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Long-term Outcome after Prophylactic Lamivudine Treatment on Hepatitis B Virus Reactivation in Non-Hodgkin's Lymphoma

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Hepatitis B virus (HBV) reactivation is the frequent complication after cytotoxic chemotherapy in HBsAg-positive non-Hodgkin's lymphoma (NHL) patients. Pre-chemotherapy viral load may be a risk factor and HBeAg-positive status is associated with increased viral load. The aim of this study was to investigate the long-term treatment outcome of lamivudine in preventing HBV reactivation and its associated morbidity according to HBeAg status. Twenty-four adult HBsAg-positive NHL patients were taken 100 mg of lamivudine daily before the initiation of chemotherapy. The median duration of lamivudine therapy was 11.5 months (range: 1-54 months) and the median number of chemotherapy cycles was 6 (range: 1-16 cycles). The steroid containing chemotherapy regimens were used in 18 patients (75%), and the anti-CD20 monoclonal antibody containing chemotherapy regimen was used in 6 patients (25%). Four patients received autologous peripheral blood stem cell transplantation without resultant HBV reactivation. Hepatitis related to HBV reactivation was developed in 1 patient among 14 HBeAg-positive patients and no one among 10 HBeAg-negative. One patient developed HBV reactivation after lamivudine withdrawal, and 4 patients developed the YMDD (tyrosine-methionine-aspartate-aspartate) mutation during lamivudine therapy. There were no statistical differences in HBV reactivation rate during chemotherapy according to the HBeAg status. Our results demonstrate that lamivudine should be considered preemptively before the chemotherapy for all HBsAg-positive NHL patients to prevent HBV reactivation, regardless of pre-chemotherapy HBeAg status. Finally, compared with the chronic hepatitis B patients, similar rate of HBV reactivation after lamivudine withdrawal and development of YMDD mutation was observed in NHL patients.

Key Words: HBV reactivation, lamivudine, non-Hodgkin's lymphoma, chemotherapy, HBeAg

INTRODUCTION

Hepatitis B virus (HBV) infection is the most common cause of chronic liver disease worldwide, and Korea is a highly endemic area of HBV infection. Some 5 to 7% of Koreans are chronic hepatic B surface antigen (HBsAg) carriers, and most of them will eventually acquire HBV infection.[1-3] Therefore, HBV reactivation in cancer patients that are HBsAg-positive and are receiving cytotoxic chemotherapy has posed serious clinical problems in Korea. HBV reactivation can give rise to acute hepatitis and even fatal fulminant hepatitis.[4-6] The risk of chemotherapy-induced HBV reactivation has been estimated from previous studies to range from 14% to 53%.[5-7] Therefore, several studies to determine prognostic factors to estimate the chance of HBV reactivation have been attempted. Some studies have reported that positive status for hepatitis B e antigen (HBeAg), which is correlated with increased viral load, is a risk factor for HBV reactivation, yet other studies demonstrate similar HBV reactivation rates regardless of HBeAg status.[5,8] Anthracyclines and glucocorticoids, ingredients of most chemotherapy

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regimens for non-Hodgkin's lymphoma (NHL), are implicated as important risk factors for HBV reactivation.[5,9]

In patients with HBV reactivation, chemotherapy is often delayed or discontinued, leading to suboptimal treatment of the underlying malignancy. Several therapeutic options have previously been attempted. Interferon, despite its antiviral properties, has not been effective in the treatment of chemotherapy-induced HBV reactivation.[10,11] Even though there were several methods previously used for the prevention of HBV reactivation, including the slow tapering of steroids in the chemotherapy regimens and using steroid-free chemotherapy regimens, these delays or modifications of therapy may reduce the chance of curing NHL.[9] Lamivudine is an oral nucleoside analogue that inhibits reverse transcription by causing chain-termination of nascent viral DNA in HBV infected cells. Numerous studies have shown that lamivudine therapy to treat chronic hepatitis B results in clinical, biochemical, and serological resolution of the HBV infection in immunocompetent individuals.[2,3,12] Several recent studies also have shown that prophylactic lamivudine usage at doses of 100 mg once daily appears to prevent HBV reactivation in HBsAg-positive patients with hematological malignancies who received cytotoxic chemotherapy.[10,12-18]

