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Low HBV-DNA Levels in End-Stage Renal Disease Patients With HBeAg-Negative Chronic Hepatitis B

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In end-stage renal disease patients treated by hemodialysis with HBeAg-negative chronic hepatitis B virus (HBV) infection, the evaluation of the presence of viral replication is essential in the assessment for renal transplantation. Data on HBV viral load, prevalence of precore mutations, as well as the influence of HCV coinfection on HBV-DNA levels in this group of patients is scarce. The aim of this study was to determine the HBV viral load in HBsAg-positive/HBeAg-negative hemodialysis patients; to compare HBV-DNA levels between isolated HBV infection carriers and HBV-HCV coinfected patients, and to evaluate the prevalence of precore mutations in these patients. Fifty hemodialysis patients with chronic HBeAg-negative HBV infection were studied. Viral load was determined by PCR (Amplicor HBV Monitor-Roche). The detection of precore mutations was made by sequencing. Of a total of 50 patients, 76% were male, with a mean age of 44 ± 11 years. Anti-HCV was positive in 56% of patients. HBV-DNA was undetectable in 58% of patients; 24% had HBV-DNA $<$10,000 copies/ml, 12% between 10,000–100,000 copies/ml, and only 6% had HBV-DNA $>$100,000 copies/ml. There was no difference in the viral load of patients infected only by HBV and HBV-HCV co-infected patients ($P = 0.96$). Precore mutations were detected in only 8% of cases. In conclusion, hemodialysis patients with HBeAg-negative HBV infection had a low viral load. Precore mutations were infrequent and the presence of anti-HCV has not influenced the levels of HBV-DNA. ***J. Med. Virol. 78:1284–1288, 2006.***

KEY WORDS: hepatitis B; HBeAg negative; hemodialysis; viral load; precore mutation

INTRODUCTION

The adoption of preventive measures and the regular use of hepatitis B vaccine have resulted in progressive reduction of the HBV prevalence of hemodialysis patients. Nevertheless, hepatitis B is still an important cause of liver disease in end-stage renal disease patients treated by hemodialysis [Wong et al., 2005].

The studies on the impact of HBV infection in hemodialysis patients are rare and were not able to demonstrate a negative effect on survival of patients [Josselson et al., 1987; Harnett et al., 1988]. The survival of HBsAg-positive renal transplanted patients is significantly lower than that of HBsAg-negative transplanted patients [Mathurin et al., 1999], making essential the assessment of the severity of liver damage during the pre-renal transplantation evaluation.

The natural history of HBV infection in hemodialysis patient is very different to that observed in non-uremic patients. The progression to chronic infection occurs around 60% of cases [London et al., 1977]. The causes of this high rate of progression to chronicity are not well established, but uremia and decrease of T lymphocyte number and/or function seem to be involved [Girndt and Kohler, 2002]. Previous studies have demonstrated a high proportion of HBeAg-positive patients among hemodialysis patients, indicating a predominance of patients with viral replication in this group of patients [Miller et al., 1978; Marchesini et al., 1981]. More recent studies have demonstrated that the majority of hemodialysis patients are HBeAg-negative, although this finding does not indicate necessarily an absence of viral replication [Fabrizi et al., 2002a, 2003].

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In fact, information regarding viral load in HBV-infected hemodialysis patients is rare in the literature and the prevalence of precore mutation is not known in this group of patients, albeit patients with serious liver disease associated with this condition have been described [Booth et al., 1995; Tanaka et al., 1995].

The majority of hemodialysis patients have normal levels of alanine aminotransferase (ALT) [Guh et al., 1995; Yasuda et al., 1995]. Thus, determination of ALT levels in hemodialysis patients is not a good test to identify the presence of viral replication in HBeAg-negative patients. Quantitative detection of HBV-DNA has been shown to be the more efficient method to evaluate viral replication in hemodialysis patients infected with HBV [Fabrizi et al., 2002b]. HBV viral load has been correlated to intensity of histological damage and higher risk of development of hepatocellular carcinoma, and can be influenced by many factors such as hepatitis C virus coinfection which is very prevalent in hemodialysis patients [Gane and Pilmore, 2002; Fattovich, 2003].

Hence, in HBsAg-positive/HBeAg-negative hemodialysis patients, the evaluation of viral replication is essential in the pre-renal transplantation assessment in order to establish the best strategy to be adopted.

The objectives of this study were to determine viral load in HBsAg-positive/HBeAg-negative patients treated by hemodialysis, to compare the levels of HBV-DNA among patients with HBV infection only and in patients with HBV-HCV coinfection, and to evaluate the prevalence of precore mutations in this group of patients.

PATIENTS AND METHODS

Hemodialysis patients with HBeAg-negative chronic HBV infection referred from different hemodialysis centers were studied. These patients were submitted to assessment for transplantion at the Kidney and Hypertension Hospital, and were directed for follow-up at Hepatitis Outpatients Clinic of the Federal University of Sao Paulo, Brazil, between January 1999 and September 2003. The exclusion criteria were the presence of HIV coinfection, alcohol abuse of more than 20 g/day, patients treated previously with interferon and/or lamivudine, and HBV and/or HCV acute infection.

