Prevention of Hepatitis B Recurrence in Liver Transplant Recipients Using Low Doses of Anti-Hepatitis B Immunoglobulin and Nucleoside Analogue

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Abstract

Introduction: Hepatitis B results in one million deaths every year and is is an important reason for liver transplantation. The use of anti-hepatitis B immunoglobulin at high doses and nucleoside analogues have reduced reinfection of the graft by 90%. **Objective:** This study evaluated the efficacy of low doses of immunoglobulin to prevent reinfection of grafts after transplantation. **Methodology:** This is a retrospective study of a series of patients who had been transplanted and who received immunoglobulin after transplantation at the Hospital Pablo Tobón Uribe between January 2004 and September 2014. Hepatitis B viral load, transaminase and serological markers were used to document relapses. Other variables studied included mortality, complications, graft dysfunction, adverse reactions and costs. **Results:** There were 18 patients with hepatitis B who had transplants: 50% had hepatocarcinoma, 22% had cirrhosis, and 22% had acute liver failure. The median follow-up time was 43.27 months with a range of 14.7 to 65.2 months. Two patients tested positive for surface antigen in the post-transplant period and one relapsed and had a positive viral load at 41 months. The graft reinfection rate was 5.5%. There were no deaths. It was estimated that the cost of using low doses of immunoglobulin was lower than that of using high doses at 6 months of therapy, but no cost-effectiveness study was done. Graft dysfunction was 10% to 33 months. **Conclusion:** Low doses of immunoglobulin prevented reinfection of grafts in a way that is similar to that reported in other series. While immunoglobulin free schemes have proven to be useful for the long term, low doses of immunoglobulin remain useful.

Key words

Liver transplantation, hepatitis B, immunoglobulin, antiviral.

INTRODUCTION

Worldwide, hepatitis B is a common infection, and about 2 billion people have had contact with the virus while 400,000 have chronic hepatitis B. (1) Colombia has intermediate endemicity with an overall incidence of 4.36 cases per 100,000 inhabitants as of November 2014. (2) Regions with higher than average incidence include Amazonas, Norte de Santander, Guaviare and Chocó with 23.8, 12.5, 9.13 and 8.89 cases per 100,000 inhabitants, respectively. (3)

Cirrhosis or hepatocellular carcinoma due to chronic infections and acute liver failure due to recent infections or to reactivation in chronic carriers result in 600,000 deaths worldwide every year. Chronic hepatitis B infections account for 10% of hepatic transplants, (1) but reinfection of the graft is a possible outcome.

Without post-transplant antiviral prophylaxis, graft infection occurs in 75% to 80% of cases, with a 50% two year mortality rate. (4) With the use of antiviral prophylaxis, recurrence is reduced to 10%. (1)

High dosages of hepatitis B immunoglobulin (HBIG) (10,000 IU/IV) were one of the first prophylaxis strategies used to reduce graft infection rates by 20% to 35%. (5) Nevertheless, reinfection occurred in more than half

of these cases due to the emergence of viruses with surface antigen (HBsAg) escape mutations. (6) Next, lamivudine showed better efficacy than did short-term administration of HBIG, (7) but, lamivudine monotherapy produced high rates of resistance which led to relapses in 40% to 50% of patients at three years. (8) Subsequently, the combination of large dosages of HBIG and lamivudine has reduced recurrences to less than 10% with a lower probability of resistance. (6, 9) Nevertheless, this scheme's high cost is a problem, especially in developing countries. To overcome the cost problem, protocols that call for low-dosage monthly administration of HBIG (400 IU/intramuscular) have been developed and have demonstrated similar effectiveness to that evidenced by high dosages. (9, 10). The aim of this study, conducted at the Hospital Pablo Tobón Uribe in Medellín , Colombia, is to describe the efficacy of the combination of low intramuscular doses of HBIG and a nucleoside analogue to prevent reinfection of the hepatic graft by hepatitis B virus after transplantation

METHODS

Population

This is a retrospective case series based on review of medical records of patients at the Hospital Pablo Tobón Uribe (HPTU) between January 2004 and September 2014. Records reviewed were those of patients with hepatitis B virus who underwent liver transplantation due to acute liver failure, cirrhosis with complications or one or more focal points of hepatocellular carcinoma and who received prophylaxis with HBIG and an antiviral agent (entecavir, lamivudine or tenofovir) during the post-transplant period. Patients who received less than 7 days of HBIG, who were followed up for less than 6 months, or whose records had insufficient data to evaluate the primary outcome were excluded. All patients had hepatitis B diagnosed positive tests for HBsAg positive and anti-core IgM antibodies (HbcIgM) or IgG type (HbcIgG) before transplantation. Co-infections with hepatitis C virus (HCV), hepatitis D virus (HDV) and human immunodeficiency (HIV) were evaluated.

