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Review

Distribution of HBV genotypes F and H in Mexico and Central America

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The distribution of HBV genotypes is associated with populations of specific geographical regions of the world. We show data from the GenBank sequence database and medical reports, which indicate that HBV genotype H (HBV/H) is mainly distributed in Mexico, whereas HBV genotype F (HBV/F) is distributed in countries from Central America. The phylogenetic analysis and historical records suggest that HBV/H has been present in Mexico even before the arrival of the Spaniards. Interestingly, occult hepatitis B is a common finding in both natives and patients with chronic liver disease in Mexico. This suggests that an immunogenic background could be important during the natural history of liver diseases. The estimated large number of HBV/H-infected patients in Mexico does not correlate with the total number of patients with chronic liver disease and cirrhosis reported in the country. This may be because of the fact that HBV infection is often masked by alcoholic liver disease, HCV coinfection and/or obesity. Here, we analyse the data concerning the distribution of HBV/F and HBV/H genotypes in Central America and Mexico. Specifically, we focus on the effect of molecular epidemiology and pathogenesis of HBV/H. These recent findings reveal new areas of study with therapeutic potential in viral liver diseases.

Introduction

Currently, 10 HBV genotypes (A–J) with a heterogeneous worldwide distribution have been recognized, and it is accepted that these genotypes are associated with particular native host populations inhabiting a specific geographical area. For instance, HBV genotypes B and C predominate in Asia, E in Africa, and A and D in Europe. However, genotype A also circulates in Africa, genotype D has worldwide distribution, and genotypes I and J are distributed in Southeast Asia. HBV genotype F (HBV/F) is mainly confined to the Amerindian population from Central to South America, and it has been suggested that HBV genotype G (HBV/G) has emerged from Africa, although most of the strains have been detected in the US and Mexico [1–3].

The HBV/F genotype has been detected mainly among the Amerindian population from Central to South America. However, although two different clinical studies, one performed in Barcelona [4] and another in Alaska [5], have associated HBV/F genotype with severe liver disease and hepatocellular carcinoma (HCC), no further studies have been described in Latin American countries. In addition, a new HBV genotype described in 2002 was designated as genotype H (HBV/H) and assigned to Central America [6]. Since then, HBV/H has been mainly detected in Mexico but not in those countries that fall within Central America. Interestingly, unlike the Asian countries which are located in geographical regions where HBV genotypes B and C are predominant, and the endemicity of both HBV infection and HCC is high [7], HBV/H predominates in Mexico, which is considered a region of low endemicity for HBV infection [8], and where the presence of HCC is rare [9,10]. Taken together, this information may be indicative of a natural course of liver diseases in Mexico different from that which is described in high endemic areas.

Furthermore, the Health Secretariat in Mexico has reported a mortality rate due to liver disease of 25.1/100,000 inhabitants per year from 2003 to 2010 [11]. Alcohol consumption, HCV infection and non-alcoholic steatohepatitis (NASH), which is currently, increasing among the population, are the main aetiological risk factors identified, whereas HBV infection may be underestimated as an aetiological risk factor to date [8].

Therefore, since the current epidemiology of HBV infection and the described association between viral

genotypes and the severity of liver disease and/or specific responses to antiviral therapy are not fully established, the aim of this study was to analyse the distribution of HBV genotypes F and H in Mexico and Central America, as well as the relationship between HBV/H and viral pathogenesis.

Distribution of HBV genotypes in Mexico and Central America

In 2002, Arauz-Ruiz *et al*. [6] described two HBV strains from Nicaragua and one from the US with a nucleotide divergence of little more than 8% compared to HBV/F, and designated it as HBV/H. At the same time, the authors suggested that the origin of this new genotype was from Central America. However, since then, new strains of HBV/H have not been detected in any country of the Central American region.

The distribution of 102 HBV DNA sequences reported in the GenBank database from Central America is as follows: HBV/F is reported in Guatemala (*n*=1), Honduras (*n*=5), El Salvador (*n*=5) and Panama (*n*=2); in Nicaragua, HBV genotype A (HBV/A; *n*=2), HBV/F (*n*=5) and HBV/H (*n*=2; the originally reported strains); and in Costa Rica, HBV/A (*n*=1), HBV genotype D (HBV/D; *n*=4) and HBV/F (*n*=75) are reported [6,12–14]. In conclusion, HBV/F is predominant among the Central American countries.

