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Da Mao Date

The Association between the Progression of Chronic Hepatitis B and Non-virus Factors: Observational Studies of Hospitalized Chinese Patients

By

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Master of Public Health

Epidemiology

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The Association between the Progression of Chronic Hepatitis B and Non-virus Factors: Observational Studies of Hospitalized Chinese Patients

By

Da Mao

Bachelor of Science
Wuhan University
2011

Thesis Committee Chair: Anne C. Spaulding, MD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Master of Public Health
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2013

Abstract

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Objective: The aim of this study was to examine the association between the changes in paired liver biopsies and nutritional status, sleep status, alcohol consumption, and antiviral therapy.

Methods: Data were collected from the medical records of 190 hepatitis B patients in the 302 Military Hospital, Beijing, China, who had two liver biopsies between January 1999 and December 2012. Univariate analysis and multivariate logistic analysis were conducted.

Results: 69 patients (36.3%) had improvements in fibrosis stage and 73 (38.4%) had improvements in inflammation grade. Drinking alcohol (OR=0.35, 95%CI 0.10-1.20, p=0.09) was a harmful factor for the progression of fibrosis. Nutritional status, sleep status, and antiviral therapy were not statistically associated with the improvement in fibrosis stage. In addition, good nutritional status (OR 3.15, 95%CI 1.43-6.96, p<0.01) and taking adefovir (OR 3.97, 95%CI 1.25-12.57, p=0.05) were significantly associated with the improvement in inflammation grade.

Conclusion: This study evaluated the risk factors for the progression of liver damage and found that alcohol consumption had a significant impact on the deterioration of fibrosis. Nutritional status and antiviral therapy were significantly associated with the improvement in inflammation grade. No significant effect was associated with sleep status. The association between the various therapeutic interventions and the serological markers of virological clearance was not evaluated. Without serological information, it is difficult to make any firm conclusions. Further study will take into account whether treatment resulted in the clearance of hepatitis B infection.

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BACKGROUND

Overview of Hepatitis B

The hepatitis B virus (HBV) infection is one of the most common chronic viral infections in the world. More than 2 billion people have been exposed to HBV and around 350 to 400 million people are chronically infected with HBV [1]. The greatest burden of hepatitis B disease is in the Asia Pacific and sub-Saharan Africa regions, where more than 60% of the population has serologic evidence of hepatitis B [2, 3]. In the United States, around 2 million people are infected with HBV, most of whom are immigrants from Asia and Africa [4].

Hepatitis B is an infectious inflammatory illness of the liver caused by the HBV. It may be either an acute infection or a chronic infection. Patients with acute infection of HBV clear the infection within weeks to months, and more than 95% of acute hepatitis B patients who are adults or older children will fully recover and develop immunity spontaneously [5]. However, younger children and infants are less likely to clear the infection; only 30% of infections in younger children, and 5% of infections in newborn resolve [5].

Natural History of Hepatitis B

According to the National Institutes of Health Consensus Conference in 2008 [6], chronic hepatitis B is divided into 4 phases: the immune-tolerant phase, the immune-active phase, the inactive phase, and the inactive carrier

phase (Figure 1). The immune-tolerant phase is the most common phase after perinatal transmission of HBV from a mother who is HBeAg-positive (hepatitis B e antigen) [7]. During the immune-tolerant phase, patients have high HBV DNA and ALT (normal alanine aminotransferase) levels. There is no obvious liver fibrosis or inflammation and the immune system cannot react to HBV in this phase [8]. Some hepatitis B patients may suffer in this phase for more than 40 years, but most patients will go into the immune-active phase [8]. During the immune-active phase, inflammation or fibrosis can be seen in the liver biopsy. Moreover, the DNA level is lower than that of the immune-tolerant phase, but the ALT level is elevated. T-cell response is active to decrease the DNA level, and HBeAg seroconversion may occur. Once HBeAg seroconversion occurs, 10% to 40% of patients will experience a reversion back to the HBeAg-positive state. About 20% of patients may remain in the immune-active phase. Most patients will go into the next phase: the inactive phase [9]. Patients who are in the inactive phase have a low level of HBV DNA, a normal ALT level, and an HBeAg-negative state [8]. During this phase, liver inflammation and fibrosis improve, and the cytotoxic T-cell response is more vigorous [9]. About 20% of patients in this phase may revert to the immune-active phase [10]. After the inactive phase, some patients will eventually go into the HBsAg (hepatitis B surface antigen) clearance phase; most will develop anti-HBs (antibody to hepatitis B surface antigen). This phase is also called the resolution phase. Some studies have reported that the

risk of cirrhosis or hepatocellular carcinoma (HCC) is reduced but still significant in this phase [11].

Factors Associated with the Progression of Chronic Hepatitis B

Alcohol is well known for its toxic effect on the liver. Several reports have indicated that chronic ethanol ingestion could alter the cellular immune responses to human viral structural proteins and may lead to persistent HBV infection [12]. Health-related quality of life, such as nutritional status and sleep quality, are also risk factors for the progression of liver disease. The prevalence of malnutrition in cirrhosis is more than 65% and has been proven to be predictive of poor survival in patients with liver cirrhosis [13]. Moreover, good nutritional status could support the therapeutic effectiveness of antiviral treatment of chronic hepatitis B [14]. Sleep disturbances occur in more than 60% of patients with chronic hepatitis C [15]. Insomnia, which is often interrelated with significant psychiatric co-morbidities such as depression, could lessen the treatment outcomes of liver disease [15]. Other risk factors associated with the progression of hepatitis B include older age (longer duration of infection), high levels of HBV DNA, obesity, and co-infection with human immunodeficiency virus (HIV) or co-infection with hepatitis C virus (HCV).

Liver Biopsy

A liver biopsy samples liver tissue to aid in the diagnosis of the severity of

liver disease and to monitor disease progression [16, 17]. It is the standard method for assessing the inflammation and fibrosis of liver. Two scores are assigned to the liver biopsy: stage and grade. The stage describes the extent and the location of fibrosis. A zero score represents the absence of fibrosis and the highest score represents cirrhosis. The grade represents the extent of histological inflammation and necrosis. The higher the score is, the more severe the lesion is.

