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HEPATITIS B VIRUS INFECTION PROFILE IN CENTRAL BRAZILIAN HEMODIALYSIS POPULATION

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SUMMARY

Hepatitis B has proved to be a major health hazard in hemodialysis patients. In order to investigate the hepatitis B virus (HBV) infection profile in the hemodialysis population of Goiânia city – Central Brazil, all dialysis patients (N=282) were studied. The prevalence of any HBV marker (HBsAg, anti-HBs, and anti-HBc) was 56.7% (95% CI: 51.1-62.7), ranging from 33.3% to 77.7% depending on dialysis unit. HBV-DNA was detected in 67.6% and 88.2% of the HBsAg-positive serum samples, in 91.3% and 100% of the HBsAg/HBeAg-positive samples, and in 18.2% and 63.6% of the HBsAg/anti-HBe-reactive sera by hybridization and PCR, respectively. The length of time on hemodialysis was significantly associated with HBV seropositivity. Only 10% of the patients reported received hepatitis B vaccination. The findings of a high HBV infection prevalence in this population and the increased risk for HBV infection on long-term hemodialysis suggest the environmental transmission, emphasizing the urgent need to evaluate strategies of control and prevention followed in these units.

KEYWORDS: Hepatitis B; HBV-DNA; Risk factors; Hemodialysis.

INTRODUCTION

Hepatitis B virus (HBV) infection is a major international public health problem affecting more than 300 million carriers worldwide, and is an important cause of morbidity and mortality from liver disease[12]. Detection of serum HBV-DNA is considered to be the best marker of active HBV replication and is usually associated with active liver disease and infectivity[2]. Hepatitis e antigen (HBeAg) has also been used as indicator of active HBV replication and infectivity. In general, the seroconversion of HBeAg to anti-HBe is associated with clearence of serum HBV-DNA. However, in some patients, HBV-DNA is detected despite the seroconversion to anti-HBe[3,16,22], and these HBeAg negative variants are responsible for an unusual form of chronic HBV infection and may be associated with fulminant hepatitis[19].

As the spread of HBV occurs mostly via parenteral, patients undergoing dialysis treatment belong to a group at high risk to acquire this infection[8,11,24]. In addition, end-stage renal disease (ESRD) patients on long-term hemodialysis have an increased tendency to become chronic HBV carriers and to be also a potential reservoir for the transmission of this virus[4,25].

In many countries, hepatitis B transmission has been controlled in hemodialysis centers by adoption of both universal precautions and the following hemodialysis-specific infection-control practices. In addition, susceptible patients are often vaccinated[1,20,30]. Nevertheless, in Brazil, considered to be an area of intermediate endemicity[13], studies carried out in São Paulo and Rio de Janeiro (Southeastern region) showed that hemodialysis patients still have high HBV prevalence rates[6,31]. As data concerning HBV infection in other Brazilian regions are still rare, we evaluated the HBV infection profile in hemodialysis population of Goiânia city, Central Brazil.

MATERIAL AND METHODS

Subjects

Our study was carried out in all dialysis units (N=10) from Goiânia city (1,000,000 inhabitants), an intermediate endemic area for hepatitis B infection. Between September and December 1995, all chronic hemodialysis patients (N=282) were interviewed for risk factors to HBV infection and hepatitis B vaccination. The studied

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population ranged in age from 13 to 79 years (average 43.3 years). One hundred fifty-seven were males (55.7%) and 125 were females (44.3%).

A standardized form was used and data on age, sex, history of hepatitis/jaundice, length of time on hemodialysis treatment, number of previous transfusion, acupuncture, tattooing, intravenous drug use, multiple partners, sexually transmitted diseases and household contact with hepatitis/jaundice were collected.

Serological Tests

Blood samples were collected from all patients and sera were stored at -20°C. The samples were screened by enzyme-linked immunosorbent assay (ELISA) for the presence of the following hepatitis B viral markers: HBsAg, anti-HBs (Hepanostika Uni-form Organon Teknika B.V., Boxtel, Holland), and anti-HBc (Fiocruz, Brazil)[26] . HBsAg- positive samples were submitted to HBeAg and anti-HBe detection (Hepanostika Uni-form Organon Teknica B. V., Boxtel, Holland).