HBV reactivation was not uncommon in patients with NHL.[10,16-18] With the increasing incidence of NHL worldwide, and the more widespread use of cytotoxic chemotherapy regimens, including anti-CD20 monoclonal antibody (rituximab) and autologous peripheral blood stem cell transplantation (PBSCT), the occurrence of HBV reactivation may increase further, especially in highly HBV-endemic areas including Korea. Several recent studies have shown that prophylactic lamivudine treatment appears to prevent HBV reactivation and reduce fatal complications in HBsAg-positive patients with hematological malignancies, including NHL, who receive cytotoxic chemotherapy.[10,14,16-18] However, these studies have been limited to small numbers of patients with NHL, and have yielded no sufficient evidence regarding the prevention of HBV reactivation in HBsAg-positive NHL patients regardless of pre-chemotherapy HBeAg status or pre-chemotherapy liver status.[14,16-18] Because these previous studies were performed in patients usually receiving first-line chemotherapy with a short follow-up period, it was hard to show sufficient evidence of the prophylactic effect of lamivudine in patients who received various intensive chemotherapies (first-line or salvage) including rituximab and autologous PBSCT. Furthermore, the long-term clinical problems associated with the use of lamivudine, such as HBV reactivation after lamivudine withdrawal and YMDD (tyrosine-methionine-aspartate-aspartate) mutation, were not properly observed.

The objectives of this study were to investigate the role of lamivudine in preventing HBV reactivation and its associated morbidity in NHL patients with chronic HBV infection who were scheduled for various intensive chemotherapies, including rituximab and autologous PBSCT. We assess the efficacy difference between HBeAg-positive and HBeAg-negative NHL patients to identify the effect of lamivudine prophylaxis according to pre-chemotherapy HBV viral load (HBeAg status). Finally, we also evaluated hepatic problems such as HBV reactivation after lamivudine withdrawal, and the development of YMDD (tyrosine-methionine-aspartate-aspartate) mutation on the long-term follow-up of NHL patients with lamivudine prophylaxis.

MATERIALS AND METHODS

Patients

From August 1999 to February 2005, 24 adult NHL patients with serological evidence of HBsAg-positive status were retrospectively reviewed at the Division of Hemato-oncology, Department of Internal Medicine, Yonsei University College of Medicine in Seoul, Korea. All NHL patients received prophylactic lamivudine (100 mg) daily before the start of various chemotherapies. No patients received a reduced dose of lamivudine due to renal insufficiency. Treatment was continued throughout the course of chemotherapy. There were no patients with decompensated liver disease as indicated by any of following: prolonged prothrombin time (> 4 seconds), low albumin level (< 20 g/L), elevated total bilirubin

(> 50 μmol/L), hepatic encephalopathy, or alanine aminotransferase (ALT) > 10 × the upper limit of normal (ULN). Furthermore, no patients showed evidence of concurrent infections with hepatitis C virus and/or anti-human immunodeficiency virus or other causes of chronic liver disease (alcoholism, autoimmune chronic hepatitis). None of the patients had been treated with a chronic antiviral therapy known to have activity against HBV (e.g., interferon-α, famciclovir, ganciclovir, and adefovir) within the previous 6 months. We divided the patients into 2 groups according to pre-chemotherapy HBeAg status.

Laboratory studies

The HBsAg and HBeAg/antibody (anti-HBe) were determined by enzyme immunoassay (Dade Behring, Marburg, Germany). HBV-DNA was measured by the Digene hybrid capture assay (Digene Diagnostics, Beltsville, MD) with a lower limit of 0.5 pg/mL (1.4×10^5 copies/mL). Antibodies against hepatitis C (anti-HCV) were detected by the third-generation enzyme-linked immunosorbent assay (Korea Greencross, Yong-In, South Korea). Before starting the study, all NHL patients underwent the following investigations: HBsAg, HBeAg/anti-HBe, HBV-DNA level, complete blood picture (hemoglobin, platelet count, white cell count, and differential count), serum biochemistry [total protein, albumin, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), ALT, and creatinine], anti-HCV, and clotting profile before cytotoxic chemotherapy. HBV serological tests for HBeAg and anti-HBe, and testing for serum HBV-DNA levels were checked every 1-3 months and every time there was clinical evidence of hepatitis aggravation of liver biochemistry during the course of chemotherapy. We also preformed the developed YMDD-variant HBV test any time during lamivudine therapy when increased serum ALT and HBV-DNA levels were present. The YMDD amino acid motif of the HBV polymerase mutant assay was performed by polymerase chain reaction (PCR)-based methods. YMDD-variant patients were those infected with > 5% YMDD-variant HBV on at least one occasion.

