Republic of Iraq

Ministry of Higher Education Scientific Research

University of Babylon

College of Science

Department of Biology

Role of Hepatitis B virus in Forensic investigation

A Research
Submitted to the Council of the College of Science and The
Committee of Postgraduate Studies University of Babylon in
Partial Fulfillment of The Requirements For The Degree Of
Higher Diploma In Forensic Evidences

By

Adnan Ali Mohsein Husain

B.Sc. Biology /University of Babylon

Supervised By

Professor

Dr. Shakir Hammad Mohammed Hassan

2021 A. D **1443 A.H**

بسم الله الرحمن الرحيم

←⬈⬩▫⬀❷⧫☼ ﴿

♊◻👌 🖮💣🕓⍓♉◆❻⍓⑩

🗏 ↓↗✏👓⧫⌖✹💧

⇪♏✍→🗎 ⬧📫⬀❑⬧▫◆❒

✌🏳▫🖫& ⬂ ❽&❶

﴾ ⇪&&⇪ &🏳⑩&🖫⧫⬂

صدق الله العلي العظيم

سورة يوسف / الآية ٧٦

Certification

I certify that this thesis was prepared under my supervision at the Department of Biology, College of Science, University Babylon, as partial fulfillment of requirement for the degree of Higher Diploma in Forensic Evidences .

Signature

Professor

Dr. Shakir Hammad Mohammed Hassan

PH.D. (Ph.D. MoleculaVirology)

Supervisor

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

Assistant Professor

Dr. Adi Jassim Abd Alrezzaq

Signature

Head of

Department of biology

College of Science/University of Babylon

Committee Certificate

We, the Examining committing, certify we have read this research and have examined the student in its content and, at our opinion, it is adequate with standing: Excellent rank as a research for the degree of Higher Diploma In Forensic Evidences

Professor

Dr.Alaa jawad Hassan

(chairman)

Assistant professor	**Professor**
Thekra Abdulaali Abed	***Dr. Shakir Hammad M.Al-Alwany***
(member)	**(supervised and member)**

Signature

Assistant Professor

Dr. Adi Jassim Abd Alrezzaq

Head of

Department of biology

College of Science/University of Babylon

Dedication

To the reason being the creator of everything, thanks and praise be to Allah for everything.

To our Prophet Mohammed

bless be upon him

To my wounded Iraq.

To the memory of my father and mother,

family (My Sons and Wife)

and

To all people whom I love .

To all persons who teach me the word

To everyone who aided me in every possible way to make this work see the light

I dedicate this work.

Adnan

Acknowledgment :-

The name of Allah before everything. I thank the lord Allah who honored and made me of the slaves, and peace and blessings be up on the best creation of our prophet Muhammad and his good pure family.

I would like to express my deepest appreciation and very honest gratitude to my supervisor Dr. Shaker Hammad Al-Alwany for their guidance, support , interest and their encouragement .

My special thanks and gratitude are to the head Department of Biology and the dean of College the Science, University of Babylon for their assistance.

My sincere thanks and gratitude are to the teaching staff members , to my family , to all friends and forensic Diploma students who helped me at the Department Biology in College of Science, University of Babylon .

Summary

Background: About half a million people die globally from chronic Hepatitis B Virus (HBV) infection. A large number of accidental virus infections occur in medical and non-medical workers exposed to infectious individuals and materials. HBV is differentiated into many genotypes, according to genome sequence. To date, eight well-known genotypes (A-H) of the HBV genome have been defined. Moreover, two new genotypes, I and J, have also been identified. Some HBV genotypes are further classified as sub-genotypes.

Objectives: To determine the infection rate of hepatitis B virus genotypes among patients infected with HBV ;blood donors attended to Blood Bank in mid- Euphrates Governorates ,Iraq.

Patients and Methods: Cross sectional study which conducted at patients infected with HBV ;blood donors attended to Blood Bank in Babylon Governorate , Iraq. Raw database were collected from General health department at Babylon; Karbala and AL_Najaf Health directorate in the last 5 years (June 2016- June 2021) and 16312 investigated person; they're aged from 16-64 years during a period from Dec. 2020 to Dec. 2021. Blood samples were collected from all participants then analyzed by use different enzyme linked immunosorrbent assay (ELISA) kits and real-time polymerase chain reaction (RT-PCR) to determine the HBV genotypes. Only 675 HBV patients was analysis by RT-PCR around the year 2020 in Babylon province.

Results : Detection of HBV –RT-PCR reactions was observed in 98 out of 675 (14.5%). The predominant genotype among patients under study was genotype A (58%), followed by genotype D (12%), and lowest frequency was found for undetermined genotype (1%). In general, there was no significant association between the different genotypes and some demographical factors, serological investigations, and liver function test.

Conclusions : The most common HBV genotype in Iraq was genotype D, followed by genotype A. In addition, there were increased disease prevalence with time , and the males were more significant than females in hepatitis infections. Finally, the north area of Babylon province had higher percentage than south and middle areas , especially in ruler area than urban.

List of Contents

List of Tables

List of Figures

List of Abbreviations.

Abbreviation	Complete Name
ANA	Anti – Nuclear Antibody
ASMA	Anti – Smooth muscle Antibody
cccDNA	Covalently closed circular DNA
ER	Endoplasmic Reticulum
HB c Ag	Hepatitis B core Antigen
HBeAg	Hepatitis B envelope Antigen
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HBx	Hepatitis X protein
IC	Internal Control
IFN- α	Interferon-alpha

LHBs	Large hepatitis B surface protein
LKM	Liver Kidney Microsome
MHBs	Middle hepatitis B surface protein
ORF	Open Read Frame
p-ANCA	Pri-nuclear Anti – Neutrophil cytoplasmic Antibody
pg RNA	Pre – Genomic RNA .
preC RNA	Pre –Core RNA
RC DNA	Relaxed Circular DNA
RT-PCR	Real Time Polymerase Chain Reaction
SLP	Specific Liver Protein
SVP	Sub viral particles
VIDRL	Victorian Infectious Diseases Reference Laboratory

Chapter One

Introduction

1.1.Introduction

Hepatitis B Virus (HBV) is chronically carried by around 400 million people worldwide and about one million die annually as result of developing liver cirrhosis and hepatocellular carcinoma (Kao *et al*., 2002 Ogawa, 2002, Chan, *et al*., 2003,) The infection is mainly present in Middle East, South-East Asia, sub-Saharan Africa, Central and South-America, and Eastern-Europe with prevalence >8% of population (Lavanchy 2004). A migratory flow that had occurred in last twenty years from these countries to the industrialized countries resulted in an increase in HBV prevalence the industrialized countries (Kao *et al*., 2002 , Nicoletta , 2002).

Hepatitis B virus is transmitted through blood and body fluids, hence certain types of behaviors increase the risk of infection, such as sharing personal items (toothbrushes, razors, etc.), use of contaminant needles for intravenous drugs or ear pricing and tattooing, and practicing unsafe sex. Hemodialysis and hemophiliacs patients as well as healthcare and emergency service workers are also at higher risk (André , 2000).

HBV genome has a high rate of mutation when compared to other DNA viruses due to the high spontaneous error rate of the viral reverse transcriptase and lack of proofreading mechanism. It is estimated approximately $1.4–3.2 \times 10^{-5}$ per genome. Accordingly, HBV can be classified into eight genotypes A-H that accounts for 8% or more in the complete nucleotide sequence on inter-sequence divergence (Stuyver, et al., 2000; Jazayeri, et al., 2004 ,). Studies on HBV genotypes show a distinct geographical distribution around the world (Huy, .2000). and India (Ding *et al*., 2004). Genotypes B and C are prevalent in Asia (Sugauchi *et al*., 2002). especially in the populations of Eastern Asia and the Far East (Ogawa, 2002). Genotype D is distributed worldwide with the highest prevalence in the Mediterranean region (Stuyver, *et al*., 2000 Ogawa, 2002,) Genotype E and F are predominant in West Africa and in the Amerindian population, respectively (Stuyver *et al*., 2000 Kao *et al*., 2002). Recently, Genotype G was identified in the USA and France (Kao et al., 2002). Also Genotype H was also recently found in Central America (Arauz Ruiz *et al*., 2002). A remarkable difference in the clinical and virologic characteristics between the patients with different genotypes has been reported (Chan, *et al*., 2003).

HBV genotypes are reported to be responsible for the differences in the natural history of chronic infection and they play a significant role in clinical manifestation of infection and response to antiviral therapy (Liaw, 2010). Therapeutically, patients that are infected with genotypes A, B, D, and F show frequent spontaneous . Hepatitis B envelope antigen (HBeAg) seroconversion when compared to genotype C. Whereas, patients that were infected with genotype E have higher frequency of HBeAg positivity and higher viral loads as compared to patients that were infected with genotype D (Kramvis, 2014).

1.2. Aim of Study

the current study was aimed to determine the prevalence of HBV infection in Babylon Province via the following objectives:

1. Investigate the prevalence of HBV genotypes, its correlation with demographic factors and impacts on hepatic biomarkers.
2. Obtaining data on the number HBV on the record kept at general health department at Babylon health director.

Chapter Two

Literature of Reviews

2. Literature of Reviews

2.1 HBV Virology

2.1.1 Classification of the hepatitis B virus

Human HBV is the prototype member of the Hepadnaviridae family, and is a species of the genus Orthohepadnavirus (infecting mammals) which also includes non-primate species such as the Ground squirrel hepatitis virus, woodchuck hepatitis virus, arctic squirrel hepatitis virus (Global Hepatitis Report 2017). HBV has been identified in non-human primates such as the apes (chimpanzees, gorillas, orangutans, and gibbons) and 'Old World monkeys' such as macaques (Lavanchy *et al.*, 2004, Global Hepatitis Report 2017). The 'New World primate' woolly monkey HBV is the most genetically divergent among the primate hepadnaviruses (MacLachlan, *et al.*, 2013). Transmission of human HBV has been demonstrated to chimpanzees who have previously been used in experimental models, and more recently baboons who were under consideration as a xenograft source for human liver transplantation (10 Kramvis et al., 2010 Croagh et al., 2015). Avihepadnaviruses are the avian species of this virus family that includes the Duck hepatitis B virus, with the recent discovery of genomic integration of the avian hepadnavirus DNA into the zebra finch genome (not a current species affected by HBV) that reveals an ancient evolutionary hepadnavirus history of more than 40 million years (Gilbert, *et al*., 2010, Littlejohn *et al*., 2016) A recent discovery of hepadnavirus in bats with reported zoonotic potential has recently raised public health concerns (Drexler *et al.*, 2013) .

2.1.2 HBV structure

HBV is an enveloped virus, with a complete virion (also known as the Dane particle) diameter of 42-45nm (Dane, D. S., Cameron, C. H., & Briggs, M. (1970). The mature virion is a double-shelled sphere consisting of an outer lipid envelope with embedded hepatitis B surface protein (HBsAg, small 22nm spherical or tubular forms), and a 27nm icosahedral inner nucleocapsid core comprised of the DNA genome and DNA polymerase surrounded by the Hepatitis B core protein (HBcAg) (Locarnini 2004). The non-infectious filamentous and spherical sub viral HBsAg (lacking a core) are produced and secreted in excess of the mature virion (Seeger and mason 2015).

2.1. 3 HBV Genome organization

The HBV genome is around 3.2kb in size and arranged into a relaxed-circular partially double-stranded DNA (RC DNA) configuration, consisting of a full-length minus strand, with the positive strand being incomplete (50-80%), held together by a short overlap at the 5’ end (Seeger and mason 2000). The positive strand gap can be repaired for replication by the endogenous covalently attached DNA polymerase, situated at the 5’ end of the minus strand (Beck and Nassal 2007). There are 4 over-lapping open reading frames (ORFs), all encoded for by the complete minus strand. The longest ORF is polymerase (Pol), with the envelope ORF contained within it, and then partially overlapping with core (C) and X ORFs. Due to the overlapping nature of the ORFs, 67% of the genome is multiple coding. The four partially overlapping open reading frames encode the envelope proteins (small, medium and large that together comprise the surface (HBsAg) protein), the core (HBcAg) protein (p21) which forms the nucleocapsid, and the polymerase protein essential for HBV replication. In addition to these proteins, two nonstructural proteins are encoded, the transcriptional transactivator hepatitis B X protein (HBx) and the 25-kDa precore protein which is processed to form the secreted Hepatitis B e antigen (HBeAg, p17) (Seeger and mason 2015). Four promotors and two enhancers (enhancer I and enhancer II) are responsible for initiating transcription: the preC/pregenomic, S1, S2 and X promotors. Additional regulatory elements, such as the negative regulatory element (NRE), act in cis to further regulate gene expression (Moolla *et al*.,2002).

