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BOTH GENE MUTATION DETECTION AND HEPATITIS B VIRUS GENOTYPING MIGHT BE USEFUL FOR GUIDING CLINICAL MANAGEMENT OF CHRONIC HEPATITIS B INFECTION IN THE AREA OF TARRAGONA

FINAL DEGREE PROJECT

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ABSTRACT

BACKGROUND: Despite the availability of a safe and effective vaccine, chronic hepatitis B virus (HBV) infection continues to be a global health concern with an estimated 350 million people infected worldwide. In HBV-infected patients, different genotypes, which show a distinct geographical distribution, influence liver disease progression and response to treatment. Therefore, detection of mutations associated to antiviral therapy and HBV genotyping are essential for monitoring treatment and an accurate diagnosis of chronic hepatitis B (CHB) patients. However, in some populations, such as the Area of Tarragona, HBV genotyping is not included in the clinical management of chronic hepatitis B.

HYPOTHESIS: Due to immigration, the prevalence of HBV mutations has increased. Thus, HBV genotyping and gene mutation detection could be included in the clinical management, in order to provide an early diagnosis and an optimized treatment.

AIM: To determine the prevalence of hepatitis B virus infection and chronic hepatitis B with precore (preC) variant and correlate it with the previous results (2009) and immigration in the Area of Tarragona.

MATERIAL AND METHODS: A total of 386 HBsAg-positive patients attending to the Joan XXIII University Hospital of the area of Tarragona during 2013, were included in this study. HBeAg, anti-HBe, ALT, AST and HBV DNA values were analysed and compared according to age, gender, clinical statuses and immigration in the recent three years.

RESULTS: In this study, precore mutants were detected in 5.7% of the infected patients. Among the preC mutant patients, males with a means age of 45.5 ± 15.6 years were predominant (77.3%). The findings revealed significantly higher incidence ($p < 0.05$) of preC mutants in CHB patients from 2009 to 2013. In addition, there were no significant differences in prevalence of HBV-infected patients.

CONCLUSION: The results in this study confirm that the distribution of HVB genotypes and the prevalence of CHB precore mutants have changed due to immigration in the area of Tarragona the recent three years. Given the potential incidence of preC mutants, we suggest a HBV genotyping as a necessity to help practicing physicians to identify those at risk of disease progression and determine optimal therapy.

KEYWORDS: Hepatitis B, HBV genotyping, mutation, immigration, CHB management

ABBREVIATIONS AND ACRONYMS

AASLD	American Association for the Study of Liver Diseases	**IDESCAT**	Instituts d'estadística de Catalunya
AE	Acridinium ester	**IFN**	Interferon alpha
ALT	Alanine aminotransferase	**IgM**	Immunoglobulin M
Anti-HBc	Antibody to the core antigen	**IQR**	Interquartile range
Anti-HBc-IgM	Immunoglobulin M antibody to the core antigen	**IU**	International units
AST	Aspartate aminotransferase	**IV**	Index value
BCP	Basal core promoter	**LC**	Liver cirrhosis
CDC	Centers for Disease Control	**M2RR**	MHC class II restricted region
CHB	Chronic hepatitis B	**MHC**	Major histocompatibility complex
dsDNA	Double stranded DNA	**MHR**	Major hydrophilic region
dUTP	2'-deoxyuridine 5'-triphosphate	**NADH**	Nicotinamide adenine dinucleotide
EASL	European Association for the Study of the Liver	**NAs**	Nucleo(t)side analogues
EM	Electron microscopy	**OHB**	Occult hepatitis B
ER	Endoplasmic reticulum	**ORF**	Overlapping open reading frame
FDA	Food and Drug Administration	**PCR**	Polymerase chain reaction
HBcAg	Hepatitis B core antigen	**PEG-IFN**	Pegylated interferon alpha
HBeAg	Hepatitis B e antigen	**PreC**	Precore
HBIG	hepatitis B immune globulin	**preS**	Presurface
HBs	Hepatitis B surface antigen	**QS**	Quantitation standard
HBV	Hepatitis B virus	**RLU**	Relative light unit
HBx	Hepatitis B X protein	**S/L/MHBs**	Short/large/middle hepatitis B surface
HCC	Hepatocellular carcinoma	**SD**	Standard deviation
HCV	Hepatitis C virus	**SPSS**	Statistica Package for Social Sciences
HDV	Hepatitis D virus	**ULN**	Upper limit of normal
HIV	Human immunodeficiency virus	**WHO**	World Health Organization
ICWR	Inactive carrier without replication		

INTRODUCTION

Hepatitis B virus (HBV) is one of the most common human pathogens that cause aggressive hepatitis, advanced liver disease and even death[1]. Therefore, Hepatitis B caused by HBV is a worldwide epidemic inflammation (irritation and swelling) of the liver that causes serious harm to human health according to World Health Organization (WHO, Geneva), which is associated with a huge spectrum of clinical manifestations[2].

HBV infection primarily affects the liver. Typically, the incubation period for hepatitis B is 90 days (range, 60–150 days). The usual signs and symptoms include malaise, fatigue, anorexia, nausea, vomiting, abdominal pain, and jaundice. In some cases skin rashes, joint pain, and arthritis may occur. On the one hand, among people aged more than 5 years, 30%–50% will develop signs and symptoms during acute infection. In the other hand, in children aged less than 5 years and immunocompromised adults, acute HBV infection is typically asymptomatic[3].

Acute hepatitis B progresses to chronic HBV infection in 30%–90% of people infected as infants or young children and in less than 5% of people infected during adolescence or adulthood. Chronic infection with HBV may result in chronic liver disease, including cirrhosis and liver cancer[2].

HEPATITIS B VIRUS VIROLOGY AND BIOLOGY

CLASSIFICATION AND MORPHOLOGY

Discovered in 1965 by Baruch S. Blumberg (1925-2011), hepatitis B virus is the smallest round DNA virus known, with a diameter of 42nm. However, pleomorphic forms, such as filamentous and spherical bodies lacking a core, also exist (Figure 1)[2,3].

HBV is the prototype member of the genus *Orthohepadnavirus* of the *Hepadnaviridae* family, which replicates in the liver and causes hepatic dysfunction. Hepadnaviruses are a continuously growing family of hepatitis enveloped viruses with an incomplete double-stranded DNA (dsDNA) genome, which can be found in both mammals (orthohepadnaviruses) and birds (avihepadnaviruses). Other well-known members of Hepadnaviruses are the woodchuck, ground squirrel and duck hepatitis viruses[1,4,5].

Structurally, HBV consists of a spherical particle (virion), also called Dane particle, which is composed of an outer lipoprotein envelope or coat (7 nm), which contains a high quantity of hepatitis B surface proteins (hepatitis B surface antigen; HBs or HBsAg), and an inner anicosahedral nucleocapsid core (27 nm) composed of core protein (hepatitis B core antigen; HBc or HBcAg) and surrounded by the envelope[3]. Whereas the envelope of HBV consists of three forms of HBsAg, which are found on the surface; the nucleocapsid of HBV bears HBcAg and hepatitis B e antigen (HBeAg) as well as partially viral dsDNA and DNA-dependent DNA polymerase, which uses reverse transcriptase in its replication cycle, that is approximated to many retroviruses detected in animals and pararetroviruses found in plants (Figure 1).

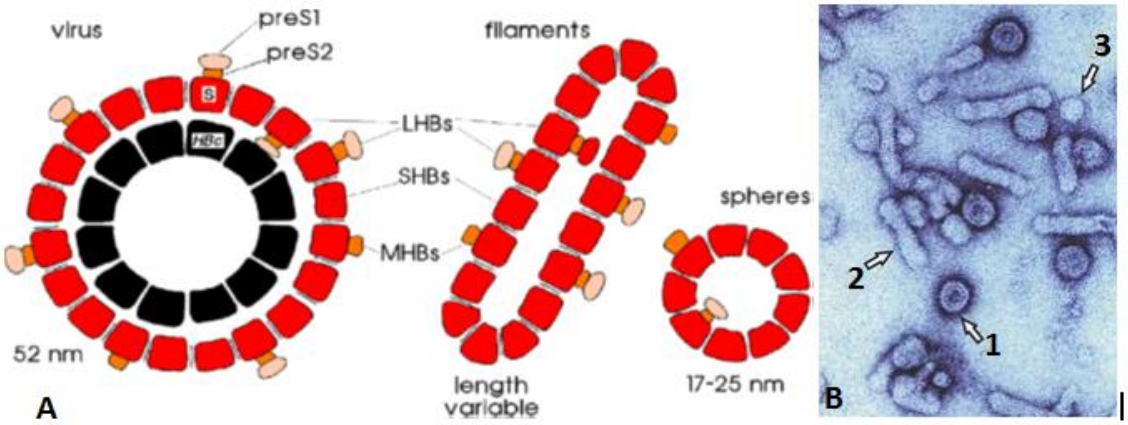

Figure 1. (A) Schematic diagram of the three types of hepadnavirus HBV particles (virus, filaments and spheres) and their surface components: with their respective length, the three envelope proteins: Large hepatitis B surface (LHBs; HBs protein plus preS1 and preS2), short hepatitis B surface (SHBs; HBs protein only) and middle hepatitis B surface (MHBs, HBs protein plus preS2), and hepatitis B core (HBc). (**B) Electron microscopy (EM) images (negative staining) of HBV associated particles**. The round 42 nm particles (1) represent infectious virions (Dane particle). The filaments (2) and the small empty spheres (3) are non-infectious. The preparation was enriched in virus particles. Figure taken from H.-W. Zentgraf)[6].

HBV replicates in the hepatocyte of humans and other higher primates. As a result of its high capacity of replication and quantity error rate of viral reverse transcriptase, HBV is able to adapt to the host's environment[7,8].

GENOME AND PROTEINS

The partly double-stranded DNA genome of HBV has only 3.2 kb and is uniquely organized in a relaxed circular pattern, whose circularity is maintained by 5' cohesive ends. It contains four overlapping open reading frames (ORFs) that are translated into seven known proteins. The minus strand of the DNA is almost complete circle and contains overlapping genes that encode both structural proteins (presurface/surface (preS/S) and precore/core (preC/C)) and the replicative proteins (polymerase (P) and a multifunctional non-structural protein called protein X (X)). The plus strand of the DNA is shorter and variable in length[9]. The negative strand of the DNA is the template for the synthesis of the viral mRNA transcripts (Figure 2).

Four mRNA transcripts of known function have been defined as being involved in HBV transcription and translation. The longest (3.5 kb) is the template for genome replication, the expression of precore/core and polymerase proteins. A 2.4 kb transcript encodes preS1, preS2 and HBs protein, while a 2.1 kb transcript encodes only preS2 and HBs protein. The last transcript, the smallest (0.7 kb), encodes the *X* protein[9-11].

Figure 2. Genome Organization of HBV and ORFs for encoding proteins in the covalently closed form of HBV DNA. The HBV virion genome is circular and approximately 3.2 kb in size and consists of partially dsDNA). It has compact organization, with four ORFs (S, P, C and X) running in one direction and no noncoding regions (left). **Schematic presentation of the S/preS1/preS2 gene, mRNA transcripts, and translational products.** Opening reading frame S has three internal AUG codons. Transcription to produce the 2.1 kb and 2.4 kb mRNAs first occurs after translation into SHBs, MHBs and LHBs proteins, ensues with different promoters (right). Figure taken from Jung ML, Hang HA[12].

