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Title

Update on epidemiology of hepatitis B in a low-endemic European country: there is still much to do.

Running title: HBV epidemiology in Spain

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List of abbreviations

HBV = Hepatitis B virus; HBsAg = Hepatitis B surface antigen; ETHON = Epidemiological sTudy of Hepatic infectiONs; HBV-DNA = Hepatitis B virus DNA; ALT = alanine

aminotransferase; anti-HBc = antibody to hepatitis B core antigen; HBeAg = hepatitis B e antigen; HCC = hepatocellular carcinoma; CHB = chronic hepatitis B; EU = European Union; HCV = hepatitis C virus; PCR = polymerase chain reaction; HUPH = Hospital Universitario Puerta de Hierro; HUMV = Hospital Universitario Marqués de Valdecilla; HUCV = Hospital Universitario Clínico de Valencia; FSU = First-stage units; SSU = Second-stage units; CI = Confidence interval; IQR = interquartile range; OR = odds ratio; IRB = Institutional Review Board; LFTs = liver function tests; HIV = human inmunodeficiency virus; HAART = highly active antiretroviral therapy; NAs = nucleos(t)ide analogs; cccDNA = covalently closed circular DNA; WHO = World Health Organization.

ABSTRACT

The latest epidemiological data in Spain were obtained a decade ago and revealed a prevalence of hepatitis B surface antigen (HBsAg) of 0.7%; hence, updated epidemiological data is necessary. Our aim was to determine the prevalence of hepatitis B virus (HBV) infection, as well as to analyse associated factors and characterize chronic infection. A population-based, cross-sectional study was performed in Spain between July 2015 and April 2017. Participants from 3 regions were selected using two-stage conglomerate sampling and stratified by age. Anthropometric and demographic data were collected, and blood samples taken to detect serological markers of HBV infection and to quantify HBV-DNA. The characterization of chronic HBV infection was based on ALT (alanine aminotransferase) values, HBV-DNA levels, and results of transient elastography. The overall prevalence rates of HBsAg and antibody to hepatitis B core antigen (anti-HBc) among 12,246 participants aged 20–74 years (58.4% females) were 0.6% [95% CI (0.4-0.7)], and 8.2% (7.7-8.7), respectively. The risk factors for HBV infection identified in the multivariate analysis were age, nosocomial risk, and non-

Spanish nationality. Moreover, most patients HBsAg positive (76.6%) presented as hepatitis B e antigen (HBeAg) negative chronic infection (formerly “inactive carriers”) and only 6 (9.4%) HBsAg carriers fulfilled current criteria for treatment. The current HBV burden in Spain remains low but virtually unchanged over the past 15 years. Increased efforts are still needed to reach the goal set forth by the World Health Organization (WHO) for HBV elimination by 2030.

Keywords

Epidemiology; HBV; hepatitis B; prevalence; screening; Spain.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a major health threat with approximately 250 million carriers worldwide many of them being at risk of developing cirrhosis and even hepatocellular carcinoma.[1] There is great variability on a global scale regarding HBV chronic infection being Spain a low-endemic southern European country.[1]

The implementation of vaccination programs against HBV, along with other primary prevention measures, including health care infection control and antenatal screening, have led to a decrease in the incidence of acute and chronic hepatitis B (CHB) in many European Union (EU) countries.[1,2] However, migration is currently changing the prevalence of HBV in several low endemic countries due to higher HBsAg prevalence rates in migrants and refugees from outside Europe.[1]

In 2016, the World Health Assembly passed a resolution to eliminate viral hepatitis as a public health threat by 2030.[3] The targets include 90% global coverage of three-dose infant vaccination by 2020, diagnosis of 90% of people infected with HBV, and antiviral treatment of 80% of those diagnosed with HBV and eligible for treatment. Timely, reliable prevalence data are needed to identify the populations that are most affected in order to improve screening and treatment programmes and to monitor the performance and impact of these activities at a strategic level. There are some recent epidemiological reports in different regions worldwide, including countries in southern and western Europe with low prevalence, that indicate a decline in average HBsAg prevalence rates to 0.6%, or even 0.3% in Spain.[1,2] However, these reports are based on estimates from systematic reviews of peer-reviewed literature on HBV prevalence in the general population. Population-based studies are necessary to confirm these estimates and update the latest epidemiological data in Spain that come from studies carried out in the first decade of this century, which showed HBsAg prevalence figures of 0.7%.[4,5]

The aim of this study was to update the current burden of HBV infection in Spain, to analyse the associated risk factors, and to characterize chronic infection.

MATERIALS AND METHODS

This observational, cross-sectional, population-based study on HBV and HCV infections was carried out in Spain between July 2015 and April 2017 (NCT02749864). The design of the study and the results regarding HCV infection have been previously published.[6] The cohort of patients with positive HbsAg was retrospectively analyzed to adequately characterize the infection. Overall 12,263 subjects aged between 20 and 74 years old were estimated to participate; they were stratified in three age cohorts (20-34 yr., 35-49 yr., and 50-74 yr.) according to the different expected prevalence. An uptake of 9−15%, via telephone invitation was estimated. Recruitment was made through a structured phone call that allowed collecting anonymous data from those who declined. Participants underwent a physical examination and were invited to complete a self-reporting questionnaire that was supervised to decrease the missing values (**Table S1**).

