HEPATOTOXICITY IN PATIENTS CO-INFECTED WITH TUBERCULOSIS AND HIV-1 WHILE RECEIVING NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR-BASED ANTIRETROVIRAL THERAPY AND RIFAMPICIN-CONTAINING ANTI-TUBERCULOSIS REGIMEN

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Abstract. To evaluate the rate of and risk factors for hepatotoxicity in tuberculosis (TB) and human immunodeficiency virus type 1 (HIV-1) co-infected patients while receiving non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART) and a rifampicin (RMP)-containing anti-TB regimen. We analyzed data from the N2R study which was an open label, randomized, comparative trial comparing treatment outcomes between 71 TB/HIV-1 co-infected patients receiving efavirenz (EFV)-based and nevirapine (NVP)-based ART; all of whom were receiving RMP-containing anti-TB treatment. Demographic data, liver function test, CD4 cell count, plasma HIV-1 RNA, hepatitis B surface antigen and anti-hepatitis C virus antibody were collected before initiating ART (week 0). Liver enzymes and total bilirubin levels were monitored at 6 weeks, 12 weeks and 24 weeks after ART initiation. All patients were followed until TB therapy was completed. Of 142 patients, 8 patients were excluded. Among the remaining 134 patients, the mean ± SD age was 36.8 ± 8.6 years and 67.2% were male. Severe hepatotoxicity (grade 3 or 4) developed in 4 patients (2.9%); 3 patients (4.6%) in the NVP group and 1 patient (1.4%) in the EFV group. Severe hyperbilirubinemia (grade 3or 4) occurred in 7 patients (5.2%); 5 patients (7.7%) in the NVP group and 2 patients (2.9%) in the EFV group. Grade 1 or 2 hepatotoxicity occurred in 34 patients (31.4%). Hepatitis C virus co-infection (adjusted OR 3.03; 95%CI 1.26-7.29) was an independent risk factor associated with grade 1-4 hepatotoxicity (p=0.013). Monitoring of hepatotoxicity should be considered in TB/HIV-1 co-infected patients who are infected with HCV and receiving NVP.

Keywords: TB-HIV-1 co-infected patients, hepatotoxicity, NNRTI-based ART, RMP

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INTRODUCTION

Tuberculosis (TB) and human immunodeficiency virus type 1 (HIV-1) are the important health threats in many developing countries where the prevalences

of TB and HIV-1 infections are high. TB/HIV-1 co-infected patients have a higher mortality rate than patients diagnosed with TB alone (Yanai *et al*, 1996). Previous studies showed that antiretroviral therapy (ART) can reduce the risk of death in patients co-infected with TB and HIV-1 (Manosuthi *et al*, 2006; Varma *et al*, 2009). The World Health Organization recommends all TB/HIV-1 co-infected patients with clinical evidence of AIDS or a CD4 count < 350 cells/µl initiate ART during TB treatment (Harries *et al*, 2004). However, some adverse reactions may be worsened by concurrent therapy for both diseases. Hepatotoxicity is a side effect of both non-nucleoside reverse transcriptase inhibitor (NNRTI) based ART and first line anti-TB drugs. Previous studies from Thailand report the rate of anti-TB drug induced hepatitis is 9.2% (Krittiyanant *et al*, 2002) and the rate of NNRTI associated severe hepatotoxicity is 14% (Law *et al*, 2003). One study from another country reported the rate of NNRTI induced severe hepatotoxicity was 20% (Ena *et al*, 2003). Co-administration of NNRTI with first line anti-TB drugs may have overlapping hepatotoxicity. Little is known about hepatotoxicity in TB/HIV-1 co-infected patients receiving NNRTI-based ART and first line anti-TB drugs. Therefore we analyzed data from the N2R study which was a prospective randomized trial comparing plasma drug concentrations and efficacies between 2 NNRTI-based regimens in Thai HIV-1 infected patients who were receiving rifampicin (RMP)-containing anti-TB drugs (Manosuthi *et al*, 2009). The aim was to determine the rate of and risk factors associated with hepatotoxicity in these patients and compare the severity of hepatotoxicity in patients who receive nevirapine (NVP) and those who receive efavirenz (EFV).

