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HEPATOLOGY

Intrafamilial prevalence of hepatitis B virus in Western Brazilian Amazon region: Epidemiologic and biomolecular study

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Abstract

Background: Hepatitis B is endemic in the Amazon region.
Methods: Serological markers for hepatitis B virus (HBV) were determined in 266 household members for hepatitis B surface antigen (HBsAg)-positive women (G1) and 395 household members for HBsAg-negative women (G2), randomly selected in Acre State Women's Medical Care Program, in order to evaluate the prevalence of HBV in this population. Before blood sample collection an epidemiological questionnaire was applied.
Results: The overall prevalence of HBV carriers (HBsAg) and exposed individuals (anti-HBc, IgG) was, respectively, 21.1% and 60.5% in G1 and 2.8% and 27.4% in G2 ($P < 0.0000001$). The frequency of HBsAg was higher among siblings from group G1 (75%) compared to the absence of any HBsAg-positive sibling in G2 ($P < 0.00006$). The HBV markers in other family members was as follows: G1 parents, 27.3% vs 4.5% ($P < 0.03$), sexual partners, 21.1% vs 2.5% ($P < 0.04$), and offspring, 10.4% vs 1.5% ($P < 0.04$). A low prevalence of HBsAg and anti-HBc (IgG) was observed for the last offspring of G2 mothers compared to the high prevalence among children of G1 mothers (0% vs 18.2%, $P < 0.01$ and 2.3% vs 59.1%, $P < 0.0000005$, respectively), with children younger than 1 year being the most affected. The frequency of the habit of sharing toothbrushes and the presence of at least one HBsAg carrier were higher in G1 than in G2 ($P < 0.0001$ and $P < 0.000002$), respectively. Genotypes A, D and G were found to be predominant by Innolipa test. There were cases that reacted to more than one genotype.
Conclusion: Intrafamilial transmission of HBV is evident in the present study and is possibly associated with the presence of more than one HBV carrier in the family and the shared use of toothbrushes among household contacts. Genotype analysis confirms intrafamilial transmission.

Introduction

The prevalence of hepatitis B virus (HBV) infection is widely heterogeneous in Brazil. The Amazon region presents the highest HBV endemicity. The prevalence of hepatitis B surface antigen (HBsAg) ranges from 3 to 10% and more than 70% of individuals have evidence of previous exposure to HBV.[1,2] However, the epidemiological aspects and transmission of HBV infection in the Amazon region are unclear, especially the conditions favoring this high endemicity.[3,4]

It has been suggested that HBV is transmitted through direct or indirect personal contact between household members, possibly through non-apparent per oral, per mucosal or percutaneous passage.[5] Other transmission routes are well-ocumented in this area such as vertical and sexual, but seroconversion after 1 year of birth strongly suggests the importance of intrafamilial transmission.[6] In this particular case, factors such as sharing of personal hygiene accessories, family size, presence of more than one HBsAg carrier among household contacts, replicative status of the carrier, and ethnic origin should be taken into account.[7]

The aim of the present study was to determine the prevalence of HBsAg in family members of HBV-infected women, as well as possible risk factors related to the intrafamilial transmission of HBV.

Methods

Study population

A total of 3789 pregnant women enrolled in the public prenatal care program of the municipality of Rio Branco, Acre State, located in Western Brazilian Amazon underwent routine prenatal exams at the Central Laboratory (LACEN, Acre). Eighty-four (2.2%) of these women were positive for HBsAg and 22 fulfilled the inclusion criteria (G1), that is, residence in Rio Branco, being found at home after three attempts, and being willing to participate in the study. The control group (HBsAg-negative women, G2) was matched for age (±5 years) and place of residence at a proportion of 1:2. Household contacts included individuals living in the same dwelling or in the peridomiciliary area. A total of 72 nuclear family household contacts of G1 and 155 of G2 were contacted. Fifteen individuals were excluded, six from G1 and nine from G2, including seven sex partners and eight children, after three negative attempts to find them at home or because they refused to participate in the study. In addition, 194 (G1) and 248 (G2) members of the family of origin, including parents, siblings, nephews, uncles and grandparents, were enrolled in this study. The addresses of the index (G1) and control (G2) cases were obtained from the simplified notification record from the prenatal unit. The individuals were visited at home, and after the objective of the study was explained to them, they signed a free informed consent form. Then a specific epidemiologic questionnaire related to demographic, personal, socioeconomic, behavioral, epidemiological, and family variables was applied.