The PreS/S gene encodes for the virus envelope and contains three in frame "start" (ATG) codons that divide the gene into three sections: preS1, preS2 and S. The major glycoprotein that forms hepatitis B surface particles is the smallest gene product (SHBs; 24 kD) coded using *S* gene. The middle protein (MHBs; 31 kD), which contains the preS2 component (coded using *preS2/*S gene), and the large surface protein (LHBs; 39 kD), which contains preS1 (coded using *preS1/S2/S* gene), are also incorporate into HBsAg particles but are found in larger proportions in the intact virus particles (Figure 2). The LHBs and MHBs proteins are expressed at levels of about 5-15% and 1-2% compared with HBS protein. The core protein (21 kD) is coded by gene *C*[2, 10]. Hepatitis B e protein (16-18 kD) is produced by proteolytic processing of the pre-core protein and is not incorporated into virions but instead is independently secreted from cells, accumulating in serum as an immunologically distinct antigen. The DNA polymerase and the terminal protein found on minus strand are encoded by gene *P*. The product of ORF *X* protein (17 kD) is a poorly understood regulatory protein that enhances the expression of heterologous and homologous cellular genes in trans[12].

HEPATITIS B VIRUS INFECTION

Hepatitis B virus infection is a global epidemic public health problem, which is associated with a huge spectrum of clinical manifestations ranging from acute, in which infected individuals can achieve complete immune clearance of virus yielding a life-long immunity, fulminant HBV infection to various forms of chronic infection, including asymptomatic or inactive carriers, with a normal liver histology, and chronic hepatitis B (CHB) infection which can develop to severe diseases, such as hepatic failure, liver cirrhosis (LC), and hepatocellular carcinoma (HCC)[13-15].

HBV EPIDEMIOLOGY, PREVALENCE AND TRANSMISSION

Even though it can be prevented through vaccination according to the World Health Organization (WHO, Geneva), nearly 2 billion people are infected by HBV and more than 350 million people worldwide are chronic carriers of the virus persistently, of whom 1 million die from the late sequelae and decompensated liver cirrhosis, liver failure or hepatocellular carcinoma per year[16]. Moreover, following acute HBV infection, 15% of infected healthy adults, 25-35% of 1-5 years infected children, and up to 90% of infected infants; become chronic carriers[2,17].

The incidence and prevalence of HBV infection vary throughout the world, ranging from 0.1% to 20%. This wide range is largely due to differences in age at the time of infection, patterns of transmission and endemicity.

Concerning to patterns of transmission, HBV is carried in the blood and other body fluid, including saliva, tears, semen and vaginal secretions[18]. Another pattern of transmission is person-to-person which is possible by various means, depending on the epidemiologic pattern within a geographic area[19]. For example, in areas of intermediate or low endemicity (less than 2%), such as Western countries, the predominant modes of infection are sexual contact and intravenous drug use[20].

Therefore, HBV infection is generally acquired in adulthood and is self-limited spontaneously. In contrast, in areas with high endemicity (8-15%), such as Asia, Pacific Islands and most African countries, the most common routes of infection are vertical mother-child transmission and horizontal transmission between children, particularly siblings[21] (Table 1, Figure 3).

Table 1. Prevalence of HBV carriers and predominant modes of transmission according to areas of endemicity.

AREAS OF ENDEMICITY	PREVALENCE OF HBV CARRIERS	PREDOMINANT MODES OF TRANSMISSION
Central Asian republics, parts of eastern Europe	High (≥8%)	Perinatal or Childhood (horizontal)
Western and northern Europe	Low (<2%)	Sexual contact or Injecting drug use (vertical)
Other countries	Intermediate (2–7%)	Early childhood (horizontal)

Different areas of endemicity and its prevalence of HV carriers (expressed in percentage) and patterns of transmission. Table taken from Custer B, et al.[22]

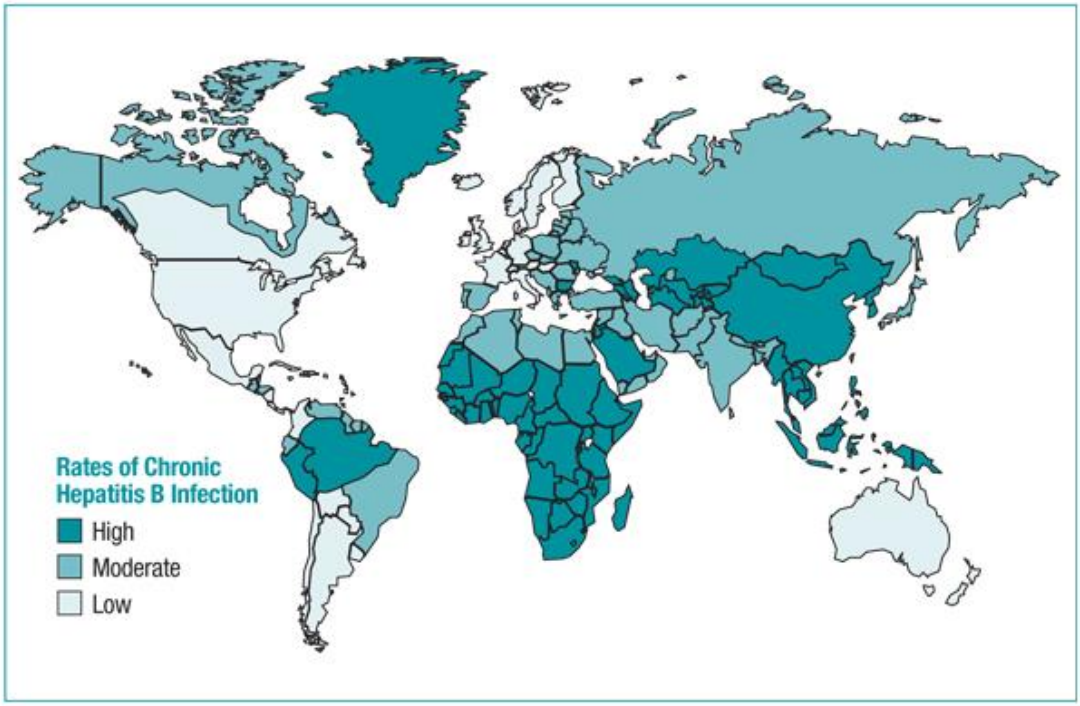

Figure 3. Global distribution of rates (high, moderate and low) of CHB infection. Figure taken from Centers for Disease Control and Prevention (CDC, Atlanta)[23].

DIAGNOSIS, PREVENTION AND TREATMENT

The clinical symptoms of HBV infection are indistinguishable from other forms of viral hepatitis, thus, definitive diagnosis of hepatitis is made by biochemical assessment of liver function and confirmed by demonstration in sera of specific antigens and/or antibodies (serological tests). Laboratory diagnosis is based firstly on the detection in sera of HBsAg and complemented by the detection of immunoglobulin M (IgM) antibody HBcAg (anti-HBc IgM), antibody to HBcAg (anti-HBc) and antibody to HBsAg (anti-HBs). These four standard blood tests for hepatitis B can determine if a person is currently infected with HBV, has recovered, is a chronic carrier, or is susceptible to HBV infection[2].

Specifically, HBsAg is the earliest indicator of acute infection and is also indicative of chronic infection if its presence persists for more than 6 months. It is useful for the diagnosis of HBV infection and for screening of blood. Anti-HBs appears 1 to 4 months after onset of symptoms indicates clinical recovery and subsequent immunity to HBV. It can also neutralize HBV and provide protection against HBV infection. Antibodies to HBc are of class IgM and IgG. They do not neutralize the virus. The presence of IgM identifies an early acute infection. In the absence of HBsAg and anti-HBs, it shows recent infection. IgG with no IgM may be present in chronic and resolved infections. Anti-HBc testing identifies all previously infected persons, including HBV carriers, but does not differentiate carriers and non-carriers[2] (Table 2).

Then, in patients with hepatitis B (HBsAg-positive), the measurement of markers of replication such as HBeAg, antibody to HBeAg (anti-HBe), HBV DNA and alanine/aspartate aminotransferases (ALT and AST) are essential for the diagnosis of the different stages of HBV infection and convalescence, decision to treat and subsequent monitoring patients. Specifically, HBeAg appearing during weeks 3 to 6 indicates an acute active infection at its most infectious period, and means that the patient is infectious. Persistence of this virological marker beyond 10 weeks shows progression to chronic infection and infectiousness. HBeAg is not incorporated into virions, but is instead secreted into the serum. Mutant strains of HBV exist that replicate without producing HBeAg. Continuous presence of anti-HBe indicates chronic active liver disease. During the acute stage of infection these seroconversion from HBeAg to anti-HBe is prognostic for resolution of infection. Its presence in the patient's blood along with anti-HBc and in the absence of HBsAg, anti-HBs and core HBV mutants indicates low contagiousness and convalescence. HBV DNA tests are generally performed for monitoring of antiviral treatment. Finally, ALT and AST assess the general health of the liver, when are elevated they indicate liver damage. ALT is the liver enzyme marker that is followed most closely in those chronically infected with hepatitis B. This test is useful in deciding whether a patient would benefit from treatment, or for evaluating how well the patient is responding to therapy.

Table 2. Interpretation of serologic test results for hepatitis B virus infection

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	INTERPRETATION
–	–	–	–	Never infected
+	–	–	–	Early acute infection; transient (up to 18 days) after vaccination
+	+	+	–	Acute infection
–	+	+	+ or -	Acute resolving infection
–	+	–	+	Recovered from past infection and immune
+	+	–	–	Chronic infection
–	+	–	–	False-positive (susceptible); past infection; occult infection or passive transfer of anti-HBc to infant born to HBsAg-positive mother
–	–	–	+	Immune after vaccine series completion; passive hepatitis B immune globulin (HBIG) transfer after administration

Abbreviations: HBsAg = hepatitis B surface antigen; anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface antigen; HBIG = hepatitis B immune globulin. Table taken from Centers for Disease Control and Prevention (CDC, Atlanta)[23].

The initial evaluation of patients with chronic HBV infection should include a through history and physical examination, with especial emphasis on risk factors for coinfection, alcohol use, and family history of HBV infection and liver cancer. Laboratory tests should include assessment of liver disease, markers of replication and hepatitis D virus (HDV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) in those at risk. Additionally, a liver biopsy is often recommended for determining the degree of necroinflammation and fibrosis since hepatic histology can assist the decision to start treatment[2,24-27].

Concerning to treatment, no specific treatment is available for acute hepatitis B, whereas antiretroviral drugs are approved to treat chronic hepatitis B. For acute hepatitis B, supportive treatment, including hospitalization, may be indicated for some people with severe clinical manifestations. The aims of treatment of CHB are to achieve sustained suppression of HBV replication and remission of liver disease. The ultimate goal is to prevent LC and HCC.

Drugs available for the treatment of CHB include interferon alpha (IFN), pegylated interferon alpha (PEG-IFN) and six nucleo(t)side analogues (NAs: Lamivudine, telbivudine, entecavir, adefovir and tenofovir). While IFNs are administered for predefined durations, NAs are usually administered until specific endpoints are achieved[28]. The difference in approach is related to the additional immune modulatory effects of IFN. However, a major concern with long-term NA treatment is the selection of antiviral-resistant mutations[2,28].

