than 60 years), which is the lowest rate of infection, the reason is due to the understanding of the smallest group who were in the study, which is the largest group within the study, and also that the proportion of those over 64 years old is 3% of the total population of Iraq (Farrell 2008) so It is natural for this percentage to be the least affected. This thesis agrees with (Opaleye *et al.* 2010), which says that the highest incidence of infection in patients over the age of 58 years, may be due to the period of marriage in this group, and perhaps the increase in the number of births in women with high marriage period, and this is also consistent with the study (Aganga *et al* 1999). And this study disagree with (Ibtahal 2012), which says that the highest incidence of infection is in the age group (30-39). Also, this study disagree with the study (Kolawole *et al.* 2012), in Nigeria and the study (Satake *et al.* 2007), which says that the most affected age group is (15-29), which proves the fact that *Hepatitis B virus* is common in all countries. age groups of life.

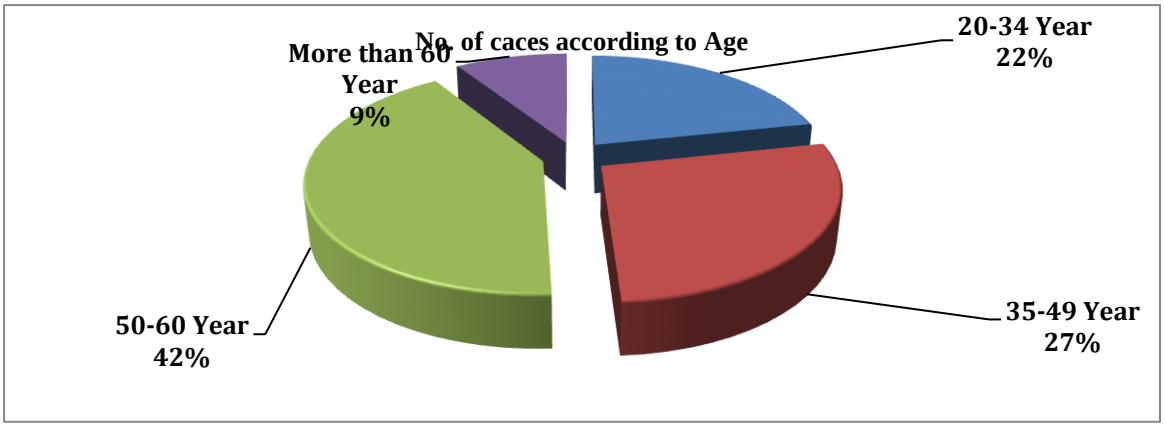

Figure 4.2. Show No. of the cases according to age.

4.2.3. The distribution of HBV patients according to habitation:

The distribution of *Hepatitis B virus* patients according to habitation. Where the number of infected rural residents (56) and the number of infected urban residents (64) (Figure 4.3). The rural percentage is 46.67% and the urban percentage is 53.33% (Table 4.1). The current study agrees with those studies that were recorded in India and Nigeria, which recorded the highest rate of HBV virus in urban areas (Chandra *et al.* 2009 ; Mabayoje *et al.* 2010) as a result of crowding of urban areas with high population density and scarcity in rural areas, and the reason may be the difference in the region, Whereas, urban areas are subject to more health control than rural areas.

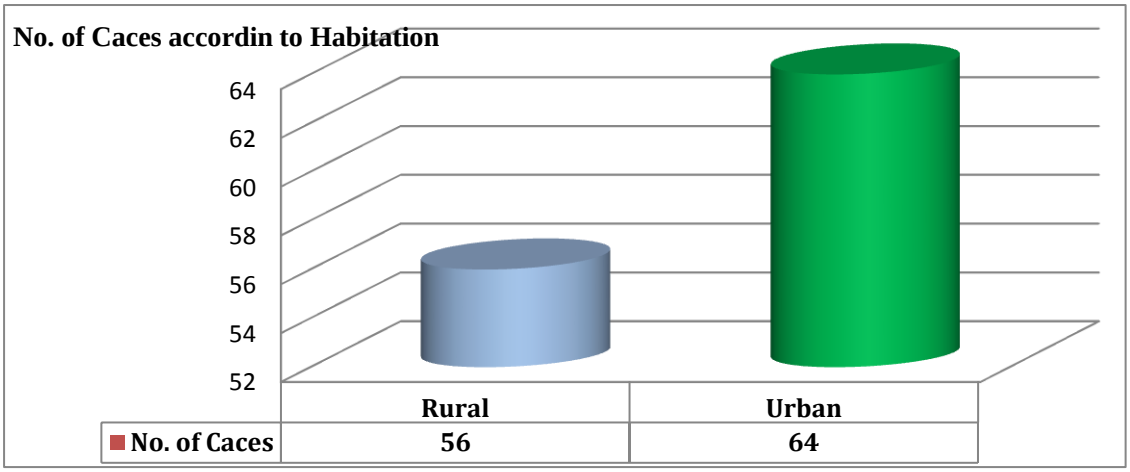

Figure 4.3. Show No. of cases according to habitation.

4.2.4. Distribution of *hepatitis B virus* patients by level of education:

Distribution of *hepatitis B virus* patients by education. The cases were divided into two groups with a high level of education and a with low level of education. It was found that the number of higher education cases amounted to 54, by 45%. The number of cases of low level of learning reached 66 cases, by 55% (Fig.4.4) (Table 4.1). The reason is due to the lack of health education and health awareness for people with a low level of education, as well as the lack of attention to personal hygiene and the failure to use special shaving tools or share them with others. This study agrees with the study conducted by (Mustafa *et al.* 2016), which says that the incidence of infection increases inversely with the decrease in the rate of learning. It differs with the study conducted by

(Kolawole *et al.* 2012) in Nigeria, where he said that the incidence of Infection with the *hepatitis B virus* increased among people with a high level of education.

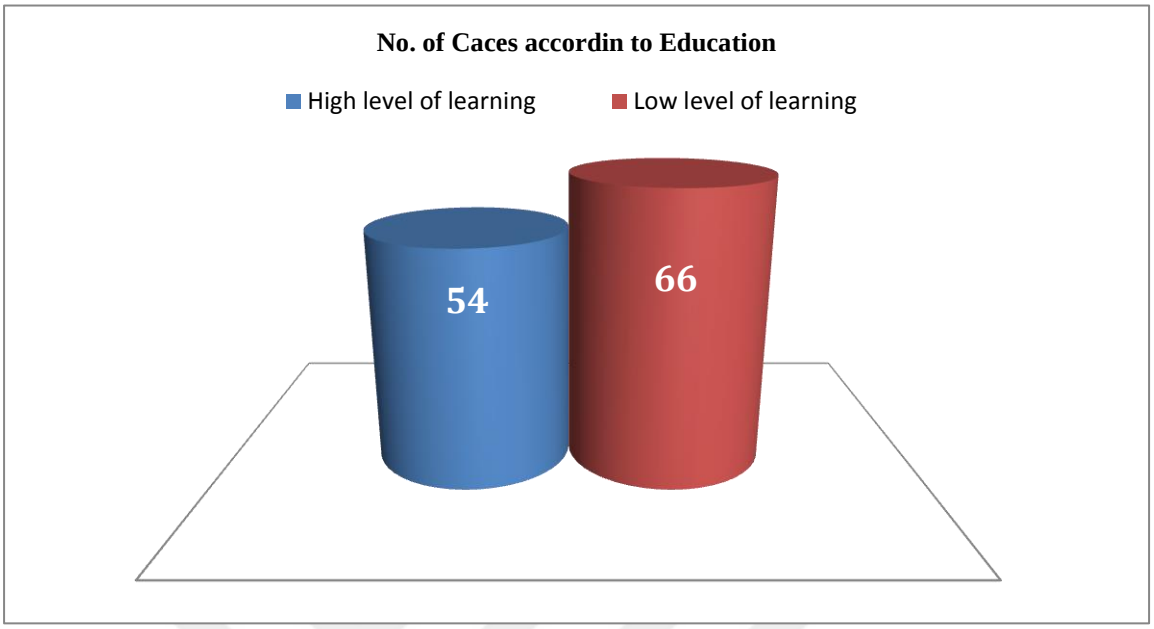

Figure 4.4. Show No. of the cases according to education .