2.1.4 Covalently closed circular DNA

Covalently closed circular DNA (cccDNA) is an essential step in the HBV life cycle as a template for replication, and in particular for genomic archiving within the hepatocyte, underpinning the viral persistence of chronic HBV infection (Yang and Kao 2014). This cccDNA "reservoir" is created from the conversion of the RC DNA into complete circles by completion of the incomplete plus strand DNA once delivered to the nucleus, where the HBV genome can therefore exist in a stable form to act as an episomal minichromosome (Beck, and Nassal, 2007). The exact mechanism of the RC DNA "repair" and modification of the terminal ends of both the plus and minus DNA strands, including removal of the covalently linked DNA polymerase from the 5 end of the minus strand, is currently unknown (Block, *et al.*, 2007). The cccDNA can persist in the nucleus of the hepatocyte for the life of the cell, and also be passed onto progeny cells during cell division (Zhang *et al*., 2003 Block, *et al*., 2007 Seeger and mason 2015). The role of the cccDNA in the HBV life cycle and transcription template of greater-than-genome length

RNAs and subgenomic RNAs is discussed further below. 1.2.3.5 Transcription of HBV RNA The nuclear cccDNA is used as a template for transcription of all HBV viral RNAs, utilizing host cellular RNA polymerase II interacting with 4 separate promotor sites (Beck, and Nassal, 2007). Four major HBV mRNA species are transcribed: pre-genomic RNA (pg RNA) and precore RNA (preC RNA), preS1 RNA, preS2/S RNA, and X RNA; all terminating with the identical polyadenylated 3’ tail. This includes the greater than genome length 3.5kb pgRNA, which encodes the viral proteins required for nucleocapsid assembly (HBcAg, HBV Pol/RT), and is the template for the reverse transcription of the HBV DNA genome. HBeAg is encoded by the 3.5 kb preC RNA that is co-linear to pgRNA with a short extension at the 5’ end. The envelope proteins are encoded by two colinear 2.4kb preS1 mRNA and 2.1kb preS2/S mRNA, resulting in the large, and middle and small surface proteins. The smallest 0.7kb mRNA encodes the transactivating factor HBx. Expression of the four mRNA transcripts are regulated by enhancer II/basal core, PreS1, PreS2, and enhancer I/X promoters (Moolla , *et al.*, 2002, Locarnini, and Zoulim , 2010). The role of spliced HBV variants is unclear, however may be related to the development of hepatocellular carcinoma in CHB infection (Bayliss *et al.*, 2013).

2.1.5 Viral proteins

2.1.5.1 Hepatitis B surface antigen (HBsAg)

The HBsAg is encoded by 2.1Kb and 2.4Kb subgenomic transcripts that produce the three envelope proteins. These mRNA are transcribed from the Pre-S/S ORF, comprised of 389-400 codons, entirely overlapped by the Pol ORF . The term HBsAg describes sum of antigenticity of all HBV surface proteins, and is classified into three individual viral proteins as either small (S), medium (M) or large (L) depending on the transcriptional inclusion of the S alone, pre-S2, or Pre-S1 upstream initiation codons respectively, with the common domain being S. Translation of these proteins occurs in the endoplasmic reticulum (ER) and exist in both glycosylated and un-glycosylated forms (Schadler, and Hildt , 2009). The nucleocapsid is surrounded by a lipid bilayer in which the S, M and L surface proteins are anchored by the S domain to the membrane to form the viral envelope. The HBV surface proteins are also secreted in excess to virions and can bud from the ER forming empty filamentous and spherical 22nm subviral particles (SVP) (Seeger and Mason 2000,). (a) Small hepatitis B surface protein The small hepatitis B surface protein (SHBs) is the most abundant of the three HBV envelope proteins, comprising around 70% of the HBsAg pool, and is made up of 226 amino acids (Seeger and Mason 2000). The ‘a’ determinant within the HBsAg is a loop structure created by cross-linked cysteine residues that is the major antigenic determinant of the HBsAg, located in the SHBs between amino acids 124 to 147. It is the major neutralization domain of the antibodies to HBsAg, with amino acid substitutions in this region resulting in conformational changes affecting binding of anti-HBs antibodies that permit immune escape (Lada, *et al.*, 2006). Minor sub-determinants “d”, “y”, “w” or “r” vary between HBV strains based on the amino acid profile in this region, designating HBV serotype. (b) Middle hepatitis B surface protein / Pre-S2 protein The middle hepatitis B surface protein (MHBs) is encoded by the 2.1kb RNA transcript and is comprised of the common S domain with the Pre-S2 domain adding 55 amino acids. It is generally present in approximately equal amounts with the large surface protein (together 30%) (Seeger *and Mason*., 2000). The membrane topology of the MHBs is the same as the SHBs (Lada, *et al.*, 2006). (c) Large hepatitis B surface protein / Pre-S1 protein The large hepatitis B surface protein (LHBs) encompasses the Pre S1, Pre S2 and S domains comprising 389-399 amino acids (depending on HBV genotype). The LHBs displays dual membrane topology on the ER, with the different orientation of the Pre S1- Pre S2 domains having important properties including viral attachment and entry, interaction with the nucleocapsid, and regulation of HBV replication (Schadler, and Hildt 2009). The Pre S1 domain on the surface of mature virions is crucial for viral entry by binding to the cellular receptor recently discovered as the sodium taurocholate co-transporting polypeptide (NTCP) receptor (or receptor complex) (Yan, *et al.*, 2012).

2.1.5.2 Hepatitis B core antigen (HBcAg)

The hepatitis B core antigen (HBcAg) is translated from the pgRNA and is comprised of 183 to 185 amino acids depending on the HBV genotype, consisting of an assembly domain (N-terminal 149 or 151 aa), and a domain responsible for the packaging of the pg RNA/Pol complex (C-terminal 34 aa) (Schadler, and Hildt ,2009). The pg RNA also encodes the Pol protein, with the ORFs for the Pol and core genes overlapping and translation occurring in a low Pol/core protein ratio important for creation of the icosahedral nucleocapsids and DNA replication with around 240 copies of the core protein encapsulating one copy of Pol bound to pgRNA triggering packaging and reverse transcription (Seeger, and Mason2015, Tong, and Revill , 2016). The C-terminal domain also contains important B and C cell epitopes (Jung and Pape, 2002). The HBV core protein is also vital a structural component of the viral minichromosome and may have a role in cccDNA transcription

(Bock, *et al*., 2001).

2.1.5.3 Hepatitis B e antigen (HBeAg)

The HBeAg is a product of the Pre C/C ORF and is transcribed from a distinct 3.5Kb mRNA. The HBV precore (PC) protein is a non-structural protein that is not essential for viral replication, however has been implicated in immune evasion and appears essential for establishment of chronic infection (Beck, and Nassal,2007). The precore protein overlaps the core protein 8 by around 90%, sharing the central core domain and C-terminal protamine-rich domain, and undergoes processing in the ER/golgi complex, producing a 22-kDa protein (p22), which can either undergo further processing by cleavage of 10 amino acids from the Nterminus to form the secreted 17-kDa HBeAg (p17), or traffic to the cytosol where it remains localized (Garcia, et al., 1988). HBeAg is a soluble protein and is traditionally a marker of active HBV replication, infectivity and response to antiviral treatment. However, mutations in the precore gene or basal core promotor region that reduce or stop HBeAg expression may be present in the presence of ongoing viral replication (Locarnini, 2004). HBeAg-negative viral variants can be transmitted perinatally or horizontally and have been reported to cause fulminant or transient acute hepatitis, although only rarely lead to establishment of chronic infection (Chang, et al., 1987,Carman, *et al*., 1991). The HBeAg is a critical protein in paediatric HBV infection. It is the only HBV protein known to cross the placenta and is proposed to act as an immune system tolerogen important for the establishment of chronic HBV infection in infants born to HBsAgpositive mothers, especially with high viral load and HBeAg positivity (Ni, 2011).The HBeAg also appears to have an important regulatory role with the host innate immune system, regulating Toll-like receptor-2 expression and NFkB signalling (Yang, 2006 ; Kramvis, 2016).

2.1.6 HBV Genotypes

There are 9 HBV genotypes defined to date (A-I) (Kim *et al.*, 2011). with a further genotype J recently proposed, discovered in one individual from Japan (Tatematsu, *et al.*,2009). Genotypes are designated by sequence divergence of more than 8% across the whole genome (Kramvis , *et al.*, 2005). These are further divided into sub-genotypes based on intra-group divergence of more than 4% in the S gene (Norder, *et al.*, 1922). Sub-genotypes have been identified for genotypes A, B, C, D, F and I. Revision of initially proposed classifications has occurred with more in-depth phylogenetic analysis with bootstrap support over the entire genome rather than partial sequences (Kramvis, 2014). HBV genotypes generally have specific geographic distribution, although this is altering with migration, and there is mounting evidence that differences in global distribution of HBV genotypes may be responsible for heterogeneity in clinical outcomes, response to antiviral therapy and vaccination (Liu, and Kao 2013).

In vitro studies of HBV genotypes Studies comparing the replication profile and protein production of HBV across genotypes have been performed mostly in transient transfection models using transformed hepatoma-derived liver cells (Huh7) or hepatoblastoma-derived liver cells (HepG2) which have been shown to support HBV replication (Lamontagne, 2013, Sozzi, *et al.*, 2016, Sozzi, et al., 2018). A major limitation with the use of these cell lines is the inability to perform infection studies due to low cellular expression of the NTCP receptor, therefore not enabling recapitulation of natural HBV infection. Although the discovery of the HBV receptor has led to the development of in vitro models of hepatocyte cell lines endogenously expressing NTCP permissive to HBV infection, (Yan, *et al*., 2012, Lamontagne *et al*., 2016). routine infection with different HBV genotypes is still not possible. There have been few in vitro studies comparing HBV replication phenotype across genotypes, likely due to the difficulties in establishing suitably representative replication competent HBV clones permitting direct comparisons. Due to the circular HBV genome, greater-than-genome length HBV plasmids are utilized to facilitate transcription of the greater than genome length pg RNA and subsequent expression of all HBV ORFs, utilizing endogenous HBV promoters. (Sugiyama *et al*. 2019). compared genotypes A, B, C and D in a transient transfection model in Huh7 cells with plasmids containing 1.24mer HBV DNA in order to directly compare Asian and European HBV genotypes [70 Sugiyama, M., *et al* 2006]. They reported higher replication in the Asian genotypes (B and C), and variation in secreted HBs Ag, and a similar profile of secreted HBe Ag and intracellular DNA seen across genotypes. Another study also utilizing 1.28mer HBV constructs, showed that replication of genotype A was better than genotype D (Bhoola, *et al*., 2014). This study by Bhoola et al. also compared African A1 genotype to A2 as well as D3, with some differences seen in replicative intermediates between the A sub-genotypes (Bhoola, *et al*., 2014). Again, the replication levels were also low in this study making interpretation of these results guarded, with the differences in construct length between the two studies also possibly affecting direct comparison. Another study by (Qin .2011) compared genotypes B and C, demonstrating higher levels of virion secretion for genotype C and higher levels of replication in genotype C with precore mutations compared to wild type (Qin, Y., *et* *al*., 2011). The Revill laboratory at the Victorian Infectious Diseases Reference Laboratory (VIDRL) has recently developed a large panel of replication-competent 1.3mer cDNA clones of the major genotypes (A2, B1, B2, B5, C1, C2 D3) as well as genotype H and the putative strain J, that have been compared in a 16 transient transfection model showing marked differences in replication profile and protein expression (Sozzi, *et al*., 2016, Sozzi, et al., 2018). They showed that these differences may in part be explained by sequence variability in the major upstream regulatory region across genotypes with observed differences in promotor activity (Sozzi, et al., 2016, Sozzi, *et al*., 2018). This important work will hopefully shed more light onto the differences observed in CHB natural history and treatment response.

2.1.7 LABORATORY DIAGNOSIS AND MONITORING OF HBV INFECTION

2.1.7.1 Serological markers of HBV infection

The diagnosis of hepatitis B infection relies on reliable serological assays to detect the viral proteins and corresponding antibodies in order to confirm exposure to HBV, ascertain chronicity (HBsAg positivity for more than 6 months), and define phase of disease. The viral proteins HBs Ag and HBe Ag or their antibodies (anti-HBs, anti-HBc IgM and IgG, or anti-HBe) are routinely measured. There is a characteristic pattern of serological markers in the natural history of acute and chronic HBV infection . HBsAg is the first viral protein to detected around 6-12 weeks after infection, with HBe Ag appearing there after (Gao, 2006). Clinical symptoms, if they occur at all in the early course of acute infection, become apparent as the serum alanine aminotransferase (ALT) become abnormal after the viral antigen titers peak, usually between 2-4 months from exposure to HBV (Hui, *et al*., 2008). Anti-HBc IgM may also be detected during flares of CHB making it serologically indistinguishable as a one-off measurement from acute HBV infection. Quantitative assays for HBe Ag and HBs Ag have more recently been developed as a surrogate for viral replicative load and have proposed for predicting treatment response (Bowden, and Thompson,2008,Nguyen, *et al*., 2009). The role of quantitative HBs Ag in particular has garnered much attention for its potential to monitor natural CHB infection as well as treatment outcomes (Martinot-Peignoux, *et al*., 2013) HBsAg titres vary significantly between the phases of natural phases of HBV infection (Karra *et al*., 2016).Ontreatment HBsAg decline kinetics in treated patients have been predictive of treatment response and even HBsAg loss, particularly with interferon-alpha (IFN- α) (Manesis, et al., 2007- Honer and Cornberg, 2014). Furthermore, correlation between serum levels of HBsAg and the intrahepatic cccDNA has been demonstrated, which may be an important clinical utility given the invasive nature of cccDNA estimation (Chan, *et al*., 2007). The dynamics of these quantitative viral proteins have not been evaluated however in the natural history of paediatric HBV infection.

2.1.7.2 HBV viral load measurement

Quantitative viral load with direct measurement of HBV DNA is an important clinical tool, representing replication activity and determining candidate eligibility for anti-viral therapy and treatment response thereafter. HBV DNA levels have become a central focus of the clinical management of CHB, with landmark studies showing the important relationship between higher viral loads and the risk of more severe liver disease and HCC development (Chen, *et al*., 2005, 90 Iloeje, *et al*., 2006). The World Health Organization (WHO) defined calibrated measurement of HBV DNA in IU/ml to ensure standardization across different assays for patient care and comparability in clinical trials (Saldanha, *et al*., 2001). Viral load is also important to monitor for antiviral drug resistance on therapy, with one of the markers of resistance being an increase in HBV DNA of more than one log10 [Locarnini, *et al* 2004]. The more sensitive viral load assays utilize target amplification, with the newer generation real-time PCR having the broadest dynamic range (Bowden, 2006, Song, and Kim, 2016).