Definition of hepatitis

Hepatitis was defined as a 3-fold or greater increase in serum ALT level that exceeded the upper limit of normal (ULN, 46 IU/L) or an absolute increase of ALT to over 100 IU/L when compared with baseline pre-chemotherapy value. Hepatitis due to HBV reactivation was defined as a 10-fold or greater increase in HBV-DNA level when compared with the baseline pre-chemotherapy HBV-DNA level in the absence of other systemic infection. The severity of hepatitis was defined as 'mild', when the rise in ALT was ≤ 2 × ULN; 'moderate', when 2 < ALT > 5 × ULN; and 'severe', when ALT > 5 × ULN. Disruptions of chemotherapy were defined as either a premature termination of chemotherapy or a delay of more than 8 days between cycles. Chronic hepatitis was defined as the presence of persistent elevated liver enzymes and past medical history of overt hepatitis lasting for at least 6 months. Liver cirrhosis was diagnosed by means of laboratory (hypoalbuminemia, thrombocytopenia, and prolonged prothrombin time with or without jaundice) and radiological findings in abdominal ultrasonography or computed tomography scan.

Statistical analysis

Clinical characteristics of patients in the 2 groups according to the pre-chemotherapy HBeAg status were compared by the Fisher's exact test for categorical variables, and the Mann-Whitney U test for continuous variables. The difference was considered significant when $p < 0.05$. Overall survival between HBeAg-positive and -negative groups was analyzed by log-rank test. Analysis of the data was performed using SPSS for Windows V. 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

Twenty-four HBsAg-positive NHL patients (17 male and 7 female) treated with prophylactic lamivudine therapy were analyzed. The median age was 47 years (range: 27-72 years). Twenty

patients (83%) had B-cell NHL and 4 patients had T or natural killer (NK) cell NHL. The pathologic subtypes were as follows: diffuse large B-cell lymphoma in 18 (75%) patients, follicular lymphoma in 1 (4%) patient, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue in 1 patient, peripheral T cell lymphoma in 2 (8%) patients, anaplastic large cell lymphoma in 1 patient, and nasal type extranodal NK/T cell lymphoma in 1 patient. According to the international prognostic index (IPI), 7 (29%) patients had low-risk disease, 8 (33%) low-intermediate-risk, 3 (13%) high-intermediate-risk, and 6 (25%) high-risk disease. Seven patients (29%) had B symptoms at presentation and 20 (83%) patients had stage III/IV (Table 1). Pre-chemotherapy HBeAg-positive status was observed in 14 out of 24 patients (58%). Their clinical characteristics are listed in Table 2 according to the pre-chemotherapy HBeAg status. There were no statistical differences between the two groups in terms of sex, age, immunophenotype, B symptoms, stage, liver involvement, or IPI score.