There are three primary scoring systems to assess the stage and grade of liver damage: the Scheuer system, Metavir system, and Ishak system [18-20]. The Scheuer system was the first biopsy scoring system (Table 1) and was established in 1991. This system uses activity grade and fibrosis stage to assess liver damage. Both activity grade and fibrosis stage are two numerical scores scaled from 0 to 4. In this system, the activity grade does not accurately define the severity of piecemeal necrosis and lobular changes, which may differ from one portal or lobular to the other. Additionally, fibrosis stage 1 (enlarged portal tracts) and stage 2 (peri-portal fibrosis) are difficult to distinguish [18]. The Metavir system was published in 1994 (Table 2). It was initially developed for hepatitis C, but is now also used for hepatitis B. In this system, there is an algorithm to describe the activity grade. The combination of piecemeal and lobular lesion is scaled from 0 (absence) to 3 (severe) and the fibrosis stage is graded on a scale from 0 to 4. Like the Scheuer system, fibrosis stage 1 represents portal fibrosis without septa and stages 2 and 3 represent rare

portal fibrosis and numerous septa, respectively [19]. The Ishak system was published in 1995 (Table 3). It is the most detailed scoring system. For this system, the elementary lesions (interface hepatitis, confluent necrosis, and focal inflammation) are separately evaluated, and the fibrosis is assessed according to a more detailed scale from 0 to 6. Ishak's method can make a clear distinction between incomplete septa and complete septa [20].

There is no general consensus about which scoring system is best; all three systems have been widely used in routine practice and for research. Liver biopsy has several disadvantages, such as cost, potentially serious complications, and hemorrhage. Furthermore, there is the risk of misclassifying the degree of liver damage if the amount of tissue is scant. No comparable noninvasive substitute exists, however [17-20].

Antiviral Treatment of Chronic Hepatitis B

The goals of antiviral therapy for hepatitis B patients are to retard the disease progression and prevent the development of severe outcomes such as HCC [6]. Some patients with chronic hepatitis B may develop these outcomes after several decades of infection. The indicators used to assess the response of antiviral therapy of chronic hepatitis B are the biochemical response, virological response, and histological response (Table 4) [21]. Measurements such as HBeAg, HBsAg, viral suppression, ALT level, and liver biopsy are used to determine the start and the end of treatment [22]. It is best to consider all

of those indicators before treatment [22, 23].

There are several antiviral therapy options for chronic hepatitis B, including interferon, lamivudine, adefovir and entecavir. Peg-interferon therapy is a short-term parenteral treatment, usually given for 12 weeks [24]. At present, Peg-interferon alfa has replaced standard interferon alfa due to improved pharmacokinetic properties, a more convenient dosing regimen and improved efficacy [24]. After treatment, 25-40% of patients experience HBeAg seroconversion and 5-10% of patients lose HBsAg [23]. Sometimes, interferon therapy is combined with another antiviral drug. Lamivudine is a potent oral nucleoside analog reverse transcriptase inhibitor; it was first approved by the U.S. Food and Drug Administration in 1994. A one-year treatment of lamivudine resulted in a 16% to 18% rate of HBeAg seroconversion [25]. The current use of lamivudine is declining due to the high prevalence of viral resistance. After five years of use, more than 60% of patients experience resistance [26]. Adefovir is an orally-administered nucleotide analog reverse transcriptase inhibitor approved for chronic hepatitis B in 2002. It works by blocking the reverse transcriptase crucial for the replication of HBV [27]. A one-year treatment of adefovir resulted in around a 12% rate of HBeAg seroconversion and adefovir treatment over five years resulted in an 83% rate of improvement of inflammation and a 75% rate of improvement of fibrosis [27]. The main advantage of adefovir over lamivudine is the slower development of drug resistance [28]. The resistance rates for adefovir were

0%, 3%, 18% and 29% at 1, 2, 4 and 5 years of treatment, respectively [29]. Entecavir was approved for chronic hepatitis B treatment in 2005. It has demonstrated virological outcomes that are superior to both lamivudine and adefovir. Chang et al. reported that long-term entecavir treatment led to virological response in 94% of patients (HBV DNA <300 copies/ml), and 88% of patients had improvements in fibrosis [30]. A report indicated that entecavir therapy had no significant effects on HBsAg loss in HBeAg-negative chronic hepatitis B patients [31]. Entecavir has a very low rate of resistance, with only 1.2% of patients developing resistance over six years of therapy [32].

MANUSCRIPT

TITLE

The Association between the Progression of Chronic Hepatitis B and Non-virus Factors: Observational Studies of Hospitalized Chinese Patients

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ABSTRACT

Objective: The aim of this study was to examine the association between the changes in paired liver biopsies and nutritional status, sleep status, alcohol consumption, and antiviral therapy.

Methods: Data were collected from the medical records of 190 hepatitis B patients in The 302 Military Hospital, Beijing, China, who had two liver biopsies between January 1999 and December 2012. Univariate analysis and multivariate logistic analysis were conducted.

Results: 69 patients (36.3%) had improvements in fibrosis stage and 73 (38.4%) had improvements in inflammation grade. Drinking alcohol (OR=0.35, 95%CI 0.10-1.20, p=0.09) was a harmful factor for the progression of fibrosis. Nutritional status, sleep status, and antiviral therapy were not statistically associated with the improvement in fibrosis stage. In addition, good nutritional status (OR 3.15, 95%CI 1.43-6.96, p<0.01) and taking adefovir (OR 3.97, 95%CI 1.25-12.57, p=0.05) were significantly associated with the improvement in inflammation grade.

Conclusion: This study evaluated the risk factors for the progression of liver damage and found that alcohol consumption had a significant impact on the deterioration of fibrosis. Nutritional status and antiviral therapy were significantly associated with the improvement in inflammation grade. No

significant effect was associated with sleep status. The association between the various therapeutic interventions and the serological markers of virological clearance was not evaluated. Without serological information, it is difficult to make any firm conclusions. Further study will take into account whether treatment resulted in the clearance of hepatitis B infection.

INTRODUCTION

Infection with the hepatitis B virus (HBV) is a worldwide health problem. According to the World Health Organization, more than 2 billion people are infected with HBV [1]. Around 350 million people have chronic infections and around 600,000 people die from HBV infection related liver diseases each year [1, 2]. China ranks number one among countries for the number of persons with hepatitis B. The latest data show that there are currently 9.3 million people who have been exposed to HBV and 2.5 million people have chronic hepatitis B in China [33].

Treatment with antiviral drugs has shown improvement in histological, virological, and biochemical markers in patients with chronic hepatitis B. Lamivudine, adefovir, and entecavir have been recommended as primary therapies for chronic hepatitis B in China. However, factors influencing the effects of antiviral therapies have not been widely studied [33]. In general, alcohol consumption is one of the major factors that have a deleterious impact on the liver. A report demonstrated that alcohol consumption increases the degree of hepatic fibrosis [34]. Demographic characteristics such as living conditions, nutritional status, and sleep quality may also influence the progression of chronic hepatitis B [33-35].