Laboratory tests

Blood samples were collected in previously identified vacutainer tubes containing separator gel, placed on ice and transported to the laboratory. Sera were shared in three aliquots and stored at –20°C. One aliquot was tested for HBsAg, antibody to hepatitis B core antigen (anti-HBc; IgG), hepatitis B e antigen (HBeAg) and antibody to hepatitis B e antigen (anti-HBe) markers using commercial kits (Hepanostika-Organon, the Netherlands). Another aliquot was tested for anti-hepatitis D, human immunodeficiency virus, Lues serology and antibody to hepatitis B surface antigen (anti-HBs) markers by ELISA using commercial kits (Hepanostika-Organon; Sanofi–Pasteur-Plus, Paris, France). A third aliquot was stored for biomolecular studies. Quantification of serum HBV-DNA was performed by commercial Kit Amplicor (Roche-Diagnostic Basel). The HBV genotypes were determined by InnoLiPA (Innogenetics, Belgium) at INSERM U271.

Statistical analysis

The data were recorded using SPSS software (SPSS, Chicago, IL, USA). Differences between proportions were analyzed using non-parametric tests (χ^2 test with Yates correction, Fisher exact test when each cell had an expected frequency of ≥5). Student's *t*-test and Mann–Whitney test were applied to the comparison of means in the case of continuous variables (Gaussian distribution or non-parametric variables, respectively). $P < 0.05$ was considered to be significant.

Ethical considerations

The present study was approved by the Research Ethics Committee of Fundação Hospital Estadual do Acre (FUNDHACRE).

Results

Comparison between G1 and G2

No significant differences in socioeconomic or demographic variables were observed between the two groups (Table 1). With respect to the gynecologic–obstetric history, only the duration of breast-feeding of the last child showed a significant difference (G1: 4.4 ± 4.5 months vs G2: 8.8 ± 5.9 months; $P < 0.004$). Both groups were comparable regarding number of offspring, abortions and sexual partners (Table 2). Regarding the risk factors for HBV infection, the shared use of toothbrushes between the HBV carriers and household contacts was identified in 72.7% (16/22) of G1 individuals and in 20.5% (9/44) of G2 individuals ($P < 0.0001$; Table 3). The presence of at least one HBV carrier among members of the family of origin (parents, brothers and sisters) was observed in 75% (15/20) and 6.5% (2/31) of G1 and G2 patients, respectively ($P < 0.001$). Shared use of the toothbrush and the presence of more than one HBV carrier in the family of origin differed significantly between the two groups (Table 3).

Table 1 Subject characteristics

Variable	G1 ($n = 22$) *n* (%)	G2 ($n = 44$) *n* (%)	*P*
Age, years (mean ± SD)	25.1 ± 3.4	24.6 ± 3.8	0.62†
Marital status			
Single	6 (27.3)	16 (36.4)	0.45‡
Other	16 (72.7	28 (63.6)	
Present occupation			
Housewife	18 (81.8)	28 (63.6)	0.21‡
Other	4 (18.2)	16 (36.4)	
Education level			
Illiterate or incomplete first grade school	13 (59.1)	22 (50.0)	0.48‡
>1 year spent in school	9 (40.9)	22 (50.0)	

G1, HBsAg-positive cases; G2, HbsAg-negative cases.
†Student's *t*-test; ‡χ^2 test with Yates' correction.

Table 2 Gynecologic–obstetric history

Variable	G1 (Mean ± SD)	G2 (Mean ± SD)	*P*
No. offspring[†]	31.2 (2.3 ± 1.7)	34.6 (2.5 ± 1.6)	0.47[‡]
No. abortions[†]	31.2 (0.4 ± 0.9)	34.6 (0.4 ± 0.8)	0.37[‡]
Age at 1st sexual intercourse (years)	16.2 ± 2.6	15.2 ± 2.0	0.09[§]
Time with a fixed partner (years)	5.6 ± 3.7	5.3 ± 5.0	0.86[§]
Duration of breast-feeding[¶] (months)	4.4 ± 4.5	8.8 ± 5.9	0.004[§]

G1, HBsAg-positive cases; G2, HbsAg-negative cases.
[†]Mean rank; [‡]non-parametric Mann–Whitney test; [§]Student's *t*-test.
[¶]Breast-feeding duration for the last child.

