



Article/Artigo

Clinical, demographic and epidemiological characteristics of patients with hepatitis B followed at a university hospital in southeastern Brazil: predominance of HBeAg negative cases

Características clínicas, demográficas e epidemiológicas dos pacientes com hepatite B em seguimento em hospital universitário no sudeste do Brasil: predominância de casos HBeAg negativos

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ABSTRACT

Introduction: Hepatitis B is common in Brazil, although there are regional differences regarding the degree of endemicity, the most frequent forms of transmission and the presence of different evolutive stages of chronic disease. The present study aimed to determine the clinical, demographic and epidemiological characteristics of patients chronically infected with hepatitis B virus (HBV) residing in the Ribeirão Preto region, southeastern Brazil. **Methods:** A total of 529 medical records of individuals with HBV monoinfection were reviewed. **Results:** More than 60% of the subjects were males, with a mean age of 38 years-old. The HBeAg-negative serological pattern was verified in 84.4% of the patients, among whom the risk of vertical/intrafamily transmission was 43.2% ($p = 0.02$). The consumption of alcohol in amounts exceeding 20g a day was observed in 21.3% of the subjects and was more frequent among men (33%) ($p < 0.001$). Among patients with cirrhosis, 54.1% were alcohol abusers ($p = 0.04$), all of them males. The presence of cirrhosis was more frequent in the HBeAg-positive group (24.4%) than in the HBeAg-negative group (10.2%) ($p < 0.001$). **Conclusions:** High proportions of HBV-infected subjects with an HBeAg-negative pattern were observed, with a higher risk of vertical/intrafamily transmission. Alcohol abuse was associated with male subjects and with cirrhosis of the liver in this group. A tendency toward an increase in the number of HBeAg-negative cases was observed over time.

Keywords: Hepatitis B. Epidemiology. Transmission. Hepatitis B E antigens.

RESUMO

Introdução: No Brasil, a hepatite B é comum. No entanto, há diferenças regionais no que diz respeito ao grau de endemidade, as formas de transmissão mais encontradas e a presença dos diferentes estágios evolutivos da doença crônica. O objetivo deste trabalho foi o de conhecer características clínicas, demográficas e epidemiológicas de pacientes cronicamente infectados pelo vírus da hepatite B (HBV), residentes na região de Ribeirão Preto, no sudeste do Brasil. **Métodos:** Foi realizada a análise retrospectiva de 529 prontuários de indivíduos com monoinfecção pelo HBV. **Resultados:** Mais de 60% eram masculinos, a média de idade foi de 38 anos. O padrão sorológico HBeAg negativo foi encontrado em 84,4% dos pacientes, entre os quais o risco para transmissão vertical/intrafamiliar foi de 43,2% ($p = 0,02$). Verificou-se uso de álcool em quantidades maiores que 20g ao dia em 21,3% dos indivíduos, sendo mais frequente entre os homens (33%) ($p < 0,001$). Entre os pacientes com cirrose, 54,1% faziam uso abusivo de bebidas alcoólicas ($p = 0,04$), sendo todos estes do gênero masculino. A presença de cirrose foi maior no grupo HBeAg positivo (24,4%) que no grupo HBeAg negativo (10,2%) ($p < 0,001$). **Conclusões:** Observaram-se elevadas proporções de indivíduos com infecção pelo HBV com padrão sorológico HBeAg negativo, entre os quais houve maior risco para a transmissão vertical/intrafamiliar. O uso abusivo de álcool esteve associado a indivíduos do sexo masculino e, neste grupo, à cirrose hepática. Observou-se tendência ao aumento no número de casos HBeAg negativo ao longo do tempo.

Palavras-chaves: Hepatite B. Epidemiologia. Transmissão. Antígenos E da Hepatite B.