This study was designed to explore the association between nutritional status, sleep status, alcohol consumption, antiviral therapy, and longitudinal

changes in liver biopsy scores in a convenience sample of chronic hepatitis B patients with paired liver biopsies in a hospital in Beijing, China. A future study will take into account whether the treatments are associated with the improvement in serological and virological markers of HBV infection.

METHODS

Null Hypothesis

In patients with chronic hepatitis B, there is no association between alcohol consumption, nutritional status, sleep status and antiviral treatment and the change in histology on a pair of liver biopsies.

Study Design

These were two no-contact case-control studies (secondary data analysis). The information for the analysis was provided by the Liver Failure Diagnosis and Treatment Research Center of the 302 Military Hospital, which is one of the largest infectious disease hospitals in China.

Research Subjects

Patients with chronic hepatitis B who had two liver biopsies between January 1, 1999 to December 31, 2012 with an interval longer than six months apart were enrolled into the study. Although the 302 Military Hospital is a military hospital, it serves the general population. All data were extracted from the medical records of patients who met the eligibility criteria. There was no contact with patients and no risk to participants as confidentiality was maintained.

The inclusion criteria included: 1) the patient had chronic hepatitis B and had been seen at the 302 Military Hospital; 2) had been hospitalized twice with at least six months between biopsies; 3) had first hospitalization after January 1, 1999 and second hospitalization before December 31, 2012; 4) had a pathology report from a liver biopsy during each hospitalization. Therefore, cases were defined as patients who had an improvement in fibrosis stage or inflammation grade between the first and second biopsies in the 302 Military Hospital, Beijing, China, between January 1, 1999 and December 31, 2012. Controls were patients who did not improve in fibrosis stage or inflammation grade between the first and second biopsies in the same hospital.

The protocol was submitted to the IRB of Emory University, and the study was judged to be exempt from needing IRB review and approval on December 3, 2012.

Data Collection

The 302 Military Hospital collected the data for the investigator. Patient information prior to December 31, 2009 came from paper-based medical records and patient information after January 1, 2010 came from electronic medical records. The identifying information was stripped before the data were supplied to the investigator. The linkage key was preserved by the 302 Military Hospital and was not disclosed to the investigator.

The outcome was the change in liver histology measured by the difference in fibrosis stage or inflammation grade between the first and second liver biopsies. The liver biopsy scoring system used in this analysis was the Scheuer Scoring System. Because lower scores were assigned to less severe pathologies, a negative change in score represented an improvement in disease, and a positive change of liver biopsy score represented a deterioration of disease.

The primary exposures of interest were nutritional status, sleep status, alcohol consumption, and antiviral therapy. Alcohol consumption was defined as any alcohol use after the first biopsy in the hospital. Sleep status was defined as good sleep status after the first biopsy. Both alcohol and sleep status were self-reported; nutritional status was evaluated by a physician during the second hospitalization. Four agents were offered during the study period: interferon, lamivudine, adefovir, and entecavir.

Additional demographic variables included sex, race, weight, and marital status. Co-morbid conditions included family history of liver disease, fatty liver, co-infection with HIV, and co-infection with HCV. Additionally, treatment variables included: treatment regimen before the first liver biopsy; continuous treatment; age at onset of hepatitis B; age when abnormal liver enzyme was detected; age at the first biopsy; interval between the disease onset and the first treatment; interval between the disease onset and the first biopsy; and interval between the first and second biopsies. All of these

variables were collected from the medical records of the second hospitalization. Weight was measured by a trained nurse, co-infection with HIV or HCV was evaluated by serological test, and the presence of fatty liver was diagnosed by B-mode ultrasound. Continuous treatment refers to whether the treatment was continuous during the therapy period after the first biopsy.

Data Analysis

Descriptive analyses were performed overall to obtain the mean, standard deviation (SD), median, and interquartile range (IQR) for continuous variables. The variables—interval between the onset and the first biopsy, interval between the onset of disease and the first treatment, and interval between the first and second biopsy—were converted to a common logarithm. To investigate the independent predictors for the change in liver biopsy score, the chi-square test or the Fisher's exact test and the Student's t-test were used to compare the categorical and continuous variables, respectively.

To investigate the association between primary exposures and the change in biopsies, crude logistic regressions were performed. Afterwards, adjusted multivariate logistic regressions were carried out. All variables were included in the first multivariate logistic model; the primary variables were fixed in the model and the backward strategy was used for other risk factors. In the backward strategy, the results of Wald tests for each variable were examined. The variable with the highest p-value and was greater than 0.1 was removed.

The process was repeated again until there was no variable in the model that met the criteria for removal. If there were any biologically reasonable predictors that were chosen by literature review, and eliminated by the backward strategy, we added them back to the models. To check the potential interaction between variables, the likelihood ratio test and the Wald Test were performed. In the final model, assumptions of homoscedasticity and independence were checked by residual plots; outliers were checked by leverage values, Cook's distance, and Jacknife residuals method. We checked if variance inflation factors (VIFs) exceeded 10 to determine if there was collinearity. All analyses were performed with SAS 9.3 (Cary, NC) and the significance level was 0.1.

RESULTS

Descriptive Analyses

Initially, 203 potential subjects were assessed for eligibility and 190 subjects were enrolled in the analysis (Figure 2). Descriptive analysis of the baseline characteristics of patients are shown in Table 5. Among all subjects, 49 patients (25.8%) were female, and the mean weight was 59.5 kg. At the time of second admission, 135 subjects (71.1%) were in good nutritional status, 119 subjects (62.2%) had good sleep status and 22 subjects (11.6%) drank alcohol during the duration of treatment. One hundred thirty-four patients (70.6%) received antiviral therapy after the first biopsy (interferon 74 (39.0%), lamivudine 16 (8.4%), adefovir 25 (13.2%), and entecavir 19 (10.0%)). All patients enrolled in analysis had two biopsies results; 69 patients (36.3%) improved in fibrosis stage and 73 patients (38.4%) had improvements in inflammation grade.

Univariate and Multivariate Analyses for Fibrosis Stage

The univariate analyses between baseline characteristics and the change of fibrosis stage are shown in Table 6. Drinking alcohol and low weight were associated with the improvement in fibrosis stage (OR=0.37, p=0.07; mean difference= -5.03 kg, p=0.07, respectively). Adhering to continuous treatment during the therapy after the first biopsy and short interval between the onset

of disease and the first biopsy were significantly associated with the improvement in fibrosis stage (OR=2.62, p=0.01; mean difference= -0.41 year, p=0.01, respectively).

