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ORIGINAL PAPER

Drug-resistant and immune-escape hepatitis B virus mutants, occult hepatitis B infection and coinfections in public hospital patients from Argentina

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Abstract

Argentina exhibits low serological prevalence for Hepatitis B virus (HBV); however, occult hepatitis B infection (OBI) has been reported in blood donors, Amerindians and individuals coinfected with hepatitis C virus (HCV), and/or human immunodeficiency virus (HIV). The aim of this study was to analyze the genetic diversity of HBV and to evaluate serological marker associations and coinfections with HCV and HIV in patients attending and treated in a public hospital in the province of Buenos Aires, Argentina. A total of 189 HBV reactive samples (HBsAg and/or anti-HBc) were analyzed for HBV DNA characterization. All reactive samples were tested for anti-HCV and HIV-antigen/antibody using CMIA assays. Thirty-six samples exhibited detectable HBV DNA, 7 of which were OBI. HBV sequences were classified as subgenotypes A1, A2, B2, D3, F1b, F3 and F4. Mutations related to the ability to escape the host's immune response, resistance to antiviral therapy and progression to disease were found in patients, partly due to the variable sensitivity of HBsAg, the reverse transcriptase, the basal core promoter and the preCore. HCV and HIV prevalence was 10% and most of the genotypes found in the sequences were genotype 1 and B/F recombinant subtype, respectively. Of the total samples analyzed, 7 exhibited coinfections. This study shows the frequency of OBI, subgenotype distribution, HBV mutations and coinfections, which may have important clinical implications in public hospital patients. Planned prevention, detection and treatment adherence are needed to reduce transmission and morbidity in vulnerable populations.

Keywords Hepatitis B virus · Occult hepatitis B infection · Subgenotypes · Hepatitis B virus mutations · Coinfections

Introduction

Hepatitis B virus (HBV) infection is a worldwide public health concern. It is estimated that 257 million individuals are chronic carriers [1]. HBV promotes liver inflammation, frequently associated with cirrhosis and/or hepatocellular carcinoma (HCC) [1]. HBV has a partially double-stranded DNA genome with four overlapping open reading frames (ORFs; PreS/S, PreCore/Core [preC/C], X and polymerase [P]) [2].

HBV infection is globally distributed, with high (≥ 8%), intermediate (2–8%) and low (≤ 2%) endemic areas [1, 2]. Most South American countries are currently low endemic areas except for Ecuador, Venezuela and southern Brazil that exhibit intermediate endemicity. There are also scattered highly endemic regions, such as Peru, southern Colombia, northern Bolivia and northern Brazil [3]. HBV is presently divided into nine confirmed genotypes (A–I) and one putative genotype (J); some of these genotypes are subdivided into numerous subgenotypes with different geographical distributions, disease progression and response to therapy [1, 2]. Occult HBV infection (OBI) is defined as the presence of HBV viral DNA in the

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liver (with or without detectable HBV DNA in serum) in HBsAg-non reactive individuals tested with currently available serum assays. A cutoff value of < 200 IU/ml was also introduced for HBV DNA in serum [4]. The prevalence of OBI varies widely throughout the world, and among the study population of patients, due to the sensitivity of HBsAg and HBV DNA detection assays [4].

Argentina exhibits low serological prevalence for HBV in blood donors [5, 6]. However, the HBV infection may vary according to the geographic region and/or the vulnerable population under study [2]. HBV different genotypes and subgenotypes have been reported in dissimilar populations in this country [5–14]. In addition, OBI has been detected in blood donors, Amerindians and patients coinfected with HCV and /or HIV from Argentina [6, 12, 15].

The objective of this study was to analyze the genetic diversity of HBV and evaluate serological marker associations and coinfections with HCV and HIV in patients attending and treated in a public hospital in the province of Buenos Aires, Argentina.

