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Hepatitis B virus infection in Haemodialysis Centres from Santa Catarina State, Southern Brazil. Predictive risk factors for infection and molecular epidemiology

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Abstract

Background: Patients under haemodialysis are considered at high risk to acquire hepatitis B virus (HBV) infection. Since few data are reported from Brazil, our aim was to assess the frequency and risk factors for HBV infection in haemodialysis patients from 22 Dialysis Centres from Santa Catarina State, south of Brazil.

Methods: This study includes 813 patients, 149 haemodialysis workers and 772 healthy controls matched by sex and age. Serum samples were assayed for HBV markers and viraemia was detected by nested PCR. HBV was genotyped by partial S gene sequencing. Univariate and multivariate statistical analyses with stepwise logistic regression analysis were carried out to analyse the relationship between HBV infection and the characteristics of patients and their Dialysis Units.

Results: Frequency of HBV infection was 10.0%, 2.7% and 2.7% among patients, haemodialysis workers and controls, respectively. Amongst patients, the most frequent HBV genotypes were A (30.6%), D (57.1%) and F (12.2%). Univariate analysis showed association between HBV infection and total time in haemodialysis, type of dialysis equipment, hygiene and sterilization of equipment, number of times reusing the dialysis lines and filters, number of patients per care-worker and current HCV infection. The logistic regression model showed that total time in haemodialysis, number of times of reusing the dialysis lines and filters, and number of patients per worker were significantly related to HBV infection.

Conclusions: Frequency of HBV infection among haemodialysis patients at Santa Catarina state is very high. The most frequent HBV genotypes were A, D and F. The risk for a patient to become HBV positive increase 1.47 times each month of haemodialysis; 1.96 times if the dialysis unit reuses the lines and filters ≥ 10 times compared with haemodialysis units which reuse < 10 times; 3.42 times if the number of patients per worker is more than five. Sequence similarity among the HBV S gene from isolates of different patients pointed out to nosocomial transmission.

Background

Hepatitis B Virus (HBV) can be detected in blood and derivatives as well as in saliva, semen, vaginal secretion and exudates from cutaneous ulcer. HBV contamination is mainly established during the first year of life in highly endemic areas (Asia, Africa and East Amazonia), while in low epidemic areas the most important infection is found among young adults belonging to risk groups (health workers, haemodialysis patients, haemophiliacs, homosexuals, prostitutes, drug abusers, Hansen's disease patients, immunosuppressed patients and contacts with HBV infected patients) [1].

During the 70's, HBV infection was recognized as a great risk to haemodialysis patients [2], as HBV infection prevalence overcame 50% in some centres [3]. Some of the factors associated with HBV propagation include blood and derivatives transfusion, duration and frequency of haemodialysis, equipment contamination and contact among patients as well among them and health-care workers [4]. Since that time, HBV incidence in haemodialysis patients has been dramatically decreased especially by selection of blood donors, HBsAg positive patients isolation during dialysis and routine vaccination of uraemic patients. In Brazil, HBV infection in haemodialysis centres varies from 7.5 to 28.0 % [1].

HBV is as ubiquitous as man and it is found in all inhabited region of the globe [5]. HBsAg heterogeneity is well established. Serological methods developed to distinguish HBsAg antigenic subtypes allowed its classification in nine different subtypes with variable frequencies in different countries [6].

Divergences of the complete genome sequence inside the same subtype is approximately 8%, similar to those found in different subtypes. Therefore, subtypes do not reflect a real genotypic variation and another classification was proposed based on the nucleotide sequence of the S gene [7]. Primarily, four genotypes were described – A, B, C and D – followed by two others, E and F, corresponding to subtypes *ayw4* and *adw4*, respectively [8,9]. Recently, two more genotypes were found: G, in North America and Europe [10] and H, in North and Central America [11].

Nucleotide sequence comparisons of HBV genome have been used to study the HBV routes of infection, like vertical or blood and derivatives accidental inoculation [12]. In our country, the viral genetic diversity of HBV has already been demonstrated [13], but the present study is the first to investigate the routes of transmission through HBV sequencing analysis.

The aims of this study were (i) to analyse the frequency of HBV serological markers (total anti-HBc, HBsAg and anti-

HBs) in haemodialysis patients, (ii) to analyse HBV DNA frequency in HBsAg positive patients submitted to haemodialysis, (iii) to characterize predictive factors to HBV infection in patients submitted to haemodialysis, and (iv) to study the epidemiology of HBV infection through molecular analysis of partial gene S sequence.