4.3. Study of the relationship of blood groups with *hepatitis B virus* infection:

This study included 120 patients infected with the hepatitis B virus. Blood groups were distributed in the following proportions: blood group A+ by 12%, A- by 13%, B+ by 11%, B- by 7%, AB+ by 8%, AB- by 5%, O+ by 27%, O- by 17%. This study found that the highest incidence of infection was for blood type O+ by 27%, and for blood type O- by 17%. While the lowest incidence was for blood group AB+ by 8% and blood group AB- by 5% (Fig.4.5). Perhaps the reason is that the highest rate of infection was in blood type O, since blood type O is the most prevalent type in the Arab world (Girma and Petros 2017).

In this thesis, we found that people with blood type O were more likely to be infected with the hepatitis B virus compared to non-O blood group members in endemic areas, which was in agreement with some previous studies conducted by (Lao *et al.* 2014 ; Liu *et al.* 2018 ; Abate *et al.* 2016), which said that the highest incidence of infection with the hepatitis B virus was in people with blood type O. And this means that more measures should be taken to ensure the safety of blood for blood type "universal" O in areas where the disease is common.

However, this thesis did not agree with the study conducted by (Mohammadali and Pourfathollah 2014), which said that HBV infection was lower in type O blood donors, possibly due to different HBV prevalence, geography, and ethnicity. Also, our this thesis says that blood type AB has the lowest incidence of the Hepatitis B virus, disagrees with the study by (Jing *et al.* 2020), which says that blood type B is associated with a reduced risk of HBV infection.

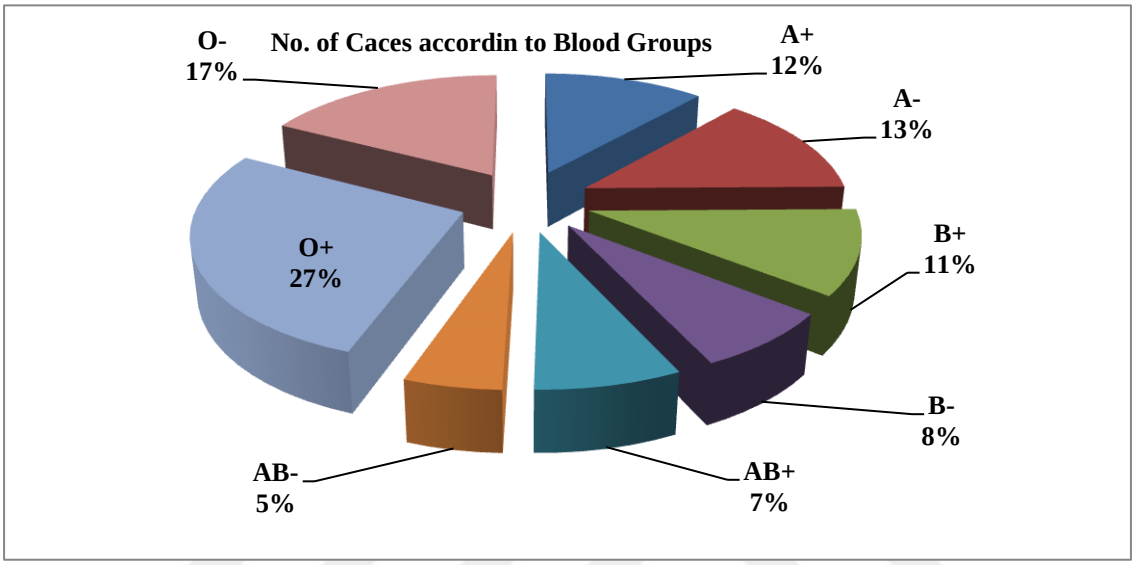

Figure 4.5. Show No. of the causes according to the blood group .

4.4. Comparisons between sex, age, and infection by Chi-Square:

Using the Chi-Square test, no moral value was found between sex, age, and infection with the hepatitis B virus, as the moral value was estimated at (P value = 0.364), and this is consistent with the study conducted by (Kolawole *et al.* 2012), who say that there is no moral value for age and gender (P value = 0.171). Also, this hypothesis does not agree with the study (Opaleye *et al.* 2010), which say that age, gender, and infection with hepatitis B have a moral value, and it also differs from the study conducted by (Abass *et al.* 2009), which says that there is a moral value to the relationship between gender and hepatitis B virus infection (P value <0.01)(Table 4.2).

Table 4.2. Comparison between gender, age and HBV infection by Chi-Square test and find Pearson Chi-Square **Cal_x^2 :** 3.185, **df:** 3, **P. value:** 0.364, **Tab_x^2:** 7.815, **α:** 0.05 .

(Gender & Age) Chi-Square Tests						
		Age Groups				Total
		20-34 Year	35-49 Year	50-64 Year	> 64 Year	
Gender	Male	21	21	33	9	84
	Female	5	12	17	2	36
Total		26	33	50	11	120
Pearson Chi-Square		Cal_x^2 : 3.185		df: 3	P. value: 0.364	
		Tab_x^2: 7.815			α: 0.05	

*Cal_x^2: The calculated chi-square value. *Tab_x^2: Tabular chi-square value.

*df: Degree of freedom. *P. value: Moral value. *α: Morale level.

4.5. Analysis of blood parameters results

Our study found that the lymphocyte increase accompanied by a decrease in neutrophil cells that was detected in each of the variants causing Hepatitis B virus in previous studies is quite expected, given that many viral infections are accompanied by an increase in the number of lymphocytes and the inverse relationship between the increase in lymphocytes and the number of decreased neutrophils (Kuo *et al.* 2014). In light of this, our current study agreed with the study (Kalyuzhin *et al.* 2017), which says that the number of lymphocytes increased in people with the Hepatitis B virus, while the number of neutrophils decreased. Also, this is consistent with the study (Yilmaz *et al.* 2014). The current study does not agree with the study (Liu *et al.* 2017), which says that the number of neutrophils increased, accompanied by a decrease in the number of lymphocytes when infected with the hepatitis B virus.

And to find the moral value (P value) and the less statistical differences (LSD) between some blood parameters (WBC, LYM, MONO) (NEUT. EOS. BASO) and the age group's statistics, we used the analysis of variance (ANOVA test). We found that there is no moral value (P. value) between the different age groups' statistics and blood parameters, as well as (LSD) non-significant between them. As shown in (Table 4.3 ; Table 4.4).