In the unadjusted logistic analysis, there were no significant differences in the improvement in fibrosis stage with primary predictors: nutritional status, sleep status, alcohol consumption, and antiviral therapy. Using the backward strategy method, 7 variables remained in the model: nutritional status, sleep status, alcohol consumption, antiviral therapy, continuous treatment during the therapy period after the first biopsy, age at the first biopsy, and interval between the onset of disease and the first biopsy. The literature guided us to consider some important predictors; age at onset of disease and co-infection with HCV were added back into the model. No interaction term was found. The assumption of independence was true. No outlier, collinearity or correlation was found.

Thus, in the adjusted multivariate analysis (Table 7), drinking alcohol (OR=0.35, 95%CI 0.10 - 1.20, p=0.09) and long interval between onset of disease and first biopsy (OR=0.45, 95%CI 0.21 - 0.95, p=0.04) were harmful factors for the progression of fibrosis. Adhering to continuous treatment during the therapy period after the first biopsy (OR=2.16, 95%CI 1.06 - 4.14, p=0.04) was significantly associated with the improvement in fibrosis stage.

Nutritional status, sleep status, and antiviral therapy were not statistically associated with the improvement in fibrosis stage.

Univariate and Multivariate Analyses for Inflammation Grade

The univariate analyses between baseline characteristics and the change of inflammation grade are shown in Table 8. Drinking alcohol (OR=0.32, p=0.04), good nutritional status (OR=2.22, p=0.02) and taking adefovir (OR 5.88, p<0.01) were significantly associated with the improvement in inflammation grade. There were significant associations between low weight (mean difference= -5.72 kg, p=0.04), young age at the first biopsy (mean difference= -5.01 year, p=0.02), and short interval between the onset of disease and the first biopsy (mean difference= -0.31 year, p=0.05) and the improvement in inflammation grade.

There was no significant difference in change of inflammation grade between the primary predictors except taking adefovir (OR=5.88, 95%CI 2.11 - 16.40, p<0.01) in the crude multivariate logistic analysis. Backward strategy was used and 7 variables remained in the model: nutritional status, sleep status, alcohol consumption, antiviral therapy, continuous treatment during the therapy period after the first biopsy, co-infection with HCV, and interval between the onset of disease and the first biopsy. Age at onset of disease was added back to model according to the literature review. No interaction term

was found. The assumption of independence was true. No outlier, collinearity or correlation was found.

Table 9 shows the adjusted multivariate logistic analysis for inflammation grade. We found that good nutritional status (OR 3.15, 95%CI 1.43 - 6.96, p<0.01) and taking adefovir (OR=3.97, 95%CI 1.25 - 12.57, p=0.05) were significantly associated with the improvement in inflammation grade. Elderly age at onset of disease (OR=0.98, 95%CI 0.95 - 1.01, p=0.08) and long interval between the onset of disease and first biopsy (OR=0.66, 95%CI 0.47 - 0.92, p=0.02) were harmful factors for the change of inflammation grade. Sleep status and alcohol consumption were not statistically associated with the improvement in inflammation grade.

DISCUSSION

In other studies, nutritional status and sleep quality have been shown to beneficially influence immunologic or inflammatory parameters [15, 37]. Qin et al. reported that the improvement of nutritional status was helpful to ameliorate the liver function of patients with severe chronic hepatitis [14]. Our data demonstrated that good nutritional status was highly associated with the improvement in inflammation grade, but there was no significant effect on the progression of fibrosis. Although sleeping late and sleep disturbance could have higher probabilities of causing liver disease, we found no significant association between sleep status and the progression of liver histology.

In general, chronic hepatitis B patients are strongly encouraged by physicians to quit drinking alcohol before antiviral therapy, but there are still some patients who continued drinking alcohol during treatment. In this study, 11.6% of patients drank alcohol during treatment. Additionally, alcohol consumption during the period after the first biopsy was a risk factor for deterioration in fibrosis stage in both univariate analysis and multivariate analysis, as well as for inflammation grade in univariate analysis. Several reports indicated that alcohol consumption could alter the cellular immune responses to virus and increase liver damage; this effect may have more influence on fibrosis than inflammatory condition [37].

Our study showed a significant association between adefovir treatment and the improvement in inflammation grade. However, there was no significant association between the type of antiviral therapy and the change of fibrosis stage. In fact, the treatment of entecavir led to deterioration in fibrosis. This result was in contrast with prior studies [30-32]. This may be due to the limitation of sample size; only 19 patients were treated with entecavir and only 9 of those patients improved in fibrosis stage. The desired effect of antiviral therapy is to reduce the level of HBV DNA, some patients who received antiviral therapy might have decreases in HBV DNA levels but the effects of therapy were not shown in the liver biopsy [23, 26]. Thus, it is difficult to make any firm conclusions between antiviral therapy and the change of liver biopsy scores based on our data.

Other factors—obesity, continuous treatment during the therapy period, younger age at infection, age at treatment, and co-infection with HIV or HCV —have been found to be associated with the prognosis of hepatitis B in prior studies [25, 26, 38-40]. Previous reports found a negative impact of significant weight loss on fibrosis in obese hepatitis B patients receiving antiviral treatment [17]. We found that heavy weight was significantly associated with the deterioration in fibrosis stage in the univariate analysis. But in our data set, more than half of the heights were missing and we could not obtain body mass index (BMI) to evaluate obesity. Continuous treatment during the therapy period after the first biopsy was significantly associated

with the improvement of fibrosis stage and inflammation grade. Moreover, the effect of co-infection with HIV or HCV has been well described [40, 41]. However, we had only one individual with HIV. No statistically significant conclusion could be made about co-infection with HIV. Short interval between the onset of hepatitis B and the first biopsy was significantly associated with both the improvement in fibrosis stage and inflammation grade. Getting effective diagnosis and treatment may beneficially affect the prognosis of hepatitis B. Furthermore, prior studies reported that younger age at the beginning of treatment predicted a positive outcome [38]. However, we found that patients who had their first biopsy at an older age were more likely to experience worsening of fibrosis and inflammation in the univariate analysis but not in the multivariate analysis. In addition, there was no significant association between age at onset of disease and the change of fibrosis stage. The age distribution of our participants had a narrow range. Most patients were 10 to 30 year old. This may help to explain why there was no significant association between age and outcome.