By contrast, among 106 HBV DNA sequences reported from Mexico, HBV/H is predominant in 58.7% of the cases, followed by A in 14.4%, G in 10.6%, D in 3.8%, C in 1.9% and B in 1% [15–17]. The remaining 9.6% belong to 11 sequences that were originally reported as HBV/F in the GenBank database, but are actually HBV/H genotype (AF369531, AF369535–AF369543 and AF369545) [15]. From a total of 81 HBV/H strains reported in the GenBank database, 76.5% correspond to Mexico [15–18], 7.4% to USA, 2.5% to Nicaragua [12], 2.5% to Argentina [19], 1.2% to Thailand and 9.9% to Japan [20–24]. Moreover, patients infected with HBV/H in countries outside of Mexico have reported to have travelled primarily to the US (San Francisco, CA) or Mexico.

The estimated maximum likelihood phylogeny of complete sequences of HBV/H genomes worldwide, including the Mexican isolates, exhibit a distinguishable genetic divergence of HBV genotypes F and H from a common ancestor. HBV/H lineage is shorter than HBV/F, highly diverse and paraphyletic (Figure 1). Further studies are required to explain the tendency of these sequences to cluster in multiple and nested clades, which could reveal the pathway of the human migrations through ancient Mexico towards South America. Therefore, with the sequence data and clinical studies, it is clear that HBV/F is predominant in Central America, including Guatemala, Honduras, El Salvador, Costa Rica, Nicaragua and Panama, whereas, in Mexico, the main HBV genotype is H (Figure 2).

Historical background of the Mexican population: Aztecs, Chichimecas and Mayas

For historical reasons, Mexico, like most of the Latin American countries, has a heterogeneous population that has risen from the admixture of Amerindian, African and Caucasian populations [25–27]. During the conquest, one of the major empires of the American continent was that of the Mexicas or Aztecs. The geographic region of this empire, considering the actual geographical definition, was Central Mexico to Guatemala, through the Maya region in southern Mexico. West Mexico was inhabited by the Chichimecas, a group of nomadic or seminomadic people that lived in a region that was not dominated by the Aztecs, nor the Spanish conquerors [28]. Once the Spaniards dominated the Aztec empire, New Spain was founded in the same territory, and the conquerors enslaved the native population and both groups mixed between each other giving rise to admixed people named 'mestizos'. The conquerors brought African slaves, who also mixed with them, originating the 'mulatos'. Thereafter, the admixture of the three races emerged into different social groups named 'castas' [29,30].

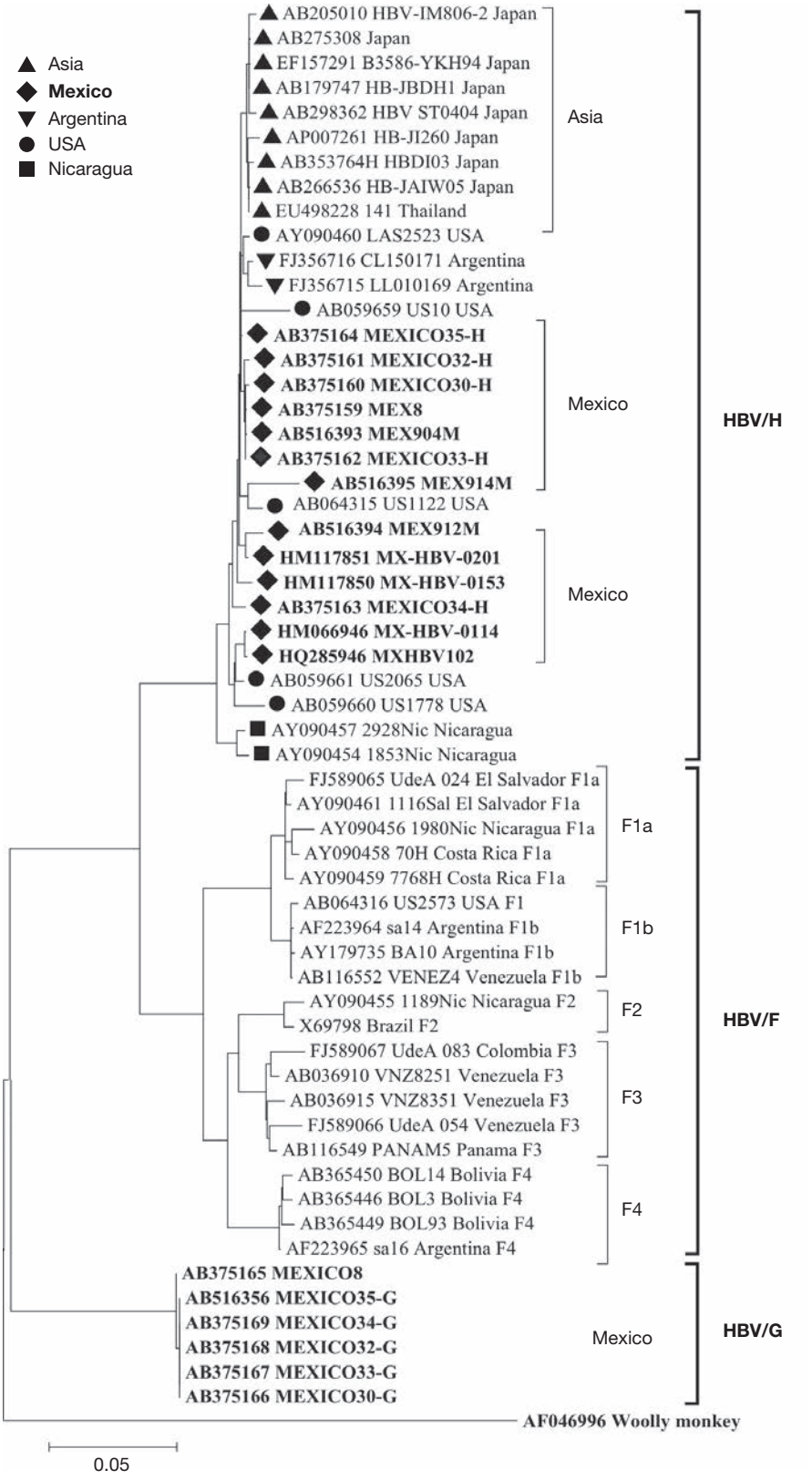
The history of west Mexico is different; the Chichimecas did not yield to the Spanish conquerors and, only after 80 years of battles, were they virtually eliminated [28]. In the mountains of west Mexico, the Huichol have survived to date as the descendents of those Chichimecas resistance fighters. At the same time, this geographical area was named New Galicia, and since then, it has been characterized as a region with a mainly White population [30] (Figure 3A).