Dot Blot Hybridization

This assay was performed as described by NIEL et al.[21]. Briefly, 10 µL of HBsAg- positive sera were incubated with 90 µL of 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) and 100 µL of NaCl 1 M, NaOH 0.1 M for 1 hour at room temperature. The denatured serum was spotted by filtration through a nitrocellulose membrane previously equilibrated with 20 x SSC. The membrane was then washed with 2 x SSC, heated at 80°C for 2 hours, prehybridized and hybridized. The probe used was the plasmid pHBV991 containing the entire genome of HBV, strain HVHEPB adw, labeled by nick translation with biotin-11-dUTP (Bionick labeling system, Gibco-BRL).

Amplification by PCR

DNA was extracted from 10 µL of serum samples, essentially as described by LOK et al.[15]. From each DNA preparation, 10 µL were denatured (3 min at 95°C) and then submitted to a first round of PCR amplification consisting of 40 cycles under the following conditions: 1 min at 95°C, 1 min at 48°C, and 1 min at 72°C in a total volume of 50 µL. From the first round of amplification, 1 µL product was amplified again with nested primers for 40 cycles. PCR products were loaded on 2% agarose gel, electrophoresed, and stained with ethidium bromide. PCR primers used were complementary to conserved areas of preS1 and precore regions of the HBV genome[27,28,29].

Statistical Analysis

Quantitative variables were expressed as mean and range. The statistical procedures used were the Chi-square test and Fisher's exact test for the comparison of categorized variables. Risk factors detected by univariate analysis were analysed by multiple logistic regression. This analysis was used to evaluate the association between each risk factor detected by univariate analysis while controlling for the effects of others. Statistical evaluations were performed using the EpiInfo 6.0 program developed by Centers for Disease Control (Atlanta, GA) and "EGRET" ("Epidemiological, Ghaphics, Estimation and Testing Package", 1991).

RESULTS

As shown in Table 1, 34 of the 282 patients (12%) were HBsAg-positive, 153 (54.2%) were anti-HBc-reactive, and 101 (35.8%) were anti-HBs-positive. Altogether, HBV markers were present in 160 patients, resulting in an overall HBV prevalence of 56.7% (95% CI: 51.1-62.7). HBV prevalence rates ranged from 33.3% to

TABLE 1
Prevalence rates of hepatitis B viral markers in ten hemodialysis units, Goiânia-Central Brazil

Unit	N_o.	HBsAg Pos. (%)	Anti-HBc Pos. (%)	Anti-HBs Pos. (%)	Any Marker Pos. (%)	95% CI
A	15	0 (0.0)	5 (33.3)	4 (26.6)	5 (33.3)	11.3 - 62.2
B	18	6 (33.3)	13 (72.2)	7 (38.8)	14 (77.7)	51.8 - 93.8
C	15	3 (20.0)	5 (33.3)	2 (13.3)	5 (33.3)	11.3 - 62.3
D	15	1 (6.6)	8 (53.3)	6 (40.0)	8 (53.3)	26.3 - 79.3
E	27	5 (18.5)	18 (66.6)	12 (44.4)	19 (70.3)	49.4 - 86.4
F	81	7 (8.6)	38 (46.9)	28 (34.5)	41 (50.6)	38.6 - 62.6
G	52	12 (23.0)	35 (67.3)	20 (38.4)	36 (69.2)	54.2 - 82.2
H	18	0 (0.0)	11 (61.1)	9 (50.0)	12 (66.6)	40.7 - 86.7
I	24	0 (0.0)	12 (50.0)	7 (29.1)	12 (50.0)	29.0 - 71.0
J	17	0 (0.0)	8 (47.0)	6 (35.3)	8 (47.0)	22.1 - 73.1
Total	282	34 (12.0)	153 (54.2)	101 (35.8)	160 (56.7)	51.1 - 62.7

TABLE 2
Serological features of the 34 HBsAg-positive-hemodialysis patients from Goiânia, Central Brazil

