Hepatitis B virus screening in contacts of blood donors with antibodies against core protein (anti-HBc), but without surface antigen (HBsAg)

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To increase blood safety Brazil introduced screening for anti-HBc among blood donors in 1993. There was a decrease in the hepatitis B virus (HBV) transmission, but this measure identified a great number of HBsAg-negative, anti-HBc-positive donors. Surveillance policy determines that contacts of HBV carriers should be screened to HBV markers, but there is no recommendation about how to guide contacts of HBsAg-negative, anti-HBc-positive donors. Aiming to evaluate whether the contacts of this group are at greater risk for HBV infection, a cross-sectional study was performed to compare prevalence of HBV infection between contacts of HBsAg-positive blood donors (group I) and contacts of HBsAg-negative, anti-HBc-positive donors (group II). Contacts were submitted to a questionnaire and blood tests for HBV markers. In group I (n = 143), 53 (37.1%) were anti-HBc-positive and 11 (7.7%) were HBsAg-positive. In group II (n = 111), there were 9 and 0.9%, respectively. HBV exposure was associated with group I, sexual activity, blood transfusion, being one of the donor's parents, and living for more than ten years with the donor. Regarding the families as sample units, it was more common to find at least one member with HBV markers (p < 0.05) among the families of group I compared to group II. Contacts of HBsAg-negative, anti-HBc-positive individuals presented a much lower risk of having already been exposed to HBV and there is no need to screen them for HBV in low to moderate prevalence populations.

Key words: blood donor screening - communicable diseases - blood-borne pathogens - horizontal transmission - vertical transmission - Brazil

Studies have shown an increasing chance of finding HBV-positive subjects among household contacts of hepatitis B virus (HBV) carriers as compared with contacts of HBV-negative individuals (Berris et al. 1973, Kashiwagi et al. 1984, Milas et al. 2000). HBV spread inside families can take place by vertical or horizontal route (Dumpis et al. 2001, Ono-Nita et al. 2004). A surveillance approach recommended by the Center for Disease Control and Prevention (US) and the Brazilian Health Ministry is to screen contacts of HBV carriers in order to identify other carriers and susceptible individuals who could benefit from prophylaxis (CDC 1991). One of the most common ways of identifying HBV carriers takes place during blood donation screening (Hadler et al. 1987, Salles et al. 2003).

In Brazil, HBV blood donors screening was extended to include analysis of antibodies against the core antigen (anti-HBc) in 1993 (Brasil, Ministério da Saúde). While improving safety, the addition of anti-HBc testing has increased the rejection of donated blood and has uncovered a large number of HBsAg-negative, anti-HBc-positive individuals considered to be unsuitable for blood donation. This serologic profile is quite c

demic areas and usually indicates a resolved HBV infection, although HBV DNA can be detected in serum from some of these individuals (Hennig et al. 2002).

These HBsAg-negative, anti-HBc-positive blood donors require counseling regarding the implications of these results and whether their relatives and sexual contacts are at increased risk for HBV infection. However there is no such policy in Brazil and it has not yet been determined if the contacts of HBsAg-negative, anti-HBc-positive individuals are at increased risk of HBV exposure (Brasil 2000). It is also not know how long these blood donors remained infective in the past or when they stopped carrying HBV. This raises the question: should the surveillance policy for contacts of HBsAg-positive individuals be broadened to include contacts of HBsAg-negative, anti-HBc-positive individuals? The aim of the present study was to clarify whether contacts of HBsAg-negative, anti-HBc-positive blood donor are at increased risk for HBV infection.

MATERIALS AND METHODS

A cross-sectional study was designed to compare HBV infection prevalence in contacts of subjects with one of two profiles of HBV markers (HBsAg-positive or only antibodies positive: HBsAg-negative, anti-HBc-positive) identified during blood donation. The study was conducted in Cuiabá, (the largest city of the state of Mato Grosso, Central Brazil) at two institutions: the local public blood bank and the main private one. Both collect blood in Cuiabá and also in other cities of the region.

Financial support: CNPq
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Received 23 November 2005
Accepted 15 February 2006

Voluntary blood donors found to be HBsAg-positive by the screening process were requested to return to blood bank in order to confirm the test result. If it was confirmed their household and sexual contacts (group I) were invited to the blood bank or if they preferred were visited in their home to answer a questionnaire and have a blood sample taken.

HBsAg-negative, anti-HBc-positive blood donors outnumbered HBsAg-positive ones in a ratio around 10:1. Thus, in order to match HBsAg-negative, anti-HBc-positive donors to HBsAg-positive ones, we identified all HBsAg-negative, anti-HBc-positive donors who had attempted to donate blood at the same week of each HBsAg-positive donor. After retaining only the subjects who matched the gender and age (within a variation of ± 3 years) of the corresponding HBsAg-positive donor, one HBsAg-negative, anti-HBc-positive donor was randomly selected. Subsequently their household and sexual contacts (group II) were invited to participate in the protocol and submitted to the same procedures described above for the contacts of HBsAg-positive donors. Children under three years of age were excluded to avoid possible stress. Blood donors living alone were excluded. Only families in which all members accepted to participate were included.