Methods

Data collection

From each patient, the following data were collected: name, age, gender, race, time in haemodialysis, change of dialysis units, number of haemodialysis sessions per week, number of times of equipment reuse, type of dialysis equipment and dialysis solution. Also, the dates of the first haemodialysis and the first haemodialysis session in the unit were recorded. A variable designated PSU (Patient always in the Same Unit) was created to distinguish patients coming from different dialysis units.

From each studied health care worker, data collected included current working unit, age, gender, professional assignment and labour time.

From each dialysis unit, the following data were collected: (i) type of dialysis equipment (proportional system, central system and tank), (ii) whether rooms were separated for patients with hepatitis B and/or C or temporal separation at the end of each day, (iii) whether separated types of reprocessing rooms for patients with hepatitis B and/or C were applied, (iv) frequency of disinfections of dialysis equipments with sodium hypochlorite (between shifts or by the end of the day), (v) and frequency of sterilization with formaldehyde or peracetic acid (daily, weekly or never).

Population

All the 813 patients and 149 (51.1%) out of 291 health-care workers from all the 22 dialysis units at Santa Catarina State, southern Brazil, were studied. Data and blood samples were prospectively collected between October 22, 1996 and December 03, 1997. As control group, 772 healthy adults – matched by sex and age (± 3 years) – were recruited from the same regions.

The studied population features were: age ranging from 14 to 86 years (47.1 ± 15.3); 349 (42.9%) females and 464 (57.1%) males; 764 (94.0%) whites, 25 (3.1%) mulattoes and 24 (3.0%) blacks. The 291 health-care workers studied included medical doctors (61), nurses (31), dialysis technicians and attendants (169) and cleaning professionals (30). All patients were submitted to four hours haemodialysis sessions three times a week using disposable needles to veno – arterial puncture and reuse of dialysis lines and equipments.

Some patients have been previously treated by peritoneal dialysis (30/813 – 3.7%), outpatient continuous peritoneal dialysis (14/813 – 1.7%) and renal transplantation (47/813 – 5.8%). Most (528/813 – 65.9%) of the patients have been previously submitted to blood transfusions: 342 (42.0%), 111 (13.7%) and 75 (9.2%) received one to five, six to ten and more than ten blood units, respectively. Previous serological data obtained from the dialysis units reported 58 (7.2%) and 246 (30.3%) out of 813 patients as HBsAg and anti-HCV positive, respectively.

The present study involved all the major dialysis units at Santa Catarina state. These units were geographically divided in: North state, units A, B, C and D; Itajaí River region, units E, H, I, R, O, P, Q, X; South state, units J, K, L, M and S; Mountain area, units F and G; and West state, units T, U and V.

Use of the different types of dialysis equipments

Among the 813 patients, proportional system, central system and tanks equipments corresponded to 161 (19.8%), 535 (65.8%) and 117 (14.4%), respectively, were used. In relation to the dialysis solution, 716 (88.1%) and 97 (11.9%) patients utilized bicarbonate and acetate, respectively.

Types of dialysis room

(i) W (white), for 268 (33%) HBsAg and anti-HCV negative patients, (ii) W/C: for 334 (41.0%) patients without HBsAg but occasionally anti-HCV positive; (iii) B: for HBsAg positive and anti-HCV negative 32 (3.9%) patients; (iv) C: for 117 (14.4%) HBsAg negative and anti-HCV positive patients; (v) B/C: for 62 (7.6%) HBsAg and/or anti-HCV positive patients.

Periodical disinfections

Frequency of disinfection of dialysis equipments was between each dialysis period for 267 (32.8%) patients and at the end of the day for 546 (67.2%) patients. No other sterilizing procedure was applied in equipments utilized by 303 (37.3%) patients, while 154 (18.9%) and 356 (43.8%) patients utilized equipments sterilized with 4% formaldehyde or peracetic acid daily or weekly, respectively.

Reuse of lines and dialysis equipments

(i) for HBsAg and anti-HCV negative patients (190 – 23.3%); (ii) for HBsAg positive patients (31 – 3.8%); (iii) for anti-HCV positive patients (102 – 12.5%); (iv) for HBsAg and/or anti-HCV positive patients (49 – 6.0%); and (v) for patients without HBsAg positive (441 – 54.2%).

Number of lines and dialysis equipments reuse

Less than 10 times for 369 (45.4%) patients, and between 10 and 20 times for 444 (54.5%) patients.