2.1.7.3 HBV Genotyping

Although not used as standard of care, HBV genotype can provide clinical support given the known differences in disease progression and response to antiviral therapy . There are various methods of HBV genotyping including restriction fragment-length polymorphism, reverse hybridization, genotype-specific PCR assays, and sequencing analysis (Sarin, *et al*., 2015). Genotype-specific PCR is suitable for detecting mixed genotype infections. While they all have costs and benefits, sequence analysis is the recognized gold standard for genotyping, and is able to identify viral recombinants (Ali, *et al*., 2010). Emerging evidence for differences in HBV natural history across HBV genotypes that alter disease progression and outcome, such as the rapid progression to hepatocellular carcinoma (HCC) associated with the African A1 subtype suggests there may be a role for genotyping in HBV standard of care in future (Karra, *et al*., 2016).

Chapter Three

Materials and Methods

3.1.Materials and Methods

3.1.1. Management Program of Hepatitis Patients

Management Program of Hepatitis patients in Babylon GIT and liver diseases center. All Hepatitis patients admitted to the center encounter with the following processes : -

- Recording and Documentation with clinical information, viral screening test for (HBV and HCV) were done and confirmed. Liver function tests, Prothrombin Time and INR.
- Radiological study required for all patients (Ultrasound, CT scan or MRI in specific cases) .
- Autoantibody profile for liver diseases should be done to exclude the autoimmune Hepatitis (Such as ANA, ASMA, LKM, SLP and p-ANCA).
- PCR technique used for all positive patients of hepatitis screen for follow up and treatment program with combination of (IFN-α) 2b and oral drugs . Statistical analysis were done by using SPSS program.

3.1.2. Patients Selection

There were 675 of known cases of Hepatitis B infection recorded in the Babylon GIT center for last year, from which 98 patients have positive viral load , while other has undetectable level of nucleic acid or (Viral load < 10 IU/ml) in which that lower level can be detected in cephid machine provided in the PCR unit of the center . Positive recoded cases were done in Babylon GIT center at the period between May (2020) up to August (2021) **.**The statistical comparison in which done between the positive cases and undetectable cases instead of health control .

Study design :- Case control study

Numbers of samples = 675

3.2. Extraction of viral genome :-

The provided lists or material were listed individually according to manual part of procedures **,** in addition to the RT-PCR and other recommended materials.

3.2.1. Methods:-

By using user manual of RNA / DNA - Ribo Virus Kit. The Kit of Ribo Virus is designed for the rapid preparation of highly pure viral nucleic acids (e.g. HCV, HIV, HAV, HDV, Enteroviruses, CMV, HBV) from fluid biological samples e.g. plasma, serum, urine, bone marrow, swabs, liquor.

3.2.1.1. Principle of assay

With the **Ribo Virus**, RNA viruses are lysed quickly and efficiently by lysis buffer RAV1 which is a highly concentrated solution . Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane in the **Ribo Virus** columns**.** Carrier RNA improves binding and recovery of the low-concentrated viral RNA. Contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps with ethanolic buffers **RAW** and finally **RAV3**. The nucleic acids can be eluted in low salt buffer or water and are ready-for use in subsequent reactions. The prepared nucleic acids are suitable for applications like automated fluorescent DNA sequencing, RT-PCR, or any kind of enzymatic manipulation.

3.2.1.2. Materials Provided .

· Buffer RAV1 , 2 x 35 ml;
· Buffer RAW , 2 x 30 ml;
· Buffer RAV3 (concentrate) , 2 x 12 ml;
· Buffer RE , 2 x 13 ml;
· Rnase -free H2O , 2 x 13 ml;
· Carrier RNA (lyophilized) , 2 x 1 mg;
· Proteinase K , 2 x 50 mg;
· Proteinase buffer , 8,0 ml;
· Ribo Virus columns with collecting tubes (2ml), 100 piece
· Collecting tubes (2ml), 8 x 50

3.2.1.3. Preparation of Working Solution :-

- **Ribo Virus** columns should be stored dry at room temperature (15–25°C) . All solutions should be stored at room temperature unless otherwise stated. Ribo Virus spin columns, all buffers and reagents can be stored for up to 2 years under the above conditions without showing any reduction in performance.

- Before use, add 1 ml lysis buffer RAV1 to the complete contents of the carrier RNA tube. Dissolve the RNA and transfer it back to the RAV1 bottle.

- Before first use of the kit, add 2,25 ml of Proteinase Buffer into the vial containing Proteinase K, to dissolve lyophilized proteinase K.

- Buffer RAV1 including carrier RNA can be stored at room temperature for 1-2 weeks. Also can be stored at 4°C for up to 4 weeks or aliquot and stored at –20°C for longer periods.

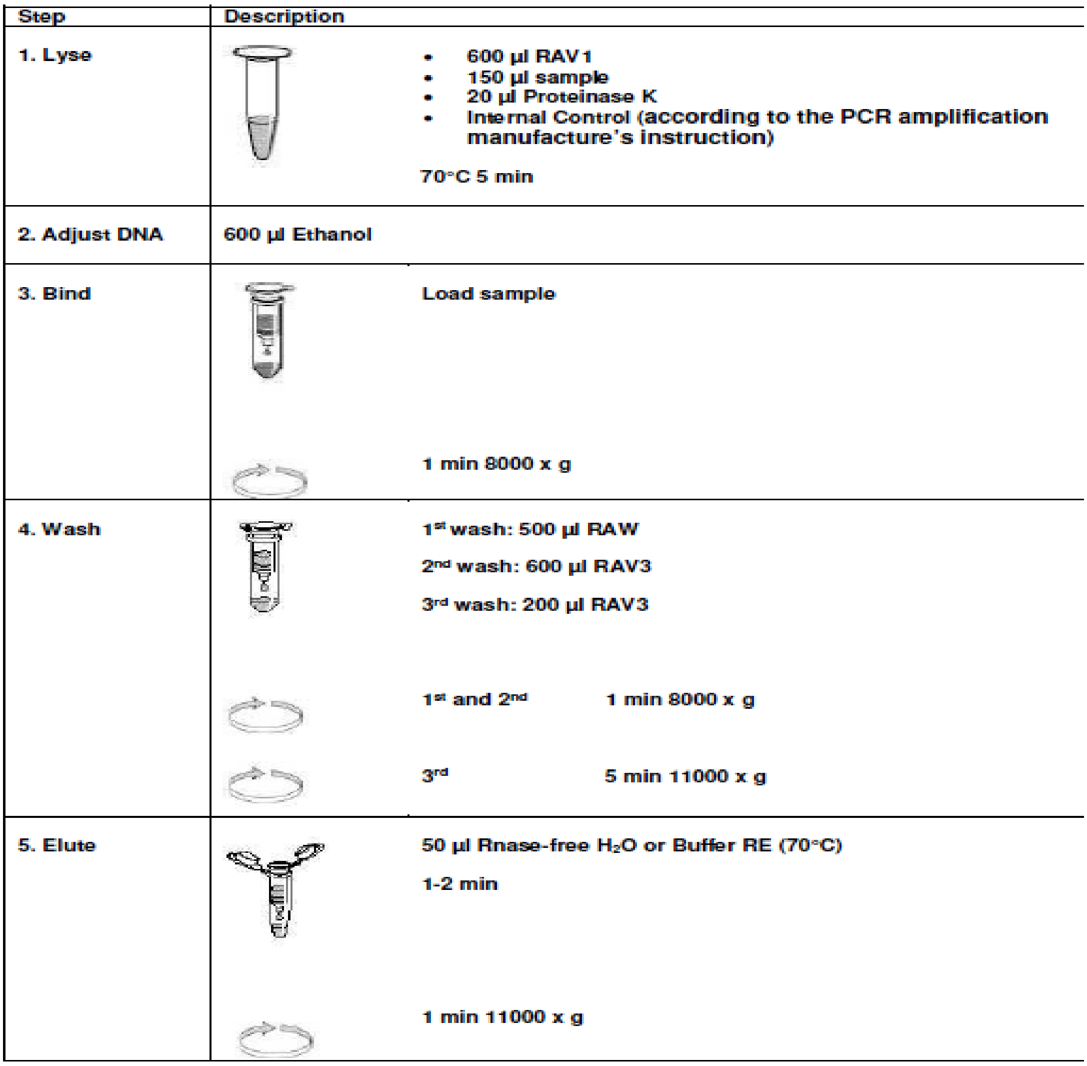
- Do not warm buffer RAV1 containing carrier RNA more than 4 times! Frequent warming, temperatures >80°C and extended heat incubation will accelerate the degradation of carrier RNA. This leads to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular if low titer samples are used.

· **Dissolved Proteinase** is stable for up to 6 months when stored at 2–8°C. Storage at –20°C is recommended to prolong the life of Proteinase, but repeated freezing and thawing should be avoided.

· **Buffer RAV3**: Add **48 ml ethanol** (96-100%) to **buffer RAV3**. Store buffer RAV3 at room temperature for up to one year.

3.2.1.4. Extraction Protocol

Viral RNA/DNA isolation from cell-free biological fluids (Plasma) with Ribo Virus. Before starting the viral RNA/DNA isolation, prepare a 70°C incubation block and preheat an aliquot of elution buffer/water. The steps were listed briefly in the following figure (3 - 1)



Step	Description
1. Lyse	• 600 µl RAV1 • 150 µl sample • 20 µl Proteinase K • Internal Control (according to the PCR amplification manufacture's instruction) 70°C 5 min
2. Adjust DNA	600 µl Ethanol
3. Bind	Load sample 1 min 8000 x g
4. Wash	1st wash: 500 µl RAW 2nd wash: 600 µl RAV3 3rd wash: 200 µl RAV3 1st and 2nd 1 min 8000 x g 3rd 5 min 11000 x g
5. Elute	50 µl Rnase-free H_2O or Buffer RE (70°C) 1-2 min 1 min 11000 x g

Figure (3 - 1) Manual procedure of RNA/DNA Extraction .

3.3. Real Time detection of Hepatitis B Virus in human plasma

The Kit **of HBV Real-TM Quant Dx** is a Real-Time Amplification test for the Quantitative detection of Hepatitis B Virus in human plasma and the simultaneous detection of a HBV-specific Internal Control (IC), by dual color detection.

3.3.1. Principle of Assay :-

HBV DNA is extracted from plasma, amplified using real time amplification and detected using fluorescent reporter dye probes specific for HBV or HBV IC. During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered.

Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of both targets (HBV and IC) takes place simultaneously in the same reaction. Monitoring the fluorescence intensities during Real Time allows the detection and quantification of the accumulating product of the reaction tube after the real time amplification.

Internal Control (IC) serves as an extraction and an amplification control for each individually processed specimen and to identify possible inhibition. IC is detected in a channel other than the HBV DNA. HBV-ICL is a lyophilized Internal Control and represents recombinant DNA-containing-structure which carried through all steps of analysis from nucleic acid extraction to PCR amplification-detection. The presence of

HBVIC allows not only to monitor the extraction procedure and to check possible PCR inhibition but also to verify possible losses of the DNA during extraction procedure thus enabling to calculate precisely the HBV viral load, and results are reported in International Units/mL (IU/mL).

3.3.2. Materials Provided

- Sacace HBV Real-TM Quant Dx Amplification Reagent Kit, RT-PCR reagent pack **(** 96vials (0,2 ml) with lyophilized amplification reagents).

- Sacace HBV Real-TM Quant Dx Control Kit1, 2 , CONTROL INT **(**4vials with lyophilized reagent HBV-IC-L)

- Control 1 (4 vials with lyophilized reagent HBV-Pos1 –L C+ , Sacace HBV Real-TM Quant Dx High Positive Control*)

- Control 2 (4 vials with lyophilized reagent HBV -Pos2 -L C+ , Sacace HBV Real-TM Quant Dx Low Positive Control*

-Control – ve **(** 4,0 vials, 4,0 ml per vial with Negative Control)

- Sacace HBV Real-TM Quant Dx Calibrator Kit 1,2

CAL 1 (4 vials with lyophilized reagent HBV Quantitative Standard 1)

CAL 2 (4 vials with lyophilized reagent HBV Quantitative Standard 2)

(1Standards' and controls' concentrations are specific for every lot. 2must be used during the sample preparation procedure).

3.3.3. Storage Reagent preparation .

All components of the HBV Real-TM Quant Dx PCR kit must be stored at +2-8ºC when not in use. All components of the HBV Real-TM Quant Dx PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated. Current studies refer to EDTA or citrate plasma as the most suitable sample materials for HBV detection.

- Before starting any HBV Real-TM Quant Dx protocol prepare the following reagents :
- Choose the requested quantity of lyophilized controls and calibrators and centrifuge briefly.
- Add Negative Control(CONTROL -) as for table (3- 1) below :

Table (3 -1) Preparation of calibration and control.

Lyophilized reagent 1	CONTROL -ve , µl
CAL 1	1100
CAL 2	1100
CONTROL 1	1100
CONTROL 2	1100
CONTROL INT	300

- Close the tubes and incubate all tubes for 2 min at room temperature. Vortex periodically.
- Centrifuge the tubes for 5 sec.
- Dissolved reagents must be stored at 2-8 °C and always protected from light up to 30days (do not freeze!) .