Twenty-one patients (88%) received prophylactic lamivudine at the beginning of the first-line chemotherapy, and 3 patients (13%) were treated with prophylactic lamivudine during salvage chemotherapy. The first-line chemotherapy regimens included: CHOP (cyclophosphamide 750 mg/m^2 iv day 1, adriamycin 50 mg/m^2 iv day 1, vincristine 1.4 mg/m^2 iv day 1, and prednisolone 100 mg po days 1-5) in 9 (38%) patients; R (rituximab 375 mg/m^2 iv day 1)-CHOP in 6 (25%) patients; ACO (cyclophosphamide 750 mg/m^2 iv day 1, adriamycin 55 mg/m^2 iv day 1, and vincristine 1.4 mg/m^2 iv day 1) in 5 (21%) patients; and CEOP (cyclophosphamide 750 mg/m^2 iv day 1, epirubicin 60 mg/m^2 iv day 1, vincristine 1.4 mg/m^2 iv day 1, and prednisolone 100 mg po days 1-5) in 1 patient (Table 1). Salvage chemotherapy regimens included: MiCMA (mitoxantrone 10 mg/m^2 iv day 1, carboplatinum 100 mg/m^2 iv days 1-4, cytosine arabinoside 2 g/m^2 iv day 5, and methylprednisolone 500 mg/m^2 iv days 1-5) in 4 (17%) patients; IMVP-16 (ifosfamide 1000 mg/m^2 iv days 1-5 plus mesna, methotrexate 30 mg/m^2 iv days 3 and 10, and etoposide 100 mg/m^2 iv days 1-3) in 3 (13%) patients; ESHAP (etoposide 40 mg/m^2 iv days 1-4, methylprednisolone 500 mg iv days 1-5, cytosine arabinoside 2 g/m^2 iv day 5, and cisplatin 25 mg/m^2 iv days 1-4) in 1 patient; paclitaxel/topotecan (paclitaxel 200 mg/m^2 iv day 1 and topotecan 1 mg/m2 iv days 1-5) in 1 patient; IVAM (ifosfamide 1500 mg/m^2 iv days 1-5 plus mesna, etoposide 150 mg/m^2 iv days 1-3, cytosine arabinoside 100 mg/m^2 iv days 1-3, and methotrexate 3 g/m^2 iv day 5 with leucovorin rescue) in 1 patient; ICE (ifosfamide 5 g/m^2 iv day 2 plus mesna, carboplatinum AUC (area under the plasma concentration-time curve) = 5 iv day 2, and etoposide 100 mg/m^2 iv days 1-3) in 1 patient; and FND (fludarabine 25 mg/m^2 iv days 1-3, mitoxantrone 10 mg/m^2 iv day 1, and dexamethasone 20 mg iv days 1-5) in 1 patient. The median number of chemotherapy cycles the patients received during lamivudine prophylaxis was 6 (range: 1-16 cycles). Initial steroid containing chemotherapy regimens were given to 18 patients (75%), and anti-CD20 monoclonal antibody (rituximab)-containing chemotherapy regimens were administrated to 6 patients (25%). There were no patients who received involved-field radiation including the liver. Four patients had received BEAM [BCNU (bischloroethyl nitrosourea) 300 mg/m^2 iv day -6, etoposide 100 mg/m^2 iv from days -5 to -2, cytosine arabinoside 100 mg/m^2 iv from days -5 to -2, melphalan 140 mg/m^2 iv from day -1] conditioning regimen followed by autologous PBSCT. There were no statistical differences between the pre-chemotherapy HBeAg status groups in terms of the numbers of patients undergoing steroid containing chemotherapy, rituximab containing chemotherapy, salvage chemotherapy, and autologous PBSCT (Table 3).

The results of a baseline hepatic function test before the start of chemotherapy showed: increased ALT (range: 51 to 105 IU/L) in 4 patients (17%); increased HBV-DNA ($\geq$ 0.5 pg/mL) in 16 patients (67%); and increased total bilirubin in 1 patient (1.7 mg/dL). Ten patients (42%) had baseline inactive HBV carrier status before initiation of chemotherapy, 10 patients (42%) had chronic hepatitis, 3 patients (13%) had liver cirrhosis, and 1 patient had hepatocellular carcinoma. All 14 HBeAg-positive patients (median HBV-DNA levels: 975.2 pg/mL, range: 0.7 to > 6000 pg/mL) and 2 (20%) of the 10 HBeAg-negative patients

Table 1. Characteristics of the 24 HBsAg-positive Non-Hodgkin's Lymphoma Patients with Lamivudine Prophylaxis during Chemotherapy

	No.	Percentage (%)
Total number	24	100
Sex		
Male	17	70.8
Female	7	29.2
Immunophenotype		
B-cell type	20	83.3
Aggressive type (DLBL)	18	75.0
Indolent type	2	8.3
T- or NK- cell type	4	16.7
Stage		
I/II	4	16.7
III/IV	20	83.3
B symptoms		
Yes	7	29.2
No	17	70.8
Liver involvement		
Yes	3	12.5
No	21	87.5
IPI score		
Low/low intermediate	15	62.5
High/high intermediate	9	37.5
Pre-chemotherapy HBeAg		
Positive	14	58.3
Negative	10	41.7
Initial chemotherapy		
First-line chemotherapy	21	87.5
CHOP	9	37.5
R-CHOP	6	25.0
ACO	5	20.8
CEOP	1	4.2
Salvage chemotherapy	3	12.5