According to the AASLD (American Association for the Study of Liver Diseases) guidelines [21], the histological response to treatment can be measured by biopsy. We used the changes in histology as the outcomes in our study. The decrease in inflammation grade at least two grades and no worsening in fibrosis stage represented the cases. According to this definition, only 9 cases were found in our data, and the 95% CIs for odds ratio between

histological response and the primary exposures were too wide. Thus, this definition of success was too narrow for our data.

There were several limitations in our study. The principle limitation was that we have not yet evaluated whether the various therapeutic interventions were associated with serological or virological improvement in the patients. The purpose of a liver biopsy is to assess the degree of liver damage. However, liver histology could improve significantly in patients who have a decrease in HBV DNA level or HBeAg seroconversion [21, 28]. Using the change of liver biopsy scores as the progression of liver histology to evaluate the effect of nutritional status, sleep status, and alcohol consumption is reasonable. However, it is meaningless to assess the association between clinical improvement between liver biopsies and disease management if the patient has virus resistant to the antiviral therapy. The serological data are available and will be explored in a later analysis.

Second, this study consists of two case-control studies performed in a single hospital. Although all Chinese are eligible for care at the 302 Military Hospital, the location and cost were barriers to care for patients from the southern part of China and patients in poverty. In China, not every patient with HBV is offered a liver biopsy. Unbiopsied patients were not included in the study. The study sample we used was a convenience sample; patients who went to other hospitals after the first hospitalization in the 302 Military

Hospital and the patients who died after first hospitalization were not included in the analysis. Moreover, some patients who got better after the first biopsy may not have received the second biopsy. The study population might be skewed.

Third, the sample size was quite small. There were small numbers of patients who received each type of antiviral therapy, and a small number of patients enrolled in the alcohol group. In addition, two-thirds of patients were male, and 75% of the study population was under age the of 30. Thus, our results might not have generalizability to women and the elderly.

Finally, this study was a secondary data analysis and the data were collected from medical records. Information reported by patients, such as the continuity of treatment, and age at onset of hepatitis B might not be accurate. Sleep status was self-reported and this may underestimate the sleep quality and lead to misclassification bias. It was not possible to assess the presence of recall bias. In addition, some earlier medical records had many missing values.

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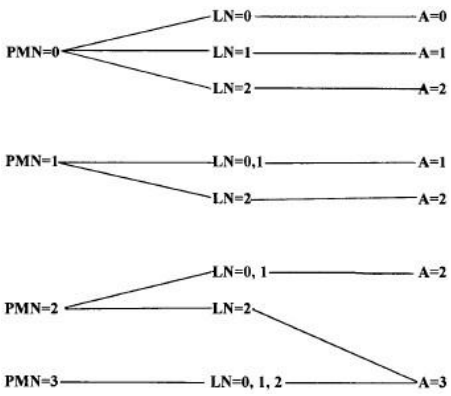
TABLES

Table 1. Scheuer System for Chronic Hepatitis

Activity Grade		
Portal activity	*Lobular activity*	
None	None	0
Portal inflammation alone	Inflammation but no necrosis	1
Mild piecemeal necrosis	Focal necrosis or acidophilic bodies	2
Moderate piecemeal necrosis	Severe focal cell damage	3
Severe piecemeal necrosis	Damage includes bridging necrosis	4
Fibrosis Stage		
None		0
Enlarged, fibrotic portal tracts		1
Peri-portal fibrosis or portal-portal septa, but intact architecture		2
Fibrosis with architectural distortion, but no obvious cirrhosis		3
Probable or definite cirrhosis		4

Table 2. Metavir System for Chronic Hepatitis

Algorithm of Activity Grade	Fibrosis Stage	
	None	0
	Portal fibrosis without septa	1
	Portal fibrosis with rare septa	2
	Portal fibrosis with numerous septa	3
	Probable or definite cirrhosis	4

Algorithm for the evaluation of histological activity. PN, piecemeal necrosis: 0 none, 1 mild, 2 moderate, 3 severe; LN, lobular necrosis: 0 none or mild, 1 moderate, 2 severe; A, activity grade; 0 none, 1 mild, 2 moderate, 3 severe. (Adapted from Neil D Theise. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach)

Table 3. Fibrosis Stage of Ishak System

None	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal-toportal (P-P) bridging	3
Fibrous expansion of portal areas with marked portal-to-portal (P-P) as well as portal-to-central (P-C) bridging	4
Fibrous expansion of portal areas with marked portal-to-portal (P-P) as well as portal-to-central (P-C) bridging	5
Probable or definite cirrhosis	6

Table 4. Definition of Response to Antiviral Therapy of Chronic Hepatitis B

Category of Response	
Biochemical Response	Decrease in serum ALT to within the normal range
Virological Response	Decrease in serum HBV DNA to undetectable levels by PCR assays, and loss of HBeAg in patients who were initially HBeAg positive
Histological Response	Decrease in histology activity index by at least 2 grades and no worsening of fibrosis score compared to pre-treatment liver biopsy
Complete Response	Fulfill criteria of biochemical and virological response and loss of HBsAg

ALT, alanine aminotransferase; HBV, hepatitis B virus; PCR, polymerase chain reaction; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.
(Adapted from AASLD Practice Guidelines. Chronic Hepatitis B: Update 2009.

Table 5. Frequency of Demographic Characteristics and Clinical Variables of Study Population

Characteristics	Patients(n=190)
Female	49 (25.8%)
Ethnic Minority	12 (6.3%)
Weight (kg, mean±SD)	59.5 ± 18.5
Good Sleep Status	119 (62.6%)
Good Nutritional Status	135 (71.1%)
Alcohol Consumption	22 (11.6%)
Family History of Liver Disease	77 (40.5%)
Fatty Liver	40 (23.7%)
HIV Co-infection	1 (0.6%)
HCV Co-infection	26 (13.68%)
Age at Onset of Hepatitis B (yr, mean±SD)	20.8 ± 13.9
Age at Abnormal Liver Function (yr, mean±SD))	24.6 ± 14.5
Age at the First Treatment (yr, mean±SD)	24.8 ± 14.7
Age at the First Biopsy (yr, mean±SD)	27.5 ± 14.6
Interval between Onset of Disease and the First Biopsy (yr, median [IQR])	4 (1-10)
Interval between Onset of Disease and the First Treatment (yr, median [IQR])	1 (1-8)
Interval between the First and Second Biopsy (yr, median [IQR])	2 (1-3)
Antiviral Therapy before the First Biopsy	62 (33.2%)
Antiviral Therapy after the First Biopsy	
No Antiviral Therapy	50 (26.3%)
Interferon	74 (39.0%)
Lamivudine	16 (8.4%)
Adefovir	25 (13.2%)
Entecavir	19 (10.0%)
Continuous Treatment after the First Biopsy	123 (64.7%)

Fibrosis Stage of the First biopsy (mean [range])	1.9 (0-4)
Inflammation Grade of the First biopsy (mean [range])	1.7 (1-4)
Fibrosis Stage of the Second biopsy (mean [range])	1.9 (0-4)
Inflammation Grade of the Second biopsy (mean [range])	1.5 (0-3)
Improvement in Fibrosis Stage	69 (36.3%)
Improvement in Inflammation Grade	73 (38.4%)

Values are presented as n (%) unless otherwise indicated.
SD, standard deviation; IQR, interquartile range; yr, year; HIV, Human immunodeficiency virus; HCV, hepatitis C virus.

