American Journal of Infectious Diseases 4 (4): 220-223, 2008
ISSN 1553-6203

Description of Hepatitis B Virus Genotypes in Selected Groups of Subjects from Paraguay and Brazil

[1,2]María Teresa Maidana Giret, [3]Maria Angélica Camargo Suarez, [4]Raquel Valdovinos, [5]Gloria Orué, [1]Denise S. Rodrigues, [6]Maria Eugenia F. Canciane and [1]Celso F. H. Granato
[1]Division of Infectious Diseases, Federal University of São Paulo, São Paulo, Brazil
[2]National Hospital of Itauguá, Ministry of Health of Paraguay, Asunción, Paraguay
[3]Blood Bank, Federal University of São Paulo, São Paulo, Brazil
[4]Haemodialysis Center, National Hospital of Itauguá, Paraguay
[5]Haemodialysis Center, Emergency Medical Hospital Dr. Luiz M. Argaña, Asunción, Paraguay
[6]Kidney and Hypertension Hospital Oswaldo Ramos,
Federal University of São Paulo, São Paulo, Brazil

Abstract: Hepatitis B virus genotyping was conducted among 11560 healthy blood donors and haemodialysis patients in Brazil and Paraguay. Genotypes A, D and F were found in Brazil and genotypes D and F in Paraguay. This is the first report showing HBV genotype diversity in individuals in Paraguay.

Key words: Hepatitis B virus, genotypes, Paraguay

INTRODUCTION

Hepatitis B Virus Infection (HBV) is a relevant public health problem, with 2 billion people infected worldwide[1]. Effective vaccines against HBV are available starting from 1982[1], however not all countries adopted those recommendations in an official way.

An important epidemiological aspect of HBV is the tendency to establish closed epidemiological cycles, hence, detailed knowledge is essential to design and improve specific prevention and control strategies[2]. However, this notion is still incomplete since the number of isolates analyzed in some parts of the world, including South America, is small[3,4].

HBV strains are classified into eight main genomic groups, designated A-H[5,6] showing a global distribution. In Brazil the most frequent genotypes were A, D and F[7].

The objective of this study was to detect and characterize HBV strains circulating among selected populations in Brazil and Paraguay, including healthy blood donors and haemodialysis patients. HBV viral diversity in Paraguay is evaluated here for the first time.

MATERIALS AND METHODS

A total of 11560 serum samples from healthy blood donors and haemodialysis patients in Brazil and Paraguay were divided into four groups. Characteristics and provenance of studied population groups are shown (Table 1).

Samples were tested for HBsAg by Enzyme immunoassay (MONOLISA® HBsAg PLUS, BIO-RAD) and "indeterminate" samples were also registered. The haemodialysis groups were also tested for anti-HBc by Enzyme immunoassay (MONOLISA® Anti HBc PLUS, BIO-RAD). Nucleic acid was extracted from serum as previously described[8]. Detection of HBV DNA and genotyping was carried out in the HBsAg positive samples, in the "indeterminate" samples and the HBsAg- / anti HBc+ samples; the methodology used was nested PCR using type-specific primers for each genotype, originally reported by Naito *et al.*[9] with slight modifications. We amplified the HBV genome using the universal primers followed by two different amplifications containing type-specific inner primers designed on the basis of the differences in size of the genotype-specific bands[9]. PCR was carried out in a tube containing 50 µl of a reaction mix with the following components: 50 ng of each outer primer, 200 mm of dNTPs, 1 U of Taq

Corresponding Author: Celso F.H. Granato, M.D., Ph.D., Laboratório de Imuno-Virologia, Disciplina de Doenças Infecciosas e Parasitárias, Escola Paulista de Medicina/ UNIFESP, Rua Pedro de Toledo, 781, 15º andar, 04039-032 - São Paulo – SP, Brazil

Table 1: HBV serology and DNA detection in studied population groups

Groups	Identify.	N	HBsAg+[a]	Indeterminate	anti-HBc+(%)	HBsAg-/anti-HBc+(%)	PCR+(%)
Group 1	Hd Py	145	12(8,3)	1(0,7)	33(22,7)	20(13,8)	14(9,6)
Group 2	Bs Py	3420	6(0,2)	0(0,0)	Not done	Not done	6(0,17)
Group 3	Bs Br	7917	33(0,4)	3(0,04)	Not done	Not done	36(0,45)
Group 4	Hd Br	78	3(3,8)	0(0,0)	10(12,8)	7(9)	5(6,4)
Total		11560	54(0,47)	4(0,03)	43(19,3)[b]	27(12,10)[b]	61(0,52)

a Numbers in parentheses are percentages.
Hd Py: Haemodialysis-Paraguay
BS Py: Blood Transfusion Service-Paraguay
BS Br: Blood Transfusion Service-Brazil
Hd Br: Haemodialysis-Brazil
b from the total of samples from the two Haemodialysis groups (223)

