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Baseline HBV Load Increases the Risk of Anti-tuberculous Drug-induced Hepatitis Flares in Patients with Tuberculosis*

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Summary: Hepatitis associated anti-tuberculous treatment (HATT) has been a main obstacle in managing patients co-infected with Mycobacterium tuberculosis and hepatitis B virus (HBV). Therefore, we evaluated the factors related to the severity of adverse effects during HATT, especially those associated with liver failure. A retrospective study was carried out at Tongji Hospital from 2007 to 2012. Increases in serum transaminase levels of >3, 5, and 10 times the upper limit of normal (ULN) were used to define liver damage as mild, moderate, and severe, respectively. Patients with elevated total bilirubin (TBil) levels that were more than 10 times the ULN (>171 μmol/L) with or without decreased (<40%) prothrombin activity (PTA) were diagnosed with liver failure. A cohort of 87 patients was analyzed. The incidence of liver damage and liver failure was 59.8% (*n*=52) and 25.3% (*n*=22), respectively. The following variables were correlated with the severity of hepatotoxicity: albumin (ALB) levels, PTA, platelet counts (PLT), and the use of antiretroviral therapies ($P<0.05$). Hypo-proteinemia and antiretroviral therapy were significantly associated with liver failure, and high viral loads were a significant risk factor with an odds ratio (OR) of 2.066. Judicious follow-up of clinical conditions, liver function tests, and coagulation function, especially in patients with high HBV loads and hypoalbuminemia is recommended. It may be advisable to reconsider the use of antiviral drugs failure during the course of anti-tuberculous treatment of HBV infection patients to avoid the occurrence of furious liver failure.
Key words: hepatitis B virus infection; anti-tuberculous treatment; Mycobacterium tuberculosis; HBV DNA loading; hypoproteinemia

Tuberculosis (TB) is still an important health problem and a leading cause of death worldwide. However, no new anti-tuberculous drug has been validated as effective in recent years. Although the use of a multi-drug regimen for the treatment of TB with a combination of isoniazid, rifampicin, and pyrazinamide is a highly-effective strategy, one of the most frequent and serious side effects of these drugs is hepatotoxicity[1], which has restricted use of this treatment regimen. The acquisition of hepatitis during anti-tuberculous treatment is a direct cause of anti-tuberculous treatment failure, resulting in modification or discontinuation of anti-tuberculous treatment in 17.7% of all TB patients[2].

The reported risk factors for the development of anti-TB drug-induced hepatotoxicity include advanced age, frequent/high alcohol consumption, malnutrition, chronic liver disease, and viral hepatitis[3–6]. There is no consensus as to which particular factors, whether alone or in combination, are involved in the development of drug-induced liver injury. The TB infection rate and HBV infection rate are high in developing countries; the combination of these two diseases is not rare. Recent reports have confirmed that chronic viral hepatitis is a risk factor for the development of anti-tuberculous drug-induced hepatotoxicity, according to the analysis of patient cohorts with and without HBV infection[7, 8]. The underlying mechanisms that cause abnormal liver function within these patients and the factors affecting the severity of hepatic injury remain unknown.

Our cross sectional study was conducted to evaluate the risk factors associated with anti-tuberculous drug-induced hepatotoxicity in patients with TB and HBV co-infection and the influence of clinical risk factors on the severity of this adverse effect.

1 MATERIALS AND METHODS

We retrospectively enrolled patients diagnosed with TB that received anti-tuberculous agents at the Department of Infectious Diseases in Tongji Hospital from June 1, 2007 to June 31, 2012. Adult patients (>14-years-old) with active or inactive tuberculosis who were treated with a combination regimen of isoniazid (INH), rifampin (RIF), and pyrazinamide (PZA) were enrolled in this study.