Please carry out the DNA extraction according to the manufacturer's instructions. Add 10µl of CONTROL INT during the DNA isolation procedure directly to the sample / lysis mixture in all samples, controls, calibrators

3.3.3.1. Assay Calibration .

The quantitative standards CAL1 and CAL2 must be treated in the same way as patients specimens. Before the first use of a new lot of HBV Real-TM Quant Dx, 6 calibrators run must be performed beginning from DNA extraction procedure to generate a calibration curve (two calibrators are run in replicates of three) : Prepare 3 sample preparation tubes for CAL 1, 3 tubes for CAL 2 for each calibration run.

- Add 10 µl of CONTROL INT to each tube;
- Add CAL1 and CAL2 to the appropriate tubes in the quantity indicated in the manual of DNA purification kit .

3.3.3.2. Real Time PCR Sample Procedure :-

1. Prepare requested quantity of reaction tubes with lyophilized reagents to perform PCR of extracted samples and controls.
2. Add **50µl** of eluted samples obtained from DNA purification step.

Close the tubes and transfer them into the Real Time PCR instrument.

Create a temperature profile on your Real-time instrument as follows in table (3- 2) :

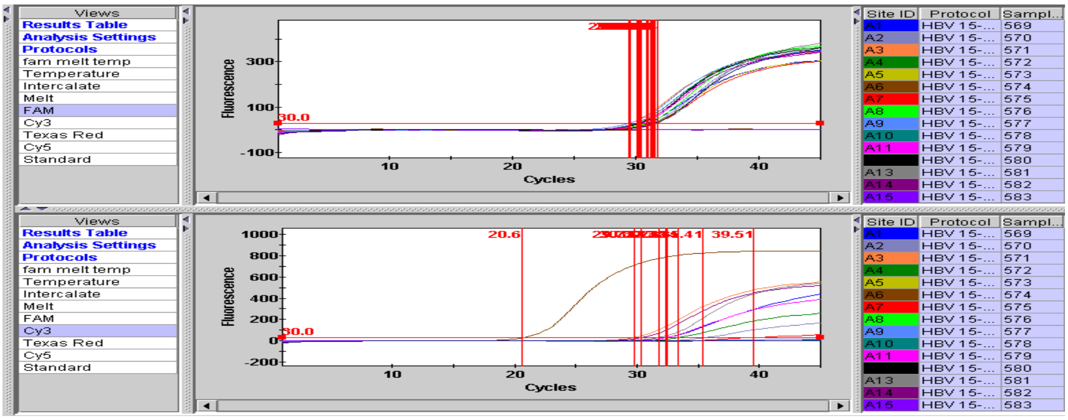
Table (3 -2) Temperature profile of HBV –viral load of RT-PCR.

Stage	Temp.°C	Time	Fluorescence Detection	Cycle Repeats
Hold	95	15min	-	1
Cycling	95	5 s	-	5
	60	20s	-	
	72	15s	-	
Cycling 2	95	5s	-	40
	60	30s	FAM(Green) / Cy3*	
	72	15s	-	

(*) FAM channel for HBV viral antigen , Cy3 for specific internal Control

Result Interpretation :-

The FAM (Green) wave length is monitor of the Internal control result , While The Cy3 refer to the positive viral load of HBV nucleic acid as in the following figure (3 - 1)



Site ID	Protocol	Sampl...
A1	HBV 15-...	569
A2	HBV 15-...	570
A3	HBV 15-...	571
A4	HBV 15-...	572
A5	HBV 15-...	573
A6	HBV 15-...	574
A7	HBV 15-...	575
A8	HBV 15-...	576
A9	HBV 15-...	577
A10	HBV 15-...	578
A11	HBV 15-...	579
	HBV 15-...	580
A13	HBV 15-...	581
A14	HBV 15-...	582
A15	HBV 15-...	583

Site ID	Protocol	Sampl...
A1	HBV 15-...	569
A2	HBV 15-...	570
A3	HBV 15-...	571
A4	HBV 15-...	572
A5	HBV 15-...	573
A6	HBV 15-...	574
A7	HBV 15-...	575
A8	HBV 15-...	576
A9	HBV 15-...	577
A10	HBV 15-...	578
A11	HBV 15-...	579
	HBV 15-...	580
A13	HBV 15-...	581
A14	HBV 15-...	582
A15	HBV 15-...	583

Figure (3 - 2) The amplification curve of HBV nucleic acid .

3.4. HBV Genotype A, B, C, D Real-TM PCR

Real Time PCR kit for detection and differentiation of hepatitis B virus genotypes A, B, C and D in HBV-positive clinical material.

HBV Genotype A, B, C, D Real-TM PCR kit is an *in vitro* nucleic acid amplification test for detection and differentiation of HBV DNA genotypes A, B, C, D in HBV-positive clinical material (blood plasma) by using real-time hybridization-fluorescence detection.

3.4.1. Principle of Assay

HBV genotypes detection includes DNA isolation from biological materials and real-time PCR amplification of HBV DNA. *HBV* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *HBV* genotype primers. In realtime PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. The real-time monitoring of fluorescence intensities during the real-time PCR allows detection of the amplified product without re-opening the reaction tubes after the PCR run. HBV Genotype A, B, C, D Real-TM PCR kit uses "hot-start" Taq polymerase, which greatly reduces the frequency of nonspecifically primed reactions.

The *HBV* genotype A DNA is detected in the Cy5/Red channel

The *HBV* genotype B DNA is detected in the Rox/Texas Red/Orange channel.

The *HBV* genotype C DNA is detected in the FAM/Green channel

The *HBV* genotype D DNA is detected in the JOE/HEX/Yellow channel

3.4.2. Materials Provided

The reagent and material were mentioned in the following table (table 3 -3) as required to complete the procedure of HBV genotyping .

Table (3 -3) Reagents of HBV genotyping procedure .

Reagent	Volume (ml)	Amount
PCR-mix-1-FRT HBV-G	0.6	1 tube
PCR-mix-2-TM	0.3	1 tube
Hot Start TaqF Polymerase	0.03	1 tube
HBV DNA B/A types (C+)	0.2	1 tube
HBV DNA C/D types (C+)	0.2	1 tube
DNA-buffer	0.07	1 tube
Negative Control (C–)*	1.2	2 tubes

*****Must be used in the isolation procedure as Negative Control of Extraction: add 100 μl of C– (Negative Control) to labeled C neg**.**

3.4.3. Sample Collection and storage .

Note: Handle all specimens as if they are potentially infectious agents.

HBV Genotype A, B, C, D Real-TM PCR kit is intended for analysis of human plasma specimens*.

· *Blood plasma:* EDTA tubes may be used with the HBV Genotype A, B, C, D Real-TM. Follow sample tube manufacturer's instructions. Whole blood collected in EDTA should be separated into plasma by centrifugation at 800-1600 x g for 20 min within six hours of collection. The isolated plasma has to be transferred into a sterile polypropylene tube. Plasma may be stored at 2-8°C for an additional 3 days. Alternatively, plasma may be stored at -18°C for up to one month or 1 year when stored at -70°C. Thaw frozen specimens at room temperature before using.

***** Serum can be used also as starting material on some occasions. In this cases the analytical sensitivity of the kit HBV Genotype A, B, C, D Real-TM is the same, but the clinical sensitivity may be significantly decreased because of the precipitation of viral particles during the clot retraction phase of serum preparation

3.4.4. DNA Isolation (DNA Extraction)

The nucleic acid extracted earlier can be used in the HBV genotyping procedure as like as viral load .

3.4.4.1. Protocol (Total reaction volume :- 25 µl)

1. Before starting work, thaw, vortex and quick spin all reagents of the kit, making sure that there are no drops on the caps of the tubes.
2. Take the required number of PCR tubes for amplification of clinical and control samples (including one negative control of extraction and two controls of amplification).
3. To prepare the reaction mixture, mix in a new sterile tube the reagents per one reaction:

    - 10 µl of PCR-mix-1-FRT HBV-G,
    - 5 µl of RT-PCR-mix-2-TM,
    - 0,5 µl of Hot Start TaqF Polymerase

    Thoroughly vortex and quick spin the mixture, making sure that there are no drops on the caps of the tubes.

4. Add 15 µl of the prepared reaction mixture to each PCR tube.
5. Add 10 µl of DNA samples isolated from the clinical samples to each PCR tube.

6. Run the control reactions:

C – - Add 10 µl of the DNA sample extracted from the Negative Control to the tube labeled C– (Negative Control of Extraction)

C + - Add 10 µl of Positive Control B/A types (C+) to the tube labeled C + B/A (Positive Control of Amplification).

- Add 10 µl of Positive Control C/D types (C+) to the tube labeled C + C/D (Positive Control of Amplification).

NCA - Add 10 µl of DNA-buffer to the tube labeled NCA (Negative Control of Amplification).

Make sure that there are no drops on the tube walls, otherwise vortex the tubes briefly and give a quick spin.

3.4.4.2. Amplification Program :-

The temperature profile and fluorescence acquiring were done on the linear RT-PCR system and listed in the table (3 – 4) in the below .

Table (3 - 4) The Temperature Profile of HBV – genotyping Method

Plate-type instruments		
Temperature, °C	Time	Cycles
95	15 min	1
95	5 s	5
60	20 s	
72	15 s	
95	5 s	40
60	30 s Fluorescence acquiring*	
72	15 s	

* Fluorescent signal is detected in the channels for the FAM, JOE, ROX and Cy5 fluorophores on Linear PCR system .

3.4.5. Data Analysis

The *HBV* genotype A DNA is detected in the Cy5/Red channel

The *HBV* genotype B DNA is detected in the Rox/Texas Red/Orange channel.

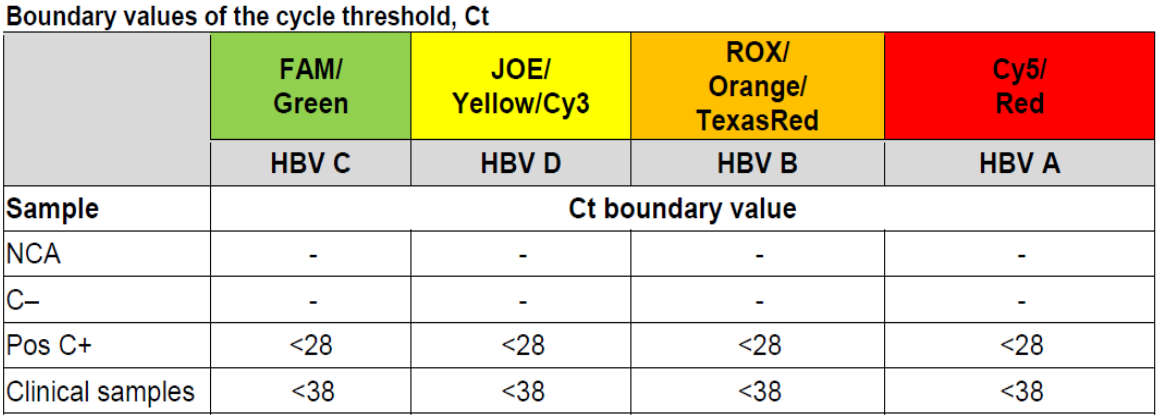
The *HBV* genotype C DNA is detected in the FAM/Green channel

The *HBV* genotype D DNA is detected in the JOE/HEX/Yellow channel .

The results are interpreted by the real-time PCR instrument software by the crossing or not crossing of the threshold line by the fluorescence curve (in the middle of the linear section of the fluorescence curve for the positive control (C+) in logarithmic coordinates).

The result of amplification is considered positive if the fluorescence curve is characteristic of real-time PCR (sigmoid-shaped) and crosses the threshold line once in the significant fluorescence increase section and if the Ct value detected in the channel is below the threshold value specified in the below table.

The result of amplification is considered negative if the fluorescence curve is not S-shaped and if it does not cross the threshold line (the Ct value is absent). As shown in Figure (3 - 3)



Boundary values of the cycle threshold, Ct

	FAM/ Green	JOE/ Yellow/Cy3	ROX/ Orange/ TexasRed	Cy5/ Red
	HBV C	HBV D	HBV B	HBV A
Sample	**Ct boundary value**			
NCA	-	-	-	-
C–	-	-	-	-
Pos C+	<28	<28	<28	<28
Clinical samples	<38	<38	<38	<38

Figure (3-3) Boundary value of the cycle threshold of HBV –genotyping

3.4.6. Result Interpretation :-

The results are interpreted by the real-time PCR instrument software by the crossing or not crossing of the threshold line by the fluorescence curve.

Sample contains ***HBV*** type A if the Ct value detected in the Cy5 channel is less than 38. As shown (figure 3 – 4)

Sample contains ***HBV*** type B if the Ct value detected in the ROX channel is less than 38. As shown (figure 3 – 5)

Sample contains ***HBV*** type **C** if the Ct value detected in the FAM channel is less than 38. As shown (figure 3 – 6)

Sample contains ***HBV*** type D if the Ct value detected in the JOE/HEX channel is less than 38. As shown (figure 3 – 7). (Sacace , 2014).

Rference (Sacace™ HBV Genotype A, B, C, D , ver. 05.11.2014)

Types of positive curve for HBV – genotypes.