HBsAg and anti-HBc tests were performed with enzyme immunoassay kits according to manufacturer's recommendation (respectively, Murex HBsAg, version III, and Eti-Ab-Corek-2 Diasorin, Saluggia, Italy).

It was assumed that the prevalence of HBV markers would be 10% in group II, based in anti-HBc prevalence among blood donors of the public blood bank between 1998 and 2002 (1998-2002 Hemomat report, Secretary of Health of Mato Grosso State, 2002). Regarding group I, we assumed that its anti-HBc prevalence would generate at least an odds ratio of 3.0. Thus, for a two-sided alpha of 0.05 and a power of 0.80, a sample size of 112 contacts on each group was calculated to be necessary.

Two dependent variables were analyzed: (a) having been exposed to HBV for the anti-HBc positive individuals; and (b) the fact of being HBsAg-positive. These dependent variables were also analyzed regarding the family rather than the individuals as the sample unit.

The Ethics Research Board at the Mato Grosso Federal University approved the protocol used in the present study. All blood donors or contacts with a positive test received counseling about the results and were directed to the Hepatology Outpatient Service of Mato Grosso Federal University Hospital. Participants still susceptible to HBV infection were oriented to start the vaccine schedule.

Data were recorded and analyzed using Epi-Info 6.04 software (CDC, Atlanta, US, 2001). The statistical analysis included Chi-square, Fisher's exact test and odds ratios with Yate's correction. Statistical significance was set at 95% confidence interval. Models of logistic regression by stepwise method were constructed to adjust for confounders using the software Stata 6.0 (Stata Corporation, Texas, US, 1999).

RESULTS

Between 2001 and 2004, 143 contacts (group I) of 47 HBsAg-positive blood donors and 111 contacts (group II) of 42 HBsAg-negative, anti-HBc-positive blood donors were included in the study. Eleven (23%) out of 47 HBsAg-positive donors were HBeAg-positive. The mean number of contacts per blood donor of group I was slightly higher than group II (3 vs 2.6; $p = 0.25$).

There was no substantial difference in the characteristics of the two groups of blood donors. The same baseline characteristics were presented between groups I and II, but the mean age was slightly higher in group I. There was a difference between groups on distribution of degree of relationship with the blood donors, since group I included more blood donors' parents than group II ($p < 0.01$) (Table I).

Sixty-three out of (24.8%) 254 surveyed contacts had HBV markers and 12 (4.7%) of these were HBV carriers. Fifty-three (84%) out of 63 subjects with HBV markers and 11 (91.6%) out of 12 carriers belonged to group I (Table II). There were only two (28%) HBeAg-positive blood donors among the seven families of group I that had HBsAg-positive contacts.

All HBV carriers belonged to one of eight families. Two of these families showed HBV infection cluster (at least four carriers in each one). In one of these families, the blood donor was a 33-year-old female carrier, HBeAg-negative, and three of her offspring were HBV carriers. Her husband and a daughter had markers of cleared infection. In the other family, the blood donor was a 20-year-old male carrier (HBeAg-negative) and his mother, a sister and a nephew were HBsAg-positive, whereas his father-in-law had markers of cleared infection.

After adjustment, the analysis to identify variables associated to exposure to HBV showed an independent association with: belonging to group I ($p < 0.0001$), having already started sexual activity ($p < 0.05$), having been a blood recipient ($p < 0.05$), being a blood donor's parent (independent of gender), and having lived longer than ten years with the respective blood donor ($p < 0.05$) (Table III). No association was found with the number of inhabitants per house. Despite the association between HBV markers and sexual activity, a higher number of sexual partners did not influence reactivity for HBV markers.

When the dependent variable analyzed was HBsAg reactivity, multivariate analysis showed independent association only with belonging to group I (Table III).

We also performed logistic regression regarding each group separately and retained all other variables utilized to adjust models above (data not shown). In group I, having already started sexual activity ($p < 0.05$), and having lived longer than ten years with the respective blood donor ($p < 0.05$) were associated to HBV exposure. On other hand, having been a blood recipient ($p < 0.05$) was the only risk factor associated to HBV exposure in group II. None association was found when HBsAg positivity was the analyzed outcome.