Table 3 Distribution of probable risk factors for HBV

Risk factor	G1 (*n* = 22) % (*n*)	G2 (*n* = 44) % (*n*)	*P*
Percutaneous exposure	0.0 (0)	2.3 (1)	1.0[†]
Blood derivatives	13.6 (3)	22.7 (10)	0.58[‡]
Injections with a glass device	31.8 (7)	43.2 (19)	0.37[§]
Puncture of the finger	50.0 (11)	52.3 (23)	0.86[§]
Dental treatment	9.1 (2)	2.3 (1)	0.26[†]
Surgery	0.0 (0)	0.0 (0)	—
Use of injectable or inhalatory drugs	13.6 (3)	4.5 (2)	0.32[†]
Tattooing	59.1 (13)	56.8 (25)	0.86[§]
Razor blade[1]	86.4 (19)	81.8 (36)	0.91[‡]
Toothbrush sharing[1]	72.7 (16)	20.4 (9)	<0.0001[§]
Nail clippers[1]	54.5 (12)	31.8 (14)	0.07[§]
Skin Wounds (old/recent)[2]	0.0 (0)	2.3 (1)	1.0[†]
Sexual exposure			
Oral sex	13.6 (3)	31.8 (14)	0.11[‡]
Anal sex	27.3 (6)	20.5 (9)	0.53[§]
Use of a condom	68.2 (15)	43.2 (19)	0.05[§]
No. of partners during the last 6 months			1.0[†]
Up to one partner	95.5 (21)	93.2 (41)	
More than one partner	4.5 (1)	6.8 (3)	
History of sexually transmitted diseases	9.1 (2)	6.8 (3)	1.0[†]
Gonorrhea	9.1 (2)	0.0 (0)	0.11[†]
Syphilis	4.5 (1)	2.3 (1)	1.0[†]
Condyloma	4.5 (1)	2.3 (1)	1.0[†]
Herpes	0.0 (0)	0.0 (0)	—
HIV	—	—	—
Family contact (family of origin)			
At least one family member	75.0 (15/20)	6.5 (2/31)	<0.0001[‡]
Genitor with hepatitis (HBsAg +)	21.1 (4/19)	7.1 (2/28)	0.2[†]
Siblings with hepatitis (HBsAg +)	75.0 (12/16)	0 (0/13)	<0.0001[†]

G1, HBsAg-positive cases; G2, HbsAg-negative cases.
[1]Shared; [2]impetico, escrofuloderms, escabiose, leishmaniose.
[†]Fisher test; [†]Yates correlation; [§]χ^2/χ^2 test with Yates' correction; [¶]Bonferroni, significant if $P < 0.002$.

Sexual partners

Evidence of HBV carrier stage of the present sexual partner was significant in the two groups, with 4/19 (21.0%) and 1/40 (2.5%) in G1 and G2, respectively ($P < 0.04$). In addition, 73.7% (14/19) of G1 partners and 45% (18/40) of G2 partners were positive for anti-HBc (IgG; $P < 0.04$).

Offspring

The frequency of HBsAg was 10.4% (5/48) in offspring of G1 and 1.9% (2/105) in offspring of G2 ($P < 0.03$). Anti-HBc (IgG) was detected in 37.5% (18/48) of G1 offspring and 6.7% (7/105) of G2 offspring. When the children of G1 and G2 mothers were separated according to age, a significant difference in anti-HBc (IgG)

Table 4 Presence of HBsAg/anti-HBc (IgG) in children from G1 and G2 mothers according to age group

	HBsAg			Anti-HBc (IgG)		
Age group (years)	G1% (*n*) (+)	G2% (*n*) (+)	*P*	G1% (*n*) (+)	G2% (*n*) (+)	*P*
<1	16.7 (3/18)	0 (0/17)	0.2†	66.7 (12/18)	0 (0/17)	<0.0002§
1–3	7.7 (1/13)	0 (0/34)	0.2†	66.7 (12/18)	2.9 (1/39)	0.4†
4–6	11.1 (1/9)	0 (0/23)	0.2†	33.3 (3/9)	4.3 (1/23)	0.05†
7–10	0 (0/5)	4.2 (1/24)	1.0†	20 (1/5)	12.5 (3/24)	0.5†
11–13	0 (0/2)	0 (0/2)	—	50 (1/2)	0 (0/2)	1.0†
14–16	0 (0/1)	0 (0/3)	—	0 (0/1)	0 (0/3)	—
>16	0 (0/0)	50 (1/2)	—	0 (0/0)	100 (2/2)	—
Total	10.4 (5/48)	1.9 (2/105)	<0.04†	37.5 (18/48)	6.77/105	<0.000002‡

G1, index group; G2, control group.
†Fisher test; ‡χ^2 test; §χ^2 test with Yates' correction.