Materials and methods

Study population

A retrospective study was conducted on 189 HBV-reactive serum samples detected by ELISA (HBsAg and anti-HBc antibodies; Architect i2000; Abbott Diagnosis); 65 were reactive for HBsAg and anti-HBc and 124 for anti-HBc. The Architect HBsAg Qualitative II assay has a sensitivity of ≤ 0.13 IU/ml. The samples were collected from the Virology Laboratory at Hospital Interzonal General de Agudos "Dr. Pedro Fiorito", Buenos Aires, Argentina, between 2015 and 2019. The samples sent to the laboratory were taken from patients with HBV serological markers of acute ($n = 57$), chronic ($n = 8$) and past ($n = 124$) infection admitted to the different hospital departments (Emergency, Internal Medicine, Infectology, Surgery, Gastroenterology and Hepatology). Antiviral therapy (entecavir or tenofovir) is available for HBV chronically infected patients with or without coinfections (HCV, HIV). Treatment indications in Argentina are based on the combination of three criteria: transaminases (> normal level), viral load (HBV DNA > 2000 IU/ml) and liver histology (inflammation and/or fibrosis), also taking into account the general condition of the patient and the availability of antiviral drugs. In Argentina, the individuals attending these public hospitals have very limited economic resources and receive free medical care. None of the individuals who participated in this study had been vaccinated for HBV.

Serological markers and load viral

Other HBV serological markers, HBeAg, IgM anti-HBc, anti-HBe and anti-HBs (Architect i2000; Abbott Diagnosis), were determined in all HBV reactive samples by ELISA. Moreover, HCV (total anti-HCV Ab; Abbott Architect i2000) and HIV (HIV-antigen/antibody; Abbott Architect i2000) serological markers were tested by CMIA tests, respectively. In HBV-positive samples, DNA quantification was performed by the COBAS Taq man HBV test (Real time; Roche Molecular Systems).

DNA and RNA extraction

DNA and RNA were extracted from all serum samples ($n = 189$) using the QIAmp DNA Mini Kit and the QIAamp Viral RNA Mini Kit (QIAGEN AG) respectively, according to the manufacturer's protocol.

HBV DNA amplification

PCR for the S/P overlapping genomic region (nucleotides [nt] 256–796) and a nested-PCR (n-PCR) for the preC/C regions (nt 1736–2471) were performed as previously described for HBV [5, 12, 16].

HCV RNA and HIV RNA amplifications

HCV and/or HIV reactive samples were amplified by n-PCR as previously described [9, 15].

Sequencing and phylogenetic analysis

PCR products HBV, HCV and HIV were sequenced directly in both directions with the corresponding amplification primers from Macrogen Inc. (Seoul, South Korea). The sequences obtained were aligned using the CLUSTALX v1.83 software. Phylogenetic trees were obtained by MEGA X using the neighbor-joining algorithm with the Kimura two-parameter model of molecular evolution.

Analysis of the regulatory regions and ORFs of HBV

The nucleotide sequences representing the basal core promoter (BCP; nt 1742–1814), the preC (nt 1814–1901) and partial S (amino acids [aa] 55–210)/P (aa 64–220) regions for HBV were translated into aa sequences according to their corresponding ORFs and compared with the consensus

sequences from the corresponding genotypes and subgenotypes using BioEdit software.

Statistical analysis

Statistical tests were performed using the Student's t-test and the Chi-square test (Epidat v3.1 software), as appropriate.

Results

Demographic features

Of the total samples analyzed, 72.5% (137/189) were from males and 27.5% (52/189) from females. The mean age ± SD of the subjects was 50 ± 14 years (range 18–70 years). Most of the individuals were Argentinians living in areas close to the hospital located in Avellaneda, Buenos Aires province. In addition, the subjects of other nationalities (Bolivians, Peruvians, Brazilians, Venezuelans and Paraguayans) were also treated. Furthermore, in the surveys most of the patients reported having engaged in risk behaviors such as the use of inhaled drugs, sexual contact without prevention measures or dangerous tattoos, among others.

Detection of HBV DNA and occult HBV infection (OBI)

One-hundred- and eighty-nine (189) HBV-reactive samples with acute ($n = 57$), chronic ($n = 8$), and past ($n = 124$) were analyzed (Supplemental Figure). Thirty-six exhibited detectable HBV DNA, 23 for the S/P region and 20 for the preC/C region (Table 1). Of the total number of positive samples for HBV DNA, 7 were classified as OBI (non-reactive HBsAg and viral load < 200 IU/ml; Table 1). All OBI samples only amplified in the preC / C region.