Blood samples were collected for complete blood count, biochemistry, and serological markers, and they were stored in the Valdecilla Biobank. The presence of HBsAg, and antibody to hepatitis B core antigen (anti-HBc) were assessed by immunoassay (ARCHITECT-i2000SR, Abbott Laboratories, Germany). In HBsAg positive patients, the HBV-DNA blood levels were measured using real-time polymerase chain reaction (PCR) and quantified using the automatic COBAS TaqMan HBV Test 2.0 equipment (15 IU/mL sensitivity). Abdominal ultrasound and transient elastography (FibroScan-Echosens) were performed to determine liver stiffness in HbsAg positive patients.

A descriptive analysis was performed. The adjusted association with the anti-HBV seroprevalence was investigated with a logistic regression analysis by introducing variables that were related to anti-HBV seroprevalence in a univariate analysis ($P<0.1$), or that were considered clinically relevant regardless of statistical significance. The strength of association was estimated using the odds ratio (OR) with 95% CI. Analyses were performed using SPSS Statistics for Windows, Version 21·0 (IBM Corp, Chicago, USA). All the P-values were two-tailed, with statistical significance defined as $P<0·05$. The Institutional Review Board (IRB) of Cantabria approved this study on March 13, 2015, and it was conducted in compliance with the Declaration of Helsinki of 1975 and revised in 2013. All the subjects gave written informed consent.

RESULTS

12,246 participants were recruited, which represents 22.8% of recruitment success (**Figure 1**). 83.5% of those who declined to participate answered a questionnaire. Significant differences between these 2 groups were observed according to the respondent's age, sex, and level of education (**Table S2**). However, only differences in the educational level seemed to discriminate between both groups, with 1.5 times more subjects presenting lower education levels in the group that declined to participate [51.6% vs. 35.0% ($p<0.001$)].

Participants were predominantly female (58.3%), Spanish nationals (93.7%), employed (56.7%), and with middle-upper levels of education (65%) [**Table S3**]. The most common, self-reported HBV risk factor among subjects was nosocomial risk (79.2%) [**Tables S3-S4**). Up to 23% of the general population presented abnormal alanine aminotransferase levels.

Overall, 67 in 12,246 individuals analysed were HBsAg positive, which accounts for a prevalence of 0.6% [95% CI (0.4-0.7)], with infection rates highest in older subjects (**Table S5; Figure S1)**; anti-HBc seroprevalence was 8.2% (7.7-8.7). 46.3% of HBsAg positive patients were already aware of their serological status (**Figure S2**). Factors that were significantly associated with HBsAg detection in the multivariate analysis were age, non-Spanish nationality, and nosocomial risk (**Table S6**). Eleven of 67 (16.4%) HBsAg positive patients were not born in Spain, which equates to a prevalence of infection of 1.4% (95% CI 0.8-2.5) in this population whereas the anti-HBc seroprevalence was 12.0 (93/772; CI 95% 9.9-14.5) (**Figures S3-S4**). **Table S7** shows the variables associated with the risk of having detectable anti-HBc.

Two HBsAg positive patients were lost to follow-up and their hepatitis B e antigen (HBeAg) status could not be assessed; another patient presented as an acute co-infection human inmunodeficiency virus (HIV)-HBV that was treated accordingly (**Table S8; Figure 1)**. Six in 64 [9.4% (95% CI 4.4-18.9)] patients were HBeAg positive/AntiHBe negative; all of them corresponded to chronic infection.[7] Alternatively, 58 in 64 [90.6% (95% CI 81.0-95.6)] patients were HBeAg negative/anti-HBe positive. Overall, 49 of these patients (84.5%) were identified in a chronic infection phase (formerly "inactive carrier"), whereas 5 (8.6%) were recruited in a chronic hepatitis phase and the remaining 4 patients could not be classified (**Figure 1**). Only 6 in 64 [9.4% (95% CI 4.4-18.9)] HBsAg

carriers fulfilled current criteria for treatment (**Figure 1**). Most patients had low-grade fibrosis (<F2: 60; 95.2%).

DISCUSSION

Updated HBV sero-prevalence rates were 0.6% and 8.2% for HBsAg and anti-HBc respectively in Spain. The risk factors for HBV infection were age, nosocomial risk, and non-Spanish nationality. Moreover, a majority of HBsAg positive patients (76.6%) presented as HBeAg negative chronic infection (formerly “inactive carriers”), and only 6 (9.4%) HBsAg carriers fulfilled current criteria for treatment.