The following epidemiological variables were considered: sex, age, history of blood transfusion, previous renal transplantation, and time on hemodialysis. The biochemical variables examined were alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT), using an automated kinetic method.

Anti-HCV reactivity was determined by the IMx HCV version 3.0 (Abbott Laboratories, Chicago, IL) and the determination of HBeAg was made by HBeAg IMx® (Abbott Laboratories).

The viral load was determined by PCR (Amplicor HBV Monitor, Roche Diagnostics, Basel, Switzerland) with a detection limit of 1,000 copies/ml. The investigation of precore mutations of HBV was made in all samples that had sufficient amplification for sequencing. The analysis of the precore region was performed by sequencing technique, allowing the identification of G1896A mutation (present in codon 28) as well as less common mutations, as deletions and mutations in codons 1, 2, and 29 [Lindh et al., 1996].

Informed Consent

The study was carried out in accordance with the Helsinki Declaration. All the patients selected for the study gave their informed consent.

Statistical Analysis

Categorical variables were analyzed by the Chi-square test and Fisher exact test. The quantitative variables were represented by mean and standard deviation. For comparison of viral load between two groups the Mann–Whitney test was applied. The level of significance of 0.05 was adopted ($\alpha = 5\%$).

RESULTS

Fifty patients were evaluated, 38 (76%) were men with a mean age of 44 ± 11 years. The mean time of treatment by hemodialysis was 6 ± 4 years. Forty-one patients (82%) had history of blood transfusion and 13 (26%) had history of previous renal transplantation. Twenty-eight (56%) patients were anti-HCV-positive. High levels of ALT, AST, and GGT were found in 26%, 10%, and 42% of the patients, respectively (Table I).

In all patients, 29 (58%) had undetectable HBV-DNA, 12 (24%) had viral load lower than 10,000 copies/ml, 6 (12%) had HBV-DNA levels between 10,000–100,000 copies/ml, and only 3 (6%) patients presented viral load higher than 100,000 copies/ml (Fig. 1). There was no difference in HBV-DNA levels between patients infected by HBV alone and those with HBV-HCV coinfection ($P = 0.96$).

A precore mutation was detected in four patients (8%), who were also anti-HCV-positive. Among patients with detectable HBV-DNA (21/50), a precore mutation was found in 4/21 (19%).

Comparative analysis between patients with low viral load (HBV-DNA $\leq$10,000 copies/ml) and high viral load

TABLE I. Characteristics of HBsAg-Positive/HBeAg-Negative Hemodialysis Patients (n = 50)

	N (%)
Male (%)	38 (76%)
Age (years)	44 ± 11
Time on hemodialysis (years)	6 ± 4
History of blood transfusion (%)	41 (82%)
Previous renal transplantation (%)	13 (26%)
Anti-HCV positive (%)	28 (56%)
Elevated ALT (%)	13 (26%)
Elevated AST (%)	5 (10%)
Elevated GGT (%)	21 (42%)

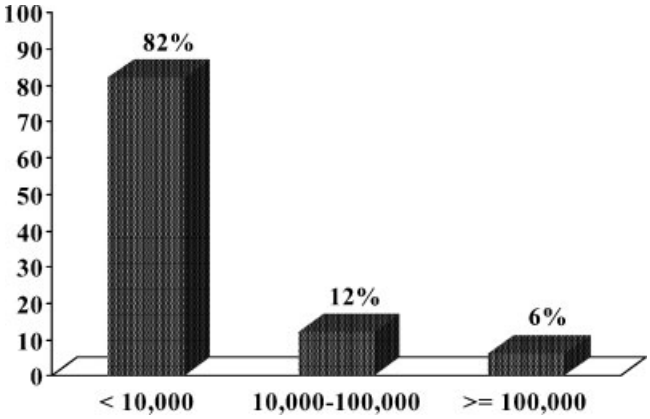

Fig. 1. Distribution of viral load in HBsAg-positive/HBeAg-negative hemodialysis patients (n = 50).

(HBV-DNA >10,000 copies/ml) regarding age, gender, time on hemodialysis, history of blood transfusion or previous renal transplant, biochemical variables, and anti-HCV positivity, showed no difference between the groups (Table II).

DISCUSSION

The diagnosis of hepatitis B, until the early 1990s, was based on the use of serological markers to evaluate viral replication. Phases of chronic HBV infection were classified, according to the serological profile, into replicative phase, characterized by the presence of positive HBeAg, high infectivity and higher frequency of biochemical and histological abnormalities, and into non-replicative phase, characterized by HBeAg clearance, decrease of viremia, lower frequency of biochemical and histological abnormalities, and low infectivity [Conjeevaram and Lok, 2003]. However, with the advent of new molecular biology techniques, the levels of viremia could be quantified and it was possible to identify significant viral replication even in HBeAg-negative patients. The study of this pattern of HBV infection has led to the identification of precore mutations [Carman et al., 1989].