Table 4.3. Using analysis of variance (ANOVA test) to compare the age group's statistics and some blood parameters (WBC, LYM, MONO) to find the least statistical differences (LSD) and moral value (P. value) for them:

Age Groups Statistics				
Parameters / Groups	No.	WBC	LYM	MONO
20-34 year	**26**	8.89 ± 3.77	0.47 ± 0.11	5.26 ± 1.61
35-49 year	**33**	8.43 ± 2.64	0.47 ± 0.12	4.92 ± 1.45
50-64 year	**50**	8.30 ± 2.90	0.41 ± 0.12	4.77 ± 1.48
> 64 year	**11**	7.58 ± 1.94	0.47 ± 0.06	4.98 ± 1.52
P. value		**0.664**	**0.096**	**0.613**
LSD		**Non-Significant**		

Table 4.4. Using analysis of variance (ANOVA test) to compare the age group's statistics and some blood parameters (NEUT, EOS, BASO) to find the least statistical differences (LSD) and moral value (P. value) for them:

Age Groups Statistics				
Parameters / Groups	No.	NEUT	EOS	BASO
20-34 year	**26**	8.36 ± 2.11	0.57 ± 0.65	0.37 ± 0.13
35-49 year	**33**	7.68 ± 2.59	0.74 ± 0.84	0.40 ± 0.11
50-64 year	**50**	7.39 ± 2.51	0.51 ± 0.67	0.37 ± 0.12
> 64 year	**11**	7.13 ± 2.24	0.51 ± 0.62	0.37 ± 0.08
P. value		**0.353**	**0.552**	**0.642**
LSD		**Non-Significant**		

4.6. Results of ELISA test:

Using the ELISA test, the number of people who were diagnosed with hepatitis B in the study was 120, out of a total of 16481 cases, and these cases represented 0.73% (Fig.4.6). This result was close to the previous study by (Othman and Abbas 2020) and lower than

the previous studies (Hussain 2010), as well as (Ibtahal 2012), all of which are considered low when compared to infection rates of up to 40% in other countries (Arora and Mann 2007). This study was also close to the study that was registered in Germany and less than the study that was registered in Amman (Gutierrez *et al.* 2011). The reason for the difference in percentages is the implementation of immunization and vaccination programs since 1989 in Iraq, as well as the introduction of the surface antigen screening system for blood donors in 1995, as well as the level of health and cultural awareness, all of which worked to reduce the incidence of infection. The percentage of positive samples of infection in the current study differs from the percentage in the previous study conducted by (Chessab and Abdul 2020), which can be attributed to the fact that the area covered by the current study is only one governorate, Thi Qar, which is smaller than the area covered by the previous study, which included four provinces. And also Iraq is a low endemicity country for HBV by study in Misan (Kadhem *et al.* 2019), because the carrier rate of HBV prevalence was found to be 0.6% among the general population (Ataallah *et al.* 2011). And from total population (37140000) Iraqi people, 3674 cases HBV(WHO 2016).

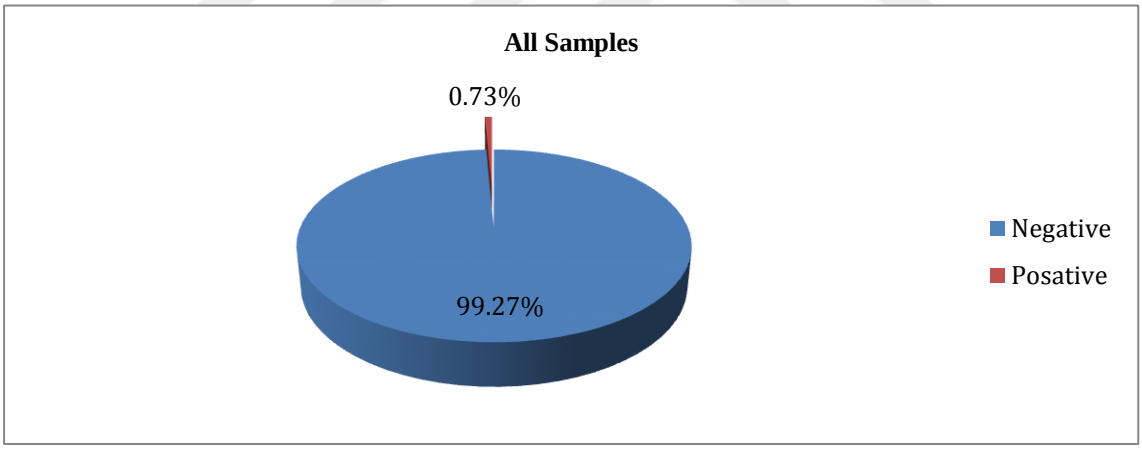

Figure 4.6. Show the percentage of positive and negative results from total samples were examined in ELISA test. Where it appeared that the percentage of positive samples was 0.73% of the total samples, and the percentage of negative samples was 99.27%.

4.7. Results of PCR test:

The cases that showed positive results for the ELISA test (120 cases) were tested by the polymerase chain reaction test (PCR) . The results of the PCR test showed 87 positive cases. These positive cases represented 72.5% of the total study samples. The negative case is 33 and represents 27.5% of the total study samples. (Table 4.5) Represents the comparison between ELISA and PCR tests.

This research demonstrates that the PCR approach is more sensitive and reliable than the ELISA technique, while PCR did not confirm HBV infection in all ELISA-positive patients. The detection of hepatitis using ELISA may be inaccurate and this is confirmed by this study. Perhaps the reason is due to the concentration of the virus in the blood, that is, if the concentration is too low, it cannot be detected by ELISA. Also the use of laboratory devices of different origins that give different degrees of sensitivity affect the results, or mutations happen in the (S gene) or (pre S gene) for some strains of the *Hepatitis B virus* give negative results for the examination of the HBs , as well as the tendency of the virus to hide and latency, which leads to negative results. It causes slow progression of the virus and difficulty in detecting it. As for the PCR test, it can determine the virus DNA in the liver and serum, and it can determine until small amount of virus DNA in the blood. Despite the importance of the PCR, it needs a longer time, more cost, and special laboratory environments to work to prevent pollution and provide qualified trainees to work (Niederhauser 2011). This study is consistent with other studies conducted by (Kurdi *et al.* 2014) as well as with the study (Chessab and Abdul 2020 ; Ibtahal 2012).

Table 4.5. Comparison between ELIZA and PCR tests :

Examination type	No. test sample	No. positive cases	Percentages %
ELISA	16481	120	0.73%
PCR	120	87	72.5%

4.7.1. The results of HBV genotyping:

Genotyping using polymerase chain reaction (PCR) revealed 87 patients. Agarose gel electrophoresis showed analysis of the PCR product for gene S in *Hepatitis B virus* serum samples. Where the ladder was (100 - 1500 bp), the lane was (1-15) the samples appeared positive at (571 bp) the size of the PCR product (Fig. 4.7).