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INTRODUCTION

Hepatitis B is one of the most common infectious diseases worldwide. More than 2 billion people have had contact with hepatitis B virus (HBV) and about 350 million people have the chronic form of infection¹⁻³. The importance of chronic infection resides in the risk of developing serious forms of hepatic disease, such as cirrhosis, liver failure and hepatocellular carcinoma (HCC)¹⁻³. Although most individuals do not develop complications during their lifetime, 15 to 40% of persons with chronic HBV infection will present sequelae related to infection, with a 10 to 20% estimate of progression to cirrhosis every 5 years¹⁻³. The relative risk of developing HCC among chronically HBV-infected individuals ranges from 5.6 to 103.0 and the age of the individual at the time of infection is important in determining this risk¹⁻⁴.

The incidence of hepatitis B, the patterns of transmission and the frequency of different stages in the natural history of chronic HBV infection varies widely throughout the world in different population subgroups¹⁻³. In Brazil, studies conducted in capitals and major cities show data consistent with low or intermediate levels of endemicity⁵⁻¹⁰. The Amazon region, some areas in the State of Espírito Santo and the western areas of the States of Parana and Santa Catarina are distinguished from other Brazilian regions by the high prevalence of HBV infection^{7,11,12}. In areas of low prevalence, transmission occurs more frequently among adolescents and adults, especially through sexual relations. In contrast, in areas of intermediate and high prevalence, data show higher rates of vertical and intrafamilial transmissions^{7,8,13-15}. The frequency of the different phases involved in the natural history of chronic HBV infection has been poorly investigated in Brazil. Few studies include frequency analysis of HBeAg negative and HBeAg positive forms of the chronic infection^{16,17}.

The objective of the present study was to determine the clinical, demographic and epidemiological characteristics of chronically HBV-infected patients followed in Ribeirão Preto, a city in the interior of the State of São Paulo.

METHODS

This work was conducted as a descriptive epidemiological study. The medical records of HBV-infected patients attended at the Hepatitis B Outpatient Clinic of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (HC-FMRP-USP) from June 2004 to June 2008 were reviewed. Of these, 529 HBV-infected patients were included in the study. Inclusion criteria were HBsAg positive patients at diagnosis, with anti-HCV and anti-HIV negative markers.

Data regarding patient identification, referral source, alcohol consumption and risk factors related to HBV transmission were collected. Abusive alcohol consumption was considered to be present when more than 20g a day were consumed for more than 10 years.

Anti-HBc IgG-positive antibodies in the mother and/or at least one sibling were considered to indicate possible vertical/intrafamilial transmission of HBV. Risky sexual contact was defined as more than three sex partners per year, a report of a sexually transmissible disease, sexual contact with an individual known to be infected with HBV, or in the case of men who had sex with men. Percutaneous exposure to potentially contaminated material was considered to have occurred when the patient reported the execution of tattoos, piercings or acupuncture.

The following determinations were performed: measurement of alanine aminotransferase (ALT), quantitative DNA values of B virus (HBV DNA), presence of HBsAg and HBeAg antigens and of anti-HBcAg IgG and IgM, anti-HBsAg and anti-HBeAg antibodies in serum and, when available, a liver biopsy. On the basis of these data, the patients were classified into different evolutive stages of the disease: acute hepatitis, immune tolerance phase, chronic HBeAg-positive hepatitis, inactive HBV or HBeAg-negative chronic hepatitis, according to the criteria of the American Association for the Study of Liver Diseases (AASLD)¹⁸. Cirrhosis was diagnosed according to the histopathological findings of the liver biopsy. When a biopsy was not available, the diagnosis was made on the basis of clinical (ascites, hepatic encephalopathy), biochemical (prothrombin time, serum bilirubin and albumin), ultrasonographic (nodules in the liver parenchyma), and endoscopic (signs of portal hypertension) parameters. HCC was diagnosed by imaging exams (ultrasonography + computed tomography and/or magnetic resonance), by the determination of serum alpha-fetoprotein and, when available, by histopathological analysis of the tumor.

The information collected was stored in a data bank using the Excel 2003 software (Microsoft Office). Data are expressed by means of descriptive statistical analysis or were analyzed by the Student t test, Chi square test and Fisher exact test using the SPSS version 17.0 software. The level of significance was set at $p < 0.05$ in all analyses.