Table 2: Primers for HBV genotyping by nested PCR

Primer	sequence (position, specificity and polarity
First round PCR	
P1	5'-TCA CCA TAT TCT TGG GAA CAA GA-3'(nt 2823-2845,universal,sense)
S1-2	5'- CGA ACC ACT GAA CAA ATG GC-3'(nt 685-704, universal, antisense)
Second round PCR	
Mix A	
B2	5'- GGC TCM AGT TCM GGA ACA GT-3' (nt 67-86, types A to E specific, sense)
BA1R	5'-CTC GCG GAG ATT GAC GAG ATG T-3' (nt113-134, type A specific, antisense)
BB1R	5'-CAG GTT GGT GAG TGA CTG GAG A-3' (nt 324-345, type B specific, antisense)
BC1R	5'- GGT CCT AGG AAT CCT GAT GTT G-3' (nt 165-186, type C specific, antisense)
Mix B	
BD1	5'- GGC AAC AAG GTA GGA GCA GCT-3' (nt 2979-2996, type D specific, sense)
BE1	5'- CAC CAG AAA TCC AGA TTG GGA CCA-3'(nt 2955-2978, type E specific, sense)
BF1	5'- GYT ACG GTC CAG GGT TCA CA-3' (nt 3032-3051,type F specific, sense)
B2R	5'-GGA GGC GGA TYT GCT TYT GCT GGC AA-3' (nt 3078-3097), type D to F specific, antisense)

An "M" represent a nucleotide that could be either an A or a C: a "Y" represents a nucleotide that could be either a C or a T. nt, nucleotide. From Naito *et al.*[9]

DNA polymerase (Life Technologies) and 1x PCR buffer containing 1.5 mm MgCl2 and 5 µL target DNA. The thermocycler. Cycling parameters were: initial incubation for 5 min at 95°C, followed by 40 cycles consisting of 94°C for 20 s, 55°C for 20 s and 72°C for 1 min. Two second-round PCRs were performed for each sample, termed mix A and mix B reactions. Mix A consisted of a PCR mix able to detect genotypes A to C and mix B D to F. A table with universal and genotype specific second-round primers is shown (Table 2). 1-µL of the first PCR product was added to two tubes containing the second sets of each of the inner primer pairs. Reactions proceed as described for first-round amplifications. HBV genotypes were determined by genotype-specific DNA band sizes in a 3% agarose gel.

RESULTS AND DISCUSSION

HBsAg was detected in 54 of 11560 individuals. Among Paraguayans samples, 12 (8.3%) belonged to the haemodialysis unit and 6 (0.2%) to the blood transfusion service. In Brazil, 3 patients (3.8%) belonged to the haemodialysis group and 33 (0.4%) to the blood transfusion service (Table 1). 4(0.03%) of all samples were considered "indeterminate". We found an anti-HBc prevalence of 22.7% in Asunción and of 12.8% in São Paulo.

HBV DNA was amplified in 61 samples, 54 HBsAg positive, 4 "indeterminate" and 3 with a HBsAg-/anti-HBc+ profile; 1 from Asunción and 2 from São Paulo. HBV genotyping was accomplished in all the 61 HBV DNA positive samples. As shown in Fig. 1, type-specific PCR products were recognized by their distinct sizes in gel electrophoresis.

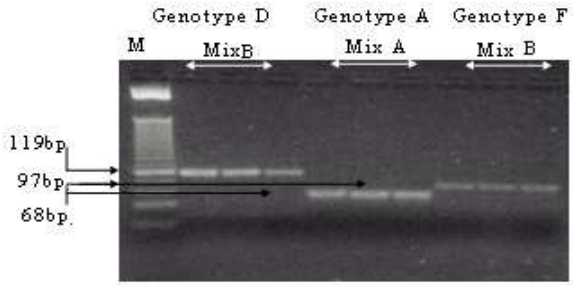

Fig. 1: The typical electrophoresis patterns of PCR products from different HBV genotypes found as determined by PCR genotyping system.. M, molecular size standards

Table 3: Genotypic distribution of HBV among different groups, determined by PCR using type-specific primers

	Group, determined by PCR using type-specific primers									
	No of samples of PCR + and genotypes									
	AgHBs+			Indeterminates			AgHBs-/anti-HBc+			
Groups	A	D	F	A	D	F	A	D	F	Total
Group 1	0	12	0	0	1	0	0	0	1	14
Group 2	0	4	2	0	0	0	-	-	-	6
Group 3	18	11	4	2	0	1	-	-	-	36
Group 4	0	2	1	0	0	0	1	1	0	5
Total	18	29	7	2	1	1	1	1	1	61

Genotypes D and F were found in Asunción, genotype D was found in 13 (93%) of the Paraguayan haemodialysis patients. Genotypes A, D and F were found in São Paulo (Table 3).

We found that 0.4 and 0.2% of the samples stored in blood banks, in the cities of São Paulo and Asunción, respectively, are HBsAg positive. These percentages fall within the expected range (between 0.3% and 13%) for HBV infections in different regions in Brazil[10]. Prevalence in blood bank samples was lower than that observed in individuals submitted to haemodialysis, being 8.3% in Asunción and 3.8% in São Paulo, probably due to the acquisition of HBV during the procedure.