Table 6. Univariate Association between Baseline Characteristics and the Improvement in Fibrosis Stage

Characteristics	Deterioration in Fibrosis	Improvement in Fibrosis	OR (95%CI)
Female	31 (16.3%)	18 (9.5%)	0.99 (0.51, 1.95)
Ethnic Minority	7 (3.7%)	5 (2.6%)	1.24 (0.38, 4.07)
Good Sleep Status	80 (45.2%)	39 (22.0%)	0.74 (0.39, 1.42)
Good Nutritional Status	86 (45.5%)	49 (25.9%)	0.90 (0.47, 1.71)
Alcohol Consumption	18 (9.7%)	4 (2.2%)	0.37 (0.12, 1.13)†
Family History of Liver Disease	50 (26.5%)	27 (14.3%)	0.90 (0.49, 1.65)
Fatty Liver	27 (16.2%)	13 (7.1%)	0.82 (0.39, 1.74)
HIV Co-infection	0 (0%)	1 (0.6%)	-
HCV Co-infection	15 (8.4%)	11 (6.2%)	1.33 (0.57, 3.10)
Antiviral Therapy before the First Biopsy	41 (21.9%)	21 (11.2%)	0.85 (0.45, 1.61)
Antiviral Therapy after the First Biopsy			
Interferon	45 (24.5%)	29 (15.8%)	1.61 (0.77, 3.39)
Lamivudine	9 (4.9%)	7 (3.8%)	1.95 (0.62, 6.11)
Adefovir	12 (6.5%)	13 (7.1%)	2.71 (1.02, 7.18)
Entecavir	15 (8.2%)	4 (2.2%)	0.67 (0.19, 2.32)
Continuous Treatment after the First Biopsy	69 (40.4%)	54 (31.6%)	2.63 (1.13, 5.64)‡
Characteristics	**Deterioration in Fibrosis**	**Improvement in Fibrosis**	**Mean Difference (95%CI)**
Weight (kg, mean±SD)	61.4 ± 17.8	56.4 ± 19.2	-5.03 (-10.59, 0.52)†
Age at Onset of Hepatitis B (yr, mean±SD)	20.9 ± 13.5	20.5 ± 14.7	-0.37 (-4.52, 3.80)
Age at Abnormal Liver Function (yr, mean±SD)	25.1 ± 14.1	23.6 ± 15.0	-1.57 (-6.12, 2.98)
Age at the First Treatment (yr, mean±SD)	24.8 ± 14.3	24.8 ± 15.6	0.08 (-4.64, 4.80)
Age at the First Biopsy (yr, mean±SD)	28.1 ± 14.1	26.4 ± 15.4	-1.68 (-6.00, 2.65)
Interval between Onset of Disease and the First Biopsy (yr,mean±SD)e	1.6 ± 1.1	1.2 ± 1.1	-0.41 (-0.74, 0.09)‡
Interval between Onset of Disease and the First Treatment (yr,mean±SD)e	1.1 ± 1.1	0.8 ± 1.1	-0.27 (-0.62, 0.09)
Interval between the First and Second Biopsy (yr,mean±SD)e	0.7 ± 0.6	0.7 ± 0.5	-0.05 (-0.24, 0.13)

Values are presented as n (%) unless otherwise indicated.

†, p-value<0.1; ‡, p-value<0.05; e, natural logarithm.

OR, odds ratio; SD, standard deviation; Mean Difference, mean of improved group minus deteriorated group

yr, year; HIV, human immunodeficiency virus; HCV, hepatitis C virus.

Table 7. Multivariate Logistic Analysis of Predictive Factors for the Improvement in Fibrosis Stage

Characteristics	OR	95%CI
Antiviral Therapy After first Biopsy (ref=no antiviral treatment)		
Interferon	1.04	0.43 - 2.53
Lamivudine	1.67	0.48 -5.90
Adefovir	1.88	0.64 - 5.55
Entecavir	0.57	0.15 - 2.17
Good Nutritional Status	1.03	0.50 - 2.13
Good Sleep Status	1.53	0.74 - 3.16
Alcohol Consumption	0.35	0.10 - 1.20†
Co-infection with HCV	1.18	0.44 - 3.20
Continuous Treatment after the First Biopsy	2.16	1.06 - 4.41‡
Age at Onset of Disease	0.91	0.81 - 1.02
Age at first Biopsy	1.10	0.97 - 1.23
Interval between Onset of Disease and the First Biopsy^{e}	0.45	0.21 - 0.95‡

†, p-value<0.1; ‡, p-value<0.05; e, natural logarithm.
OR, odds ratio; CI, confidence interval; HCV, hepatitis C virus.

Table 8. Univariate Association between Baseline Characteristics and the Improvement in Inflammation Grade

Characteristics	Deterioration in Inflammation	Improvement in Inflammation	OR (95%CI)
Female	30 (15.8%)	19 (10.0%)	1.02 (0.52, 1.99)
Ethnic Minority	9 (4.7%)	3 (1.6%)	0.51 (0.13, 1.97)
Good Sleep Status	69 (36.3%)	50 (26.3%)	1.51 (0.82, 2.80)
Good Nutritional Status	76 (40.2%)	59 (31.2%)	2.22 (1.10, 4.45)†
Alcohol Consumption	18 (9.7%)	4 (2.2%)	0.32 (0.10, 0.99)†
Family History of Liver Disease	46 (24.3%)	31 (16.4%)	1.17 (0.64, 2.12)
Fatty Liver	27 (16.2%)	13 (7.8%)	0.69 (0.33, 1.47)
HIV Co-infection	1 (0.6%)	0 (0%)	-
HCV Co-infection	19 (10.7%)	7 (3.9%)	0.53 (0.21, 1.35)
Antiviral Therapy before the First Biopsy	42 (22.1%)	23 (12.1%)	0.82 (0.44, 1.53)
Antiviral Therapy after the First Biopsy			
Interferon	46 (24.2%)	28 (17.7%)	2.01 (0.93, 4.38)
Lamivudine	9 (4.7%)	7 (3.7%)	2.57 (0.80, 8.26)
Adefovir	9 (4.7%)	16 (8.4%)	5.88 (2.11, 16.40)‡
Entecavir	10 (5.3%)	9 (4.7%)	2.98 (1.00, 8.89)
Continuous Treatment after the First Biopsy	69 (40.4%)	54 (31.6%)	1.72 (0.85, 3.49)