Patient Number	HBeAg	Anti-HBe	Hybridization	PCR			
				1st round		2nd round	
				PreS1	Precore	PreS1	Precore
16	+	-	-	-	-	+	+
18	-	+	-	-	-	-	-
25	+	-	+	+	+		
27	+	-	+	+	+		
30	-	+	-	-	-	-	-
32	+	-	+	+	+		
33	-	+	-	-	-	+/-	+/-
43	-	+	-	-	-	-	-
46	-	+	+	+	+		
50	+	-	+	-	-	+	+
82	+	-	+	+	+		
83	+	-	+	+	+		
84	+	-	-	-	-	+	+
85	+	-	+	+	+		
106	+	-	+	+	+		
145	+	-	+	+	+		
159	+	-	+	+	+		
160	+	-	+	+	+		
168	+	-	+	+	+		
169	+	-	+	+	+		
170	+	-	+	+	+		
175	-	+	-	-	-	-	+
176	-	+	-	-	-	-	+
180	+	-	+	+	+		
189	-	+	-	-	-	-	+
191	-	+	-	-	-	-	-
192	-	+	+	+	+		
195	-	+	-	-	-	+	+
197	+	-	+	+	+		
198	+	-	+	+	+		
208	+	-	+	+	+		
210	+	-	+	+	+		
217	+	-	+	-	-	+	+
277	+	-	+	+	+		

77.7% depending on the dialysis unit studied. The rates for HBsAg, anti-HBc and anti-HBs ranged from 0% to 33.3%, 33.3% to 72.2%, and 13.3% to 50.0%, respectively.

Out of 34 HBsAg-positive patients studied, 23 (67.6%) and 11 (32.3%) serum samples were HBeAg- and anti-HBe-positive, respectively. HBV-DNA was detected in 67.6% (23/34) and 88.2% (30/34) of the HBsAg-positive serum samples, in 91.3% (21/23) and 100% (23/23) of the HBsAg/HBeAg-positive samples, and in 18.2% (2/11) and 63.6% (7/11) of the HBsAg/anti-HBe-reactive sera by hybridization and PCR, respectively (Table 2). Among the HBeAg-reactive samples, the majority were PCR positive after the first round. However, most of anti-HBe sera were PCR positive after the second round.

Analysis of all risk factors studied showed that length of time on hemodialysis, sex, and history of hepatitis/jaundice were significantly associated with HBV seropositivity by univariate analysis (Table 3).

In addition, the multivariate analysis revealed that patients under treatment for more than three years had a 11.2 fold (95% CI: 4.7-26.6) greater risk of HBV positivity compared to subjects who had less than one year of treatment. Only 28 (10%) patients reported vaccination against HBV. Among these, 3 (10.7%) were anti-HBs-positive (data not shown).

DISCUSSION

The present investigation showed high prevalence rates of hepatitis B viral markers in hemodialysis patients from Goiânia, Central Brazil, when compared to those found in blood donors and female population from the same region[5,17]. Nevertheless, with reference to other hemodialysis populations, these rates were similar to those obtained in São Paulo and Rio de Janeiro, Brazil[6,31], but generally higher than prevalences observed in Europe and the USA[9,10,25]. This demonstrates that HBV infection continues to be a significant problem in Brazilian dialysis units.

Increased prevalence rates of any HBV serologic markers were

TABLE 3
Risk factors for HBV infection in the hemodialysis population of Goiânia- Central Brazil

Risk factor	HBV Positive /Total *	(%)	Odds ratios (95% CI)+
Sex			
Female	58 / 125	(46.4)	1.0
Male	99 / 157	(63.0)	2.0 (1.2-3.3)
History of hepatitis/jaundice			
No	112 / 221	(50.6)	1.0
Yes	42 / 50	(84.0)	5.1 (2.2-12.4)
Length of time on hemodialysis			
< 1 year	37 / 94	(39.3)	1.0
1-3 years	61 / 120	(50.8)	1.6 (0.9-2.8)
> 3 years	59 / 68	(86.7)	10.1 (4.5-22.8)
Number of previous transfusion			
0	20 / 31	(64.5)	1.0
<5	71 / 154	(46.1)	0.5 (0.2-1.0)
5-10	21 / 32	(65.6)	1.1 (0.4-3.0)
>10	34 / 48	(70.8)	1.3 (0.5-3.5)
Acupuncture			
No	152 / 273	(55.6)	1.0
Yes	4 / 7	(57.1)	1.1 (0.2-6.1)
Tattooing			
No	156 / 276	(56.5)	1.0
Yes	1 / 5	(20.0)	0.2 (0.01-1.8)
Intravenous drug use			
No	153 / 276	(55.4)	Indefined
Yes	2 / 2	(100.0)	
Multiple partners			
No	130 / 244	(53.2)	1.0
Yes	24 / 34	(70.5)	2.1 (0.9-4.9)
Sexually transmitted diseases			
No	114 / 213	(53.5)	1.0
Yes	38 / 62	(61.2)	1.4 (0.7-2.5)
Household contact with hepatitis/jaundice			
No	105 / 197	(53.3)	1.0
Yes	35 / 63	(55.5)	1.1 (0.6-2.0)