This history defines most of the real Mexican society today. The western part of Mexico is characterized by mestizos, with a predominance of White people; in the centre are the mestizos with a preponderance of Amerindian population, descendants of the Mexicas or Aztecs (also named Nahuas), and the south is characterized by a native Amerindian population.

Distribution of HBV genotypes in native populations

Recently, we have identified the HBV genotypes in two groups of native Mexican populations [16]; the Huichol from the west, descendents of the Chichimecas, and Nahuas, descendents of Mexicas or Aztecs from central Mexico. In both native groups, a high endemicity of HBV infection and a high prevalence of occult hepatitis B infection (OBI) were detected. OBI is

Figure 1. Maximum likelihood phylogeny of HBV genotype H and F genomes



A total of 31 complete genomes of HBV genotype H (HBV/H; 18 worldwide and 13 Mexican isolates) and 20 representative sequences of HBV genotype F (HBV/F; subgenotype F1–F4) were retrieved from GenBank to compare their genotypic phylogeny. Each isolate is labelled by its accession number and country of origin. HBV/G, HBV genotype G.

defined as low titres of HBV genomes detectable in the serum (<200 IU/ml) or liver tissue of hepatitis B surface antigen (HBsAg)-negative subjects that are either positive or negative for serological markers of previous HBV infection [31]. In addition, no clinical or laboratory data evidence of overt liver damage was found in these groups. In the Nahuas, the HBV/H genotype is predominant in 62.5% followed by genotypes C and D (12.5% each), and genotypes A and B (6.5% each). Therefore, this study may indicate that HBV/H was present among the Aztec population before the arrival of the Spaniards. By contrast, in the Huichol, HBV/A is predominant in 55.6% of the cases, followed by D in 22.2%, with G and H accounting for 11.1% each [16]. The explanation may be that, during the colonial period, African slaves could have fled into the mountain range, home of the past Chichimecas population. However, due to the practice of cannibalism among the Chichimecas, the fugitive slaves might have been hunted down rather than mixing or intermingling with the native population. Further studies are needed to investigate whether HBV genotypes other than HBV/H were already in America or were incorporated into the continent more recently. Also, it would be interesting to investigate, whether outbreaks of HBV could have been part of the many different epidemics that afflicted the Amerindian population leading to their significant decrease during the first century of the conquest [25].

Epidemiology of HBV in Mexico

Mexico has >110 million inhabitants, of which 12 to 14 million are part of the native population distributed among different ethnic groups. The remaining population corresponds to mestizos living in the large cities or urban areas [32]. High endemic areas of HBV infection among the native population have been detected, as 40% to >80% of individuals from native study groups are positive for antibody against hepatitis B core antigen (anti-HBc) [8,33]. Therefore, we could extrapolate that ≥6 to 7 million of the native people have been exposed to HBV.