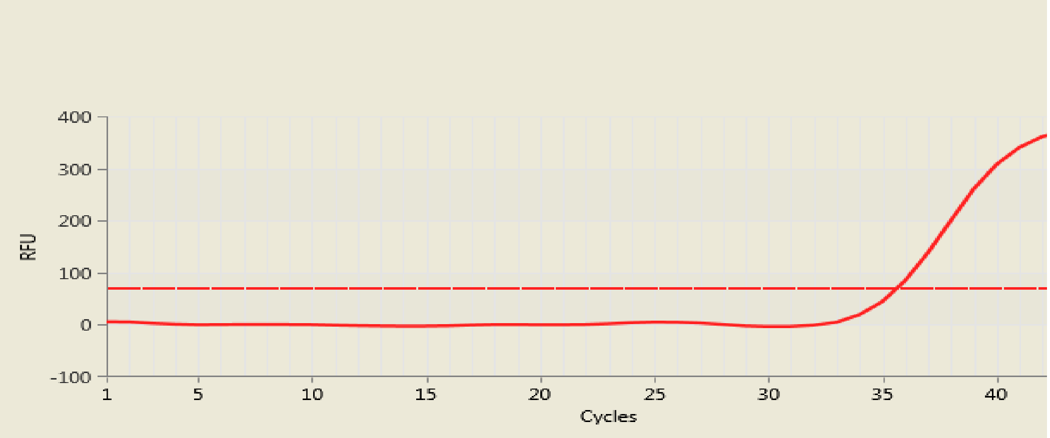
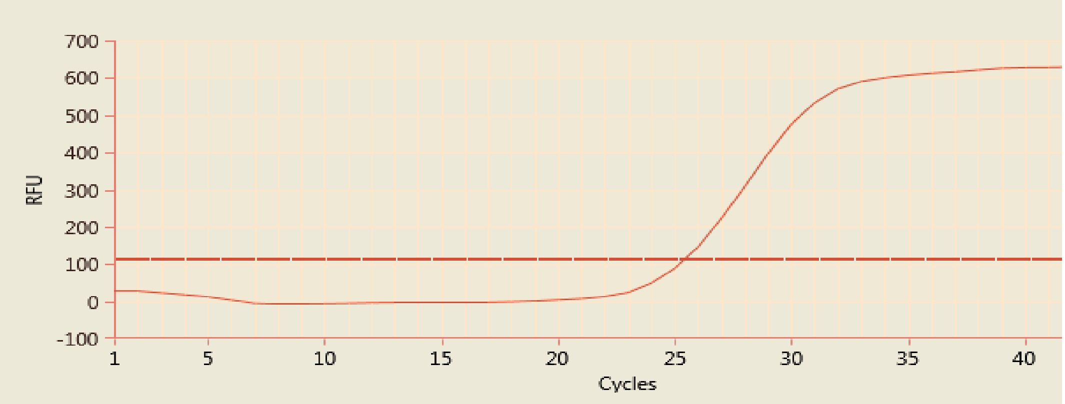


Figure (3 – 4) The Cy5 – Red channel for HBV- A genotype .



The Figure (3 -5) The Rox /Texas Red – Orange channel for HBV- B genotype .

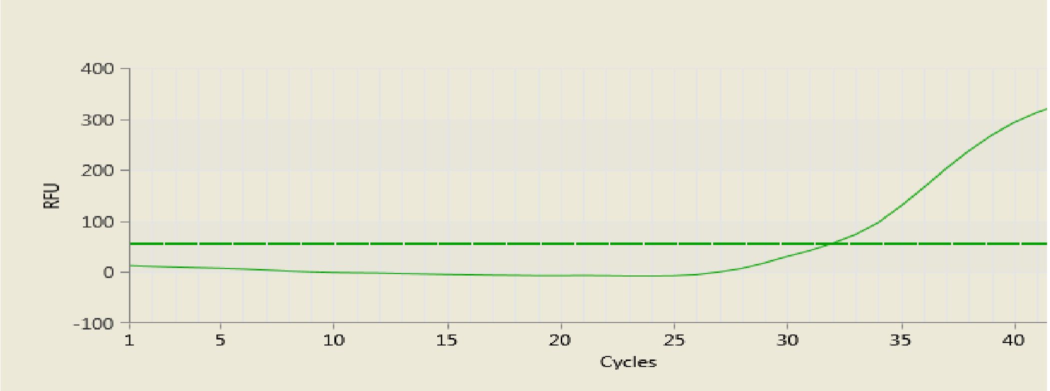
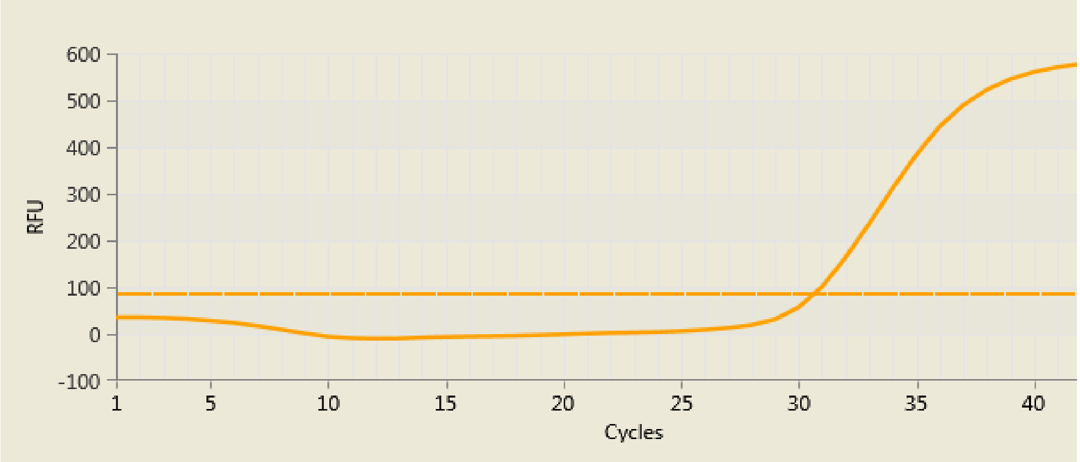


Figure (3 – 6) The FAM – Green Channal, or HBV- C genotype



The Figure (3 - 7) The Joe / Hex – Yellow channel for HBV –D genotype .

3.5 :- Statistical Analysis :-

According to study design , the statistical analysis were done by using SPSS program (Version 25) , as frequency of data , percentage , mean comparison , and assessment of P. Value of studied parameters (Armonk. 2017).

Chapter Four

Results

4. Results :-

4.I. Distribution and incidence rate of HBV infection among screened patients in Babylon province for past 5 years (June 2016 to June 2021).

Total of (877573) cases were investigated for IgG and IgM - HBV using rapid test and ELISA assays to confirm infections. The results revealed that , total positive cases were 5285 for HBV. The highest number. of cases for HBV recorded in 2020 year was 985 (0.676%).The incidence rate of HBV was varies worldwide. The results revealed that the incidence rate (Mean ± SD) of HBV was (0.627±0.134) asshown (Table 4.1)

Table 4-1: Distribution of incidence % of HBV infections in Babylon Province (2016-2021).

YEAR	TESTED	Positive	HBV %
2016	94970	782	0.823
2017	207654	893	0.430
2018	154321	971	0.629
2019	142532	979	0.686
2020	145675	985	0.676
2021	132421	675	0.677
TOTAL	877573	5285	0.627
		(Mean ± SD) 0.568 ± 0.152	

4.1.1 The Distribution of Patients that Infected with HBV in Regarding of Geographical Area of Babylon Province

The north area have higher number of patients 52.1% (2870 out of 5507 cases) rather than middle 21.8% (1200 out of 5285 cases) and south area 26.1% (1437 out of 5285 cases) as illustrated in table (4-2).

The statistical analysis showed significant difference ($P > 0.05$) among the geographical area of Babylon Province.

Table 4.2: Distribution of Patients that Infected with HBV in Regarding of Geographical Area of Babylon Province

District	HBV POSTIVE	%	P-Value
North Babylon Mahaweel , Musaib and Al – Eskanderia	2870	52.1	Significant P> 0.05
Middle area , the center area of province	1200	21.8	
South Babylon Al – Hashimia , Alkefel and Al –Shomaly	1437	26.1	
Total	5507	100	

4.1.2 Distribution of patients according Ruler and Urban area

The Results revealed area have higher number of patients rather than urban area rural in which have enrolled as shown in figure (4-1).

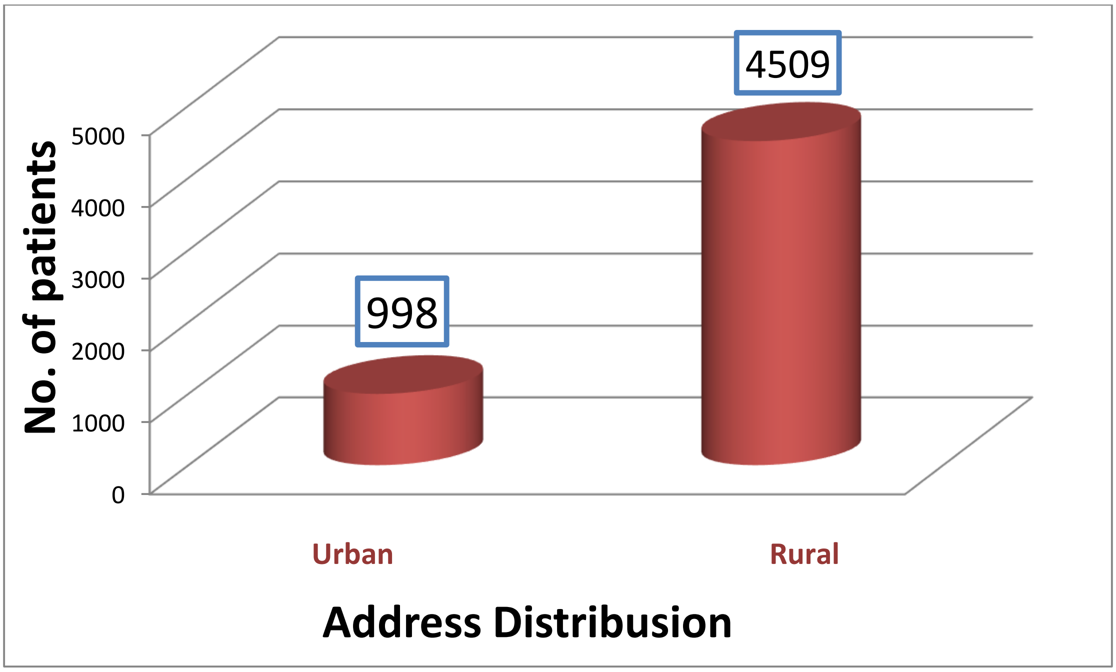


Figure 4.1: Address distribution of Hepatitis patients.

Gender distribution of HBV recorded patients

The figure (4.2) was showed that the gender distribution of HBV patients , that male patients have higher number than female.

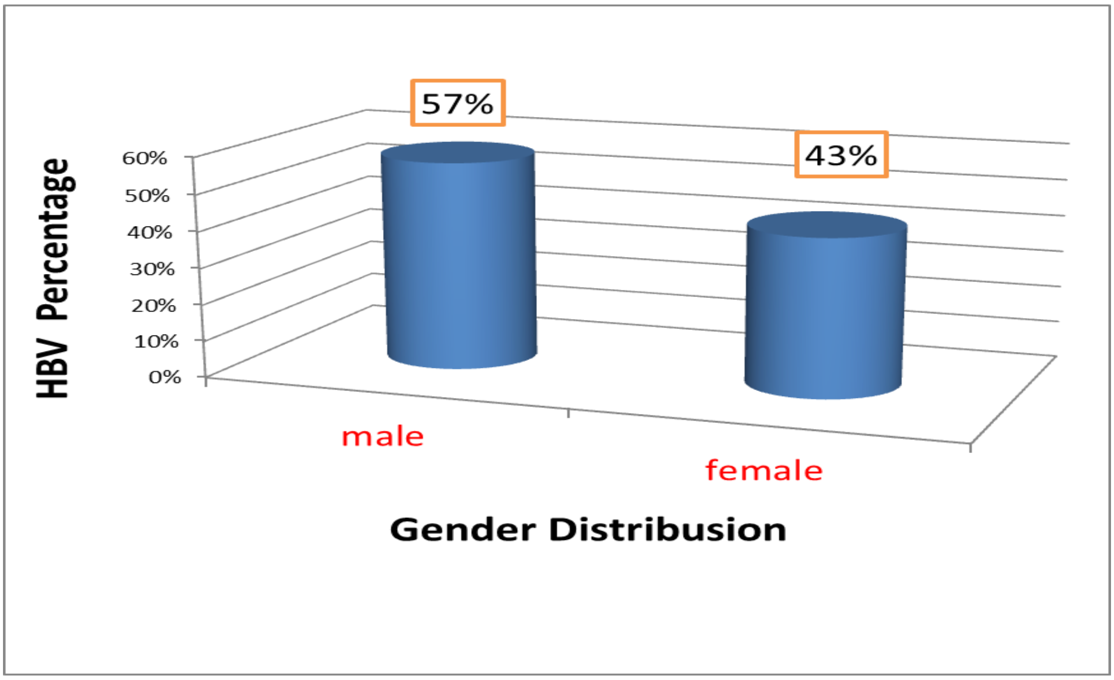


Figure 4.2: Gender distribution of HBV recorded patients.