Phylogenetic analysis and HBsAg subtype

Most of the S/P region sequences from individuals belonged to genotype F (15/23), 9 to subgenotype F1b, 1 to subgenotype F3 and 5 to subgenotype F4. Of the 6 sequences containing genotype A, 2 were assigned to subgenotype A1 and 4 to subgenotype A2. Subgenotypes B2 and D3 were described in one sequence, respectively (Fig. 1). Moreover, HBsAg subtype of the S gene sequence were described in Table 1 [17].

Subgenotypes in the preC/C region could not be validated in 13 sequences that only amplified for this region.

Analysis of the S and P proteins

The S sequences ($n = 23$) of the patients showed specific aa polymorphisms that are characteristic of genotype A, B, D and F, respectively. Of the total genotype A sequences ($n = 6$), 5 exhibited mutations inside and outside the main hydrophilic region, Y100C, M103I, T131N, T143M and W182Stop among them. No mutations were observed in sequence B2, whereas sequence D3 showed mutations I110L, T125M and T189I. Twelve of the total genotype F sequences did not detect aa mutations, whereas 2 subgenotype F4 sequences showed P62L, S167L and V177A mutations and one sequence exhibited L98V and S140T. In the P protein, resistance-related mutations rtS106C, rtW153R, rtL180M, rtM204V and rtV191I were observed. The remaining S and P protein variants are shown in Table 1.

Analysis of the BCP and PreC

Of the total preC/C sequences ($n = 20$), 11 exhibited mutation 1764, two mutation 1896 and two mutation 1899. Furthermore, C1766T, T1768A, C1773T, C1799G, A1846T, T1850A, and T1858C mutations were observed in some sequences (Table 1, MT448618-MT44863).

HCV and HIV analysis

Prevalence, molecular detection and phylogenetic analysis

Of the total samples analyzed ($n = 189$), 20 were anti-HCV reactive with a prevalence rate of 10.58% (20/189; 95% CI 5.9–15.2). Of the 20 reactive samples, HCV RNA from 9 samples was amplified. HCV sequences were classified as genotype 1 (103P, 116P, 128P, 141P and 175P), 2 (145P) and 3 (38P, 72P and 168P; data not shown; MT902919-MT902927; Table 2a).

A total of 19 samples were HIV Antigen/Ab reactive with an overall prevalence of 10.05% (19/189; 95% CI 5.5–16.4) and HIV RNA were detected in 11 reactive samples. All HIV sequences were classified: 2 as subtype B (165P and 222P), 1 as subtype F (204P) and BF recombinant subtype (1P, 14P, 27P, 38P, 71P, 82P, 127P, 195P; data not shown; MT902909–MT902918; Table 2a).

Serological marker associations and coinfections

Of the total samples analyzed, 20.6% (39/189; 95% CI 14.6–26.6) showed more than one serological marker of viral infection (HCV and/or HIV): two (HBV-HCV and HBV-HIV) were observed in 18.0% (34/189; 95% CI

Table 1 HBV serological markers, genotypes and mutations from 36 HBV DNA positive samples from hospital patients