Figure 2. Distribution of HBV genotypes in Central America and Mexico as reported in GenBank

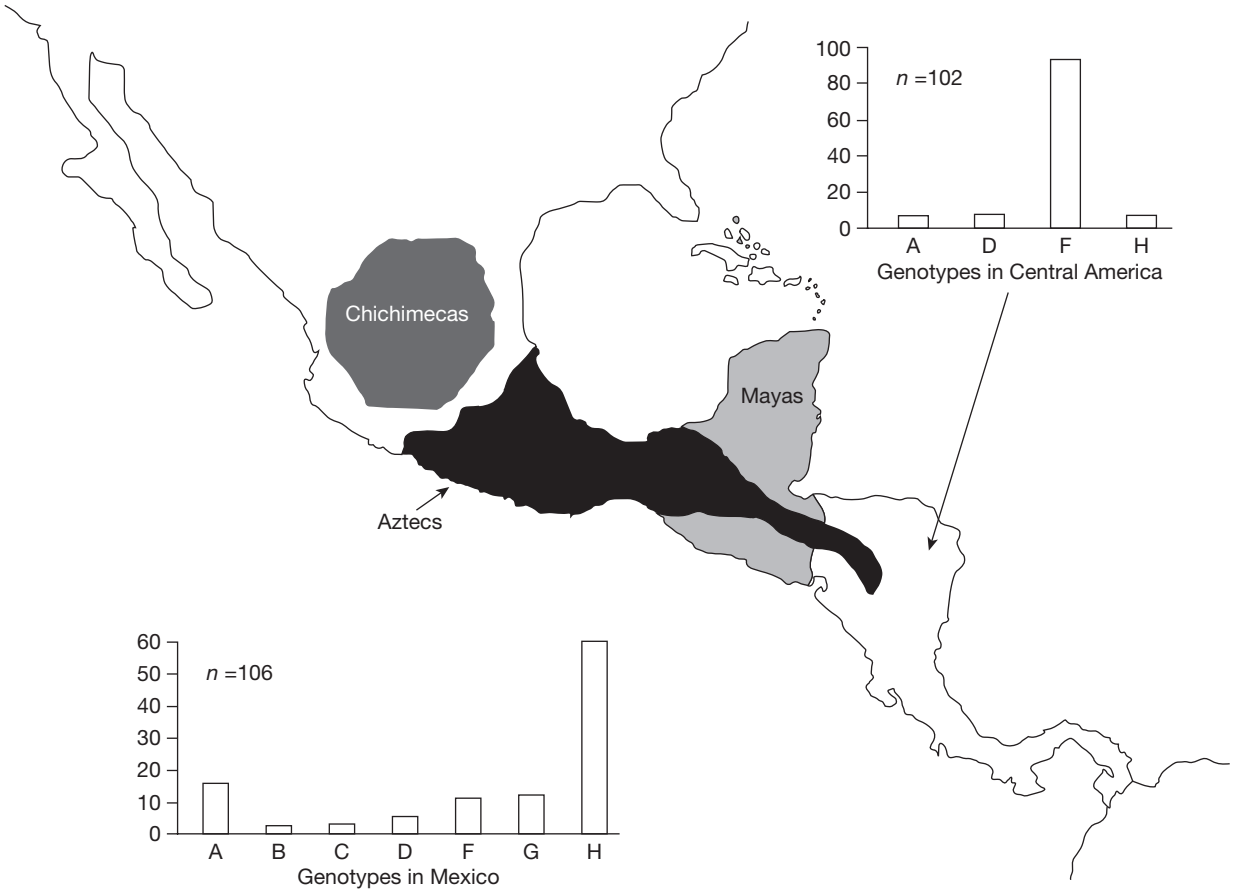
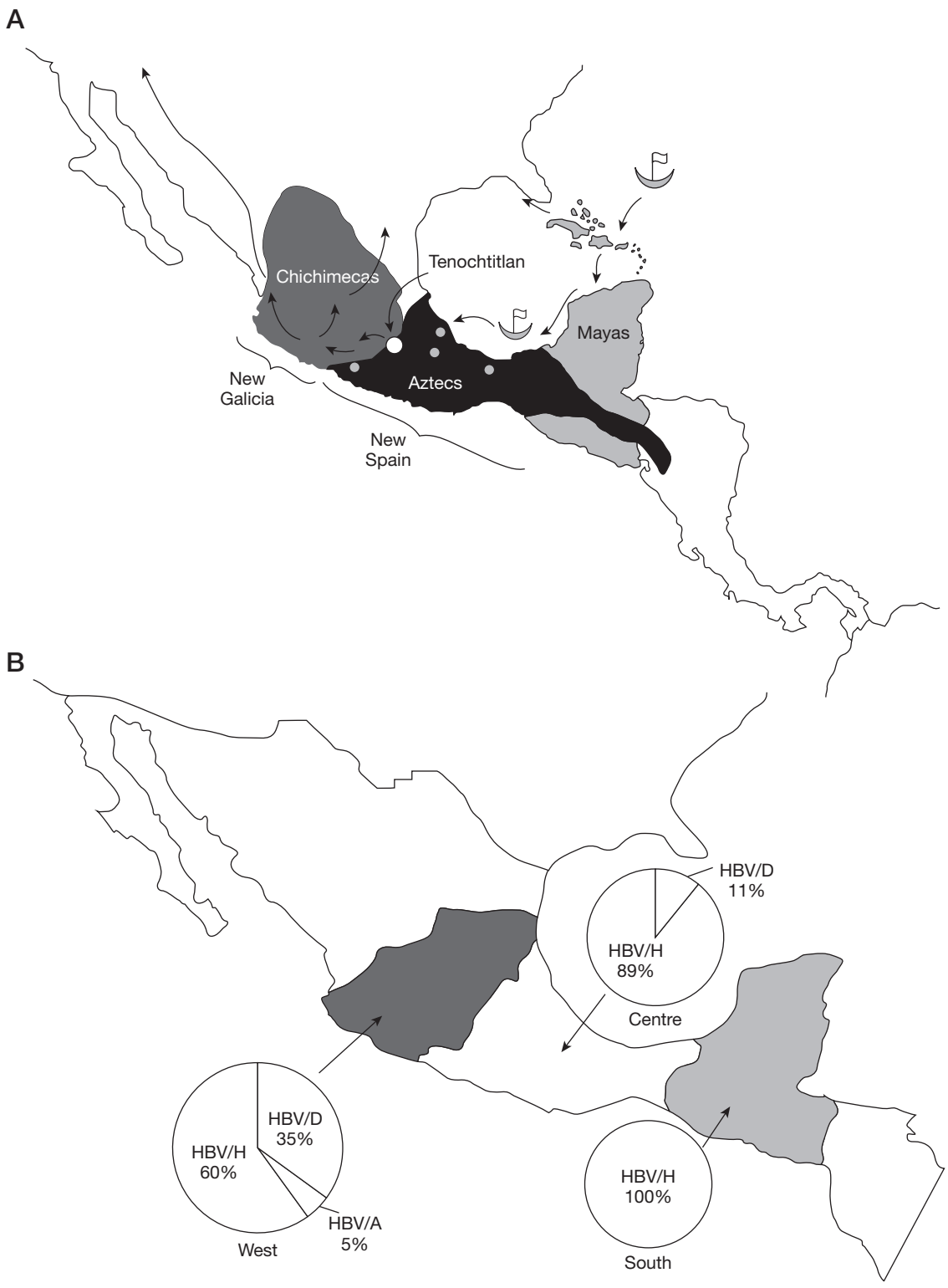