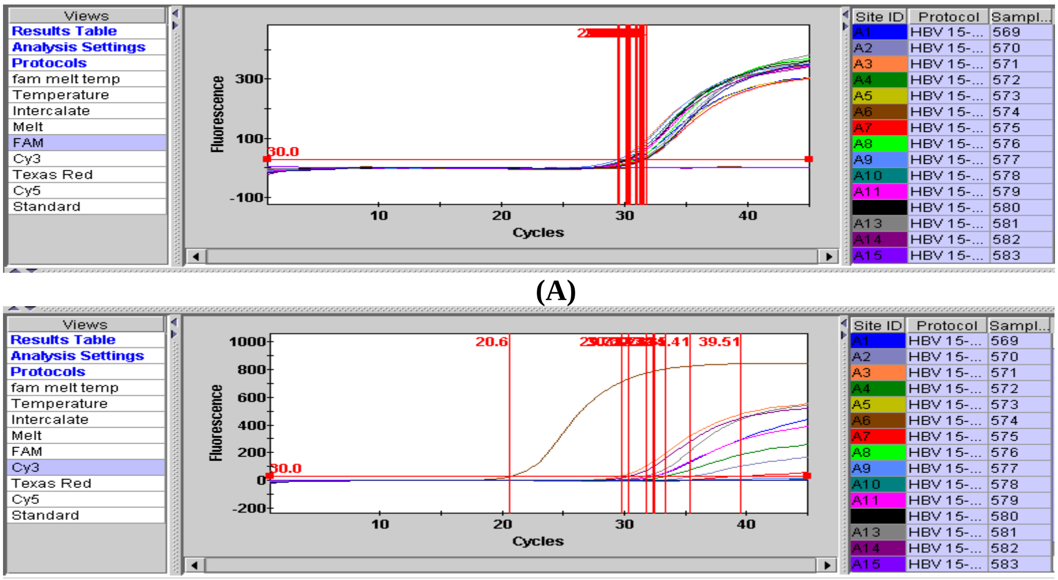
4.1.3 Detection of HBV Genome By RT.PCR:

The positive result according to RT-PCR shows 14.5% (98 out of 675 cases) as positive while 85.5% (577 out of 675 cases) as negative, as shown in table (4-3) as well as figures (4-3-A and B). Statistically significant differences (p = 0.04) among patients group.

Table 4.3: Percentage of HBV Positive Signals by Using qRT.PCR Technique.

Total Viral genome	No.	%	P value
Positive	**98**	14.5	**P=0.04** **S** **>0.05**
Negative	577	85.5	
Total	675	100	

Images (4.3) both of them shows line positive control and line negative control , also positive samples and negative samples, according to detection kit (RT-PCR) of HBV which used in this study the curves that represent positive samples appear between the line positive control and line negative control.



Site ID	Protocol	Sampl...
A1	HBV 15-...	569
A2	HBV 15-...	570
A3	HBV 15-...	571
A4	HBV 15-...	572
A5	HBV 15-...	573
A6	HBV 15-...	574
A7	HBV 15-...	575
A8	HBV 15-...	576
A9	HBV 15-...	577
A10	HBV 15-...	578
A11	HBV 15-...	579
	HBV 15-...	580
A13	HBV 15-...	581
A14	HBV 15-...	582
A15	HBV 15-...	583

(A)

Site ID	Protocol	Sampl...
A1	HBV 15-...	569
A2	HBV 15-...	570
A3	HBV 15-...	571
A4	HBV 15-...	572
A5	HBV 15-...	573
A6	HBV 15-...	574
A7	HBV 15-...	575
A8	HBV 15-...	576
A9	HBV 15-...	577
A10	HBV 15-...	578
A11	HBV 15-...	579
	HBV 15-...	580
A13	HBV 15-...	581
A14	HBV 15-...	582
A15	HBV 15-...	583

(B)

Figure (4-3 A and B): Detection of HBV by RT-PCR.

4.2. Genotype and Sociodemographic Data

Table (4-4) states the distribution of genotypes with respect to gender and age groups as well as viral load. Percentage of treated HBV patients high and low viral load shall be repeated treatment program, while stoping treatment for undetectable load , this figure revealed the percentage of patients in regarding to hepatitis viral load .

The prevalence of HBV infection for each genotype differs for gender, but there is no significance difference in terms of genotype prevalence and genders ($p = 0.4$; $p > 0.05$). Overall, the genotype A (62.5%), A/D (66.7%), B (66.7%), C (100%), and D/E (100%) are more related to males, whereas genotype E (100%) and Undetermined genotype (60%) are more related to females . Individuals of different age groups were enrolled in the study, there was no significance differences in terms f genotype prevalence and age ($p = 0.4$; $p > 0.05$).

Table (4-4) : the distribution of genotypes among patient with HBV.

Genotype HBV	**Viral load (IU/ml)**	**Percent**	**Gender**		**Age Groups**			
			Male	**Female**	**<20**	**21–40**	**41–60**	**>61**
A	>100 million	4.3	60%	40%	0.0%	23.0%	24.0%	11.5%
A\D	50 -100 million	6.8	65%	35%	0.0%	75.6%	18.4%	0.0%
B	1 – 50 million	10.4	66%	34%	0.0%	0.0%	37.0%	36.3%
B\D	< 1 million	23.7	45%	55%	0.0%	0.0%	90.0%	0.0%
C	Undetectable	54.8	90.0%	10.0%	6.0%	23.0%	40.0%	0.0%
E			57.0%	43.0%	0.0%	21.0%	24.0%	13.0%

The results of this study as shown in Table (4-5) , The presence of IgG antibody against surface marker of HB virus , revealed that (256 : 38%) have past infection antibody , while there are (133: 19.8 %) have Total Anti –HBc antibody (IgM and IgG) as well as (5 : 2%) listed as Anti –IgM anti core antibody .The other serological marker is (141 : 21%) as anti-envelope antibody were detectable.

Table (4-5) Serological differentiation of hepatitis B-virus antigens

HBs (Surface)		**Anti-HBc (Core)**		**HBe (envelope)**
HBs Ag	Anti –HBs- IgG Ab	Total IgG and IgM)	Anti –HBc-IgM	Anti HBe Ab.
675	256 (38%)	133 (19.8%)	5 (2%)	141 (21%)

The overall detection of hepatitis patients (675) , were classified as serological finding by using ELISA manual and principle of (Foresight company)

Chapter Five

Discussion

5.1. Discussion

Almost 90% of HBV infections occur during the perinatal period and within 6 months after birth. The HBV vaccine first went on sale in 1982. In many countries, HBV vaccination for newborn babies and medical workers started in the 1980s (Nannini and Sokal 2016)Therefore, the HBV-positive rate of blood donors is low, at approximately 0.1% (Thomas et al., 1993). Nowadays, the infection rate of HBV is lower than that of HCV (Murokawa *et al*., 2005). However, despite vaccination, there are some people whose anti-HBs titer are negative or less than 10 IU/mL. Although it is said that immunological memory persists in such cases after vaccination, it is an issue that merits consideration (Zanetti *et al* , 2005).

In the United States, 6.2% of medical workers are positive for Anti-HBc, which is higher than the rate in blood donors, which is 1.8% (Thomas *et al*., 1993) , indicating that medical workers are at a high risk of infection. Approximately 75% of HBV-related transmissions in healthcare workers are via percutaneous injury with a scalpel or needle; the remaining mode of transmission in these workers is via mucosal-cutaneous exposure. When an individual is positive for both HBsAg and HBeAg, there is a 22–31% risk of hepatitis (Public Health Service , 2001). Even in a high-risk working environment, medical workers have existing knowledge about infectious diseases and the appropriate use of guards such as gloves and masks. However, there is a higher risk of infection (and becoming Anti-HBc-positive) when non-medical workers, who lack this medical expertise, attend a disaster. The risk of HBV infection has been reduced by universal vaccination in several countries (Zanetti , *et al*., 2008 , Mele et al., 2008) , however the infection risk, not only in the medical field but also in the general population remains high, therefore it is advisable to extend universal vaccination to the rest of the world.

In this study, although it was only with a single case, HBV in postmortem blood successfully infected the HepG2 cell line (Table 3). HepG2 cells are a human hepatoblastoma cell line derived from a 15-year-old male with a well-differentiated carcinoma. HepG2 cells differ morphologically from primary hepatocytes. Recently, the sodium taurocholate cotransporting polypeptide (NTCP) was identified as a receptor for HBV (Mele et al., 2008) ; however, it is not expressed in HepG2 cells (Kullak-Ublick et al., 2009).

HBV infection is an important global problem that places a continuously increasing burden on developing countries. As the HBV genotype can be classified into different genotypes, the classification has to be cost-effective and clinically relevant (Ministry of Cabinet, 2010). Research on the relationship between HBV genotypes, their pathogenicity in chronic liver disease, including hepatocellular carcinoma, and their therapy are of great interest, as this allows for understanding the spread and risk of HBV infection around the world (Miyakawa , and Misokami 2003). On the other hand, HBV infection is a major health problem in the Middle East. The majority of the countries in the region have an intermediate or high endemicity of HBV infection (Qirbi and Hall 2001]. Despite the low prevalence of HBV in Bahrain, it is important to investigate the frequency of HBV genotypes and its association with various sociodemographic factors, hepatic biomarkers, and mode of transmission, which is essential for fine tuning the control of the disease.

According to a study by Janahi (2014), completed on 877,892 individuals, Bahrain has low HBV endemicity for the period (2000–2010). The prevalence of hepatitis B virus infection in Bahrain was found to be 0.58% (Janahi , *et al.*, 2010). This study reports for the first time in

Bahrain, the correlation of HBV genotypes frequency with the demographic characteristics and hepatic biomarker. The results showed that there were no significant differences of genotype frequency in relation to the demographic characteristics as well as hepatic biomarkers. Out of the 82 screened patients in this study, 58.5% were male, while the remaining 41.5% were females. There was a significant increased risk of HBV infection in male as compared to females (**Table 1**). 53.7% of HBV positive patients had Bahraini nationality, while the remaining 46.3% belonged to other eleven nationalities, such as Pakistan, Sudan, Egypt, Yamen, Syria, Kuwait, Bangladesh, India, Ethiopia, Indonesia, and Philippines, which are known to be highly endemic for HBV. Relationship between genotype and age-group indicates that HBV prevails 4.9% in < 21 years, 28% in 21–30 years, 25.6% in 31–40 years, 13.4% in 41–50 years, 15.9% in 51–60 years, and 12.2% in >61 years. age groups.

The frequency of mode of transmission was highly unknown (59.8%), followed by blood/blood products (19.5%), sexual contact (7.3%), vertical transmission (7.3%), and finally organ transplant (3.7%). HBV and HCV have common modes of transmission; therefore, their coinfection is quite frequent. This particularly occurs in areas where the two viruses are endemic and among subjects with high risk of parental infection (Hui *et al*., 2006). According to a study that was conducted in Bahrain, dental procedures and surgical operations account for 37.2% and 35.6%, respectively, of the HBV transmission routes. Followed by the blood transfusion (24.6%), the sexual contact and intervenors drug abuse were the least possible routes of transmission (Janahi , *et al*., 2010). There was some significant difference in the HBV genotype prevalence with respect to some investigated variables. For example, the frequency of HBV genotype is more related to males and the risk of HBV infection increased with older age.

The dominant genotype in our study was genotype D with 61% frequency, which is similar to some countries in the Middle East, like Saudi Arabia (81%) (Al Ashgar *et al*., 2008). UAE (79.5%) (Alfaresi *et al*., 2010), Iran, and Jordan (≈100%) (Alavian *et al*., 2007 , Ghazzawi *et al*., 2016) The dominance of this genotype might be attributed to different factors, such as the presence of high number of workers from countries that are known to have dominant D genotype, such as India, Pakistan, Yemen, Syria, and Bangladesh. These infected workers are a principal source for the transmission of hepatitis B. As most of them belong to highly endemic countries with low educational and socio-economical backgrounds, they positively contribute to the transmission of the disease. Living in small houses and having unhygienic behaviors (such as sharing same razors and toothbrushes) put such workers at high risk of contracting HBV. A poor hygiene system in hospitals of such countries is known as a high-risk factor for HBV transmission, as the same syringe is used for vaccination of different people (Fung, *et al*., 2006).

The quasi-species nature of HBV infection indicates that the variation and evolution of Hepatitis B virus has been influenced by the recombination between genotypes. Hence, a high prevalence of more than one dominant genotype in a certain region is common (Zanetti , *et al*., 2008, McMahon *et al*., 2008). It is documented that mixed infection with different HBV genotypes is not uncommon and it is of great virological and clinical interest. For example, a study done by Chen *et al*. (2007) showed that the prevalence of mixed HBV genotype infection was 16.3% for HBsAg positive and 34.4% in occult HBV-infected intravenous drug users .

HBV is non-cytopathic virus, which highlights the complex and important interaction between the virus and host in causing HBV-related liver disease. Bilirubin, Direct Bilirubin, ALT, and AST are the most common liver enzymes that are measured to investigate the condition of liver due to HBV or HCV infection (Hadziyannis *et al*., 2006). Elevated ALT levels, elevated AST level, elevated serum bilirubin, and decreased serum albumin might be indicative of advanced liver disease and even cirrhosis (Limdi *and* Hyde. 2003) in current study,

Finally, this study showed that the overall HBV prevalence among males' patients to be 58.5%, while it was 41.5% among females..

Conclusions and Recommendations

Conclusions:-

The following conclusions are obtained from the present study:-

1. High incidence rate were investigated among prisoners and foreigners so must focusing on those sectors and place regulation and strategies for mitigation.
2. The most common HBV genotype in Iraq was genotype D, followed by genotype A.
3. There were increased disease prevalence with time , and the males were more significant than females in hepatitis infections.
4. The north area of Babylon province had higher percentage than south and middle areas , especially in rular area than urban.

Recommendations

1.Studying the genotypes of HBV in middle Euphrates-Iraq
2. Studying the mortality rate of HBV .