Samples	Age-Sex	Serological markers				Infection	Antiviral therapy HBV[a]	Viral load (IU/ml)	S/P regions			pC/C region	
		HBsAg	Total/ IgM anti-HBc	HBeAg	anti-HBe				Genotype	Subtype HBsAg	S/P mutations[b]	n-PCR	BCP (1742–1814) / Precore (1814–1901)[c]
1P	36-M	R	R/R	R	NR	Acute[e]	–	1.0×10^6	F1b	*a4d*	–	NA	–
3P	45-M	R	R/R	R	NR	Acute[e]	–	4.1×10^6	NA	–	–	Positive	A1846T, G1896A
4P	50-M	R	R/R	R	NR	Acute[e]	–	4.5×10^6	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
10P	26-F	R	R/NR	R	NR	Chronic	No	3.0×10^7	B2	*a2d*	–	Positive	C1799G
14P	62-M	R	R/NR	NR	R	Acute	–	5340	F1b	*a4d*	–	NA	–
16P	40-M	R	R/NR	NR	R	Acute	–	6105	F1b	*a4d*	–	NA	–
22P	26-F	R	R/R	R	NR	Acute[e]	–	3.0×10^5	NA	–	–	Positive	C1773T, A1846T, T1850A, T1858C
24P[d]	56-M	NR	R/NR	NR	R	OBI[e]	–	<200	NA	–	–	Positive	C1773T, A1846T, T1850A, T1858C
27P	37-M	R	R/R	R	NR	Acute[e]	–	3.5×10^6	F4	*a4d*	–	NA	–
29P	44-M	R	R/R	R	NR	Acute[e]	–	1.2×10^6	F1b	*a4d*	–	NA	–
36P	26-F	R	R/R	R	NR	Acute[e]	–	3.4×10^6	F4	*a4d*	L98V, S140T / rtS106C, rtF148Y	NA	–
40P	35-F	R	R/R	R	NR	Acute[e]	–	4.5×10^5	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
44P	30-M	R	R/R	R	R	Acute	–	2.1×10^4	A1	*a2d*	Y100C, A194P	NA	–
48P	51-M	R	R/R	R	NR	Acute[e]	–	3.8×10^6	F1b	*a4d*	rtL91I	NA	–
49P	43-M	R	R/NR	R	NR	Chronic	ETV	5.4×10^6	A2	*a2d*	M103I, Y161F, S174N, I195M / rtV112I, rtL180M, rtM204V	NA	–
58P	49-M	R	R/R	R	NR	Acute[e]	–	2.8×10^6	F1b	*a4d*	–	Positive	–
62P	68-M	R	R/NR	NR	R	Acute	–	7640	NA	–	–	Positive	G1757A, G1764A, C1766G, A1846T, G1896A
71P	56-M	R	R/NR	NR	R	Chronic	TDF[f]	5.2×10^6	A2	*a2d*	Y161F, W182Stop / rtV191I	Positive	G1764A, C1766T, T1768A, C1773T, A1846T, T1850A, T1858C, G1899A
79P	35-M	R	R/R	R	NR	Acute[e]	–	2.1×10^6	F3	*a4d*	rtS109P	NA	–
84P	40-F	R	R/NR	R	NR	Chronic	No	4×10^7	A1	*a2d*	Y100C, N131T, T143M, E164G L173P / rtQ139H, rtW153R	Positive	C1773T, A1846T, T1850A, T1858C
106P	37-M	R	R/R	R	NR	Acute[e]	–	1.1×10^6	F1	*a4d*	–	NA	–

Table 1 (continued)

Samples	Age-Sex	Serological markers				Infection	Antiviral therapy HBV[a]	Viral load (IU/ml)	S/P regions			pC/C region	
		HBsAg	Total/ IgM anti-HBc	HBeAg	anti-HBe				Genotype	Subtype HBsAg	S/P mutations[b]	n-PCR	BCP (1742–1814) / Precore (1814–1901)[c]
110P	62-M	R	R/NR	NR	R	Acute	–	4309	F4	*a4d*	P62L, S167L, V177A / rtY122N, rtQ149K, rtL151F	NA	–
121P	44-F	R	R/R	R	NR	Acute[e]	–	4.0×10^7	F1b	*a4d*	–	Positive	A1846T, T1850A, T1858C
138P	70-M	R	R/R	R	R	Acute[e]	–	2.3×10^6	F4	*a4d*	P62L, S167L, V177A/ rtY122N, rtQ149K, rtL151F	NA	–
148P	43-M	R	R/R	R	NR	Acute[e]	–	IS	F4	*a4d*	–	NA	–
151P	35-M	R	R/NR	R	R	Acute	–	4300	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
173P	44-F	R	R/R	R	NR	Acute[e]	–	1.4×10^7	A2	*a2d*	–	Positive	C1773T, T1850A, T1858C
185P	50-M	R	R/NR	R	NR	Chronic	ETV[f]	3×10^5	A2	*a2d*	Y161F, V168A, W182Stop, V184G / rtV191I	Positive	A1846T, T1850A, T1858C
192P	26-M	R	R/R	R	NR	Acute[e]	–	5.6×10^6	F1b	*a4d*	–	NA	–
204P[d]	40-F	R	R/NR	NR	NR	Chronic	No	IS	D3	*a3y*	Q101R, I110L, T125M, T189I / rtN118T, rtF122I, rtS135Y		A1846T, G1899A
215P	60-M	NR	R/NR	NR	NR	OBI	–	< 200	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
216P	46-M	NR	R/NR	NR	R	OBI	–	< 200	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
218P	55-M	NR	R/NR	NR	NR	OBI[e]	–	< 200	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
219P	39-M	NR	R/NR	NR	R	OBI[e]	–	< 200	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
220P[d]	56-M	NR	R/NR	NR	R	OBI[e]	–	< 200	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C
222P	28-M	NR	R/NR	NR	NR	OBI[e]	–	< 200	NA	–	–	Positive	G1764A, C1766T, C1773T, A1846T, T1850A, T1858C