Figure 3. Geographic distribution of the ethnic population before 1519 and HBV genotypes in the Mexican poulation at present



(A) The distribution of the Mexican population before the period of the Spanish conquest (1519 to 1541). **(B)** Geographic distribution of the HBV genotypes among the mestizo population with liver disease in 2010. HBV/A, HBV genotype A; HBV/D, HBV genotype D; HBV/H, HBV genotype H.

The HBsAg seroprevalence among the general population in Mexico has remained low (0.3%) since the first studies were carried out in 1974, to date, despite the recent HCV and HIV epidemics, reported in Mexico City during the 1980s and 1990s [8]. However, unlike the native population, the prevalence of anti-HBc among the general population is very low during the first decade of life, increasing in adulthood, so that almost 15 million adults have been exposed to HBV during their lifetime [34]. This increase of HBV infection in adulthood is associated with the main risk factors detected in the general population, such as sexual relationships and promiscuity, as well as exposure to HBV-containing biological fluids [35]. Other risk factors that have been identified include nosocomial infections via blood transfusions and surgical interventions [35]. Up until 2006, the use of intravenous drugs was not established in Mexico, since the main recreational drugs were inhalants and marihuana [8,35,36]. However, since 2006, the number of cases of intravenous drug abuse has increased, as well as the number of people with tattoos; both significant risk factors associated with the increase of drug violence and spread of drug dealers [36]. In summary, considering both the native and urban populations, current estimations are that approximately 20 million Mexicans have been exposed to HBV. However, this information is not concordant with those reported by the Mexican Health Secretariat.