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All patients enrolled were positive for hepatitis B surface antigen (HBsAg), and HBV infection was diagnosed. Liver ultrasonography and fibroscans were carried out to exclude patients with liver cirrhosis. Pregnant patients or patients with abnormal baseline liver function tests (LFT) due to congestive heart failure, cancer, diabetes mellitus, severe chronic kidney disease, autoimmune disease, hepatic malignancy or alcohol abuse were excluded from this study. Serological screening of antibodies against hepatitis C virus (HCV), HAV, HEV, and human immunodeficiency virus (HIV) was carried out for all patients to eliminate positive cases. This case report was compiled after obtaining the written informed consent of the closest relatives of the patient.

A standardized case record form was used to delineate patient characteristics (age, sex, height, weight, daily drinking habits, baseline levels of albumin, pulmonary or extrapulmonary TB, underlying diseases, and concurrent use of other hepatotoxic drugs), anti-tuberculous agents (regimen and dosage), serological results for HBV, HCV, and HIV infection before treatment, baseline and monthly biochemical tests during treatment [including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT), and total and direct bilirubin], associated symptoms/signs of hepatitis (including poor appetite, nausea, vomiting, fatigue, lower leg edema, abdominal discomfort, and jaundice), and management of hepatitis (including observation and modification or discontinuation of antituberculotics).

Increased serum transaminase levels, which were 3 or 5 times above the upper limit of normal (ULN) were defined as mild and moderate hepatitis flares, respectively. Severe hepatitis flares were defined when ALT levels were elevated to 10 times the ULN (>400 U/L)[9]. Liver failure was identified when total bilirubin was elevated more than 10 times the ULN (>171 μmol/L) with/without decreased (<40%) PTA levels, which also included cases with any degree of hepatic encephalopathy.

Following data collection, a χ^2 test was used for categorical variables and a T test was used for non-categorical variables to evaluate the risk factors associated with hepatitis flares and liver failure. Logistic regression analysis was used to determine the risk factors associated with liver failure with OR and a 95% confidence interval (95% CI). All analyses were performed using SPSS for Windows (v17.0, SPPS Inc, USA) and P<0.05 (for two-tailed tests) was considered statistically significant.

2 RESULTS

Eighty-seven HBV infected patients treated with anti-tuberculous agents were enrolled in this study. Their demographics are presented in table 1. The mean age was 44.38±13.82 years old (range, 17–80) with 68 (75.7%) males and 19 (24.3%) females, 12 (13.8%) and 75 (86.2%) of which were older than 60 years and under 60 years, respectively. The number of patients positive for HBV infection was 49 (56.3%), and the number of patients negative for HBV infection was 38 (43.7%). The baseline HBV-DNA levels could be detected (>500 copies/mL) in 45 (51.7%) cases, of which 24 (53.3%) had high viral loads (>10^5 copies/mL), while the remaining 42 (48.3%) were negative for HBV-DNA (<500 copies/mL). Icterus occurred in 44 (50.6%) patients, jaundice occurred in 42 (48.3%) patients, hypoproteinemia developed in 45 (51.7%) patients, and other laboratory characteristics are shown in table 1. Thirty-six (41.4%) patients were given antiviral treatment, and the remaining 51 (58.6%) did not receive antiviral treatment. Routine biochemical tests showed that 45 (51.7%) patients in this cohort presented with hypoproteinemia, while the remaining 42 patients (48.3%) did not. Ten patients died in the hospital, despite the fact that appropriate and timely measures were taken. Detailed data are summarized in table 1.

Table 1 Summary of clinical and laboratory characteristics

Variables	$\bar{x}\pm s$	Minimum	Maximum
Age (years)	44.38±13.82	17.00	80.00
ALB (g/L)	34.69±6.75	20.50	50.10
TBil (μmol/L)	149.25±171.18	2.70	621.20
DBil (μmol/L)	90.06±104.93	1.00	306.0
PT (s)	17.99±10.90	10.40	70.90
PTA (%)	73.51±30.54	11.00	135.00
Scr (μmol/L)	78.39±52.94	24.70	393.50
UA (μmol/L)	249.69±135.16	37.30	607.70
ALT (U/L)	245.94±373.49	7.00	2301.00
AST (U/L)	190.89±264.2	5.00	1447.00
WBC (×10^9/L)	7.53±4.50	1.46	27.32
Hb (g/L)	119.86±23.11	53.20	174.00
PLT (×10^{12}/L)	157.25±89.79	11.00	442.00