Characteristics	Deterioration in Inflammation	Improvement in Inflammation	Mean Difference (95%CI)
Weight (kg, mean±SD)	61.7 ± 18.2	56.0 ± 15.8	-5.72 (-11.25, -0.19)†
Age at Onset of Disease (yr, mean±SD)	22.1 ± 13.9	28.7 ± 13.9	-3.36 (-7.45, 0.73)
Age at Abnormal Liver Function (yr, mean±SD)	26.4 ± 14.5	21.8 ± 14.1	-4.67 (-9.10, -0.24)
Age at the First Treatment (yr, mean±SD)	26.4 ± 14.7	22.3 ± 14.5	-4.08 (-8.68, 0.52)
Age at the First Biopsy (yr, mean±SD)	29.4 ± 14.7	24.4 ± 13.9	-5.01 (9.25, 0.78)†
Interval between Onset of Disease and the First Biopsy (yr,mean±SD)^{e}	1.5 ± 1.1	1.2 ± 1.1	-0.31 (-0.64, -0.01)†
Interval between Onset of Disease and the First Treatment (yr,mean±SD)^{e}	1.1 ± 1.2	0.8 ± 1.0	-0.22 (-0.57, 0.13)
Interval between the First and Second Biopsy (yr,mean±SD)^{e}	0.7 ± 0.6	0.7 ± 0.5	0.06 (-0.12, 0.25)

Values are presented as n (%) unless otherwise indicated.

†, p-value<0.05; ‡, p-value<0.01; e, natural logarithm.

OR, odds ratio; SD, standard deviation; Mean Difference, mean of improved group minus deteriorated group; yr, year; HIV, human immunodeficiency virus; HCV, hepatitis C virus.

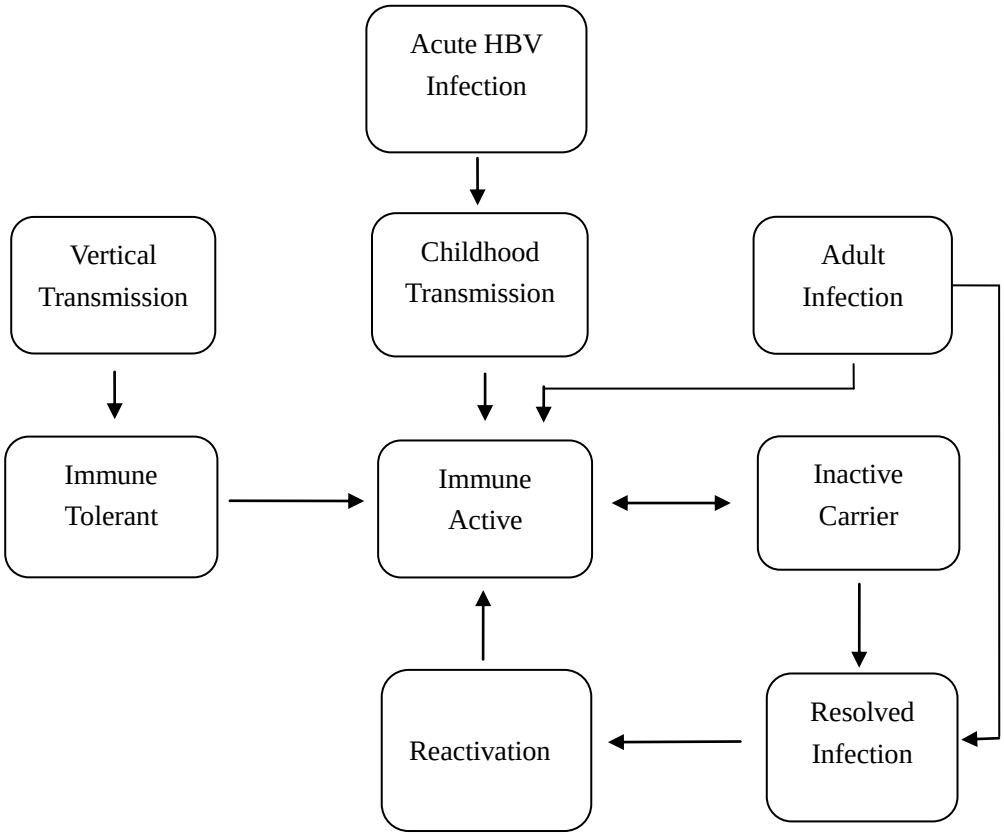
Table 9. Multivariate Logistic Analysis of Predictive Factors for the Improvement in Inflammation Grade

Characteristics	OR	95%CI
Antiviral Therapy After first Biopsy (ref=no antiviral treatment)		
Interferon	1.21	0.47 - 3.09
Lamivudine	1.52	0.43 -5.33
Adefovir	3.97	1.25 - 12.57‡
Entecavir	2.61	0.80 - 8.51
Good Nutritional Status	3.15	1.43 - 6.96§
Good Sleep Status	1.33	0.66 - 2.69
Alcohol Consumption	0.51	0.15 - 1.71
Co-infection with HCV	0.62	0.21 - 1.83
Continuous Treatment after the First Biopsy	1.73	0.84 - 3.57
Age at Onset of Disease	0.98	0.95 - 1.01†
Interval between Onset of Disease and the First Biopsy[e]	0.66	0.47 - 0.92‡

†, p-value<0.1; ‡, p-value<0.05; § , p-value<0.01; e, natural logarithm.
OR, odds ratio; CI, confidence interval; HCV, hepatitis C virus.

