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DE NOVO HEPATITIS B VIRUS INFECTION AFTER LIVER TRANSPLANTATION

A.D. Nikogosova[1], *D.V. Umrik*[1], *O.M. Tsirulnikova*[1, 2]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Sechenov University, Moscow, Russian Federation

De novo hepatitis B virus (HBV) infection developing after liver transplantation (LTx) is the development of infection in a patient with liver disease etiologically unrelated to HBV infection and who had no preoperative HBV markers. **Objective:** to analyze the clinical features and characteristics of *de novo* HBV infection and evaluate the efficacy of nucleos(t)ide analogue therapy in liver transplant recipients. **Materials and methods.** The study involved 247 adult patients who underwent deceased donor LTx from 2016 to 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs and who had no pre-transplant HBV markers. **Results.** Twenty-two (7%) of 247 patients had *de novo* HBV markers from 5 to 69 months. At the time HBV DNA was detected, the mean alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the patients was 53.3 ± 36.4 IU/L and 54.5 ± 33.0 IU/L, respectively. All patients received nucleos(t)ide analogues (NAs). The therapy led to a statistically significant decrease in the mean ALT level to 31.5 ± 24.2 IU/L ($p = 0.049$) and AST to 33.33 ± 21.5 IU/L ($p = 0.025$). In most cases (18 persons, 81%), no serum HBV DNA was detected after treatment (6 ± 3 months). **Conclusion.** Timely detection of *de novo* HBV risk factors, early diagnosis and immediate treatment can prevent severe graft damage.

Keywords: HBV infection de novo, liver transplantation, nucleos(t)ide analogues, entecavir, tenofovir, immunoglobulin.

INTRODUCTION

De novo HBV infection arising after LTx is the development of infection in a patient/recipient with liver disease that is etiologically unrelated to HBV infection and who had no preoperative HBV markers. According to reports, *de novo* HBV infection after orthotopic LTx in patients without viral replication and even in patients without markers of previous infection with HBV is between 1.7% and 5% [1]. Untreated HBV infection leads to severe liver disease, rapid graft dysfunction, graft cirrhosis, and risk of hepatitis D virus (HDV) co-/superinfection.

OBJECTIVE

To analyze the clinical features and characteristics of *de novo* HBV infection and evaluate the efficacy of therapy with nucleos(t)ide analogue therapy in liver recipients.

CLINICAL CASES AND RESEARCH METHODS

The study involved 247 adult patients who underwent deceased donor LTx from 2016 to 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs and who had no pre-transplant HBV markers. After LTx, the patients underwent standard clinical examination at least once every 3 months, including interview and examination, routine laboratory – total blood count, biochemical blood count, coagulogram, total urine count, measurement of immunosuppressive drug levels in blood, serological blood test – hepatitis C antibodies, hepatitis B surface antigen (HBsAg) – and instrumental examinations (abdominal ultrasound. When a positive HBsAg was detected, we performed qualitative and quantitative detection of HBV DNA by polymerase chain reaction (PCR), and examined the HBV profile (HBeAg, anti-HBe, HbcAg, anti-HBc IgM) and HDV antibodies. All patients received immunosuppressive therapy in various combinations. When HBV infection markers were detected, patients were prescribed high-barrier NAs – entecavir (ETV) and tenofovir (TDF).

Statistical analysis was performed using Statistica 12.6 software. Differences were considered statistically significant at the $p < 0.05$ level.

RESULTS

Of 247 patients, 22 (7%) (8 men and 14 women) showed *de novo* HBV infection markers (HBV DNA, HBsAg) at 5 to 69 months (mean was 21.4 ± 17.3 months, median was 17 months). No cases of HDV co/superinfection were identified.

The most common indications for LTx were cirrhosis resulting from autoimmune liver diseases (autoimmu-

Corresponding author: Anna Nikogosova. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (958) 828-32-09. E-mail: benevolenskaya.a@gmail.com

ne hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC)), hepatitis C virus (HCV) and toxic liver disease. Other indications for transplantation were renal allograft dysfunction (retransplantation due to recurrent underlying disease – 1 AIH and 1 PSC), hepatic alveolar echinococcosis (HAE), Byler disease and neuroendocrine liver metastases (NELM). One patient underwent simultaneous liver and kidney transplantation due to polycystic liver/kidney disease (Table 1).