TABLE II. Comparison of General Characteristics Between Patients With Low Viral Load (HBV-DNA ≤10,000 copies/ml) and High Viral Load (HBV-DNA >10,000 copies/ml)

	HBV-DNA ≤10,000 copies/ml (n = 41)	HBV-DNA >10,000 copies/ml (n = 9)	*P*
Males	73%	89%	0.43
Age (years)	45 ± 12	43 ± 8	0.60
Time of hemodialysis (years)	6 ± 4	6 ± 6	0.99
History of renal transplant	26%	33%	0.69
History of blood transfusion	89%	89%	1.00
anti-HCV positive	56%	56%	1.00
Elevated ALT	24%	33%	0.68
Elevated AST	8%	22%	0.26
Elevated GGT	42%	43%	0.99

In uremic patients with chronic HBV infection, who are candidates for renal transplantation, the evaluation of viral replication needs a judicious approach, being essential to establish the existence or absence of significant levels of viral replication in HBeAg-negative patients. Nevertheless, only few studies have evaluated the levels of viral load in HBeAg-negative hemodialysis patients and there are no studies on the prevalence of precore mutations in this population.

The level of HBV-DNA with clinical significance in HBeAg-negative non-uremic patients remains controversial. In a recent study, Manesis et al. [2003] have suggested a cut-off of 30,000 copies/ml to differentiate the inactive carriers from the patients with HBeAg-negative chronic hepatitis. An even lower cut-off level was suggested by Keeffe et al. [2004], who proposed a limit of 10,000 copies/ml as indication to treat patients with HBeAg-negative chronic hepatitis B. However, American, European, and Asian consensus indicate treatment of patients with HBeAg-negative chronic hepatitis with a viral load equal or higher than 100,000 copies/ml [Lok and McMahon, 2001; EASL, 2003; Liaw et al., 2003].

Studies on viral load in hemodialysis patients are rare, and different methods of HBV-DNA detection are factors that make comparison difficult. Studies using PCR for determination of the presence of HBV-DNA in HBeAg-negative hemodialysis patients showed different results, varying form 35% [Teles et al., 2002] to 64% [Teles et al., 1998]. Fabrizi et al. [2002a], analyzing the median level of HBV-DNA in 20 HBeAg-negative hemodialysis patients, have found low levels of viral load and in another study evaluating the dynamics of viral load in hemodialysis patients, the same author observed that the median of viremia found in HBeAg-negative patients was only 409 copies/ml [Fabrizi et al., 2003].

The present study has demonstrated that the majority of HBeAg-negative hemodialysis patients had a low viral load (82% of viral load <10,000 copies/ml), and only 6% had a viral load higher than 100,000 copies/ml. This low prevalence of high HBV-DNA levels in hemodialysis patients is a relevant information of this study and was not reported previously. The factors that explain this low viral load, despite immune system abnormalities observed in uremic patients [Cohen et al., 1997], are not well elucidated. The passage of virus into the dialysate compartment or to the peritoneal fluid during dialysis, and the lower clearance of endogenous interferon in uremic population seem to be involved [Salo et al., 1980; Kroes et al., 1994]. Demographic or clinical variables were not associated with viral load levels and the same results were also observed in the recent study [Fabrizi et al., 2003].

In this study, 8% of patients were precore mutation carriers. In non-uremic patients, rates changing from 29% to 58.6% have been described in our population [Pacheco et al., 2000; Rezende et al., 2005]. There are

no studies on the prevalence of precore mutations in hemodialysis patients. The presence of precore mutation has been described only in case reports of patients with fulminant hepatitis and fibrosing cholestatic hepatitis [Booth et al., 1995; Tanaka et al., 1995].

Precore mutations occur or become dominant during the late phase of HBV infection, and it is related to the moment of HBeAg to anti-HBe seroconversion [Okamoto et al., 1990; Locarnini et al., 2003]. The mean time of treatment by dialysis in the group studied that can reflect theoretically the time of infection, was low (6 years), and could explain the low frequency of precore mutations found in this population. Other characteristics of precore mutations are the association with genotypes B, C, and D [Lin et al., 2002]. In Brazil, in non-uremic populations, genotype A is predominant [Sitnik et al., 2004]. Genotype determination was not undertaken in this study, and it was not possible to establish the correlation between precore mutations and HBV genotype in this sample. Another factor that can be involved in the rate of precore mutations was the low level of viremia found in hemodialysis patients. The existence of mutations has also been related to high viral loads, and in patients with low viral load generally a low prevalence of precore mutations can be observed [Niitsuma et al., 1997; Peng et al., 2005]. The four patients with precore mutations were anti-HCV carriers. The reason for this finding needs to be elucidated.

In conclusion, hemodialysis patients with HBeAg-negative HBV infection presented low viral load and the occurrence of precore mutations was an uncommon event in this group of patients. These findings suggest that viral load determination, although useful, is not mandatory in this group of patients.

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