

Hepatitis B and hepatocellular carcinoma recurrence after living donor liver transplantation: The role of the Milan criteria

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ABSTRACT

Background/Aims: The aim of this study was to evaluate the effect of the Milan criteria on the hepatitis B virus (HBV) and hepatocellular carcinoma (HCC) recurrence in patients who underwent living donor liver transplantation due to HBV-induced cirrhosis and HCC.

Materials and Methods: We evaluated a total of 142 patients, 88 who underwent transplantation due to HBV-induced cirrhosis and 54 due to HCC, between 2009 and 2014. In the posttransplant period, after the HBsAg seroconversion, 400 IU of hepatitis B immunoglobulin were applied intramuscularly every 2 weeks, and daily nucleos(t)ide analogs were continued as prophylaxis. The HBV recurrence was defined as the presence of HBsAg in serum. Patients were screened for alpha-fetoprotein levels and imaging for evaluation of HCC recurrence.

Results: The average follow-up period was 26 (2-65) months. Fifty-four patients had HCC. The HCC recurrence was observed in 12 patients during the follow-up period. The HBV recurrence was observed in four patients. Three of the patients who developed HBV recurrence had liver transplantation due to HCC. Tumor recurrence was observed 1.4-12 months following the HBV recurrence. The HCC recurrence within the Milan criteria and beyond the Milan criteria was 0% vs. 28.4% in the first year and 3.4% vs. 47.5% in the third year. The cumulative incidence of the HBV recurrence was 2.8% and 3.7% for the first year and 3.7% for the third year. The HBV recurrence was more frequently detected in patients with HCC ($p=0.048$), especially with HCC beyond the Milan criteria ($p=0.044$).

Conclusion: The HBV recurrence should be evaluated as a predictor of the HCC recurrence in patients who underwent liver transplantation due to HCC with exceeding Milan criteria.

Keywords: Living liver transplantation, Milan criteria, HBV recurrence, HCC recurrence

INTRODUCTION

Liver transplantation is a curative treatment for decompensated cirrhosis and hepatocellular carcinoma (HCC). However, the most common problem in the world is an inadequate number of donated organs (1). Therefore, the use of a living donor is an alternative to expand the organ pool (2). There are some studies suggesting that living donor liver transplantation (LDLT) should be the preferred method for patients with HCC because of a shorter waiting time for the liver, shorter cold ischemia time, and virtually no warm ischemia damage (3,4). However, some of the researchers suggest that the results of deceased donor liver transplantation (DDLT) are better (5,6).

The most important factors affecting the survival after transplantation are postoperative complications, the effect of immunosuppressive agents, graft rejection, and

recurrence of primary disease (7). Especially, the HCC recurrence is higher in the patients with HCC larger than 5 centimeters. The Milan criteria have started to be used in predicting the HCC recurrence in the last decades. According to the Milan criteria, transplantation can be performed for HCC with no macrovascular invasion, a single tumor ≤ 5 cm, or up to three tumors ≤ 3 cm each. The posttransplant survival rates for HCC were disappointing before the use of the Milan criteria (8). The results have been shown to be better in patients undergoing transplantation for HCC according to the Milan criteria (8,9).

In patients with hepatitis B virus (HBV), the HBV recurrence also affects the posttransplant mortality, in addition to other effects. There are some publications showing that the HBV recurrence is more common in patients undergoing transplantation due to HCC (10,11).

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The aim of this study was to evaluate the effects of the Milan criteria on the HBV and HCC recurrence in patients who underwent LDLT due to HBV-induced cirrhosis and HCC.

MATERIALS AND METHODS

We retrospectively evaluated a cohort of 142 (123 M/19 F) patients from a liver transplantation center, who underwent liver transplantation due to hepatitis B-related liver cirrhosis and HCC between July 2009 and February 2014. The mean age of the patients was 51.4 ± 9.4 (15-71) years.

Patients over the age of 15 who underwent liver transplantation due to the diagnosis of decompensated cirrhosis and/or HCC due to hepatitis B were included in the study.

Patients who had HBV-related acute liver failure, were coinfecting with HCV, and were HIV positive were excluded from the study.