In one patient, HBV infection developed 248 months after LTx, which resulted in graft injury requiring retransplantation. In the remaining patients, HBV infection proceeded without severe clinical manifestations.

Patients continued to receive immunosuppressive therapy to the same extent as before the detection of *de novo* HBV infection markers. Most patients received double immunosuppressive therapy (10 patients, 45%) or tacrolimus (TAC) monotherapy (8 patients, 36%); 18% had a triple immunosuppressive protocol (Table 2). Mean whole blood TAC concentration was 6.05 ± 2.01 ng/mL.

At the time HBV DNA was detected, patients had 53.3 ± 36.4 IU/L and 54.5 ± 33.0 IU/L as mean ALT and AST, respectively. All patients with positive HBV DNA were prescribed the high-barrier NAs – ETV and TDF; three patients were initially treated with ETV, then converted to TDF disoproxil fumarate due to persistent viremia. One patient received lamivudine (LVD), which was subsequently changed to ETV because of resistance to LVD (Table 3).

The therapy led to a statistically significant decrease in the mean ALT level to 31.5 ± 24.2 IU/L ($p = 0.049$) and AST to 33.33 ± 21.5 IU/L ($p = 0.025$). In most cases (18 persons, 81%), no serum HBV DNA was detected after 6 ± 3 months of treatment. Also, 10 (45%) patients had HBsAg seroconversion after 19.7 ± 9.5 months. Of these, 7 received ETV therapy and 3 received TDF. Twelve patients (54%) remained HBsAg-positive in the absence of viremia. Two patients (9%) were treated with NAs for no more than 4 months and had a viral load of 8.0×10^3 IU/mL.

Table 1

Underlying diseases leading to liver transplantation in *de novo* HBV infection

Underlying disease	Patient count
AIH	4
HCV	3
PBC	3
PSC	3
Toxic hepatitis	2
RAD	2
HAE	1
Polycystic liver disease	1
Wilson–Konovalov disease	1
NELM	1
Byler disease	1

Table 2

Immunosuppressive therapy in patients with *de novo* HBV infection

Therapy regimen	Patient count
TAC	8
TAC + MMF	7
TAC + MMF + Methylprednisolone	4
TAC + Everolimus	2
TAC + Methylprednisolone	1

Note: MMF, mycophenolic acid / mycophenolate mofetil.

Table 3

Antiviral therapy in patients with *de novo* HBV infection

Drug	Patient count
ETV	14
TDF	3
ETV → TDF	3
Tenofovir alafenamide (TAF)	1
LVD → ETV	1

DISCUSSION

As in the general population, the source of HBV infection may be blood transfusions, surgical interventions, including dental surgeries, etc. Accordingly, HBV infection markers can be detected during the whole life of a recipient, which our study demonstrates – the average time before the onset of infection was almost two years. In our sample, the prevalence of *de novo* HBV infection was consistent with literature data [2].

HBcAb- and HBsAb-negative recipients are at the highest risk of *de novo* HBV infection [3]. A recent study reported that of 1,458 patients, 21 (1.4%) were found to have *de novo* HBV infection. The time to detection of infection varied, ranging from 8 to 55 months. HBcAb-negative recipients had a higher risk of *de novo* HBV infection than HBcAb-positive recipients (22.6% versus 9.1%). The incidence of *de novo* HBV infection did not differ depending on the recipient's HBs-antibody status [4].

There are three main approaches to prevent *de novo* HBV infection: active immunization (vaccination of recipients before liver transplantation), passive immunization (administration of human hepatitis B immune globulin, HBIg) and therapy with direct antiviral drugs (nucleos(t)ide analogues) for preventive purposes at high risk of infection and for treatment when infection markers are identified.

There are ongoing studies looking at active immunization of liver transplant recipients before and after LTx with monitoring of HBs antibody titers as a measure

to prevent *de novo* HBV infection. One was presented in 2017; in this study, Wang et al. looked at a group of 71 liver recipients who received HBV vaccination before and after transplantation from HBcAb-positive donors. The mean follow-up period was 8 years, with only 3 (4%) cases of *de novo* HBV infection reported. All patients belonged to the group with insufficient immune response to vaccination (anti-HBs titer of <100 IU/L). The detected infection had no significant abnormalities in the biochemical blood count that would have required liver biopsy, and it had no effect on the transplant outcome. Throughout the study, a fairly large number of vaccine injections were required to maintain immunity (average of 4 doses; range 1–9 doses), and 9 patients were never vaccinated after transplantation because of contraindications. Thus, the researchers note that the approach described is cheaper, but the vaccines are less effective in cirrhosis and require careful monitoring of response after vaccination. Vaccination timing is difficult to predict, and vaccination can take months, and some patients fail to achieve target anti-HBs levels for a variety of reasons. This approach is more applicable in the context of living donor liver transplantation, when surgery is performed routinely [5].