Although there is treatment available for chronic hepatitis B, Immunization with hepatitis B vaccine is the most effective means of preventing HBV infection and its consequences[29]. World Health Organization (WHO Geneva) recommends that all infants receive the hepatitis B vaccine as soon as possible after birth[2].

CHRONIC HEPATITIS B INFECTION

In particular, chronic HBV infection poses serious health problems because of the high prevalence rates in many high endemic parts of the world, and for his adverse long-term clinical outcomes, including high risk of developing end-stage liver diseases and premature deaths from cirrhosis, hepatic failure and hepatocellular carcinoma[30-32].

The natural history and spectrum of chronic HBV infection are diverse and variable, ranging from an inactive carrier state to progressive chronic disease. Furthermore, this infection is a dynamic process which can be divided into several replicative or non-replicative (or low replicative) phases based on virus-host interaction which is pivotal to the pathogenesis of liver disease. Therefore, understanding this dynamic nature of CHB infection is crucial in the management of HBV carriers[11].

NATURAL HISTORY OF CHB

The natural history of chronic HBV infection is classified in several phases or stages. However, not all CHB infected patients go through all CHB stages[3].

Immune tolerant phase

This first phase is more common and prolonged in patients with perinatal or early childhood-acquired HBV infection. It is characterized by HBeAg positivity, anti-HBe negativity, high levels of HBV replication (very high HBV DNA levels), normal or low levels of aminotransferases (ALT and AST), mild or no liver necroinflammation and no or slow progression of fibrosis[15,33].

Immune reactive HBeAg-positive phase

This stage is characterized by HBeAg-positivity, anti-HBe negativity, relatively lower level of replication compared to the immune tolerant phase (high HBV DNA levels), fluctuating levels of aminotransferases, moderate or severe liver necroinflammation and more rapid progression of fibrosis compared to previous phase. It might occur after several years of immune tolerance and is more common in subjects infected during adulthood. This phase ends with the seroconversion[i] to anti-HBe[3,34].

Inactive HBV carrier state

This state may follows seroconversion from HBeAg to anti-HBe. It is characterized by very low or undetectable serum HBV DNA levels and normal or elevated levels of serum aminotransferases. However, some inactive carriers may have slightly elevated HBV DNA levels accompanied by persistently normal aminotransferases levels. On the other hand, progression to CHB, usually with HBeAg-negativity, may also occurs[3,15,33].

HBeAg-negative CHB

The last phase, HBeAg-negative CHB might follow seroconversion from HBeAg o anti-HBe during the immune reactive phase or may develop after years of the inactive carrier state. It represents a later immune reactive phase in the natural history. It is also characterized by periodic reactivation with pattern of fluctuating levels of HBV DNA and aminotransferases. Some HBeAg-negative CHB, called precore mutants, patients can develop an HBV variant, a consequence of emerging mutations in the preC region that disrupts HBeAg production. These precore and core mutant viruses develop under selective immune pressure and are able to retain high levels of HBV replication and aminotransferases. It is important to distinguish true inactive HBV carriers from patients with active HBeAg negative CHB, in whom phases of spontaneous remission may occur because they have different prognosis, and from preC mutants because of its different clinical management and treatment[20,34].

HBsAg-negative phase

After HBsAg loss, low-levels HBV replication may persist with detectable HBV DNA in the liver, but generally is not detectable in the serum. It is associated with improvement of the outcome with reduced risk of LC and HCC. However, occult HBV (OHB) infection, which is defined as the existence of HBV DNA in serum without HBsAg can exists. Although it is not considered as a phase of CHB, it can be a source of virus contamination in blood or organs donations[1,35-39].

[i] HBeAg seroconversion is the loss of HBeAg and detection of anti-HBe in a person who was previously HBeAg positive and anti-HBe negative[31].

HBV GENOTYPES AND SUBGENOTYPES

Owing to the high genetic variability of HBV, it is classified into several viral genotypes which differ by at least 8% of divergence or heterogeneity of the S gene sequence greater than 4%, according to phylogenetic analysis with alignments of whole genomes of the HBV in human hosts[1,39]. The sequence variation between the distinct HBV genotypes suggests that the spliced RNAs should be different from each one[40-42]. Since the first definition of the phylogenetically separable genotypes named by capital alphabet letters (A, B, C and D) in the order of discovery, four more well-known genotypes (E, F, G and H) have been defined[5]. In addition, two new genotypes have been currently recognized (I and J)[43]. The formation of the ninth genotype I has been suggested by HBV isolated in Vietnam and Laos[40,44]. Currently, a HBV strain isolated from a Japanese patient has been provisionally designated as HBV/J. HBV genotype J is closer to gibbon/orang-utan than human genotypes in the P and large S genes[40,43].

Up to date, 34 related subgenotypes belonging to some of these genotypes have recently been described[45,46]. Systematic nomenclature is proposed for designating the HBV subgenotypes using Arabic numbers, such as A1-5 of genotype A, B1-8 of genotype B, D1-7 for genotype D and F1-4 for genotype F[41]. For HBV/C genotype, more than ten subtypes have been detected (C1-C6) and two C/D recombinant subgenotypes (CD1 and CD2)[47]. However, inconsistent knowledge of these subtypes still exists. These subgenotypes have distinct virological and epidemiological properties and their sequences are characterized by 4-8% nucleotide differences for each sequence[18]. Moreover, recombination among HBV genotypes increases the diversity of the virus[48].

HBV genotypes B, C, F and H have a prototypic genome length of 3215 nucleotides (nt)[8,45]. The other viral genotypes differ a little bit in length because of deletions and insertions. Genotype G has a length of 3248 nt, 66 more than genotype D with 3182 nt (table 3)[43].

Table 3. Different genome length of genotypes and their differences of ORFs.

GENOTYPE	GENOME LENGHT (bp)	ORF-DIFFERENCES
A	3221	Insertion of aa 153 and 154 in HBc
B	3215	Prototype
C	3215	Prototype
D	3182	Deletion of aa 1 – 11 in preS1
E	3212	Deletion of aa 11 in preS1
F	3215	Prototype
G	3248	Insertion of 12 aa in HBc
H	3215	Prototype
I[40]	3215	Prototype
J[43]	3182	Deletion of aa 1 – 11 in preS1

Abbreviations: bp = Base Pair; ORF = Open Reading Frame; HBc = Hepatitis B Core; aa = amino acid. Table adapted from Mustafa S[43].

GLOBAL DISTRIBUTION

Some current studies have attested that genotypes and most subgenotypes reflect a different geographic distribution of HBV between and even within regions[47-49] and often present demographic characteristics[13,16]. This area-specific localization of HBV is strong evidence that suggests that viral genotypes and subgenotypes are a priceless tool in tracing the molecular evolution, patterns and modes of spread of HBV[49]. In other words, it is associated with anthropologic history. HBV genotypes and subgenotypes distinct geographical distributions are presented in table 4[1,43]. Subenotypes of genotypes HVBA are found predominantly in Sub-Saharan Africa, especially in Western Africa (A1, A3, A4 and A5), whereas A2 is prevalent in Northern Europe.

Genotypes B and C are highly prevalent in Asian and Pacific Islanders. Among them, B2 and C2 are endemic in most parts of Asia. Subtype B1 is primarily observed in Japan, subtype C4 is encountered in Aborigines from Australia, and frequently termed as the Australian aboriginal strain. Subgenotypes B3-8, C1, C3 and C5-8 are widespread in Southeast Asia, in particular in Indonesia and the Philippines. B6 is also isolated in indigenous populations living in the Artic (Alaska, Canada and Greenland)[39,50]. Genotype D is prevalent in the entire Old World including Africa, India, the Mediterranean region and most European countries. HBV/D1 mainly exists in Moslen ethnicity, HBV/D2 in Russia and he Baltic region, subgenotypes D2, D3 and D5 have been found in India too, and D4 and D6 are prevalent in Oceania and Indonesia, respectively. The new HBV/D7 has been detected in Tunisia. Genotype E is endemic in West and Central Africa. HBV/F, HBV/G and HBV/H are prevalent in America. Genotype F (F1-4), which is one of the most divergent and it is closely related to HBV/H, is restricted to Central and South America[8]. Genotype G is commonly encountered in France, Germany and the United States. The eighth genotype H is dominant in Central America[51]. The novel inter-genotypic recombination between A, C and G, genotype I, is found in Vietnam and Laos[40]. The last and newest viral genotype, J, which has a close relationship with gibbon/orangutan genotypes and human genotype C, has recently been reported in the Ryukyu Islands in Japan[44] (Figure 4).

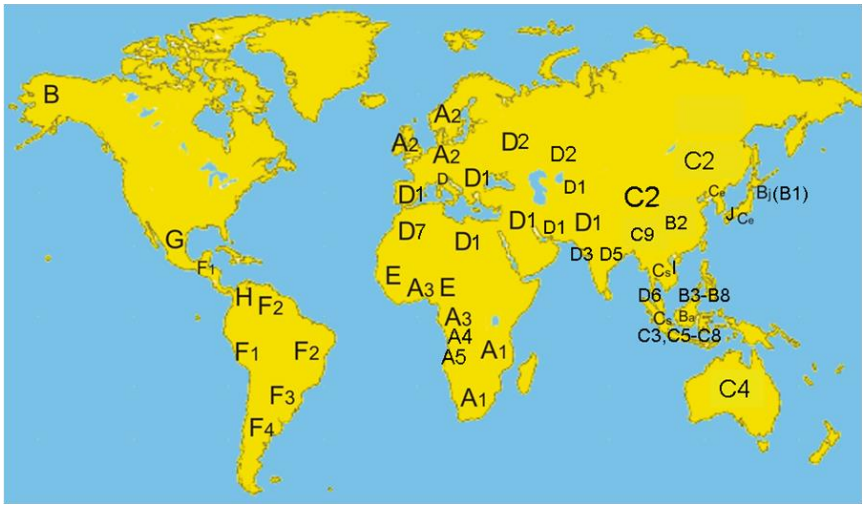

Figure 4. Geographic distribution of hepatitis B virus genotypes and subgenotypes (A-J) worldwide. HBV genotypes A-J; Ba=B2; Bj=B1; Ce=C2; Cs=C1. Figure taken from Zhang Q, Cao GW[53].

Table 4. Geographic location of HBV genotypes and subgenotypes

GENOTYPE	SUBGENOTYPE	GEOGRAPHIC LOCATION
A	A1	Sub-Saharan Africa
	A2	Northern Europe
	A3-5	Western Africa
B	B1	Japan
	B2	East Asia, Taiwan, China, Indonesia, Vietnam, Philippines
	B3	Alaska, Northern Canada, Greenland, Indonesia
	B4	Indonesia, Vietnam
	B5	Indonesia, Philippines
	B6-8	Indonesia
C	C1	Taiwan, Southern China, Korea, Southeast Asia
	C2	Northeast Asia, China
	C3	Indonesia, Oceania
	C4	Australia
	C5, C6	Philippines, Indonesia
	C7	Indonesia
	C8	Philippines
	C9	Tibet, China
D	D1 (mainly D)	Mediterranean countries, Middle East
	D2	Russia, the Baltic region, India
	D3	Indonesia, India
	D4	Oceania
	D5	India
	D6	Indonesia
	D7	Tunisia
E		Restricted to West and central Africa
F	F1	Argentina, Japan, Venezuela, USA, Chile, Alaska
	F2	Brazil, Venezuela, Nicaragua
	F3	Brazil, Venezuela, Panama, Columbia
	F4	Argentina, Bolivia, France
G		Mexico, Canada, France, Germany and USA
H		Mexico (Central America)
I		Vietnam, Laos
J		Ryukyu, Japan

Table adapted from Cao GW[1] and Mustafa S[43].