Clinical and demographic variables collected and recorded included follow-up times, months of immunoglobulin treatment, graft dysfunction defined as transaminase elevation twice normal values, mortality, HBsAg, pretransplant HBeAg, post-transplant anti-e antigen, hepatitis B viral load, transaminases, prophylaxis and long-term immunosuppression.

Prophylaxis for Reinfection

A low dose HBIG regimen of daily intramuscular injections of 400 IU doses were administered for seven consecutive days starting in the anhepatic phase and thereafter with 400 IU intramuscular injections once a month. The first patients received indefinite prophylaxis with HBIG, then a duration of 6 months was established. All patients also received an oral nucleoside analog indefinitely. Those receiving lamivudine prior to transplantation continued with the same treatment if the viral load at the time of transplantation was negative. Those who had received no treatment or had viral replicative activity received 1 mg of entecavir daily in the post-transplant period. Patients with positive viral loads at the time of transplantation received HBIG for a longer period of time.

Immunosuppression Scheme

The Gastrohepatology Group's immunosuppression protocol, used for these patients, calls for descending IV administration methylprednisolone beginning at of 1 gram the first day and continuing for six days. This is followed by daily doses of 20 mg of prednisolone for 3 months. Patients with renal impairment or risk of renal failure receive 1,000 mg of mycophenolate every 12 hours and those with normal renal function or low risk of renal failure receive 1-2 mg/kg of azathioprine. A calcineurin inhibitor, either cyclosporine or tacrolimus, is started within 6 to 18 hours, up to 72 hours post-transplant depending on renal function. All patients have are strictly followed up on an outpatient basis.

Virological Follow-up

HBsAg was measured in the post-transplant period. For those who tested positive or had elevated transaminases, HBV viral loads were measured in real time. Reinfection of the graft was defined as positive HBV viral load in the post-transplant period.

Statistical Analysis

Data collection and analysis was done in SPSS® version 20 (SPSS Inc., Chicago, Illinois, USA). Quantitative variables are expressed as means or medians, with their respective dispersion measures according to the distribution of the variable using the Shapiro-Wilk test. Kaplan-Meier survival curves were used to assess long-term graft dysfunction.

Ethical Issues

The study was approved by the Ethics Committee of the Hospital Pablo Tobón Uribe and adhered to Resolution 008430 of 1993 of the Ministry of Health of the Republic of Colombia regarding ethical issues in research on human beings.

RESULTS

Eighteen transplant patients with hepatitis B virus infections who met the inclusion criteria were treated between January 2004 and September 2014. The baseline characteristics of the population are described in Table 1. Of these patients, 77.8% were men, the average age was 52.6 ± 11.28 years, and 83% of the patients had one or more comorbidity. The most frequent were hypertension which affected 40%, and diabetes which affected 26.6% of the cases. The median follow-up time was 43.27 months (14.7 to 65.2). Indications for transplantation were hepatocellular carcinoma (50%), cirrhosis with complications (22.2%), and acute liver failure (22.2%). Seven out of fourteen patients had positive HBV viral loads at the time of transplantation with a median of 2,832 IU/mL (609-19762 IU/mL). 94% of the patients received antiviral agents before transplantation, mean time of 17.9 ± 17.7 months in cases of chronic infection versus 0.28 ± 0.31 months in cases of acute liver failure. Entecavir was the most frequently used antiviral agent (67%).

Table 1. Baseline characteristics of the population

Variable	Total N (%)
Men	14 (78.7%)
Age in years, average, SD	52.6±11.28
Comorbidities	15 (83%)
Arterial hypertension	6 (40%)
Diabetes mellitus	4 (26.6%)
Cardiovascular pathology	2 (13.3%)
Indication for transplantation	
Hepatocellular carcinoma	9 (50%)
Cirrhosis	4 (22.2%)
Acute hepatic insufficiency	4 (22.2%)
Fibrolamellar tumor	1 (5.6%)
Pretransplant HBsAg	18 (100%)
Positive pre-transplant HBeAg test (evaluated in 12 patients)	5 (41.6%)
Positive HBV viral load pretransplant (Evaluated in 14 patients)	7 (50%)
Median HBV viral load at transplant. Median p25-75 in international units/mL	2,832 (609-19,762)
Antiviral agent administered immediately prior to transplantation.	17 (94.4%)
Antiviral agent administered long-term prior to transplantation.	18 (100%)
Lamivudine	6 (33.3%)
Entecavir	12 (66.7%)
Time of antiviral agent administration prior to transplantation. median. range. In months	
Acute insufficiency	0.5 (0.94-0.6)
Chronic Insufficiency	19 (1.75-27)
HCV Coinfection	0
HIV Coinfection	1 (5%)
Post-transplant Complications	15 (83.3%)
Post-transplant Sepsis	4 (26.6%)
Opportunistic infection	4 (22.2%)
Vascular thrombosis	2 (13.3%)
Postoperative bleeding	2 (11.1%)
Cell rejection	2 (13.3%)