Table 5 Presence of HBsAg and/or total anti-HBc

	HBsAg			Anti-HBc (IgG)		
Family	G1% (*n*) (+)	G2% (*n*) (+)	*P*	G2% (*n*) (+)	G1% (*n*) (+)	*P*
Parents¶	27.3 (6/22)	4.5 (2/44)	<0.03§	86.4 (19/22)	54.5 (24/44)	<0.03§
Mother	21.1 (4/19)	7.1 (2/28)	0.2†	100 (19/19)	71.4 (20/28)	<0.04‡
Father	33.3 (3/9)	0 (0/10)	0.09†	100 (9/9)	60 (6/10)	0.09†
Sexpartner	21.1 (4/19)	2.5 (1/40)	<0.04†	73.7 (14/19)	45.0 (18/40)	<0.04‡
Siblings¶¶	75 (12/16)	0 (0/13)	<0.00006†	100 (19/19)	81.3 (13/16)	0.08†
Offsprings	10.4 (5/48)	1.9 (2/105)	<0.04†	37.5 (18/48)	6.7 (7/105)	<0.000002‡
Lastchild	18.2 (4/22)	0 (0/44)	<0.01†	59.1 (13/22)	2.3 (1/44)	<0.0000005§
Others¶¶¶	22.2 (4/18)	9.7 (3/31)	0.3†	72.2 (13/18)	51.6 (16/31)	0.1§

G1, index group; G2, control group.
†Fisher test; ‡χ^2 test; §χ^2 test with Yates' correction.
¶Among those studied; ¶¶at least one brother or sister; ¶¶¶other family members (nephews, uncles, grandparents).

positivity was observed for children aged <1 year in G1 (66.7%, 12/18) compared to G2 (0%, 0/17; $P < 0.0002$), while for children aged 4–6 years, this proportion was 33.3% (3/9) and 4.3% (1/23) in G1 and G2, respectively ($P = 0.05$). No significant differences in anti-HBc (IgG) positivity were observed for the other age groups, and there was no difference in the frequency of HBsAg between G1 and G2 children when stratified by age (Table 4). Of a total of 81.8% (54/66) of children who had completed vaccination for HBV, 83.3% (45/54) showed no evidence of HBV infection (HBsAg and anti-HBc [IgG] negative, with 77.8% [35/45] of the children presenting post-vaccination seroconversion [anti-HBs]; 57.1% in G1 vs 81.6% in G2, $P > 0.1$). Only 18% (4/22) of G1 children received hyperimmune hepatitis B immunoglobulin (HBIG) and a vaccine during the first day of life. Of the children aged <6 months who had not completed the vaccination scheme, 66.7% (2/3) were HBsAg positive and 100% (3/3) were anti-HBc (IgG) positive (Table 4).

Others household contacts

Overall positivity for HBsAg was 24.3% (42/173) in G1 and 3.2% (10/315) in G2 ($P < 0.0000000002$). Anti-HBc (IgG) was present in 70.5% (124/176) of G1 family members and in 33.1% (105/318; $P < 0.000003$) of G2 members ($P < 0.0000001$; Table 4). The HBsAg positivity in G1 was observed for all age groups and was higher for individuals aged 11–30 years and >50 years. However, in G2, HBsAg positivity was observed only in individuals >21 years ($P < 0.0000001$), with individuals >26 years having the highest positivity ($P < 0.0000001$). No significant difference in HBsAg or anti-HBc (IgG) positivity was observed between male and female subjects in the same group (G1, $P > 0.23$ and $P > 0.47$; G2, $P > 0.49$ and $P > 0.92$), but the difference was highly significant when the two groups were compared ($P < 0.05$). In summary the frequency of HBV carriers and of individuals exposed to the virus was higher among siblings, followed by parents, sexual partners and offspring (Table 5).

Hepatitis B virus genotypes

From the 72 HBsAg-positive individuals, HBV-DNA was positive in only 31, while 17 samples had insufficient material and 24 had undetectable HBV-DNA by polymerase chain reaction (PCR). Although the analysis was restricted to 31 samples, there was a trend for concentration of the same genotypes in the same families. Remarkably, few cases with genotype F were found, contrasting with a higher frequency of genotype A and D. Surprisingly, genotype G was found in some cases (Table 6).