+ CI = Confidence interval
* Variations in denominators reflect incomplete questionnaire response

observed among the ten dialysis units, ranging from 33.3% to 77.7%, while high rates of HBsAg were found in six of them, probably important measures for prevention and control of hepatitis B infection were not adopted by these units such as monthly screening for HBsAg and an appropriate isolation of infected patients.

The findings that 67.6% and 88.2% of HBsAg-positive patients had detectable serum HBV-DNA by dot-blot hybridization and PCR, respectively, demonstrate that the majority of them are infectious. In additon, all patients with HBsAg and HBeAg in their sera were PCR-positive, and most had HBV-DNA present in such a high titers that it was detectable by hybridization, indicating an important association between the detection of HBeAg and HBV-DNA. On the other hand, the presence of anti-HBe may not correspond a complete interruption of viral replication, as viral DNA was detected in 63.6% of HBsAg and anti-HBe-reactive samples by PCR. However, only 18.2% of the samples were positive by hybridization. Thus, a low level of HBV replication persists in most patients after anti-HBe seroconversion. These data emphasize the need for rapid recognition and isolation of HBsAg-positive patients.

Previous studies reported that the duration of dialysis treatment is clearly correlated with HBV seropositivity[4,6,23]. In the present study, this association was also observed. The multivariate analysis revealed that patients under treatment for more than three years had a 11.2 - fold (95% CI: 4.7-26.6) greater risk of HBV positivity compared to subjects who had less than one year of treatment.

Regarding previous blood transfusion, our results revealed that, in contrast to other studies[6,18,23], this risk factor was not an important mechanism of HBV transmission in hemodialysis patients in Central Brazil. This may be due to the obligatory screening of both HBsAg and anti-HBc in Brazilian blood donors, and the administration of recombinant human erythropoietin (rHuEPO) in these hemodialysis patients.

Some possible causes of HBV dissemination in hemodialysis units have been described, which included failure to identify and isolate HBV-infected patients; sharing of staff, equipment, and supplies among patients; and lapse to vaccinate susceptible patients against hepatitis B [7,8]. In fact, at the time we started our study, some units did not isolate HBsAg-positive patients by room, machine, supplies and staff. Moreover, although the most effective means of preventing hepatitis B is through vaccination, a measure recommended for suscetible dialysis patients since 1982 [14], only 10% of these patients had been vaccinated.

In conclusion, in addition to the high endemicity of HBV in hemodialysis units of Goiânia, Central Brazil, the length of time on hemodialysis treatment seems to be the main risk factor, suggesting the environmental transmission of hepatitis B virus. These data emphasize the need for stricter adherence to infection control measures and reinforce the importance of hepatitis B vaccination in dialysis centers.

RESUMO

Perfil da infecção pelo vírus da hepatite B na população de hemodiálise, Brasil Central

A hepatite B tem sido uma grande ameaça aos pacientes de hemodiálise. Para investigar o perfil da infecção pelo vírus da hepatite B (VHB) na população de hemodiálise de Goiânia – Brasil Central, 282 pacientes foram estudados. A prevalência de marcadores do VHB (AgHBs, anti-HBc e anti-HBs) foi de 56,7% (IC 95%: 51,1 – 62,7) variando de 33,3% a 77,7% entre as unidades de diálise. O VHB-DNA foi detectado nas amostras AgHBs positivas em 67,6% e 88,2%, nas AgHBs e AgHBe em 91,3% e 100%, e nas AgHBe e anti-HBe soro reativas em 18,2% e 63,6% por hibridização e PCR, respectivamente. O tempo de tratamento hemodialítico mostrou-se estatisticamente associado à soropositividade ao VHB. Somente 10% dos pacientes relataram vacinação para a hepatite B. Assim, uma prevalência elevada para infecção pelo VHB nesta população e o risco aumentado do tempo de tratamento hemodialítico sugerem a transmissão ambiental deste vírus, enfatizando a necessidade urgente de reavaliação das medidas de controle e prevenção adotadas nestas unidades.

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