SD: standard deviation, HBeAg: HBV antigen *e*, HBsAg: HBV surface antigen, HBV: hepatitis B virus, HCV: hepatitis C virus, HIV: human immunodeficiency virus

Immunoglobulin prophylaxis

Of the 18 patients, 16 received HBIG for 23.68 ± 14.8 months accompanied by lamivudine in 27.7% of the patients and by or entecavir in 72.2% of the patients. Of the 18 patients, two received HBIG only for the first 7 days after transplantation and the continued with entecavir (Table 2).

Serological follow-up

HBeAg seroconversion was found in 11 of the 14 patients evaluated. During follow-up, two of the 18 patients (11.1%) were HBsAg positive. One relapsed at 41 months post-transplant and three months after HBIG suspension. His viral load was 464,977,202 copies/mL despite the continued use of lamivudine. This patient had received lamivudine for 19 months prior to transplantation. After the relapse was detected, entecavir therapy was modified without response. The patient was then switched to tenofovir which resulted in better control of viral load and better graft functioning. There were unexplained recurrences of hepatocellular carcinoma through viral replication in the post-transplant period in two of the 18 cases (Table 3). The overall relapse rate was 5.5% (1 out of 18 patients).

Graft dysfunction

There were 8 cases of graft dysfunction during follow-up: two due to bile duct stenosis, one due to hepatotoxicity, two due to mild acute cellular rejection, one due to chronic periportal hepatitis, one due to HBV relapse, one due to relapse of a fibrolamellar tumor. All cases of dysfunction were transient, except in the patient with fibrolamellar tumor, in whom the disease progressed. Overall graft dysfunction was 10% at 33 months (Figure 1), and there were no deaths during follow-up.

Table 2. Treatment received by 18 patients during the follow-up

Variable	Total N (%)
Antiviral treatment post-transplant	18 (100%)
Lamivudine	5 (27.7%)
Entecavir	13 (72.2%)
HBIG	18 (100%)
1 week	2 (11%)
More than 1 week	16 (88%)
Average duration of HBIG in months, SD	23.68±14.8

SD: standard deviation, HBIG: anti-hepatitis B immunoglobulin

Table 3. Outcomes of 18 patients with hepatitis B virus infections who underwent liver transplantation

Variable	Total (n/%)
Follow-up time in months, median, range	43.27 (14.7-65.2)
HBeAg Seroconversion (Evaluated in 14 patients)	11 (78.5%)
HBsAg positive post-transplant	2 (11.1%)
Positive viral load post-transplant (Evaluated in 15 patients)	1 (6.6%)
Recurrence	1 (5.5%)
Recurrence of hepatocellular carcinoma	2 (11%)
Loss of graft or retransplantation	0 (0)
Graft dysfunction	8 (44.4%)
Total recovery from dysfunction	7 (87.5%)
Mortality	0

HBeAg: HBV antigen *e,* HBsAg: HBV surface antigen

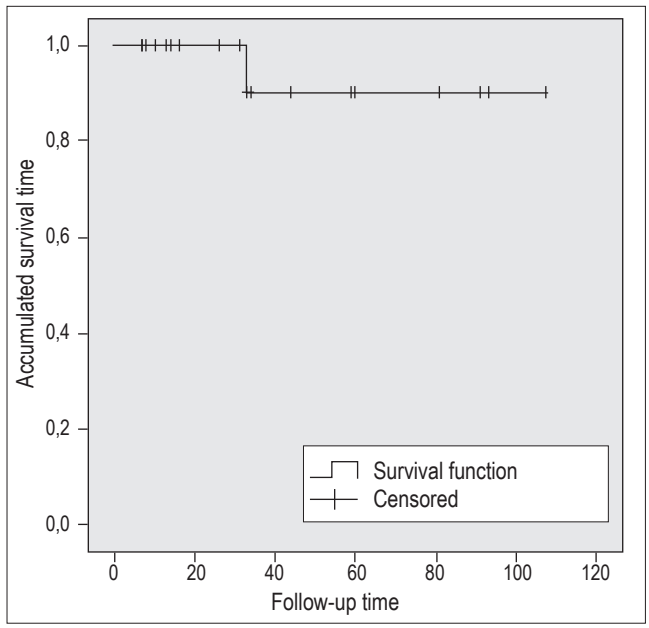

Figure 1. Kaplan-Meier curve of Graft dysfunction during follow-up.