DLBL, diffuse large B-cell lymphoma; NK, natural killer; IPI, international prognostic index; HBeAg, hepatitis B e antigen; CHOP, cyclophosphamide, adriamycin, vincristine, and prednisolone; R-CHOP, rituximab, cyclophosphamide, adriamycin, vincristine, and prednisolone; ACO, cyclophosphamide, adriamycin, and vincristine; CEOP, cyclophosphamide, epirubicin, vincristine, and prednisolone.

Table 2. Clinical Characteristics of Non-Hodgkin's Lymphoma Patients According to Pre-chemotherapy HBeAg Status

	HBeAg status		*p* value
	Positive (n = 14)	Negative (n = 10)	
Age	47 (27 - 64)	49 (31 - 72)	NS
Sex (male : female)	9 : 5	8 : 2	NS
Immunophenotype			NS
B-cell type	12	8	NS
Aggressive type (DLBL)	11	7	
Indolent type	1	1	
T- or NK- cell type	2	2	
Stage			NS
I/II	1	3	
III/IV	13	7	
B symptoms			NS
Yes	4	3	
No	10	7	
Liver involvement			NS
Yes	2	1	
No	12	9	
IPI score			NS
Low/low intermediate	8	7	
High/high intermediate	6	3	

DLBL, diffuse large B-cell lymphoma; NK, natural killer; IPI, international prognostic index; NS, no significance.

had increased HBV-DNA levels (13 and 212.8 pg/mL, respectively). Underlying liver status and baseline serum HBV-DNA level before chemotherapy were significantly different between HBeAg-positive and HBeAg-negative patients (Table 4, $p = 0.003$, $p < 0.001$, respectively).

Hepatitis during chemotherapy with lamivudine prophylaxis

The median duration of lamivudine therapy was 11.5 months (range: 1-54 months). The duration of lamivudine therapy was not different between the two pre-chemotherapy HBeAg status groups. Though twelve patients (50%) developed hepatitis during chemotherapy, there was no difference in the incidence of hepatitis according to HBeAg status (Table 5). Of the 12 patients who developed hepatitis during chemotherapy, only 1 diffuse large B-cell lymphoma patient with pre-chemotherapy HBeAg-positive status and a high HBV-DNA level (> 6000 pg/mL) developed hepatitis associated with HBV reactivation, resulting in 4% of the HBV reactivation rate occurring during cytotoxic chemotherapy with lamivudine prophylaxis. The causes of hepatitis other than HBV reactivation were drug (chemotherapeutic agent or antibiotics) induced hepatitis (N = 9) and systemic sepsis (N = 2). These 11 patients had no elevation of HBV-DNA level at the time of hepatitis. None of the 10 HBeAg-negative patients developed hepatitis due to HBV reactivation. There were no statistical differences between HBeAg-positive and HBeAg-negative patients in

Table 3. Treatment of Patients during Lamivudine Prophylaxis According to Pre-chemotherapy HBeAg Status

	HBeAg status		*p* value
	Positive (n = 14)	Negative (n = 10)	
Initial chemotherapy			NS
First-line chemotherapy	13	8	
Salvage chemotherapy	1	2	
Steroid containing chemotherapy	10	8	NS
Rituximab containing chemotherapy	3	3	NS
Salvage chemotherapy performed			NS
Yes	3	5	
No	11	5	
Autologous PBSCT			NS
Yes	2	2	
No	12	8	
Median number of chemotherapy (range)	6 (1 - 16)	6 (2 - 12)	NS

PBSCT, peripheral blood stem cell transplantation; NS, no significance.