References

References

Al Ashgar, H.; Peedikayil, M.; Imambaccus, H.; Althawadi, S. Prevalence of hepatitis B virus genotype in Saudi Arabia: A preliminary report. *Indian J. Gastroenterol.* **2008**, *2008*. [Google Scholar]

Alavian, S.M.; Fallahian, F.; Lankarani, K.B. The changing epidemiology of viral hepatitis B in Iran. *J. Gastrointest. Liver Dis.* **2007**, *16*, 403–406. [Google Scholar]

Alfaresi, M.; Elkoush, A.; Alshehhi, H.; Alzaabi, A.; Islam, A. Hepatitis B virus genotypes and precore and core mutants in UAE patients. *Virol. J.* **2010**, *7*. [Google Scholar] [CrossRef] [PubMed]

Ali, M.M., et al., Comparative evaluation of INNO-LiPA HBV assay, direct DNA sequencing and subtractive PCR-RFLP for genotyping of clinical HBV isolates. Virol J, 2010. 7: p. 111.

André, F. Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine* **2000**, *18*, S20–S22. [Google Scholar] [CrossRef]

Arauz-Ruiz, P.; Norder, H.; Robertson, B.H.; Magnius, L.O. Genotype H: A new Amerindian genotype of hepatitis B virus revealed in Central America. *J. Gen. Virol.* **2002**, *83*, 2059–2073.

Armonk, NY (2017) IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0.

Barker, L.F., et al., Hepatitis B virus infection in chimpanzees: titration of subtypes. J Infect Dis, 1975. 132(4): p. 451-8.

Barrera, A., et al., Mapping of the hepatitis B virus pre-S1 domain involved in receptor recognition. J Virol, 2005. 79(15): p. 9786-98.

Bayliss, Julianne, et al. "Hepatitis B virus splicing is enhanced prior to development of hepatocellular carcinoma." *Journal of hepatology* 59.5 (2013): 1022-1028. *Beck, J. and M. Nassal, Hepatitis B virus replication. World J Gastroenterol, 2007. 13(1): p. 48-64.*

Belloni, L., et al., Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. Proc Natl Acad Sci U S A, 2009. 106(47): p. 19975-9.

Benn, J. and R.J. Schneider, Hepatitis B virus HBx protein activates Ras-GTP complex formation and establishes a Ras, Raf, MAP kinase signaling cascade. Proc Natl Acad Sci U S A, 1994. 91(22): p. 10350-4.

Block, T.M., Guo, H. and Guo, J.T. Molecular virology of hepatitis B virus for clinicians. Clin Liver Dis, 2007. 11(4): p. 685-706, vii.

Bock, C.T., et al., Structural organization of the hepatitis B virus minichromosome. J Mol Biol, 2001. 307(1): p. 183-96.

Bowden, Scott. "Serological and molecular diagnosis." *Seminars in liver disease*. Vol. 26. No. 02. Copyright© 2006 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA., 2006.

Bruss, V., et al., Myristylation of the large surface protein is required for hepatitis B virus in vitro infectivity. Virology, 1996. 218(2): p. 396-9.

Carman, W.F., et al., Association of a precore genomic variant of hepatitis B virus with fulminant hepatitis. Hepatology, 1991. 14(2): p. 219-22.

Chan, H.; Wong, M.; Hui, A.; Hung, L.; Chan, F.; Sung, J.2003. Hepatitis B Virus Genotype C Takes a More Aggressive Disease Course Than Hepatitis B Virus Genotype B in Hepatitis B e Antigen-Positive Patients. *J. Clin. Microbiol.* 2003, *41*, 1277–1279. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Chan, H.L., et al., Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol, 2007. 5(12): p. 1462-8.

Chang, M.H., et al., Fulminant hepatitis in children in Taiwan: the important role of hepatitis B virus. J Pediatr, 1987. 111(1): p. 34-9.

Chen, C.; Hung, C.; Lee, C.; Hu, T.; Wang, J.H.; Wang, J.C.; Changchien, C.S. Pre-S Deletion and Complex Mutations of Hepatitis B Virus Related to Advanced Liver Disease in HBeAg-Negative Patients. *Gastroenterology* 2007, *133*, 1466–1474. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Chen, C.J., et al., Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA, 2006. 295(1): p. 65-73.

Croagh, C.M., P.V. Desmond, and S.J. Bell, Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. World J Hepatol, 2015. 7(3): p. 289-303.

Dane, D.S., C.H. Cameron, and M. Briggs, Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet, 1970. 1(7649): p. 695-8.

Ding, X.; Mizokami, M.; Yao, G.; Xu, B.; Orito, E.; Ueda, R.; Nakanishi, M. Hepatitis B Virus Genotype Distribution among Chronic Hepatitis B Virus Carriers in Shanghai, China. *Intervirology* 2001, *44*, 43–47. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Drexler, J.F., et al., Bats carry pathogenic hepadnaviruses antigenically related to hepatitis B virus and capable of infecting human hepatocytes. Proc Natl Acad Sci U S A, 2013. 110(40): p. 16151 6.

Dupinay, T., et al., Discovery of naturally occurring transmissible chronic hepatitis B virus infection among Macaca fascicularis from Mauritius Island. Hepatology, 2013. 58(5): p. 1610-20.

Fernholz, D., et al., Infectious hepatitis B virus variant defective in pre-S2 protein expression in a chronic carrier. Virology, 1993. 194(1): p. 137-48.

Fung, S.K.; Wong, F.S.; Wong, D.K.; Hussain, M.T.; Lok, A.S. Hepatitis B virus genotypes, precore and core promoter variants among predominantly Asian patients with chronic HBV infection in a

Canadian center. *Liver Int.* 2006, *26*, 796–804. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Garcia, P.D., et al., Targeting of the hepatitis B virus precore protein to the endoplasmic reticulum membrane: after signal peptide cleavage translocation can be aborted and the product released into the cytoplasm. J Cell Biol, 1988. 106(4): p. 1093-104.

Ghazzawi, I.; Hamoudi, M.; Hamoudi, W. Hepatitis B Genotypic and Serologic Characteristics in Jordan. *J. R. Med. Serv.* **2016**, *23*, 17–22. [**Google Scholar**] [**CrossRef**]

Giboney, P.T. Mildly elevated liver transaminase levels in the asymptomatic patient. *Am. Fam. Phys.* **2005**, *71*, 1105–1110. [**Google Scholar**]

Gilbert, C. and Feschotte, C(2010).Genomic fossils calibrate the long-term evolution of hepadnaviruses. PLoS Biol, 2010. 8(9).

Global Hepatitis Report 2017, Geneva: World Health Organization; . 2017.

Gripon, P., I. Cannie, and S. Urban, Efficient inhibition of hepatitis B virus infection by acylated peptides derived from the large viral surface protein. J Virol, 2005. 79(3): p. 1613-22.

Hadziyannis, S.J.; Papatheodoridis, G.V. Hepatitis B e Antigen-Negative Chronic Hepatitis B: Natural History and Treatment. *Semin. Liver Dis.* 2006, *26*, 130–141. [**Google Scholar**] [**CrossRef**]

References

Honer Zu Siederdissen, C. and M. Cornberg, The role of HBsAg levels in the current management of chronic HBV infection. Ann Gastroenterol, 2014. 27(2): p. 105-112.

Hui, C.; Lau, E.; Monto, A.; Kim, M.; Luk, J.; Poon, R.; Wright, T.L. Natural History of Patients with Recurrent Chronic Hepatitis C Virus and Occult Hepatitis B Co-Infection after Liver Transplantation. *Am. J. Transplant.* 2006, *6*, 1600–1608. [**Google Scholar**] [**CrossRef**]

Huovila, A.P., A.M. Eder, and S.D. Fuller, Hepatitis B surface antigen assembles in a post-ER, pre-Golgi compartment. J Cell Biol, 1992. 118(6): p. 1305-20.

Huy, T.; Abe, K. Molecular epidemiology of hepatitis B and C virus infections in Asia. *Pediatr. Int.* 2004, *46*, 223–230. [**Google Scholar**] [**CrossRef**]

Iloeje, U. H., H. I. Yang, and J. Su. "Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-In HBV (the REVEAL-HBV) Study Group. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load." (2006): 678-686.

Janahi, E. Prevalence and Risk Factors of Hepatitis B Virus Infection in Bahrain, 2000 through 2010. *PLoS ONE* 2014, *9*, e87599. [**Google Scholar**] [**CrossRef**]

Jazayeri, M.; Basuni, A.; Sran, N.; Gish, R.; Cooksley, G.; Locarnini, S.; Carman, W. HBV core sequence: Definition of genotype-specific variability and correlation with geographical origin. *J. Viral Hepat.* 2004, *11*, 488–501. [**Google Scholar**] [**CrossRef**]

Jones, S.A. and J. Hu, Hepatitis B virus reverse transcriptase: diverse functions as classical and emerging targets for antiviral intervention. Emerg Microbes Infect, 2013. 2(9): p. e56.

Jung, M.C. and G.R. Pape, Immunology of hepatitis B infection. Lancet Infect Dis, 2002. 2(1): p. 43-50.

Karra, Vijay K., et al. "Clinical significance of quantitative HBsAg titres and its correlation with HBV DNA levels in the natural history of hepatitis B virus infection." *Journal of clinical and experimental hepatology* 6.3 (2016): 209-215.

Kann, M., A. Bischof, and W.H. Gerlich, In vitro model for the nuclear transport of the hepadnavirus genome. J Virol, 1997. 71(2): p. 1310-6. 60. Will, H., et al., Replication strategy of human hepatitis B virus. J Virol, 1987. 61(3): p. 904-11.

Kao, J.; Chen, P.; Lai, M.; Chen, D. (**2002**) Genotypes and Clinical Phenotypes of Hepatitis B Virus in Patients with Chronic Hepatitis B Virus Infection. *J. Clin. Microbiol.* *40*, 1207–1209. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Keasler, V.V., et al., Enhancement of hepatitis B virus replication by the regulatory X protein in vitro and in vivo. J Virol, 2007. 81(6): p. 2656-62.

Kedda, M.A., et al., Susceptibility of chacma baboons (Papio ursinus orientalis) to infection by hepatitis B virus. Transplantation, 2000. 69(7): p. 1429-34.

Kim, B. K., Revill, P. A., & Ahn, S. H. (2011). HBV genotypes: relevance to natural history, pathogenesis and treatment of chronic hepatitis B. *Antiviral therapy*, *16*(8), 1169.

Kramvis, A. and M.C. Kew, Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. Hepatol Res, 2007. 37(s1): p. S9-S19.

Kramvis, A. Genotypes and Genetic Variability of Hepatitis B Virus. *Intervirology* **2014**, *57*, 141–150. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Kramvis, A., The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. Rev Med Virol, 2016. 26(4): p. 285-303.

Kullak-Ublick GA, Ismair MG, Kubitz R, Schmitt M, Häussinger D, Stieger B, Hagenbuch B, Meier PJ, Beuers U, Paumgartner G. Stable expression and functional characterization of a Na^+-taurocholate cotransporting green fluorescent protein in human hepatoblastoma HepG2 cells. *Cytotechnology*. 2009;34:1–9. doi: 10.1023/A:1008152729133. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Kuroki, K., et al., Epitope mapping of the PreS1 domain of the hepatitis B virus large surface protein. Virology, 1990. 176(2): p. 620-4.

Lada, O., et al., Coexistence of hepatitis B surface antigen (HBs Ag) and anti-HBs antibodies in chronic hepatitis B virus carriers: influence of "a" determinant variants. J Virol, 2006. 80(6): p. 2968-75.

Lamontagne, R.J., S. Bagga, and M.J. Bouchard, Hepatitis B virus molecular biology and pathogenesis. Hepatoma Res, 2016. 2: p. 163-186.

Lamontagne, Jason, et al. "Screening and identification of compounds with antiviral activity against hepatitis B virus using a safe compound library and novel real-time immune-absorbance PCR-

based high throughput system." Antiviral research 98.1 (2013): 19-26.

Lanford, R.E., et al., Isolation of a hepadnavirus from the woolly monkey, a New World primate. Proc Natl Acad Sci U S A, 1998. 95(10): p. 5757-61.

Lavanchy, D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J. Viral Hepat.* 2004, *11*, 97–107. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Li, M.R., et al., Kinetics and prediction of HBsAg loss during long-term therapy with nucleos(t)ide analogues of different potency in patients with chronic hepatitis B. PLoS One, 2014. 9(6): p. e98476.

Liaw, Y.; Brunetto, M.; Hadziyannis, S. The natural history of chronic HBV infection and geographical differences. *Antivir. Ther.* 2010, *15*, 25–33. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Limdi, J.K.; Hyde, G.M. Evaluation of abnormal liver function tests. *Postgrad. Med. J.* **2003**, *79*, 307–312. [**Google Scholar**] [**CrossRef**]

Littlejohn, M., S. Locarnini, and L. Yuen, Origins and Evolution of Hepatitis B Virus and Hepatitis D Virus. Cold Spring Harb Perspect Med, 2016. 6(1): p. a021360.

Liu, C. J., & Kao, J. H. (2013, May). Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J. In *Seminars in liver disease* (Vol. 33, No. 02, pp. 097-102). Thieme Medical Publishers.

Locarnini, S. and F. Zoulim, Molecular genetics of HBV infection. Antivir Ther, 2010. 15 Suppl 3: p. 3-14.

Locarnini, S., Molecular virology of hepatitis B virus. Semin Liver Dis, 2004. 24 Suppl 1: p. 3-10.

Lupberger, J. and E. Hildt, Hepatitis B virus-induced oncogenesis. World J Gastroenterol, 2007. 13(1): p. 74-81. 155

MacLachlan, J.H., et al., The burden of chronic hepatitis B virus infection in Australia, 2011. Australian and New Zealand journal of public health, 2013. 37(5): p. 416-22.

