Polymorphism of *PXR* gene associated with the increased risk of drug-induced liver injury in Indonesian pulmonary tuberculosis patients

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SUMMARY

What is known and objective: Tuberculosis is still a major infectious disease in Indonesia. Patients are treated mostly using fixed-dose combination treatment in primary public health facilities. The incidence of antituberculosis drug-induced liver injury (AT-DILI) is approximately 10% among Indonesian tuberculosis patients who used standard fixed combination regimens during the intensive phase of treatment. However, information regarding genetic polymorphism associated with the increase risk of drug-induced liver injury is still limited. The aim of this study was to investigate pregnane X receptor (*PXR*) gene polymorphisms as one of the risk factors of AT-DILI.

Methods: In this prospective cohort study, we recruited 106 adult patients diagnosed with pulmonary tuberculosis and treated with category I FDC (fixed-dose combination). The identification of SNP -25385C>T (rs3814055) was conducted by ARMS (amplification refractory mutation system). Hepatotoxicity was defined as ALT and/or AST levels above the normal threshold on the second, fourth and sixth months of monitoring during tuberculosis treatment.

Results and discussion: The logistic regression analysis showed that patients with the TT genotype of PXR gene (rs3814055) significantly had a greater risk of AT-DILI (OR 8·89; 95% CI 1·36–57·93, $P < 0.05$), compared with those of wild-type CC genotype.

What is new and conclusion: The result suggests that in Indonesian patients with tuberculosis, the risk of having AT-DILI was associated with TT genotype of the *PXR* gene.

WHAT IS KNOWN AND OBJECTIVE

Tuberculosis (TB) is an infectious disease that is still a major problem in developing countries, including Indonesia. In 2014, the World Health Organization categorized Indonesia as a high-TB-, multi-drug resistant (MDR)-TB-, and human immunodeficiency virus (HIV)-burdened country. There are an estimated 9·0 million incident cases of TB and 1·5 million people died of the disease each year (1·1 million deaths were HIV negative, and 360 000 were HIV positive). The corresponding figures for Indonesia are 325 582, and 7964 cases of TB have been identified as new and relapse cases,

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respectively.[1] Given this high burden of disease, the government of Indonesia is focusing on the control and elimination of TB. One of the efforts is through the directly observed treatment, short-course strategy (DOTS) programme.

One of the main concerns in antituberculosis drugs (ATDs) is drug-induced liver injury (DILI) or hepatotoxicity caused by drugs.[2] Of the first-line anti-TB drugs, isoniazid, pyrazinamide and rifampicin can all cause liver damage (drug-induced hepatitis).[3] In addition, rifampicin can cause asymptomatic jaundice without evidence of hepatitis.[3] A previous publication has shown that the incidence of antituberculosis drug-induced liver injury (AT-DILI) is around 10% among Indonesian TB patients treated with standard fixed combination regimens during the intensive phase of treatment.[4] Many researchers have reported on specific gene polymorphisms as risk factors for those hepatotoxic effects. Some of the reported genes are N-acetyltransferase 2 (*NAT2*),[5–10] cytochrome P450 2E1 (*CYP2E1*),[9,11,12] glutathione S-transferase mu-1 (*GSTM1*)[9,10,13] and glutathione S-transferase theta (*GSTT*).[9,13] Other genes also reported to be potential predictors of ATDs-DILI are pregnane X receptor (*PXR*), glutathione S-transferase alpha-1 (*GSTA1*), manganese superoxide dismutase (*MnSOD, SOD2*), UDP-glucuronosyltransferase (*UGT*), nitric oxide synthase 2A (*NOS2A*), BTB and CNC homology 1 (*BACH1*), Maf basic leucine zipper protein (*MAFK*), and human leucocyte antigen (*HLA*).[14–18]