12.2–23.7) and three (HBV-HCV-HIV) were detected in 2.7% (5/189; 95% CI 0.8–6.0; Table 2a).

Of the total amplified samples ($n = 189$), 56 showed some of the three viruses analyzed (HBV, HCV and HIV) and 7 exhibited coinfections. HBV–HIV and HIV–HCV coinfections were found in 6 samples (1P, 14P, 27P,71P, 204P and 222P) and 1 (38P), respectively. Sample 222P with HBV–HIV coinfection was classified as OBI. Detailed data of the genotypes, subgenotypes and subtypes associations markers are shown in Table 2b.

Discussion

The aim of this study was to analyze the genetic diversity of HBV and evaluate serological marker associations and coinfections in patients with acute, chronic and past infection attending and treated in a public hospital in the province of Buenos Aires, Argentina. These results show the frequency of OBI, HBV subgenotype distribution, HCV and HIV serological marker associations and coinfections. Mutations related to the ability to escape the host's immune response, resistance to antiviral therapy and progression to disease were found in these patients, which may have important clinical implications.

OBI is a common condition among infected individuals belonging to endemic regions, to high-risk groups or patients with chronic liver disease [4, 18]. The causes of HBV suppression are not yet well clarified, although different factors might be associated: elapsed time and recovery from infection as a result of host immune response, low viral load, difference in sensitivity of immunoassays, formation of immune-complexes, epigenetic factors, coinfection with other infectious agents and/or variability of the HBV genome or S gene mutations [4, 18]. In this study, all OBI samples were negative in the PCR assay targeting the S-gene probably due to mutations in the S region and could not be genotyped. Moreover, OBI may impact several different clinical contexts, including the possible transmission of the infection, the risk of reactivation, the contribution to liver disease progression and the development of HCC [4]. In this regard, 3 out of 7 OBI case studies showed HCV (215P and 216P) and HIV (222P) infection. Prospective studies and meta-analyses have reported a higher incidence of HCC in patients with HCV and OBI compared with patients without OBI [19]. This status may play a synergistic role in the occurrence of HCC in HCV coinfected patients, especially in patients with advanced fibrosis and cirrhosis [19]. In HIV-positive subjects, the OBI condition has been associated with a low number of CD4-positive lymphocytes, elevation of alanine aminotransferase and more frequent AIDS-defining illnesses [20]. However, there is a lack of studies to better evaluate HBV infection progression and liver disease

Table 1 (continued)

M male, *F*: female, *NR* non-reactive, *R* reactive, *IS* insufficient sample. [a]Antiviral therapy: entecavir (ETV); tenofovir (TDF). [b]Parcial S (aa 55–210)/P (aa 64–220) proteins. [c]Basal core promoter (BCP; nt 1742–1814) and Precore (nt 1814–1901). Lower detection limit PCR S/P and preC/C regions: 500 copies/ml. [d]Samples with anti-HBs antibodies reactive. [e]First visit. [f]Non-adherence to therapy (drug addiction)