Taking families as the sampling units, 21 (44.7%) out of 47 group I families presented at least one member with HBV markers, while 9 (21.4%) out of 42 group II families (p

TABLE I

Characteristics of contacts, according to serologic profile of the respective blood donor

	254 (%)	Group I [a] 143 (%)	Group II [b] 111 (%)	P value
Gender				
male	103 (40.9)	61 (42.7)	42 (38.7)	0.62
female	151 (59.1)	82 (57.3)	69 (61.3)	
Mean age (years)	25.7	27.5	23.3	0.11 [c]
By age group				
≤ 10	54 (21.3)	27 (18.9)	27 (24.3)	0.37
11-20	56 (22.0)	33 (23.1)	23 (20.7)	0.76
21-30	51 (20.1)	24 (16.8)	27 (24.3)	0.18
31-40	35 (13.8)	21 (14.7)	14 (12.6)	0.77
41-50	31 (12.2)	18 (12.6)	13 (11.7)	0.98
50 >	27 (10.6)	20 (14.0)	7 (6.3)	0.08
Degree of relationship to the blood donor				
Sexual partner	65 (26.7)	33 (23.7)	32 (30.8)	0.28
Offspring	98 (40.3)	53 (38.1)	45 (43.3)	0.49
Parent	36 (14.8)	29 (20.9)	7 (6.7)	< 0.01
Sibling	31 (12.8)	19 (13.7)	12 (11.5)	0.76
Other	13 (5.3)	5 (3.6)	8 (7.7)	0.26
Previous hepatitis	26 (10.2)	14 (9.8)	12 (10.8)	0.65

a: contacts of HBsAg-positive blood donors; *b*: contacts of HBsAg-negative, anti-HBc-positive blood donors; *c*: Mann-Whitney's test

TABLE II

Prevalence of hepatitis B virus (HBV) markers of the blood donors contacts, according to serologic profile of respective donor

	Group I [a] (%) 143	Group II [b] (%) 111	OR (CI95%)	P
Anti-HBc				
Positive	53 (37.1)	10 (9.0)	5,9 (2.7; 13.4)	< 0.0001
Negative	90 (62.9)	101 (91.0)		
HBsAg				
Positive	11 (7.7)	1 (0.9)	9,1 (1.5; 200)	< 0.05
Negative	132 (92.3)	110 (99.1)		

a: contacts of HBsAg-positive blood donors; *b*: contacts of HBsAg-negative, anti-HBc-positive blood donors.

< 0.05). Regarding to have at least one HBsAg-positive member, there are seven (14.9%) group I families with this condition and only one (2.4%) out of group II (p = 0.06). Families belonging to group I (p < 0.05) and time living with the blood donor (p < 0.05) were significantly associated with the existence of at least one HBV exposed subject in household, according to logistic models adjusted for the mean age of household contacts and gender (Table IV). When the dependent variable was the existence of at least one HBV carrier in the household, the only variable associated was time living with the blood donor (p < 0.05). However, group I families were marginally associated (p = 0.07).

DISCUSSION

When countries with intermediate HBV endemicity, such as Brazil, introduced anti-HBc on blood donation screening they increased the safety of blood transfused (Tobler & Busch 1997, Martinez 1998). However, this policy identifies a large group of HBsAg-negative, anti-HBc-positive individuals that require assistance and counseling. It is not known whether their household and sexual contacts are at increased risk for HBV infection, and consequently if they should be screened for HBV, as is in the case of HBV carriers' contacts. Similar reports comparing relatives of HBV carriers and healthy subjects have been published, nevertheless no reports were found comparing contacts of HBsAg-positive subjects with contacts of HBsAg-negative, anti-HBc-positive ones (Berris et al. 1973, Kashiwagi et al. 1984, Milas et al. 2000).

In this analytical cross-sectional study, refusing to participate was more common among HBsAg-negative, anti-HBc-positive blood donors and their contacts. This was probably in consequence of less interest among these people after having been clarified about the implications of such serologic profile, i. e. that they are probably not HBV carriers. Furthermore, families of group I were slightly larger than group II. As a consequence HBsAg-positive

TABLE III

Risk factors analyzed for hepatitis B virus (HBV) exposure (model 1) and for HBsAg carrier status (model 2) among contacts by multivariate analysis

		Model 1 Dependent variable: HBV exposure (anti-HBc-positive) [a]			Model 2 Dependent variable: contact HBsAg-positive [a]		
	N	OR	CI95%	p	OR	CI95%	p
Contact of							
Group II	111	1.0	-		1.0	-	
Group I	143	5.5	2.4; 12.1	< 0.0001	9.3	1.2; 73.2	< 0.05
Sexual activity							
None	108	1.0	-		1.0	-	
Already started	146	3.3	1.5; 7.3	< 0.005	3.0	0.3; 26.7	= 0.32
Time living with the donor (years)							
<10	108	1.0	-		1.0	-	
10 ≥	146	3.3	1.4; 7.7	< 0.01	3.2	0.3; 35.2	= 0.33
Degree of relationship							
Other	218	1.0	-		1.0	-	
Parent	36	2.6	1.1; 6.5	< 0.05	1.7	0.2; 15.6	= 0.64
Transfusion							
No	241	1.0	-		1.0	-	
Yes	11	6.7	1.6; 29.1	< 0.05	4.4	0.6; 32.4	= 0.15

a: logistic model included 254 observations. Both models were adjusted for gender and age group.