All factors were compared among the three defined levels of hepatotoxicity as shown in table 2. Of the 87 patients, the liver function of 35 cases (40.2%) remained normal, while 18 (20.7%) cases suffered mild to moderate hepatitis flares, and 35 (39.1%) patients were diagnosed as having severe hepatitis flares or even hepatic failure. Interestingly, age, gender, HBeAb, and HBV-DNA loads did not significantly correlate with the severity of the hepatitis flare. We found that patients with more severe liver damage had higher viral loads, yet this difference was not statistically significant (P=0.178), whereas antiviral treat-

ment and albumin levels were significantly related to the severity of liver impairment (P<0.001). Moreover, the ratio of patients who received antiviral treatment was astonishingly higher in the normal liver function group than in the hepatitis flare groups, while the results were the opposite, as far as the incidence of hypoproteinemia.

Table 2 Potential factors associated with different levels of hepatotoxicity on the baseline

Variables *n* (%)	Total (*n*=87)	Normal 35 (40.2%)	Mild to moderate hepatitis flare 18 (20.7%)	Severe hepatitis flare hepatic failure 34 (39.1%)	*P*
Age					0.060
<60 years old	75 (86.2%)	30 (85.7%)	16 (88.9%)	29 (85.3%)	
≥60 years old	12 (23.8%)	5 (4.3%)	2 (11.1%)	5 (14.7%)	
Gender					0.932
Male	68 (78.2%)	24 (68.6%)	13 (72.2%)	31 (91.2%)	
Female	19 (21.8%)	11 (31.4%)	5 (27.8%)	3 (8.8%)	
HBeAb					0.928
Positive	38 (43.7%)	16 (45.7%)	8 (44.4%)	14 (41.2%)	
Negative	49 (56.3%)	19 (54.3%)	10 (55.6%)	20 (58.8%)	
HBV-DNA					0.627
≥500 copies/mL	45 (51.7%)	18 (51.4%)	11 (61.1%)	16 (47.1%)	
<500 copies/mL	42 (48.3%)	17 (48.6%)	7 (38.9%)	18 (52.9%)	
High-virus					0.178
Yes	24 (27.6%)	6 (17.1%)	7 (38.9%)	11 (32.4%)	
No	63 (72.4%)	29 (82.9%)	11 (61.1%)	23 (67.6%)	
ALB					<0.001
<35 g/L	45 (51.7%)	8 (22.9%)	9 (50%)	28 (82.4%)	
≥35 g/L	42 (48.3%)	27 (77.1%)	9 (50%)	6 (17.6%)	
Anti-HBV					<0.001
Yes	36 (41.4%)	24 (68.6%)	5 (27.8%)	7 (20.6%)	
No	51 (58.6%)	11 (31.4%)	13 (72.2%)	27 (79.4%)	

By comparing blood test results among the three groups, we found that age was not significantly correlated with the severity of the hepatitis flare. PTA was notably lower in severe group than in the other two groups (mild and moderate). On the other hand, there was a significant association between serological ALB levels and the degree of liver damage; the concentration of albumin in the severe hepatitis flare group was lower than that in the mild and moderate hepatitis flare groups. In addition, serum creatinine (sCr) and uric acid (UA) levels could be used to predict the severity of hepatotoxicity. Among routine blood and liver function tests, PLT was correlated with the level of liver damage, along with TBil, direct bilirubin (DBil), PTA, and white blood cell counts (WBCs).