From 223 studied patients belonging to the two haemodialysis groups, 12.1% were negative for HBsAg and positive for anti-HBc. A recent study showed that at least 35% of patients with hepatocellular carcinoma were HBsAg negative but harbored HBV DNA in the liver[11].

A study about HBV variant distribution done in haemodialysis units in the cities of Rio de Janeiro and São Paulo demonstrated that genotypes A and D are prevalent in Rio de Janeiro, while only genotype D was detected in São Paulo[12]. We found genotypes A, D and F in São Paulo. The most prevalent genotype in this city was A followed by D and F. In Paraguay, we detected the genotypes D and F on the Blood Bank and genotype D in the haemodialysis units. In this case, the result may reflect an mechanism of infection in patients undergoing the procedure. Additional studies done in the general population would clarify if genotype D in Paraguay is linked to haemodialysis. More detailed studies using DNA sequence data will help to evaluate these questions. Since this is the first study showing the HBV genotype distribution in Paraguay, we believe that this study may contribute to the understanding of the HBV epidemiology in Paraguay in those groups.

ACKNOWLEDGEMENT

This work was supported by DIPA/EPM-UNIFESP. M.T.Maidana has a CAPES PEC/PG scholarship.

REFERENC

1. Organization, W.H., posting date. Hepatitis B. World Health Organization Fact Sheet 204 (Revised October 2000). WHO Web site. 2000. http: // who. Int /inf -fs/ en /fact 204. html. [Online.]
2. Echevarria, J.M.L.P., 2003. Epidemiology of viruses causing chronic hepatitis among populations from the Amazon Basin and related ecosystems. Cad. Saúde Pública, Rio de Janeiro, 19:1583-1591. Doi: 10.1590/S0102-311X2003000600003
3. Norder, H., B. Hammas, S.D. Lee, K. Bile, A. M. Courouce, I.K. Mushahwar and L.O. Magnius. 1993. Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. J. Gen. Virol., 74: 1341-1348. DOI: 10.1099/0022-1317-74-7-1341
4. Tanaka, J., 2000. Hepatitis B epidemiology in Latin America. Vaccine, 18: S17-9. Doi: 10.1016/S0264-410X(99)00455-7
5. Norder, H., B. Hammas, S. Lofdahl, A.M. Courouce and L.O. Magnius, 1992. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. J. Gen. Virol., 73(Pt 5):1201-1208. DOI: 10.1099/0022-1317-73-5-1201
6. Okamoto, H., F. Tsuda, H. Sakugawa, R.I. Sastrosoewignjo, M. Imai, Y. Miyakawa and M. Mayumi, 1988. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J. Gen. Virol., 69(Pt 10): 2575-2583. DOI 10.1099/0022-1317-69-10-2575

7. Teles, S.A., R.M. Martins, B. Vanderborght, L. Stuyver, A.M. Gaspar and C.F. Yoshida, 1999. Hepatitis B virus: genotypes and subtypes in Brazilian hemodialysis patients. Artif. Organs, 23:1074-1078. Doi:10.1046/j.1525-1594.1999.06310.x
8. Boom, R., C.J. Sol, R. Heijtink, P.M. Wertheim-van Dillen and J. van der Noordaa, 1991. Rapid purification of hepatitis B virus DNA from serum. J. Clin. Microbiol., 29: 1804-1811. http://jcm.asm.org/cgi/content/abstract/29/9/1804
9. Naito, H., S. Hayashi and K. Abe, 2001. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. J. Clin. Microbiol., 39: 362-364. DOI: 10.1128/JCM.39.1.362-364.2001
10. Bittencourt, A.L., 1995. Freqüência da transmissão congênita. In: Infecções congênitas transplacentárias, R., Rio de Janeiro, Bittencourt A.L. (Ed.). Freqüência da transmissão congênita. Revinter, Rio de Janeiro. pp: 3-7.
11. Ding, X., Y.N. Park, T.C. Taltavull, S.N. Thung, X. Jin, Y. Jin, N.S. Trung, Y. Edamoto, T. Sata and K. Abe, 2003. Geographic characterization of hepatitis virus infections, genotyping of hepatitis B virus and p53 mutation in hepatocellular carcinoma analyzed by in situ detection of viral genomes from carcinoma tissues: comparison among six different countries. Jpn. J. Infect. Dis., 56: 12-18. http://www.nih.go.jp/JJID/56/12.pdf
12. De Castro, L., N.M. Araujo, R.R. Sabino, F. Alvarenga, C.F. Yoshida and S.A. Gomes, 2000. Nosocomial spread of hepatitis B virus in two hemodialysis units, investigated by restriction fragment length polymorphism analysis. Eur. J. Clin. Microbiol. Infect. Dis., 19: 531-537. Doi: 10.1007/s100960000302