MATERIALS AND METHODS

In the N2R study, 142 Thai patients (71 patients per group) with combined TB and HIV-1 infection were enrolled between December 2006 and October 2007 at Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health. The inclusion and exclusion criteria have been previously described (Manosuthi *et al*, 2009). All patients received oral lamivudine (150 mg every 12 hours) and oral stavudine (30 mg every 12 hours for those who weighed ≤ 60 kg and 40 mg every 12 hours for those who weighed >60 kg). Patients were randomized to receive either EFV 600 mg at bedtime while fasting or NVP 200 mg every 12 hours. In patients who received NVP, the starting dosage was 200 mg every 24 hours for the first 2 weeks then 200 mg every 12 hours thereafter. The dosage of rifampicin was 450 mg per day for patients who weighed ≤50 kg and 600 mg per day for those who weighed >50 kg. The anti-TB regimen included isoniazid (INH), RMP, ethambutol and pyrazinamide for the first 2 months followed by INH and RMP for the subsequent 4-7 months. Demographic characteristics, baseline liver function test, CD4 cell count, plasma HIV-1 RNA, HBV surface antigen (HBsAg) and anti-HCV antibody (anti-HCV) were collected at the time of ART initiation. Complete blood counts, blood chemistries and liver function tests were routinely monitored at 6, 12 and 24 weeks after starting ART.

In this study, patients who had no liver function test monitoring after ART initiation were excluded. Any hepatotoxic events noted at follow-up and any changes in medication due to hepatotoxicity were also obtained from the patient's medical records. All patients were followed until TB therapy was completed.

Hepatotoxicity was defined as an elevation in serum AST or ALT level from the normal range and was categorized according to a modified, standardized toxicity grade scale (Sulkowski *et al*, 2000). Patients with normal serum AST and ALT levels (AST = 0-37 and ALT< 41 IU/l) before ART initiation were classified based on changes relative to the upper limit of normal (ULN): grade 1, 1.25-2.5xULN; grade 2, 2.6-5xULN; grade 3, 5.1-10xULN; grade 4, greater than 10xULN. To avoid selection bias favoring the inclusion of persons with chronic viral hepatitis, patients with elevated serum AST and ALT levels before ART initiation were classified based on changes relative to the baseline value rather than to the ULN: grade 1, 1.25-2.5xbaseline; grade 2, 2.6-3.5x baseline; grade 3, 3.6-5xbaseline; grade 4, greater than 5x baseline. If the AST and ALT grades were discordant, the higher of the two grades was used for classification. Changes in serum total bilirubin level were classified based on changes relative to the ULN: grade 1, 1.1-1.5xULN, grade 2, 1.6-2.9xULN; grade 3, 3-5xULN; grade 4, greater than 5xULN. Severe hepatotoxicity was defined as grade 3 or 4 increases in serum AST or ALT levels. Severe hyperbilirubinemia was defined as grade 3 or 4 increases in serum total bilirubin levels.

Data collected in the study were analyzed using the Statistical Package for Social Sciences, version 15 (SPSS Inc, Chicago, IL). Categorical variables, such as patient gender, were expressed in frequencies and percentages, whereas numerical variables, such as patient age, were expressed in means and standard deviations or medians with inter-quartile range. The various risk factors in the study population were analyzed using the independent *t*-test and chi-square test to evaluate their association with the development of hepatotoxicity. The independent variables were then evaluated with binary logistic regression to identify significant factors in the development of hepatotoxicity. A p-value < 0.05 was considered to be statistically significant. The study was conducted in accordance with the guidelines of the Helsinki Declaration of 2000. The institutional ethics committees of the Bamrasnaradura Infectious Diseases Institute and the Thai Ministry of Public Health approved this study and all patients provided written, informed consent before enrollment.

RESULTS

Among 142 participants in the main study, 8 cases were excluded because no liver function tests were available. The reasons for lack of monitoring were death (5 cases), lost to follow-up (2 cases) and transferred out (1 case). All events occurred before week 6 after ART initiation. The baseline characteristics of the remaining 134 cases are shown in Table 1. The mean ± SD age was 36.8 ± 8.6 years and 67.2% of patients were male. HCV co-infection was found in 23.9% of patients. The median (IQR) time from TB diagnosis to ART initiation was 5.6 (4.8-9.2) weeks. The duration of TB therapy after ART initiation ranged from 6 to 92 weeks. The median (IQR) duration was 24 (18-36) weeks.