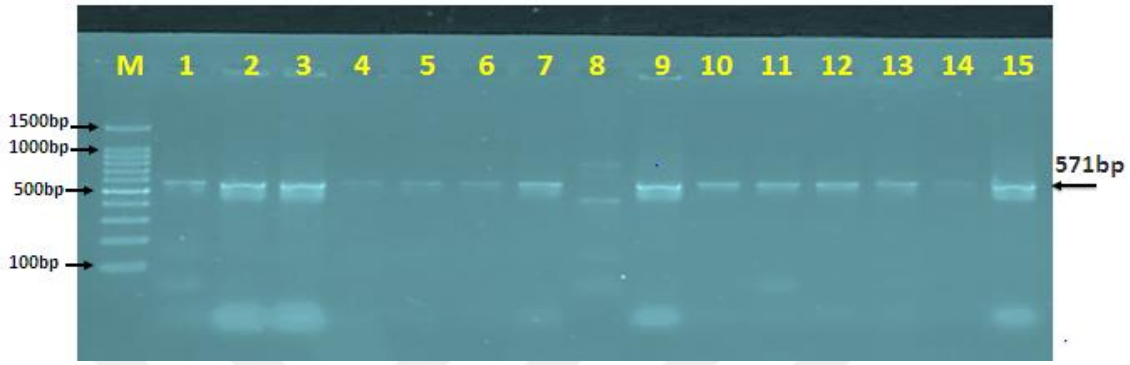

Figure 4.7. Agarose gel electrophoresis image that show the PCR product analysis of S gene in *hepatitis B virus* serum samples. Where ladder (100 – 1500 bp), the lane (1-15) were showed positive samples at (571bp) PCR product size.

Genotyping using polymerase chain reaction (PCR) revealed 87 patients. Agarose gel electrophoresis showed analysis of the PCR product for the pol gene in *Hepatitis B virus* serum samples. Where the ladder was (100–1500 bp), the lane was (1–15) the samples appeared positive at (692 bp) the size of the PCR product (Fig. 4.8).

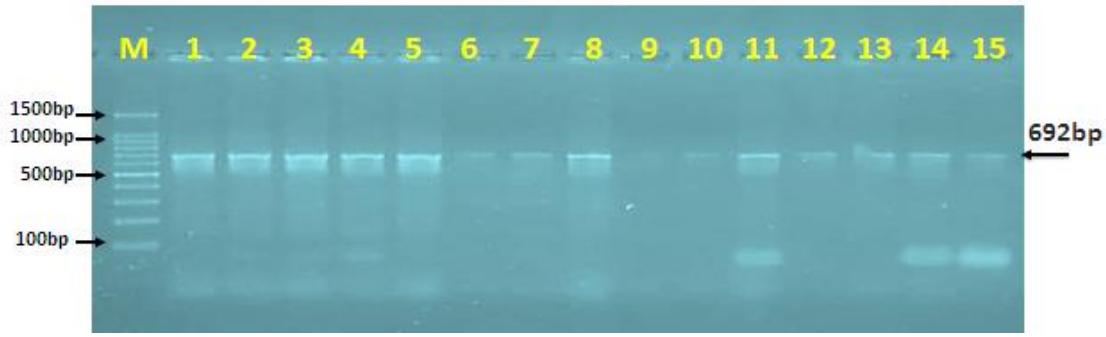

Figure 4.8. Agarose gel electrophoresis image that show the PCR product analysis of pol gene in *hepatitis B virus* serum samples. where ladder (100 - 1500 bp), the lane (1-15) were showed positive samples at (692bp) PCR product size.

4.7.2. DNA sequence results analysis :

The DNA sequencing method was carried out to identification genetic relationship and genetic variation (substitution Mutations) analysis in large S protein and polymerase protein gene of local Iraqi *hepatitis B virus* (IQ.No.1 - IQ.No.2) that compared with NCBI-Blast related global *hepatitis B virus* isolates.

The phylogenetic tree genetic relationship analysis was showed large S protein (S) gene partial sequence in *hepatitis B virus* isolate (IQ-1-IQ-2) were showed closed genetic related into NCBI-BLAST *hepatitis B virus* China isolates **(JX036332.1)** at total genetic change as (0.0200-0.0050%). Whereas the phylogenetic tree genetic relationship analysis was showed polymerase (pol) gene partial sequence in *hepatitis B virus* isolate (IQ-1-IQ-2) were showed closed genetic related into NCBI-BLAST *hepatitis B virus* China isolates **(KX354997.1)** at total genetic change as (0.0150-0.0050%).

The homology sequence identity of large S protein gene in local *Hepatitis B virus* isolates (IQ.No.1 - IQ.No.2) and NCBI BLAST related *hepatitis B virus* isolates China isolate were showed genetic homology sequence identity ranged from (0.22%-99.78%). Whereas, the homology sequence identity of polymerase gene in local *hepatitis B virus* isolates (IQ.No.1 - IQ.No.2) and NCBI BLAST related *hepatitis B virus* isolates China isolate were showed genetic homology sequence identity ranged from IQ.No.1(0.20% - 99.80%) and IQ.No.2(0.40% - 99.60%).

The genetic variation (substitution Mutations) analysis in large S protein *gene* were find one to IQ.No.1

(Query 408 GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA 467

Subject 361 .....C..... 420)

and one to IQ.No.2

(Query 408 GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA 467

Subject 361 .....C..... 420)

substitution mutations at total genetic variation percentage ranged (0.23-0.70%). Whereas, the polymerase gene were find one to IQ.No.1

(Query 301 CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACCTTCCA 360

Subject 301 .....A..... 360)

and two to IQ.No.2

(Query 241 GCCAGCAGTACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT 300
Subject 241 .....**C**..... 300

Query 301 CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACCTTCCA 360
Subject301 .....**A**..... 360)

substitution mutations at total genetic variation percentage ranged (0.23-0.70%). As showed in **(**Table 4.6)(Table 4.7). And figure (Fig 4.9)(Fig 4.10) and (Appendix 2)(Appendix3). Finally, the local *Hepatitis B virus* isolates (IQ.No.1 - IQ.No.2) were submitted into NCBI Genbank and identified by accession numbers to large S protein gene (OK359035 into OK359036). And to the polymerase gene (OK359037 into OK359038).

Table 4.6. The NCBI-BLAST Homology Sequence identity (%) between *hepatitis B virus* isolate (IQ-1-IQ-2) isolate and NCBI-BLAST submitted related *hepatitis B virus* isolate. Polymerase (pol) gene.

Hepatitis B virus isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Number Mutation	Type of Mutation	Mutation Percent (%)	Identity (%)
IQ-1	OK359037	1	T/A	0.20%	99.80%
IQ-2	OK359038	2	T/C, T/A	0.40%	99.60%

Table 4.7. The NCBI-BLAST Homology Sequence identity (%) between *Hepatitis B virus* isolate (IQ-1-IQ-2) isolate and NCBI-BLAST submitted related *Hepatitis B virus* isolate. large S protein (S) gene.