Adverse reactions

There were no adverse events related to therapy.

Costs

Because the recurrence rates reported in this study are similar to those reported in studies using high doses of HBIG, it can be assumed that both protocols coincide in effectiveness. Nevertheless, even though we did not do a cost-effectiveness study and these are only observational data, the two protocols costs are very different.

DISCUSSION

Currently, liver transplantation is the best alternative for patients with chronic complications of HBV infections. (11, 12) In the early 1990s, high-dose HBIG prophylaxis became the best therapeutic alternative for decreasing graft reinfections and mortality. (4) Markowitz optimized prophylaxis by combining HBIG with lamivudine, (13) but the cost of high doses of immunoglobulin has been a limiting factor and has led to the emergence of protocols with lower doses. Nevertheless, ability to generate neutralizing antibodies and to avoid recurrence with low doses has been questioned.

McCaughan et al. used low doses of HBIG in 9 patients to 17 months after transplantation without relapses. (14) Angus et al. used low doses of HBIG in 32 patients without recurrences up to 18 months after transplantation. (10) Finally, in 2007, Gane et al. used low doses of HBIG with lamivudine in an Australian and New Zealand cohort and found a reinfection rate of 4% up to 5 years. The one year survival rate was 82%, and the five-year survival rate was 88% which is similar to those reported with high dose protocols. (9) We report our experience using a low-dose HBIG protocol combined with lamivudine or entecavir in transplant patients with hepatitis B at a liver transplant referral center in Colombia. The results agree with those of other series. With a recurrence rate of infection only 5.5% (One out of 18 patients). The viral load at the time of transplantation of the patient who relapsed is unknown, even though he had received 19 months of lamivudine monotherapy previously. The relapse occurred 3 months after discontinuing HBIG. We suspect resistance to the nucleoside analogue, but he also relapsed with entecavir and showed improvement only after starting tenofovir and reducing immunosuppression to control viremia.

Most patients became negative for HBsAg during follow-up, so it is considered to be a good parameter for follow-up. Only one patient was positive for HBsAg during follow-up but without correlation with a positive viral load. Similar to other cohorts in which there are very few reports of adverse events with low doses of HBIG, we found no adverse effects related to the administration of HBIG. Nevertheless, more similar studies are required to support the use of low-dose immunoglobulin as a prophylactic alteration in low-income countries. This is due to the fact that duration of prophylaxis can be long-term or indefinite (15% to one year and 8% to two years). (15) In this regard, studies have been published suggesting a limited time use of HBIG combined with a high genetic antiviral barrier (tenofovir or entecavir) (13 16 17) HBIG-free schemes have even been suggested. (18).

A study by Choudhary et al. that used HIBG for 12 months followed by tenofovir or entecavir reported a relapse rate of 4.3% at an average of 43 months of follow-up (range: 12-117). (19) Similarly, Tanaka et al. found no relapses at 29 months after using HBIG for 12 months followed by tenofovir combined with lamivudine. (20) An HBIG-free protocol using entecavir monotherapy once viral load has been controlled has also been described with a relapse rate of 1.26% up to 26 months. (21). The mean time of HBIG administration in our patients was 23 ± 14 months. The reason for this is that patients transplanted in the early years used to use HBIG indefinitely, but in recent years with the advent of new antivirals we decided to shorten the time of HBIG treatment, especially when seroconversion of HBeAg and HBsAg was achieved. (22) Use of nucleoside analogues in monotherapy or in combination therapy with another analogue is progressively increasing for prophylaxis. One result is decreasing use of HBIG. Nevertheless, long-term studies of the safety of HBIG-free schemes are still required. In the meantime, many transplant centers worldwide continue to use HBIG as the optimal treatment for preventing reinfection.

This study has several limitations. Among them are its retrospective character and the absence of viral load measurements at the time of transplantation for four patients. Consequently, it was not possible to make associations between pretransplant HBV viral loads and viral loads after relapse. Another limitation was the absence of systematic evaluation of viral loads in the post-transplant period. Nevertheless, extensive follow-up allowed evaluation of graft functioning as well as mortality for more than 3 years after transplantation. Although we did not perform any cost-effectiveness analysis, we can infer that results similar to those reported with the administration of high dosages can be obtained at lower costs through usage of low dosages of HBIG.

While studies of whether HBIG-free prophylaxis can result in sufficient long-term safety continue, low-dose prophylaxis of HBIG will continue to be useful for preventing recurrences of infections following transplantation.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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