While receiving concomitant therapy, 4 patients (2.9%) developed severe hepatotoxicity. Of these, 2 patients had associated severe hyperbilirubinemia and the remaining patients had isolated elevations of AST or ALT levels. Severe hyperbilirubinemia occurred in 7 patients (5.2%). Of these, 2 patients had associated severe hepatotoxicity and 5 patients had associated grade 1 or 2 hepatotoxicity. The characteristics

Table 1
Baseline characteristics of 134 TB/HIV-1 co-infected patients.

Characteristics	Patients (*n*=134)
Males	90 (67.2)
Age, years, mean ± SD	36.8 ± 8.6
Body weight, kg, mean ± SD	53.3 ± 9.5
CD4 cell count, cells/mm^3, mean ± SD	69.1 ± 67.4
Plasma HIV-1 RNA level, $\log_{10}$ copies/ml, median(IQR)	5.75 (5.51-5.75)
ALT level, IU/l, mean ± SD	29.5 ± 19.1
ALT elevation (≥ 41 IU/l)	30 (22.6)
Site of TB	
Pulmonary	74 (55.2)
Extrapulmonary	27(20.1)
Combined	33 (24.6)
Positive acid-fast stain	80 (59.7)
HBV co-infection	7 (5.2)
HCV co-infection	32 (23.9)

Table 2
Characteristics of 9 TB/HIV-1 co-infected patients who developed severe hepatotoxicity or severe hyperbilirubinemia during receiving concomitant therapy.

Patient (age/gender)	Viral hepatitis co-infection	NNRTI use	Baseline ALP/AST/ALT/BILI	Elevated ALP/AST/ALT/BILI	Wks
27/male	HBV	NVP	62/63/52/0.4	85/185/239/0.8	6
37/male	HBV	EFV	74/45/36/0.3	142/366/456/1.0	24
30/male	HCV	EFV	174/44/35/0.7	222/71/100/4.8	6
30/male	HCV	NVP	770/30/9/0.8	1,176/96/47/4.3	6
37/male	HCV	NVP	497/41/21/1.1	180/162/125/11.8	12
44/male	HCV	NVP	91/21/24/2.5	72/133/87/6.4	12
36/male	HCV	EFV	151/61/17/3.5	130/139/41/15.1	6
33/male	HCV	NVP	141/31/21/0.8	94/272/178/9.3	24
34/female	no	NVP	154/21/63/0.7	510/115/269/7.3	6

ALP, alkaline phosphatase; BILI, total bilirubin; Wks, weeks
HBV, hepatitis B virus; HCV, hepatitis C virus; NVP, nevirapine; EFV, efavirenz

and patterns of liver function changes of these patients are summarized in Table 2. Severe hepatotoxicity occurred in 3 of 65 patients (4.6%) in the NVP group and 1 of 69 patients (1.4%) in the EFV group. Severe hyperbilirubinemia occurred in 5 patients (7.7%) in the NVP group and 2 patients (2.9%) in the EFV group. Grade 1 or 2 hepatotoxicity occurred in 43 patients (31.4%). Hepatotoxicity grades at weeks 6, 12 and 24 are shown in Fig 1. Grade 1-2 hepatotoxicity was found at weeks 6,

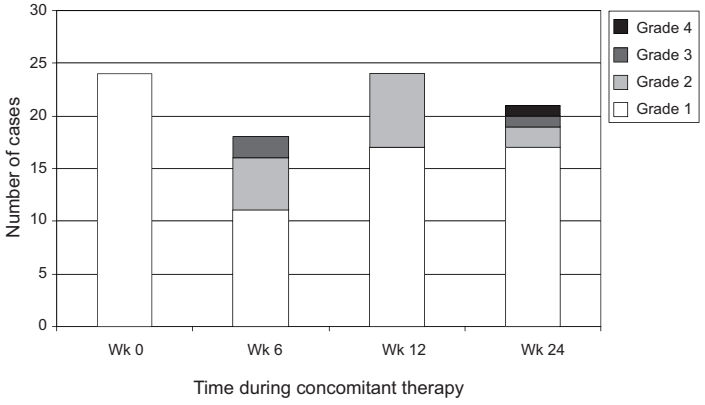

Fig 1–Hepatotoxicity grades during concomitant use of NNRTI and rifampicin in TB/HIV-1 co-infected patients.