Table 4. Baseline Hepatic Function before Chemotherapy According to Pre-chemotherapy HBeAg Status

	HBeAg status		*p* value
	Positive (n = 14)	Negative (n = 10)	
Baseline serum ALT			NS
Increased	3	1	
Mild (ALT $\leq \times 2$)	3	0	
Moderate (ALT $> \times 2$ and $\leq \times 5$)	0	1	
Within normal range	11	9	
Baseline serum HBV-DNA			< 0.001
Positive (≥ 0.5 pg/mL)	14	2	
Negative (< 0.5 pg/mL)	0	8	
Baseline serum total bilirubin			NS
Increased	1	0	
Within normal range	13	10	
Underlying liver status			0.003
Inactive carrier	2	8	
Chronic hepatitis	9	1	
Liver cirrhosis	2	1	
Hepatocellular carcinoma	1	0	

ALT, alanine aminotransferase; HBV, hepatitis B virus; NS, no significance.

Table 5. The Hepatic Profiles in Patients Using Prophylactic Lamivudine According to HBeAg Status

	HBeAg status		*p* value
	Positive (n = 14)	Negative (n = 10)	
Hepatitis during chemotherapy			NS
Yes	9	3	
No	5	7	
Hepatitis reactivation during CTx			NS
Due to HBV reactivation	1	0	
Due to other causes	8	3	
Severity of hepatitis during CTx			NS
Mild (ALT $\leq \times 2$)	3	2	
Moderate (ALT $> \times 2$ and (≤ 5)	3	1	
Severe (ALT $> \times 5$)	3	0	
Hepatitis reactivation after withdrawal of lamivudine	1	0	NS
Documentation of YMDD mutation	3	1	NS

CTx, chemotherapy; ALT, alanine aminotransferase; HBV, hepatitis B virus; NS, no significance; YMDD, tyrosine-methionine-aspartate-aspartate.

HBV reactivation rate during chemotherapy (Table 5). There were 3 patients who had pre-chemotherapy HBV-DNA levels more than 1.0×10^9 copies/mL (3530 pg/mL), and the 1 patient who developed HBV reactivation in this study belonged to this group. All 4 patients finished the total course of chemotherapy and autologous PBSCT, with no evidences of HBV reactivation. The severity of hepatitis was mild in 5 patients, moderate in 4 patients (1 due to HBV reactivation), and severe in 3 patients. Of the 3 patients who developed severe hepatitis during chemotherapy, only 1 patient received salvage chemotherapy. There was no correlation between the severity of hepatitis and salvage chemotherapy. Disruption in chemotherapy occurred in 1 patient (4%), who developed moderate hepatitis associated with HBV reactivation.

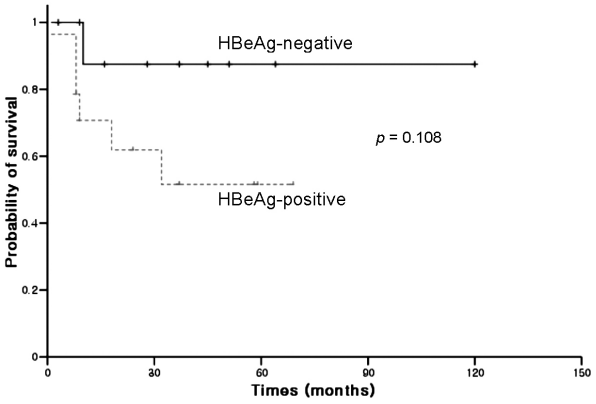

Fig. 1. Overall survival of lymphoma patients by pre-chemotherapy HBeAg status.

Hepatic problems on the long-term follow-up periods and survival

Among 11 lamivudine-treated patients, one patient developed HBV reactivation 2 months after lamivudine withdrawal. Four patients (17%, 3 pre-chemotherapy HBeAg-positive and 1 HBe Ag-negative) were observed to have the YMDD mutation during lamivudine therapy (6, 7, 36, and 52 months, respectively), and among them, two patients are currently being treated with adefovir. Seven patients (29%) died during the follow-up period, 6 patients due to progressive NHL and 1 patient due to severe infection. There was no mortality related to HBV reactivation. Overall

survival rate of HBeAg-negative patients was higher than that of HBeAg-positive patients, but observation was without statistical significance (p = 0.108) (Fig. 1).

DISCUSSION

Our study demonstrated that prophylactic use of lamivudine to prevent HBV reactivation in HBsAg-positive NHL patients during various intensive cytotoxic chemotherapies, including rituximab and autologous PBSCT, was effective regardless of pre-chemotherapy HBeAg status or pre-chemotherapy liver status.