Samples

Blood samples were collected through venopuncture in dry tubes (9.5 ml) with vacuum. After clot retraction, samples were centrifuged at 1500 to 2000 rpm and stored in 1 mL aliquots at -20°C.

Serological reactions

Serological markers of hepatitis B virus were detected by ELISA using commercially available kits (Murex Biotech Ltd, United Kingdom): total anti-HBc (ICE* HBc Detection Pack), HBsAg (Murex HBsAg) and anti-HBs (Murex anti-HBs).

All samples were tested for anti-HBc and the positive ones were also tested for HBsAg. Finally, anti-HBs was tested in the HBsAg negative samples.

All samples were also investigated for contact with hepatitis C virus (HCV); samples repeatedly reactive to anti-HCV ELISA test (INNOTEST HCV Ab III[®] Innogenetics, Belgium) were further submitted to a confirmatory third generation immunoblot test (IB-III, INNO-LIA HCV Ab III[®] – Innogenetics, Belgium).

Polymerase chain reactions (PCR)

HBV DNA was detected by PCR using specific primers covering the core (C) and surface (S) genes of the HBV genome. In all HBsAg positive samples, extraction and amplification of HBV-DNA were carried out by nested PCR, as described by Kaneko *et al.* [14]. Samples with positive PCR result were further submitted to another PCR reaction covering a fragment of 417 bp of the S gene as developed by Sitnik *et al.*, 1999 [15]. HCV RNA was detected by a commercial RT-PCR kit following the manufacturer recommendations (INNO-LiPA HCVII PCR amplification, Innogenetics, Ghent, Belgium).

Sequencing reactions

PCR products covering the S gene were submitted to cycle sequencing reactions, using the second round primers and a method derived from Sanger *et al.* [16] with dideoxynucleotides (ddNTPs) labelled with fluorescent markers (ABI Prism[®] BigDye[™] Terminator Cycle Sequencing Ready Reaction Kit – Applied Biosystems, Foster City, CA, USA).

Genotyping

Genotype analysis was carried out through the comparison of the obtained sequences with sequences of the different HBV genotypes from the Genbank. For this

Table 1: Frequency of HBV serological and molecular markers in the 4 different studied subgroups.

Group	n	Anti-HBc +ve	HBsAg +ve	Anti-HBs +ve	PCR core +ve	PCR surface +ve
Haemodialysis Patients (HP)	813	262 (23.2%)	81 (10.0%)	126 (15.4%)	62 (7.6%)	49 (6.0%)
HP Controls	620*	150 (24.2%)	24 (3.8%)	89 (14.3%)	15 (2.4%)	8 (1.3%)
Health care Workers (HCW)	149	30 (20.1%)	4 (2.7%)	20 (13.4%)	4 (2.7%)	4 (2.7%)
HCW Controls	149	21 (14.1%)	4 (2.7%)	11 (7.4%)	3 (2.0%)	2 (1.3%)

*Three samples were not available for HBV markers detection. +ve = positive

Table 2: Frequency of anti-HCV ELISA reactivity and HCV infection in the 4 different studied subgroups.

Group	n	Anti-HCV +ve	HCV infection*
Haemodialysis Patients (HP)	813	276 (33.9%)	274 (33.4%)
HP Controls	623	7 (1.1%)	5 (0.8%)
Health care Workers (HCW)	149	7 (4.7%)	4 (2.7%)
HCW Controls	149	0	0

* anti-HCV positive by ELISA and immunoblot or PCR positive. +ve = positive

purpose, EditSeq and Megalign softwares from the DNAs-tar package (Lasergene Inc., USA) were used.

Phylogenetical analysis

Phylogenetic and molecular evolutionary analyses were conducted using the MEGA version 2.1 program (17). Sequences were aligned using CLUSTAL X version 1.81 (18) and then analyzed by the Neighbor-joining method using a distance matrix calculated according to the Kimura-2-parameter model (19) and gamma distribution.

Statistical analysis

For statistical analysis, HBsAg and anti-HBc positive cases were considered as HBV carriers, while concomitant anti-HBc and anti-HBs positive and HBsAg negative results were appointed as previous HBV infection.

All variables were descriptively analysed for patients, health-care workers and healthy controls using frequency and percentage for qualitative variables or, for quantitative variables, maximum, minimum, median, mean and standard deviation values.

For univariate analysis, Student's *t* and χ^2 tests were used to compare means and distributions among different groups. For multivariate analysis, a statistical logistic regression with stepwise method of variables selection was used to evaluate the significance of the variables obtained from the univariate analysis to predict HBV infection [20]. Through this procedure, it was possible to evaluate the contribution of each variable in diagnosis probability of HBV infection. Significance level was estab-

lished at 5%. All calculations were carried out using the Statistical Analysis System (SAS) [21].