From 2000 to 2007, the Mexican Health Secretariat reported a total of 192,588 cases of viral hepatitis in Mexico, of which 79% corresponded to HAV, 6% to HCV, 3.3% to HBV and 12% corresponded to hepatitis with unknown origin. HBV is predominant in juvenile and adult populations. HAV infection is frequent in children, whereas HCV is mainly prevalent in adults [35,37].

Several epidemiological studies have shown that, in Mexico, 400,000 to 1.4 million people may have been infected with HCV [38–41], and other studies suggest that the prevalence of HBV infection could be higher than HCV [35]. This discrepancy could be due to missed HBV diagnosis, a strong and effective immunological response to HBV and HCV [42], and specific genomic characteristics of HBV itself due to the particular genotype or to the intriguing interrelationship between the host and HBV among the Mexican population.

The fact that HBsAg is not always detected in tested samples and the high prevalence of OBI among the Mexican population without overt liver disease may indicate a lack of the sensitivity of HBsAg detection kits. This is feasible given that the available kits used for diagnosis were designed according to genotypes distributed in different geographical regions that do not correspond to the genotypes prevalent in Mexico [8,43]. Alternatively, a strong and effective immunological response against the virus thereby reducing the overall prevalence of HBV infection in the population could be responsible for the undetectable levels of HBsAg observed [16]. In this latter regard, a robust T-cell response for a protective memory has been suggested during OBI development, supporting an essential role of T-cell response during the infection [44]. Thus, the immune characteristics of the Mexican population may result in an effective T-cell response able to maintain a low viral load in OBI patients. Recent studies from our laboratory show a distinctive cytokine profile in OBI genotype H native Mexican patients, where the OBI patients displayed an increased interleukin-2 secretion along with a typical inflammatory profile. Our data suggest that viral transcription repression that results in HBsAg negativity and undetectable levels of serum HBV DNA may be interleukin-2-modulated in OBI genotype-H patients [42].

By contrast, preliminary virological evidence of OBI cases in the Mexican population has shown nonmutations in the X region, responsible for viral replication, and in only one case was a stop codon mutation detected (unpublished observations). Further studies on the genetic background and viral characteristics should be addressed in order to determine their effect on the development of OBI in specific populations.

Molecular diagnosis of HBV and genotypes in patients with risk factors for viral hepatitis in west Mexico

Our Department of Molecular Biology in Medicine, at the Hospital Civil de Guadalajara 'Fray Antonio Alcalde' (Guadalajara, Mexico), has become a National Reference Center for the clinical and molecular analysis of viral hepatitis. In an attempt to explain the actual molecular epidemiology of HBV and HCV infections in Mexico, we first selected from our reference database 265 serum samples of patients from western Mexico with any risk factor for viral hepatitis. Of these, 119/265 (45%) were positive for antibody against hepatitis C (anti-HCV), whereas 40/265 (15%) were seropositive for HBsAg. However, when molecular diagnostics was performed for both viruses, the number of HCV-RNA-positive cases decreased to 85/265 (31.6%), whereas the positive cases for HBV DNA increased to 70/265 (26.4%). Therefore, OBI was detected in 30/265 (11.32%); by contrast, among 42.8% (30/70) of the HBV-infected patients, the diagnosis of HBV infection was missing [45,46].

Regarding HCV infection, most of the HCV-infected patients had chronic hepatitis (54%) and cirrhosis (38%) with a lower portion of acute hepatitis, asymptomatic hepatitis or associated alcoholic liver disease. By contrast, approximately 60% of the HBV-infected

patients had chronic liver disease (cirrhosis 25% and chronic hepatitis 35%) and a higher frequency of asymptomatic hepatitis (12%), acute hepatitis (15%) or associated alcoholic liver disease (13%) than in the HCV-infected patients. HCC was not detected in patients with HCV or HBV infection.

HBV genotypes were determined in 51/70 HBV-infected patients [17,45,46]. The preponderant genotype was H (*n*=40), followed by D (*n*=9) and A (*n*=2). Of these patients, 25/51 were seropositive for HBsAg, and the remaining 26/51 had OBI. HBV/H was the prevailing genotype in both OBI versus HBsAg-positive patients (*n*=19 versus 21, respectively), followed by HBV/D (*n*=7 versus 2, respectively), and to a lesser extent HBV/A (*n*=0 versus 2, respectively). Most of the HBV/D cases were prevalent among the OBI patients.