We examined *PXR* gene polymorphism because ligand formation between rifampicin and PXR could trigger the expression of various genes, including various cytochromes and carboxylesterases that may be relevant to the metabolism of isoniazid in producing hepatotoxic metabolites. *PXR* plays a role in the regulation of a number of hepatic and intestine genes involved in detoxification and elimination of xenobiotics.[19] The polymorphisms are generally located in the 5′-flanking region of the target genes.[20] PXR activates various genes through binding and heterodimer formation with the retinoid X receptor (RXR). PXR ligands stimulates the expression of genes involved in xenobiotics oxidation (phase I), conjugation (phase II) and transport (phase III) in the liver. PXR is involved in phase I metabolism through the expression of *CYP2B6, CYP2C8, CYP2C9, CYP2B9* and *CYP2C19*.[21–25] Moreover, PXR is involved in phase II metabolism through the expression of glutathione S-transferase (GST), sulfotransferase, UDP-glucuronosyltransferase and carboxylesterase.[26–31] PXR is involved in phase III through the expression of organic anion transporting polypeptide 2(OATP2) and multiple drug resistance protein 2 (MRP2).[32–34] This study aimed to investigate possible associations of *PXR* gene polymorphisms with the increased risk of ATDs-DILI.

METHODS

Patients

A total of 106 patients with active lung TB who visited selected primary public health facilities in Bandar Lampung and Yogyakarta were enrolled consecutively and followed up in this prospective cohort study. Inclusion criteria were as follows: (i) adult patients (over 18 years of age) newly diagnosed with active lung TB, (ii) treated with fixed-dose combination of antituberculosis drugs (FDC-ATD) category I using directly observed treatment, short-course strategy (DOTS) and (iii) have agreed to the terms in the written informed consent. Patients with any of the following conditions were excluded from the study: (i) positive HIV/AIDS, (ii) abnormal serum ALT and AST levels over twice upper limit of normal (ULN) value before treatment with ATD, (iii) with hepatitis or have a history of hepatitis, (iv) haemoglobin serum <8 mg/dL, (v) not having ATDs for over 2 weeks, (vi) have a history of kidney disease and (vii) refusal to blood collection.

All patients received FDC-ATD category I intensive phase (rifampicin 150 mg, isoniazid 75 mg, pyrazinamide 400 mg and ethambutol 275 mg per tablet) and FDC-ATD category I continuation phase (rifampicin 150 mg and isoniazid 150 mg) in 6 months of therapy. The dosage of ATDs was selected according to the patients' weight. ALT and AST serum levels were obtained five times: before ATD treatment, after completing the second, the fourth, the sixth months of ATD therapy and 1 month after the patient finished the last ATDs therapy. Antituberculosis drug-induced liver disease (ATD-DILI) was designated as an increase in serum ALT and AST levels above the ULN after ATDs treatment, according to the criteria of drug-induced liver injuries developed by Drug-Induced Liver Injury Network (DILIN), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and Division of Specialized Information Services of the National Library of Medicine (NLM), and National Institutes of Health and Common Toxicity Criteria for Adverse Events 4.0 version (CTCAE v.4.0, Bethesda, MD, USA).[35]

The written informed consent was obtained from each patient enrolled in this study. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee for Health Research, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia.

Single Nucleotide Polymorphism genotyping

DNA was extracted from the peripheral blood sample collected from each patient using the standard proteinase K digestion and GeneJET Genomic DNA Purification Kit (Thermo Scientific®, Walthman, MA, USA.). Polymerase chain reaction–amplification refractory mutation system (PCR-ARMS) was used to detect the -25385C>T (rs3814055) PXR polymorphisms located in the 5′UTR promoter. The two primer sets were as follows: forward 5′-TTTTGGCAATCCCAGGTTT-3′ to detect PXR polymorphic gene (F-primer – 1) and 5′-TTTTGGCAATCCCAGGTTC-3′ to confirm normal fragment gene (F-primer – 2); reverse 5′-CGAATGTGGTG-GATACCAG-3′. PCR was carried out using 5x FIREPol® Master Mix Ready from Solis BioDyne® (Tartu, Estonia), according to the manufacturer's instruction. Initial denaturation at 95 °C for 4 min was followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 59·7 °C, extension at 72 °C for 1 min, with final extension at 72 °C for 7 min. The products obtained were electrophoresed on 2% agarose gels. Polymorphic gene TT showed 219-bp PCR product only when it was identified using F-1 forward primer, non-polymorphic gene CC showed 219 bp only when it was identified using F-2 reverse primer, and heterozygote gene CT showed 219 bp when it was identified using both forward primers.

Statistical analysis

Allele frequencies were calculated, and the agreement between the allele frequencies and Hardy–Weinberg equilibrium was tested using a chi-square test (d.f. = 1) for each locus. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using binomial logistic regression analysis, adjusting for sex and age. The baseline ALT and AST differences among genotype groups were determined using the Kruskal–Wallis test. Statistical analyses were performed using a data analysis freeware.