Results

The results of HBV serological and molecular markers in haemodialysis patients and health-care workers and their respective controls are shown in Table 1.

Anti-HCV was detected in 276 (33.9%) out of 813 patients. Among these 276 patients, immunoblot confirmatory test was positive in 271 (98.2%) and inconclusive in five (1.8%). Among the latter, HCV RNA was detected by PCR in 171 (61.9%) patients. HCV infected cases were considered those with positive ELISA and at least another positive test (immunoblot and/or PCR); Table 2 shows the positive cases meeting these criteria.

Patient variables according to the presence (HBsAg and anti-HBc positive) or absence of HBV infection are shown in Table 3. Statistical significant differences were found for haemodialysis total time ($p = 0.0002$) and anti-HCV detection ($p < 0.0001$).

The relation between characteristics of haemodialysis units and HBV infection is shown in Table 4. Statistical significant differences were found for the type of dialysis equipment ($p = 0.0071$), frequency of disinfection with sodium hypochlorite ($p = 0.0002$), frequency of sterilization with 4% formaldehyde or peracetic acid ($p = 0.0043$), dialysis units rooms ($p < 0.0001$), type of reuse of lines and dialysis equipments ($p = 0.0009$) and patients/HCW ratio ($p < 0.0001$).

Table 3: Univariate analysis of patient features according to HBV infection

	HBV carriers	HBV non-carriers	P
Age			
mean	45.7	47.2	0.4026
standard deviation	13.6	15.4	
Gender			
Male	54 (11.6%)	410 (88.4%)	0.0660
Female	27 (7.7%)	322 (92.3%)	
Time (months) on haemodialysis			
mean	44.3	30.8	0.0002
standard deviation	39.1	32.7	
Previous renal transplantation			
Yes	5 (10.6%)	42 (89.4%)	0.8735
No	76 (9.9%)	690 (80.1%)	
Number of previous blood or derivates transfused			
Zero	24 (8.4%)	261 (91.6%)	0.5017
1 – 5	34 (9.9%)	308 (90.1%)	
6 – 10	15 (13.5%)	96 (86.5%)	
> 10	8 (10.7%)	67 (89.3%)	
Patients always in the same unit (PSU)*			
Yes	64 (9.6%)	601 (90.4%)	0.1891
No	17 (13.5%)	109 (86.5%)	
Anti-HCV			
Reactive	44 (16.0%)	232 (84.0%)	<0.0001
Non-reactive	37 (6.9%)	500 (93.1%)	

HBV carrier = HBsAg and anti-HBc positive; HBV non-carrier = anti-HBc negative. * data not available for 22 patients

Stepwise logistic regression analysis

Seven independent variables were submitted to a multivariate analysis by a logistic regression model [20] with the purpose of determining the probability of HBV infection among Haemodialysis patients. Variables with statistical significant results are shown in Table 5.

It was observed that total time in haemodialysis, number of reuse of lines and dialysis equipments and the patient/employee relation adjusted a probabilistic model that allows predicting HBV infection among patients with 60.5% sensitivity, 71.2% specificity and 70.1 percentage of correctly classified patients.

Consequently, the probability of a haemodialysis patient to have a HBV infection is given by:

$$P = \frac{\left[\exp(-4,5952 + 0,3963 \times T + 0,6731 \times R + 1,2297 \times P) \right]}{\left[1 + \exp(-4,5952 + 0,3963 \times T + 0,6731 \times R + 1,2297 \times P) \right]}$$

– where, T = time on haemodialysis; R = Reuse number of lines and dialysis equipments; and P = Patients / Health-care workers proportion.

The following data could be drawn from the above equation: (i) the chance of HBV infection increases 1.47 times monthly whilst the patient is submitted to haemodialysis; (ii) the chance of HBV infection in a patient that is submitted to haemodialysis in a unit where the number of reuse of lines and dialysis equipments is > 10 times multiplies by 1.96 the chance of a patient that goes to a unit where the reuse is inferior to 10 times; (iii) the chance of HBV infection in a patient that is submitted to haemodialysis in units where the patient/employee ratio is higher than five is 3.42 times the chance of a patient that goes to a unit where this proportion is lower than five.