Research involving human subjects (including human material or human data) reported in the manuscript is in compliance with the Helsinki Declaration. This study was approved by the Ethical Committee of the İzmir Katip Çelebi University Atatürk Training and Research Hospital, decision number 2018-55.

Immunosuppressive regimen

A total of 500 mg of methylprednisolone was administered on the day of the operation. Starting from the postoperative first day, a daily dose of 100 mg was given. The dose was reduced by 10 mg each day, until it reached a minimum daily dose of 20 mg. This dose of 20 mg/day was given for a month, and then it was decreased by 5 mg every month. After 4-6 months, prednisolone application was discontinued. On the first postoperative day, 1 mg of tacrolimus 2x1 po was started, and the dose was augmented to maintain a blood level of 5-15ng/mL; 15 ng/mL to begin with and to reach 5 ng/mL at the end of the first year and beyond.

Sirolimus or everolimus was started in the patients who showed an increase in the creatinine level or in whom specific side effects developed against tacrolimus. Mycophenolate mofetil was also applied in patients in whom immunosuppressive therapy remained insufficient. Each patient was given an oral antiviral treatment (lamivudine, adefovir, entecavir, tenofovir, telbivudine), and HBV-DNA was negative prior to transplantation in all patients. The preoperative oral antiviral treatment was also continued postoperatively for all patients.

HBV prevention strategy

The patients were administered 5000 IU HBIG intravenously (IV) during the anhepatic phase. During the posttransplant period, HBIG was continued at 400 IU intramuscularly (IM) daily. Three days a week (Monday, Wednesday, and Friday), HBsAg and anti-HBs levels were examined. After HBsAg became negative (HBsAg < 1.0 S/CO [negative]), and anti-HBs (anti-HBs > 10 IU/L [positive]) became positive, the daily HBIG practice was terminated. In addition to the antiviral treatment, prophylaxis was continued with HBIG 400 IU IM once every 2 weeks. HBsAg values were studied using the ARCHITECT HBsAg Qualitative II device. After HBsAg became negative, it was followed up at the intervals of 3-6 months. The HBV recurrence was evaluated as the positivity of HBsAg with/without detectable serum HBV-DNA.

Statistical analysis

In the analysis of the relevant data, the Statistical Package for Social Sciences 17.0 Statistics Program (SPSS Inc.; Chicago, IL, USA) was used. All numeric values are given as the mean \pm SD (standard deviation) or as the median. The chi-square test was used for the categorical variables. The normality of the groups and their compliance with homogeneity were evaluated as well. The data incompatible with the normal distribution were evaluated using the Mann-Whitney U test, whereas those compatible with the normal distribution were evaluated using the Student's t-test. The Kaplan-Meier method was used to estimate the Survival Curves. The Survival Curves were compared across the ordered categories with the Log-Rank Test for trend. The relationship between the dependent and independent variables was analyzed through the regression correlation analysis. A p-value < 0.05 was accepted as having statistical significance.

RESULTS

Demographic data and characteristics of the study group are shown in Table 1. The mean follow-up period was 26 months. Liver transplantation was performed in 54 patients who suffered from HBV-related HCC. Twenty-two of these patients (40.7%) with HCC were those that exceeded the Milan criteria. The HDV co-infection was observed in 50 (35.2%) patients.

During the follow-up period, the HBV recurrence was found in four patients, and all recurrences were within the first year (Table 2). The HBV recurrence was deter-