RESULTS AND DISCUSSION

Results

The result of UV gel visualization is shown in Fig. 1. Homozygotes TT and CC showed single bands on F-primer 1 and F-primer 2, respectively. Heterozygote CT showed double bands with the F-primer 1 and F-primer 2.

Seven patients (6·6%) were TT homozygotes, 32 patients (30·19%) were CT heterozygotes, and most patients (67; 63·21%) were wild-type CC homozygotes. The genotype frequencies were not different from those predicted by the Hardy–Weinberg equation (Table 1).

Table 2 shows that there was no statistical difference in ALT and AST baseline levels among both male and female patients with different genotypes ($P > 0·05$). The mean of ALT and AST levels increased at the end of the first 2 months of ATD therapy or after completion of the intensive phase and decreased during the continuous phase (Table 3). Most of the patients (71 patients; 66·98%) experienced no hepatotoxicity, but 35 patients showed elevated transaminase levels. The transaminase elevation was seen in 33 patients (31·13%) with mild hepatotoxicity (1+) and 2 patients (1·89%) with moderate hepatotoxicity (2+) (Table 4).
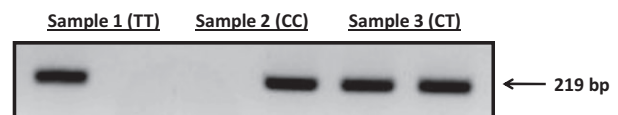

Fig. 1. UV visualization result of 25385C>T (rs3814055) polymorphisms.

Table 1. Comparison of observed and expected genotype frequency based on Hardy–Weinberg equilibrium

Genotypes	Observed	Expected	P value
CC	67	65	0·25
CT	32	36	
TT	7	5	

Table 2. Characteristics of patients according to genotype

	Genotype						
	TT		CT		CC		
	n (%)	Mean ± SD	*n* (%)	Mean ± SD	*n* (%)	Mean ± SD	*P* value
Sex							
Male	5 (71·43)		18 (56·25)		49 (73·13)		
Female	2 (28·57)		14 (43·75)		18 (26·87)		
Total	7 (100·00)		32 (100·00)		67 (100·00)		
Age group (years)							
18–45	4 (57·14)		23 (71·88)		46 (68·66)		
46–55	1 (14·29)		6 (18·75)		13 (19·40)		
>55	2 (28·57)		3 (9·38)		8 (11·94)		
Total	7 (100·00)		32 (100·00)		67 (100·00)		
ALT baseline							
Male		31·20 ± 8·55		24·56 ± 2·87		21·22 ± 1·96	0·195
Female		12·00 ± 2·00		17·07 ± 3·13		14·94 ± 1·38	0·767
AST baseline							
Male		33·60 ± 8·70		26·00 ± 3·00		21·49 ± 1·35	0·053
Female		17·50 ± 2·50		21·21 ± 2·63		20·67 ± 1·40	0·754

Table 3. Serum aminotransferase level cohort data according to sex

	Min (g/dL)	Max (g/dL)	Mean ± SE (g/dL)
Male			
ALT			
Baseline	5·00	91·00	22·75 ± 1·63
2nd month	9·00	218·00	25·43 ± 3·18
4th month	5·00	46·00	18·00 ± 0·97
6th month	5·00	77·00	19·31 ± 1·48
8th month	7·00	76·00	20·96 ± 1·39
AST			
Baseline	8·00	70·00	23·46 ± 1·36
2nd month	10·00	167·00	27·76 ± 2·27
4th month	11·00	46·00	23·81 ± 0·87
6th month	9·00	63·00	24·12 ± 1·03
8th month	9·00	51·00	22·93 ± 0·88
Female			
ALT			
Baseline	8·00	55·00	15·65 ± 1·47
2nd month	8·00	49·00	18·91 ± 1·79
4th month	5·00	59·00	17·35 ± 2·19
6th month	6·00	74·00	19·74 ± 3·05
8th month	5·00	64·00	20·74 ± 2·24
AST			
Baseline	11·00	52·00	20·71 ± 1·30
2nd month	10·00	49·00	22·91 ± 1·49
4th month	10·00	53·00	23·18 ± 1·63
6th month	8·00	67·00	23·47 ± 2·13
8th month	13·00	44·00	23·12 ± 1·19