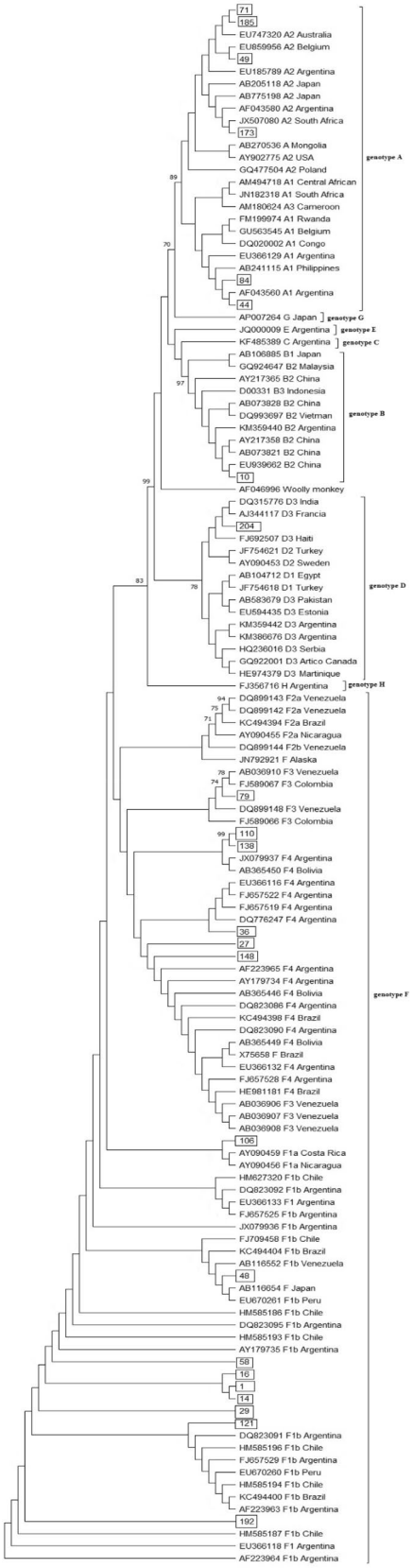

Fig. 1 Phylogenetic relationships of 23 HBV sequences (black frame) shown in this study were compared to representative sequences belonging to all reportedly known genotypes and subgenotypes by using the neighbor-joining method. Bootstrap statistical analysis was performed using 1000 data sets and the numbers of the nodes indicate the percentage of the number of substitutions per site (bootstrap). All sequences deposited in the GenBank are named with their corresponding accession number. This tree represents the partial S region (nt 320–775) of HBV sequences. The GenBank accession numbers of those sequences reported in this study are MT439874–MT439896

evolution in HIV-positive patients with OBI [20]. On the other hand, OBI status was unknown in all the patients because the diagnosis and follow-up of OBI in Argentina are not routinely performed in "anti-HBc only" individuals.

HBV genotype F is the most frequent genotype in South America. Nevertheless, global human migrations affect the pattern of genotype distribution, introducing genotypes and subgenotypes differing from those circulating in the original inhabitants [21]. In this work, A, B, D and F genotypes were documented, while subgenotypes F1b and F4 were the most common in this population (Table 3). Other studies have demonstrated the previous circulation in Argentina and border countries of the subgenotypes found in hospital patients, with the exception of subgenotype F3 that was detected for the first time in our country [Table 3; 5–14, 21–27]. The presence of this subgenotype is due to the recent migrations of individuals from Venezuela and Colombia looking for better job, health and educational opportunities. or undergraduate, graduate and postgraduate university programs [21]. Furthermore, the observed HBsAg subtype was consistent with the phylogenetic analysis [17].