HBV genotypes

The frequency of HBV genotypes in haemodialysis patients and their controls is shown in Table 6. Genotype D was the most frequent in both groups, but genotypes A and F were found exclusively in haemodialysis patients.

HBV genotypes frequency in health-care workers and their controls at haemodialysis units is shown in Table 7. Genotype A was the most prevalent among health-care workers. All controls were infected with genotype D.

Table 4: Univariate analysis of haemodialysis-units variables and HBV infection

	HBV carriers	HBV non-carriers	P
Type of haemodialysis equipment			
proportional system	8 (4.9%)	153 (95.1%)	0.0071
central system	66 (12.3%)	469 (87.7%)	
double tank	7 (5.9%)	110 (94.1%)	
Hygiene frequency			
Between shifts	11 (4.1%)	256 (95.9%)	0.0002
End of the day	70 (12.8%)	476 (87.2%)	
Sterilization frequency			
daily	8 (5.2%)	146 (94.8%)	0.0043
weekly	30 (8.4%)	326 (91.6%)	
never	43 (14.2%)	260 (85.8%)	
Dialysis Unit Rooms			
B	26 (86.7%)	4 (13.3%)	<0.0001
B/C	15 (28.3%)	38 (71.7%)	
W	4 (1.5%)	265 (98.5%)	
C	13 (12.5%)	91 (87.5%)	
W/C	23 (6.4%)	334 (93.4%)	
Reuse of lines and dialysis equipments			
< 10 times	21 (5.7%)	348 (94.3%)	0.0009
≥ 10 times	60 (13.5%)	384 (86.5%)	
Patients/ HCW ratio			
1 – 4	19 (4.6%)	396 (95.4%)	<0.0001
5 – 8	60 (13.5%)	223 (86.5%)	
>9	17 (14.8%)	98 (85.2%)	

Where: B = for HBsAg positive and anti-HCV negative patients, B/C = for HBsAg and/or anti-HCV positive patients, W = for HBsAg and anti-HCV negative patients, C = for HBsAg negative and anti-HCV positive patients, W/C = for patients HBsAg negative but occasionally anti-HCV positive; HCW = health-care workers

Table 5: Data from the adjusted model of stepwise logistic regression

Variable	Estimative	Error	P	95% Confidence Interval		
				Odds ratio	Inferior	Superior
Intercept	-4.5952	0.4653	0.0001			
Time on haemodialysis (T)	0.3863	0.1138	0.0007	1.472	1.177	1.839
Reuse number of lines and dialysis equipments (R)	0.6731	0.2730	0.0137	1.960	1.148	3.347
Patients / Health-care workers Proportion (P)	1.2297	0.2784	0.0001	3.420	1.982	5.903

Table 6: HBV genotypes frequency in haemodialysis patients and controls

HBV Genotypes	Haemodialysis Patients		Controls	
	N (+)	Total (%)	N (+)	Total (%)
A	15/49	(30.6)	-	
D	28/49	(57.1)	8/8	(100.0)
F	6/49	(12.2)	-	

Table 7: Frequency of HBV genotypes in health-care workers and controls at haemodialysis units

HBV Genotypes	Health-care workers	Controls
	N (+) / Total (%)	N (+) / Total (%)
A	2/4 (50.0)	-
D	1/4 (25.0)	2/2 (100.0)
F	1/4 (25.0)	-

HBV phylogenetic tree

The phylogenetic tree drawn from the analysis with the HBV S-gene sequences (PCR-positive samples) shows that the branches of the different genotypes are clearly individualised (Figure 1).

Genotype A was found in haemodialysis groups from units H (Itajaí River region), K, M and S (South region). All cases were clustered in the same branch of the tree with a 100 bootstrap value. Genotype D was found in haemodialysis groups from units A, B, D, (North region), J, K (South region), O, R, (Itajaí River region), T, U and V (West region). Most of the cases were clustered in the same tree branch with a bootstrap value of 88. The two cases from unit J clustered in the same branch (bootstrap value = 98). Genotype F was found only in units F (Mountain region) and V (West state). All cases clustered in the same branch with a 79 bootstrap value. The sequences from the control population, being all genotype D, were scattered through different branches of the tree.

Discussion

Many studies on HBV infection in dialysis units have been published; nonetheless the landmark of the present investigation is the inclusion of all patients from all dialysis units of the Santa Catarina State.

All of the 813 patients of the 22 dialysis units have accepted to enrol on this study, allowing us to investigate 100% of the patients that received assistance at that time in the state of Santa Catarina. Besides, 51.1% of the health-care workers from the dialysis units and, as controls, 772 healthy workers matched by sex and age (\pm 3 years) from companies in the same area have volunteered to take part.