HBV MUTATIONS

On the one hand, many data demonstrated that HBV exhibits a mutation rate more than 10 fold higher than that of other DNA viruses and exists as quasispecies[ii], due to a lack of proofreading capacity during reverse transcription[1,4]. On the other hand, In addition to HBV genotypes, emerging studies reveal that naturally occurring mutant strains or genetic mutations are closely related with long-term outcomes of chronic liver disease and could be risk markers for liver injury during the progression of liver disease[9,54].

[ii] It is a distribution of mutants that is generated by an equilibrated mutation-selection process. Recently, virus populations have been called quasispecies to indicate their extensive genetic heterogeneity[55].

MUTATIONS IN PRESURFACE/SURFACE REGION

HBV genomic variations in the preS/S regions which are selected during the infection course are of clinical and public health importance[56]. Firstly, because of preS domain is the essential binding site for virion assembly and for transporting of virions out of hepatocyte, and also plays an important role in the interaction with the immune response because they contains several epitopes for T or B cells; mutants occurring naturally in this region may be a direct influence with more progressive forms of liver disease and HBV infection[57]. Moreover, preS mutations, particularly deletions which affect the ratio between the SHBs and LHBs envelope proteins resulting endoplasmic reticulum (ER) stress, were found in CHB infection, fulminant and acute hepatitis B. Some studies have defined a link between preS mutation (W4P/R mutation) and HCC, especially in those infected with HBV/C[56]. Furthermore, another deletion (preS1 start codon deletion) which leads to the deletion of 11 aa at the N-terminus of the LHBs protein, characteristic of HBV/D, also exhibited a higher prevalence in HCC patients. Therefore, it is suggested that preS deletion and nucleotide substitution mutations at the promoters side of preS1/2, which are more predominant in HBV/C and HBV/D genotypes, might serve as useful biomarkers for predicting the clinical outcomes, concretely for predicting HCC[57]. Additionally, mutations such as T207A and T770C, in the small *S* region, were observed in cirrhosis and HCC patients, respectively[58]. Recently, variation and deletion in the 3' terminus of preS1 and a C695 mutation was found in liver tissue of occult HBV infection patients, which indicated that it could be responsible for the defect in the secretion of virions or production leading to undetectability of HBsAg[59].

Secondly, owing to the *S* gene contains a dominant neutralizing epitope ("a" determinant) in the major hydrophilic region (MHR) which is the main target for viral neutralization, either by natural or vaccine-induced anti-H; mutations in the immunodominant "a" determinant of MHR, are related with the generation of vaccine escape variants or persistent infection by reducing the binding affinity between the HBsAg and anti-HBs. Therefore, it is crucial that the incidence and types of *S* gene variants, found in endemic population, should be controlled, because this affect to vaccine and diagnostic reagents design[60]. Despite mutations in the *S* region are found in MHR, currently, a novel mutation type in the outside MHR, called *sW182**, which resulted in a premature stop at codon 182 in the *S* gene of genotype C, is defined. It is demonstrated that this mutations is higher prevalent in progressive forms of the disease, such as hepatocellular carcinoma and cirrhosis[61,62].

MUTATION IN PRECORE/CORE REGION

HBV preC/C region encodes HBeAg and HBcAg, which are the indicators of viral replication. On the one hand, HBcAg is the principal target for the host immune response, in particular cytotoxic T lymphocyte attacks, in which non-synonymous mutations may lead to the production of immune escape variants, resulting in HBV persistence.

On the other hand, since the mutation in HBcAg can lead to simultaneous mutations in HBeAg, which is a key HBV immune-regulatory protein, they can profoundly affect the natural course of HBV chronic infection. It should be noticed that mutations in the C region were mainly distributed in major histocompatibility complex (MHC) restricted region[63,64].

Particularly, mutations in the MHC class II restricted region (in M2RR), such as C1653T, T1753V, T1766/A1768 and A1762T/G1764A were associated to HCC and affected to HBeAg serostatus. For example, C1653T, T1753V and A1762/G1764A are more prevalent as CHB infection progresses from the asymptomatic HBsAg carrier state to liver cirrhosis or HCC, indicating that these mutations accumulate before the diagnosis of HCC[65]. It should be noted that, these HBV mutations have been associated mainly in genotype B, C and D and have defined to be a useful biomarker for predicting clinical outcomes of the patients with CHB, for identifying a subset of male HBsAg carriers who are not extremely high risk of HCC and consequently for predicting whether they will develop HCC[66-68].

HBV mutations in the preC/C region, alone or in combination and especially in the M2RR, are associated to the HCC progress in chronic patients infected with genotype C via immune evasion of the CD4 T cell-mediated immune response[69]. Moreover, it is found that the accumulation of preC/C mutations during the natural course of CHB is associated to old age and to the persistent infection in areas where vertical infection is predominant and may be reasonably arranged as predictive biomarkers for HCC prediction[60].

MUTATIONS IN POLYMERASE REGION

The HBV genomic variations in polymerase gene region have clinical importance. Many studies have been reported and characterized that HBV variants in the viral *P* gene confer resistance to drug antiviral therapies with five nucleoside analogues (lamivudine, adefovir, entecavir, telvivudine and tenofovir), approved at the present by European Association for the Study of Liver (EASL, Geneva), in HBV carrier patients or chronically infected[33]. Therefore, several mutations have been associated to each nucleoside analogue resistance.

It should be noted that analysis of HBV *P* gene from infected or carrier patients could be useful to reveal abundant information including mainly the presence of HBV drug resistant mutants which could lead to prediction of effectiveness of antiviral therapy as well as severity of the disease. Therefore, it is of importance to evaluate antiviral therapy by surveillance of the significant sites of mutations. Thus, early detection of HBV drug resistance is primordial for clinicians to decide on the choice of antiviral treatment and further management of hepatitis B carriers[70].

MUTATIONS IN THE PROTEIN X REGION

Hepatitis B *X* protein is a 154 aa multifunctional protein with an N-terminal negative regulatory domain and a C-terminal transactivation domain. Due to its multifunctionality, *X* protein plays an important role in: gene transcription, viral replication, signalling pathways, genotoxic stress response, cell-cycle control and apoptosis. So, thus far, it is known that specific point mutations in the HBx gene, including M1386, T1485, B1499, A1613, T1653, G1727, A1757/T1764/G1766, T1773, G or C1753, and T1766/A1768 mutations, are strongly implicated in pathogenesis of HBV related diseases, in particular LC and HCC in chronic patients and in hepatocarcinogenesis[7-,72].

It is known that an accumulation of mutations in the *X* region, particularly the subsequent mutations in specific codons following the basal core promoter (BCP) double mutations (A1762T/G1764A), contributes to disease progression in patients with chronic genotype C infections. Moreover, other mutations have been reported to be predictive markers for hepatocarcinogenesis[73].

CLINICAL SIGNIFICANCE OF GENOTYPES AND MUTATIONS

Clinical outcomes of chronic hepatitis B virus infection vary widely. In addition to host factors, several viral factors including HBV genotype, viral load, specific viral mutations and quantitative HBsAg levels, have been associated with disease outcomes. Among viral factors, HBV genotype correlates with not only the clinical outcomes, but also with the response to interferon treatment[17]. In a study comparing genotypes B and C, ALT levels were higher in patients with genotype C[74].

There are differences in date obtained from studies that reviewed genotypes and chronicity. Some recent studies showed that progression to chronic infection was increased in individuals with acute infection due to HBV genotype A[74-76]. However, a study conducted in China reported that chronic infection developed more frequently in patients with C2 sub-genotype than in those with sub-genotype B2, and genotype C2 was an independent risk factor for chronicity development[77].

HBeAg seroconversion and HBsAg seroclearance are the most important steps in the natural progression of HBV infection, and the annual incidence of these are 12% and 2%, respectively. Compared with genotype A and B cases, patients with genotypes C and D have lower rates and usually delayed onset of spontaneous HBeAg seroconversion. HBV-genotype C has a higher frequency of precore, basal core promoter (BCP) A1762T/G1764A mutation and preS deletion, and a higher viral load than genotype B. Similarly, genotype D has a higher prevalence of BCP A1762T/G1764A mutation than genotype A[7].

LC and HCC are the most severe complications of hepatitis B. Therefore, studies conducted in the past decade have focused particularly on the correlation between HCC and HBV variants, and correlations between two important mutations of the HBV virus, PC mutant in nucleotide 1896 and BCP mutants in nucleotides 1762 and 1764[78].

Moreover, previous papers, reported that for example, HBeAg seroconversion appears earlier in HBV/B infections than in genotype HBV/C infections, and fewer patients with HBV/B progress to chronic hepatitis, cirrhosis or liver cancer[79,80]. Therefore, more advanced liver disease and progression to HCC is more habitual in patients who are chronically infected with genotypes HBV/C and HBV/D than HBV/B and HBV/A. Interferon therapy is also more useful or has a higher response rate in subjects with genotype HBV/B than HBV/C and HBV/A than HBV/D.

In addition, HBV/B genotype has a lower mutation rate than HBV/C. In short, patients with genotype HBV/B infections have better clinical outcomes than patients with genotype HBV/C and HBV/D infections. Specifically, HBV genotypes and subgenotypes A1, C, B2-5 and H seem to be related with more severe complications than A2, B1 and B6[80].

The primary clinical and virological features among HBV genotypes are shown in Table 5.

Table 5 Comparison of clinical and virological features among hepatitis B virus genotypes (A-J).

GENOTYPE	B	C	A	D	E-J
CLINICAL CHARACTERISTICS					
Modes of transmission	Perinatal/vertical	Perinatal/vertical	Horizontal	Horizontal	Horizontal
Tendency of chronicity	Lower	Higher	Higher	Lower	ND
Positivity of HBeAg	Lower	Higher	Higher	Lower	ND
HBeAg Seroconversion	Earlier	Later	Earlier	Later	ND
HBsAg seroclearance	More	Less	More	Less	ND
Histological activity	Lower	Higher	Lower	Higher	ND
Clinical outcomes (LC, HCC)	Better	Worse	Better	Worse	Worse in genotype F
Response to INF-α	Higher	Lower	Higher	Lower	Lower in genotype G
Response to nucleos(t)ide analogs	No significant differences among genotypes A to D				ND
VIROLOGICAL CHARACTERISTICS					
Serum HBV DNA level			ND	ND	ND
Frequency of PC A1896 mutation	Higher	Lower	Lower	Higher	ND
Frequency of basal core promoterT1762/A1764 mutation	Lower	Higher	Higher	Lower	ND
Frequency of preS deletion mutation	Lower	Lower	Higher	ND	ND

Abbreviations: ND: No data available; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus.
Table taken from Allain JP[81]

HYPOTHESIS AND OBJECTIVES

Up to now, at least 10 hepatitis B virus genotypes and 34 subgenotypes have been reported. HBV genotypes and its subtypes have also been linked with some mutations, such as A1762/G1764A mutation in precore region. They have been defined with different geographical distribution and have been associated with variable impacts on the course of disease pathogenesis, prognosis, response to treatment and drug tolerance. These observations suggest that these pathogenic differences between HBV genotypes, subgenotypes and mutations play an important role in the virus-host relationship and disease outcomes[(1)].