Ethical considerations

The study was approved by the Research Ethics Committee of the Clinics Hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto (HC-FMRP-USP) (protocol n 14339/2006).

RESULTS

Of the 529 patients included in the study, 320 (60.5%) were males. Age at the beginning of follow-up ranged from 12 to 79 years-old, with a mean of 38 years-old. The age of 84% of the patients ranged from 20 to 59 years-old. Mean age was similar for men (38.0 years-old) and women (37.8 years-old) ($p = 0.85$).

Of the total number of patients included in the study, 208 (39.3%) were referred by clinicians, 181 (34.2%) were referred by the Ribeirão Preto Blood Center and 103 (19.5%) were included after family screening. Information regarding the source of referral was not available in 37 (7%) medical records.

Two hundred and ninety-six (56%) patients reported that they had not consumed alcohol in any phase of life. The absence of alcohol consumption was more common among women than among men; i.e., 88.5% and 34.4%, respectively ($p < 0.001$). Data regarding the amount of alcohol consumed by the men and women included in the study are listed in **Table 1**.

TABLE 1 - Consumption of alcoholic beverages by HBV-infected patients.

Quantity of alcohol consumed per day in grams	Sex				p
	male		female		
	n	%	n	%	
Absent	110	34.4	185	88.5	<0.001
Less than 20	79	24.8	13	6.3	<0.001
20 to 40	43	13.4	4	1.9	<0.001
More than 40	63	19.6	3	1.4	<0.001
Unknown		25.0	7.8	4.0	1.9
Total	320	100.0	209	100.0	

Eight patients (1.5%) presented symptomatic acute hepatitis on the occasion of the first visit. One of them developed acute hepatic insufficiency and died, another developed chronification of the infection and six progressed to spontaneous cure. The remaining 521 (98.5%) patients included in the study presented chronic HBV infection since the beginning of follow-up.

Of the 521 patients with chronic HBV infection, 440 (84.5%) had the HBeAg-negative and anti-HBeAg-positive serological pattern. Of these, 238 (54.1%) presented active chronic hepatitis and 202 (45.9%) were inactive HBV carriers. Eighty-one (15.5%) individuals had the HBeAg-positive and anti-HBeAg-negative serological pattern. Fifty-nine (72.8%) of the latter presented active chronic hepatitis and the remaining 22 (27.2%) were in the immune tolerance phase. Of the 521 patients with chronic HBV infection, 297 (57%) presented active chronic hepatitis.

Analysis of the HBeAg and anti-HBeAg serological pattern at the beginning of patient follow-up revealed a tendency toward an increased number of HBeAg-negative and anti-HBeAg-positive individuals over time; i.e., between January 1992 and December 2007 (**Figure 1**). Data for 2008 were excluded from this analysis, because the collection did not include the second half of the year in question.

The clinical, demographic and epidemiological characteristics of patients with chronic HBV infection with HBeAg-positive and HBeAg-negative patterns (age, sex, risk factor, presence of cirrhosis and HCC) are presented in **Table 2**.