The 22 dialysis units were heterogeneous when comparing the use of disposable needle for artery-vein fistula, number of reuse of lines and dialysis equipments, type of dialysis machine, machine hygiene, machine sterilisation, type of dialysis rooms regarding infected patients, lines and dialysis equipments reprocessing rooms and the

patients/care workers ratio. In fact, after literature review, no related reference was found with such a large coverage.

The ideal human resources for dialysis units established by decree (number 2,042) from the Brazilian Ministry of Health's [23] define one technician or attendant per four patients. The reality disclosed by this study was quite different, though; the lack of qualified personnel and, sometimes, under qualified care workers was the main cause for the low human resource/patient ratio found at Santa Catarina State. Due to this distortion, this variable was adapted by taking into consideration the total number of technicians, auxiliaries and nursery attendants per attended patients.

As determined by a third-generation ELISA method in a previous work with hepatitis C virus [22], anti-HCV was found in 33.9% (276/813) of the present patient population, and confirmed by immunoblot in 98.2% (271 patients). Polymerase chain reaction for the 271 immunoblot-positives and five inconclusive results was positive in 171 (62%) patients. Anti-HCV serological marker was detected in 44 of the 813 patients infected by HBV, representing 5.4% of HBV + HCV co-infection.

In this study, the definition of present or past HBV infection was based on total anti-HBc reactivity. In our casuistic, anti-HBc was positive in 32.2% of the patients, 10.0% with HBsAg and 15.4% with anti-HBs. Such high frequency of HBsAg positive was observed in several investigations, not only in Brazil, but also in other countries. Hepatitis B serology was positive in 72.7% of the haemodialysis patients in Saudi Arabia – a hyperendemic area –, of which 10.9% was HBsAg positive [24]. On the other hand, HBsAg frequency was 1.6% and anti-HBc 36.7%, in non-hyperendemic areas in Japan [25]. In Brazil, the reported HBV-infection frequency varied from 14.1% in Porto Alegre city [26], 7.8 to 28.0% in São Paulo city [27-31], 12.0% in Goiania city [32] and up to 20.0% in Salvador city [33].

In a three-year prospective study carried out in São Paulo, the appearance of HBsAg in 17 patients submitted to