The natural history of chronic HBV infection ranges from the replicative phase with active liver disease (HBeAg-positive hepatitis) to low or non-replicative phase with HBeAg seroconversion and remission of liver disease (inactive carriers). In far east Asia, including Korea, HBV genotype C is predominant and associated with delayed HBeAg seroconversion, more active hepatitis, more advanced liver disease, and a higher risk of hepatocellular carcinoma, compared with HBV genotype B.[1,2,19] Therefore the number of patients with HBeAg expression (increased HBV viral load) at the pre-chemotherapy stage in Korea might be larger than in other countries.

The two possible mechanisms explaining HBV reactivation during chemotherapy are an exaggerated activated T-cell-mediated immunological response to the initial increase in HBV infected hepatocytes due to increased viral replication during immunosuppression periods, and immunosuppression-induced augmentation of HBV replication that is directly toxic to host hepatocytes.[5,14,18] Therefore, the patients with HBeAg expression (increased HBV viral load) at the pre-chemotherapy stage would be more easily reactivated during the following chemotherapy periods. Some studies have found that HBeAg-positive status and high HBV viral load prior to cytotoxic chemotherapy are significant predictive factors for HBV reactivation.[16,20] In a previous study with no lamivudine prophylaxis, 53 percent of the HBV-reactivated patients during cytotoxic chemotherapy were pre-chemotherapy HBeAg-positive, as compared with 13% in the non-reactivated patients.[21] Therefore, evaluating the prophylactic effect of lamivudine in HBeAg-positive patients might be important, especially in Korea, because of the high prevalence of HBeAg-positive individuals.

However, several previous studies have not shown that prophylactic lamivudine appears to prevent HBV reactivation in HBeAg-positive (high viral load) patients with NHL who receive cytotoxic chemotherapy because these studies included limited numbers of HBeAg-positive patients (8-29%).[10,14,16-18] In this study, prophylactic lamivudine treatment was performed on 24 HBsAg-positive NHL patients. Moreover, there was a high rate of HBeAg-positive patients (58%) in contrast to the incidence of previous studies. In the HBeAg-positive group, there was a significantly higher baseline of serum HBV-DNA-positive status (100% vs. 20%, $p < 0.001$), and fewer incidences of inactive carrier on the underlying pre-chemotherapy liver status (14.3% vs. 80.0%, p = 0.003). Even though 1 pre-chemotherapy HBeAg-positive patient with a high HBV-DNA level (> 6000 pg/mL) developed HBV reactivation during cytotoxic chemotherapy in spite of lamivudine prophylaxis, there were no statistical differences between HBeAg-positive and HBeAg-negative patients in HBV reactivation rate during chemotherapy and overall survival in this study. These results suggest that the prophylactic lamivudine effects observed in HBeAg-positive NHL patients with baseline HBV-DNA levels could comparable to HBeAg-negative NHL patients.

Several previous clinical studies demonstrate that steroid-containing chemotherapy regimens increased the risk of hepatitis flares in HBV carriers.[6,9] We also evaluated the differences in prophylactic lamivudine effect between NHL patients who received steroid-free chemotherapy and those who received steroid-containing chemotherapy. In this study, 18 patients (75%) received the initial steroid-containing chemotherapy, and 6 patients (25%) received the initial steroid-free chemotherapy regimen, such as ACO (n = 5) and paclitaxel/topotecan (n = 1). No patients had HBV reactivation during the steroid-free chemotherapy, and there were no statistical differences in HBV reactivation with the use of steroid containing chemotherapy. These results suggest that lamivu-