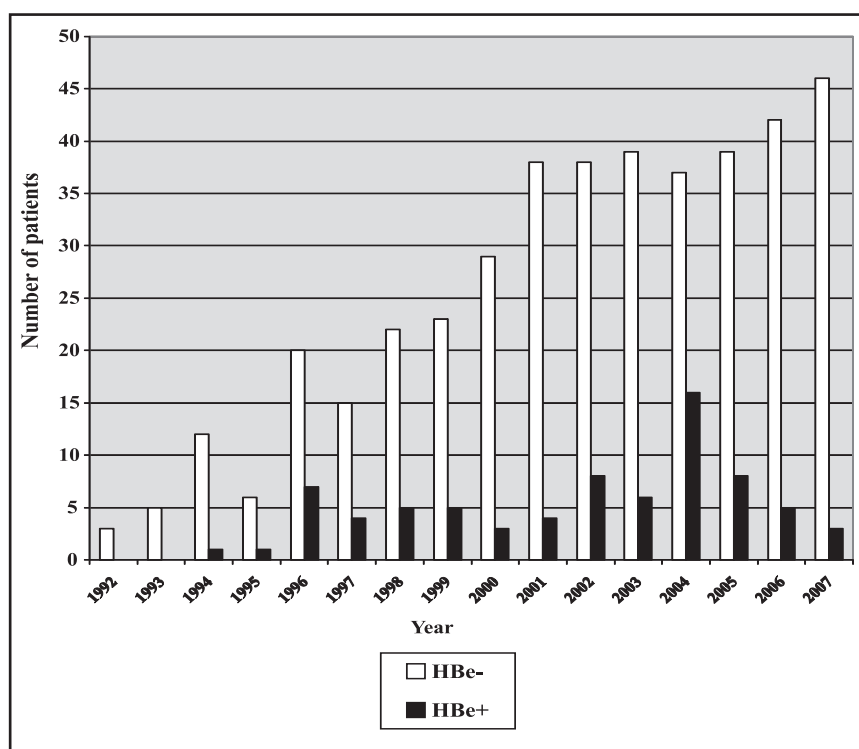


FIGURE 1 - Distribution of the number of new cases of patients with hepatitis B attended at HC-FMRP-USP between 1992 and 2007, according to the serological pattern of the HBe antigen.

TABLE 2 - Clinical, demographic and epidemiological characteristics of patients with chronic hepatitis B infection according to the serological pattern of the HBe antigen.

	HBeAg positive		HBeAg negative		p
	n	%	n	%	
Mean age (years)	36.7		38.4		0.40
	n	%	n	%	
Male sex	59	72.8	256	64	0.02
Possible mode of transmission					
vertical/intrafamilial	24	29.3	190	43.2	0.02
sexual	27	33.0	94	21.4	0.02
transfusion of blood products	13	16.0	48	10.9	0.19
percutaneous	8	9.8	10	2.3	<0.001
risky injections	-	-	12	2.7	0.23
hemodialysis	2	2.4	7	1.6	0.64
health professionals	1	1.2	16	3.6	0.49
unidentified	21	26.5	138	31.4	0.95
more than one factor	15	18.5	66	15.0	0.42
Presence of cirrhosis	20	24.4	45	10.2	<0.001
Presence of HCC*	3	3.6	13	2.9	0.72

*HCC: hepatocellular carcinoma

Table 3 lists the clinical, demographic and epidemiological data of HBeAg-positive and HBeAg-negative patients with active chronic hepatitis. Patients in the immune tolerance phase and patients with inactive HBV infection were not included in this table. Data were analyzed statistically in a univariate manner.

Of the total of 521 patients with chronic HBV infection, 65 (12.5%) received a diagnosis of cirrhosis during the study period. The mean age of these cirrhotic patients was greater than that of the group of non-cirrhotic patients (48.7 versus 36.5 years-old)

TABLE 3 - Clinical, demographic and epidemiological characteristics of patients with active chronic hepatitis B, according to the serological pattern of the HBe antigen.

	HBeAg positive		HBeAg negative		p
	n	%	n	%	
Mean age (years)	37.4		38.8		0.48
	n <td>%</td> <td>n <td>%</td> <td></td> </td>	%	n <td>%</td> <td></td>	%	
Male sex	44	74.5	146	61.1	0.06
Possible mode of transmission					
vertical/intrafamilial	19	32.2	99	41.6	0.18
sexual	19	32.2	48	20.1	0.04
transfusion of blood products	12	20.3	32	13.4	0.18
percutaneous	6	10.1	5	2.1	0.01
risky injections	-	-	6	2.5	0.60
hemodialysis	1	1.7	7	2.9	1.00
health professionals	1	1.7	7	2.9	1.00
unidentified	12	20.3	74	31.1	0.10
more than one factor	12	20.3	31	13.0	0.15
Alcohol intake (> 20g/day)	20	33.9	51	21.4	0.04
Presence of cirrhosis	20	33.9	39	16.3	<0.001
Presence of HCC*	3	13.6	9	3.7	0.71