Hepatitis B virus isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Number Mutation	Type of Mutation	Polymorphism Percent (%)	Identity (%)
IQ-1	OK359035	1	A/C	0.22%	99.78 %
IQ-2	OK359036	1	A/C	0.22%	99.78%

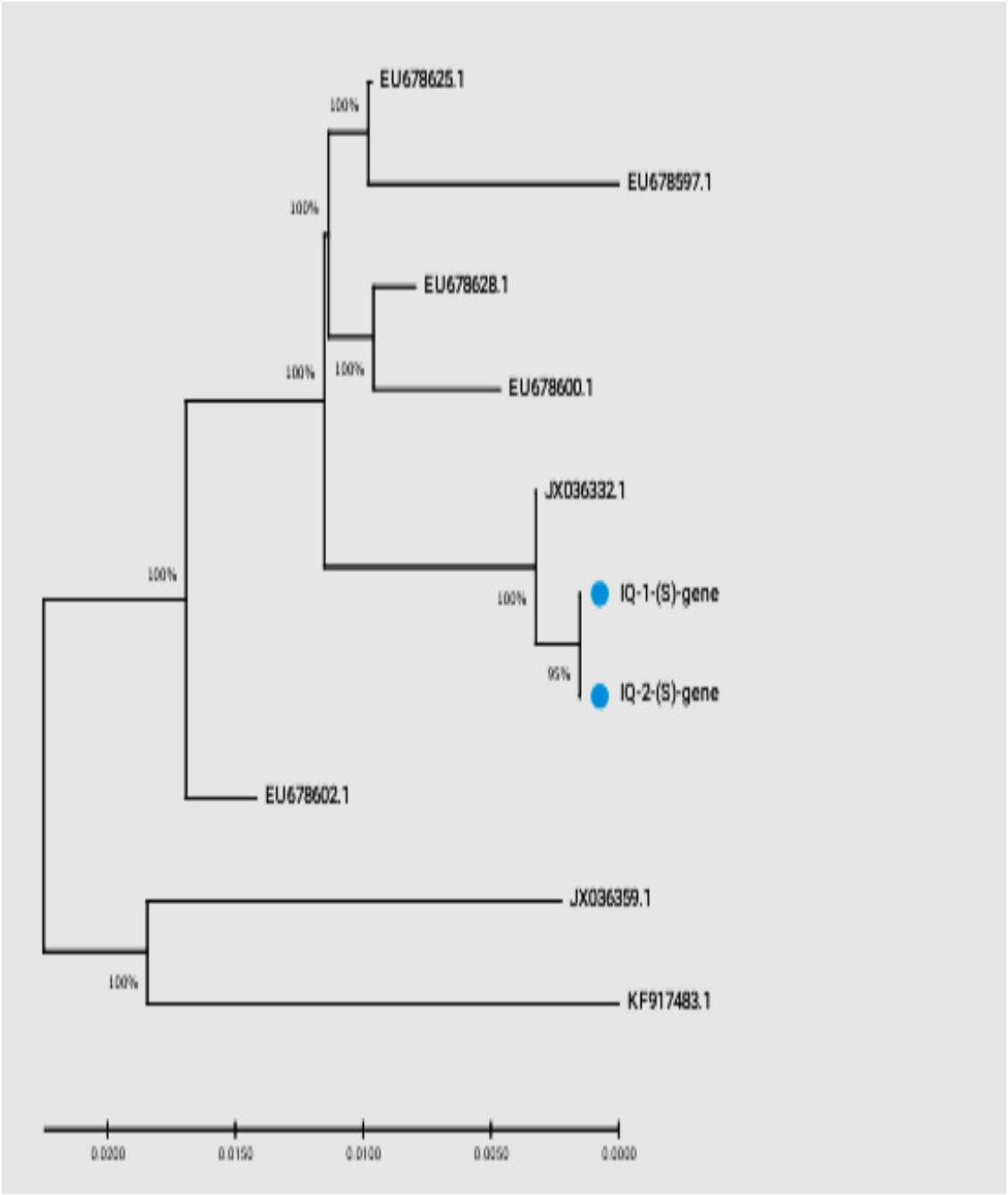

Figure 4.9. Phylogenetic tree analysis based on large S protein (S) gene partial sequence in *hepatitis B virus* isolate (IQ-1-IQ-2) isolate that used for genetic relationship analysis. The phylogenetic tree was constructed using The evolutionary history was inferred using the Neighbor-Joining method in (MEGA X version). The *hepatitis B virus* isolate (IQ-1-IQ-2) were showed genetic related into NCBI-BLAST *hepatitis B virus* China isolates (JX036332.1) at total genetic changes (0.0200-0.0050%).

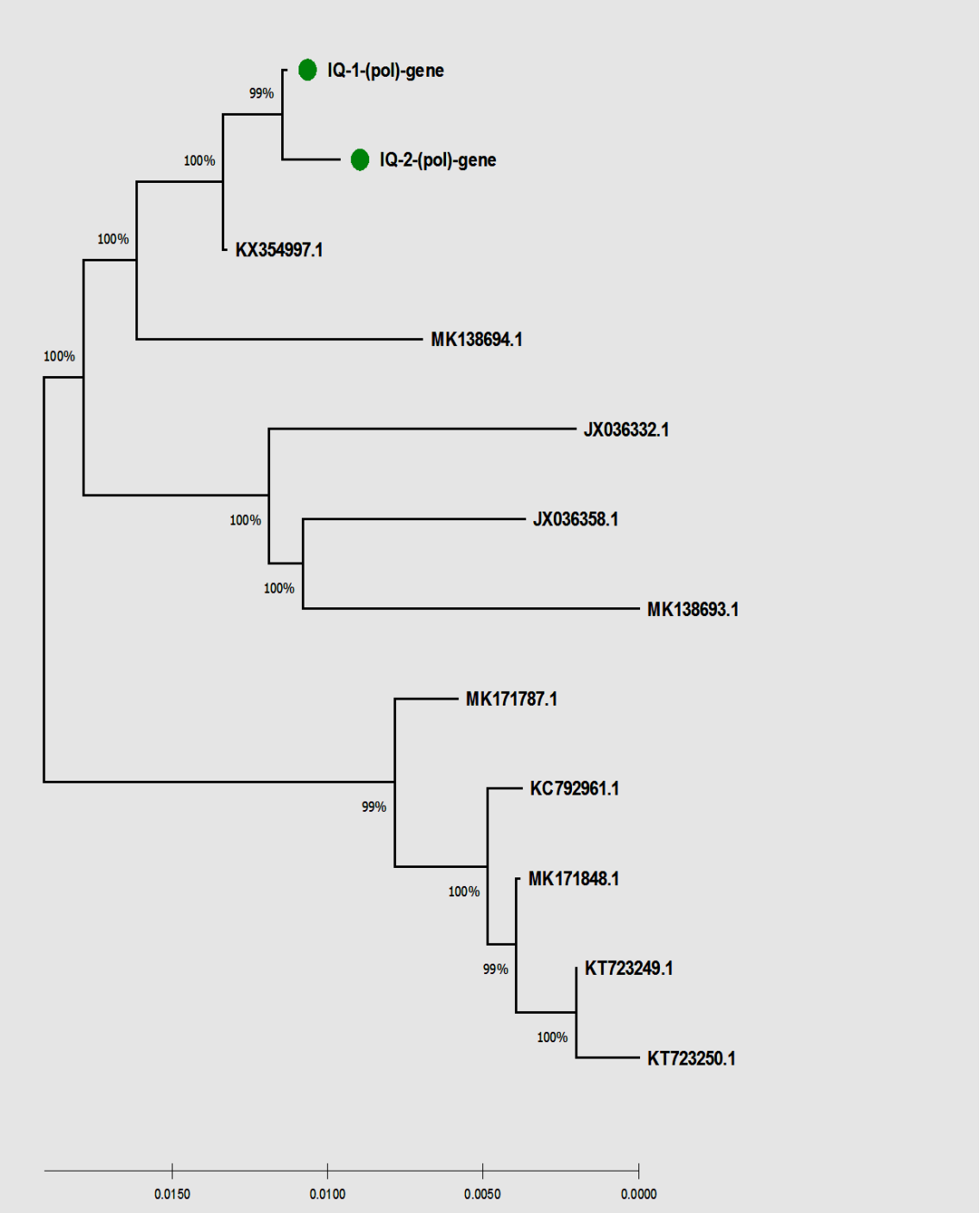

Figure 4.10. Phylogenetic tree analysis based on polymerase (pol) gene partial sequence in *hepatitis B virus* isolate (IQ-1-IQ-2) isolate that used for genetic relationship analysis. The phylogenetic tree was constructed using The evolutionary history was inferred using the Neighbor-Joining method in (MEGA X version). The *hepatitis B virus* isolate (IQ-1-IQ-2) were showed closed genetic related into NCBI-BLAST *hepatitis B virus* China isolates (KX354997.1) at total genetic changes (0.0150-0.0050%).