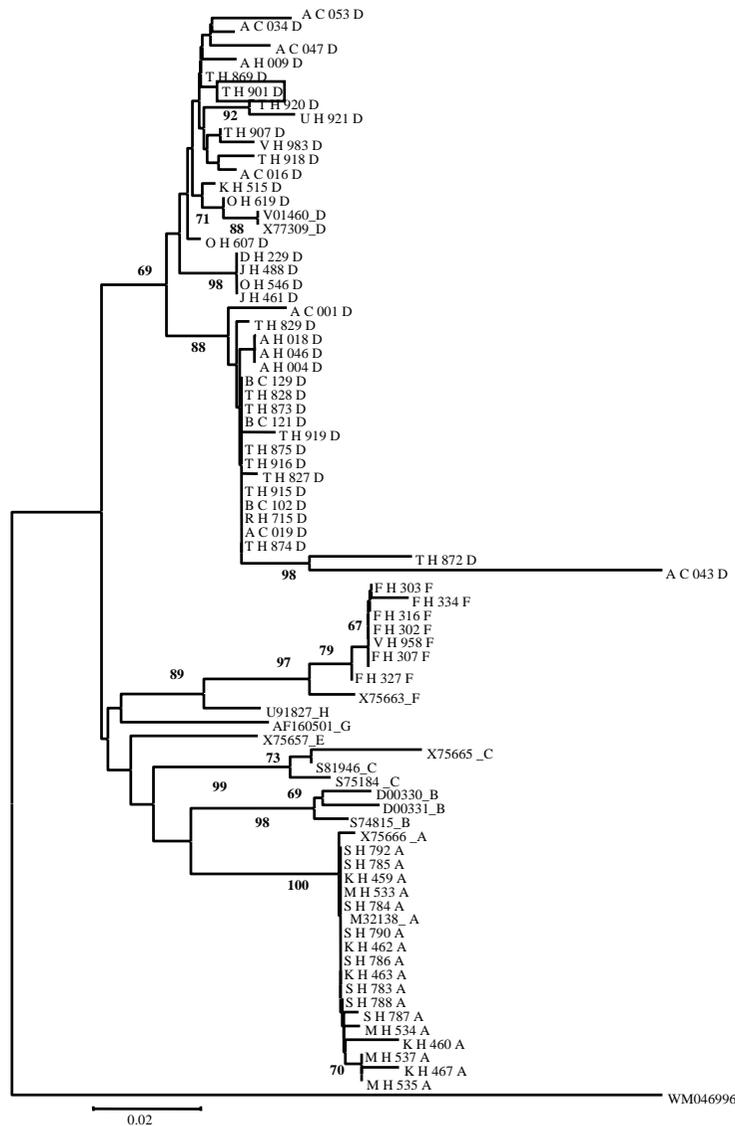


Figure 1
Neighbor-joining tree of a 348 nt fragment (including gaps) of the HBV S gene from isolates of the present studied population and Genbank sequences representing genotypes A to H. Tree was constructed with the distance matrix calculated with the Kimura 2-parameter method and γ distribution using MEGA version 2.1. Bootstrap test of phylogeny was performed with 100 replications and values equal or greater than 69 are indicated on the branches. Sequences from the present study are named as followed R_P_NNN_G where: R = region of haemodialysis unit (A to V); P (H = haemodialysis groups and C = controls); NNN = number of the sequence; and G = genotype (A, D or F). Genotype representative sequences from the Genbank (loci and accession numbers) are: A (HHVBA – X75666, HPBSAG – M32138); B (HPBADW2 – D00330, HPBADW3 – BD00331, S74815 – S74815); C (HHVBC – S75184, HHVCCHA – X75665; S81946 – S81946); D (RXXHEPA – V01460 , HPVPS12SA – X77309); E (HHVBASS – X75657); F (HBVADW4A – X69798; HHVBF – X75663); G (IG9227 – AF160501) and H (U91827 – U91827). The Woolly Monkey Hepatitis B Virus (WM046996) was utilized as outgroup.

haemodialysis corresponded to 0.19 patients a year [34]. Seroconversion rates of 2% were similar to other dialysis unit in São Paulo during a six-month study [30].

HBV genotyping use as an epidemic marker allowed the demonstration that the infection was introduced by chronic patients and disseminated in a haemodialysis unit, probably due to equipment contamination and the unit environment [4].

In relation to the health-care workers and controls, HBsAg prevalence varied between 2.7% and 3.8%, lower than the one observed in haemodialysis patients. A similar HBsAg figure (2.9%) was observed in hospital workers in other Brazilian regions [35]. HBsAg frequency in health-care workers in the USA was 1.0%, thus lower than the observed in our country [36].

HBV-DNA was detected by PCR in 62 (7.6%) of the 813 patients and in 76.5% of the 81 positive HBsAg patients, confirming the high viral replication frequency in these patients. This figure was similar to the one observed by other authors in haemodialysis populations in Goiania city, in which the PCR-positive frequency was between 67.6% and 88.2% of the HBsAg positive patients [32]. The observations above show that HBV infection continues to be an important problem in Brazilian haemodialysis units.

Univariate analysis of age and sex has not shown significant differences for presence or absence of HBV infection. Likewise, renal transplant background, number of blood and/or derivatives transfusions and patients always in the same unit (PSU) were not significantly associated with this infection.

On the other hand, significant differences among patients without HBV infection were observed with the following variables: total haemodialysis time in months and anti-HCV serological marker. Significant association between HBV and anti-HCV markers was also observed in São Paulo by other authors. It was reinforced the role of haemodialysis duration, previous background of haemodialysis, renal transplant background and blood transfusions background [27,34].

The presence of total anti-HBc in patients' serum has been associated to a higher prevalence of HCV infection, but this is still controversial. Several authors found such relation [35-46], while others denied [46,47].

All variables related to dialysis units showed, by univariate analysis, significant differences for HBV infection; those were (i) type of dialysis equipment, (ii) hygiene and sterilization frequency, (iii) patients distribution accord-

ing to the group of dialysis rooms, (iv) number of reuse of lines and dialysis equipments and (v) patients distribution according to the number of health-care workers (technicians, auxiliary and nursery attendants).

Reuse of dialysis equipments was initially proposed to reduce costs [48], as guidelines supporting this practice claimed no increase in HBV infection [49]. On the other hand, a previous case-control study had shown the importance of poor function of dialysis machines due primarily to rupture of dialysis membranes [50].

As observed by univariate analysis, HBV infection and the following variables were statistically significant: haemodialysis total time, machine hygiene frequency, machine sterilization frequency, number of reuse of lines and dialysis equipments, number of patients per number of health-care workers and HCV infection.