*HCC: hepatocellular carcinoma

($p < 0.001$). Seventy-eight percent were men (51/65), demonstrating an association between male sex and this form of hepatic disease ($p = 0.001$). Information concerning alcohol consumption was available for 59 cirrhotic patients. In the male group, 26 (54.1%) out of 48 cirrhotic patients consumed more than 20g of alcohol a day, and the association was significant ($p = 0.004$). None of the seven women who consumed alcohol had cirrhosis and therefore it was not possible to determine associations between alcohol abuse and cirrhosis in this

group. Among HBeAg-negative patients with chronic hepatitis who abused alcoholic beverages, 31.4% were cirrhotic (16/51), and the association between alcohol and cirrhosis was significant in this group ($p = 0.001$). Among HBeAg-positive patients with chronic hepatitis who abused alcoholic beverages, 45% (9/20) were cirrhotic, although in this group no significant association was observed between alcohol abuse and cirrhosis ($p = 0.197$).

Sixteen patients (3%) had HCC, 14 of them with underlying cirrhosis and two without cirrhosis. The mean age of patients with HCC exceeded that of patients without HCC (50.8 versus 37.5 years-old) ($p = 0.001$). No association was detected between the presence of HCC and alcohol consumption ($p = 1.000$).

During the study period, six patients were submitted to a liver transplant at HC-FMRP-USP: one due to acute hepatic insufficiency, two due to HCC in a cirrhotic liver and three with decompensation of cirrhosis. Three transplanted patients died. Of the total number of individual studied, 15 died of causes related to HBV infection during the study period.

DISCUSSION

The Clinics Hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto (HC-FMRP-USP) belongs to the XIII Regional Health Department of the Health Secretariat of the State of São Paulo and is the principal tertiary reference center in the Ribeirão Preto region, which includes 26 municipalities and approximately 1,130,000 inhabitants¹⁹. The hepatitis B outpatients clinics of this hospital treats patients with an indication of drug treatment and more severe cases of hepatic disease caused by HBV. This clinic also represents a reference center for cases detected during the donation of blood products at the Ribeirão Preto Blood Center. As observed in the current study, most (98.5%) of the care provided by this service involves patients with chronic HBV infection. Cases of symptomatic acute infection are believed to be retained in the primary and secondary health networks.

Analysis of cases of chronic infection revealed a predominance of individuals with the HBeAg-negative/anti-HBeAg-positive serological pattern, with a tendency toward an increase in new HBeAg-negative cases in relation to new HBeAg-positive cases. Studies conducted in France in 1994 and 2003 demonstrated that cases of HBeAg-negative chronic hepatitis also became the most frequent in this region of the European continent over the last decade^{20,21}. Cases of HBeAg-negative hepatitis B also predominate in Greece, with the proportion of Greek patients showing an HBeAg-negative and anti-HBeAg-positive pattern reaching 93.3%²². Studies conducted in Italy, Portugal and Spain have also demonstrated high rates of this serological pattern of chronic HBV²³⁻²⁵. Studies conducted in Brazil have shown differences in the prevalence of HBeAg-negative chronic hepatitis. In northeastern Brazil, in the State of Bahia, Ribeiro et al¹⁶ studied 76 individuals with chronic HBV infection and did not identify any patients with HBeAg-negative chronic hepatitis. In contrast, in a study involving 139 HBV-infected patients in the City of Campinas, in the southeast region of the State of São Paulo, Tonetto et al¹⁷ detected high proportions (70%) of HBeAg-negative individuals. Similar to that observed in the Campinas study, HBeAg-negative cases predominated in the present study, a fact possibly reflecting greater circulation of HBV originating from the European continent, particularly the Mediterranean region. In contrast, regarding northeastern Brazil, the data suggest that there is a greater prevalence of HBV originating from the African continent^{16,17,26}.