Magnius, L.; Norder, H. Subtypes, Genotypes and Molecular Epidemiology of the Hepatitis B Virus as Reflected by Sequence Variability of the S-Gene. *Intervirology* 1995, *38*, 24–34. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Manesis, E.K., et al., Prediction of treatment-related HBsAg loss in HBeAGnegative chronic hepatitis B: a clue from serum HBsAg levels. Antivir Ther, 2007. 12(1): p. 73-82.

McMahon, B. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. *Hepatol. Int.* 2008, *3*, 334–342. [**Google Scholar**] [**CrossRef**]

Mele A, Tosti ME, Mariano A, Pizzuti R, Ferro A, Borrini B, Zotti C, Lopalco P, Curtale F, Balocchini E, Spada E, National Surveillance System for Acute Viral Hepatitis (SEIEVA)

Collaborating Group Acute hepatitis B 14 years after the implementation of universal vaccination in Italy: areas of improvement and emerging challenges. *Clin Infect Dis*. 2008;46:868–875. doi: 10.1086/528687. [PubMed] [CrossRef] [Google Scholar]

Milich, D.R., et al., Enhanced immunogenicity of the pre-S region of hepatitis B surface antigen. Science, 1985. 228(4704): p. 1195-9.

Milich, D.R., et al., Immune response to the pre-S(1) region of the hepatitis B surface antigen (HBsAg): a pre-S(1)-specific T cell response can bypass nonresponsiveness to the pre-S(2) and S regions of HBsAg. J Immunol, 1986. 137(1): p. 315-22. 154

Ministry of Cabinet Affairs, Kingdom of Bahrain. *Basic Results Population, Housing, Buildings and Establishment Census*; Ministry of Cabinet Affairs, Kingdom of Bahrain: Manama, Bahrain, 2010. [**Google Scholar**]

Manesis, Emanuel K., et al. "Quantitative analysis of hepatitis D virus RNA and hepatitis B surface antigen serum levels in chronic delta hepatitis improves treatment monitoring." *Antiviral therapy* 12.3 (2007): 381.

Miyakawa, Y.; Mizokami, M. Classifying Hepatitis B Virus Genotypes. *Intervirology* 2003, *46*, 329–338. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Moolla, N., M. Kew, and P. Arbuthnot, Regulatory elements of hepatitis B virus transcription. J Viral Hepat, 2002. 9(5): p. 323-31.

Mphahlele, M.J., et al., Transmission of a homogenous hepatitis B virus population of A1896-containing strains leading to mild resolving *acute hepatitis and seroconversion to hepatitis B e antigen antibodies in an adult. Hepatology, 1997. 26(3): p. 743-6.*

Murakami, S., Hepatitis B virus X protein: a multifunctional viral regulator. J Gastroenterol, 2001. 36(10): p. 651-60.

Murokawa H, Yoshikawa A, Ohnuma H, Iwata A, Katoh N, Miyamoto M, Mine H, Emura H, Tadokoro K, Japanese Red Cross NAT Screening Research Group Epidemiology of blood donors in Japan, positive for hepatitis B virus and hepatitis C virus by nucleic acid amplification testing. *Vox Sang.* 2005;88:10–16. doi: 10.1111/j.1423-0410.2005.00581.x. [PubMed] [CrossRef] [Google Scholar]

Nannini P, and Sokal EM. Hepatitis B: changing epidemiology and interventions. *Arch Dis Child.* 2016 [PubMed] [Google Scholar]

Newbold, J.E., et al., The covalently closed duplex form of the hepadnavirus genome exists in situ as a heterogeneous population of viral minichromosomes. J Virol, 1995. 69(6): p. 3350-7.

Ni, Y.H., Natural history of hepatitis B virus infection: pediatric perspective. J Gastroenterol, 2011. 46(1): p. 1-8.

Nicoletta, P.; Daniel, L. *Hepatitis B.*; World Health Organization: Geneva, Switzerland, 2002. [**Google Scholar**]

Ogawa, M.(2002) Clinical features and viral sequences of various genotypes of hepatitis B virus compared among patients with acute hepatitis B. *Hepatol. Res.* 2002, *23*, 167–177. [**Google Scholar**] [**CrossRef**]

Persing, D.H.,Varmus H.E., and Ganem, D. The preS1 protein of hepatitis B virus is acylated at its amino terminus with myristic acid. J Virol, 1987. 61(5): p. 1672- 7.

Public Health Service (2001) Updated US public health service guidelines for the management of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis. MMWR Recomm 50 (RR-11), pp 1–52 [PubMed]

Qirbi, N.and Hall, A.J. Epidemiology of hepatitis B virus infection in the Middle East: A Review. *La Revue de Sante de la Mediterranee Orientale* 2001, *7*, 1034–1045. [**Google Scholar**]

Qin, Yan, et al. "The newsvendor problem: Review and directions for future research." *European Journal of Operational Research* 213.2 (2011): 361-374.

Sarin, S.K., et al., Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int, 2016. 10(1): p. 1-98.

Schadler, S. and E. Hildt, HBV life cycle: entry and morphogenesis. Viruses, 2009. 1(2): p. 185-209.

Schaefer, S., Hepatitis B virus taxonomy and hepatitis B virus genotypes. World J Gastroenterol, 2007. 13(1): p. 14-21.

Seeger, C. and MasonW.S.(2000) Mason, Hepatitis B virus biology. Microbiol Mol Biol Rev, 2000. 64(1): p. 51-68. 153

Seeger, C. and Mason, W.S. Molecular biology of hepatitis B virus infection. Virology, 2015. 479-480: p. 672-86.

References

Song, J.E. and D.Y. Kim, Diagnosis of hepatitis B. Ann Transl Med, 2016. 4(18): p. 338.

Sozzi, Vitina, et al. "In vitro *studies show that sequence variability contributes to marked variation in hepatitis B virus replication, protein expression, and function observed across genotypes." Journal of virology 90.22 (2016): 10054-10064.*

Stuyver, L.; Rossau, R.; Zoulim, F.; Fried, M.; De Gendt, S.; Schinazi, R.; Van Geyt, C. A new genotype of hepatitis B virus: Complete genome and phylogenetic relatedness. *J. Gen. Virol.* 2000, *81*, 67–74. [**Google Scholar**] [**CrossRef**]

Sugauchi, F.; Chutaputti, A.; Orito, E.; Kato, H.; Suzuki, S.; Ueda, R.; Mizokami, M. Hepatitis B virus genotypes and clinical manifestation among hepatitis B carriers in Thailand. *J. Gastroenterol. Hepatol.* 2002, *17*, 671–676. [**Google Scholar**] [**CrossRef**] [**PubMed**]

Tatematsu, Kanako, et al. "A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J." *Journal of virology* 83.20 (2009): 10538-10547.

Terre, S., M.A. Petit, and C. Brechot, Defective hepatitis B virus particles are generated by packaging and reverse transcription of spliced viral RNAs in vivo. J Virol, 1991. 65(10): p. 5539-43.

Thomas DL, Factor SH, Kelen GD, Washington AS, Taylor E, Jr, Quinn TC. Viral hepatitis in health care personnel at The Johns Hopkins Hospital. The seroprevalence of and risk factors for hepatitis B virus and hepatitis C virus infection. *Arch Intern Med.* 1993;153:1705–1712. doi: 10.1001/archinte.1993.00410140095011. [PubMed] [CrossRef] [Google Scholar]

Med. 1993;153:1705–1712. doi: 10.1001/archinte.1993.00410140095011. [Google Scholar]

Tong, S. and Revill, P.(2016). Overview of hepatitis B viral replication and genetic variability. J Hepatol, 2016. 64(1 Suppl): p. S4-S16.

Van Geyt, C.; De Gendt, S.; Rombout, A.; Wyseur, A.; Maertens, G.; Bartholomeusz, A.; Schinazi, R.F.; Locarnini, S.A. Significance of mutations in the hepatitis B virus polymerase selected by nucleoside analogues and implicationfor controlling chronic disease. *Viral Hepat. Rev.* 1998, *4*, 167–187. [**Google Scholar**]

Visvanathan, K., et al., Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. Hepatology, 2007. 45(1): p. 102-10.

Wilson, R., et al., The hepatitis B e antigen suppresses IL-1beta-mediated NFkappaB activation in hepatocytes. J Viral Hepat, 2011. 18(10): p. e499-507.

Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J, Li W. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife*. 2012;1:e00049. doi: 10.7554/eLife.00049. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Yan, H., et al., Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife, 2012. 1: p. e00049.

Yang, C.Y., T.H. Kuo, and L.P. Ting, Human hepatitis B viral e antigen interacts with cellular interleukin-1 receptor accessory protein and triggers interleukin-1 response. J Biol Chem, 2006. 281(45): p. 34525-36.

Yang, H.C. and J.H. Kao, Persistence of hepatitis B virus covalently closed circular DNA in hepatocytes: molecular mechanisms and clinical significance. Emerg Microbes Infect, 2014. 3(9): p. e64.

Zanetti AR, Mariano A, Romanò L, D'Amelio R, Chironna M, Coppola RC, Cuccia M, Mangione R, Marrone F, Negrone FS, Parlato A, Zamparo E, Zotti C, Stroffolini T, Mele A, Study Group Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet*. 2005;366:1379–1384.doi: 10.1016/S0140-6736(05)67568-X. [PubMed] [CrossRef] [Google Scholar]

Zanetti AR, Van Damme P, Shouval D. The global impact of vaccination against hepatitis B: a historical overview. *Vaccine*. 2008;26:6266–6273. doi: 10.1016/j.vaccine.2008.09.056. [PubMed] [CrossRef] [Google Scholar]

Zhang, X.; Zoulim, F.; Habersetzer, F.; Xiong, S.; Trépo, C. Analysis of hepatitis B virus genotypes and pre-core region variability during interferon treatment of HBe antigen negative chronic hepatitis B. *J. Med Virol.* 1996, *48*, 8–16. [**Google Scholar**] [**CrossRef**]

Zhang, Y.Y., et al., Single-cell analysis of covalently closed circular DNA copy numbers in a hepadnavirus-infected liver. Proc Natl Acad Sci U S A, 2003. 100(21): p. 12372-7.

Zuckerman, A.J., Hepatitis Viruses, in Medical Microbiology, th and S. Baron, Editors. 1996: Galveston (TX).

الخلاصة

الخلفية:-

تعتبر العدوى المزمنة بفيروس التهاب الكبد B احد الاسباب لوفيات حوالي نصف مليون شخص في العالم . يتم تمييز العديد من الأنماط الجينية الى HBV ، وفقًا لتسلسل الجينوم. تم تحديد ثمانية أنماط وراثية معروفة (A-H) من جينوم التهاب الكبد B. علاوة على ذلك ، تم تحديد نوعين وراثيين جديدين ، I و J. ولذلك صنفت بعض الأنماط الجينية الى HBV أيضًا على أنها طرز وراثية فرعية.

الاهداف:-

أجريت تلك الدراسة على المرضى المصابين بفيروس التهاب الكبد B ؛ حيث تم جمع الدم من المتبرعون الذين حضروا إلى بنك الدم في محافظة بابل . تم جمع قاعدة البيانات الخام من دوائر الصحة العامة في بابل، كربلاء والنجف في فترة خمس سنوات من (حزيران ٢٠١٦ - حزيران ٢٠٢١) وكان عدد المراجعين ١٦٣١٢ مراجع. تراوحت أعمارهم بين ١٦ و ٦٤ عامًا خلال الفترة من ديسمبر ٢٠٢٠ إلى ديسمبر ٢٠٢١. تم تقدير معايير الدراسة باستخدام تقنية الاهتزاز المناعي المرتبط بالإنزيم (ELISA) وتفاعل البلمرة المتسلسل في الوقت الفعلي (RT-PCR) لتحديد الأنماط الجينية HBV. تم تحليل عينات ٦٧٥ مريضًا من مرضى التهاب الكبد الوبائي بواسطة RT-PCR في محافظة بابل.

النتائج:-

بينت النتائج بواسطة تقنية HBV –RT-PCR ان ٩٨ فردا من أصل ٦٧٥ فردا كان مصابين بالتهاب الكبد الفيروسي - ب (١٤,٥٪). وكان النمط الجيني السائد بين المرضى الخاضعين للدراسة هو النمط الجيني (58% A)، يليه النمط الجيني (12% D)، في حين وجد نمط جيني غير المحدد بنسبة (١٪). بشكل عام ، لم يكن هناك ارتباط معنوي بين الطرز الوراثية المختلفة وبعض العوامل الديموغرافية ، والاختبارات المصلية ، واختبار وظائف الكبد.

ونستنتج من هذه الدراسة ان النمط الجيني لفيروس HBV الأكثر شيوعًا في العراق هو النمط الجيني D ، يليه النمط الجيني A. بالإضافة إلى ذلك ، كان هناك زيادة في انتشار المرض مع مرور الوقت ، وكان الذكور أكثر تعرضا للعدوى مقارنة مع الإناث في عدوى الالتهاب الكبدي. أخيرًا ، كان للمنطقة الشمالية من محافظة بابل نسبة أعلى من المناطق الجنوبية والوسطى ، خاصة في المناطق الريفية منها في المناطق الحضرية.

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

دور الالتهاب الكبدي الفيروسي- ب- في التحقيقات الجنائية

بحث مقدم الى

مجلس كلية العلوم – جامعة بابل

كجزء من متطلبات نيل درجة الدبلوم العالي في العلوم/ أدلة جنائية

من قبل

عدنان علي محيسن حسين

بكالوريوس علوم حياة/جامعة بابل

اشراف

أ. د شاكر حمادي محمد حسن

١٤٤٣هـ **٢٠٢١م**