Table 4. Frequency of hepatotoxicity according to severity of drug-induced hepatotoxicity

Drug-induced Hepatotoxicity Level	*n* (%)
1+, Mild (<2·5 × ULN)	33 (31·13)
2+, Moderate (2·5–5 × ULN)	2 (1·89)
No hepatotoxicity	71 (66·98)
Total	106 (100·00)

the wild-type (CC genotype) (Table 5). The results also show that the combined (CT + TT) genotype had a tendency for higher incidence of ATD-DILI in comparison with the wild-type CC genotype (OR: 1·32; 95% CI: 0·56–3·14; *P* = 0·525). We also found that the risk of an ATD-DILI in females was 2·64-fold (OR: 2·64; 95% CI: 1·06–6·57; *P* = 0·036) that of males.

Discussion

We found that there was a significant association between the variant TT homozygote of *PXR* (-25385C>T; rs3814055) and the risk of AT-DILI. This result is consistent with results reported by Andrews *et al.*[36] who reported that a different *PXR* polymorphism at the same location was significantly associated with liver injury caused by flucloxacillin, a PXR agonist. Andrews *et al.* concluded that the CC genotype was associated with the risk of flucloxacillin-induced liver injury. However, a previous study conducted in Europe and South Asia reported no association between ATDs and PXR gene polymorphism in patients.[37]

As genetic polymorphism is only one of the many factors that may contribute to drug-induced hepatotoxicity, its significance requires further testing.[38] Rifampicin is a PXR agonist. PXR is involved in the regulation of bile acid and cholesterol homeostasis.

The risk of having ATD-DILI was significantly associated with the TT genotype. For this genotype, the risk of having an ATD-DILI was 8·89-fold (OR: 8·89; 95% CI: 1·36–57·93; *P* = 0·022) that of

Table 5. Results of logistic regression analysis

	Non-hepatotoxic n (%)	Hepatotoxic		
		n (%)	OR (CI 95%)	P value
Genotype				
CC	47 (66·2)	20 (57·14)	1·00[a]	
TT + CT	24 (33·8)	15 (42·86)	1·32 (0·56–3·14)	0·525
TT	2 (2·82)	5 (14·29)	8·89 (1·36–57·93)	0·022*
CT	22 (30·99)	10 (28·57)	0·87 (0·33–2·27)	0·770
Sex				
Male	53 (74·65)	19 (54·29)	1·00[a]	
Female	18 (25·35)	16 (45·71)	2·64 (1·06–6·57)	0·036*
Age			0·97 (0·93–0·99)	0·044*

[a]Reference category.
*Statistically significant $P < 0.05$.

Some of the bile acids are toxic. Therefore, the regulation by PXR of the expression of proteins involved in both phase I and phase II drug metabolism and transport requires further investigation.[39] Murine studies with the humanized pregnane X receptor (hPXR) suggested that rifampicin and isoniazid combination therapy leads to accumulation of protoporphyrin IX, an endogenous hepatotoxin through a mechanism involving PXR's effect on the haem biosynthesis pathway.[40]

This study also indicates that females are significantly more likely to show ATD-DILI than males. This is consistent with some previous studies which showed that female patients were more likely to show hepatotoxicity.[41–43] They also reportedly show a higher risk of hepatotoxicity with nitrofurantoin, erythromycin, flucloxacillin, minocycline and isoniazid.[44] Drug-induced hepatotoxicity in females tends to be hepatocellular with vascular damage on the centrilobular vein.[44,45] The presence of genetic abnormality in the mitochondria is also said to be a major determinant of idiosyncratic drug-induced hepatotoxicity especially in women and the elderly.[46]

Our study has some limitations because we did not use other liver function parameters such as total serum bilirubin, serum ALP, INR and GGT. Their measurements would have been useful in defining liver function and the severity of hepatotoxicity better. We were also not able to assess other confounding factors, such as exposure to hepatotoxic agents including commonly encountered items such as acetaminophen, potentially hepatotoxic herbal and food products, and other chemicals in the patient's environment. The small number of PXR T homozygotes calls for a larger confirmatory study.

WHAT IS NEW AND CONCLUSION

We found a significant association between the PXR TT variant (-25385C>T; rs3814055) and the AT-DILI. Whether testing for PXR genotype will provide reliable predictions of AT-DILI requires further validation.

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CONFLICT OF INTEREST

None declared.

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