IQ-1-(pol)-gene:

Score	Expect	Identities	Gaps	Strand
924 bits(500)	0.0	502/503(99%)	0/503(0%)	Plus/Plus

```
Query    1   TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA 60
Subject  1   ............................................................ 60

Query    61 CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA 120
Subject  61 ............................................................ 120

Query    121 CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC 180
Subject  121 ............................................................ 180

Query    181 CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT 240
Subject  181 ............................................................ 240

Query    241 GCCAGCAGTACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT 300
Subject  241 ............................................................ 300

Query    301 CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACCTTCCA 360
Subject  301 ..................................A......................... 360

Query    361 CCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTATATTTCCCTGCTGGTGGCTCCAGTTC 420
Subject  361 ............................................................ 420

Query    421 AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT 480
Subject  421 ............................................................ 480
Query    481  TGGGGACCCTGCGCTGAACATGG  503
Subject  481  ........................  503
```

IQ-2-(pol)-gene:

Score	Expect	Identities	Gaps	Strand
905 bits(490)	0.0	494/496(99%)	0/496(0%)	Plus/Plus

```
Query    1   TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA  60
Subject  1   ............................................................  60
```

```
Query   61 CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA  120
Subject 61 ............................................................  120

Query   121 CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC 180
Subject 121 ............................................................ 180

Query   181 CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT 240
Subject 181 ............................................................ 240

Query   241 GCCAGCAGTACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT 300
Subject 241 .....................C.................................... 300

Query   301 CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACCTTCCA 360
Subject 301 ................................A..........................  360

Query   361 CCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTATATTTCCCTGCTGGTGGCTCCAGTTC 420
Subject 361 ............................................................ 420

Query   421 AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT 480
Subject 421 ............................................................ 480

Query    481  TGGGGACCCTGCGCTG  496
Subject  481  ................  496
```

IQ-1-(S)-gene:

Score	Expect	Identities	Gaps	Strand
837 bits(453)	0.0	455/456(99%)	0/456(0%)	Plus/Plus

```
Query   48  TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG 107
Subject 1   ............................................................ 60

Query   108 ATTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG 167
Subject 61  ............................................................ 120

Query   168 GGCCAGGGTTCACTCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA 227
Subject 121 ............................................................ 180

Query   228 TATTGACAACAGTGCCAGCAGCACCTCTTCATGCCCCCACCAATCGGCAGTCAGGAAGAC 287
Subject 181 ............................................................ 240
```

```
Query   288 AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT 347
Subject 241 ............................................................ 300

Query   348 CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG 407
Subject 301 ............................................................ 360

Query   408 GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA 467
Subject 361 .......................C................................... 420

Query   468 TCTTCTTGAGGATTGGGGACCCTGCGCCGAACATGG 503
Subject 421 ................................... 456
```

IQ-2-(S)-gene:

Score	Expect	Identities	Gaps	Strand
837 bits(453)	0.0	455/456(99%)	0/456(0%)	Plus/Plus

```
Query   48  TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG 107
Subject 1   ............................................................ 60

Query   108 ATTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG 167
Subject 61  ............................................................ 120

Query   168 GGCCAGGGTTCACTCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA 227
Subject 121 ............................................................ 180

Query   228 TATTGACAACAGTGCCAGCAGCACCTCTTCATGCCCCCACCAATCGGCAGTCAGGAAGAC 287
Subject 181 ............................................................ 240

Query   288 AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT 347
Subject 241 ............................................................ 300

Query   348 CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG 407
Subject 301 ............................................................ 360

Query   408 GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA 467
Subject 361 .......................C................................... 420

Query   468 TCTTCTTGAGGATTGGGGACCCTGCGCCGAACATGG 503
Subject 421 ................................... 456
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5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions:

The number of people detected using the ELISA test, which includes 120 cases out of a total of 16481 cases, and these positive cases represented 0.73%. Cases that showed positive results for the ELISA test (120 cases) were tested by the polymerase chain reaction (PCR) test. The results of the polymerase chain reaction (PCR) test showed 87 positive cases. These positive cases represented 72.5% of the total study samples, while the negative cases constituted 33 cases and represented 27.5% of the total study samples.

The results showed that the infection rate of males with Hepatitis B virus in the study area is greater than the number of females, as the number of males was (84) by 70%, while the number of females was (36) by 30%. The results also showed that the rate of infection among those who live in rural areas is lower than that among those who live in urban areas. The percentage in the countryside is 46.67% and the percentage in the cities is 53.33%. The results also showed that the age ranged between (20−69 years). It was also distributed over four age groups: (20–34 years) by 22%, (35–49 years) by 27%, (50–60 years) by 42%, and (above 60 years) by 9%. At first, it was found that most of the patients were in the age category of (50–60 years) by 42%, and at least 9% were found in the category (over 60 years old). As for the level of learning of the injured, the results were divided into two groups: those with a high level of education, with an infection rate of 45%, and those with a low educational level, with an infection rate of 55%. With regard to blood groups, the results showed that the highest incidence of infection was for blood type O+ at 27% and blood type O- by 17%, while the lowest percentage of infection was for blood type AB+ at 8% and blood type AB- at 5%.

In this study DNA sequencing method was carried out to identification genetic relationship and genetic variation (substitution Mutations) analysis in large S protein and polymerase protein gene of local Iraqi Hepatitis B virus (IQ.No.1 - IQ.No.2) that compared with NCBI-Blast related global Hepatitis B virus isolates. The phylogenetic tree genetic relationship analysis showed that large S protein (S) gene partial sequences in Hepatitis B virus isolates (IQ-1-IQ-2) were shown to be closed genetically related to NCBI-BLAST Hepatitis B virus China isolates (JX036332.1) at total genetic change of (0.0200-0.0050%). Whereas the phylogenetic tree genetic relationship analysis showed

that polymerase (pol) gene partial sequences in Hepatitis B virus isolates (IQ-1-IQ-2) were shown to be closed genetically related to NCBI-BLAST Hepatitis B virus China isolates (KX354997.1) at total genetic change of (0.0150-0.0050%). Finally, the local Hepatitis B virus isolates (IQ.No.1-IQ.No.2) were submitted to the NCBI Genbank and identified by accession numbers to the large S protein gene (OK359035−OK359036). and to the polymerase gene (OK359037−OK359038).

This thesis found that the PCR method has high sensitive comparing to ELISA technique, because in the positive cases of ELISA infection were not confirmed by PCR. Detection of hepatitis using ELISA may be inaccurate and this is confirmed by this study. This thesis also found that males are more susceptible to infection than females, and that most of the infected patients are residents of the city, due to the population density and the lack of health awareness.

According to this thesis,

1. The ELISA examination is considered a preliminary examination and not a confirmatory examination.

2. The PCR test is an important test for the purpose of diagnosing hepatitis compared to ELISA.

3. The presence of genetic mutations in the viral DNA sequence in the research area was compared to the same virus in other countries by NCBI GenBank.

4. The areas most affected by HBV infection in this study were the urban areas. Most of the injured were in the age group (50–60 years of age).

5. It was also found that most of the patients had a low educational level.

5.2. Recommendations:

1. Creating a database for collecting all the information on viral *Hepatitis B virus* type in Thi Qar Governorate. As well as providing a database of people infected with *Hepatitis B virus* of age, sex and residential address.

2. Emphasis on the application of the polymerase chain reaction (PCR) technique in blood tests in Iraqi hospitals and blood transfusion banks, because it detects early infection, specificity and sensitivity, in a more accurate way than other tests currently available.

3. Conducting periodic examinations for all health care workers in health institutions to investigate Infection with viral hepatitis.

4. Emphasis on spreading health awareness of the disease and its risks as well as its symptoms and how to prevent it.

5. Avoid direct contact with surfaces, especially in hospitals and other health units, because viruses can live for a period in a dry environment, and precautions are required in handling and disposing of objects and materials contaminated with blood or blood components.

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APPENDICES

Appendix1. Information form patient.

Appendix 2. Multiple sequence alignment analysis of large S protein (S) gene partial sequence in *Hepatitis B virus* isolate (IQ-1-IQ-2) and NCBI-Genbank related *Hepatitis B virus* isolate related isolates. The multiple alignment analysis was constructed using NCBI BLAST alignment tool and showed the nucleotide alignment similarity as (*) and substitution mutations in *large S protein* (S) gene.

Appendix3. Multiple sequence alignment analysis of polymerase (pol) *gene* partial sequence in *Hepatitis B virus* isolate (IQ-1-IQ-2) and NCBI-Genbank related *Hepatitis B virus* isolate related isolates. The multiple alignment analysis was constructed using NCBI BLAST alignment tool and showed the nucleotide alignment similarity as (*) and substitution mutations in polymerase (pol) gene.

Appendix 1: Information form patient

Hospital:	Date:	Sample No.:
Patient name:	Age:	Blood group:
Gender:	Male:	Female:
Address:	City:	
Residence:	Urban:	Rural:
Telephone No.:		
Notice:		

Appendix 2: Multiple sequence alignment analysis of large S protein (S) gene partial sequence in *Hepatitis B virus* isolate (IQ-1-IQ-2) and NCBI-Genbank related *Hepatitis B virus* isolate related isolates. The multiple alignment analysis was constructed using NCBI BLAST alignment tool and showed the nucleotide alignment similarity as (*) and substitution mutations in *large S protein* (S) gene.

large S protein (S gene):