12 and 24. Grade 3 hepatotoxicity was found at weeks 6 and 24 and grade 4 hepatotoxicity was found at week 24.

NVP was switched to EFV and RMP was discontinued in 4 patients due to grade 2-3 hepatotoxicity associated with severe hyperbilirubinemia. RMP was discontinued in 1 patient due to grade 2 hepatotoxicity associated with grade 4 hyperbilirubinemia. EFV was switched to lopinavir/ritonavir and RMP was discontinued in 1 patient due to grade 2 hepatotoxicity associated with

Table 3
Comparison between grade 1-4 hepatotoxicity group and non- hepatotoxicity group.

Characteristics	Grade 1-4 hepatotoxicity (*n*=46)	Non-hepatotoxicity (*n*= 88)	OR (95%CI)	*p*-value
Age, years, mean ± SD	35.5±7.0	37.8±9.2		0.140
Male sex	38 (82.6)	52 (59.1)	3.29 (1.37-7.87)	0.006
Baseline body weight, kg, mean ± SD	55.47±10.28	52.26±9.02		0.069
Baseline CD4 cell count, cells/mm^3, mean ± SD	56.89±57.45	75.51±71.52		0.129
Baseline HIV-1 RNA level, $\log_{10}$copies/ml, median (IQR)	5.70 (5.38-5.75)	5.75 (5.56-5.81)		0.091[a]
Baseline ALT level, IU/l, mean ± SD	27.41±16.00	30.57±20.58		0.367
Baseline ALT elevation (≥ 41 IU/l)	9 (20)	22 (25)	0.75 (0.31-1.80)	0.519
Baseline albumin, mg/dl, mean ± SD	3.62±0.90	3.55±0.78		0.666
Site of TB				0.271
Pulmonary	21 (45.7)	53 (60.2)		
Extrapulmonary	11 (23.9)	16 (18.2)		
Combined	14 (30.4)	19 (21.6)		
Positive acid-fast stain	26 (5.5)	54 (61.4)	0.82 (0.40-1.69)	0.587
Positive HBsAg	4 (8.7)	3 (3.4)	2.70 (0.58-12.61)	0.231[b]
Positive anti-HCV	18 (39.1)	14 (15.9)	3.40 (1.49-7.73)	0.003
Cotrimoxazole use	40 (87.0)	63 (71.6)	2.65 (0.99-7.02)	0.045
Fluconazole use	30 (65.2)	45 (51.1)	1.79 (0.86-3.74)	0.119
Nevirapine use	21 (45.7)	44 (50.0)	0.84 (0.41-1.72)	0.633

OR, odds ratio; CI, confidence interval; [a] Mann-Whitney *U* test; [b] Fisher's exact test

Table 4
Univariate and multivariate analysis of risk factors for grade 1-4 hepatotoxicity in TB/HIV-1 co-infected patients while receiving concomitant therapy.

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*p*-value	AOR (95%CI)	*p*-value
Positive anti-HCV	3.40 (1.49-7.73)	0.003	3.03 (1.26-7.29)	0.013
Male sex	3.29 (1.37-7.87)	0.006	2.25 (0.90-5.66)	0.084
Cotrimoxazole use	2.65 (0.99-7.02)	0.045	2.46 (0.87-6.98)	0.090

OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

grade 4 hyperbilirubinemia. Changing medication resulted in improvement of hepatotoxicity and hyperbilirubinemia in these patients. TB therapy was completed when grade 4 hepatotoxicity without hyperbilirubinemia occurred in 1 patient in the EFV group. On follow-up, the elevated liver enzymes improved despite continuation of EFV.

The baseline characteristics, CD4 cell count, plasma HIV-RNA level, HBV co-infection, HCV co-infection and other drugs used were compared between the grade 1-4 hepatotoxicity group and the non-hepatotoxicity group. On univariate analysis, Cotrimoxazole use (OR 2.65; 95%CI 0.99-7.02), male sex (OR 3.29; 95%CI 1.37-7.87) and HCV co-infection (OR 3.40; 95%CI 1.49-7.73) were associated with grade 1-4 hepatotoxicity (Table 3). On multivariate analysis, HCV co-infection (AOR 3.03; 95%CI 1.26-7.29) was the only independent risk factor associated with grade 1-4 hepatotoxicity (Table 4).