Coinfection of HCV and HBV was detected in 3.9% of patients with risk factors for viral hepatitis (9/265). In the HCV-infected patient group, 7.5% (9/119) were coinfected with HBV, whereas in the HBV-infected patients, 12.87% (9/70) were also infected with HCV. The initial diagnosis in these patients was HCV infection, followed by HBV in the form of occult hepatitis. In summary, these studies show that the diagnosis of HBV infection is apparently missed in an important number of cases. Furthermore, OBI is common in HBV-infected patients and most of the chronic liver diseases such as cirrhosis, and chronic hepatitis is commonly associated with HCV-infected patients rather than in the former. By contrast, in HBV-infected patients, asymptomatic hepatitis, acute hepatitis and alcoholic liver diseases are associated with infection and are more frequent than in the HCV-infected patients.

Distribution of HBV genotypes, mixtures of HBV genotypes and viral load in HBsAg-positive samples from west, central and south Mexico

To investigate the prevalence and geographical distribution of HBV genotypes in Mexico, we selected 182 serum samples from HBsAg-positive patients from west, central and south Mexico analysed at our laboratory to date. HBV genotypes were determined by DNA sequencing and multiplex PCR techniques [15–17,47]. HBV/H was the predominant genotype in 67.4% followed by HBV/D in 25%, HBV/A in 4.9% and then HBV/G in 2.7%.

From a total of 145 HBV strains obtained from patients with chronic liver disease and seropositive for HBsAg, HBV/H was predominant in three different geographical regions of Mexico. In the west, besides HBV/H, both HBV/D and HBV/A were detected (*n*=60, 36 and 5, respectively). In central Mexico, HBV/H was detected in 25 cases, and HBV/D in 3 cases, whereas in south Mexico, all the samples tested were HBV/H (*n*=16). Such a distribution of HBV genotypes is concordant with the ethnic characteristics of the population, in which Caucasian inhabitants reside in the west, whereas the native population predominately lives in central and south Mexico (Figure 3B).

To test for mixed infection with distinct HBV genotypes, 39 HBV strains previously sequenced [17] or first-time sequenced were analysed by multiplex PCR [48]. By DNA sequencing, 20 strains were detected as HBV/H, 9 as HBV/D and 2 as HBV/A. However, with multiplex PCR, an increase in the number of HBV/D was found, suggesting a low viral load of HBV/D compared to HBV/H and HBV/A. Mixtures of HBV genotypes were further confirmed by cloning the positive strains [3,47]. Next, from a total of 147 samples analysed by multiplex PCR, 39 (26.5%) had HBV genotype mixtures with the following combinations: D/H 77%, D/G/H 7.6%, A/H 5.1%, A/D/H 5.1%, and A/D and A/D/G with 2.6% each (Figure 4A).

Viral load was determined in 82 serum samples of which 80% had a low viral load (<4,000 IU/ml), whereas <10% had viral loads >100,000 IU/ml. In 42% of the cases, viral load was undetectable, 38% had <4,000 IU/ml, 4% had 4,000–10,000 IU/ml and 6% had 10,000–100,000 IU/ml. When HBV genotypes were analysed by their viral load, the cases with HBV genotype mixtures had the highest viral load (>100,000 IU/ml), while HBV/D genotype was prevalent in cases with undetectable viral load and HBV/H in those that were <4,000 IU/ml (Figure 4B).

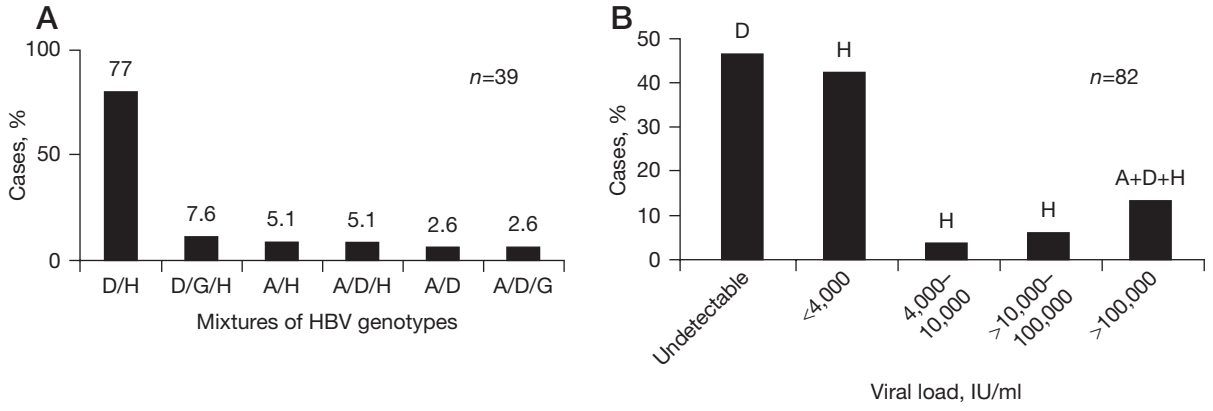
Clinical characteristics associated with HBV genotypes