Discussion

Transmission of HBV between household contacts seems to be important in areas of high endemicity. In the present study, the

Table 6 Genotype analysis with Innolipa test

Family	HBsAg positive	HBV-DNA by PCR	Genotype
01	03	NR	NR
02	03	02	A, D + G
03	05	NR	NR
04	01	NR	NR
05	01	01	D + G
06	10	04	All A
07	02	02	F, A
08	02	01	A
09	04	01	A
10	04	NR	NR
11	04	NR	NR
12	02	01	A
13	04	01	A
14	01	01	D
15	02	01	A
16	01	02	A, A +D
17	03	02	A, D,
18	01	NR	NR
19	01	02	A, D
20	13	08	All D + G
21	06	01	A + F
22	05	01	D

NR, non-reacted; PCR, polymerase chain reaction.
Samples excluded: 17 insufficient material; 24 HBV-DNA (PCR) negative; 31 HBV-DNA by PCR positive.

overall prevalence of HBsAg was 10.1%, a value similar to those reported in other studies carried out in the Amazon region, which showed a high prevalence of HBV infection.[8,9] The frequency of infection among household contacts was 10-fold higher in family members and sexual partners of index cases (G1) compared to the control group (G2), Furthermore the difference was approximately twofold lower in the case of exposed individuals (anti-HBc IgG).

The prevalence of HBV markers increased progressively with age.[10–12] This finding points to an early exposure during life in the Brazilian Amazonian region. It is in agreement with our observation in hospitalized patients, of young patients with cirrhosis due to HBV. Considering only the family members who lived in the same house, in the present study a higher frequency of anti-HBc (IgG) was found among household contacts, although no statistical significance between genders was observed in the same group. This is in contrast to other studies that found a trend towards higher positivity among men.[13] This difference may be explained by the higher number of female siblings and offspring.

In the present study, intrafamilial dissemination was clearly observed. This aspect has yielded conflicting results in other studies.[14–16] Lok and other authors did not confirm these results in China. Again the selection methods could explain opposite results.

The results of the present study are also in agreement with those reported in the literature in terms of the elevated positivity for HBV markers in families consisting of a larger number of members and more than one HBV carrier.[17]

Various factors are related to the risk of intrafamilial transmission of HBV, including exposure to the body fluids of an infected person with skin wounds, and the shared use of personal hygiene items such as toothbrushes, towels, nail scissors and shavers.[18,19] In the present study, the shared use of toothbrush was found to be strongly associated with HBV transmission through household contacts. Sharing a toothbrush was considered when the individual reported this habit at least once in the last 6 months with any household member. It is a common habit among family members of poorer populations of the region, indicating the need for the establishment of preventive and educational measures in order to prevent this practice. Unfortunately, our databank did not permit cross-referencing of this variable with sexual partnership. If an individual who shared toothbrushes was, at the same time, a sexual partner, we could not determine which one was involved in the viral transmission. In any case, only few adults reported sharing of toothbrushes, which makes sexual partnership less probable as a confounding variable.

Interestingly, in the present study the frequency of HBsAg positivity of the last child was higher in infants who were not breast-fed. However, due to the small sample size, we cannot exclude breast-feeding as a risk factor for HBV transmission in this group, although, as a rule, breast-feeding should not be discontinued even when the mothers are HBsAg positive because the risk of transmission is very low.[20] This is an important question in developing countries.

Genotype analysis was performed in only 31 samples, probably because of storage and transportation troubles and/or very low viral load. Despite the small number of samples our results are provoking and deserve further study. Few cases of genotype F were seen, in contrast with the high prevalence of this genotype in this area.[21] This could be because the present study was conducted in a big Amazonian city where native (Amerindians) compose the minority of the population. Because genotype F is considered prevalent in native populations of the Americas (Alaska and the Amazonian), then its presence in cosmopolitan cities could be modest. In contrast, we had 24 HBV-DNA (PCR)-negative samples. Taking into consideration anecdotal experience regarding genotype F (data not published) and a trend to a low viral load, some of these samples could indeed be F genotypes. New sample collection with more careful storage is needed to better clarify this point.

Another surprising result regarding genotype analysis was the presence of genotype G, which has never been described in this area before. In addition, double genotype was recognized by Innolipa test in the same patient. These results should be confirmed with full sequencing of the viral genome, to confirm genotype G in the Amazonian, as well as to observe the possibility of genome recombination.[22]

Another aspect that deserves further clarification is the role of viral load in the efficiency of intrafamilial transmission of HBV. Unfortunately, there are no HBV-DNA commercial kits in Brazil so these data were not available in the present study.

In conclusion, the present study provided strong evidence for the intrafamilial transmission of HBV, with the frequency of HBV markers being higher among families with more than one HBsAg carrier and among those who habitually share toothbrushes. Further studies are necessary to better establish the route of dissemination of HBV in the Brazilian Amazon region.

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