The selection pressure of HBV by host immunity and/or antiviral therapies can generate strains with amino acid variants in or around the determinant a (*det a*) of HBsAg, the main target of neutralizing Ab. Amino acid mutations have been observed in the deduced sequences of HBsAg, among which Y100C, M103I, T131N, T143M and W182Stop stand out for genotype A, I110L and T125M for genotype D and L98V for genotype F. The Y100C mutation (44P and 84P) has been associated with OBI individuals, although in vitro studies demonstrate that it alone does not reduce the amounts of HBsAg or the affinity of HBsAg for ELISA assays, as well as both samples analyzed in this study [28]. M103I (49P) and T131N (84P) mutations have been detected together with other variants of the S region, hindering the recognition of HBsAg by the humoral immune response and favoring its escape [29, 30]. Mutation T131N was found even in children with HBV prophylaxis [30]. The T143M variant (84P) was documented to decrease the sensitivity of ELISA assays; in turn it was observed by protein modeling that it alters the antigenicity of HBsAg [31, 32]. The W182Stop found in two chronic patients (71P and 185P) generates the defective secretion of HBsAg. This mutation

Table 2 HCV and HIV analysis in the total population studied and positive HBV DNA samples

(a) HIV and HCV infection

Infection	Acute	Chronic	Past
Total	*57*	*8*	*124*
Anti-HCV (R)	1	3	16
HCV RNA (+)	0	1	8
Genotypes	–	3	1, 2, 3
Antigen/Ab HIV (R)	9	2	8
HIV RNA (+	4	2	5
Genotypes	BF	BF, F	BF, B
Positive HBV DNA	*23*	*6*	*7*
Anti-HCV (R)	0	2	2
HCV RNA (+)	0	0	0
Anti-HIV (R)	5	2	1
HIV RNA (+)	3	2	1
Genotypes	BF	BF, F	B

(b) HBV, HCV and HIV serological markers and genotypes of the 7 coinfected samples

Samples	ge-Sex	HBV			HCV		HIV		Coinfection
		HBsAg	anti-HBc	Genotype	anti-HCV	Genotype	Ag/anti-HIV	Genotype	
1 P[a]	36-M	R	R	F1b	NR	–	R	BF	HBV–HIV
14 P[a]	62-M	R	R	F1b	NR	–	R	BF	HBV–HIV
27 P[a]	37-M	R	R	F4	NR	–	R	BF	HBV–HIV
38P[b]	47-M	NR	R	–	R	1	R	BF	HCV–HIV
71P[b]	56-M	R	R	A2	R	–	R	BF	HBV–HIV
204P[b]	40-F	R	R	D3	NR	–	R	F	HBV–HIV
222 P[a,c]	28-M	NR	R	A1	NR	–	R	B	HBV–HIV

M male, *F* female, *NR* non-reactive, *R* reactive, [a]First visit, [b]Non-adherence to therapy (drug addiction), [c]occult hepatitis B infection (OBI)

has carcinogenic properties and was found to induce apoptosis in vitro, exacerbating the progression of the disease [33, 34]. With regard to genotype D, mutation T125M has been previously described in this genotype in patients monoinfected with HBV and coinfected with HBV/HIV [35]. This mutation is found in sample 204P that has co-circulation of HBsAg/anti-HBs, showing evasion of the humoral immune response. Furthermore, the L98V variant (36P) was observed in genotype F in patients with chronic HBV infection or HBV reactivation [36, 37].

In the P protein, several mutations were related to antiviral therapy resistance. Mutation rtS106C (36P; acute infection) was found to be associated with resistance to tenofovir, although it has been observed in naïve patients with necroinflammation and an increased development of cirrhosis [38]. The rtW153R mutation (84P; chronic infection without therapy) is frequent in genotype A and was described in association with adefovir resistance, while rtV191I - W182Stop in ORF S-occurs more frequently in individuals with chronic HBV (71P and 158P; chronic infection) [33, 34, 39]. Mutation rtV191I/sW182* is resistant to lamivudine, and remains sensitive to adefovir and tenofovir [33]. The 71P (HCV and HIV coinfection) and 185P patients received tenofovir and entecavir antiviral therapy, respectively. However, the detectable viral load in them was related to low adherence to therapy, probably due to their socioeconomic status and addiction to inhaled drugs. Mutations rtL180M and rtM204V—in the YMDD motif of viral polymerase—are responsible for resistance to lamivudine, telbivudine, entecavir and clevudine [33, 40]. Resistance to entecavir was observed in the 49P patient and the addition of adefovir or tenofovir to the therapy was recommended (Ministry of Health of Argentina recommendations).