The multivariate analysis (stepwise-logistic regression) selected the variables haemodialysis total time, number of reuse of lines and dialysis equipments and the proportion patients per health-care workers. This analysis adjusted a probabilistic model that allows predicting HBV infection in the haemodialysis patients with 60.5% sensitivity, 71.2% specificity and 70.1% of correctly classified patients.

Other investigators, by means of multivariate analysis, observed an association of three variables only – haemodialysis duration, type of dialysis (haemodialysis or ACPD – ambulatory continuous peritoneal dialysis) and blood transfusion background – with HBV infection markers [27,34]. These results contradict the present work, as no relation between the number of blood/derivates transfusion and HBV infection was observed.

So far, the importance of the variables related to dialysis units and HBV infection has not been well emphasized in literature. The present investigation emphasizes some aspects of strong epidemiological importance: (i) the HBV chance of infection increases 1.47 times a month in haemodialysis patients; (ii) the chance of a haemodialysis patient to become HBV infected in a unit that reuses lines and dialysis equipments more than 10 times is 1.96 times the chance of a patient in a unit that reuses the same material less than 10 times; and (iii) the chance of HBV infection in a patient that does haemodialysis in a unit in which the patient/health-care worker ratio is higher than 5 is 3.42 times the chance of a patient that does so in units with lower ratio values.

With the purpose of a better understanding of the HBV spreading among haemodialysis patients, HBV genotyping and sequencing were applied. In 65 positive HBsAg,

the following frequency was found: genotype A – 28%, genotype D – 57% and genotype F – 15%. Therefore, it can be observed that genotypes B, C and E were not represented in the present study. As for genotypes B and C, frequently detected in patients from Southeast Asia, this result was expected due to the absence of Japanese in the studied patients. Likewise, genotype E – usually found in African populations – was not detected. In this study, 94% of the patients were white and only 3% black, but other studies did not show any evidence of genotype E in Brazil [15].

Due to the high miscegenation in Brazil and lack of data, the present study points out the need of similar epidemiological researches in other dialysis units with more heterogeneous populations for a comprehensive and significant genotype characterisation. Genotype F was found at a low frequency in 10/65 (15.4%) and 6/84 (7.1%), respectively to haemodialysis and chronic hepatitis patients [15].

It is worth noting that the HBV DNA-sequencing disclosed 100% similarity among isolates of various genotypes from 17 patients belonging to different dialysis units. This similarity found in a same unit strongly suggests contamination by the same viral strain and, consequently, nosocomial transmission. These findings represent important advances in HBV epidemiology and similar studies, from other regions of Brazil, are awaited.

Despite the knowledge of the risk factors related to haemodialysis, the high frequency of HBV infection observed in our dialysis environment shows that these units represent a closed system for this viral transmission.

Many governmental and institutional recommendations have been set to reduce HBV dissemination in haemodialysis patients. Unfortunately, those recommendations have not been followed by all units, as shown by HBV infection surveys [51] and the appearance of recent infection outbreaks [52].

The present investigation enabled us to suggest more rigorous measures to be adopted: (i) maintenance of nationwide debates on the subject; (ii) active immunoprophylaxis in ureamic patients before their admission in dialysis units; (iii) reduction on reuse of lines and dialysis equipments; and (iv) reduction in the number of patients per health-care worker.

Conclusions

In face of the present results, it can be concluded that: (i) HBV serological markers were highly frequent in haemodialysis patients as in comparison to health-care workers and controls; (ii) HBV DNA was detected in most positive

HBsAg patients; (iii) predictive HBV infection factors included haemodialysis total time, number of reuse of lines and dialysis equipments and patients/health-care workers ratio; (iv) HBV genotypes found in these patients were A, D and F, with A and D predominance; (v) HBV DNA sequencing revealed 100% similarity in isolates from different patients, bounding them to a common and nosocomial source of infection; and (vi) the need for strict observance of the rules for correct technical functioning with qualified personnel and service surveillance.

Competing interests

None declared.

Authors' contributions

FJC: conceived and coordinated the study from its design to the manuscript confection; CRM: participated in the study design and collected clinical samples and data; JRRP: coordinated the molecular biology experiments and participated in the manuscript writing; IMVGC, DAB, MFL, RCM, LCB: carried out the laboratory determinations; RAC: participated in the study design and performed the statistical analysis; GRS: participated in the manuscript writing and revision; LCS: participated in the study design and in the manuscript writing. All authors read and approved the final manuscript.

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