Therefore, the variability of HBV genotypes leads to the hypothesis that due to immigration in the area of Tarragona since the past three years, the predominance and distribution of HBV genotypes and mutations has changed. Furthermore, the population incidence of hepatitis B virus infection and chronic HBV with preC mutations infection has been altered too. Thus, understanding the characteristics of HBV and including HBV genotyping assay and sequencing for detecting mutations it may help to identify chronic hepatitis B patients at risk of disease progression and therapeutic outcome. Moreover, it can also provide an early diagnosis and optimized treatment.

In order to prove this hypothesis, the aim of this study is to determine the incidence and frequency of hepatitis B infection and chronic hepatitis B with preC mutations among patients who attended to the of Joan XXIII University Hospital of the Area of Tarragona during 2013, compare it with the past results (2009) according to age and gender, and correlate it with the immigration of the area of Tarragona the past three years.

MATERIALS AND METHODS

STUDY DESIGN, PATIENTS AND DATA COLLECTION

This study includes a total of 405 blood samples with HBsAg-positive, which were collected from patients attending the Laboratory of Clinical Analysis of Joan XXIII University Hospital of the Area of Tarragona during the time period January 2013 to December 2013. The data of all these samples were obtained from specific database of the clinical documentation services of the laboratory by using the database Synergy® (Synergy Software, PA) of the laboratory.

Among them, after excluding samples of the same patients and insufficient samples for the HBV-DNA quantification, which is an important parameter to diagnose; a cohort of 386 patients were included in this final retrospective study. The criteria inclusion was being HBsAg-positive and having results for HBeAg/anti-HBe profile, aminotransferases levels and viral load. This information was obtained from clinical records and was included in a common database, specifically created for this study. The main characteristics of patients were further analysed. For these analyses, the following variables were: age, gender, HBeAg/anti-HBe profile, AST and ALT levels, and DNA HBV viral load, which were established from database and clinical records.

DETECTION OF SEROLOGIC MARKERS

Serological tests for HBV markers were routinely performed in the Laboratory of Clinical Analysis of Joan XXIII University Hospital using direct chemiluminescence using Acridinium Ester (AE) Technology from ADVIA Centaur immunoassay system® (Bayer Diagnostics, USA) according to the manufacturer's instructions[82].

The serological assessment includes the following three HBV markers: hepatitis B surface antigen or HBsAg, hepatitis B e antigen or HBeAg, and antibodies to hepatitis B e antigen or anti-HBe. These assays employ magnetic particle separation technology with direct chemiluminescence[83].

HEPATITIS B SURFACE ANTIGEN (HBsAg)

Hepatitis B surface antigen was analysed by the commercial kit ADVIA Centaur® HBsAg Assay which is an *in vitro* diagnostic immunoassay for the qualitative detection of HBsAg in human serum and plasma using the ADVIA Centaur® system (Bayer Diagnostics, USA) according to the manufacturer's instructions[84].

The relative light units (RLUs) detected are used to calculate the index value from the master curve. A result of reactive or nonreactive is determined according to a cut-off of 1.0 Index Value (IV) established with the calibrators. Samples with an IV less than 1.0 are nonreactive. Samples of greater than or equal to 1.0 but less than or equal to 50 are considered initially reactive for HBsAg, but they are to be repeated or supplemented with other tests. Finally, patients with a greater IV than 50 are reactive and they do not need to be repeated[84].

HEPATITIS B E ANTIGEN (HBeAg)

Hepatitis B e antigen was determined by the commercial kit ADVIA Centaur® HBeAg assay according to the manufacturer's instructions[85]. It is an *in vitro* diagnostic immunoassay for the qualitative determination of the HBeAg in human serum and plasma from individuals who have signs and symptoms of hepatitis or who may be at risk for HBV infection, using the ADVIA Centaur® Systems (Bayer Diagnostics, USA).

A direct relationship exists between the amount of HBeAg activity present in the patient sample and the amount RLUs detected by the system. A result of reactive or nonreactive is determined according to a cut-off of 1.0 IV established with the calibrators. Results should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings. The system reports HBeAg results in IV's and as reactive (positive), samples with an initial value greater than 10.0 IV; nonreactive (negative), samples with an initial value of less than 0.80 IV, or as needing retest, which are samples on the rested zone with an initial value more than 0.8 and less than 1.0 IV[85].

ANTIBODY TO HEPATITIS B E ANTIGEN (ANTI-HBe)

Anti-HBe was tested by the ADVIA Centaur® HBe assay according to the manufacturer's instructions[86]. This commercial kit is an *in vitro* diagnostic test for the qualitative determination of total antibodies to anti-HBe in human serum or plasma using the ADVIA Centaur® System (Bayer Diagnostics, USA).

A direct relationship exists between the amount of anti-HBe activity present in the patient sample and the amount RLUs detected by the system. A result of reactive or nonreactive is determined according to a cut-off of 1.0 IV established with the calibrators. Samples with an initial value greater than 1.2 IV are reactive (positive); with an initial value of less than 0.8 IV are nonreactive (negative) and samples on the rested zone with an initial value greater than 0.8 and less than 1.2 IV need to be retested[86].

DETECTION AND QUANTIFICATION OF HEPATITIS B VIRAL DNA

HBV DNA detection and quantification are essential to diagnose and treat chronic HBV infection. The use of real-time polymerase chain reaction (PCR) assays for HBV DNA quantification is strongly recommended.

Following serological tests, human plasma and serum HBV-DNA was quantified by using *COBAS AmpliPrep/COBAS® TaqMan® HBV Test, v2.0* (Roche Diagnostics, Germany)[87] in the Laboratory of Clinical Analysis of Joan XXIII University Hospital. This HBV test is an Food and Drug Administration (FDA, Maryland)-approved *in vitro* nucleic acid amplification test for the quantification of HBV DNA in human serum using the COBAS® AmpliPrep[88] instrument for automated viral nucleic acid extraction (generic silica-based capture technique) and the COBAS® TaqMan[88] analyser for automated amplification and detection of the viral nucleic acid target. This assay targets the highly conserved precore/core region of the HBV genome and generates amplification products that are detected real-time by a sequence-specific TaqMan probe during amplification.

The procedure processes 1050 µL of plasma or serum and consists of subsequent steps of lysis with chaotropic agents and proteinase K, DNA capture by use of glass particles, and purification. After DNA elution at high temperature (80°C), a robotic arm loads nucleic acids in microvials containing the PCR master mix prepared for each sample by the same robotic arm.

An internal quantitation standard (QS) is added to each sample during the processing step. After HBV DNA extraction with the COBAS® AmpliPrep instrument, a real-time PCR test is performed by the COBAS® TaqMan analyser with a multiplex TaqMan assay. Two targets are amplified: HBV DNA and the internal QS. The QS is a non-infectious construct containing fragments of HBV sequences with primer binding regions identical to those of the HBV target sequence but with detection probe different from that for HBV.

Prevention of carryover contamination and sample integrity is provided by the use of the Amperase system based on uracil-*N*-glycosylase and 2'-deoxyuridine 5'-triphosphate (dUTP) incorporation[89,90].

HBV DNA levels were expressed in international units per millilitre (IU/mL) (Conversion factor: 1 UI/mL = 5.6 copies/mL). According to the instructions, the quantification range of this assay is 20 to 1.7×10^8 IU/mL (1.30-8.23 log IU/mL). An HBV DNA level above 20 IU/mL was considered a positive result. The sensitivity of the assay was 99%[90].

DETECTION OF BIOCHEMICAL MARKERS

Analysis of blood biochemical parameters (ALT and AST) was routinely performed using ADVIA® 2400 Clinical Chemistry System (Bayer Diagnostics, Tarrytown, NY) in the Laboratory of Clinical Analysis of Joan XXIII University Hospital. They were determined by measuring kinetic enzymatic activity in serum samples.

ALANINE AMINOTRASFERASE (ALT)[91]

Alanine aminotransferase (EC 2.6.1.2)[92] was analysed by ADVIA® 2400 Clinical Chemistry System (Bayer Diagnostics, Tarrytown, NY). The system monitors the rate of change in absorbance at 340nm over a fixed time interval between 6 and 10 minutes after sample's addition. The rate of change in absorbance is directly proportional to the AST activity in the sample.

The detection range for ALT was from 0 to 1100 IU/L. According to the instructions, an ALT level above 41 IU/L for men and 31 IU/L for women were considered upper limit of normal (ULN) levels[91].

ASPARTATE AMINOTRANSFERASE (AST)[93]

Aspartate aminotransferase (EC 2.6.1.1)[92] was determined by ADVIA® 2400 Clinical Chemistry System (Bayer Diagnostics, Tarrytown, NY). AST activity in the sample is determined by assaying the rate of nicotinamide adenine dinucleotide (NADH) oxidation, which is proportional to the reduction in absorbance at 340nm over a fixed time interval between 3 and 5 minutes after sample's addition.

The detection range for AST was from 0 to 1000 IU/L. According to the instructions, an AST level above 38 IU/L for men and 32 IU/L for women were considered upper limit of normal[93].

DEFINITION OF THE CLASSIFICATION GROUPS

For analyses, these patients were further classified to different groups based on: gender (male and female), age (10-20 to 71-80 years), and clinical status in which each subject was assigned founded on the reactive or positive (+) or the nonreactive or negative (-) HBsAg, anti-HBe and HBeAg; the HBV-DNA more or less than 20 IU/mL; the ULN or normal levels of AST and ALT; and was not influenced by additional laboratory or clinical information.

The group of clinical status was divided into five categories (stages or forms of chronic hepatitis B), according to the recommendations from the American Association for the Study of Liver Diseases (AASLD, Virginia) 2009 practice guidelines[31] (Table 6).

Table 6. Classification of clinical status group and definition of each five category, according to the recommendations from AASLD[28]

CLINICAL FEATURES	STAGES OR FORMS OF CHRONIC HEPATITIS B							
	HBeAg+ CHB	ICWR	HBeAg- CHB	PreC CHB	Others			
HBeAg	POS	POS	NEG	NEG	POS	NEG	POS	NEG
Anti-HBe	NEG	NEG	POS	POS	NEG	NEG	POS	POS
ALT, AST	ULN	N	N	ULN	N	N or ULN	N or ULN	N
HBV DNA (IU/mL)	>20	< 20	>20	>20	< or >20	< or >20	< or >20	< 20

HBeAg and anti-HBe are expressed as positive (POS) or negative (NEG); ALT and AST are indicated as ULN or normal (N) levels; HBV DNA is presented as < or > 20 IU/mL.

Abbreviations: CHB = Chronic Hepatitis B; IU = international units; ICWR = inactive carrier without replication; PreC = precore; ALT = alanine aminotrasnferase; AST = aspartate aminotransferase.