```
IQ-1-_S_-gene   TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
IQ-2-_S_-gene   TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
JX036332.1      TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
EU678625.1      TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
EU678597.1      TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
EU678628.1      TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
EU678600.1      TCTTTCCCGATCACCAGTTGGACCCTGCATTCGGAGCCAACTCAAACAATCCAGATTGGG
EU678602.1      TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
JX036359.1      TCTTTCCCGATCACCAGTTGGACCCTGCGTTTGGAGCCAACTCAAACAGTCCAGATTGGG
KF917483.1      TCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGG
                **************************** ** ********************** ***********

IQ-1-_S_-gene   ATTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
IQ-2-_S_-gene   ATTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
JX036332.1      ATTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
EU678625.1      ACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGTGGGAGCATTCG
EU678597.1      ACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGTGGGAGCATTCG
EU678628.1      ACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
EU678600.1      ACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
EU678602.1      ACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
JX036359.1      ACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
KF917483.1      ACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCG
                * ****************************************** ***********

IQ-1-_S_-gene   GGCCAGGGTTCACTCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
IQ-2-_S_-gene   GGCCAGGGTTCACTCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
JX036332.1      GGCCAGGGTTCACTCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
EU678625.1      GGCCAGGGTTCACTCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
EU678597.1      GGCCAGGGTTCACTCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
EU678628.1      GGCCAGGGTTCACTCCACCGCACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
EU678600.1      GGCCAGGGTTCACTCCACCGCACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
EU678602.1      GGCCAGGGTTCACCCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
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JX036359.1      GGCCAGGGTTCACCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
KF917483.1      GGCCAGGGTTCACCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCA
                ************* ***** ****************************************

IQ-1-_S_-gene   TATTGACAACAGTGCCAGCAGCACCTCTTCATGCCCCACCAATCGGCAGTCAGGAAGAC
IQ-2-_S_-gene   TATTGACAACAGTGCCAGCAGCACCTCTTCATGCCCCACCAATCGGCAGTCAGGAAGAC
JX036332.1      TATTGACAACAGTGCCAGCAGCACCTCTTCATGCCCCACCAATCGGCAGTCAGGAAGAC
EU678625.1      TATTGACAACAGTGCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGAC
EU678597.1      TATTGACAACAGTGCCAGCAGCACCTCCTCCTGCCTCCGCCAATCGGCAGTCAGGAAGAC
EU678628.1      TATTGACAACAGTGCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGAC
EU678600.1      TACTGACAACAGTGCCAGTAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGAC
EU678602.1      TATTGACAACAGTGCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGAC
JX036359.1      TATTAACAACAGTGCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGAC
KF917483.1      TATTGACAACAGTGCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGAC
                ** * ************* ******** ** **** ** **********************

IQ-1-_S_-gene   AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
IQ-2-_S_-gene   AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
JX036332.1      AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
EU678625.1      AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
EU678597.1      AGCCTACTCCCATTTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
EU678628.1      AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
EU678600.1      AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
EU678602.1      AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
JX036359.1      AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAATT
KF917483.1      AGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACT
                ************* ****************************************** *

IQ-1-_S_-gene   CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG
IQ-2-_S_-gene   CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG
JX036332.1      CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG
EU678625.1      CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG
EU678597.1      CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAAAGGCCTGTATTTCCCTGCTG
EU678628.1      CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG
EU678600.1      CCACAACCTTCCACCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTG
EU678602.1      CCACAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTATATTTCCCTGCTG
JX036359.1      CCACGACATTCCACCAAGCTCTGCTAGATCCCAGAGTAAGGGGCCTATATTTTCCTGCTG
KF917483.1      CCACAACATTCCACCAAGCTCTGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTG
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IQ-1-_S_-gene   GTGGCTCCAGTTCAGGAACAGTAACCCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA
IQ-2-_S_-gene   GTGGCTCCAGTTCAGGAACAGTAACCCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA
JX036332.1      GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA
EU678625.1      GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA
EU678597.1      GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA
EU678628.1      GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTATTGCCTCTCCCATATCGTCAA
EU678600.1      GTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA
EU678602.1      GTGGCTCCAGTTCAGGGACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAA
JX036359.1      GTGGCTCCAGCTCCGGAACAGTAAACCCTGTTCCGACTACTGTCTCTCCCATATCGTCAA
KF917483.1      GTGGCTCCAGTTCCGGAACAGTAAACCCTGTTCCGACTACTGCCTCACCCATATCGTCAA
                ********** ** ** ******* ************** ** *** *************