Table 3 Clinical test results correlated with levels of hepatotoxicity

Variables (*n*=87)	Normal 35 (40.2%)	Mild to moderate hepatitis flare 18 (20.7%)	Severe hepatitis flare/ hepatic failure 34 (39.1%)	*P*
Age (years)	42.40±15.20	43.30±15.60	47.00±11.00	0.362
ALT (U/L)	32.54±21.51	348.67±369.60$^{\#}$	88.06±462.17$^{\#}$	＜0.001
AST (U/L)	34.09±21.29	305.17±278.36$^{\#}$	286.00±314.16$^{\#}$	＜0. 001
TBil (μmol/L)	13.78±8.82	44.37±41.60	339.39±112.94$^{\#*}$	＜0.001
DBil (μmol/L)	5.66±5.96	26.21±30.46	207.00±64.93$^{\#*}$	＜0.001
PTA (%)	97.60±16.70	86.80±16.10	45.50±26.90$^{\#*}$	＜0.001
ALB (g/L)	38.64±6.00	35.03±6.48$^{\#}$	30.39±4.73$^{\#}$	＜0.001
Cr (μmol/L)	66.21±20.08	68.56±28.42	96.59±76.73$^{\#}$	0.036
UA (μmol/L)	288.46±177.08	277.42±114.78	193.75±139.82$^{\#}$	0.026
WBC（$\times 10^9$/L)	7.34±5.38	5.81±3.62	8.82±4.70*	0.096
PLT（$\times 10^{12}$/L)	210.29±107.71	148.72±88.32$^{\#}$	120.29±74.47$^{\#}$	<0.001
Hb (g/L)	119.27±21.95	118.60±27.11	120.31±20.50	0.930

$^{\#}P$<0.05 *vs*. normal group, $^{*}P$<0.05 *vs*. mild to moderate hepatitis flare group

Based on the aforementioned results and previous research, possible risk factors, which included advanced age, gender, positive HBeAg, high HBV-DNA loads, anti-HBV treatment, and hypoproteinemia were put into a logistic regression analysis. According to this analysis, we found that anti-HBV treatment was inversely correlated with the occurrence of liver failure with an OR of 0.27, which highlights its function as a protective factor in HBV infection among patients undergoing anti-tuberculous treatment, while hypoproteinemia was identified as a positive risk factor for liver failure with an OR of 6.546. Although high

HBV-DNA loads were not statistically significant, this might also be recognized as a risk factor for liver failure with an OR value of 2.066. There was no correlation between liver failure and gender, age, or HBeAg.

Table 4 Correlation between risk factors and occurrence of acute hepatic failure

Factors	Acute hepatic failure *n* (%)	OR	95% CI	*P*
Age		1.059	0.184–6.098	0.949
≥60 years old	4 (33.3)			
<60 years old	18 (24.0)			
Sex		1.442	0.329–6.323	0.628
Male	19 (27.9)			
Female	3 (15.8)			
HBeAb		0.837	0.271–2.584	0.757
Positive	10 (26.3)			
Negative	12 (24.5)			
HBV-DNA		2.066	0.546–7.822	0.286
$\geq 10^5$ copies/mL	8 (33.3)			
$<10^5$ copies/mL	14 (22.2)			
Anti-virus treatment		0.270	0.076–0.960	0.043
Yes	5 (13.9)			
No	17 (33.3)			
ALB		6.546	1.863–23.00	0.003
<35 g/L	18 (40.0)			
≥35 g/L	4 (9.5)			

OR: odds ratio; CI: confidence interval; ALT: alanine transaminase; AST: aspartate transaminase

3 DISCUSSION

TB is an important disease that threatens human health worldwide, especially in developing countries. Although the combination of multi-drug regimens, like INH, RIF, and PZA is a highly effective therapy, it has been reported that hepatitis B carriers that are given anti-tuberculous treatment have an increased incidence of hepatic dysfunction compared to noncarriers (34.9% *vs*. 9.4%)[10]. Therefore, the development of drug-induced hepatitis should be considered in HBV patients that become infected with TB, because it may necessitate the cessation or modification of treatment in patients with chronic liver disease.