OBI and low viral load appear to be the two main characteristics of patients infected with HBV/H. This is found not only in the high endemic areas, in the native population, but also in patients with chronic liver disease. By contrast, HBV/A has a major distribution in west Mexico and is associated with sexual promiscuity, including men who have sex with men. In addition, HBV/A genotype is more likely to be detected in acute cases as well as in those which reveal mixtures of HBV strains of different genotypes. HBV/D is also predominant in west Mexico and is typically found associated with a low viral load in mixtures of HBV genotype strains and in the setting of OBI.

In general, HBV/F is not commonly detected in Mexico; isolated strains had been detected in central and south Mexico, suggesting that this genotype is more predominant in Central and South America than in Mexico itself.

Although HBV/G has been suggested to have an African origin [49], most of the strains reported to date are

Figure 4. Mixtures of HBV genotypes and low viral load: common characteristics of HBV-infected Mexican patients



(A) Mixtures of HBV genotypes among 147 HBV-infected patients, of which 39 were coinfected with two or more distinct genotypes. **(B)** Distribution of viral load among Mexican patients infected with distinct HBV genotypes.

from the Americas, particularly from Mexico (unpublished observations and [3]). HBV/G has been linked mainly to men who have sex with men, as mentioned previously by Sánchez *et al*. [17] and Tanaka *et al*. [18] suggesting the anal–genital transmission as the main source of infection.

With regard to the clinical impact of OBI on HCV-infected patients, although the presence of OBI in HCV-infected patients has been established, its clinical significance is under investigation. Some studies have suggested that OBI could be responsible for the acceleration of chronic HCV progression and could interfere with antiviral therapy, and higher fibrosis stages in HCV-patients with OBI have been documented [44]. However, conclusive data, particularly those associated with Mexican and Central American OBI cases, have not been established.

Role of HBV infection on the current burden of liver disease in Mexico

In a preliminary study performed by our group, 600 patients from west Mexico with liver disease were included. The main aetiological factors found in this group included alcoholism in 44%, followed by HCV in 35% and NASH in 9%, whereas HBV was found in <2%, and other risk factors accounted for the remaining 10%. Interestingly, when patients with only cirrhosis at diagnosis were studied, the main aetiological factors included alcoholism in 62%, HCV in 20%, NASH in 12% and other factors totalled 6% (unpublished observations). Thus, if we consider the data on HBV infection, including OBI, we would expect that ≥6% of the mortality in the group could be due to HBV infection. The lack of detection of HBV may be because infection is being masked by alcoholism, obesity and HCV infection.

To summarize, if we consider the number of people infected with HBV in both native and urban populations in Mexico, we would estimate that almost 20 million individuals have been infected with HBV; of these, ≥1 million would have HBV-associated liver disease. This makes it potentially plausible to estimate approximately 1,500 deaths per year due to HBV.

Conclusions

Sequence data from GenBank, as well as medical reports, show that HBV/F predominates in countries located in Central and South America whereas HBV/H is confined to Mexico. The HBV/H genotype is predominant in both natives and mestizos suggesting that this genotype has been present in Mexico or among the descendants of the Aztec population, even before the arrival of Caucasians to the Americas.

HBV/F is uncommonly detected in Mexico, whereas HBV/H predominates in high endemic areas among the natives and in patients with risk factors for viral hepatitis and with liver disease. HBV/H is commonly detected in these areas as OBI, and in acute hepatitis or asymptomatic hepatitis. Although HBV is present in patients with liver disease, it is masked by other aetiological agents, which are common in Mexico, such as alcoholism, coinfection with HCV and obesity. When HBV/H is detected, it usually has a low viral load. This is plausibly due to an effective host immune response, except in those cases of acute hepatitis or chronic liver disease coexisting with mixtures of other HBV genotypes.

Future studies that involve distinct ethnic groups from Central America are necessary to determine the distribution of HBV genotypes and how host innate and adaptive responses, as well as immunogenetics, may influence HBV infection and the development of significant clinical sequelae.

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Disclosure statement

The authors declare no competing interests.

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