DISCUSSION

Hepatitis developing in TB/HIV-1 co-infected patients while receiving both therapies concurrently causes difficulty in management because both NNRTI and first line anti-TB drugs can induce hepatotoxicity. Disseminated forms of TB or underlying chronic viral hepatitis may complicate the problem. In our study, hepatotoxicity in 6 patients led to discontinuation of two drugs, RMP and the NNRTI. RMP was considered to be the offending agent because severe hyperbilirubinemia associated with grade 1 or 2 hepatotoxicity, in 5 patients with this problem, is characteristic of RMP induced hepatitis.

Previous studies report the rates of severe hepatotoxicity in TB/HIV-1 co-infected patients receiving both NNRTI based ART and RMP containing anti-TB drugs concurrently range from 0.6% to 13% (Breen *et al*, 2006; Sathia *et al*, 2008; Shipton *et al*, 2009; Moses *et al*, 2010). In our study the rate of severe hepatotoxicity was 2.9% which is within the range of previous studies. Wide variation in the rates of hepatotoxicity reported in our study and previous studies is probably due to differences in population characteristics, different definitions of severe hepatotoxicity, and the frequency of monitoring. Compared with the rate of severe hepatotoxicity in HIV-1 patients who

receive NNRTI based ART alone (Ena *et al*, 2003; Law *et al*, 2003) or the rate of severe hepatotoxicity in TB/HIV-1 co-infected patients who received anti-TB drugs alone (Ozick *et al*, 1995; Pukenyte *et al*, 2007), the rate of severe hepatotoxicity in TB/HIV-1 co-infected patients receiving therapy for both infections concurrently in this study was not higher than when both treatments were given separately.

Currently available information regarding the frequency of hepatotoxicity related to NVP and EFV shows NVP causes hepatotoxicity more frequently than EFV (Rivero *et al*, 2007). Our study also found that severe hepatotoxicity and severe hyperbilirubinemia occurred more frequently in the NVP group than in the EFV group. NVP should be used with caution in TB/HIV-1 co-infected patients who are receiving RMP-containing anti-TB drugs.

HBV or HCV co-infection is a known risk factor for NNRTI induced hepatotoxicity in HIV-1 infected patients (Sulkowski *et al*, 2002; Ena *et al*, 2003; Law *et al*, 2003) and HCV co-infection is a risk factor for anti-TB drug induced hepatitis in TB/HIV-1 co-infected patients (Ungo *et al*, 1998). In this study HCV co-infection was the only independent risk factor associated with grade 1-4 hepatotoxicity. HBV co-infection was found more frequently in the hepatotoxicity group than in the non-hepatotoxicity group, but the difference was not statistically significant. The mechanism of liver injury mediated by HBV infection is related to immune restoration after ART, but the mechanism by which HCV leads to elevated liver enzymes is unclear. It may induce liver damage by a direct cytotoxic effect or by stimulation of immune response (Jain, 2007).

There were some limitations to this study. First, monitoring for hepatotoxicity covered 24 weeks of concomitant therapy in most patients; however, some patients received more than 24 weeks of concomitant therapy and late-onset hepatotoxicity might have been missed. Second, a history of alcohol intake, illicit drug use and herbal medicine use was not obtained in this study. These factors may contribute to hepatotoxicity. Lacking these variables makes the study of risk factors for hepatotoxicity imperfect.

In conclusion, our findings suggest the incidence of severe hepatotoxicity is not increased in TB/HIV-1 co-infected patients who receive NNRTI based ART and rifampicin containing anti-TB drugs concurrently. HCV co-infection is an independent risk factor for any grade of hepatotoxicity in TB/HIV-1 co-infected patients. Due to higher rates of severe hepatotoxicity and severe hyperbilirubinemia in patients who receive NVP than in patients who receive EFV, we recommend monitoring for hepatotoxicity should be considered in TB/HIV-1 co-infected patients who are infected with HCV and receive NVP.

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