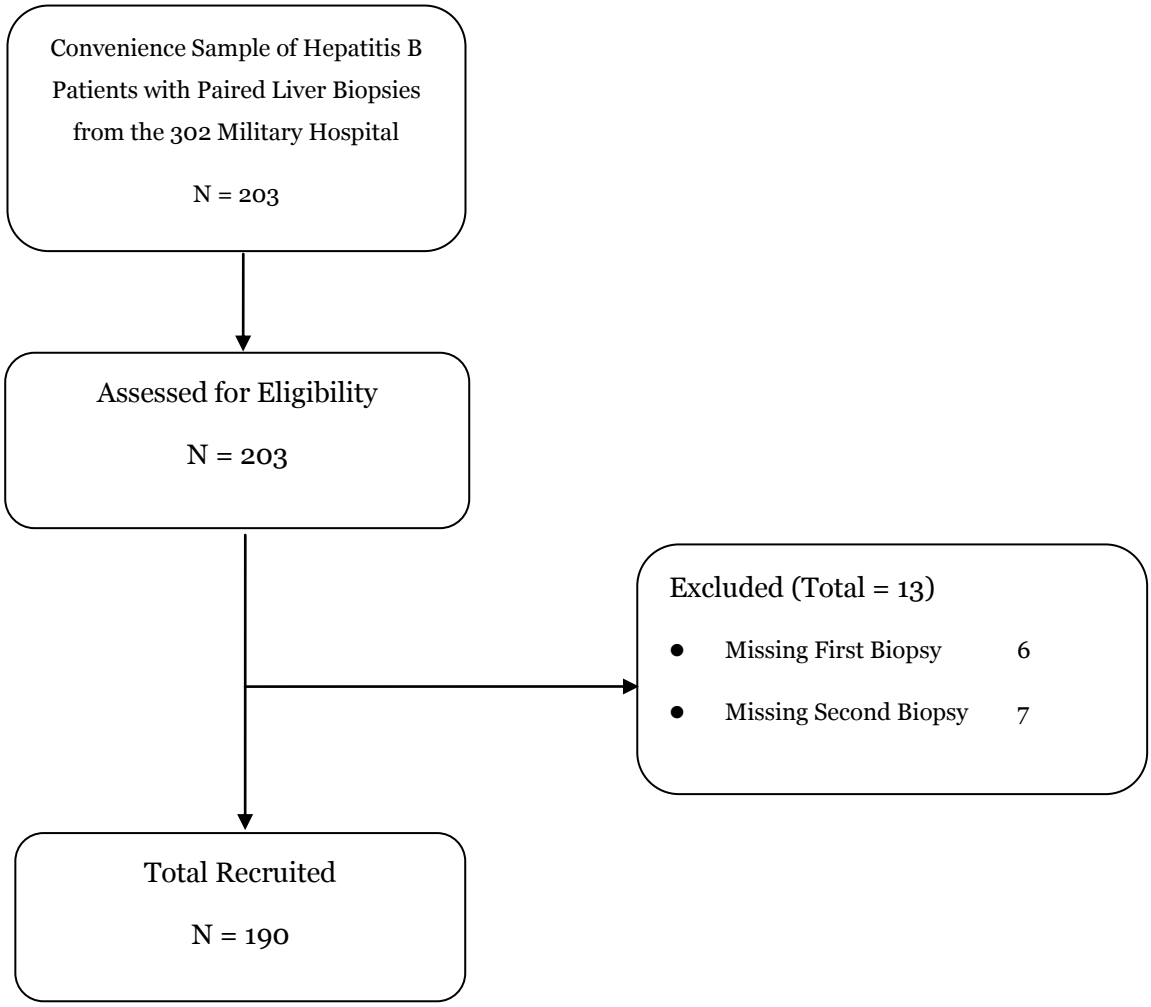
FIGURES

Figure 1. Natural History of Acute Hepatitis B Infection



Vertical transmission usually results in immune tolerant infection; childhood transmission usually results in immune-active infection; adult infection usually results in resolved infection and immune-active infection. Transition between the immune tolerant, immune-active and inactive carrier phases of chronic infection is dynamic. (Adapted from Alexander Kuo. Chronic Hepatitis B Infection [7].)

Figure 2. Flow Diagram in Analysis of the Association between the Progression of Chronic Hepatitis B and Non-virus Factors, 1999 - 2012



SUMMARY

In conclusion, our results support the importance of good nutritional status and treatment with adefovir to achieve improvement in liver inflammation. Also the study showed the significant association between alcohol consumption and the worsening of fibrosis stage. There were no statistically significant associations between sleep status and the improvement in fibrosis stage or inflammation grade. These findings provide useful information to our understanding of health-related life status during the chronic hepatitis B treatment. For chronic hepatitis B patients, it is very important to maintain good nutrition and strictly refrain from drinking alcohol during the treatment.

The use of convenience samples limited the generalizability to all hepatitis B patients. The small sample size may result in the lack of statistical representation. Finally, the association between antiviral therapy and virological clearance was not evaluated. Without this information, it is difficult to conclude whether a particular patient's hepatitis B virus was susceptible to the agent used.

A future study will include serological and virological information, such as HBV DNA level and HBeAg seroconversion, to assess whether there is resistance to various therapeutic agents. In that study, we will take into account the association between treatment induced improvement in serological markers and improvement in liver histology.

APPENDIX

IRB letter

EMORY UNIVERSITY | Institutional Review Board

12/3/2012

Da Mao
Principal Investigator
Public Health

Dear Mr. Mao:

Thank you for submitting an application to the Emory IRB for the above-referenced project. Based on the information you have provided, we have determined on 12/3/2012 that although it is human subjects research, it is exempt from further IRB review and approval.

This determination is good indefinitely unless substantive revisions to the study design (e.g., population or type of data to be obtained) occur which alter our analysis. Please consult the Emory IRB for clarification in case of such a change. Exempt projects do not require continuing renewal applications.

This project meets the criteria for exemption under 45 CFR 46.101(b)(4). Specifically, you will be receiving deidenfitied data from medical records in the 302 Military Hospital in China.

- Protocol, version date 3/11/2012

Please note that the Belmont Report principles apply to this research: respect for persons, beneficence, and justice. You should use the informed consent materials reviewed by the IRB unless a waiver of consent was granted. Similarly, if HIPAA applies to this project, you should use the HIPAA patient authorization and revocation materials reviewed by the IRB unless a waiver was granted. CITI certification is required of all personnel conducting this research.

Unanticipated problems involving risk to subjects or others or violations of the HIPAA Privacy Rule must be reported promptly to the Emory IRB and the sponsoring agency (if any).

In future correspondence about this matter, please refer to the study ID shown above. Thank you. Sincerely,

Scott Jenkins, BS
Research Protocol