dine prophylaxis during steroid-free chemotherapy also should be performed, because HBV reactivation during steroid-free chemotherapy was reported in about 38% of cases.[9] Recently, rituximab, a chimeric mouse-human anti-CD20 monoclonal antibody, has been generally used in the treatment of B-cell NHL, and the prophylactic use of lamivudine also prevented HBV reactivation during rituximab-containing chemotherapy.[22,23] In this study, 6 HBsAg-positive NHL patients (3 patients with HBeAg-positive and 3 patients with HBeAg-negative) received initial chemotherapy including rituximab (R-CHOP), and no patients had HBV reactivation during the R-CHOP chemotherapy. We also concluded that administration of prophylactic lamivudine might have a preventive effect on HBV reactivation during rituximab-containing chemotherapy regardless of pre-chemotherapy HBeAg status. Generally, first-line chemotherapy regimens used in NHL (usually CHOP regimen) were less toxic and less immunosuppressive than other salvage chemotherapy regimens or conditioning chemotherapy (usually BEAM regimen) using autologous PBSCT. Therefore the patients treated with salvage chemotherapy or autologous PBSCT would be easily reactivated compared to the patients who received the first-line chemotherapy. A previous study has shown that approximately half of the HBsAg-positive malignant lymphoma patients developed hepatitis due to HBV reactivation after autologous PBSCT and no prophylactic lamivudine therapy.[24] In this study, 8 NHL patients received prophylactic lamivudine therapy and overcame the salvage chemotherapy periods without HBV reactivation. Moreover, 4 patients completed the total course of chemotherapy and autologous PBSCT with no evidences of HBV reactivation.

Despite the safety and effectiveness of lamivudine in suppressing HBV replication during chemotherapy, the optimum duration of lamivudine therapy is not yet determined. Previous reports on post-immunosuppression HBV reactivation suggest maintenance of lamivudine therapy for at least 4 to 6 months following chemotherapy.[13,14,18] Thus, we treated NHL patients for the median duration of 11.5 months (range: 1-54 months) during the median 6 (range: 1-16 cycles) chemotherapy cycles. The patients in this study who completed the total course of chemotherapy received a median of 6 months (range: 1-34 months) of lamivudine therapy after chemotherapy withdrawal.

In this study, the median follow-up from NHL diagnosis until the end of follow-up was 26 months (range: 1-120 months). Because this study had relatively long-term follow-up data, there were two emerging problems during the follow-up periods, HBV reactivation after lamivudine withdrawal and lamivudine resistance (YMDD mutant). Among 11 patients treated with lamivudine, one patient developed HBV reactivation 2 months after lamivudine withdrawal, but the patient responded to the reintroduction of lamivudine. HBV reactivation after withdrawal of pre-emptive lamivudine (median 3 months after completion of chemotherapy) showed in about 24% of hematologic malignancy patients with HBV-positive according to the recent data.[25] Thus, long-term (more than 6 months after completion of chemotherapy) lamivudine prophylaxis in HBsAg-positive NHL patients in Korea (usually HBV genotype C) seems to be reasonable, because HBeAg seroconversion after lamivudine therapy was not durable in patients with chronic hepatitis B located in a HBV endemic area, including Korea.[26]

Lamivudine-resistant mutants have been reported in chronic hepatitis B patients who underwent 6-9 months of lamivudine therapy. The cause of the resistance was revealed as a point mutation in the YMDD motif within the polymerase domain.[2,12,27] Newer nucleoside analogues such as adefovir, entecavir, and a combination of them have been developed to overcome lamivudine-resistant mutants.[2,12] In this study, 4 patients (17%) were documented with the YMDD mutation during lamivudine therapy, which is similar to results previously reported in general HBsAg-positive populations (15-17% at 1 year).[2,3] Among the four patients, two were documented to have the YMDD mutation just after the completion of chemotherapy (6 months and 7 months after initiation of chemotherapy, respectively). Because the YMDD mutations were detected within a few weeks during lamivudine therapy in Korea,[27] routine evaluation for YMDD mutation should be performed any time during lamivudine therapy in

individuals with increased serum ALT and HBV-DNA levels.

In conclusion, lamivudine should be considered preemptively before the initiation of chemotherapy for all HBsAg-positive NHL patients, regardless of pre-chemotherapy HBeAg status (HBV viral load), undergoing various intensive chemotherapies, including rituximab and autologous PBSCT, to prevent chemotherapy-related HBV reactivation. Compared with the general HBsAg-positive population, a similar rate of hepatic problems (HBV reactivation after lamivudine withdrawal and YMDD mutation) in the long-term evaluation were observed in the NHL patients, with no difference in the overall survival rate of NHL patients according to pre-chemotherapy HBeAg status.

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