On the other hand, BCP and preC/C mutations have been associated with significant virological or clinical events, such as the failure to form a nucleocapsid, liver disease progression or HBeAg seroconversion [41]. In particular, mutations G1896A, A1762T, G1764A and the A1762T/G1764A may prevent the production of HBeAg by introducing a premature stop codon into the ORF or may increase the transcription of pregenomic ribonucleic acid by removing of the nuclear receptor-binding motif, contributing to an

Table 3 The circulation in Argentina and border countries of the HBV subgenotypes

Countries	Regions	Populations	HBV Subgenotype*	Reference
Argentina	Cuidad Autónoma de Buenos Aires	Blood donors	A2, B2, **C2**, F1b, F4	[5]
	Cuidad Autónoma de Buenos Aires	HBV-monoinfected and HIV/HBV-coinfected patients	A1, **A2**, D3, F1b	[7]
	Cuidad Autónoma de Buenos Aires	Hospital patients	A1, A2, D, **F1b**, F4	[8]
	Cuidad Autónoma de Buenos Aires and Buenos Aires, Córdoba, Santa Fé and Santiago del Estero provinces	Trans Sex Workers	A2, C	[9]
	Ciudad Autónoma de Buenos Aires and Buenos Aires, Salta, Jujuy, Chaco and Formosa provinces	HBV chronically infected individuals	A1, A2, **D1-4, F1b, F4**	[10]
	Mendoza province	Hospital patients	A1, A2, F2a	[11]
	Misiones and San Juan provinces	Ameriandians	A2, **C2**, **F1b, F4**	[12]
	Misiones province	Blood donors	F1b, **D3**	[5]
	Misiones province	Blood donors	A1, A2, D2, **D3**, F1b, F4	[13]
	Córdoba province	HBV-monoinfected and HIV/HBV coinfected patients	**A2,** C, D2, D, **F1b, F4**	[14]
	Córdoba province	Blood donors	A2, C, D2, F1b, **F4**	[6]
Paraguay	All cities	Blood donors	A1, A2, B2, C2, D3, **F4**	[22]
Chile	Santiago City	Hospital patients	A2, B2, C1, C2, D2, D3, **F1b**	[23]
Uruguay	Montevideo	Blood donors and patients	A2, **F1b**	[24]
Bolivia	Eastern Bolivia	Japanese immigrants and native Bolivian	B2, C2, F4	[25]
Brazil	Rio Grande do Sul state South	HBV chronically infected individuals	A, **D1-3**, A1,A2, **D1, D2, D3**	[26, 27]

*Bolded subgenotypes are reported as the most frequent

inefficient immune response that ultimately leads to hepatocarcinogenesis. [42]. A meta-analysis study revealed that the G1896A ($n=2$) and G1764A ($n=11$) mutations observed in this work, correlate with a statistically significant increase in the risk of HCC, even this latter mutation alone plays a significant role similar to A1762T and A1762T/G1764 [43]. This may indicate that any one site of mutation of A1762T or G1764A constitutes a danger signal [43]. Moreover, other mutations such as C1766T, A1846T and G1899A are correlated with an increased risk of acute chronic liver failure [44].

Of the total samples analyzed, 20% showed associations with HCV and/or HIV serological markers and 7 exhibited coinfections, highlighting the importance of coinfection detection in HBV-reactive samples. With regard to the genomic characterization of HCV and HIV in HBV-reactive samples, our study allowed to determine the presence of different genotypes and OBI in coinfections in this population [9, 15].

In conclusion, this study shows the presence of OBI in patients with and without HCV and HIV coinfection. This is important because OBI screening or "anti-HBc only" patient follow-up is not routinely performed in the hospital. Furthermore, HCV and / or HIV serological markers and genotype-subtype associations were found in subjects with past and active HBV infection. Drug-resistant in naïve and treated patients together with immune-escape HBV mutants have important clinical implications in the vulnerable population. Finally, planned prevention, detection and treatment adherence are needed to reduce transmission and morbidity in this vulnerable population.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

Ethics approval This study was reviewed by the Bioethics Committee of "Fundación Huésped" and conducted in compliance with all federal regulations governing the protection of human subjects.

Informed consent All participants 18 years of age or older were required to sign an informed consent.

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