Table 1. Characteristics of patients

	Total n=142	HCC n=54	non-HCC n=88	p*
Age	51.4±9.4	55.6±8.2	48.9±9.2	<0.001
Gender (F/M)	19/123	4/50	15/73	0.101
Follow-up period (months)	26 (2-65)	20 (2-63)	27 (2-65)	0.016
MELD	14 (6-35)	11 (6-29)	16 (6-35)	<0.001
CHILD	8 (5-15)	8 (5-13)	9 (5-15)	0.001
Weight of patients (kg)	78±13	81±16	76±11	0.067
Weight of graft (g)	781±142	760±147	793±138	0.2
Weight of explanted liver (g)	1165±320	1268±262	1100±274	0.003
Log HBV-DNA before treatment	4.7±1.7	5.0±1.7	4.5±1.6	0.249
HBeAg	11 (8.5%)	7 (13.0%)	4 (4.5%)	0.206
Anti-Delta	50 (35.2%)	11 (20.4%)	39 (44.3%)	0.001
HBsAg negativity (days)	11 (2-63)	11 (2-63)	11 (2-36)	0.959
Anti-HBs positivity (days)	9 (1-31)	9 (2-24)	9 (1-31)	0.731
Anti-HBs >100 IU/ml (days)	11 (2-36)	11 (2-36)	10.5 (2-26)	0.968
Antiviral (TDF/ENT)	49 (34.5%)	18 (33.3%)	31 (35.2%)	0.818
Antiviral (LAM/ADF/LdT)	93 (65.5%)	36 (66.7%)	57 (64.8%)	0.818

*Comparison of the patients with HCC and non-HCC.

**Patients with the anti-HBs level above 100 IU/mL

HCC: hepatocellular carcinoma; HBV: hepatitis B virus; MELD: Model for End Stage Liver Disease; LAM: lamivudine; ENT: entecavir; ADF: adefovir; TDF: tenofovir; LdT: telbivudine

Table 2. Characteristics of the patients with HBV recurrence

Age	Gender	Etiology	Antiviral Drug	HCC Existence	HBV Recurrence (months)	HCC Recurrence (months)	Outcome (months)
55	male	HBV-HDV	LAM	Yes	3.0	10.1	14 (died)
52	male	HBV	ENT	Yes	5.0	6.4	7.3 (died)
46	male	HBV-HDV	LAM	No	10.9	-	64 (alive)
62	male	HBV	ADE+LAM	Yes	8.0	20.0	34 (died)

HCC: hepatocellular carcinoma; HBV: hepatitis B virus; HDV: hepatitis D virus; LAM: lamivudine; ENT: entecavir; ADE: adefovir

mined to be 3.7% in the first year, and 3.7% in the third year. Three of these patients had a transplant due to HCC and exceeded the Milan criteria. During the follow-up period, 12 patients experienced HCC recurrence, and 10 of them had exceeded the Milan criteria (Figure 1). Prominent HBV recurrence was detected more frequently in patients with HCC than in those without HCC ($p=0.048$) (Figure 2).

In the groups with and without HCC, the HBV recurrence rates were found to be 10.8% vs. 2.0% and 10.8% vs. 2.0%, respectively, at the first and third years.

An association between hepatitis delta with HBV recurrences was not determined ($p=0.435$).

A significantly higher rate of HBV recurrence was found in patients with HCC exceeding the Milan criteria ($p=0.044$) (Figure 3).

The survival rate was similar between the patients within and those who exceed the Milan criteria without the HCC recurrence ($p=0.962$) (Figure 4).

The mean HBIG use by the patients was 20,885±2,898 IU in the first year and 42,362 ±3,028 IU in the third year. With the use of our HBIG treatment regimen, the anti-HBs titration in 12 (8.5%) patients did not exceed 100 IU/L. The HBV recurrence was found in only one of these patients. This patient was not diagnosed with HCC. None of the patients died because of liver failure

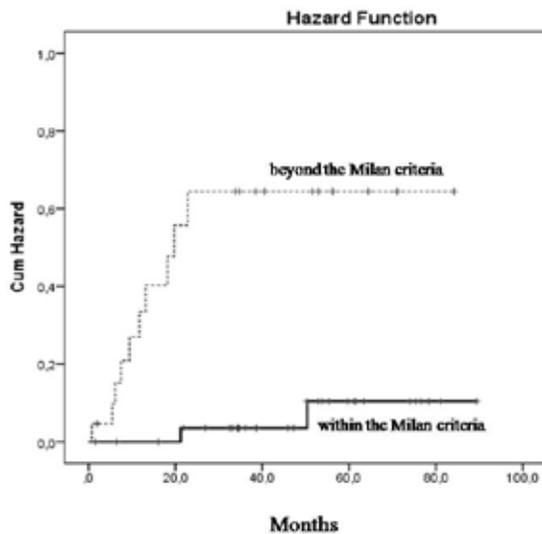


Figure 1. Recurrence of HCC within and beyond the Milan criteria (p<0.0001)