The first group was formed by patients with "Immune reactive HBeAg-positive chronic hepatitis B (HBeAg+ CHB)". These patients are characterized by remain HBeAg-positive with HBV DNA levels more than 20 IU/mL after a 3-6 month period, and with ULN alanine aminotransferase levels (ALT; more than 41 IU/L for men and 31 IU/L for women) and aspartate aminotransferase levels (AST; more than 38 IU/L for men and 32 IU/L for women).

The second group was compound by "Inactive HBV carrier without replication (ICWR)" patients. Their serological profile was HBeAg-negative, normal ALT and AST levels and HBV DNA less than 20 IU/mL.

The third clinical group were patients with "HBeAg-negative chronic hepatitis B (HBeAg-CHB)". This group have HBeAg-negative, normal ALT and AST levels and HBV DNA greater than 20 IU/mL.

The fourth group were elaborated by "Precore variant CHB (PreC CHB)" patients who remain HBeAg-negative with HBV DNA levels more than 20 IU/mL after a 3-6 month period of elevated ALT and AST levels.

The last group, "Other states of hepatitis B infection (Others)", were a mix of several different states. For examples, it includes HBeAg-positive patients with high serum HBV DNA but normal ALT and AST levels, patients who had neither HBeAg nor anti-HBe or both with high or non-detected DNA load and with high or normal AST/ALT levels; or were HBeAg-negative, anti-HBe-positive, HBV DNA-negative, and elevated aminotransferases; or were HBeAg-negative, anti-HBe-positive, HBV DNA-negative, and ALT/AST in normal levels.

Additionally, all patients were further classified into patients infected with hepatitis B virus without preC mutation and patients chronically infected with preC mutation. Finally, the study includes the incidence of these two groups of disease in the area of Tarragona in 2009[94] and 2013 and was analysed according to age, gender and percentage of immigration.

STATISTICAL ANALYSIS

All the statistical analyses of the data were performed using Statistica Package for Social Sciences (SPSS) program for Windows version 19.0 (SPSS Inc., Chicago, IL, USA).

Descriptive values were presented as counts and percentages for the entire study group and groups with different demographic characteristics, continuous variables are expressed as mean ± standard deviation (SD) and as medians (interquartile range [IQR]). Using contingency tables, associations between categorical variables were analysed using $\chi 2$ and Fisher exact tests and the comparison between groups (mean values) were compared by two tailed Student's t-test. A p-value of <0.05 was considered as statistically significant.

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION

The demographic features of the 386 infected patients, who attended to the laboratory of the Area of Tarragona during 2013, were analysed in order to determine the prevalence, according to the five clinical statuses or forms of chronic hepatitis B (Immune reactive HBeAg-positive chronic hepatitis B, inactive HBV carrier without replication, HBeAg-negative chronic hepatitis B, precore variant CHB and other states of hepatitis B infection).

Therefore, Table 7 summarizes the classification of clinical statuses based on gender (male and female) and age ranged from 10-20 to 70-80 years; and compares these baseline characteristics among the five clinical status groups.

In general, of the 386 patients, the most common corresponded to Others group (39.1%), followed by HBeAg- CHB (28.0%), ICWR (25.1%), PreC CHB (5.7%) and HBeAg+ CHB (2.1%).

Among the patients, 37.8% were women and 62.2% were men. The male to female ratio (ratio M/F) was 240/146 (1.6), i.e., for each 1.6 men infected with HBV there is 1 woman with hepatitis B infection. The age ranged from 12 to 80 years, with a mean age ± SD of 48.1 ± 13.9 years.

Concretely, the demographic features for each group are illustrated in Table 7. With regard to age, the inactive HBV carriers without replication patients (ICWR) were significantly older (54.0 ± 11.8 years) than patients in the other clinical status groups ($p < 0.05$). In contrast, HBeAg+ CHB patients are significantly younger (33.8 ± 9.6 years) than PreC CHB, HBeAg- CHB and Others patients ($p < 0.05$). About the rest of groups (HBeAg- CHB, PreC CHB and Others) no statistical difference ($p > 0.05$) was observed for mean age between each of them (46.3 ± 13.7, 45.5 ± 15.6 and 48.2 ± 13.9 years, respectively) and the overall population (48.1 ± 13.9 years). Concerning to gender, the ratio M/F of HBeAg+ CHB (3.0) and preC CHB (3.4) groups compared to ICWR (1.8) , HBeAg- CHB (1.3) and Other patients (1.6), have significant higher proportion of patients who are men than women ($p < 0.05$). However, in general, patients who are women are significantly lower in all five groups (Table 7).

Table 7. Demographic characteristics of the study population according to clinical statuses (n = 386)

DEMO-GRAPHIC FEATURES	CLINICAL STATUSES (n, %)					
	Overall population (386, 100%)	HBeAg+ CHB (8, 2.1%)	ICWR (97, 25.1%)	HBeAg- CHB (108, 28.0%)	PreC CHB (22, 5.7%)	Others (151, 39.1%)
Ratio M/F	240/146 (1.6)	6/2 (3.0)[a]	62/35 (1.8)	61/47 (1.3)	17/5 (3.4)[a]	94/57 (1.6)
Mean age ± SD, years	48.1 ± 13.9	33.8 ± 9.6[a]	54.0 ± 11.8[a]	46.3 ± 13.7	45.5 ± 15.6	48.2 ± 13.9

Frequency (number of cases) and percentage are showed in parenthesis below of each clinical status group at the top. Comparison of mean age ± SD and gender is represented between the total of patients, HBeAg+ CHB, ICWR, HBeAg- CHB, PreC CHB and Others group. Gender is defined by the ratio male to female (M/F) and the result of relation in parenthesis. Mean age ± SD is expressed in years. Continuous variables are expressed as mean ± SD. Analysis is determined using a 2-tailed Student's t-test and descriptive statistics.

Abbreviations: CHB = chronic hepatitis B, ICWR = inactive carrier without replication, SD = Standard deviation, M = male, F = female, PreC = precore

[a] A p value <0.05 is considered as statistically significant for each group.

To sum up, these results indicate that the prevalence of chronically infected patients is more predominant in males than females. The most frequent form of CHB is HBeAg- CHB, excluding "Others" group, whereas HBeAg+ CHB is the less frequent. Additionally, Table 7 also indicates that the mean age of being chronically infected in the Area of Tarragona in 2013, was more frequent in population aged 48.1 ± 13.9 years.

HBV SEROLOGY, AMINOTRANSFERASES LEVELS AND VIRAL LOADS ACCORDING TO CLINICAL STATUS

Initially, the entire population of infected patients were classified based on the criteria of American Association for the Study of Liver Diseases (AASLD, Virginia) as established by the updated AASLD practice guidelines[(2)].

Following the study of demographic features, this present study is based on the clinical characteristics of the different forms of chronic hepatitis B. The main factors to consider are ALT, AST and HBV DNA values, which were performed in relation to the clinical status so as to classify the 386 infected patients.

Accordingly, biochemical (ALT and AST) and virological (HBV DNA) mean values for each clinical status are depicted in Table 8. Patients should be considered for treatment when they have HBV DNA levels above 20 IU/mL and serum ALT and AST above the upper limit of normal. Usually, aminotransferases levels indicate the assessment of the severity of the liver disease and were associated with severe inflammation. Therefore elevated levels for ALT (more than 41 IU/L for male and 31 IU/L for female) and AST (greater than 38 male and 32 IU/L for female) were present in HBeAg+ CHB, PreC CHB and Others; whereas ICRW and HBeAg- CHB patients were associated with normal levels.

Table 8. Biochemical and virological characteristics of patients according clinical statuses (n = 386).

BIOCHEMICAL AND VIROLOGICAL FEATURES	CLINICAL STATUSES (n, %)					
	Overall population (386, 100%)	HBeAg+ CHB (8, 2.1%)	ICWR (97, 25.1%)	HBeAg- CHB (108, 28.0%)	PreC CHB (22, 5.7%)	Others (151, 39.1%)
ALT, mean (IQR) (IU/L)	52.8 (35.8-77.9)	397.4 (177.0-874.3)	22.0 (20.6-23.5)	23.0 (21.7-24.4)	79.6 (64.8-96.5)	81.7 (44.0-161.1)
AST mean (IQR) (IU/L)	37.7 (30.1-48.6)	177.5 (53.0-416.6)	22.7 (21.6-23.7)	22.4 (21.0-23.7)	60.8 (47.6-78.7)	53.0 (36.4-85.5)
HBV DNA mean (IQR) (IU/mL)	$4.2 \cdot 10^6$ ($1.7\text{-}7.0 \cdot 10^6$)	$1.3 \cdot 10^8$ ($7.2 \cdot 10^7\text{-}1.7.10^8$)	<20.0 (<20.0)	2812 (1395-4800)	$2.2 \cdot 10^5$ ($6.4 \cdot 10^4\text{-}3.9 \cdot 10^5$)	$3.9 \cdot 10^6$ ($8.2 \cdot 10^4\text{-}9.7 \cdot 10^6$)

Frequency (number of cases) and percentage are showed in parenthesis below of each clinical status group. Comparison of ALT, AST and HBV DNA mean and interquartile range (presented in parenthesis) levels, are represented between the total of patients, HBeAg+ CHB, ICWR, HBeAg- CHB, PreC CHB and Others group. The results are expressed in IU/L for ALT and AST and in IU/mL for HBV DNA. Analyses are performed using descriptive statistics.

Abbreviations: CHB = chronic hepatitis B, ICWR = inactive carrier without replication, ALT = alanine aminotransferase, AST = aspartate aminotransferase, HBV = hepatitis B virus, PreC = precore, IU = International units, IQR = interquartile range.

With regard to HBV DNA, detection and HBV DNA measurement are essential for the diagnosis, decision to treat and subsequent monitoring patients. HBV DNA levels above 20 IU/mL correspond to high levels of HBV replication. All CHB phases are characterized by high HBV DNA levels, except inactive carrier patients (ICWR). However, HBeAg-negative patients (HBeAg- CHB and PreC CHB) are characterized by relatively lower level of replication compared to HBeAg+ CHB patients.

In summary, the three factors studied show the different classification and prevalence of chronic hepatitis B stages of infected patients, following the criteria of AASLD, which have significant differences between each group.

PREVALENCE OF HBV INFECTION WITH AND WITHOUT PRECORE MUTATION IN 2013

It has been previously reported that precore mutations in chronic hepatitis B infected patients may lead to more severe liver disease than patients who have not (HBeAg+ CHB, ICWR, HBeAg- CHB and Others). Thus, in the present study independently factors associated with preC mutations in CHB infected patients, such as gender and age intervals, were analyzed and compared with the rest of patients who do not have preC mutations and the overall population (Figure 5 and 6).

Therefore, HBV-infected patients were grouped into three groups (wild, mutant and total) to investigate different results depending on gender, age and the presence or absence of mutation.

As shown in Figure 5A, concerning to gender, in the three categories (mutant, wild and total) males (77.3%, 61.3% and 62.2%) predominate more than double than females (22.7%, 38.7% and 37.8%). It should note that in patients with chronic hepatitis B with preC mutations (mutant) the difference is more significant. As represented in Figure 5B, relating to age, there are not statistical significantly differences between the three groups. Despite CHB infection starts between 21-30 years for mutants (18,2%), wild (10,7%) and total (11.1%) population, the more frequently chronically infected with hepatitis B with or without preC mutations are mutant (27.3%), wild (29.7%) and total (29.5%) population between 31-40 years. As expected, due to mortality, the prevalence of CHB infection has been decreased year after year. Furthermore, patients less than 20 years for mutant population (0%) do not present CHB and for wild population only 2.5%.