Additionally reported risk factors for the development of anti-tuberculous drug-induced hepatotoxicity include advanced age, frequent/high alcohol consumption, and malnutrition[5, 6]. However, until recently no studies were published focusing on the HBV patients that become infected with TB, despite the fact that liver dysfunction is more likely to occur in this group during anti-tuberculous treatment. Such patients experience a high mortality rate; the prognosis of these patients is usually very poor, as in our research, 10 of 22 patients suffering from liver failure died during treatment, despite prompt and reasonable treatment. Our results suggest that these patients suffered from enhanced hepatotoxicity due to anti-tuberculous treatment. Therefore, the identification of hepatotoxicity risk factors in these patients will be particularly helpful to prevent/limit hepatotoxicity and thereby reduce the morbidity and mortality of this condition.

In our study, we tried to explore whether anti-HBe positivity was a protective factor that might help to prevent liver dysfunction during anti-tuberculous drug therapy in hepatitis B patients. However, our results showed that it did not confer any advantage to these patients, which agrees with the findings of other reports[11]. This might be explained by the fact that HBV DNA is usually detectable by polymerase chain reaction in most patients with positive anti-HBe serology.

At the same time, we did not find significant associations between the severity of liver damage and age, gender, or HBV-DNA loads. However, high HBV-DNA loads (more than 10^5 copies) were closely associated with liver dysfunction episodes. Furthermore, the use of antiviral drugs was confirmed to reduce the occurrence of liver failure. These findings agree with those reported by Sun *et al*[12], who proposed that antiviral drugs may be therapeutically beneficial in preventing the development of liver dysfunction in HBV patients while undergoing anti-tuberculous treatment. Taking this a step further, the risk of HATT-induced adverse effects is similar in HBV-infected and uninfected HBV patients, if the baseline viral titer is low, consistent with other reports[6].

Additionally, our research verified that hypoalbuminemia was another risk factor that contributes to liver failure in HBV-infected patients during anti-tuberculous treatment. This result is similar to previously published findings[10]. We therefore propose that in patients undergoing anti-TB treatment, hypoalbuminaemia may ultimately influence the hepatic metabolism of drugs, such as INH and RIF, thereby enhancing their hepatotoxicity.

Monitoring ALT levels was necessary to monitor hepatotoxicity, while detecting coagulation functions, sCr, UA, PLT, and ALB[13], which was necessary to estimate the severity of the patient's condition because of their significant correlations with the level of liver damage. Besides these, as the incidence of liver dysfunction increased dramatically in patients with a high viral load, the HBV DNA load is another important risk factor for liver failure. It may be advisable to con-

sider the use of antiviral drugs, such as a nucleoside analog to decrease HBV-DNA.

There are some limitations with regards to interpreting our study results. First, because this was a retrospective study, the liver function at the very beginning of anti-tuberculous treatment and other factors, such as the occurrence of single nuclear polymorphisms (SNPs) in the CYP2E1 gene or other drug-metabolizing enzymes could not be evaluated, as has been done in other articles[14]. Second, due to the lack of a pathological examination, we could not determine the exact cause of liver damage, whether it was HATT-induced or caused by a HBV hepatitis relapse, which can only be determined by re-challenge with anti-tuberculous therapy. We also had a limited number of cases enrolled in our study, which makes it more difficult to extend our observations to a larger population. Without a control group that included co-infected patients not receiving antiviral treatment, we cannot conclusively determine if the liver damage was induced by drug or virus. The lack of liver histology and quantitative comparison of HBsAg and HBeAg is a key limiting factor. Further study is mandatory to explore the therapeutic use of antiviral treatment in this subpopulation.

Nonetheless, routine measurements of HBsAg and viral loads should be considered in patients beginning antituberculous treatment due to the high prevalence of HBV infection and the likelihood that these measures may be helpful in planning the frequency of visits and liver function testing for TB patients. Judicious follow-up of the patient's clinical condition, liver function, and coagulation function, especially in patients with high HBV loads and hypoalbuminemia, should be carried out. It may be advisable to consider the use of antiviral drugs to prevent or limit liver damage during courses of anti-tuberculous treatment in HBV patients.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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