TABLE IV

Risk factors analyzed for at least one hepatitis B virus (HBV) infected (model 1) and HBsAg carrier (model 2) contact considering families as sampling unit by logistic regression

	Model 1 HBV markers in contacts [a]			Model 2 HBsAg in contacts [a]		
	OR	CI95%	P	OR	CI95%	p
Donor						
HBsAg-negative, anti-HBc-positive	1.0	-		1.0	-	
HBsAg-positive	3.2	1.2; 8.7	< 0.05	8.1	0.8; 79.4	= 0.07
Time living with the donnor (years)						
< 10	1.0	-		1.0	-	
10 ≥	2.3	1.1; 5.2	< 0.05	4.8	1.2; 18.9	<0.05
Donor's gender						
female	1.0	-		1.0	-	
male	0.3	0.1; 1.2	= 0.20	0.2	0.1; 1.2	= 0.07
Household contacts mean age						
< 20	1.0	-		1.0	-	
20 ≥	0.8	0.2; 2.5	= 0.68	0.3	0.1; 2.6	= 0.29

a: both models included all 89 families.

donors and group I contacts outnumbered HBsA-negative donors and group II contacts, respectively.

Both groups of blood donors and contacts had similar characteristics, although the mean age was higher in group I. This was due to the fact that more HBsAg-positive blood donors still lived with their parents compared to the other blood donors. This characteristic may have influenced the prevalence difference between the groups.

HBV exposure was increased fourfold and HBsAg-positivity eightfold in group I when compared to group II. HBV markers were more common among group I subjects even after adjustment for age and gender of contacts and other variables. Besides living with an HBsAg-positive blood donor, factors associated with HBV exposure after adjustment were a longer period living with the donor and having already started sexual activity (in group I), having been a blood recipient (in group II), and parenthood. A longer domestic exposure to the HBsAg-positive donor reinforces the importance of intra-familial spread in contacts of HBsAg-positive subjects, such as pointed out

elsewhere (Dumpis et al. 2001). Transfusion route is a classical risk factor for HBV infection, suggesting that extrafamilial horizontal transmission also took place. Since it was a risk factor only in group II, this finding suggest that contacts of HBsAg-negative, anti-HBc-positive were more likely infected in a nosocomial set than by household contact.

Sexual activity is the main risk factor for HBV infection in populations with low or moderate endemicity (Wilkinson 1984, Brabin & Brabin 1985) and may have influenced the exposure to HBV in the present study, since having begun sexual activity was associated with anti-HBc positivity. However, this route can only explain spread between sexual partners, who accounted for only a fraction of infected subjects. In a recently published Brazilian study, sexual transmission was a frequent route for HBV spread in the Western-descendant individuals (Ono-Nita et al. 2004). A higher number of previous sexual partners were not associated in the multivariate analysis, as reported elsewhere (Alter et al. 1986).

It is not possible to accurately determine the sequence of epidemiological events of a silent and sometimes long lasting disease such as HBV infection and it is beyond the scope of a cross-sectional study (Van Damme et al. 1995). Certainly some of the individuals acquired HBV infection from sources outside of the household. Nevertheless, the present study reinforces that HBV tends to cluster in households and screening contacts of HBsAg-positive individuals identify families that are more susceptible to chronic HBV infection, as shown by other authors (Berris et al. 1983, Lindberg & Lindholm 1988, Davis et al. 1989, Hsu et al. 1993).

On other hand, contacts of group II were at a very low risk of having a family member exposed to HBV even less of having a carrier in the household. The HBsAg and anti-HBc prevalence in this group (0.9 and 9%, respectively) was very similar to those reported for the general population of blood donors in this region, 1 and 10%, respectively (1998-2002 Hemomat report, Secretary of Health of Mato Grosso State, 2002). Analysis using family as the sampling units showed that it would be need to screen nearly six-fold more families of HBsAg-negative, anti-HBc-positive blood donors than families of HBsAg-positive blood donors in order to identify a HBsAg-positive subject. Thus it can be assumed that there is no need to screen HBV infection among contacts of HBsAg-negative, anti-HBc-positive individuals. Finally, health care workers attending populations with low or intermediate HBV prevalence should be oriented to reassure HBsAg-negative, anti-HBc-positive blood donors about the low risk of HBV infection among their relatives. The present data indicate that they are not under increased risk for this agent compared to general population.

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