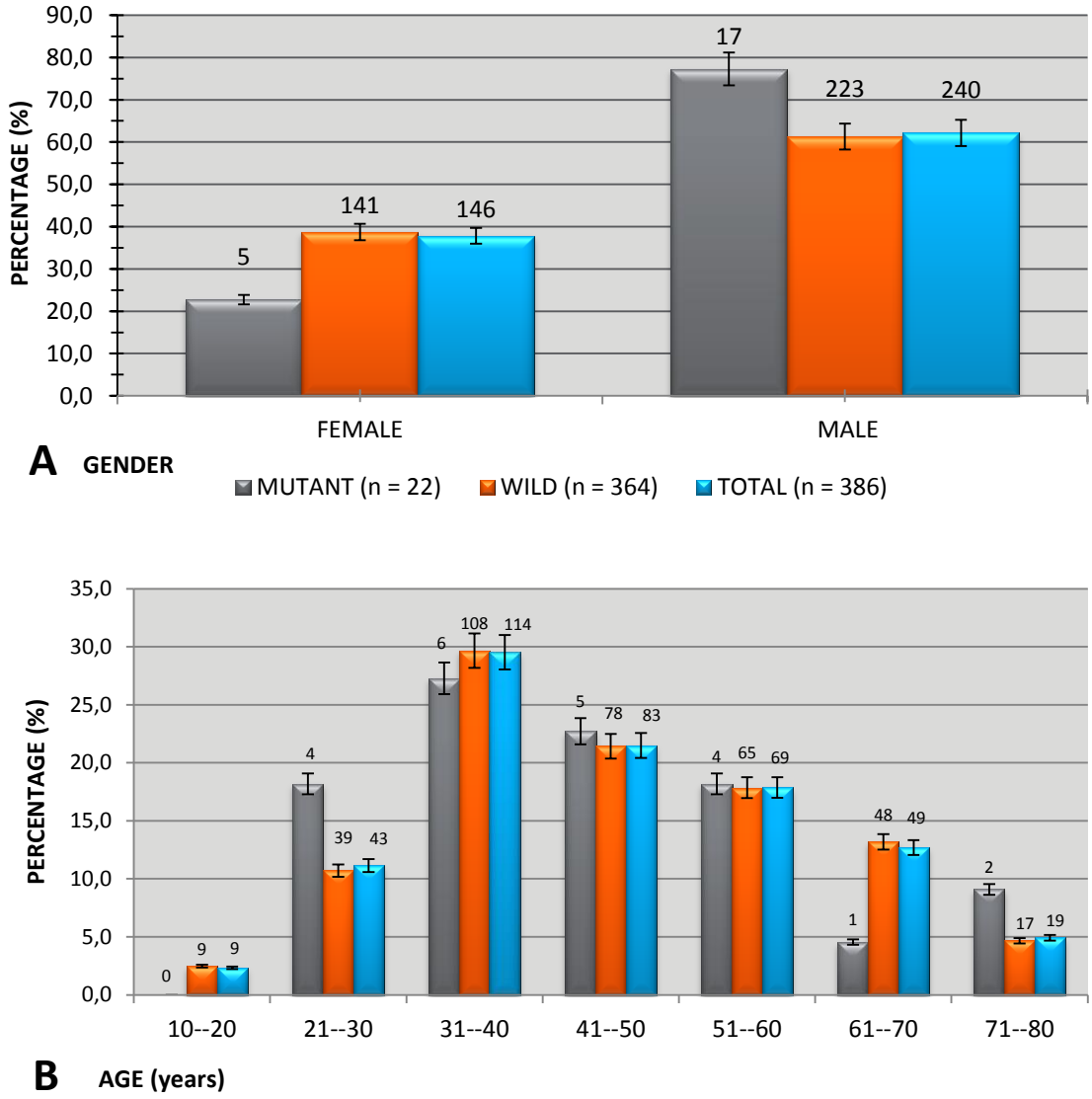

Figure 5. Prevalence of CHB with precore mutation (mutant: n = 22; *grey*), without precore mutation (wild: n = 364; *Orange*) and total CHB infection (total: n = 386; *blue*) according to gender (A) and age (B). The number of cases is indicated on the top of each column.

Particularly, the 22 mutant patients were summarized in the following Figure 6, to study the prevalence of only patients who are chronically infected with HBV with precore mutations (mutant population) in relation to demographic features.

Generally, it should be noted that prevalence of preC mutations in relation to the demographic (gender and age) features of patients presents significantly differences. Of a total of 22 mutants, a percentage of 22.7% was found in females, whereas 77.3% was found in males (ratio M/F = 3.4). As a result, these percentages indicates that preC mutations were significantly more prevalent in men than women (p = 0.021). Moreover, mean age ± SD was 44.6 ± 16.8 years and 50.6 ± 11.5 years for male and female respectively. In comparison with gender, a similar mean age (p = 0.470) of males and females was observed in precore mutant patients (Table 9).

Table 9. Characteristics of mutant population (n = 22)

GENDER	Mean age ± SD, years	Nº cases/total (%)
Male	44.6 ± 16.8	17/22 (77.3%)
Female	50.6 ± 11.5	5/22 (22.7%)

Comparison of frequency (number of cases between the total) and percentage, which is showed in parenthesis, and mean age ± SD, which is expressed in years, according to mutant males and females. Continuous variables are expressed as mean ± SD (years) and the number of cases in percentage. Analysis is determined using a 2-tailed Student's. A p-value less than 0.05 is considered as statistically significant

Abbreviations: SD = Standard deviation
a p-vale = 0.021
[b] *p*-vale = 0.470

As shown in Figure 6, precore variant occurred the most frequently (5/17; 29.4%) in the age interval of 41-50 years in males, whereas the most frequently age interval in females was 51-60 years (3/5; 60%). This mutation was also extensive in the age interval of 21-30 and 31-40 years (4/17; 23.5%) in men, and 31-40 years (2/5; 40%) in women. Both male and female patients with less than 20 years did not present chronic hepatitis B. As expected, due to mortality, the prevalence of preC mutation in CHB has been decreased year after years.

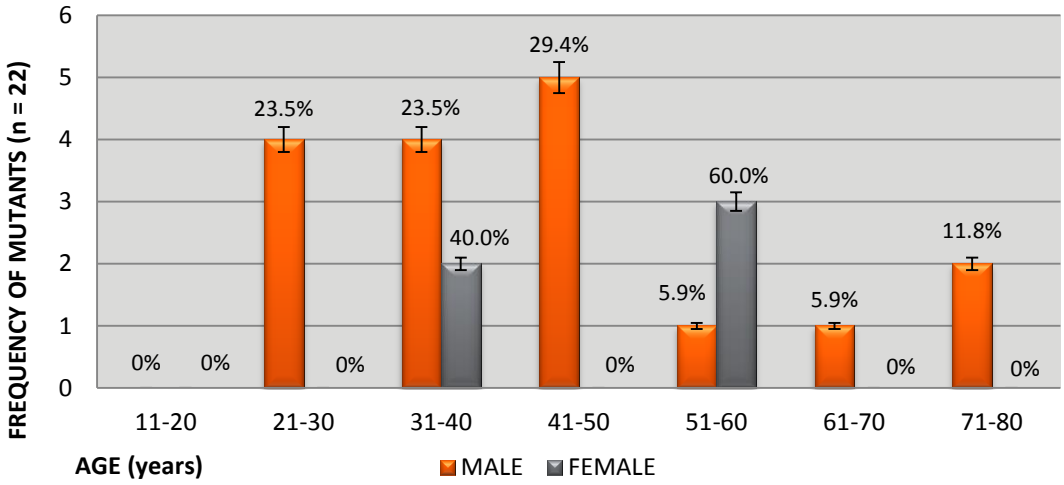

Figure 6. Comparison of precore mutation incidences in different age intervals, according to gender (n = 22). Female (n = 5) is represented in *grey* and male (n = 17) in *orange*. The percentage % of each group is indicated on the top of each column.

In summary, patients chronically infected with HBV with precore mutations are more prevalent in men aged, between 30-50 years, whereas patients without precore mutations are more frequent in men (but less that precore mutants) aged between 31-40 years.

PREVALENCE OF HBsAg-POSITIVE AND PRECORE VARIANT PATIENTS IN THE LAST THREE YEARS

Until now, all studied data was from 2013. In the present work, the frequency of patients infected with HBV (HBsAg-positive) and patients with precore mutations, in the Area of Tarragona, was studied in relation to the last three years in order to determine and compare if the prevalence has been changed. Therefore, to compare it, the prevalence and percentage of adults currently infected with HBV or with HBV-precore variant in 2009[94] and 2013 are summed up in Figure 7.

As shown in Figure 7, with regard to precore mutants, a percentage of the 5.7% was found in subjects infected with HBV in 2013, whereas it was only the 2.5% in 2009. It should be noted that, the prevalence of HBsAg-positive patients has not increased or has slightly decreased (p >0.05) from 2009 (391) to 2013 (386). In contrast, a percentage of 3.2% of patients chronically infected with precore mutation, has increased in the last three years.

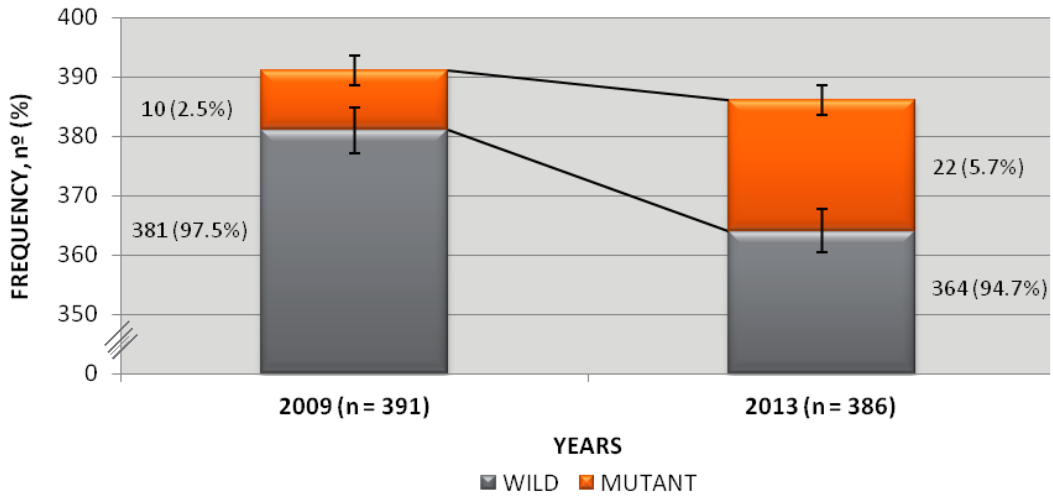

Figure 7. Comparison of frequency (*x* axis), which is expressed with the number of cases and the percentage in parenthesis, of patients with HBV infection without preC mutations (WILD, grey) and patients with chronic hepatitis B with preC mutations (MUTANT, *orange*) in years 2009 and 2013 (*y* axis). The total of HBV infected population is indicated below of each column.