As in patients with initial HBV infection, human anti-HBsAg immune globulin (HBIg) is used in patients with *de novo* HBV infection. Some studies indicate the effectiveness of HBIg monotherapy with a very low risk of *de novo* HBV infection in recipients who have received a transplant from an anti-HBc-positive donor and who have an anti-HBs titers of >100 IU/L [5]. However, the lack of long-term data, the risk of decreasing anti-HBs titers over time, and the need for concomitant antiviral prophylaxis in nonresponders have led to a significant reduction in the use of this strategy. According to several studies, extra addition of HBIg to NAs administration did not enhance treatment efficacy [6, 7]. The American Association for the Study of Liver Diseases and the European Association for the Study of the Liver recommended nucleos(t)ide analogue monotherapy for prevention and treatment of *de novo* HBV infection, given the absence of differences in treatment outcomes with and without HBIg, low frequency of *de novo* HBV infection, high cost of immunoglobulin, and the need for intravenous routes for administration of this medication [8].

For many years, LVD was the standard treatment for HBV infection, with approximately 3% of patients developing the infection despite taking the drug [9]. Subsequently, various primary and secondary mutations leading to resistance to LVD treatment have been identified. The most common primary mutations associated with LVD resistance occur in codon 204 in the tyrosine-methionine-aspartate-aspartate (YMDD) site and result in amino acid substitution – rtM204V/I (replacement of methionine with valine or isoleucine). These changes cause >100-fold decrease in sensitivity to LVD [10]. Resistance to LVD develops gradually during treatment: with a rate of 14% to 32% in the first year of treatment and exceeding 70% after 48 months of therapy [11]. There was also data in the literature on the effectiveness of tenofovir [12]. However, currently, due to the high rate of resistance, the need for long-term prevention/treatment and the development of a number of side effects, high-barrier NAs – ETV, TDF and TAF – are used in clinical practice [6, 7, 13].

In our study, we used the most modern treatment regimen for HBV-infected patients – high-barrier reverse transcriptase inhibitors, NAs – ETV and TDF salts. Thanks to timely administration of these preparations, *de novo* HBV infection was, in the overwhelming majority of cases, mild, without clinical manifestations and with minimal changes in laboratory values (ALT and AST increased to 2–2.5 norms at most). The therapy led to a statistically significant decrease in hepatic aminotransferases, and all patients had an undetectable level of HBV DNA by PCR. HBsAg seroconversion was observed in 45% of cases. Our results correlate with other studies. One of the most voluminous works was published in 2021 by Saidy et al. out of 2686 liver transplant recipients, 32 patients (1.2%) demonstrated a *de novo* HBV infection without an obvious source of infection. Additionally, 78 (2.9%) received a HBcAb-positive graft without having undergone HBV-infection prior to LTx. In this subgroup, 14 (17.9%) patients were recorded with *de novo* HBV infection. After the diagnosis, the patients were treated with either ETV or tenofovir. The authors noted a significant reduction in inflammation signs and no progression of steatosis on graft biopsy after initiation of therapy; no difference in survival between patients with and without *de novo* HBV infection was found [14]. Consequently, timely detection of risk factors for *de novo* HBV infection, early diagnosis and immediate initiation of treatment can prevent serious damage to the graft, which has been confirmed in various studies.

CONCLUSIONS

1. The clinical course of *de novo* HBV infection in the examined patients was mild with minimal clinical and laboratory manifestations. Therefore, timely detection and initiation of antiviral therapy will increase graft survival in this patient cohort.
2. Antiviral therapy with NAs is effective against the background of immunosuppressive therapy and is accompanied by disappearance of replication markers in the majority of patients (81%) 6 ± 3 months after the beginning of antiviral therapy; HBsAg seroconversion was observed in 45% after 19.7 ± 9.5 months.
3. High-barrier NAs are effective enough; additional therapies are not required.

The authors declare no conflict of interest.

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