The most recent data regarding HBV prevalence in Spain based on systematic reviews or modelling studies reveal discrepant rates (0.34% to 0.60%) that may impact the planning of health and financial resources.[1,2] Our results are closer to the latest estimation from the recent modelling study of the Polaris Observatory, which accounts for approximately 190,000 chronically infected patients in the age range studied.[2] Interestingly, data from our study are practically identical to the data obtained 10-15 years ago in two large-scale population-based studies carried out in Spain.[4,5] They revealed a substantial reduction in the global prevalence of HBsAg (from 1.5% to 0.7% in the period 1989-2002) and later a stabilization (2002-2010). Indeed, measures like the introduction of the vaccination calendar in the 90s, the systematic control of donations of blood, or the screening in pregnants have contributed to the decreased incidence of HBV in Spain as in other developed countries in recent years.[4,5] However, a plateau phase (or at least a slowdown) in the dropping curve of HBsAg prevalence appears to have occurred. We could hypothesize that the beneficial effect of vaccination was “discounted” in the latest aforementioned epidemiological studies and there are still a considerable amount of people who have not benefited from this program due to their age. Furthermore, an increase in the immigrant population could partially account for this effect, which appears to be reinforced in this study where the prevalence of HbsAg in the immigrant population was more than twice as high as in the general population. Finally, it is possible that facilitation in the access to better antiviral therapies that preclude the evolution to more advanced stages of the disease can explain greater survival rates and, therefore, the maintenance of prevalence rates. Taking into account the demographic evolution of the European population towards aging and an increase in the immigrant population these results support the need to intensify screening programs in these groups.

A complete characterization of CHB according to levels of serum ALT and HBV-DNA, as well as liver fibrosis, are important predictors of long-term outcome that aid decision-

making regarding treatment initiation as well as treatment response. In Spain, an evaluation from 396 HBV mono-infected HBsAg positive patients in 1999 revealed that 67% of these were “inactive carriers”, whereas 33% presented as chronic hepatitis.[8] The study also showed that most CHB infections were antiHBe positive (86%), and only 16% of these fulfilled criteria to initiate treatment. Our study confirms the predominance of anti-HBe positive patients and also that only a very small percentage of patients require treatment based on current guidelines.[7] These guidelines do not support generalized treatment for all carriers due to the slow rate of progression of the disease, relative low likelihood of carcinogenesis in occidental countries, and possibly due to the cost and limitations of current treatments. Meanwhile, increasing the efforts in universal vaccination worldwide, increasing the number of educational campaigns to avoid new infections, screening people at risk (i.e. older than 45-50 years old and immigrants from countries of high prevalence), and possibly expanding current treatment criteria could help to eliminate this viral infection.

The study has some limitations that could involve selection and participation bias that have been previously discussed.[6] We cannot rule out some misclassification in the stage of infection due to the study design. The sample size calculation was based on estimated slightly higher values of the prevalence figures for each age stratum;[6] therefore, a larger number of subjects was studied, which helps provide a more accurate estimation of the parameters.

In conclusion, the current HBV burden in In Spain remains relatively low but virtually unchanged in the last 15 years. Many efforts are still needed (educational, preventive, screening in at-risk populations, treatment and drug research) to reach the WHO commitment of HBV elimination by 2030.

REFERENCES

1. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet.* 2015;386(10003):1546-1555.
2. Polaris Observatory C. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol.* 2018.
3. World Health Organization. Combating hepatitis B and C to reach elimination by 2030. Available at: . http://appswhoint/iris/bitstream/handle/10665/206453/WHO_HIV_201604_engpdf;jsessionid=5F7B95D01C898DF2C9C537EDF5ED25D3?sequence=1 *(Accessed November 2018).*
4. Calleja-Panero JL, Llop-Herrera E, Ruiz-Moraga M, et al. Prevalence of viral hepatitis (B and C) serological markers in healthy working population. *Rev Esp Enferm Dig.* 2013;105(5):249-254.
5. Salleras L, Dominguez A, Bruguera M, et al. Declining prevalence of hepatitis B virus infection in Catalonia (Spain) 12 years after the introduction of universal vaccination. *Vaccine.* 2007;25(52):8726-8731.
6. Crespo J, Cuadrado A, Perello C, et al. Epidemiology of hepatitis C virus infection in a country with universal access to direct-acting antiviral agents: data for designing a cost-effective elimination policy in Spain. *J Viral Hepat.* 2019.
7. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol.* 2017;67(2):370-398.
8. Buti M, Costa X, Valdes A, et al. [Study of hepatitis B virus replication and infection by other hepatitis viruses in patients with chronic hepatitis B virus infection]. *Gastroenterol Hepatol.* 2002;25(5):295-298.

TABLES

FIGURE LEGENDS

Figure 1. Flow-chart of the study and characterization of HBsAg infected patients.
FSU, first-stage units; IC, informed consent; and SSU, second-stage units.
†HBeAg status could not be assessed; ‡The patient presented an acute co-infection HIV and HBV at the time of recruitment. He showed HBsAg seroconversion on HAART treatment that included tenofovir. §HBeAg patients could not be fully characterized due to the unavailability of either HBV-DNA or ET (Fibroscan®). ¶4 in 5 patients HBeAg negative with chronic hepatitis B were already undergoing treatment at the time of recruitment, whereas the other patient started the treatment after being diagnosed during the study. Another patient with HBeAg negative chronic infection was being treated due to the risk of HBV transmission as a health profesional worker.

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