IQ-1-_S_-gene   TCTTCTTGAGGATTGGGGACCCTGCGCCGAACATGGAGAACATCACATCAGGATTCCTAG
IQ-2-_S_-gene   TCTTCTTGAGGATTGGGGACCCTGCGCCGAACATGGAGAACATCACATCAGGATTCCTAG
JX036332.1      TCTTCTTGAGGATTGGGGACCCTGCGCCGAACATGGAGAACATCACATCAGGATTCCTAG
EU678625.1      TCTTCTCGAGGATTGGGGACCCTGCGCCGAACATGGAGAACATCACATCAGGATTCCTAG
EU678597.1      TCTTCTCGAAGATTGGGGACCCTGCACCGAACATGGAGAACATCATATCAGGATTCCTAG
EU678628.1      TCTTCTCGAGGATTGGGGACCCTGCGCCGAACATGGAGAACATCACATCAGGATTCCTAG
EU678600.1      TCTTCTCGAGGATTGGGGACCCTGCGCCGAACATGGAGAACATCACATCAGGATTCCTAG
EU678602.1      TCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCACATCAGGATTCCTAG
JX036359.1      TCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCACATCAGGATTCCTAG
KF917483.1      TCTTCTCGAGGACTGGGGACCCTGCACCGAACATGGAGAACACAACATCAGGATTCCTAG
                ****** ** ** ************ * **************  * **************

IQ-1-_S_-gene   GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
IQ-2-_S_-gene   GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
JX036332.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
EU678625.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
EU678597.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
EU678628.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
EU678600.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
EU678602.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
JX036359.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGC
KF917483.1      GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCAC
                ************************************************************     *
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Appendix 3. Multiple sequence alignment analysis of polymerase (pol) *gene* partial sequence in *Hepatitis B virus* isolate (IQ-1-IQ-2) and NCBI-Genbank related *Hepatitis B virus* isolate related isolates. The multiple alignment analysis was constructed using NCBI BLAST alignment tool and showed the nucleotide alignment similarity as (*) and substitution mutations in polymerase (pol) gene.

polymerase (pol) gene

```
IQ-1-_pol_-gene TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
IQ-2-_pol_-gene TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
KX354997.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
MK138694.1      TCGACAAGGCATGGGGACGAATCTATCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
JX036358.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
MK138693.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
JX036332.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
KT723249.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
KT723250.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
MK171848.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
KC792961.1      TCGACAAGGCATGGGGACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
MK171787.1      TCGACAAGGCATGGGGACGAATCTATCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCA
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IQ-1-_pol_-gene CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
IQ-2-_pol_-gene CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
KX354997.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
MK138694.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
JX036358.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAG
MK138693.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
JX036332.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGATTTCAACCCCAA
KT723249.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
KT723250.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
MK171848.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
KC792961.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
MK171787.1      CCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCAA
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IQ-1-_pol_-gene CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
IQ-2-_pol_-gene CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
KX354997.1      CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
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MK138694.1      CAAGGATCATTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
JX036358.1      CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
MK138693.1      CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
JX036332.1      CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
KT723249.1      CAAGGATCATTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
KT723250.1      CAAGGATCATTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
MK171848.1      CAAGGATCATTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
KC792961.1      CAAGGATCAATGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
MK171787.1      CAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATTCGGGCCAGGGTTCAC
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IQ-1-_pol_-gene CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
IQ-2-_pol_-gene CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
KX354997.1      CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
MK138694.1      CCCACCACACGGCGGTCTTTTAGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
JX036358.1      TCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
MK138693.1      TCCACCGCACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCCCAGGGCATATTGACAACGGT
JX036332.1      TCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
KT723249.1      CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
KT723250.1      CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
MK171848.1      CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
KC792961.1      CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
MK171787.1      CCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACAACAGT
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IQ-1-_pol_-gene GCCAGCAGTACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
IQ-2-_pol_-gene GCCAGCAGTACCTCCTCCTGCCCCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
KX354997.1      GCCAGCAGTACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
MK138694.1      GCCAGCAACACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
JX036358.1      GCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
MK138693.1      GCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
JX036332.1      GCCAGCAGCACCTCTTCATGCCCCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
KT723249.1      GCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
KT723250.1      GCCAGCAGCACCTCCTCCTGTCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
MK171848.1      GCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
KC792961.1      GCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
MK171787.1      GCCAGCAGCACCTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACTCCCAT
                ******* ***** ** ** * ************************************

IQ-1-_pol_-gene CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCAAGCAGTGGAACTCCACAACCTTCCA
IQ-2-_pol_-gene CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCAAGCAGTGGAACTCCACAACCTTCCA
KX354997.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACCTTCCA
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MK138694.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCTCAACCTTCCA
JX036358.1      TTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACCCCACAACTTTCCA
MK138693.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCA
JX036332.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACCTTCCA
KT723249.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCA
KT723250.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCA
MK171848.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCA
KC792961.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCA
MK171787.1      CTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCA
                 ********************************** ********** ** **** *****

IQ-1-_pol_-gene CCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTATATTTCCCTGCTGGTGGCTCCAGTTC
IQ-2-_pol_-gene CCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTATATTTCCCTGCTGGTGGCTCCAGTTC
KX354997.1      CCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTATATTTCCCTGCTGGTGGCTCCAGTTC
MK138694.1      CCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTATATTTCCCTGCTGGTGGCTCCAGTTC
JX036358.1      CCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTGGTGGCTCCAGTTC
MK138693.1      CCAAACTCTCCAAGATCCCAGGGTGAAAGGCCTGTATTTCCCTGCTGGTGGCTCCAGTTC
JX036332.1      CCAAACTCTGCAAGATCCCAGGGTGAGAGGCCTGTATTTCCCTGCTGGTGGCTCCAGTTC
KT723249.1      CCAAGCTCTGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTGGTGGCTCCAGTTC
KT723250.1      CCAAGCTCTGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTGGTGGCTCCAGTTC
MK171848.1      CCAAGCTCTGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTGGTGGCTCCAGTTC
KC792961.1      CCAAGCTCTGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTGGTGGCTCCAGTTC
MK171787.1      CCAAGCTCTGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTGGTGGCTCCAGTTC
                **** **** * ********* ****  ***** ***** ********************

IQ-1-_pol_-gen  AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT
IQ-2-_pol_-gene AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT
KX354997.1      AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT
MK138694.1      AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT
JX036358.1      AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT
MK138693.1      AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAT
JX036332.1      AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTTGAGGAT
KT723249.1      CGGAACAGTAAACCCTGTTCCGACTACTGCCTCACCCATATCGTCAATCTTCTCGAGGAC
KT723250.1      CGGAACAGTAAACCCTGTTCCGACTACTGCCTCACCCATATCGTCAATCTTCTCGAGGAC
MK171848.1      CGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAC
KC792961.1      CGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAC
MK171787.1      AGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGAC
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CURRICULUM VITAE

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Undergraduate Thi-Qar University
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