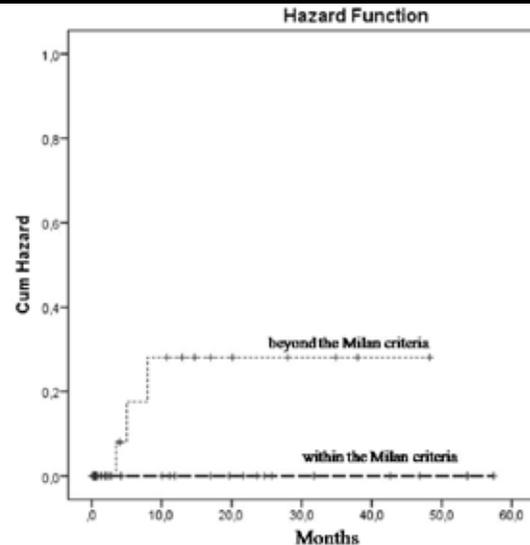


Figure 3. Recurrence of hepatitis B within and beyond the Milan criteria (p=0.044)

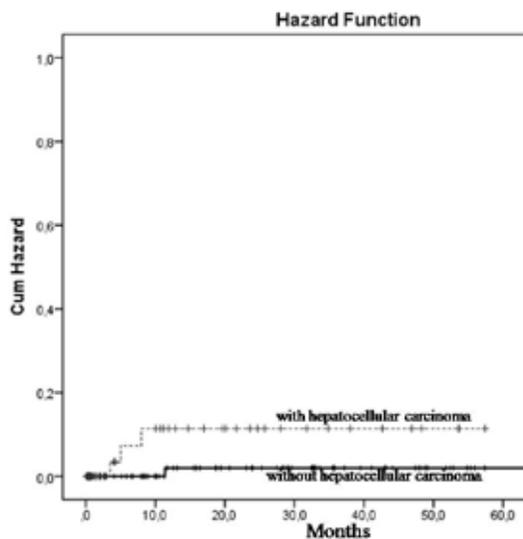


Figure 2. HBV recurrence in patients with HCC and without HCC (p=0.048)

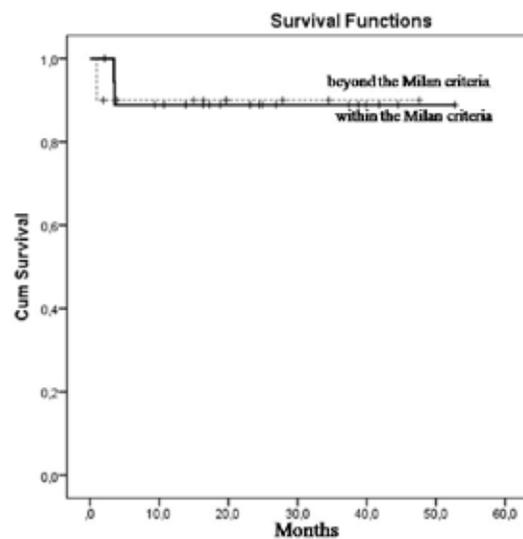


Figure 4. Survival of patients with HCC without HCC recurrence (p=0.962)

due to HBV recurrence. An elevation of liver enzymes due to the recurrence was found in one patient. Antiviral treatment of this patient was changed to a potent antiviral agent, TDF.

No relationships were found between patients' weight and the HBsAg negativity (p=0.221, r=0.056), the anti-HBs positivity (p=0.599, r=0.013), and anti-HBs that were >100 IU/L (p=0.708, r=-0.042).

DISCUSSION

Hepatitis B virus is the most common cause of HCC and liver transplantation in many countries (12). After the use of the Milan criteria, it has been observed that the 5-year survival rate exceeds 70% and that the 5-year tumor recurrence rates decrease to 5%-9.7% (13,14). The University of California, San Francisco; Shanghai Fudan; and Hangzhou criteria are also used for patient selection in liver transplantation. All of these expanded criteria have a

higher HCC recurrence after transplantation than the Milan criteria (15,16). Therefore, the Milan criteria have been accepted as the main criteria in patients undergoing liver transplantation for HCC (15).