In addition, if we divide the total population infected with HBV between patients with chronic hepatitis B with precore mutation (ratio= total/precore), a ratio of 39.1 was associated in 2009, while it was only of 17.5 in 2013. That is, for every 39.1 or 17.5 patients infected with HBV there was one patient with chronic hepatitis B with preC mutation in 2009 and 2013 respectively.

To sum up, the prevalence of general population infected with HBV has not changed significantly, whereas the prevalence of preC mutants has ramped up from 2009 to 2013.

DISCUSSION

The prevalence and outcomes of chronic viral hepatitis B in the Area of Tarragona may have varied because of the immigrants coming from countries having an elevated incidence with a higher endemicity of hepatitis B. Thus, in this study, the prevalence of stages or forms of CHB was investigated in 386 samples of HBsAg-positive patients, who attended in the laboratory during 2013, by categorizing the infected patients into five clinical statuses of CHB according to the criteria of American Association for the Study of Liver Diseases (AASLD, Virginia). The majority of patients corresponded to Others patients (39.1%), followed by HBeAg- CHB, ICWR and PreC CHB patients (28.0%, 25.1% and 5.7%, respectively) and only 2.1% of the overall population corresponded to HBeAg+ CHB patients. This prevalence was relatively similar to those reported and carried out in the area of Tarragona in 2009 (data not shown)[94]. These findings were supported by the results of previous studies suggesting that the prevalence of the HBeAg-negative forms of the disease has been increasing over the last decade as a result of aging on the HBV infected population and predominance of specific HBV genotypes and represents the majority of cases in many areas[33,95].

Furthermore, the demographic features (age and gender) were analysed to evaluate their relationship with the prevalence of clinical statuses of CHB, among the 386 infected patients. Interestingly, as it has been previously reported, the analyses showed that the inactive HBV carriers without replication patients, were significantly older than HBeAg+ CHB, HBeAg- CHB, PreC CHB and Others patients; with the HBeAg+ CHB patients being significantly younger[96]. This differs from HBeAg- CHB, PreC CHB and Others patients who have the same mean age ± SD than overall population.

In addition, no gender differences (ratio male/female) in ICWR, HBeAg- CHB and Others patients were detected, whereas a significant difference was found among PreC CHB and HBeAg+ CHB patients. However, males were slightly more frequent to being infected than females in overall population. These findings were supported by the results of previous report suggesting that male dominance was found to be consistent in all categories of patients. There may be an influence of estrogen in the protection and defence of hepatic cells against the development of chronic liver disease[97]. These observations are similar to the prevalence of the population of immigrants (52.9% of males and 47.1% females) in the Area of Tarragona during 2013. This data was obtained from the Statutory Reporting System of the Department of the Generalitat of Catalonia (IDESCAT, Barcelona)[98].

In agreement with the AASLD 2009 practice guidelines, the HBeAg+ CHB group had the highest median of viral load, ALT and AST levels, followed by Others and PreC CHB group[31]. These observations are similar from previous studies which report that HBV DNA and ALT levels are significantly lower in HBeAg-negative than HBeAg-positive patients. However, spontaneous recovery is rarer, long-term prognosis is poorer, and histological lesions are more severe in HBeAg-negative patients than in HBeAg-positive patients[99,100]. High viral loads have been implicated as a risk factor for HCC development[101]. Moreover, the majority of the HBeAg-negativity with high levels of ALT was as a result of the classical G1896 mutation, which abolishes HBeAg expression and occurs frequently in genotype D or E.

Other mutations including transcriptional A1762T/G1764A and translation initiation mutations were responsible of HBeAg-negativity in some infected patients. Thus, HBV genotype may influence the association between HBV load and HCC risk [13,102].

Additionally, for geographical and historical reasons, Catalonia and particularly, the Area of Tarragona has often acted as a gateway for the entrance of immigrants into Europe. Therefore, the constant migratory flow over the last decades may have contributed to the wide and heterogeneous spread of unusual infections and variants of common infectious agents such as HBV. It is known that HBV genotypes, subgenotypes and variants display a characteristic geographical distribution; with subgenotype D1and A2 predominating in Catalonia, D7 in Morocco, genotype F in South America and subgenotype C2 in China[103]; and occasionally, unique biological behaviour[1].

In the 2013, up to 17.83% of the 520200 people living in the Area of Tarragona were foreigners, Moroccans being the largest group (29.38%), followed by Romanians (14.63%), Colombians (4.78%), Italians (3.36%), Senegalese people (2.73%) and Chinese people (2.42%) (Data provided by IDESCAT)[98]. Thus, in the Area of Tarragona, where HBV infections are heterogeneous due to immigration, little is known about the prevalence of different genotypes and mutants of HBV in liver disease diagnosis, such as chronic hepatitis B. In the present work, prevalence of HBV infection with and without precore mutation was analysed in the recent three years.

The results of this study showed that the frequent age of being HBV-infected is more than 20 years approximately and perinatal population is not infected. These findings are consistent with HBV transmission and endemicity. According to World Health Organization (WHO, Geneva), in areas of intermediate or low endemicity, such as the Area of Tarragona, the predominant modes of HBV infection are sexual contact and intravenuous drug use, particularly in immigrant groups who are not immunized; whereas in areas with high endemicity, such as most countries of Africa or Asia, the high frequency of HBeAg-positivity in mothers infected with HBV, would be to its vertical mother-child transmission and horizontal transmission between children, particularly siblings. Although the increased incidence of immigration of areas with high endemicity, the incidence of hepatitis B in babies born is negligent due to the coverage of vaccination programmes. However, despite the impact of disease prevention measures and maintenance of high vaccination coverages are important; this impact might be offset by an increase in cases in adult immigrants. For this reason, vaccination strategies for risk groups as immigrants should be reinforced[104,105]. These recommendations should also be applicable to susceptible immigrants (children and adolescents) coming from countries with high or intermediate prevalence where hepatitis B vaccines have still not been launched or where coverages are still low[2].

An interesting finding in this study, concerning to gender, is that the prevalence of HBV-infected patients and patients infected with HBV-precore variant was significantly higher in males than females. The frequent occurrence of high prevalence in men aged from 20 to 50 years might in part be explained by the incidence of immigration. However, precore variant occurred the most frequently in the age interval of 41-50 years, whereas the most frequently age interval in wild infected patients was 31-40 years.

This finding was supported by the results of a previous study suggesting that, with regard to the precore mutations, older aged patients (more than 40 years) were more prevalent to be precore mutants[103].

The majority of immigrants, in both male and female, corresponded to immigrants aged to 30-34 years (13.81%), followed by 35-39 years (12.74%), 25-29 years (10.59%) and 40-44 years (10.15%). Moreover, according to gender, immigrant males were relatively higher than immigrant females. Of the overall immigrant population in the Area of Tarragona in 2013, a percentage of 52.95% were men, whereas a percentage of 47.05% were women. (Data not shown from IDESCAT[98]).

Particularly, comparable prevalence of chronic hepatitis B infection, with wild HBV or precore HBV variant, have been analysed in HBsAg-positive patients and precore mutants between 2009 and 2013. The prevalence of HBsAg-positive patients in 2013 was relatively similar to those reported in the study in 2009[94]. The reason that may account for the similar prevalence of HBV-infected population in both years is the prevention measures or that the percentage of immigrants has decreased a 1.40% from 2009 to 2013. However, discrepant results were obtained in the prevalence of chronic HBV-precore variant infection because, while the prevalence of HBsAg-positive population has not varied, of the 386 samples analysed in 2013, close to 6% belonged to HBV infected patients with precore variant, whereas of the 391 studied in 2009, only 2.5% corresponded to precore mutants. That is, the prevalence of precore mutants has increased 3.2%. Taking into account other studies, this difference among populations might be due to the fact that not only does immigration influence the prevalence of the disease but it also influences the HBV genotype and subgenotype. This affirmation was supported by the difference in immigrant incidence from 2009 to 2013 (Table 10). Moreover, because this chronic HBV infected population were not vaccinated for HBV, these mutations at precore region may have emerged as a result of host immune pressure.

Table 10. Percentage of the six more prevalent immigrant countries of 2009 and 2013

2009		2010	
COUNTRY	**%**	**COUNTRY**	**%**
MOROCCO	26.46	MOROCCO	29.38
ROMANIA	13.85	ROMANIA	14.63
COLOMBIA	5.85	COLOMBIA	4.78
ARGENTINA	3.45	ITALY	3.36
ITALY	3.22	SENEGAL	2.94
SENEGAL	2.76	XINA	2.61

Table adapted from IDESCAT[97]

In particular, an HBV prevalence of 10% was observed in the general population of China, meaning hepatitis B was an endemic in China[104,105]. Moreover, several reports regarding HBV precore mutations from chronic patients have led to the conclusion that the more predominant genotypes prone to precore mutations are genotype B, C and D with the exclusive predominance of subgenotype C2, which is the most frequent in Northeast Asia and particularly, in China. Thus, whereas China was not in the first six prevalent countries in the Area of Tarragona in 2009, it was the sixth country more prevalent in 2013.

These findings may give an insight into the mechanism of hepatitis B. In all, understanding the characteristics of HBV, in particular genotypes and mutations, is crucial for early diagnosis, optimized treatment and enables clinicians to identify those patients at increased risk of disease progression whilst aiding the selection of appropriate antiviral therapy, as detecting for resistance should be done as quickly as possible. Furthermore some precore mutation might be used as a biomarker for the prediction of future disease activity and might be helpful in the identifications of patients that should be regularly followed for HBV DNA level or require therapeutic intervention. Therefore, genotyping, monoclonal subtyping, and HBV sequencing to detect HBV mutations, can provide useful information and help practicing physicians to identify CHB patients at risk of disease progression and to decide on the choice of antiviral treatment and further management of hepatitis B carriers. Thus, an accurately assigning for an HBV strain is of clinical, therapeutical and epidemiological importance. However, the guidelines from two regional bodies, AASLD and EASL, all stop short of recommending genotyping as part of the management of chronic hepatitis B. The recommendations, however, from several national organizations as well as from individual reviewers suggest that genotyping is essential to detect patients in whom the use of pegylated interferon will give a high likelihood of response with a finite course of therapy and avoid the disadvantages of nucleoside analogues with their viral resistance.

Finally, the results show that the overall incidence of HBV infection has declined or has not increased owing to the universal vaccination programme of infants and preadolescents and given the seriousness of HBV infection for individuals and for community. However, it also indicates that HBV precore mutations, in our environment, occur mainly in middle-aged men patients and has increased. Vaccination is one way to prevent it, and several strategies can be used depending on endemicity, the main pattern of HBV transmission and the demographic structure of the population. This finding may give an insight into universal vaccination programmes, which should be continued and measures to increase vaccination of risk groups, such as middle-aged immigrant men among others, should be reinforced.

CONCLUSION

In conclusion, the results in this study confirm that during the recent three years, the incidence of chronic hepatitis B infection with precore mutations has increased in the Area of Tarragona due to immigrant population and variability of HBV genotypes that are more prone to precore mutations.

Thus, it could be suggested that determination of HBV genotype and gene mutations should form part of the clinical management in treating chronic hepatitis B. Moreover, some mutations could be useful as biomarkers to predict HCC risk.

It would be worthwhile to examine the genotype of HBV infecting people living in the Area of Tarragona for mapping the epidemiology of genotypes and finding any clinical relevance.

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