A previous study conducted in Ribeirão Preto between 1999 and 2002 showed that, of the total number of HBV-infected patients studied, 36% presented precore and core-promoter mutations: HBV variants that affect the production of the HBe antigen²⁷. In that study, although the number of patients was small ($n = 50$), it was possible to observe that no difference in age was verified between the groups of HBeAg-positive and HBeAg-negative patients²⁷. Similarly, in the present study, no difference occurred between the groups of HBeAg-positive and HBeAg-negative patients. These data disagree with results reported in other studies^{3,17,20,22}. Since this is a later stage of infection, greater values for age were expected for the group of HBeAg-negative patients³. The explanation for these data is not clear, but we suggest that transmission of previously mutant HBV forms, defective for HBeAg synthesis, may have occurred among younger individuals. This hypothesis is supported by the increase in the proportion of HBeAg-negative cases over the last 15 years, a fact that may reflect greater circulation of mutant viruses in our region. However, the fact that HBeAg-negative patients submitted to antiviral treatment were not excluded from this study should be taken into consideration, with the possibility of bias in the data obtained due to the presence of these patients in whom seroconversion of the HBe antigen was induced by medications.

Regarding the possible mode of transmission, high proportions of sexual risk were verified among the patients with the HBeAg-positive serological pattern, with a predominance of male patients in this group. On the other hand, a high proportion of patients in general with the possibility of vertical/intrafamilial transmission of HBV were also verified, in particular among patients with the HBeAg-negative forms (43.2%). Studies conducted in Brazil have demonstrated high frequencies of this type of transmission among people living in the same household¹³⁻¹⁵. In a study conducted in Acre, a region of high endemicity for HBV infection in the northern Brazil, Lobato et al¹⁵ detected a high frequency of HBV transmission in the domestic environment among the relatives of HBsAg-positive pregnant women. Motta-Castro et al¹⁴ also demonstrated a high frequency of HBV transmission in the family environment, especially among children, in an Afrodescendant community with a high prevalence of HBV infection in the central-western region of Brazil. This last study and others have suggested that dissemination of the virus in the family environment may be more important among individuals of lower socioeconomic level and living in poor hygiene conditions^{13,14}. Although the HBeAg-negative serological pattern and the vertical/intrafamilial form of transmission are known to be more frequent in regions of greater endemicity of infection, studies conducted in Ribeirão Preto on different population groups have suggested a low prevalence of HBV infection^{9,10,28}. However, since in general the patients treated at HC-FMRP-USP are of low socioeconomic level, this result may support the hypothesis raised by Motta-Castro¹⁴.

In the study model used here, it was not possible to estimate the rates of progression to cirrhosis and to HCC, but it was possible to observe that the proportion of individuals with these complications was similar to that detected in other studies^{3,20,22}. Regarding the possible factors related to progression to cirrhosis, this work was in agreement with published data regarding the predominance of these forms of hepatic disease in older individuals¹⁻³. Higher rates of cirrhosis were observed in the HBeAg-positive group with chronic hepatitis. Although some studies conducted in European countries have detected greater proportions of cirrhotic individuals in the group of HBeAg-negative patients, there is no consensus regarding the

influence of the HBe antigen on the progression to cirrhosis^{1-3,20,22,29}. Several studies have highlighted the role of the abusive consumption of alcoholic beverages in the progression of hepatic damage and in the presence of cirrhosis and HCC among patients with chronic hepatitis B^{2,3,30}. It should be emphasized that, in the present study, the proportion of abusive consumption of alcoholic beverages by the subjects under study was low, especially among women. This fact agreed with the analyses showing that no association was determined between alcohol consumption and cirrhosis in the female group or in the group with HCC. Similarly, the reduced number of patients with HCC may also have impaired the analyses performed. Among males and among individuals with HBeAg-negative chronic hepatitis, an association between abusive alcohol consumption and cirrhosis was verified, in agreement with published data. However, it should be pointed out that multivariate analyses were not performed and that no information was available regarding other factors that might influence the progression of hepatic disease, such as time of infection, viral load and HBV genotype.

Over the last 15 years, an increase in the proportion of cases of chronic HBV infection presenting the HBeAg-negative form has occurred in the Ribeirão Preto region compared to the HBeAg-positive form. High proportions of HBV-infected individuals with the HBeAg-negative serological pattern were observed, with a higher risk of vertical/intrafamilial transmission. Abusive alcohol consumption was associated with male subjects and with cirrhosis of the liver in this group.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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