In the study by Xiao et al. (1), a total of 360 HBV patients were evaluated. The overall survival of patients with LDLT and DDLT was similar. According to the Milan criteria, recurrence-free survival rates at the first and fifth years were 94.7% and 78.7% for LDLT, 89.2% and 74.5% for DDLT, respectively. Although the results were better for LDLT, there was no statistically significant difference ($p=0.50$).

In our study, the HBV and HCC recurrence after transplantation were significantly higher in patients who exceeded the Milan criteria. There was no difference between the HCC recurrence-free survival rates in patients who did not exceed and who did exceed the Milan criteria in the first and third years ($p=0.952$).

Another study found that the Milan criteria did not affect the recurrence-free survival of HCC, but that the survival was significantly higher in patients without the HBV recurrence than in patients with the HCC recurrence (17).

In our study, we found that the HBV recurrence was especially found in patients with HCC recurrence. A close association between the HBV and HCC recurrence after transplantation has also been shown in other studies.

Li et al. (18) evaluated the exceeding of the Milan criteria to predict the HCC recurrence in patients who underwent transplantation for HCC due to HBV, and they found the sensitivity at 81.4% and specificity at 72.4%. Posttransplantation HBV recurrence was found to be 9.7%. In addition, the HBV-DNA levels more than $5 \log_{10}$ copies/mL, and the HBV recurrence was significant for the HCC recurrence. Mortality rates were found to be high in patients with HCC recurrence. They estimated that the HCC recurrence increased the risk of HBV recurrence 4.58-fold. They also noted that a missed metastatic lesion or remnant tumor in patients exceeding the Milan criteria may cause this.

In a study evaluating 738 patients with HBV who underwent transplantation, the HCC recurrence was significantly affected by the Milan criteria. In patients with HBV recurrence, the recurrence of HCC increased 3.6-fold (11).

Faria et al. (19) showed that there was ccc DNA in both HCC cells and in non-tumor cells in the explanted livers from the patients who had recurrent HCC, which suggested that the HBV replication might also appear in tumor cells. Since the proliferation ability of immortal tumor cells is rather high, the replication is also high, indicated by the presence of HBsAg within the blood (20,21). So, in this study, it was observed that the HCC recurrence appeared 1.4-12 months after the HBsAg became positive; the HBV recurrence should be evaluated as a predictor of HCC recurrence (19).

Kıyıcı et al. (10) found that the total HBV recurrence after transplantation was 10.1%, 23.6% in the HCC group and 5.5% in the non-HCC group. In the Cox analysis, only the HCC recurrence was found to be a significant predictor of HBV recurrence. The authors found that the HBV recurrence was 26.94 times higher in patients with HCC recurrence. They suggest that undetectable micrometastases may be the cause. In our study, we think that the cause of HBV recurrence before the HCC recurrence may be micrometastases.

The limitations of the study are that the number of patients and the number of patients in the subgroup analyzes was insufficient. A higher number of patients may provide more striking results. In addition, the antiviral treatment was not homogeneous, and we did not study the HBV-DNA polymerase gene mutation in patients with HBV recurrence.

In conclusion, the HBV and HCC recurrence are closely related in patients who underwent transplantation due to HBV-associated HCC. The HBV recurrence after transplantation also increases the risk of HCC recurrence. Therefore, the HBV recurrence may be used as a predictor in forecasting the HCC recurrence in HBV-related HCC patients who underwent transplantation and who have exceeded the Milan criteria.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the İzmir Katip Çelebi University Atatürk Training and Research Hospital. (Decision No: 2018-55).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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- S.V., M.K., Z.B.P.; Literature Search - Z.B.P.; Writing Manuscript - Z.B.P., M.Z., D.V.; Critical Review - M.K., M.Z.

Conflict of Interest: The authors have no conflict of interest to declare.

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